The physiological basis of vigour control by apple rootstocks – an unresolved paradigm

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Abstract

For millennia, scions have been grafted onto dwarfing apple rootstocks to reduce final tree size. However, it is unclear how scion architecture is first modified by the dwarfing apple rootstock, the time from grafting when this occurs and the endogenous hormonal signalling mechanisms that may cause the initial modifications in growth that then define the future architecture of the scion. In this study, the dwarfing (M.9) rootstock significantly decreased the mean total shoot length and node number of 'Royal Gala' apple scions by the end of the first year of growth from grafting when compared with rootstock(s) of greater vigour (MM.106, M.793 and a 'Royal Gala' rootstock control). Similarly, the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) applied to the stem of vigorous rootstocks significantly decreased mean total shoot length and node number of the scion, and the architectural changes imposed were generally similar to those imposed by M.9. For example, both treatments decreased the mean length and node number of the primary shoot, reduced the formation of secondary axes on the primary shoot and caused a greater proportion of primary and secondary shoots (if present) to terminate growth early. Decreased formation of secondary axes imposed by both treatments was reversed by applying the cytokinin benzylaminopurine (BAP) repeatedly to the scion, whilst applications of gibberellins (GA_{4+7}) reduced the proportion of primary and secondary shoots that terminated growth early, therefore increasing the final mean length and node number of these shoot types. Both M.9 and NPA also significantly decreased the final mean dry mass and length of the root system. Given these general similarities, it is proposed that the basipetal IAA signal is of central importance in rootstock-induced scion dwarfing, and that a shoot/root/shoot signalling mechanism may exist whereby the stem tissue of the M.9 rootstock decreases the basipetal transport of IAA to the root during summer, thereby decreasing root growth and the amount of root-produced cytokinin and gibberellin transported to scion. Reduced amounts of cytokinin transported to the scion may decrease branching, whilst reduced amounts of gibberellins may decrease the duration for which a large proportion of primary and secondary shoots grow. Analysis of endogenous hormones for newly grafted composite 'Royal Gala' apple trees on rootstocks of different vigour provided some additional support for these ideas. It is recommended that future studies elucidate what unique properties of the M.9 bark act to restrict IAA transport, whilst it is concluded that gene(s) regulating rootstock-induced

scion dwarfing are likely to control processes within the rootstock that modify the metabolism of IAA, its basipetal transport and the subsequent synthesis of root-produced vigour-inducing hormones including cytokinins and gibberellins.

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Extended thesis summary

Recent research has identified a dwarfing locus (*DW1)* involved in the dwarfing mechanism of the M.9 rootstock (Rusholme-Pilcher et al., 2008) and a new genetic map has been constructed of apple rootstock progeny derived from a cross between M.9 and 'Robusta 5' (Celton et al., 2009). These are important scientific advancements in the attempt to breed new and improved dwarfing apple rootstocks. For example, the identification of genetic markers linked to genes involved in rootstock-induced scion dwarfing, and other important traits like pest and disease resistance, will enable desirable progeny to be selected from large populations of tree material at a very young age. Therefore, efficiency and effectiveness of rootstock breeding programmes will be increased. Genetic maps constructed for rootstock progeny derived from a M.9 x 'Robusta 5' cross (Celton et al., 2009) also have potential application in further elucidating the genetic control of rootstock-induced dwarfing of the scion.

For the dwarfing apple rootstock, the fundamental biological processes that dwarfing gene(s) control are poorly understood, particularly the underlying physiological mechanism(s) that are first modified within the composite apple tree growing on a dwarfing rootstock, and their consequent expression in scion architecture during early tree phenology. Important physiological mechanisms by which dwarfing apple rootstocks decrease scion vigour may involve restricting the endogenous transport of nutrients, water and hormones. The most plausible of these is the modification of shoot/root/shoot signalling of endogenous plant hormones because alterations in their transport appear to explain some architectural changes imposed on the scion by the dwarfing apple rootstock. However, it is poorly understood how the modification of hormonal signals by a dwarfing apple rootstock may change scion architecture soon after grafting of the composite tree. Understanding this is essential to clearly identify those signals and processes that are the first physiological causes of scion dwarfing from those that are subsequent developmental effects.

Therefore, 'Royal Gala' apple scions were grafted onto rootstocks of M.9 (dwarf), MM.106 (semi-vigorous), M.793 (vigorous) and 'Royal Gala' (self-rooted, very vigorous; control) to determine how each rootstock type initially modified scion architecture after propagation of the composite tree. These modifications were also

compared with those of root restriction and plant growth regulators applied to either the scion (± gibberellins (GA_{4+7}), ± benzylaminopurine (BAP)) or an auxin transport inhibitor (± 1-N-naphthylphthalamic acid (NPA)) applied to the stem of the rootstock.

In four different experiments, M.9 decreased the mean total shoot length and node number of 'Royal Gala' apple scions by the end of the first year of growth from grafting when compared with rootstocks of greater vigour (Chapters 3, 4, 5, and 6 and see Figure B for a typical example). The mean cumulative length and node number of the primary shoot was initially similar amongst rootstocks prior to December. Thereafter, cumulative growth of the primary shoot on M.9 was decreased compared with rootstocks of greater vigour. This occurred because a greater proportion of primary shoots either underwent a bicyclic pattern of growth in December (Chapter 4) or February (Chapter 5) and then terminated growth earlier in April (Chapter 4), or grew from a continuous season-long growth flush before terminating growth earlier in April (Chapter 3). The general effect of these growth patterns imposed by M.9 was to decrease the final mean length and node number of the primary shoot (Figure A) by complete growth cessation (Chapters, 3, 4 and 5). The mean internode length of the primary shoot was unaffected by rootstock type, hence the primary shoot on M.9 was shorter (i.e., Chapters 3, 4 and 5) because of fewer neoformed nodes. In Chapter 6, however, cumulative growth of the primary shoot on M.9 was similar to rootstocks of greater vigour, and the dwarfing effect mostly resulted from the development of fewer secondary shoots. The M.9 rootstock also decreased the formation of secondary axes on the primary shoot during the first year of growth from grafting, particularly secondary shoots (Figure B), and this was an important architectural change that also contributed to reduced total shoot growth of the 'Royal Gala' scion growing on M.9 (Chapters 3, 5, and 6 and see Figure B). In Chapter 4, however, the scion on M.9 produced a greater mean number of secondary axes compared with that on MM.106, although this was not a typical effect of M.9, and may have been imposed by transplanting the tree material into the field in December.

In each experiment, M.9 formed proportionally more secondary spurs (i.e., < 25 mm) and short secondary shoots (i.e., ≥ 25 mm but with ≤ 10 nodes) compared with rootstocks of greater vigour. Both of these shoot types may have formed solely from preformed primordia within the vegetative axillary bud and terminated very soon after

their outgrowth. The M.9 rootstock also decreased the proportion of secondary shoots with more than 10 nodes, or those that had produced neoformed nodes, particularly very long secondary shoots with more than 20 nodes. This was a likely result of proportionally more secondary shoots completely terminating growth early in February and March when the scion was grown on M.9. Regardless of rootstock type, secondary shoots with the same node number were of almost identical length. Hence, M.9 did not affect internode length of 'Royal Gala' shoots, and this result supports the findings of other previous studies (Selezynova et al., 2003, 2008).

The application of NPA to the rootstock stem of MM.106, M.793 and 'Royal Gala' significantly decreased the final mean total length and node number of the scion, and the architectural modifications were most similar to those that occurred for untreated 'Royal Gala' trees growing on M.9 (compare Figures B and F). For example, following an application of NPA, the shoot apical meristem (SAM) on the primary shoot slowed, and/or, terminated its growth temporarily, thereby decreasing the final mean length and node number of the primary shoot. For both M.9 and NPA-treated trees, reduced cumulative growth of the primary shoot was reversed with GA_{4+7} applied repeatedly to the scion (Figure A), however few additional secondary and tertiary axes developed on the scion without applications of BAP (Figure B, D, F and H). The NPA treatment also decreased the formation of secondary axes on the primary shoot, and like M.9, the formation of secondary axes on the primary shoot was reinstated with exogenous BAP (Figures B, C, F and G). However, new secondary axes that formed for the BAP-treated scion on M.9 or NPA-treated rootstocks generally terminated without exogenous GA_{4+7} (compare C and E or G and I for M.9 or NPA, respectively).

Sequential applications of BAP followed by GA_{4+7} to the scion on M.9 increased branch formation and decreased the proportion of primary and secondary shoots that terminated growth early during summer. Consequently, the dwarfing effect of M.9 was reversed to some extent, particularly for the BAP x GA_{4+7}-treated scion on M.9 and MM.106 that developed very similar total shoot growth (Figure E). In addition, total shoot length and node number of the BAP x GA_{4+7}-treated scion on M.9 was much greater than that of the untreated tree grown on the 'Royal Gala' rootstock. However, the BAP x GA_{4+7}-treated scion on M.9 was still markedly smaller than the BAP x GA_{4+7}-treated scion on M.793 or 'Royal Gala' (Figure E). Similarly, BAP x GA_{4+7} applied to the scion on

NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' increased total shoot growth of the scion (Figures F and I). However, BAP x GA_{4+7} applied to the scion on NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' stimulated markedly less total shoot extension growth when compared with the BAP x GA_{4+7}-treated scion on the same rootstock type that was not treated with NPA (compare Figures E and I). Hence, BAP x GA_{4+7} could not fully reverse reductions in total scion growth whilst IAA transport from shoot to root was impaired by the NPA treatment, and this may explain why the BAP x GA_{4+7}-treated scion on M.9 was smaller than the BAP x GA_{4+7}-treated scion on M.793 and 'Royal Gala' (Figure E).

Treatments that decreased the size of the root system, such as M.9, NPA and root restriction, also decreased the total shoot growth or size of the scion. This indicated that a functional relationship existed between the size of the root and shoot, and that part of the dwarfing effect imposed by M.9 may be explained because of its smaller root system. However, some physiological mechanisms regulating scion vigour for M.9 and root restriction differ because, unlike M.9, decreased formation of axillary axes imposed by root restriction was not fully reversed with BAP applied repeatedly to the scion, and root restriction tended to decrease the size of leaves.

Results from this study, based on both application of plant growth regulators and analysis of endogenous hormones, have led to the conclusion that the basipetal IAA signal is of central importance in rootstock-induced scion dwarfing. A shoot/root/shoot signalling mechanism may exist whereby the stem tissue of the M.9 rootstock decreases the basipetal transport of IAA to the root during the summer, thereby decreasing root growth and the amount of root-produced cytokinin and gibberellin transported to scion, which consequently decreases either branch formation or the duration for which primary and secondary shoots grow, respectively. In partial support of this hypothesis, the M.9 rootstock had a significantly lower concentration of GA_{19} in the xylem sap during March (Chapter 6). However, further research would be required to show more convincingly that the above model of growth regulation is reflected in the endogenous transport of hormones. In particular, it would be important to demonstrate that decreased shoot/root basipetal transport of IAA by the M.9 rootstock reduces root/shoot transport of either cytokinins or gibberellins, and that decreased root/shoot transport of cytokinins precede the period(s) in the growing season when axillary bud outgrowth

occurs, whilst decreased root/shoot transport of gibberellins precedes the predominant time when shoot termination first occurs on the scion. It is also recommended that future studies elucidate what unique properties of the M.9 bark act to restrict IAA transport because it is likely that primary gene(s) regulating rootstock-induced scion dwarfing control processes within the rootstock that affect the transport and metabolism of IAA.

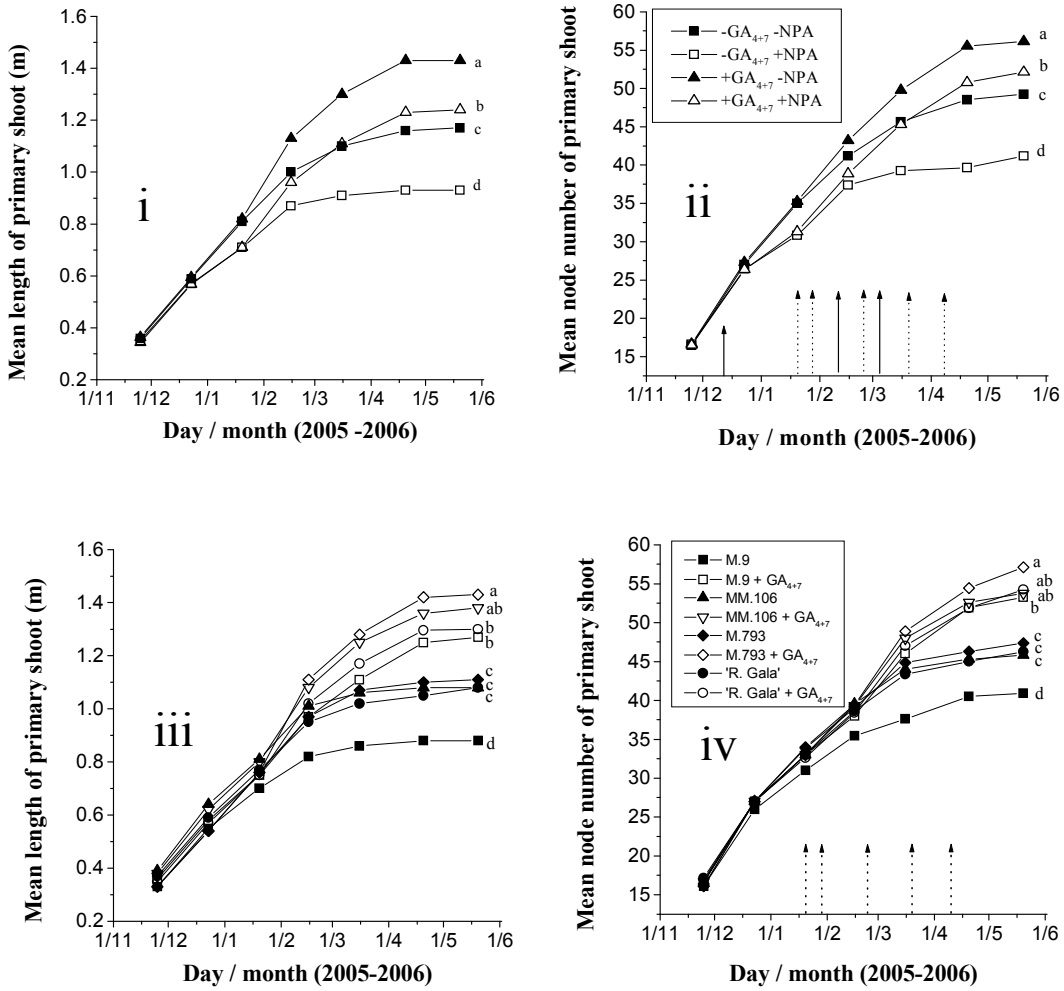

Figure A. Gibberellin (GA_{4+7}) x 1-N-naphthylphthalamic acid (NPA) (i and ii) and rootstock x GA_{4+7} interactions (iii and iv) for the mean cumulative length and node number of 'Royal Gala' primary shoots during their first growing season from grafting. Arrows on ii or iv with a dotted line denotes the timing of GA_{4+7} treatments to the scion, whilst arrows with a solid line on ii denote the timing of NPA treatment to the rootstock stem. On a single graph, means sharing the same letter are not significantly different. Mean separation in May is at $P \leq 0.11$ for i and $P \leq 0.05$ for ii, iii and iv (lsmeans tests with Tukey's adjustment, SAS). Data for i and ii are averaged over BAP and rootstock treatments, whilst iii and iv are averaged over BAP and NPA treatments.

Figure B. Effect of M.9 (dwarfing), MM.106 (semi-vigorous), M.793 (vigorous) and 'Royal Gala' (very vigorous control) rootstocks (from left to right, respectively) on the architecture of 'Royal Gala' apple scions by the end of their first growing season from grafting. Yellow rule is 1 m. **The M.9 rootstock typically imposed scion dwarfing by decreasing branch formation and reducing the final length of the primary and secondary shoots by increasing the proportion of these shoots that terminated growth early.**

Figure C. Effect of benzylaminopurine (BAP) applied repeatedly to 'Royal Gala' apple scions on scion architecture by the end of the first growing season after grafting the scion onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (left to right, respectively). Yellow rule is 1 m. **Decreased branching imposed by M.9 (see Figure B) was reversed by applying BAP to the scion, therefore indicating the dwarfing apple rootstock may decrease the transport of endogenous cytokinins to the scion, which reduces branching.**

Figure D. Effect of gibberellins (GA_{4+7}) applied repeatedly to 'Royal Gala' apple scions on scion architecture by the end of the first growing season after grafting the scion onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (left to right, respectively). Yellow rule is 1 m. **Earlier shoot termination imposed by M.9 was prevented by applying GA_{4+7} to the scion, therefore indicating that the dwarfing rootstock decreases the transport of endogenous root-produced gibberellins to the scion, which increases shoot termination.**

Figure E. Effect of sequential applications of BAP followed by GA_{4+7} on scion architecture of 'Royal Gala' apple scions by the end of the first growing season after grafting the scion onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (left to right, respectively). Yellow rule is 1 m. **Applying cytokinin increased branching whilst gibberellin reduced the proportion of shoots that terminated growth early, but scion dwarfing still occurred on M.9 when compared with the BAP x GA_{4+7}-treated scion on M.793 and 'Royal Gala'. A likely difference that still existed amongst these trees was their capacity to transport auxin to the roots, with the stem tissue of the M.9 rootstock having a reduced capacity to do so. This result suggests that auxin transport differences amongst these rootstocks may be the primary cause of scion dwarfing.**

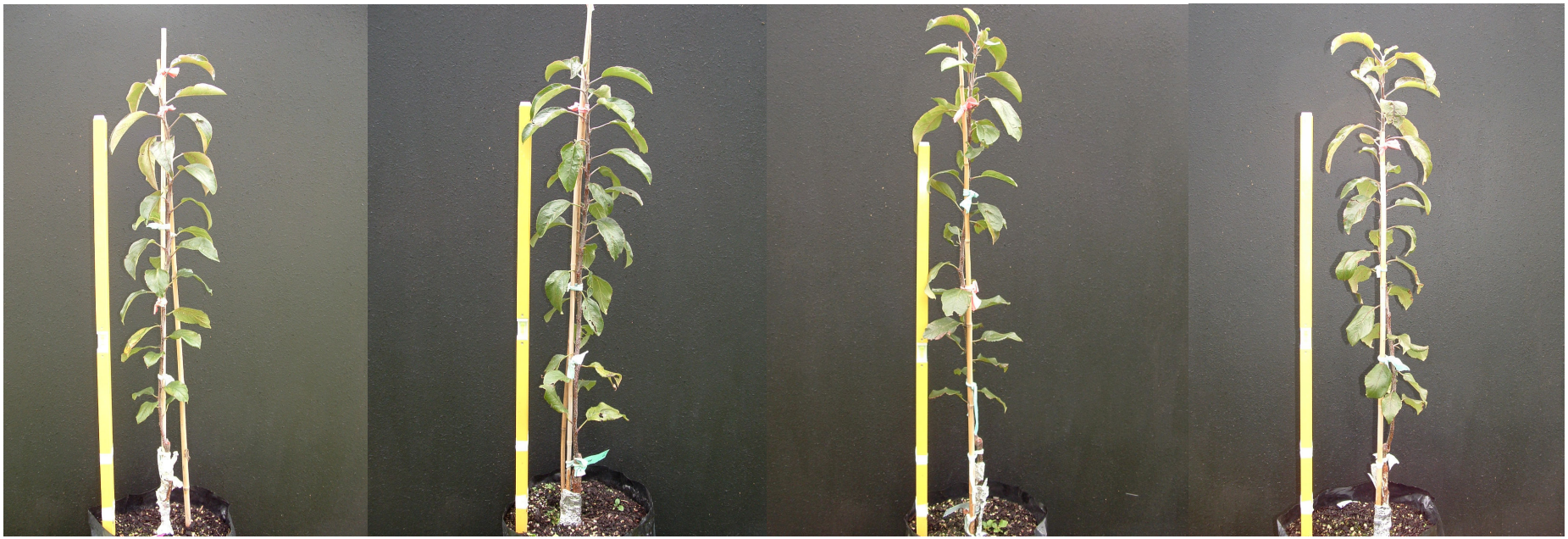

Figure F. Effect of the auxin transport inhibitor 1-N-naphthylphthalamic acid (NPA) applied to the stem of M.9, MM.106, M.793 and 'Royal Gala' rootstocks (left to right, respectively) on scion architecture of composite 'Royal Gala' apple trees by the end of their first growing season from grafting. Yellow rule is 1 m. The **'Royal Gala' scion on the vigorous rootstocks could be made to behave like the scion grafted onto M.9 (compare with Figure B), and the architectural changes were generally similar to those imposed by M.9 (i.e., reduced branching and increased shoot termination), thereby indicating the basipetal transport of auxin is an important physiological signal regulating rootstock-induced scion dwarfing.**

Figure G. Effect of 1-N-naphthylphthalamic acid (NPA) applied to M.9, MM.106, M.793 and 'Royal Gala' rootstocks (left to right, respectively) plus exogenous benzylaminopurine (BAP) applied to the scion on the architecture of 'Royal Gala' apple scions by the end of their first growing season from grafting. Yellow rule is 1 m. **Decreased branching in response to treatment of the rootstock stem with the auxin transport inhibitor 'NPA' could be reinstated by applying cytokinin to the scion, and the scion phenotype on MM.106, M.793 and 'Royal Gala' was similar to the BAP-treated scion grafted onto the M.9 rootstock that was not treated with NPA (see Figure C). Therefore, decreased shoot/root transport of IAA and reduced root/shoot transport of cytokinin may cause decreased branch formation of the scion on M.9.**

Figure H. Effect of 1-N-naphthylphthalamic acid (NPA) applied to M.9, MM.106, M.793 and 'Royal Gala' rootstocks (left to right, respectively) plus exogenous gibberellin (GA_{4+7}) applied to the scion on the architecture of 'Royal Gala' apple scions by the end of their first growing season from grafting. Yellow rule is 1 m. **Exogenous GA_{4+7} prevented the primary shoot from terminating growth in response to NPA treatment of the rootstock stem, thereby alleviating the effects of probable impaired auxin transport, and indicating that decreased shoot/root transport of auxin and reduced root/shoot transport of endogenous gibberellin may cause earlier shoot termination of the scion on the M.9 rootstock.**

Figure I. Effect of 1-N-naphthylphthalamic acid (NPA) applied to M.9, MM.106, M.793 and 'Royal Gala' rootstocks (left to right, respectively) plus benzylaminopurine (BAP) followed by gibberellin (GA_{4+7}) applied sequentially to the scion on the architecture of 'Royal Gala' apple scions by the end of their first growing season from grafting. Yellow rule is 1 m. **Exogenous BAP reinstated branching whilst GA_{4+7} prevented the primary and secondary shoots from terminating growth early, thereby indicating the basipetal transport of IAA interacts with both root-produced cytokinin and gibberellin.**

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List of abbreviations

AMU	Atomic mass unit
ANOVA	Analysis of variance
BAP	Benzylaminopurine
cv.	Cultivar
^{14}C-IAA	Carboxyl-labelled indole-3-acetic acid
^{14}C-IAA-Me	Carboxyl-labelled indole-3-acetic acid methyl ester
DPM	Disintegrations per minute
LSD	Least significant difference
lsmeans	Least square means
HPLC	High performance liquid chromatography
GA_n	Gibberellin $_n$ – denotes the number
[$^2H_2$]GA_n-MeTMSi	Deuterium gibberellin methyl ester trimethylsilylether
GA_{19}-MeTMSi	Gibberellin A19 methyl ester trimethylsilylether
GC-MS	Gas chromatography-mass spectrometry
GLM	General linear model
MES	2-(N-morpholino)ethanesulphonic acid
M.9	Malling 9
MM.106	Malling Merton 106
M.793	Merton 793
MPa	Mega pascal(s) (1 MPa = 10 bars)
MSD	Tukey's minimum significant difference
MSTFA	N-Methyl-N(trimethyl-silyl) trifluoroacetamide
n	Number
NPA	1-N-naphthylphthalamic acid
IBA	Indole butyric acid

IAA	Indole-3-acetic acid
$[^2H_5]$IAA	Pentodeuterium indole-3-acetic acid
IAA-Me	Indole-3-acetic acid methyl ester
$[^2H_5]$IAA-MeTMSi	Pentodeuterium indole-3-acetic acid methyl ester trimethylsilylether
IAA-MeTMSi	Indole-3-acetic acid methyl ester trimethylsilylether
IPA	Isopentenyladenosine
^{3}H-IPA	Tritiated isopentenyladenosine
2iP	Isopentenyladenine
ODS	Octadecyl Silica
RIA	Radioimmunoassay
SAM	Shoot apical meristem
SARD	Specific apple replant disorder
SAS	SAS system for statistical analysis
SCA	Shoot cross-sectional area
SEM	Standard error of the mean
SEOD	Standard error of the difference
TDR	Time domain reflectometry
TEA	Acetic acid (40 mmol L^{-1}) adjusted to pH 3.38 with triethylamine
TIBA	2,3,5,-Triiodobenzoic acid
T	Transpiration
^{3}H-ZR	Tritiated zeatin riboside
UV	Ultraviolet
θ	Volumetric water content (m^3 m^{-3})
Z	Zeatin
ZR	Zeatin riboside

1. General introduction

1.1 Taxonomy of apple

Domesticated apples belong to the family *Rosaceae,* subfamily *Maloideae,* genus *Malus* and subgenera *Malu*s. The subgenera *Malus* is further divided into two series including series *Malus* and series *Baccatae* (Phipps et al., 1990). Series *Malus* includes the domesticated apple or *Malus domestica* Borkh., whose fruit have five carpels and mostly persistent calyces. In contrast, fruit of series *Baccatae* have three to five calyces that are deciduous (Phipps et al., 1990; Ferree and Warrington, 2003).

1.2 History of apple cultivation

The apple was thought to originate from the forests of central Asia (Harris et al., 2002; Ferree and Warrington, 2003). Archaeological and molecular evidence indicate that travellers on the silk roads from central China took apple seeds in their travels west some time in the late Neolithic or early Bronze age (Harris et al., 2002). With time, the apple was transported to Persia, Greece and then to Rome (Harris et al., 2002; Jackson, 2003). Subsequently, the Romans distributed the apple throughout Europe, from which, apple cultivation spread to many other temperate regions around the world including parts of North and South America, South Africa and Australasia (Harris et al., 2002; Ferree and Warrington, 2003; Jackson, 2003).

From ancient times, the apple tree has been propagated as a composite tree where a desirable fruiting cultivar or scion is budded or grafted onto a rootstock (see Section 1.5). The practice of grafting was first recorded approximately 3800 years ago for *Vitis* (Harris et al., 2002). For apple, the precise time when grafting was first used is not clear. However, Theophrastus described grafting of apple trees around 320 BC (Ferree and Warrington, 2003). Apple trees grown from seed are not true to type and typically exhibit a long period of juvenility where vegetative growth predominates and flowering does not occur for several years. In addition, scion cultivars are often very hard to root (Hatton et al., 1923; Hartmann and Kester, 1983). Thus, it is highly likely that the practice of grafting a scion onto a rootstock was developed as a convenient way of asexually multiplying a desirable fruiting cultivar, and presumably a means by which

the juvenile phase of tree development could be shortened. In more recent times, rootstocks have also been selected to provide resistance to various diseases (often soil borne) and insect pests.

The precise time during history when dwarfing apple rootstocks were first utilised is unknown. Theophrastus described tree care and dwarf types of apple trees that were later used as rootstocks (Ferree and Warrington, 2003). Roman horticulturists also made use of dwarfing apple rootstocks that were easy to root (Hartmann and Kester, 1983). In the 16th Century, the name 'Paradise' was given to dwarfing apple rootstocks (Jackson, 2003). Dwarfing apple rootstocks were later classified as the very dwarfing 'French Paradise' or the less dwarfing 'Doucin' or 'English Paradise' (Ferree and Carlson, 1987; Jackson, 2003).

In 1912, Wellington initiated a rootstock selection programme at East Malling Research Station in England and later Hatton gathered rootstocks from 71 collections and 35 sources (Jackson, 2003). Hatton (1917) renamed nine rootstocks (numbers 1 to 9), although unfortunately numbers did not pertain to the potential dwarfing capability of each rootstock. Rootstocks from East Malling were originally given Roman numerals. For example, the dwarfing rootstock 'Malling IX' is today known as 'Malling 9' (M.9) (Ferree and Carlson, 1987; Jackson, 2003). With time, additional rootstocks were renamed or bred at East Malling giving rise to numbers M.1 to M.27 (Ferree and Carlson, 1987; Jackson, 2003).

In an attempt to breed rootstocks resistant to woolly apple aphid, the John Innes Institute at Merton in England crossed M.2 with the woolly apple aphid resistant 'Northern Spy' to produce the Merton Immune (M) series, which were numbered from 778 to 793 (Ferree and Carlson, 1987; Jackson, 2003). East Malling and the John Innes Institute at Merton then bred the Malling-Merton (MM) series, which included numbers 101 to 115. The MM.106 rootstock was bred by crossing M.1 and 'Northern Spy' (Ferree and Carlson, 1987). In contrast, the M.9 rootstock was thought to be selected from a chance seedling in 1879 and was originally named 'Jaune de Metz' (Ferree and Carlson, 1987).

1.3 Present utilisation of apple rootstocks in New Zealand

Since the 1960's, the New Zealand apple industry has used intermediate density planting systems incorporating composite apple trees propagated on MM.106 or M.793 rootstocks (Palmer, 1999) planted at a density of 650 to 900 trees per hectare (Tustin and Palmer, 2008). In the growing environment of New Zealand, intermediate density planting systems utilising precocious rootstocks like MM.106 are very productive once mature. For example, Warrington (1994) quoted a yield of 163 t ha^{-1} for mature 'Granny Smith'. High productivity of intermediate density planting systems in New Zealand occurs from an almost ideal growing environment that provides sufficient winter chilling, a long growing season with high solar radiation and moderate air temperatures during fruit growth (Palmer et al., 2002).

Rootstocks of 'Northern Spy', MM.106 and M.793 have also been used by the New Zealand apple industry because they are resistant to woolly apple aphid (*Eriosoma lanigerum*). In contrast, the more dwarfing rootstocks historically available for use in New Zealand, including M.9, M.26 and Mark, are susceptible to this pest (Palmer, 1999; White and Tustin, 2000). Collectively, lack of woolly apple aphid resistant dwarfing apple rootstocks and the high productivity obtained from the use of MM.106 and M.793 planted at intermediate densities has meant that the New Zealand apple industry has been slower than northern hemisphere regions to adopt intensive planting systems (Palmer, 1999; White and Tustin, 2000; Tustin and Palmer, 2008). This is further compounded by a scarce supply of dwarfing rootstocks, poor quality nursery trees with few feathers and a general lack of experience in the management of high-density plantings of composite apple trees on dwarfing rootstocks (Palmer, 1999; Tustin and Palmer, 2008).

Currently, 80% of New Zealand's apple crop is produced from intermediate density planting systems (Tustin and Palmer, 2008). However, the majority of recent apple plantings are intensive utilising either semi-dwarf or dwarf rootstocks (Tustin and Palmer, 2008). The reason for the adoption of intensive planting systems in New Zealand is largely economic. International markets have been heavily over-supplied with apples for a number of years. In addition, important apple cultivars like 'Royal Gala' and 'Braeburn', which have been an integral part of the variety mix grown and

exported internationally from New Zealand, are now grown extensively around the world by other competitors. Hence, New Zealand apple growers have received low returns for export-oriented fruit for more than a decade.

To remain competitive in international markets, New Zealand apple growers will need to reduce production costs, strengthen their capability to rapidly introduce new apple cultivars and improve marketable quality whilst maintaining high yields (Palmer, 1999). This could be achieved by planting intensive growing systems utilising dwarfing apple rootstocks. For example, dwarfing apple rootstocks reduce scion vigour and final tree size (Hatton, 1928; Warne and Wallace, 1935; Tubbs, 1951; Preston, 1958a, b; Ferree and Knee, 1997; Tustin et al., 2001) that enables a higher planting density per hectare. Increased tree density greatly increases light interception in the early life of the orchard (Robinson, 1992) and reduces the time taken for the scion canopy to fill its allotted space. Combined with greatly increased floral precocity of the scion when grown on dwarfing apple rootstocks (Hatton, 1928, 1931; Warne and Wallace, 1935; Preston, 1958a, b; Ferree, 1976; Hirst and Ferree, 1995; Tustin et al., 2001; Selezynova et al., 2008), planting composite apple trees on dwarfing rootstocks at an increased density per unit land area greatly increases cumulative yield, especially in the early life of the orchard (Hatton, 1931; Preston, 1958a; Tustin et al., 2001).

Consequently, the initial investment of capital to establish a high-density planting of composite apple trees on a dwarfing rootstock is regained much earlier in the orchard life cycle compared with trees on a semi-vigorous rootstock planted at an intermediate density. Once mature, and with correct management, the low vigour and compact nature of the scion on dwarfing rootstocks can decrease the cost of fruit picking and pruning (Palmer, 1999; Tustin and Palmer, 2008). In addition, dwarfing apple rootstocks improve some important components of marketable fruit quality including skin blush (Tubbs, 1951; Gyuro et al., 1986; Tustin et al., 2001; Palmer et al., 2001), total soluble solids (Warne and Wallace, 1935; Tubbs, 1951; Sansavini et al., 1986; Autio et al., 1996; Palmer et al., 2001) and, in some instances, fruit size (Tubbs, 1951; NC-140, 1996; Atkinson et al., 2000; Atkinson and Else, 2001).

1.4 The need to improve dwarfing apple rootstocks through breeding

Despite the potential of dwarfing apple rootstocks to increase production efficiency of New Zealand apple orchards, there are some disadvantages in their adoption, particularly with those dwarfing apple rootstocks that have historically been available. For example, M.9 and M.26 are highly susceptible to both woolly apple aphid and fire blight (*Erwinia amylovora*) (Ferree and Carlson, 1987). The widely used semi-vigorous MM.106 rootstock is also susceptible to *Phytophthora* (Ferree and Carlson, 1987).

However, recently the semi-dwarf 'Geneva 202' and 'Cornell-Geneva 6210' rootstocks were commercialised in New Zealand. These rootstocks are resistant to woolly apple aphid and are an alternative for growers wishing to produce a composite tree in the size range of M.26 (Tustin and Palmer, 2008). The Japanese dwarfing rootstock JM7, which is also resistant to woolly apple aphid, was commercially licensed in 2006 and provides a composite tree intermediate in size between that of M.9 and M.26 (Tustin and Palmer, 2008).

Despite the recent introduction of these new size-controlling rootstocks into New Zealand, there is still a need for a dwarfing apple rootstock that produces a composite tree in the size range of M.9, and that provides resistance to woolly apple aphid and fire blight. In addition, Tustin and Palmer (2008) reported that many new high-density orchards are being replanted into land that has previously grown pipfruit and, therefore, soil fumigation with chloropicrin is required to prevent Specific Apple Replant Disorder (SARD). Thus, there is also a strong need to breed new dwarfing apple rootstocks with tolerance to SARD, especially given that the use of chloropicrin may be deemed unacceptable by international markets in the future.

1.4.1 Difficulties associated with breeding new dwarfing apple rootstocks

The major difficulty with breeding new dwarfing apple rootstocks is that it is very time consuming. Progeny must undergo extensive selection for pest and disease resistance, performance in the nursery and, subsequently, the orchard. Consequently, it may take between 25 to 30 years to commercially release a new rootstock selection (Johnson,

2000; Webster, 2002). However, recent genetic advances have great potential to assist in the breeding and selection of new dwarfing apple rootstocks, which traditionally has been largely empirical.

Gene mapping of apple rootstock progeny derived from a cross of M.9 x 'Robusta 5' parents identified a major locus (*DW1*) involved in the dwarfing trait of the M.9 rootstock (Rusholme-Pilcher et al., 2008) and genetic markers for woolly apple aphid resistance in the 'Robusta 5' rootstock (Bus et al., 2008). Identification of genetic markers that are linked to genes involved in rootstock-induced dwarfing of the scion (Rusholme-Pilcher et al., 2008) and other important traits like disease resistance (Bus et al., 2008) will greatly improve rootstock breeding programmes. Marker assisted selection enables desirable progeny to be selected from large populations of tree material at a very young age leading to greater efficiency and effectiveness of rootstock breeding programmes (Fazio and Mazzola, 2004; Rusholme-Pilcher et al., 2008). Recently, the first genetic map of apple rootstock progeny derived from a cross between M.9 and 'Robusta 5' was constructed and has potential application in further elucidating the genetic control of rootstock-induced dwarfing of the scion by the M.9 rootstock (Celton et al., 2009).

The biological processes that dwarfing gene(s) control are poorly understood, particularly the underlying physiological mechanism(s) that are modified within the composite tree on a dwarfing apple rootstock, and their consequent expression in scion architecture and scion precocity. Remarkably, following propagation of the composite apple tree it is presently unclear how dwarfing apple rootstocks initially modify scion architecture and the precise stage of tree phenology when this occurs. Elucidation of the initial architectural modifications imposed on the scion by a dwarfing apple rootstock following propagation is essential to clearly identify processes that are the first physiological causes of rootstock-induced dwarfing of the scion from those that are subsequent developmental effects.

1.5 Propagation of the composite tree in the nursery

The following briefly introduces the most common methods used to propagate composite apple trees and a general description of terminology to describe their growth in the first year from propagation. Section 1.6 then provides examples of how different

methods of tree propagation reported in the literature may confound attempts to understand how a dwarfing apple rootstock initially modifies scion architecture and the time during tree phenology when this may occur.

1.5.1 Budding

Budding is the most common method used to propagate composite apple trees because it is quick to perform, provides a successful union between stock and scion and is an efficient method of using limited bud wood (Hartmann and Kester, 1983). Prior to budding, one-year-old rootstock stools are planted into the field during early spring and rootstocks are usually budded with a scion in late summer while the cambium is still active and the rootstock bark 'slips' or readily separates from the wood. In the following spring, the two-year-old rootstock stem is cut back just above the inserted scion bud, which is then allowed to regrow to form the primary axis or shoot of the composite tree.

With T-budding, a 'T' shape incision is made into the rootstock that enables the bark to be peeled back. A single bud piece of the desired scion cultivar is then inserted inside the bark flaps, which are then closed by binding with tape. The bud union then forms parenchyma cells or callus tissue, which is mainly derived from the exposed surface of the xylem cylinder and, to a much lesser extent, the inside of the bark flaps and the sides of the bud shield (Mosse and Labern, 1960; Hartmann and Kester, 1983). Callus tissue of rootstock and scion interlock and then proliferate completely filling the bud cavity from approximately 14 to 21 days after budding. Subsequently, a continuous cambium forms between the scion bud and the rootstock, which is followed by the vascular connection of bud to rootstock as new xylem and phloem are produced by the cambium (Hartmann and Kester, 1983). In contrast, chip budding involves inserting an oval shield containing a central scion bud into an exact matching area where the bark and wood has been removed from the rootstock stem. Chip budding, therefore, enables the cambium layers of the two graft partners to be directly aligned and healing of the union is much quicker than T-budding.

1.5.2 Grafting

Propagation of composite trees by grafting is more laborious than budding. Most commonly, a dormant annual vegetative shoot of the scion, which is approximately one-year-old at the time of propagation, is bench grafted onto the dormant stem of a newly

harvested one-year-old rootstock stool. In the spring, trees are closely planted into the nursery, or sometimes directly into the orchard at a desired tree density. Because the new shoot system of the scion and a large proportion of the root system establish in the same year, scion growth of bench grafted trees is generally moderate in the first year compared with the aforementioned methods of budding. Therefore, the one-year-old primary shoot of bench grafted trees is often headed back at 50 to 80 cm in the winter and trees are grown for second year in the nursery where they grow very vigorously and typically form axillary shoots (Wertheim and Webster, 2003).

The most common methods of bench grafting include whip and tongue and cleft grafting. The latter method was used in this thesis (Section 2.2) and involves cutting a one-year-old piece of scion stem, usually with two dormant vegetative buds, and positioning it into a cleft cut into the one-year-old rootstock stem so that both graft partners make intimate contact (Figure 1.1) and the cambium regions are in close proximity. Between one to seven days after grafting, callus tissue is produced by both the rootstock and scion from the parenchyma cells of the phloem rays and the immature parts of the xylem (Sass, 1932; Hartmann and Kester, 1983). Callus tissue of the rootstock and scion then mingle and interlock (Sass, 1932), whilst new parenchyma adjacent to the original cambium region of the graft partners differentiates into new cambium cells after approximately three weeks, which then form a new cambium connection between rootstock and scion (Hartmann and Kester, 1983). New xylem and then phloem are produced towards the inside or outside, respectively, of the newly formed cambium layer. Good vascular connections are a prerequisite for vigorous extension growth of the scion (Hartmann and Kester, 1983).

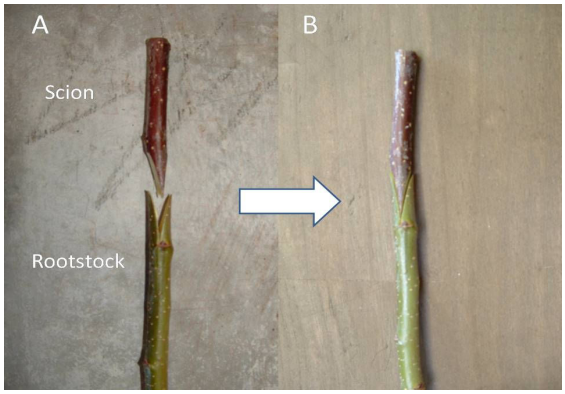

Figure 1.1. Example of a cleft cut into a one-year-old rooted stool of MM.106 and the matching wedge cut into a one-year-old 'Royal Gala' scion prior to (A) and after positioning of the scion into the cleft cut into the rootstock (B).

1.5.3 Extension growth of the scion in the first year after propagation and general terminology to describe growth

Following budding or grafting, the primary axis or shoot of an apple scion begins growth in the spring from a single vegetative bud. According to Pratt (1990), the first 9 to 10 nodes produced along a shoot axis of apple in early spring are preformed in the overwintering vegetative bud. Subsequent nodes produced over the growing season are newly formed (neoformed) in the peripheral region of the shoot apical meristem (SAM) (Pratt, 1990; Evert, 2006). The elongation of internodes occurs in the sub-apical region of the SAM (Pratt, 1990; Evert, 2006), which is the major region of cell division and elongation (Sachs, 1965). The meristematic activity of the subapical region may extend several internodes below the apical region of the SAM (Sachs, 1965; Evert, 2006).

Once formed, axillary buds on the primary shoot may remain latent or grow out for a short time to form a secondary spur (< 25 mm with minimal internode extension) or for a longer duration to form a secondary shoot (≥ 25 mm with internode extension) (Seleznyova et al., 2003). In this thesis, secondary spurs and shoots are collectively called 'secondary axes'. Nodes developed by a secondary shoot may be solely preformed within the axillary bud or formed from a combination of preformed and neoformed nodes (Pratt, 1990). Although 9 to 10 preformed primordia exist in the one-year-old overwintering vegetative bud, it is unclear at exactly what stage during the first year from propagation an axillary vegetative bud has fully developed 9 to 10 primordia.

Therefore, for newly grafted trees studied in this thesis, it is unknown whether secondary shoots with 10 or less nodes have formed solely from preformed nodes. However, secondary shoots with more than 10 nodes have presumably undergone node neoformation.

Over the growing season, primary or secondary shoots may form from either a season-long flush of growth by the SAM or by two or more growth flushes caused when growth temporarily ceases before resuming at some later point in the season. Primary or secondary shoots formed from two or more growth flushes are identifiable by the presence of bud scale scars, and/or, compressed internodes at some position(s) along the shoot axis (Seleznyova et al., 2003). The two distinct flushes of growth are each called growth units. Shoots with two growth units are also referred to as having undergone a 'bicyclic' pattern of growth.

The different possible types of shoots that may form on the scion in the first year of growth after grafting are presented in Chapter 2 (Figure 2.29).

1.5.4 Propagation methods may modify early growth of the composite tree

Studies investigating the effects of different size-controlling rootstocks on the initial growth of the scion following propagation are often difficult to compare because the propagation methods used are often different. For example, studies have used budding (Cannon, 1941; Tukey and Brase, 1941; Ferree, 1976), or grafting (Rao and Berry, 1940; Cannon, 1941; Ferree, 1976; Costes et al., 2001; Seleznyova et al., 2005, 2007, 2008; Costes and Garcia-Villanueva, 2007) or have not stated how composite trees were propagated (Avery, 1969). In contrast, Costes and Garcia-Villanueva (2007) compared two own-rooted scion varieties propagated by tissue culture with the same scion variety grafted onto the M.9 rootstock. Thus, comparisons of rootstock effects in that study were made between trees with and without graft unions.

For budded trees, two studies used summer-budding (Cannon, 1941; Ferree, 1976), whereas Tukey and Brase (1941) did not state when budding occurred. As previously described, summer-budding involves planting one-year-old rootstock stools into the field during spring and the rootstock stem is then budded with a scion variety in the summer. In the following spring, the rootstock stem, which is now two-years-old, is cut

back just above the inserted scion bud, which is then allowed to regrow. With this method of propagation, growth of the scion is vigorous because the root system is in its second year of growth and therefore is well established and sizeable, and the bud union will have formed vascular connections in the previous year. Although strong vigour of budded trees is very desirable in the commercial tree nursery, in physiological studies it could potentially mask any subtle changes in scion architecture that a dwarfing rootstock may modify in the first year of growth.

Studies assessing the initial effects of different size-controlling rootstocks on scion vigour have used very different propagation and cultural practices to produce grafted experimental tree material. In some studies, one-year-old rootstock stools were planted into the field in spring and grown for one season prior to grafting of the scion onto the two-year-old rootstock stem in the following spring (Rao and Berry, 1940; Cannon, 1941). Others have established newly grafted trees in the nursery or field for one season and then headed back the one-year-old scion in the second spring from propagation prior to assessing rootstock effects on scion vigour (Ferree, 1976; Costes et al., 2001). The use of trees that are headed back (i.e., Ferree, 1976; Costes et al., 2001) would cause very strong vigour and probably impose different architectural responses by the scion, particularly through increased sylleptic shoot formation. Furthermore, the graft union would have healed prior to growth, therefore making comparisons difficult with other studies where a one-year-old vegetative shoot of the scion was either bench grafted onto newly harvested one-year-old rootstock stools (Seleznyova et al., 2005, 2007, 2008) or grafted onto two-year-old established rootstocks grown in the field (Rao and Berry, 1940; Cannon, 1941).

The propagation of experimental tree material by summer budding (Cannon, 1941; Ferree, 1976) or by spring grafting onto two-year-old established rootstocks (Rao and Berry, 1940; Cannon, 1941) resulted in a composite tree where the scion bud began its outgrowth on a rootstock that was two-years-old. In contrast, Seleznyova et al., (2008) propagated experimental tree material by grafting dormant one-year-old ‘Royal Gala’ scions onto newly harvested dormant one-year-old rootstock stools of M.9 and MM.106. Composite trees were then planted into the field prior to spring bud break and a single primary shoot was allowed to grow without pruning. This methodology enabled the propagation of a composite tree where the starting age of the graft partners (i.e.,

rootstock and scion) were identical, thereby preventing any confounding effects on scion vigour that may have been imposed by using two-year-old rootstocks of different vigour (Rao and Berry, 1940; Cannon, 1941; Avery, 1969; Ferree, 1976; Costes et al., 2001), which may have already developed very different physiological and morphological characteristics.

In summary, studies understanding the initial effects of dwarfing apple rootstocks on scion vigour are difficult to compare because there is no standardised methodology for propagating tree material. The most appropriate method for propagating tree material for such studies is that reported by Seleznyova et al., (2008) because rootstock and scion graft partners are the same age. In addition, the initial disparities between the size of the root system and the scion are minimised. Therefore, this method is likely to provide a more accurate representation of when and how dwarfing apple rootstocks initially modify scion architecture following propagation of the composite tree and was, therefore, the method of propagation used in this thesis (Section 2.2).

1.6 Alteration of scion architecture by dwarfing apple rootstocks

1.6.1 Types of dwarfism in apple

The term ‘rootstock-induced dwarfing’ is used throughout this thesis to describe the unique form of size control that dwarfing apple rootstocks impose on the scion. Rootstock-induced dwarfing of the scion is very different from compact dwarf apple cultivars where shoot length is decreased because the scion has inherently reduced internode length (Steffens et al., 1989; Steffens and Hedden, 1992). In contrast, annual shoots of ‘Royal Gala’ with the same node number were of similar length regardless of whether the scion was grown on M.9 or MM.106 (Selezynova et al., 2003). Hence, the dwarfing apple rootstock did not decrease internode length of annual shoots, but rather reduced node neoformation (Selezynova et al., 2003).

Other unique architectural effects of compact dwarf apple cultivars include marked reductions in shoot length in the first year of growth in the tree nursery and delayed flowering compared with non-dwarf cultivars (Steffens and Hedden, 1992). In contrast, the growth of the scion grafted onto a dwarfing apple rootstock (M.9) was similar to a

more vigorous rootstock (MM.106) in the first year of growth from grafting. However, floral precocity of the scion was greatly increased on M.9 in the spring of year two compared with MM.106 (Selezynova et al., 2008).

Therefore, rootstock-induced dwarfing of the scion is architecturally very different from that imposed by compact dwarf apple cultivars. There are, however, non-rootstock forms of scion dwarfing that are very similar to that imposed by dwarfing apple rootstocks. For example, root restriction of apple trees increased floral precocity (Myers, 1992) and reduced scion vigour (Atkinson et al., 2000; Webster et al., 2000; Webster et al., 2003) of young apple trees. However, it is largely unknown whether both the architectural and physiological mechanisms are the same for dwarfing apple rootstocks and root-restriction treatments. In this thesis, a comparison was made between the architectural changes imposed on the scion by M.9 and by root restriction (Chapters 3 and 4).

1.6.2 The initial modification of scion architecture by dwarfing apple rootstocks

Following the propagation of the composite tree, the initial modifications to scion architecture that occur on a dwarfing apple rootstock are not clearly understood. Therefore, this has been the focus of recent research (Seleznyova et al., 2003; 2005, 2007, 2008; Costes and Garcia-Villanueva, 2007). Elucidation of how a dwarfing apple rootstock initially modifies scion architecture, and the time from propagation when this occurs, is essential to clearly identify processes that are the first physiological causes of rootstock-induced dwarfing of the scion from those that are subsequent developmental effects.

In the first year of growth from grafting, Seleznyova et al., (2007) reported that the rate of plastochron did not differ for the primary shoot of ‘Royal Gala’ scions on rootstocks of M.9 and MM.106. In addition, the final mean node number, length and internode length of the primary shoot was not different for these rootstocks at growth cessation (Seleznyova et al., 2007, 2008). In the spring of year two from grafting, the first observable change imposed on the scion by M.9 was increased flowering (Seleznyova et al., 2005, 2007, 2008). Compared with MM.106, the greater incidence of flowering along the primary shoot of the scion on M.9 increased the proportion of floral axillary annual shoots that formed, and reduced the number of annual shoots that developed

extension growth units, particularly annual axillary shoots comprised of two growth units (Seleznyova et al., 2005, 2008). In addition, the vigour of annual axillary vegetative shoots was reduced in the second year of growth for the scion on M.9 when compared with MM.106 (Seleznyova et al., 2008). The scion of 'Ariane' grafted onto M.9 also produced slightly more flowers in the spring of year two than own-rooted trees of 'Araine' propagated by tissue culture (Costes and Garcia-Villanueva, 2007). However, increased floral precocity of the scion on M.9 and the subsequent reduction in vigour were not highly prominent until the third year of growth (Costes and Garcia-Villanueva, 2007). In the latter study, data were not reported for rootstock effects on scion growth in the first year from propagation.

Similar to the study of Seleznyova et al., (2008), some earlier research has shown that apple scions grown on a range of different rootstocks formed a primary shoot of comparable mean length by the end of the first growing season from budding (Tukey and Brase, 1941) or grafting (Ferree, 1976). In addition, the floral precocity of the scion on the dwarfing rootstock was increased in the spring of year two from propagation compared with rootstocks of greater vigour (Hirst and Ferree, 1995). The increased incidence of scion flowering in year two (Hirst and Ferree, 1995; Seleznyova et al., 2005, 2007, 2008) must result from a physiological change induced by the dwarfing rootstock in year one, particularly because floral evocation of apple buds occurs during the previous year (Buban and Faust, 1982; Pratt, 1988; Forshey and Elfving, 1989). Year one, therefore, would appear to be the critical year of development when the first physiological changes, as modified by the dwarfing rootstock, occur in the scion.

Dwarfing apple rootstocks may, however, reduce scion vigour in the first year of growth following propagation of the composite tree and, hence, architectural changes can precede the first occurrence of flowering in year two. For example, M.9 reduced the mean length of the primary shoot of 'Cox's Orange Pippin' or 'Grenadier' scions compared with rootstocks of greater vigour by the end of the first year of growth in a tree nursery (Cannon, 1941). In addition, newly grafted scions of 'Crawley Beauty' on M.9 had slower cumulative extension growth from late summer compared with the same scion variety on M.13 (Rao and Berry, 1940). Consequently, the final mean length of the primary shoot was reduced on the M.9 rootstock by the end of the first year of growth. In the latter study, the experimental material was reasonably uniform at the

beginning of the experiment and the M.9 rootstock had decreased the mean length of the primary shoot by approximately 200 mm by autumn. Therefore, initial changes in the mean length of the primary shoot imposed by M.9 were very small. Hence, subtle differences in growth of the primary shoot may not be detected amongst different size-controlling rootstocks if large variation exists in the tree material at the commencement of growth. In this thesis, considerable effort was made to propagate and grow experimental tree material of high-uniformity (Section 2.2 and Figures 2.3 and 2.4).

A more recent study reported that M.9 reduced both the mean length and node number of the 'Rome Beauty' primary shoot that regrew after the one-year-old scion was headed back (Costes et al., 2001). In that study, there was also a trend that M.9 reduced the mean number of secondary axes on 'Starkrimson' scions compared with the same scion cultivar on M.7. Similarly, there were trends that the mean number of secondary shoots on scions of 'Gala' (Volz et al., 1994) and 'Cortland' 'Gloster', 'Jonagold' or 'Melrose' (Jaumien et al., 1993) were reduced by M.9 in a tree nursery during the first year of growth from propagation. For older trees, the M.9 rootstock reduced the mean number of axillary shoots on 'Skyline Supreme Delicious' that were longer than 100 mm (Ferree, 1976), whilst the size of the 'Worcester Pearmain' scion growing on M.9 was reduced compared with M.16 partly because of fewer growing points (Avery, 1969). Dwarfing rootstocks were also reported to reduce the size of the scion by decreasing the proportion of shoots that underwent node neoformation (Selezynova et al., 2003; Selezynova et al., 2008), which may result because dwarfing apple rootstocks increase the proportion of shoots that terminate growth early compared with more vigorous rootstocks (Swarbrick, 1929; Colby, 1935; Tubbs, 1951; Avery, 1969; Robitaille and Carlson, 1976). Earlier shoot termination of the scion on a dwarfing apple rootstock may consequently decrease the proportion of long shoots that form (Ferree, 1976; Costes et al., 2001; Selezynova et al., 2008).

In summary, dwarfing apple rootstocks may modify scion architecture and decrease vigour in either year one or two of growth after propagation of the composite tree. Architectural modifications imposed on the scion by a dwarfing apple rootstock during the first year of growth could include decreased mean length (Rao and Berry, 1940; Cannon, 1941; Costes et al., 2001) and node number (Costes et al., 2001) of the primary shoot and the formation of fewer secondary shoots per scion (Jaumien et al., 1993; Volz

et al., 1994). However, the scion on the dwarfing apple rootstock may also grow similarly to the scion on a vigorous rootstock in the first year of growth from propagation (Tukey and Brase, 1941; Ferree, 1976; Selezynova et al., 2008). Subsequently, reductions in scion vigour may occur in year two of growth after increased flowering of the scion (Selezynova et al., 2008). The reasons for differing results concerning when and how the dwarfing apple rootstock initially modifies scion architecture are not known, but could include differences in growing environments and the wide range of methods used to propagate and establish composite apple trees. Therefore, an overall aim of this thesis was to determine the time from propagation when rootstock-induced dwarfing of the scion was first expressed, and elucidate how scion architecture was initially modified by rootstocks of different vigour.

1.7 Physiology of rootstock-induced dwarfing of the scion

Identifying when and how dwarfing apple rootstocks initially modify scion architecture after propagation of the composite tree may provide important insights into the first physiological causes of rootstock-induced dwarfing of the scion. Remarkably, the physiological mechanisms by which dwarfing apple rootstocks reduce scion vigour are unclear and largely hypothetical despite at least 70 years of research.

1.7.1 Nutrients

Dwarfing apple rootstocks may reduce scion vigour by restricting the uptake and transport of mineral nutrients to the scion. For example, composite trees of 'McIntosh' grown in solution culture and treated with ^{32}P and ^{45}Ca had greatly decreased transport of radioactivity from the root to the scion when grown on M.9 and compared with the M.16 rootstock (Bukovac et al., 1958). Jones (1971) also reported that concentrations of nitrogen, phosphorous and potassium in the xylem sap collected from root pressure exudate was reduced by M.7 compared with the MM.104 rootstock.

In contrast, Warne and Wallace (1935) measured the concentrations of mineral anions and cations in two scion varieties on a range of different size-controlling rootstocks and concluded that many beneficial attributes imposed by the M.9 rootstock, such as reduced scion vigour, increased floral precocity and fruit quality, could not be explained by differences in mineral composition within the leaves, bark, wood and fruit. Similarly,

the root system of M.9 grown in nutrient culture tended to absorb more water, nitrogen and potassium over the growing season than the M.12 root system when data for absorption by each rootstock was expressed as a proportion to the annual gain in fresh weight of the scion (Pearse, 1940). More recently, Atkinson and Else (2001) reported that the M.9 rootstock was actually capable of transporting greater amounts of mineral ions and solutes to the scion than MM.106 when differences in sap flow rate, root mass and leaf area imposed by each rootstock type were appropriately considered.

1.7.2 Altered plant water status

Dwarfing apple rootstocks may reduce scion vigour by restricting the movement of water from the root to the scion (Rogers and Beakbane, 1957; Olien and Lasko, 1986; Atkinson et al., 2003). Early anatomical studies reported that roots of dwarfing apple rootstocks had a reduced ratio of xylem to phloem and much smaller xylem vessels compared with roots of vigorous rootstocks (Beakbane and Thompson, 1947; Beakbane, 1956). Collectively, these anatomical differences of the root for dwarfing apple rootstocks were thought to limit axial water movement through the root system, hence restricting the transport of water to the scion.

In further support of this theory, the hydraulic conductivity of M.27, M.9 and MM.106 roots (1.5 mm in diameter) decreased with decreasing rootstock vigour, therefore indicating that the root system of dwarfing apple rootstocks may provide a greater resistance to axial water movement than rootstocks of greater vigour (Atkinson et al., 2003). However, the hydraulic conductivity of the entire M.9 root system was similar to the MM.106 root system when hydraulic conductivity was expressed relative to the total dry mass of the root system (Atkinson et al., 2003).

The increased axial resistance to water movement in the roots of M.9 (Atkinson et al., 2003) is unlikely to reduce water uptake by the root system, which is influenced solely by radial resistances to water movement (Steudle and Peterson, 1998). Arguably, some architectural differences in the M.9 root system might enhance the extraction of soil water when soil moisture is non-limiting. For example, a large proportion of the M.9 root system was comprised of roots less than 1 mm in diameter (Atkinson et al., 2003), which are typically produced on a seasonal basis (Eissenstat et al., 2001) and new white

roots are important for water and nutrient acquisition by apple trees (Atkinson, 1974; Eissenstat et al., 2001). Despite these probable benefits to water absorption, decreased axial water movement through the xylem of dwarfing root systems may explain why they sometimes decrease the stem water potential of the scion.

Olien and Lasko (1986) reported that M.9 and M.26 decreased the midday stem water potential of six-year-old 'Empire' scions compared with rootstocks of M.7, MM.106 and MM.104. Differences in stem water potential for M.9 and M.26 were not due to differences in stomatal conductance or transpiration but were attributed to reduced water flow through the rootstock portion of the tree (Olien and Lasko, 1986). Indeed, the two-year-old union of the 'Bramley' scion budded onto M.9 contained xylem vessels of reduced diameter and little xylem was produced in the rootstock tissue (Soumelidou et al., 1994b). In addition, the area of functional xylem at the bud union and in the above scion stem was reduced by M.27 and M.9 compared with MM.106, and this may have decreased the hydraulic conductance of the bud union for trees on M.27 and M.9 (Atkinson et al., 2003). Therefore, differences in graft union morphology for dwarfing apple rootstocks may impede water movement into the stem of the primary shoot, thereby explaining why the scion on dwarfing apple rootstocks developed more negative midday water potentials (i.e., Olien and Lasko, 1986).

However, dwarfing apple rootstocks are not consistently reported to decrease midday water potential of the scion. For example, changes in diurnal leaf water potential and stomatal conductance were almost identical when three-year-old 'Queen Cox' grafted onto M.9 and MM.106 rootstocks were grown without root-restriction and supplied with sufficient irrigation (Atkinson et al., 2000). It is important to note that in the study of Olien and Lasko (1986), decreased midday stem water potentials for the scion growing on M.9 or M.26 were, biologically, very small compared with rootstocks of greater vigour (i.e., -0.1 to -0.2 MPa). In addition, stem water potential of the scion in the canopy exterior did not exceed -1.0 MPa at midday for any rootstock type. For deficit irrigated apple trees, a midday leaf water potential greater than -1.5 MPa was required to significantly decrease shoot extension growth of the scion compared with fully irrigated controls (Irving and Drost, 1987), whereas photosynthesis was reduced as midday leaf water potential approached -1.8 to -2.5 MPa (Kilili et al., 1996; Mills et al., 1996). Arguably, relatively small reductions in stem water potential imposed by M.9 in

the study of Olien and Lasko (1986) may have been insufficient to decrease shoot extension growth and, therefore, are probably not the primary cause of scion dwarfing. Similarly, Webster (2004) reported that reductions in the hydraulic conductance at or above the graft union might not be physiologically sufficient to reduce shoot extension growth of the scion growing on dwarfing apple rootstocks.

In summary, dwarfing apple rootstocks do not consistently decrease midday water potential of the scion compared with rootstocks of greater vigour. Differences that occur amongst different size-controlling rootstocks are physiologically very small, and therefore are probably not the primary cause of rootstock-induced dwarfing of the scion.

1.7.3 Alterations in shoot/root/shoot transport of endogenous plant hormones

1.7.3.1 Effect of dwarfing apple rootstocks on shoot to root signalling of indole-3-acetic acid

Dwarfing apple rootstocks may reduce scion vigour by modifying the transport of endogenous hormones (Rogers and Beakbane, 1957; Lockard and Schneider, 1981; Webster, 1995; Kamboj and Quinlan, 1997; Atkinson and Else, 2001; Webster, 2004). The endogenous control of scion vigour by dwarfing apple rootstocks is most convincingly explained by hormonal signalling between endogenous indole-3-acetic acid (IAA) and cytokinin. Lockard and Schneider (1981) hypothesised that dwarfing apple rootstocks reduced scion vigour by decreasing the basipetal transport of IAA within the phloem and cambial cells of the rootstock stem, therefore limiting the amount of IAA transported from scion to root. Sub-optimal amounts of IAA transported to the root system may decrease root growth, the synthesis of root-produced cytokinins and their consequent transport in the xylem sap to the scion where cytokinins may be required to stimulate shoot extension growth (Lockard and Schneider, 1981). In agreement with this hypothesis, the stem of dwarfing apple rootstocks reduced the basipetal transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj et al., 1997), whilst the concentration of endogenous IAA was decreased in their cambial sap (Michalczuk, 2002) when compared with rootstocks of greater vigour. In addition, composite trees of ‘Fiesta’ grafted onto M.9 transported less ^{3}H to the root system

compared with rootstocks of greater vigour when ^{3}H-IAA was applied to a mature basal leaf on the scion (Kamboj, 1996).

The mechanism by which the tissue of the rootstock stem decreases the basipetal transport of IAA is not known, but the bark of dwarfing apple rootstocks exhibited an increased capacity to destroy IAA (Gur and Samish, 1968) and contained higher concentrations of growth inhibiting phenols and lower concentrations of growth promoting phenols (Martin and Stahly, 1967) that may act to enhance or suppress the oxidative decarboxylation of IAA, respectively (Lockard and Schneider, 1981). In addition, dwarfing rootstocks may have an abnormal arrangement of efflux proteins that facilitate active polar IAA transport out of the cell (Soumelidou et al., 1994a; Kamboj, 1996; Kamboj et al., 1997). Although Kamboj (1996) showed that M.9 transported less ^{3}H to the root system compared with rootstocks of greater vigour when ^{3}H-IAA was applied to a mature basal leaf on the scion, the proportion of ^{3}H in the root system that was still associated with IAA was not determined for rootstocks of different vigour. Therefore, it remains untested experimentally whether the transport of 'IAA' to the root is actually decreased by M.9, and whether this is causal in decreased root growth of dwarfing apple rootstocks as proposed by Lockard and Schneider (1981).

Assessment of other literature for apple suggests that the basipetal transport of IAA from scion to root is an important physiological signal regulating root growth. For example, exogenous auxins promoted the initiation of apple roots (Jones and Hatfield, 1976; Delargy and Wright, 1979) and composite apple trees on dwarfing rootstocks generally have smaller root systems than vigorous rootstocks (Hatton et al., 1923; Rogers and Vyvyan, 1934; Colby, 1935; Vyvyan, 1955; Beakbane and Rogers, 1956; Tubbs, 1980; Abod and Webster, 1989). Smaller root systems of dwarfing apple rootstocks typically decrease the size and vigour of the scion (Tubbs, 1980) and similar non-rootstock induced vigour reductions can be imposed for apple scions by root pruning (Ferree, 1992; Ferree et al., 1992) or root restriction (Webster et al., 2000; Atkinson et al., 2000; Webster et al., 2003). However, it is unknown whether the physiological mechanisms modified by root restriction are the same as those regulating rootstock-induced dwarfing of the scion.

The stem tissue of dwarfing apple rootstocks can also reduce the growth of vigorous root systems, possibly by restricting the basipetal transport of IAA. For example, the size of the M.12 root system was greatly reduced when budded with a M.9 scion and the resulting composite tree was much smaller than M.12 budded onto the M.12 rootstock (Vyvyan, 1955). Similarly, budding or grafting a length of dwarfing shoot or interstem between the scion and a vigorous rootstock reduced root and scion growth (Tukey and Brase, 1943), and the dwarfing effect on the scion was increased with increasing length of the interstem used (Parry and Rogers, 1972). In addition, the grafting of bark inserts of M.26 into composite trees of 'Gravenstein' on M.111 greatly reduced scion vigour and the dwarfing effect was markedly increased by inverting the bark (Lockard and Schneider, 1981), presumably because reversing tissue polarity greatly decreases the basipetal transport of radio-labelled IAA (Antoszewski et al., 1978).

In summary, dwarfing apple rootstocks may decrease the basipetal transport of IAA within their rootstock stem to the root system. Sub-optimal amounts of IAA transported to the root system of a dwarfing apple rootstock might limit root growth and, consequently, scion vigour. As discussed below, reduced scion vigour on a dwarfing apple rootstock may result from reduced basipetal IAA transport to the root modifying root-produced hormonal signals transported to the scion in the xylem vasculature.

1.7.3.2 Effect of dwarfing apple rootstocks on root to shoot signalling of cytokinin and gibberellin

Lockard and Schneider (1981) hypothesised that reduced basipetal transport of IAA from scion to root decreased root growth and the consequent amount of cytokinin transported to the scion in the xylem sap, thereby limiting extension growth of the scion. This hypothesis is physiologically feasible because auxin is important for the initiation of apple roots (Jones and Hatfield, 1976; Delargy and Wright, 1979). In addition, it has long been known that root tips are important sites of cytokinin biosynthesis, which was convincingly demonstrated in a recent study of *Arabidopsis* (Nordstrom et al., 2004). Therefore, decreased basipetal transport of IAA from scion to root of trees growing on dwarfing apple rootstocks may reduce cytokinin biosynthesis by decreasing the number of root tips. Furthermore, inhibition of the basipetal IAA signal may down-regulate cytokinin metabolism by the root directly (Lockard and

Schneider, 1981). However, short-term decapitation studies for other plant species have shown that decreased basipetal transport of IAA from shoot to root increased the concentration of cytokinin transported in the xylem sap (Currie, 1997; Bangerth et al., 2000; Nordstrom et al., 2004). Therefore, decreased basipetal transport of IAA may not reduce cytokinin biosynthesis directly.

Nevertheless, cytokinins are present in the xylem sap of apple trees (Jones, 1973; Young, 1989; Cutting et al., 1991; Skogerbo and Mage, 1992; Tromp and Ovaa, 1994; Kamboj, 1996; Kamboj et al., 1999) suggesting that they are produced by the root and transported to the scion in the xylem vasculature (Jones, 1964, 1967, 1973). In addition, the 'Fiesta' scion grafted onto M.9 had lower total concentrations of zeatin plus zeatin riboside in the xylem sap than MM.106 (Kamboj, 1996; Kamboj et al., 1999). Therefore, dwarfing rootstocks do reduce the amount of cytokinin transported from root to scion. Jones (1973) showed that cytokinin stimulated the outgrowth of isolated apple shoots *in vitro,* and subsequently it was postulated that increased endogenous concentrations of cytokinin within the xylem sap might explain the increased rates of scion growth for composite trees on vigorous rootstocks (Kamboj et al., 1999).

However, it has long been known that exogenous cytokinin does not increase the mean shoot length of primary (Wertheim and Estabrooks, 1994) or secondary shoots (Forshey, 1982; Elfving, 1984, 1985; Cody et al., 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988) of young composite apple trees. Furthermore, the cytokinin benzylaminopurine (BAP) applied to young apple scions primarily stimulated axillary buds along the primary shoot to break and form secondary shoots (Williams and Stahly, 1968; Kender and Carpenter, 1972; Forshey, 1982; Elfving, 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988; Volz et al., 1994; Wertheim and Estabrooks, 1994). In contrast, in a tree nursery there were trends that M.9 reduced the mean number of secondary shoots that formed on different scion cultivars in their first year of growth (Jaumien et al., 1993; Volz et al., 1994). Similarly, the 'Worcester Pearmain' scion on M.9 was smaller than that on M.16 partly because of fewer growing points (Avery, 1969). Therefore, the literature indicates that a decreased supply of cytokinin transported to the scion growing on a dwarfing apple rootstock (Kamboj et al., 1999) may predominantly cause a scion phenotype with fewer growing points.

The inability of exogenous cytokinin to stimulate additional growth of the primary (Wertheim and Estabrooks, 1994) and secondary shoots (Forshey, 1982; Elfving, 1984, 1985; Cody et al., 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988) for scions growing on young composite apple trees strongly indicates that other endogenous hormones are necessary to stimulate shoot extension growth of the scion on a dwarfing apple rootstock. A recent study at East Malling reported that gibberellins were virtually undetectable in the xylem sap of scions grown on different size-controlling rootstocks (East Malling, 2005). In addition, the most recent review into the physiological causes of rootstock-induced dwarfing of the scion reported that gibberellins might not be important in the dwarfing mechanism (Webster, 2004).

In contrast, other evidence suggests that gibberellins may indeed be important in rootstock-induced dwarfing of the scion. For example, gibberellins are transported within the xylem sap of apple trees (Jones and Lacey, 1968; Ibrahim and Dana, 1971; Motosugi et al., 1996) indicating that the apple root may synthesise and supply gibberellins to the scion (Jones and Lacey, 1968). Dwarfing compared with vigorous rootstocks decreased endogenous concentrations of gibberellins within the root (Yadava and Lockard, 1977), xylem sap (Ibrahim and Dana, 1971) and leaves or shoots (Yadava and Lockard, 1977; Fontana-Degradi and Visai, 1978). More importantly, exogenous gibberellin(s) stimulated shoot extension growth of apple (Sironval et al., 1962; Marcelle, 1963; Martin, 1967; Robitaille and Carlson, 1971, 1976; Luckwill and Silva, 1979; Tromp, 1982; Steffens et al., 1985; Popenoe and Barritt, 1988). Exogenous GA_3 did not increase the rate of node emergence by the apple shoot apical meristem, but increased the proportion of shoots that were growing late in the season (Luckwill and Silva, 1979). In contrast, an important way in which dwarfing apple rootstocks decreased shoot growth compared with vigorous rootstocks was to increase the proportion of shoots that terminated early in the growing season (Swarbrick, 1929; Colby, 1935; Tubbs, 1951; Avery, 1969; Robitaille and Carlson, 1976). Therefore, earlier termination of SAMs for a scion growing on a dwarfing apple rootstock may result from the dwarfing rootstock transporting lower amounts of root-produced gibberellin to the scion in the xylem vasculature.

The literature for apple also indicates that the basipetal transport of IAA from scion to root is important for the growth of shoot apical meristems. For example, the application

of the auxin transport inhibitor 'TIBA' to the root/shoot transition region of 'Antonovka' apple seedlings reduced the basipetal transport of ^{14}C-IAA and caused the eventual termination of the SAM on the primary shoot (Grochowska et al., 1994). In a similar manner, the bark of the dwarfing apple rootstock metabolised more IAA (Martin and Stahly, 1967; Gur and Samish, 1968) and its stem reduced the basipetal transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj et al., 1997), particularly as shoot growth slowed late in the season (Kamboj et al., 1997).

Therefore, shoot/root/shoot signalling mechanisms may exist whereby dwarfing apple rootstocks decrease the basipetal transport of IAA from scion to root that in turn reduces gibberellin synthesis by the root system and its consequent transport to the scion, therefore limiting extension growth of the scion by increasing shoot termination. In addition, reduced basipetal transport of IAA from scion to root may reduce root growth, the biosynthesis of cytokinins and their transport to the scion, thereby causing a scion phenotype with fewer growing points.

1.8 Summary, rationale and thesis objectives

Adoption of high-density orchard systems planted on dwarfing apple rootstocks has potential to greatly improve production efficiency of New Zealand apple orchards. However, there is a strong need to breed new dwarfing apple rootstocks that are resistant to woolly apple aphid, fire blight, phytophthora and SARD. At present, breeding and selection of new apple rootstocks for commercial release is largely empirical and, therefore, time consuming.

Recent research has identified a dwarfing locus (*DW1)* involved in the dwarfing mechanism of the M.9 rootstock (Rusholme-Pilcher et al., 2008) and a genetic map has been constructed of apple rootstock progeny derived from a cross between M.9 and 'Robusta 5' (Celton et al., 2009). These are important scientific advancements because the identification of genetic markers linked to dwarfing gene(s) will enable desirable progeny to be selected from large populations of tree material at a very young age. Efficiency and effectiveness of rootstock breeding will, therefore, be improved. In addition, detailed genetic maps have potential application in further elucidating the genetic control of rootstock-induced dwarfing of the scion.

Despite recent advances in the genetic elucidation of rootstock-induced dwarfing of the scion, the fundamental biological processes that dwarfing gene(s) control are poorly understood, particularly the underlying physiological mechanism(s) that are first modified within the composite apple tree growing on a dwarfing rootstock, and their consequent expression in scion architecture during early tree phenology. Important physiological mechanisms by which dwarfing apple rootstocks decrease scion vigour may involve restricting the endogenous transport of nutrients, water and hormones. The most plausible of these is the modification of shoot/root/shoot signalling of endogenous plant hormones because alterations in their transport appear to explain some architectural changes imposed on the scion by dwarfing apple rootstocks. However, it is poorly understood how the modification of shoot/root/shoot signalling of endogenous hormones by a dwarfing apple rootstock may change scion architecture soon after grafting of the composite tree.

Recent studies showed that the first observable effect of a dwarfing apple rootstock was to increase the floral precocity of the scion in the spring of year two after grafting of the composite tree, thus causing subsequent alterations in shoot development and morphology. However, earlier studies indicated that dwarfing apple rootstocks may impose small reductions in scion growth in the first year from propagation. Unfortunately, it is very difficult to compare scion architecture amongst studies in the literature because of different methodologies used to propagate or grow the experimental tree material. These may have caused very different scion architecture in early tree development. In some instances, excessive vigour stimulated by heading back, or initial disparities between the size of the scion and the root, may have masked subtle changes imposed on the scion by the dwarfing apple rootstock in the first year of growth from budding or grafting. Therefore, the first objective of this thesis was to elucidate how the dwarfing apple rootstock initially modified scion architecture after grafting and the precise stage of tree phenology when this first occurred (objective one).

Elucidation of objective one was essential to clearly identify processes that were the first physiological causes of rootstock-induced dwarfing of the scion from those that were subsequent developmental effects. Knowledge of the initial architectural modifications imposed on the scion by a dwarfing apple rootstock would also provide important information on which hormone groups were initially causal in scion dwarfing.

Hypotheses generated were then tested in a number of growth manipulation studies designed to understand whether endogenous shoot/root/shoot signalling of hormones may have caused the initial changes in scion architecture on the dwarfing apple rootstock (objective two). Elucidation of objectives one and two then provided the rationale to quantify endogenous hormones that may have caused the initial changes in scion architecture on the dwarfing apple rootstock (objective 3). The latter objective may provide insights into the likely physiological processes that dwarfing gene(s) control within the composite apple tree.

1.8.1 Summary of major objectives

The overall aim was to understand rootstock dwarfing effects on the scion in relation to tree phenology, architecture and plant hormones. Specific objectives were to:

1) Elucidate how the dwarfing apple rootstock initially modified scion architecture compared with rootstocks of greater vigour and the precise stage of tree phenology when this occurred following grafting of the composite tree.

2) Use exogenous growth regulators and root restriction treatments to determine the likely endogenous hormonal shoot/root/shoot signalling mechanisms that caused the initial changes in scion architecture on the dwarfing apple rootstock.

3) With a knowledge of the above, quantify fluxes of endogenous auxin, cytokinins and gibberellins to assess whether their differential transport by the dwarfing apple rootstock may have caused the initial architectural modifications that will subsequently define the future architecture of the scion.

2. General materials and methods

2.1 Propagation of self-rooted 'Royal Gala' rootstocks

2.1.1 Hardwood cuttings

The modified method of Child and Hughes (1978) was attempted for propagating hardwood cuttings of apple. Annual shoots (10 mm in diameter) of 'Royal Gala' were collected while dormant in June, 2004. The basal end of the shoot was cut below a node and dipped for 10 sec into a solution of 25% ethanol:75% water containing 1000 mg L^{-1} indole-3-butyric acid (IBA). Shoots were then cut back to a bud at a height of approximately 250 mm above the cutting base and the tip of the cutting was sealed with pruning paste (BacSeal® Super, Bayer, New Zealand). The basal ends of the cuttings were buried 100 mm into moist perlite. The medium surrounding the base was maintained at 25°C using bottom heat controlled by a thermostat probe placed into the medium at the same depth as the cutting base. To prevent leafing out, cuttings were propagated in a cool-store maintained at an air temperature of 7°C. Callus development at the shoot base was observed within 12 days, but less than 1% of the cuttings formed root initials.

2.1.2 Aerial layering

Following the failure of hardwood cuttings to form roots, aerial layering of actively growing shoots was carried out at both Massey University and HortResearch orchards located in Palmerston North and in the Hawke's Bay, respectively. In late November, the bases of shoots formed in the previous season (i.e., 1-year-old) were girdled to remove a 10 mm ring of bark. For half the shoots, the girdle was treated with IBA (1000 mg L^{-1}) prepared in lanolin. Shoot bases were wrapped in moist sphagnum moss and covered with plastic film to hold the moss in place. The plastic was pierced with small holes to aid aeration and then covered with tinfoil to prevent overheating from solar radiation. By autumn, 60% and 40% of the shoots treated with (Figure 2.1) or without IBA, respectively, had formed roots. Aerial layering was, therefore, substantially better in promoting root formation of 'Royal Gala' shoots than hardwood cuttings. Although aerial layering produced adequate rooting percentages, the shoots were variable in their diameters, with a large proportion outside the preferred 8 to 10 mm for bench grafting.

2.1.3 Stool bed system

A stool bed system was also used to promote rooting and to try and increase the number of stools produced with diameters between 8 to 10 mm. In August 2005, 'Royal Gala' scions were cleft grafted at a height of 100 mm onto MM.106 rootstocks. New grafts were bedded into moist sawdust until spring. During October, trees were planted in the field leaving 50 mm of rootstock above the soil level. In early January, the lower leaves on the primary shoot (approximately 300 mm) were removed and the axillary buds on half the scions were treated with IBA (1000 mg L^{-1}) in lanolin. Sawdust was mounded around the base of the scions and was kept moist by daily trickle irrigation. Root initials began emerging in late March and rooted scions were harvested in late July (Figure 2.2). Irrespective of IBA treatment, rooting percentage was 95% with 80% of the stools falling within the preferred 8 to 10 mm grade for bench grafting. To provide a yearly supply of stools, only half the stools within a bed were harvested. The remaining 'Royal Gala' shoots were cut back to a bud close to the ground and allowed to re-grow in the following spring.

Figure 2.1. Aerial layering of a 'Royal Gala' shoot in November 2004 resulted in good root development by April, 2005.

Figure 2.2. An example of a high-quality 'Royal Gala' stool produced in a stool bed system.

2.2 Preparation and management of experimental tree material

Grafting of tree material was conducted during winter dormancy. Rooted stools (8 to 10 mm in diameter) of M.9, MM.106 and M.793 were sourced from Pattullo's Nurseries Ltd, Hawke's Bay, New Zealand. The clone of M.9 used in all studies was NZ.9 (DSIR, heat-treated to be free of specified viruses). 'Royal Gala' rootstocks were produced in stool beds set up at the Fruit Crops Unit Palmerston North (Section 2.1.3). Grafting was completed by mid August unless otherwise stated. A 'Royal Gala' scion (i.e., one-year-old vegetative shoot with two buds) was grafted onto each rootstock using a cleft graft cut with a bench mounted grafting machine (Ragget Industries, Gisborne, New Zealand). Grafting height of the scion onto the rootstock was standardised at 350 mm for all experimental tree material. Graft unions were taped and the tip of the scion was sealed with pruning paste.

Newly grafted trees were planted into black 4 L polythene planter bags containing a 70:30 bark/pumice medium. The following nutrients were added per 100 L of medium: 300 g of six-month controlled release fertiliser (N:15, P:4, K:7.5, Mg:1.8) plus trace elements (Osmocote®, Scotts, USA), 100 g of Aglime and 300 g of dolomite. Trees were establishing in a tunnel house (Figure 2.3) and the scion was debudded to a single shoot during October. In late October, trees were moved outside to a sheltered standing out area to harden off (Figure 2.4) before transplanting into larger growing bags (Chapter 3) or the field (Chapter 4). In Chapters 5 and 6, following dormant grafting the experimental trees were bedded into moist sawdust until the first signs of scion bud movement (20/9/05), at which, trees were planted into 50 L black polythene planter bags and grown in the tunnel house before being moved outside to harden off in November. For all experiments, scions were not pruned to prevent any confounding influences on scion architecture. Shoots that grew out from the rootstock stem were debudded as required over the growing season.

Figure 2.3. Grafted tree material establishing in a tunnel house in mid September, 2004.

Figure 2.4. Tree material hardening off outside in November, 2004.

2.3 Extraction procedures for hormone quantification

2.3.1 Collection of xylem sap from 'Royal Gala' apple scions for the analysis of cytokinins and gibberellins

Sap was extracted from 'Royal Gala' scions following the methods of Bollard (1953). Between 0:700 to 0:800 hr, primary shoots were stripped of their leaves and branches. Primary shoots were then cut above the graft union, immediately wrapped in moist newspaper, sealed in plastic bags and stored at 2^{o}C. Sap extraction from the harvested primary shoots was completed within 8 hr from decapitation. A 200 mm section of bark was removed from the basal end of the primary shoot to avoid contamination from any phloem exudates. To extract xylem sap, the base of the primary shoot was placed into a sealed vacuum flask connected to a vacuum pump. From the distal end, 100 mm of shoot was cut every 5 sec to keep a constant flow of xylem sap into the sample vial. Sap was immediately filtered through filter paper (Whatmans No. 1) and stored at -20^{o}C until analysis. In the first season of scion growth following grafting, approximately 2, 4 and 6 mL of sap was extracted per primary shoot during February, March and April, respectively.

2.3.2 Diffusion of indole-3-acetic acid from the primary shoot apex of 'Royal Gala' apple scions

To measure the diffusion of indole-3-acetic acid (IAA) out of shoot apices, the apex of the primary shoot was excised 20 mm from the shoot tip. The lower 5 mm of the apex base was immediately placed into 700 µL of 2-(N-morpholino)ethanesulphonic acid (MES, M3671, Sigma, USA) buffer (50 mmol L^{-1}, pH 6.2) (Li and Bangerth, 1999) contained in a 1.5 mL polypropylene micro-centrifuge tube. The tubes/apices were then placed in a dark room with an air conditioning unit (Daikin, FTX 515) operating to maintain a constant air temperature of 25^{o}C. An automated misting unit (505 Defensor, Switzerland) was used to maintain high humidity. At 10 hr of incubation, the base of each apex was re-cut with a sharp scalpel and placed into a new buffer solution. At 20 hr of incubation, the apices were removed from the buffer solutions. IAA diffusate was stored at -75^{o}C until analysis.

2.4 Chromatography of endogenous plant hormones

2.4.1 Development of a preliminary purification method for cytokinins and gibberellins within the xylem sap of apple

Preliminary purification of sap utilised Sep-Pak® C_{18} columns (Waters, Ireland). This method enabled cytokinins and gibberellins to be conveniently concentrated before separation by high performance liquid chromatography (HPLC). Initially, validation of the method for cytokinins was carried out using authentic cytokinin standards and a spectrophotometer (Hitachi U-2000) to determine whether there were any significant losses of cytokinins from the C_{18} during sample loading and on washing of the column with 5 mL of H_20:0.5% formic acid.

Four C_{18} columns were preconditioned sequentially with 5 mL each of 100, 50, 10 and 0% methanol / H_20:0.5% formic acid. A single cytokinin standard (10 μg) of zeatin (Z), zeatin riboside (ZR), isopentenyladenosine (IPA) or isopentenyladenine (2iP) (Sigma, Germany) was dissolved in 1 mL of H_20:0.5% formic acid and adsorbed onto one of the four-preconditioned columns. Columns were then eluted with 5 mL of H_20:0.5% formic acid followed by six 1 mL fractions of 100% methanol. Fractions were measured for the presence of each cytokinin using the spectrophotometer. No cytokinins were detected within the H_20:0.5% formic acid fraction, but were completely eluted in the first 5 mL of 100% methanol (data not shown).

To ensure that the more polar cytokinins, particularly Z and ZR, were not lost within the H_20:0.5% formic acid fraction, additional checks were made using either radioimmunoassay (RIA) or tritiated zeatin riboside (^{3}H-ZR). For RIA, 100 ng of Z was adsorbed onto a preconditioned column that was then eluted with 5 mL of H_20:0.5% formic acid, followed by three 2 mL fractions of methanol. Fractions were quantified by radioimmunoassay (RIA) (Section 2.5.2) for the presence of Z. Notably, no Z was detected in the H_20:0.5% formic acid fraction (Figure 2.5). Instead, Z was completely recovered in the methanol fractions. The same result was also obtained using 20,000 disintegrations per min (DPM) of ^{3}H-ZR partitioned through a Sep-Pak® column (data not shown), therefore indicating the suitability of the method for the preliminary purification of cytokinins prior to HPLC.

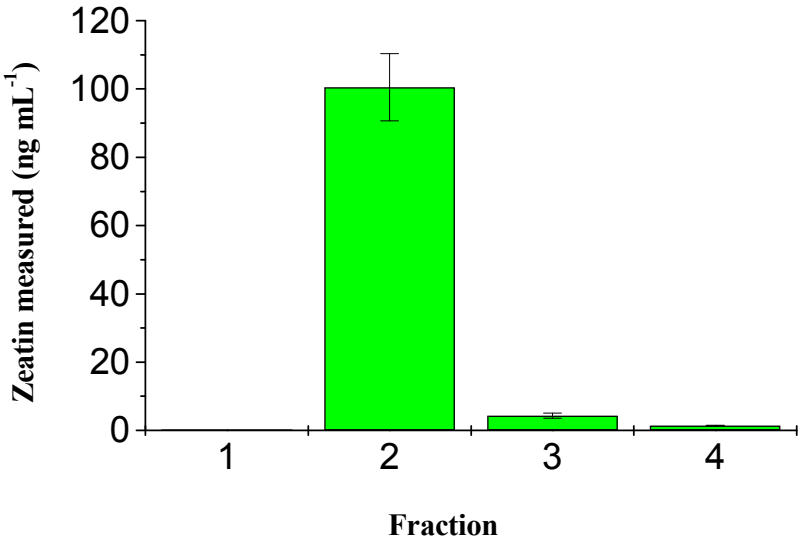

Figure 2.5. Mean concentration of zeatin measured by radioimmunoassay within fractions eluted off a Sep-Pak® C_{18} column. Zeatin (100 ng) was adsorbed onto the column before eluting with 5 mL of H_20:0.5% formic acid (fraction 1), followed by three 2 mL washes (fractions 2 to 4) of 100% methanol. Vertical bars represent the standard error, n=2.

2.4.2 Pre-purification of cytokinins from xylem sap using Sep-Pak® C_{18} columns before separation of cytokinins by high performance liquid chromatography (HPLC)

2.4.2.1 Zeatin, zeatin riboside, isopentenyladenosine and isopentenyladenine

Xylem sap (2 to 5 mL per tree) was spiked with 20,000 DPM of repurified ^{3}H-ZR and ^{3}H-IPA as internal standards. Deuterium-labelled gibberellin A19 ([$^2H_2$]GA_{19}, 50 ng) (Olchemim Ltd, Czech Republic) was also added when gibberellins were collected from the HPLC gradient. Sap was acidified with 25 µL of formic acid before loading onto a preconditioned Sep-Pak® C_{18} column. The column was eluted with 5 mL of H_20:0.5% formic acid, which was collected for the analysis of cytokinin ribotides. The column then was washed with 6 mL of methanol that was evaporated to dryness in a vacuum freeze dryer before redissolving the cytokinins in HPLC starting solvent (6% acetonitrile:94% 40 mmol L^{-1} acetic acid solution adjusted to pH 3.38 with triethylamine (TEA)).

2.4.2.2 Cytokinin ribotides

For cytokinin ribotides, the H_20:0.5% formic acid phase was incubated with a phosphatase to convert zeatin-riboside-5-monophosphate and isopentenyladenosine-5-monophosphate to ZR and IPA, respectively. In short, the H_20:0.5% formic acid phase was spiked with 20,000 DPM of ^{3}H-ZR and ^{3}H-IPA, taken to dryness in a vacuum freeze drier, resuspended in 1 mL of 20 mmol L^{-1} Tris buffer (pH 9.8) containing 5 units of alkaline phosphatase (Sigma, U.S.A, P5521) and incubated at 37^{o}C for 4 hr in a water bath. Following incubation, samples were acidified with 5 µL of formic acid before separation on Sep-Pak® C_{18} columns as described in Section 2.4.2.1. The H_20:0.5% formic acid phase was collected for the analysis of cytokinin glycosides. The methanol fraction was dried and subjected to HPLC separation. The peaks coinciding with ZR, ^{3}H-ZR, ^{3}H-IPA and IPA were collected (Section 2.4.6). Cytokinin and trialcohol peaks were either radioimmunoassayed (Section 2.5.2) or counted for the recovery of radioactivity (Section 2.4.6.3), respectively.

2.4.2.3 Cytokinin glycosides

A β-glucosidase was used to convert any cytokinin glycosides remaining in the H_20:0.5% formic acid phase to Z or 2iP. The H_20:0.5% formic acid phase, spiked with 20,000 DPM of ^{3}H-ZR and ^{3}H-IPA, was dried and resuspended in 1 mL of 20 mmol L^{-1} ammonium acetate buffer (pH 5.5) containing 5 units of beta-glucosidase (Fluka, 49290). Samples were incubated at 37^{o}C for 4 hr in a water bath. Following incubation, samples were separated on Sep-Pak® C_{18} columns as previously described. The methanol phase was subjected to HPLC separation and the peaks coinciding with Z, ^{3}H-ZR, ^{3}H-IPA and 2iP were collected (Section 2.4.6). Cytokinin and trialcohol peaks were either radioimmunoassayed (Section 2.5.2) or counted for the recovery of radioactivity (Section 2.4.6.3), respectively.

2.4.3 Development of a purification method for indole-3-acetic acid diffused from the shoot apices

Prior to HPLC separation, IAA diffused from the shoot apices was subjected to purification on C_{18} columns. To validate the method, a C_{18} column was preconditioned with 100%, 50% and 15% methanol / 0.5% formic acid solution. Carboxyl-labelled indole-3-acetic acid (^{14}C-IAA, Sigma, USA, I-8262) was adsorbed onto the C_{18}

(100,000 DPM) in 10% methanol. The column was washed with 6 mL of 15% methanol (3 x 2 mL fractions) followed by 6 mL of methanol (3 x 2 mL fractions). Collected fractions were resuspended in scintillation fluid (Ultima Gold, PerkinElmer, USA) and counted for radioactivity in a scintillation counter (Tri-Carb 2900TR, PerkinElmer, USA). The ^{14}C-IAA was recovered in the first 4 mL of methanol and no significant losses of radioactivity resulted from eluting the column with 6 mL of 15% methanol (Figure 2.6).

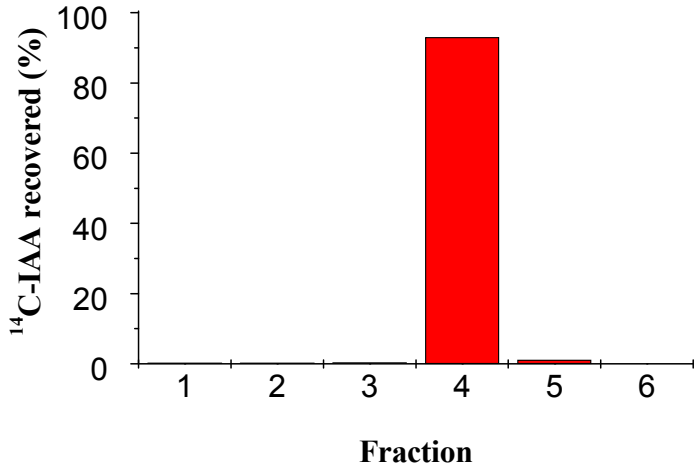

Figure 2.6. Recovery of carboxyl-labelled indole-3-acetic acid (^{14}C-IAA) in fractions eluted off a Sep-Pak® C_{18} column. Following the adsorption of ^{14}C-IAA onto the C_{18}, the column was eluted with three 2 mL fractions of 15% methanol:0.5% formic acid (fractions 1, 2 and 3) followed by three 2 mL fractions of 100% methanol (fractions 4, 5 and 6).

2.4.4 Synthesis of carboxyl-labelled indole-3-acetic acid methyl ester

Carboxyl-labelled indole-3-acetic acid methyl ester (^{14}C-IAA-Me) was synthesised for use as an internal standard for determining the recovery of IAA within the samples after chromatography steps on Sep-Pak® C_{18} columns and by HPLC.

2.4.4.1 Synthesis of diazomethane for methylating indole-3-acetic acid

Diazomethane was synthesised within a fumehood using the modified methods of Schlenk and Gellerman (1960). Extreme care was taken because diazomethane synthesis can result in explosions. N-methyl-N-nitroso-p-toluenesulfonamide (2.14 g)

(Diazald®, Scientific Supplies Ltd, Auckland, New Zealand) was dissolved in 40 mL of ether that had been chilled on ice. Potassium hydroxide (0.4 g) was dissolved in 10 mL of 95% ethanol, added to the Diazald®/ether solution, and stirred for 5 min with a magnetic stirrer. The ether solution was then heated to 90°C in a water bath and diazomethane was collected by distillation into a collection vial that was cooled with liquid nitrogen. This synthesis yielded 25 to 30 mL of highly yellow coloured diazomethane, which was able to be stored in a sealed glass bottle at -20°C for four weeks.

2.4.4.2 Efficacy of diazomethane to methylate indole-3-acetic acid

The diazomethane synthesised was tested to ensure that it did methylate IAA effectively. Excess IAA dissolved in methanol (1000 ng/tube) was dried within 5 mL glass test tubes and 500 µL of diazomethane was added at 1:1, 1:5, and 1:10 dilutions in ether. Tubes were sealed, vortexed and left to stand at 4°C for 30 min. Acetic acid (50 µL, 0.2 mols L^{-1}) was added to each tube and the ether was dried under vacuum. IAA-Me was redissolved into 80% TEA:20% acetonitrile and the samples were subjected to HPLC using gradient elution (Table 2.2). The presence of IAA and IAA-Me peaks were assessed by UV and fluorescence detection (Section 2.4.6.2). For the 1:1 and 1:5 dilutions only, no peak was detected at the retention time of IAA (14.20 min) (Figure 2.7). Instead, UV and fluorescence methods detected a single large peak at the retention time of IAA-Me (24.30 min), thereby indicating that all of the IAA added was methylated. A 1:5 dilution of diazomethane in ether was used for routine methylation of samples.

2.4.4.3 Methylation of carboxyl-labelled indole-3-acetic acid to carboxyl-labelled indole-3-acetic acid methyl ester

Carboxyl-labelled indole-3-acetic acid (5,000,000 DPM) was dried and then methylated as described previously. The sample was resuspended in 80% TEA:20% acetonitrile and injected into an HPLC running isocratically at 65% TEA:35% acetonitrile. The retention time of ^{14}C-IAA-Me was established with a UV detector and with a flow-through radioactivity monitor (Section 2.4.6.2). The retention time measured by UV for the synthesised ^{14}C-IAA-Me was identical to standards of IAA-Me, and a single radioactive peak eluted close to the peak of ^{14}C-IAA-Me in the UV (Figure 2.8). Once collected, the fraction containing ^{14}C-IAA-Me was dried and resuspended in 5 mL of

methanol. A 10 μL sub-sample was counted for radioactivity, the ^{14}C-IAA-Me fraction was then diluted with methanol to 15,000 DPM 100 μL^{-1} and stored at -20^{o}C.

2.4.5 Routine procedure for pre-purification of indole-3-acetic acid using Sep-Pak® C_{18} columns before HPLC separation

Prior to sample loading, IAA diffusate from shoot apices was acidified (10 μL formic acid) and spiked with 15,000 DPM of ^{14}C-IAA-Me as an internal standard. The sample was adsorbed onto a preconditioned Sep-Pak® C_{18} column before washing the column with 6 mL of 15% methanol: 85% H_20:0.5% formic acid. IAA was then eluted off the column with 5 mL of methanol. Methanol was dried under vacuum and the sample was redissolved in 500 μL of 80% TEA:20% acetonitrile ready for injection into the HPLC.

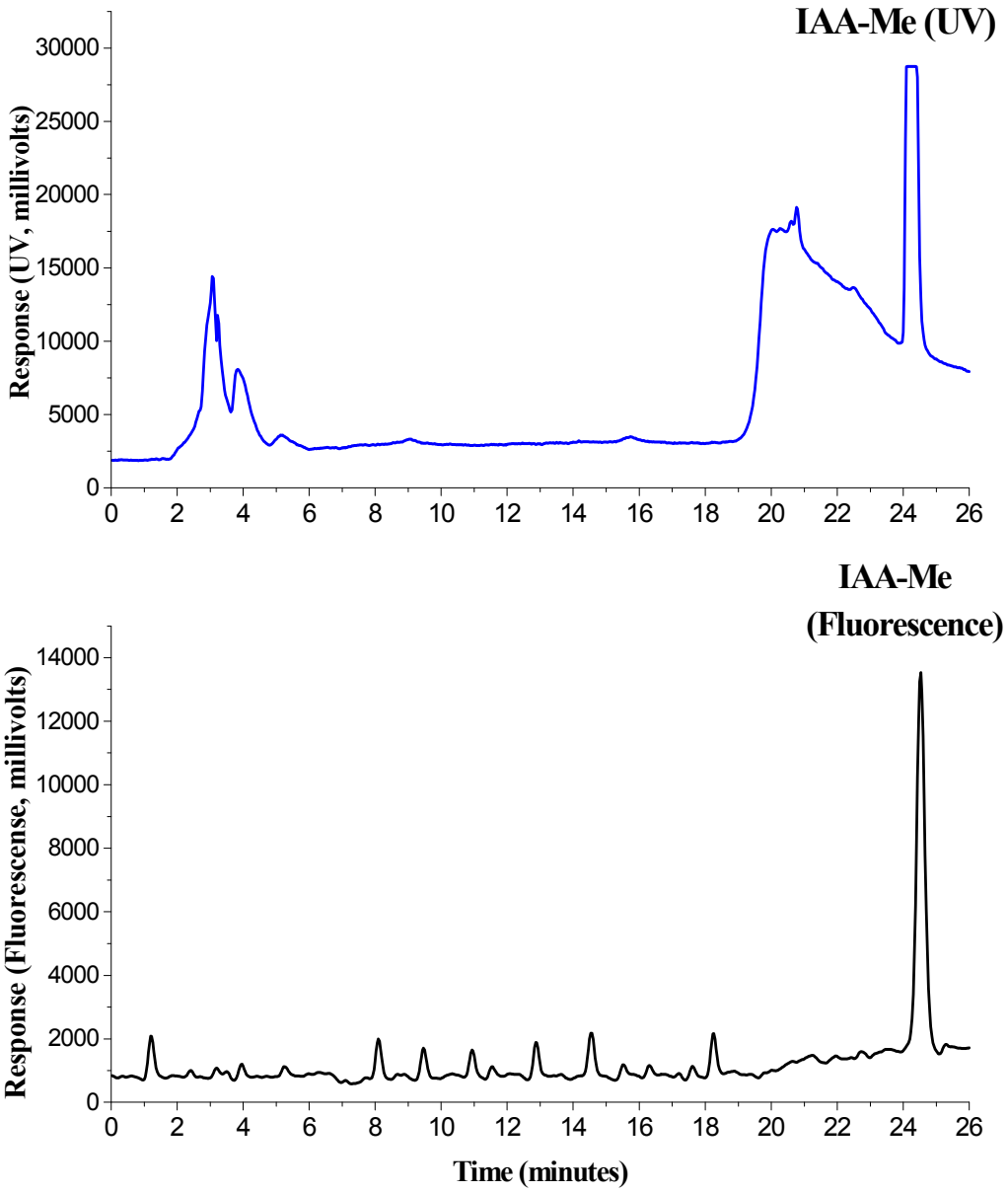

Figure 2.7. Response of UV (top) and fluorescence (bottom) detectors to 500 ng of indole-3-acetic acid (IAA) after methylation with diluted diazomethane (1:5 dilution in ether) and separation by HPLC. The absence of a peak for IAA at 14.20 min indicates all of the IAA was methylated to indole-3-acetic acid methyl ester (IAA-Me) (24.30 min).

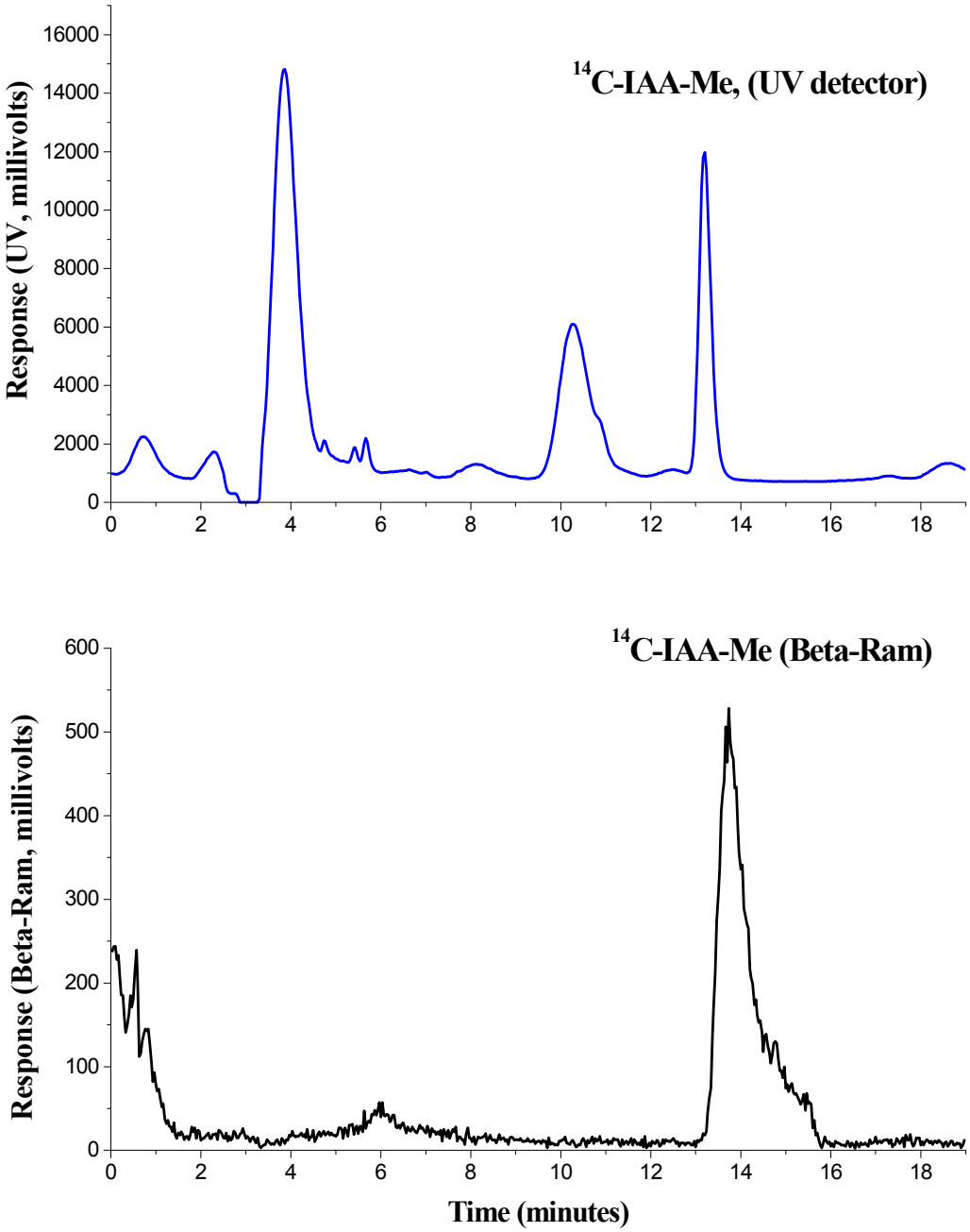

Figure 2.8. Response of UV (top) and radioactivity (bottom) detectors to carboxyl-labelled indole-3-acetic acid methyl ester (^{14}C-IAA-Me) after carboxyl-labelled indole-3-acetic acid (^{14}C-IAA) was methylated with diazomethane. The peak of radioactivity (bottom) was delayed by approximately 30 sec because the radioactivity detector was downstream of the UV detector.

2.4.6 High performance liquid chromatography of plant hormones

2.4.6.1 HPLC system

Separation of cytokinins by HPLC was modified from the methods of Currie (1997). Separation was made on a 220 x 4.6 octadecyl silica (ODS) column in line with a 3 x 4.6 ODS guard column (Applied Biosystems Inc., Brownlee Spheri-5). Solvent delivery to the column was via a Waters™ 501 and a Waters™ 510 HPLC pump controlled by a Waters™ automated gradient controller. Solvents were HPLC grade acetonitrile and TEA (40 mmol L^{-1} acetic acid solution adjusted to pH 3.38 with triethylamine). Solvents were made prior to each sample run, filtered through a 0.2 µm teflon filter and further degassed by sonicating for 30 min.

2.4.6.2 Determination of retention times

Injections (500 µL) were manually made into a Rheodyne injection port. Cytokinins and gibberellins were separated by a solvent gradient (Table 2.1) at a flow rate of 1 mL min^{-1}. Separation of IAA used the same flow rate but a different solvent gradient (Table 2.2). The retention times of cytokinins (Figure 2.9) and IAA (Figure 2.10) standards were identified with a programmable UV detector (Waters™ 490E) at 268 and 280 nm, respectively. IAA was also detected using a scanning fluorescence detector (Waters™ 474). Excitation and emission maxima for IAA were 280 and 350 nm, respectively (Crozier et al., 1980). Excitation and emission bandwidths were 10 and 18 nm, respectively. The retention times for ^{3}H-ZR, ^{3}H-IPA, ^{14}C-IAA and ^{14}C-IAA-Me were established using a flow through radioactivity monitor (βRam, In/Us systems N.J., USA) connected downstream of the UV detector. The hold up volume of the radioactivity detector was 1.48 mL. Inflow-3™ liquid scintillant (IN/US Systems, Inc., USA) was pumped at a flow rate of 1 mL min^{-1} or at a 1:1 ratio with the HPLC solvent. Hence, the total flow through the radioactivity detector was 2 mL min^{-1}.

Table 2.1. HPLC solvent gradient used for the separation of cytokinins and gibberellins. Curves 6 and 7 are linear and concave, respectively (Waters, 1985).

Minute	TEA (%)	Acetonitrile (%)	Curve
0	91	9	*
15	91	9	6
20	73	27	6
30	73	27	6
35	5	95	7
40	5	95	7
45	91	9	6

Table 2.2. HPLC solvent gradient used for the separation of indole-3-acetic acid.

Minute	TEA (%)	Acetonitrile (%)	Curve
0	80	20	*
14	80	20	6
15	60	40	6
22	60	40	6
23	2	98	6
25	2	98	6
30	80	20	6

To determine the elution times of gibberellins, 500 ng of GA_{19}, $[^2H_2]GA_{19}$ and GA_{20} (Olchemim Ltd, Czech Republic) were injected into the HPLC gradient (Table 2.1) and 1 min fractions were collected. Fractions were dried under vacuum and resuspended in 100 μL of 50% acetonitrile:50% 0.01 mol L^{-1} ammonium acetate. To identify which fractions contained gibberellin, fractions were injected directly into a single quadrapole mass spectrometer with an electrospray interface (Waters™ MicroMass, United Kingdom). Flow rate of sample into the mass-spectrometer was 100 μL min^{-1}. Tune settings were optimised manually and were as follows: cone and capillary voltage were

+30 V and +3 kV, respectively; cone and N_2 dissolvation gas were 50 and 400 L hr^{-1} respectively; source and dissolvation temperatures were 100°C and 250°C, respectively. Fractions were scanned between 100 to 400 atomic mass units (AMU, scan speed 500 AMU sec^{-1}) in negative ionisation mode. GA_{19} and GA_{20} eluted off the HPLC gradient between 27:30 and 31 min and were collected as a single fraction for quantification by gas chromatography-mass spectrometry (GC-MS) (Section 2.5.5).

2.4.6.3 Routine HPLC separation of endogenous cytokinins, gibberellins and indole-3-acetic acid within samples

Injections (500 µL) of prepared sample were made manually into the HPLC system. Cytokinin, gibberellins, IAA and their internal standards were collected manually into test tubes based on their retention times. Retention times for cytokinins and IAA were checked daily prior to sample separation and were very consistent. To determine recoveries of cytokinins and IAA, the fractions containing tritium or ^{14}C-IAA-Me internal standards were dissolved in scintillant (Ulitima Gold, PerkinElmer, USA) and counted for radioactivity using a scintillation counter (Tri-Carb 2900TR, PerkinElmer, USA). Fractions containing endogenous hormone were dried in a vacuum freeze drier in preparation for quantification (Section 2.5).

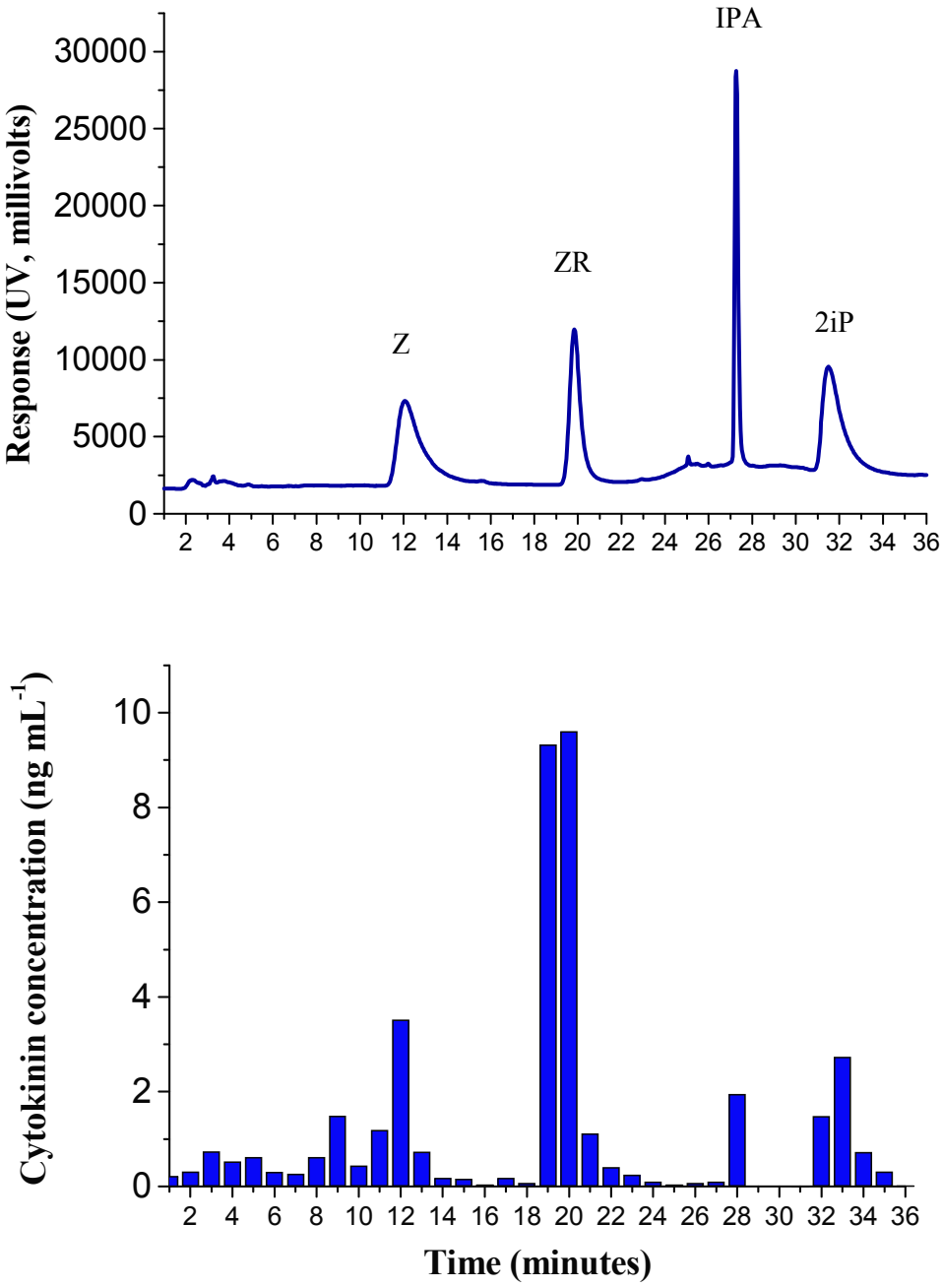

Figure 2.9. (Top) Chromatogram of authentic cytokinin standards separated by HPLC and detected by UV at 268 nm. (Bottom) An immuno-histogram of putative cytokinins within the xylem sap of apple that were separated by HPLC and quantified by radioimmunoassay (see Section 2.5).

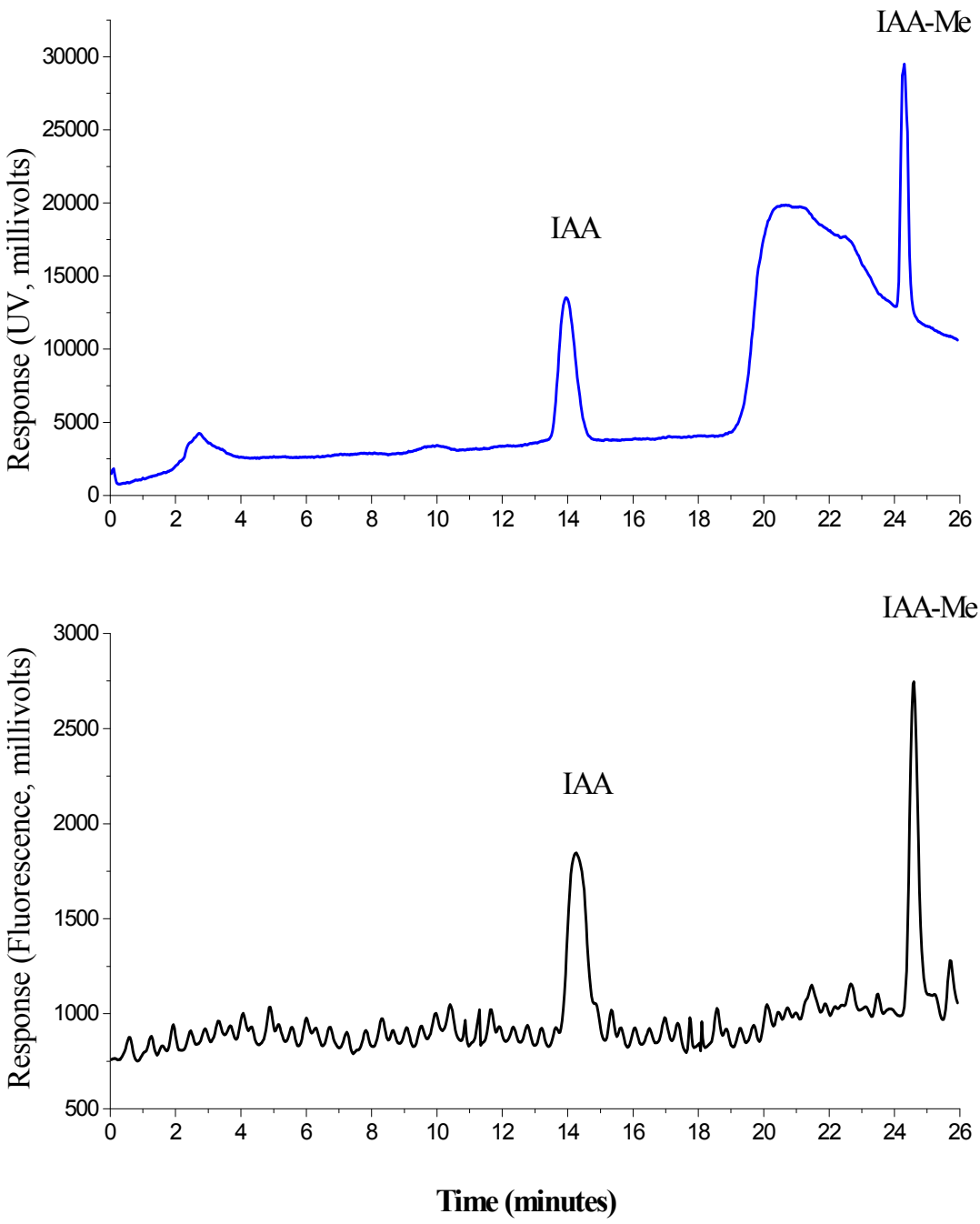

Figure 2.10. Retention times for indole-3-acetic acid (IAA) and indole-3-acetic acid methyl ester (IAA-Me) established with a UV (top) and fluorescence (bottom) detector using an HPLC elution gradient (Table 2.2).

2.5 Hormone quantification

2.5.1 Repurification of cytokinin trialcohols by High Performance Liquid Chromatography

Over time, trialcohols used for radioimmunoassay (RIA) can breakdown (Figure 2.11) causing high levels of non-specific binding and poor sensitivity of the RIA (Currie, 1997). Initial attempts to produce a standard curve using the unpurified trialcohol were unsuccessful. Therefore, trialcohols synthesised by Currie (1997) were repurified using HPLC. The purification protocol for the zeatin riboside trialcohol (^{3}H-ZR) is presented. The ^{3}H-ZR stock was evaporated to dryness under a gentle stream of N_2 gas, re-dissolved in 2 mL of 94% TEA:6% acetonitrile, injected into the HPLC and chromatographed using an isocratic gradient (92% TEA:8% acetonitrile). Six radioactive peaks were identified and collected based on their retention times (Figure 2.11) established with a flow-through radioactivity monitor (Section 2.4.6.2).

2.5.1.1 Immunological identification of the ^{3}H-ZR trialcohol within HPLC fractions

HPLC fractions were dried on a rotary evaporator to remove the solvents and redissolved in 5 mL of methanol. A 10 µL sub-sample of each fraction was counted for radioactivity. A sub-sample of each fraction was subsequently diluted with methanol to 20,000 DPM 100 μL^{-1} and subjected to RIA (Section 2.5.2) at an excess (1000 times dilution) of polyclonal zeatin riboside antisera. For a single peak at the retention time of 12:40 min (Figure 2.11), the antisera bound 75% of the radioactivity added. This level of binding between antisera and the putative trialcohol was similar to the level of binding reported when the antibody and trialcohol were first synthesised (Currie, 1997).

The repurified ^{3}H-ZR was then radioimmunoassayed at different antisera dilutions to determine a suitable dilution where 50% of ^{3}H-ZR was bound in the absence of cytokinin standard or sample. For routine assay, a 2500 or 3000 times dilution of the ZR or IPA antiseras were used, respectively. Standard curves of authentic cytokinin standards were then generated for the repurified trialcohols with their appropriate antibody. The assays were most sensitive between 0.1 to 10 ng per 100 μL^{-1} for Z, ZR, IPA and 2iP, which was very adequate for cytokinin detection in the xylem sap of apple.

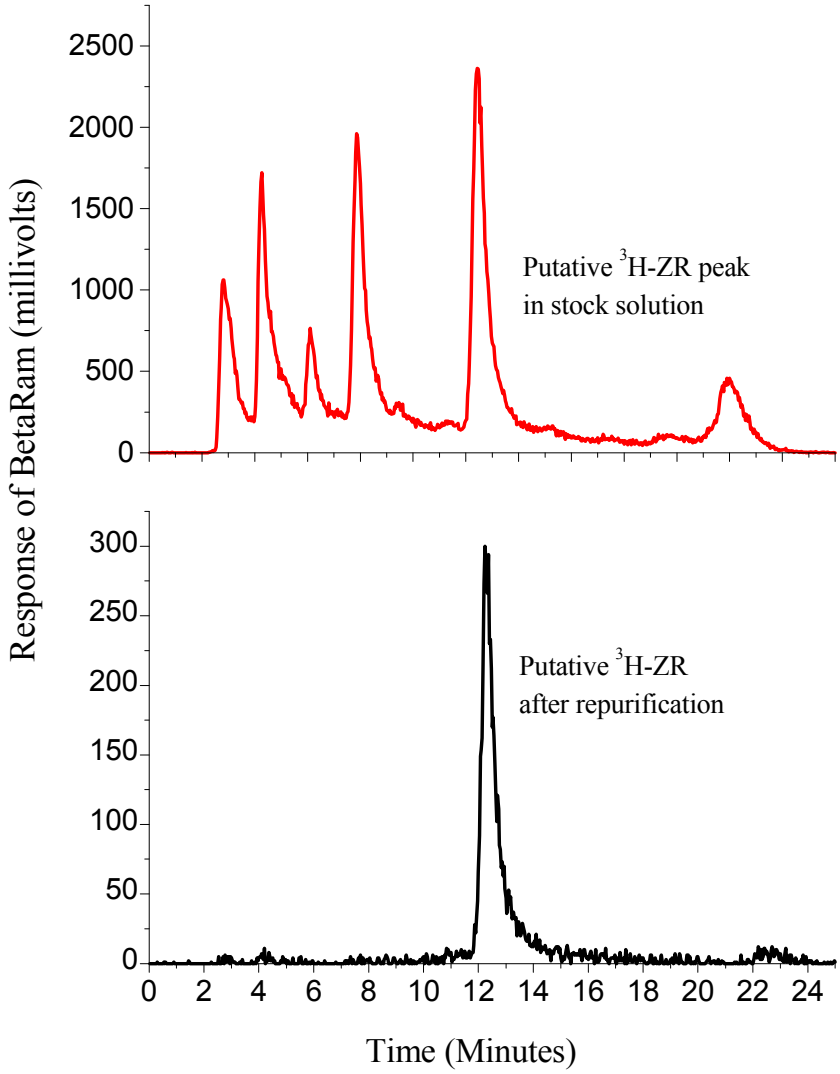

Figure 2.11. Radioactive peaks identified using a radioactivity detector (βRam) for unpurified ^{3}H-ZR stock (top) and a sub-sample of the putative ^{3}H-ZR peak after repurification by HPLC (bottom).

2.5.2 Radioimmunoassay of cytokinins

Cytokinins were quantified by radioimmunoassay using the methods of Currie (1997). HPLC fractions of purified xylem sap were dried in a vacuum freeze drier, resuspended in methanol and then transferred to 1.5 mL polypropylene mini-centrifuge tubes containing 20,000 DPM of trialcohol per tube dissolved in methanol (100 µL). For each cytokinin, a standard curve was generated by adding a fixed volume of serially diluted cytokinin standard to individual tubes containing only the trialcohol. Samples and

standards were assayed in duplicate or triplicate tubes, respectively. Methanol was evaporated from each tube before adding 400 µL of phosphate buffered saline (PBS, pH 7.4) containing antisera diluted to bind 50% of the trialcohol (see Section 2.5.1.1) and 5 µL mL^{-1} of new-born calf serum. After 20 min, the tubes were vortexed and then left to incubate for a further 40 min. The antibody was then precipitated by adding 500 µL of 90% saturated NH_4SO_4 solution per tube. Tubes were vortexed, left to stand for 20 min and then centrifuged for 9 min (13000 rpm at 5°C). To enable the pellet to be coated along the tube wall beneath the hinge, the hinges of the tubes were placed facing outwards within the centrifuge (Currie, 1997). A syringe needle attached to a vacuum was slid down the tube wall opposite the hinge to aspirate the supernatant. The pellet left was dissolved in 100 µL of 50% methanol:water, mixed with 1 mL of scintillation fluid (Ultima Gold, PerkinElmer, USA) and counted for radioactivity (DPM).

Estimation of putative cytokinins within HPLC fractions were calculated from the appropriate standard curve of Z, ZR, IPA or 2iP after logit transformation:

$$Logit(B/Bo) = \ln\left(\frac{B / Bo}{1-B / Bo}\right),$$

where B = tracer bound in the presence of sample or standard and Bo = tracer bound in the absence of cytokinin (Currie, 1997). For each sample, the recovery of radioactivity associated with the ^{3}H-ZR or the ^{3}H-IPA internal standard was used to correct for losses of Z and ZR or IPA and 2iP, respectively, incurred during purification.

2.5.2.1 Validation of zeatin riboside and isopentenyladenosine radioimmunoassays

Collected HPLC fractions from apple xylem sap were tested for non-specific interference (Jones, 1987). HPLC fractions of ZR and IPA from the xylem sap were spiked with different concentrations of authentic ZR and IPA standards, respectively (Figure 2.12). These fractions of sap were subjected to RIA using the appropriate antiseras for ZR and IPA. For both cytokinins, curves generated for sample plus standard were parallel to the curve generated for the authentic cytokinin standard, indicating that non-specific interference from the samples was not present (Figure 2.12).

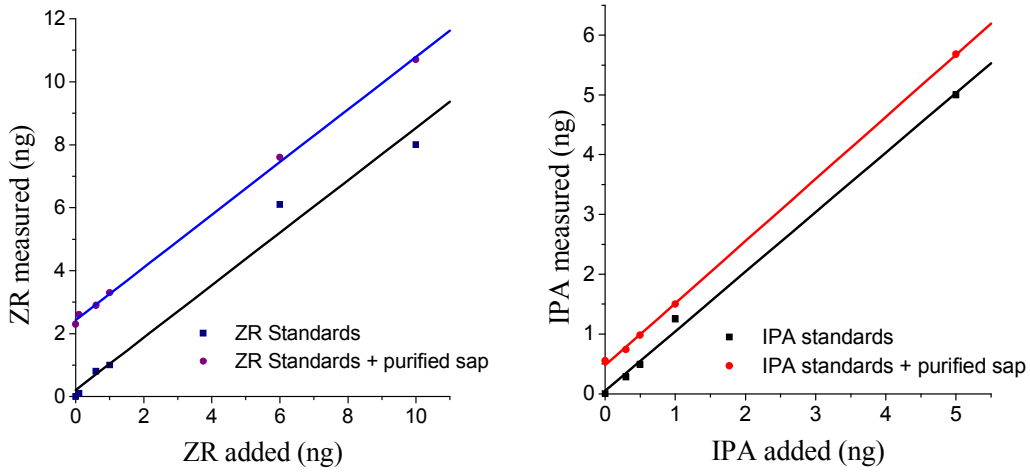

Figure 2.12. Validation curves produced for radioimmunoassay (RIA) of zeatin-riboside (ZR) and isopentenyladenosine (IPA). Parallel lines between the cytokinin standard and cytokinin standard plus purified sap fraction indicate no interference to the RIA from the sample. Along the y-axis, the distance between the lines reflects cytokinin concentration within the sample.

2.5.3 Quantification of indole-3-acetic acid by HPLC-spectrofluorimetry

Indole-3-acetic acid was quantified during separation by HPLC (Section 2.4.6). To determine IAA concentration within the samples, a standard curve was generated by injecting serially diluted standards of IAA into an HPLC gradient (Table 2.2). The integrated peak area of each IAA concentration was plotted against the concentration of standard injected (Figure 2.13). Unknown IAA concentrations within the samples were then calculated from the standard curve after integrating their peak areas coinciding with the same retention time as authentic IAA (Figure 2.14). For each sample, the recovery of radioactivity associated with the ^{14}C-IAA-Me internal standard was used to correct its IAA concentration for losses incurred during purification.

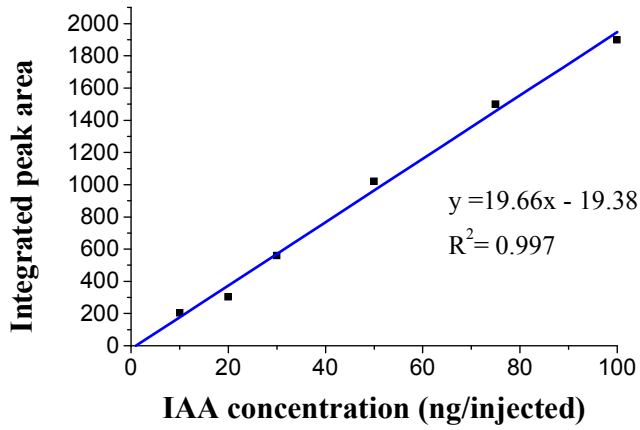

Figure 2.13. Standard curve of indole-3-acetic acid (IAA) injected into the HPLC and quantified by fluorescence detection.

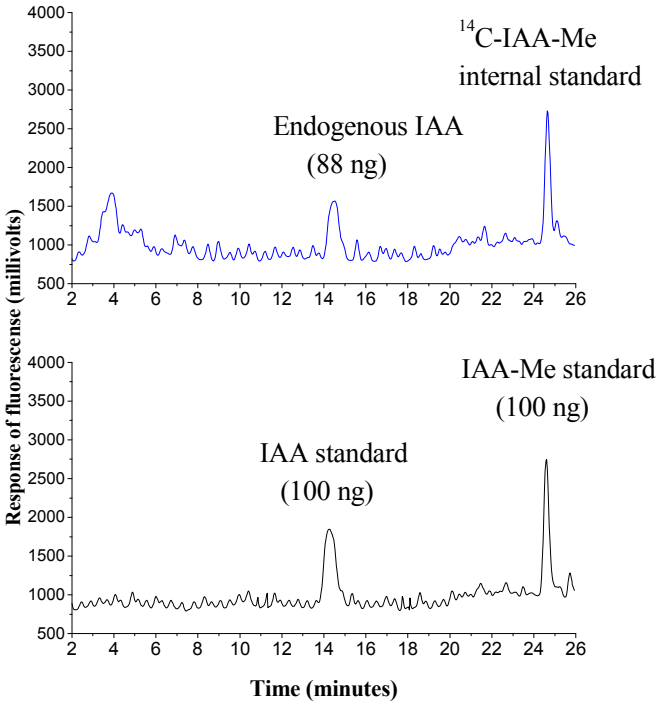

Figure 2.14. Response of fluorescence detector to standards of indole-3-acetic acid (IAA) and indole-3-acetic acid methyl ester (IAA-Me) (below, 100 ng injection) and endogenous IAA within a sample of diffusate from the shoot apices of 'Royal Gala' apple scions (top) after injection into an HPLC. The sample was quantified by fluorescence as containing 88 ng of IAA. Subsequent quantification of the collected HPLC fraction by GC-MS confirmed a similar concentration of 92 ng of IAA (see M.9 January, Table 2.3).

2.5.4 Confirmation of indole-3-acetic acid concentrations determined by HPLC-fluorescence with quantification by gas chromatography-mass spectrometry

Gas chromatography-mass spectrometry (GC-MS) was used to validate IAA concentrations quantified by HPLC-fluorescence, and to determine whether the peak quantified by fluorescence was in fact IAA. In January and March, diffusate from shoot apices was collected from 'Royal Gala' scions grafted onto rootstocks of M.9 and 'Royal Gala'. As previously described, IAA within the meristem diffusate was quantified by HPLC-fluorescence and the recovery of ^{14}C-IAA-Me was used to correct for losses of IAA within the sample. For validation by GC-MS, the IAA peak was collected from the HPLC, spiked with 50 ng of [$^{2}H_5$]indole-3-acetic acid ([$^{2}H_5$]IAA) (Olchemim Ltd, Czech Republic) as an internal standard and taken to dryness in a vacuum freeze drier. Fractions were resuspended in 500 µL of methanol and transferred to smaller 1.5 mL polypropylene microcentrifuge tubes. The methanol was dried off in a vacuum concentrator (miVac Quattro, Genevac, England), the sample was resuspended in 25 µL methanol and vortexed to dissolve any sample dried to the side of the tube. The sample was transferred to a 150 µL tapered glass insert housed inside a 2 mL autosampler bottle (12 x 32 mm with screw top lid and silicone septa). The methanol was dried off within the glass insert under low vacuum in a vacuum freeze drier.

Samples were methylated and derivatised within the glass insert. IAA-methyl esters were produced by adding 25 µL of diazomethane into the glass insert, immediately sealing the vial and allowing it to stand for 30 min at 4^{o}C. Ether was then removed by allowing the opened vials to stand for 20 min within a fumehood. N-Methyl-N-(trimethyl-silyl) trifluoroacetamide (25 µL) (MSTFA, M7891, Sigma, Germany) was added to form IAA-methyl ester trimethylsilyl ether (IAA-MeTMSi) and [$^{2}H_5$]methyl ester trimethylsilyl ether ([$^{2}H_5$]IAA-MeTMSi). The vials containing MFSTA were incubated within a water bath at 70^{o}C for 30 min prior to injection.

2.5.4.1 Gas chromatography-mass spectrometry (GC-MS) of indole-3-acetic acid

Samples of IAA-MeTMSi and [$^{2}H_5$]IAA-MeTMSi (1 µL) were automatically injected (Shimadzu AOC-5000 Autoinjector) into a GC (Shimadzu, GC-2010) connected to a mass spectrometer (Shimadzu, GC-MSQP2010). The carrier gas was helium at a flow rate of 1.3 mL min^{-1}. Sample separation was made on a capillary column (30 m, 0.25 mm id, 0.25 µm film thickness, ZB-5, Phenomenex, New Zealand). Injection

temperature was 270^{o}C and the column start temperature was 40^{o}C with the injection splitter (10:1) closed for 1 min. After 1 min, the temperature was increased to 180^{o}C at 10^{o}C min^{-1}, and then to 290^{o}C at 6^{o}C min^{-1}. The interface temperature, source temperature and ionisation voltage of the mass spectrometer were 200^{o}C, 270^{o}C and 70 eV, respectively.

Prior to quantification by single ion monitoring, the retention times and the mass spectra for standards of IAA-MeTMSi and [$^{2}H_5$]IAA-MeTMSi were determined by GC-MS in scan mode (scan speed 2500 AMU sec^{-1}) using GCMSsolutions software (Version 2, Shimadzu). The retention times of IAA-MeTMSi and [$^{2}H_5$]IAA-MeTMSi were identified as 20.67 and 20.63 min, respectively, as indicated by the mass spectra of each peak (Figure 2.15). The fragment ions for IAA (ions 202 and 261) and their ratio with each other were similar to those reported by Gaskin and MacMillan (1991). In addition, these were similar for the IAA standard and IAA within the sample of diffusate separated by HPLC, therefore indicating that IAA was present in the sample peak quantified by HPLC-fluorescence.

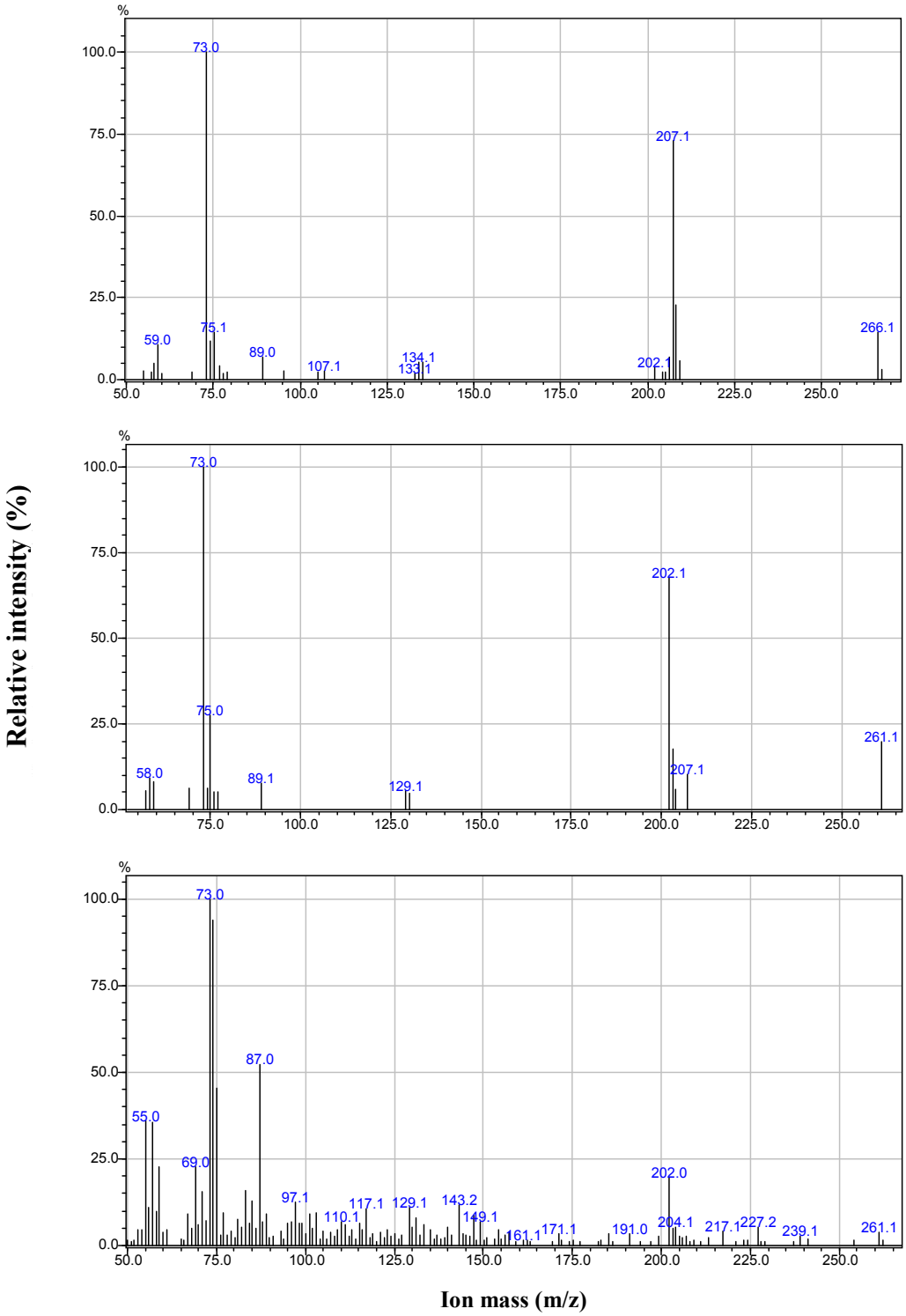

Figure 2.15. Mass spectra of methyl ester trimethylsilyl ethers of [$^2H_5$]indole-3-acetic acid ([$^2H_5$]IAA-MeTMSi) (top), indole-3-acetic acid (IAA-MeTMSi) standard (middle) and indole-3-acetic acid (IAA-MeTMSi) within the sample (bottom) corresponding to the retention times of 20.63, 20.67, and 20.67 min, respectively.

2.5.4.2 Quantification of indole-3-acetic acid by single ion monitoring

A calibration curve was generated by injecting known concentrations of IAA-MeTMSi standards spiked with 50 ng of [$^2H_5$]IAA-MeTMSi as an internal standard. As previously identified by a scan of the IAA standards, the target ions of 207 and 202 were quantified by single ion monitoring (SIM) at the retention times of 20.63 and 20.67 min for [$^2H_5$]IAA-MeTMSi and IAA-MeTMSi, respectively. As a check of peak purity, ions 261 and 266 were also quantified to check that their ratios with the target ions of 202 and 207, respectively, were similar between standards and sample (Figure 2.16). Peak areas were integrated using GCMSsolutions software and a standard curve was created by plotting the ratio of peak areas between the ions 202 (IAA-MeTMSi) and 207 ([$^2H_5$]IAA-MeTMSi) against the concentration of IAA-MeTMSi standard to [$^2H_5$]IAA-MeTMSi internal standard injected (Figure 2.17). Chromatograms for samples were integrated for their peak areas at the appropriate retention time for IAA-MeTMSi and [$^2H_5$]IAA-MeTMSi. Peak area ratios of IAA-MeTMSi to internal standard were then used to calculate concentrations of endogenous IAA from the standard curve. The endogenous concentrations of IAA quantified by HPLC-fluorescence and GC-MS were comparable (Table 2.3). Therefore, HPLC-fluorescence was deemed a suitable, convenient and cost effective method for the quantification of IAA, particularly given the high endogenous concentrations present within the samples.

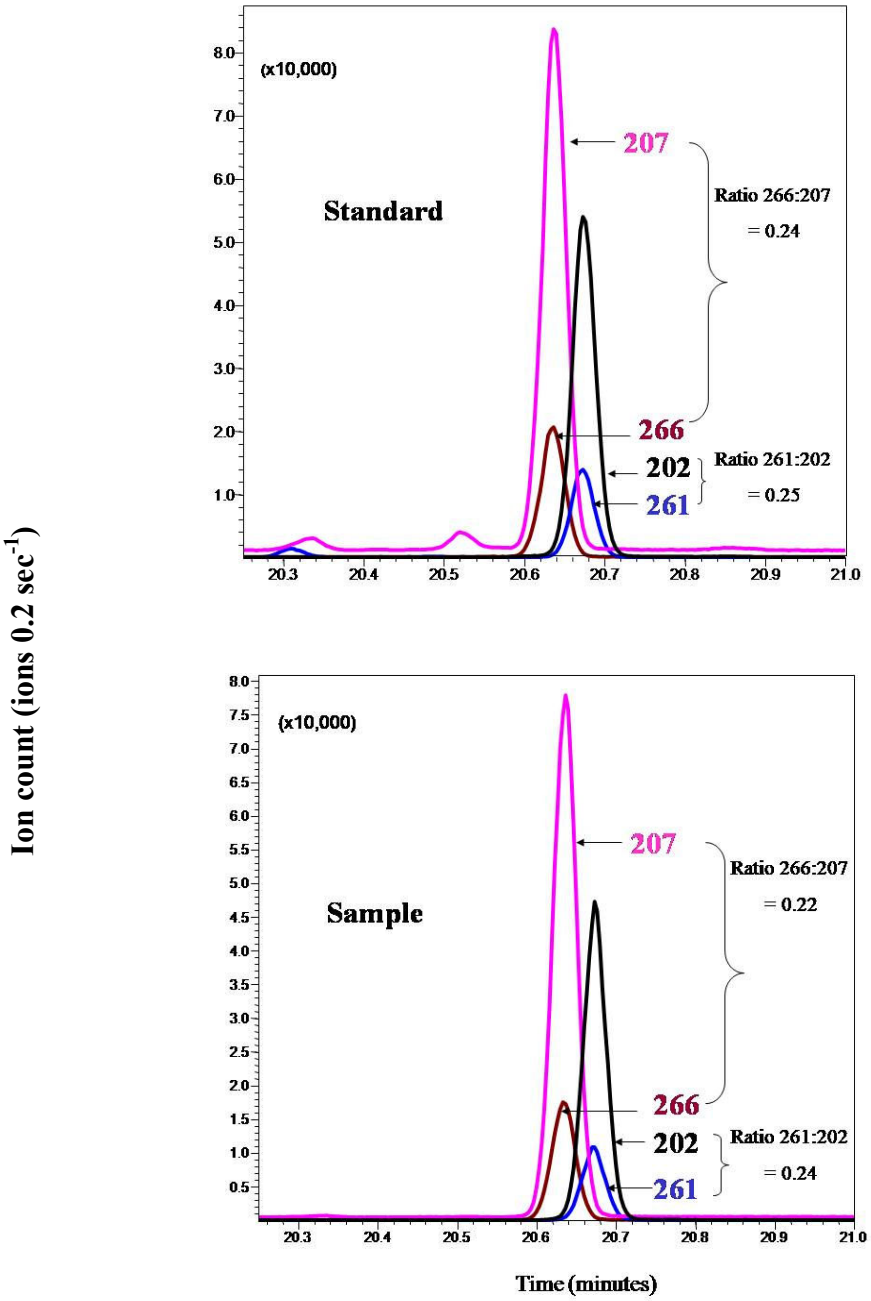

Figure 2.16. Single ion monitoring of ions 207/266 and 202/261 for methyl ester trimethylsilyl ethers of [$^2H_5$]indole-3-acetic acid ([$^2H_5$]IAA-MeTMSi) and indole-3-acetic acid (IAA-MeTMSi), respectively. The [$^2H_5$]IAA-MeTMSi internal standard chromatographed close to the IAA-MeTMSi standard (top) and endogenous IAA (IAA-MeTMSi) diffused from the shoot apices of 'Royal Gala' scions (bottom). Peak area ratios for ions 266:207 or 261:202 were similar between the standard (top) and sample (bottom).

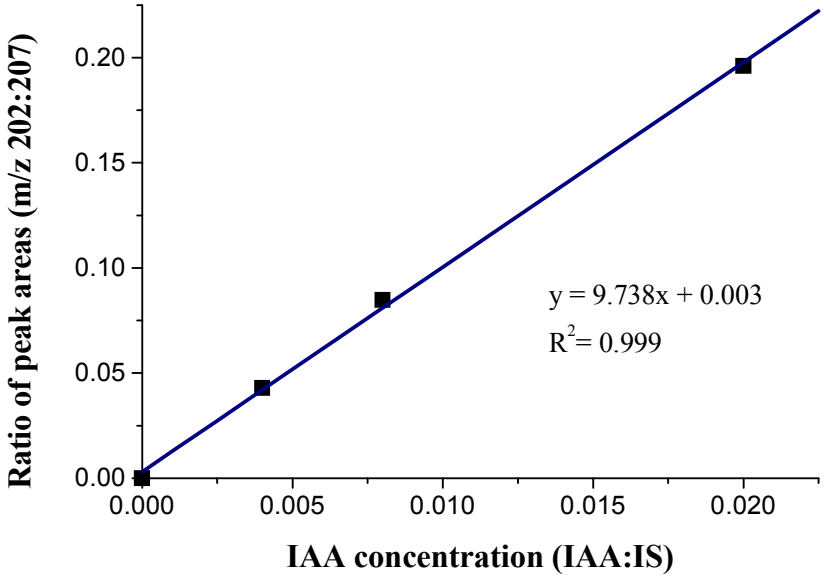

Figure 2.17. Standard curve of indole-3-acetic acid methyl ester trimethylsilyl ether (IAA-MeTMSi) (m/z 202) standards injected into the gas chromatograph (GC) and quantified by single ion monitoring using mass spectrometry. From left to right, data points represent a single injection of 0, 0.2, 0.4 and 1 ng of IAA-MeTMSi into the GC. Each standard was spiked with 50 ng of [$^2H_5$]indole-3-acetic acid methyl ester trimethylsilyl ether (m/z 207) as an internal standard (IS).

Table 2.3. Comparison of indole-3-acetic acid concentrations quantified by HPLC-fluorescence and gas chromatography-mass spectrometry for a single replicate of meristem diffusate collected during January and March from 'Royal Gala' apple scions grafted onto M.9 and 'Royal Gala' rootstocks.

		IAA in sample (ng)	
Rootstock	Month	Fluorescence[1]	Mass-spec[2]
M.9	January	88	92
'Royal Gala'	January	95	100
M.9	March	38	51
'Royal Gala'	March	46	49

[1] corrected for losses using ^{14}C-IAA-Me.

[2] corrected for losses using [$^2H_5$]IAA-MeTMSi.

2.5.5 Quantification of gibberellins in the xylem sap of apple by gas chromatography-mass spectrometry

2.5.5.1 Sample preparation

Apple sap was spiked with 50 ng of [$^2H_2$]GA_{19} (Olchemim Ltd, Czech Republic) and endogenous gibberellins A19 and A20 (GA_{19} and GA_{20}) were separated by HPLC as previously described (Section 2.4.6). Fractions were collected into 40 mL glass tubes, dried under vacuum, redissolved in 500 μL of methanol and then transferred to smaller 1.5 mL polypropylene microcentrifuge tubes. The preparation of gibberellin methyl esters trimethylsilyl ethers ([$^2H_2$]GA_{19}-MeTMSi, GA_{19}-MeTMSi and GA_{20}-MeTMSi) followed a similar method as previously described for IAA (Section 2.5.4). The only difference was that 20 μL of diazomethane and MSTFA were used for derivatisation steps.

2.5.5.2 Gas chromatography of gibberellins

The modified methods of Croker et al., (1990) were used for the chromatography of gibberellin-MeTMSi. Standards of [$^2H_2$]GA_{19}-MeTMSi, GA_{19}-MeTMSi and GA_{20}-MeTMSi (Olchemim Ltd, Czech Republic) were injected (2 μL) into the gas chromatograph. Injection temperature was 270^{o}C and the column start temperature was 60^{o}C with the injection splitter (50:1) closed for 1 min. After 1 min, the temperature was increased to 200^{o}C at 20^{o}C min^{-1} and then to 290^{o}C at 4^{o}C min^{-1}. Mass-spectrometer settings were the same as those used for IAA.

Prior to quantification by single ion monitoring, the retention times and the mass spectra for gibberellin standards of [$^2H_2$]GA_{19}-MeTMSi, GA_{19}-MeTMSi and GA_{20}-MeTMSi were determined in scan mode (scan speed 2500 AMU sec^{-1}). The retention times of [$^2H_2$]GA_{19}-MeTMSi, GA_{19}-MeTMSi and GA_{20}-MeTMSi were identified as 24.68, 24.70 and 22.75 min, respectively, as indicated by the mass spectra of each peak (Figure 2.18). The mass spectra and the ratios of fragment ions for each compound were very similar to those reported by Binks et al., (1969) and Gaskin and MacMillan (1991).

2.5.5.3 Quantification of gibberellins by single ion monitoring

Concentrations of gibberellins within the samples were determined as previously described for IAA. A standard curve was generated by injecting known concentrations

of GA_{19}-MeTMSi and GA_{20}-MeTMSi spiked with 50 ng of [$^2H_2$]GA_{19}-MeTMSi as an internal standard. As previously identified by a scan of the gibberellin standards (Figure 2.18), the target ions of 436, 434 and 418 were quantified by single ion monitoring at their retention times. As a check of peak purity, ions of 376, 374 or 375 were also quantified to check that their ratios with the target ions of 436, 434 or 418, respectively, were similar between standards and sample (Figures 2.19 and 2.20).

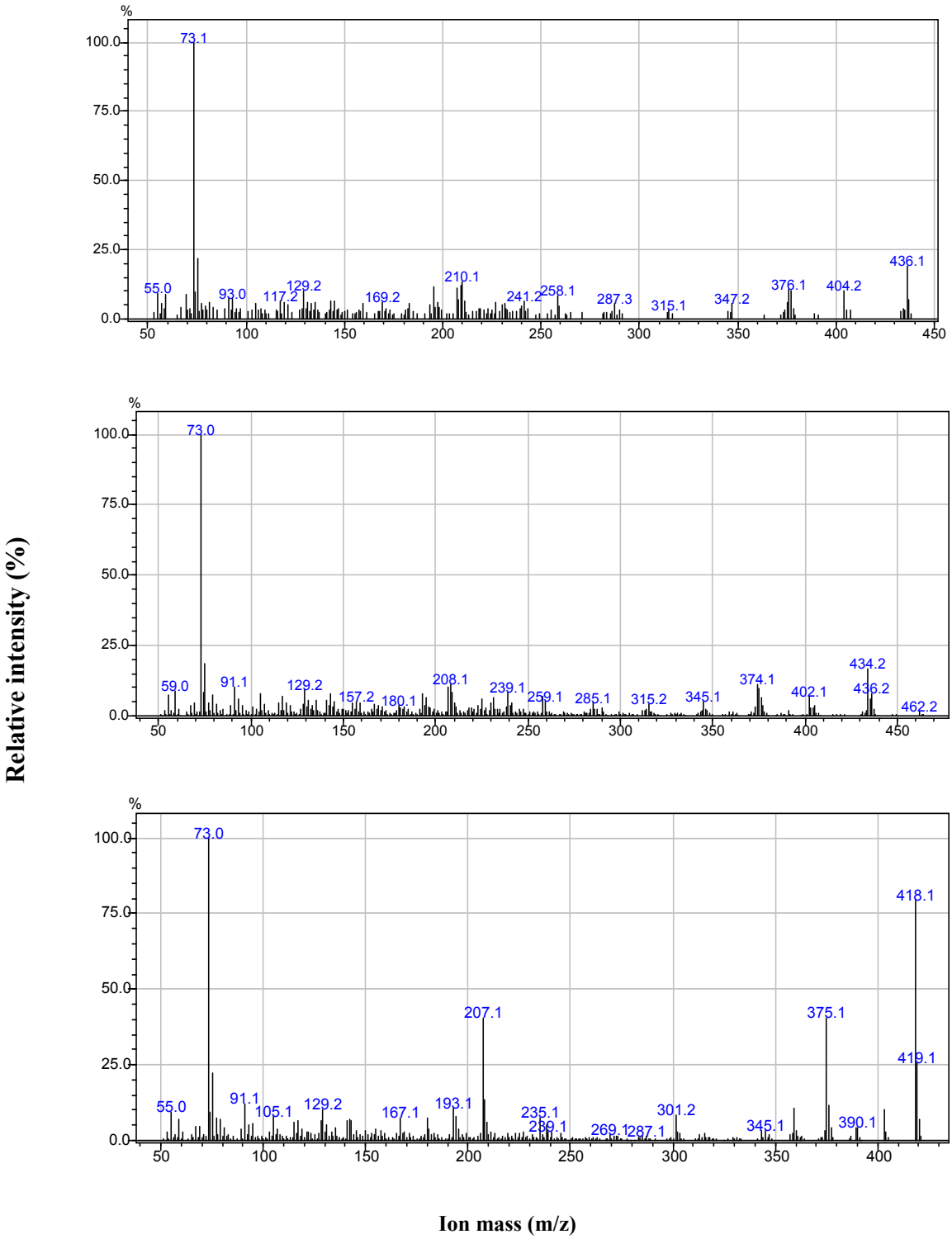

Figure 2.18. Mass spectra of methyl ester trimethylsilyl ethers of the [$^2H_2$]GA_{19} internal standard (top), GA_{19} standard (middle) and the GA_{20} standard corresponding to the retention times of 24.68, 24.70 and 22.75 min, respectively.

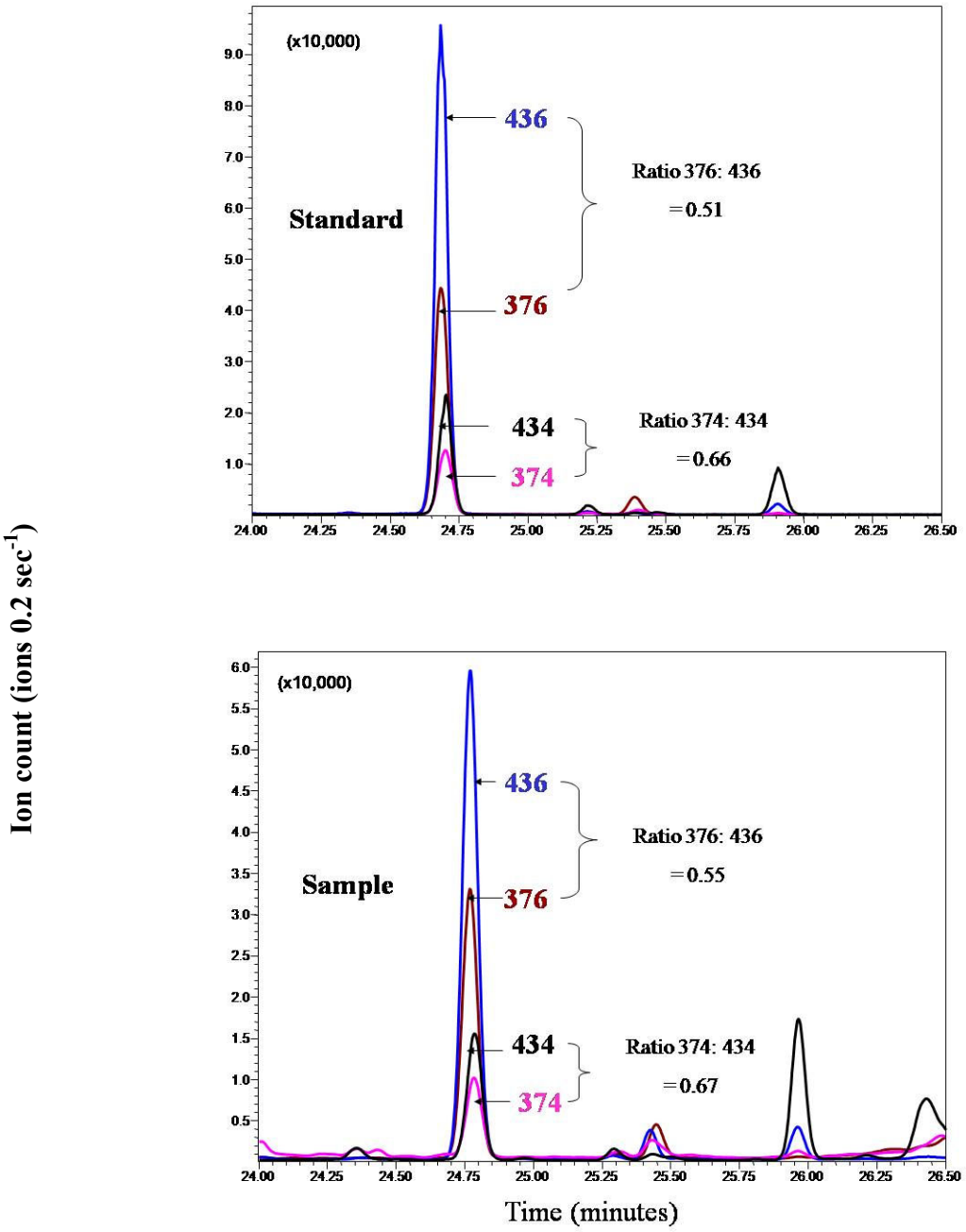

Figure 2.19. Single ion monitoring of ions 436/376 and 434/374 for methyl ester trimethylsilyl ethers of [$^2H_2$]GA_{19} ([$^2H_2$]GA_{19}-MeTMSi) and GA_{19} (GA_{19}-MeTMSi), respectively. The [$^2H_2$]GA_{19}-MeTMSi internal standard co-chromatographed with the GA_{19}-MeTMSi standard (top) and with GA_{19} (GA_{19}-MeTMSi) within the xylem sap of apple (bottom). Peak area ratios between ions 376:436 or 374:434 were similar between the standard (top) and sample (bottom).

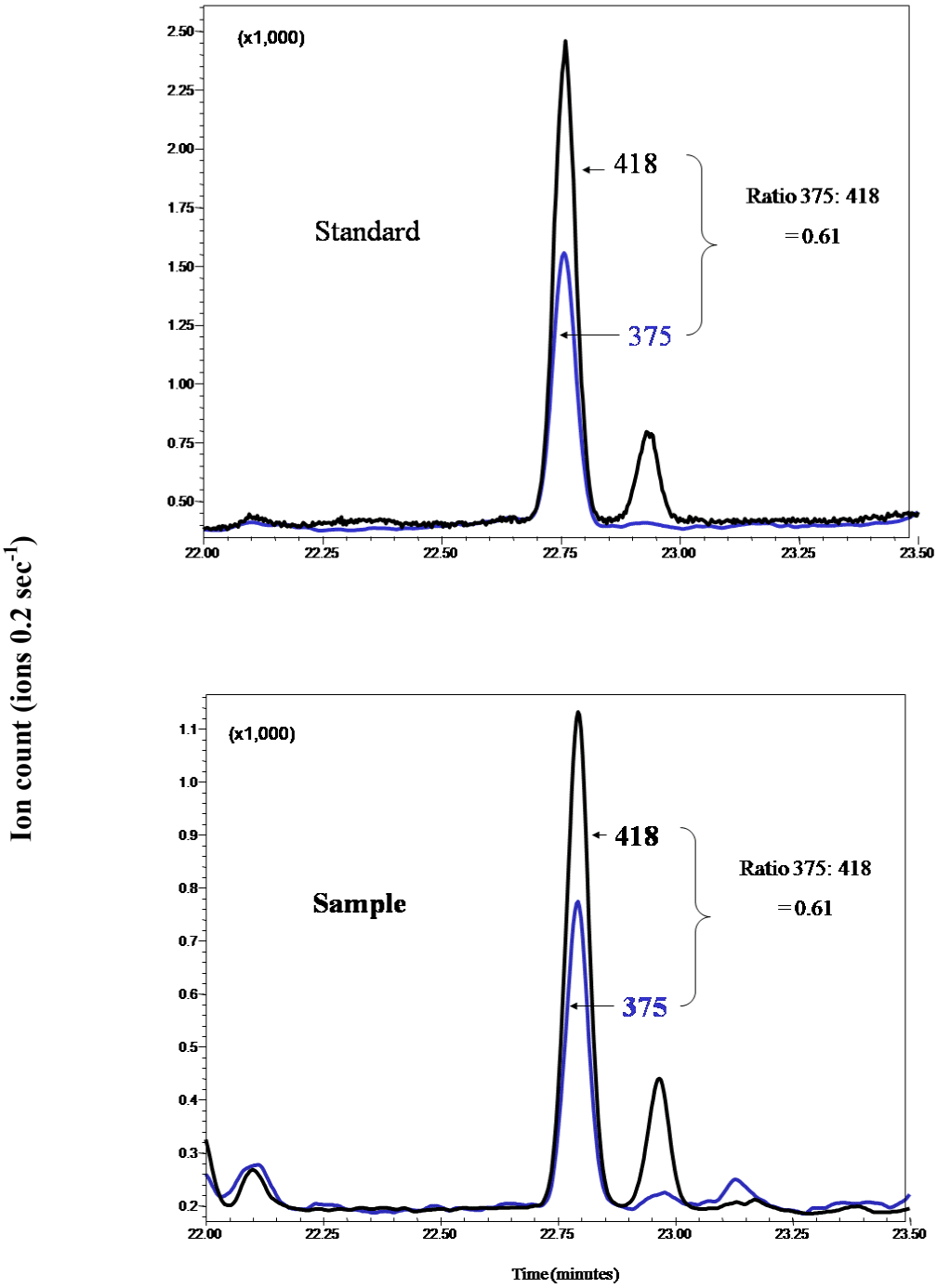

Figure 2.20. Chromatogram for single ion monitoring of ions 418 and 375 for methyl ester trimethylsilyl ethers of the GA_{20} standard (top) and GA_{20} within the xylem sap of apple (bottom). Peak area ratios between ions 375:418 were similar between the standard (top) and sample (bottom).

2.6 Synthesis of the auxin transport inhibitor 1-N-naphthylphthalamic acid

2.6.1. Synthesis of 1-N-naphthylphthalamic acid

The methods of Thomson et al., (1973) and Currie (1997) were used to synthesise 1-N-naphthylphthalamic acid (NPA). 1-Naphthylamine (5 g) was dissolved in 500 mL of toluene, and phthalic anhydride (5 g) was dissolved in a further 500 mL of hot toluene (Currie, 1997). The two-toluene solutions were mixed and then stored at 25^{o}C for 24 hr to enable precipitation. Precipitated NPA was then filtered through filter paper (Whatmans No. 1). The precipitate was washed five times in clean toluene to remove any unreacted reagents (Currie, 1997). Toluene was removed from the NPA by evaporation within a fumehood.

2.6.1.1 Determination of mass spectra

NPA (10 ug) was dissolved in 500 µL of 50:50 acetonitrile/water (0.01 mol L^{-1} ammonium acetate) and injected directly into a mass-spectrometer in negative ionisation mode as previously described for gibberellins (Section 2.4.6.2). A quasi-molecular ion at 290 m/z (Figure 2.21) matched the molecular ion of NPA (291 m/z).

2.6.2 Testing of the biological efficacy of synthesised 1-N-naphthylphthalamic acid using a lettuce root growth bioassay

The biological efficacy of NPA was measured in a lettuce root bioassay. Lettuce seedlings (cv. 'Marksman') were germinated for 12 hr at 24^{o}C in distilled water to promote radical emergence from the seed coat. Upon radical emergence, 12 lettuce seedlings per petri-dish were grown in 2 mL of NPA solution, with each petri-dish containing a different mol L^{-1} concentration (Figure 2.22) of NPA dissolved as an ammonium salt. Seedlings were grown 500 mm beneath fluorescent light (4 x 18 W Cool White tubes, 80 µmol sec^{-1} m^{-2}) within an incubator maintained at 25^{o}C. After 72 hr, root lengths were measured (Figure 2.22). As found by Katekar and Geissler (1980) for various phytotropins, NPA reduced the growth of roots as concentration increased (Figure 2.22), and resulted in loss of root gravitropism between 10^{-4} to 10^{-6} mol L^{-1} NPA (Figure 2.23).

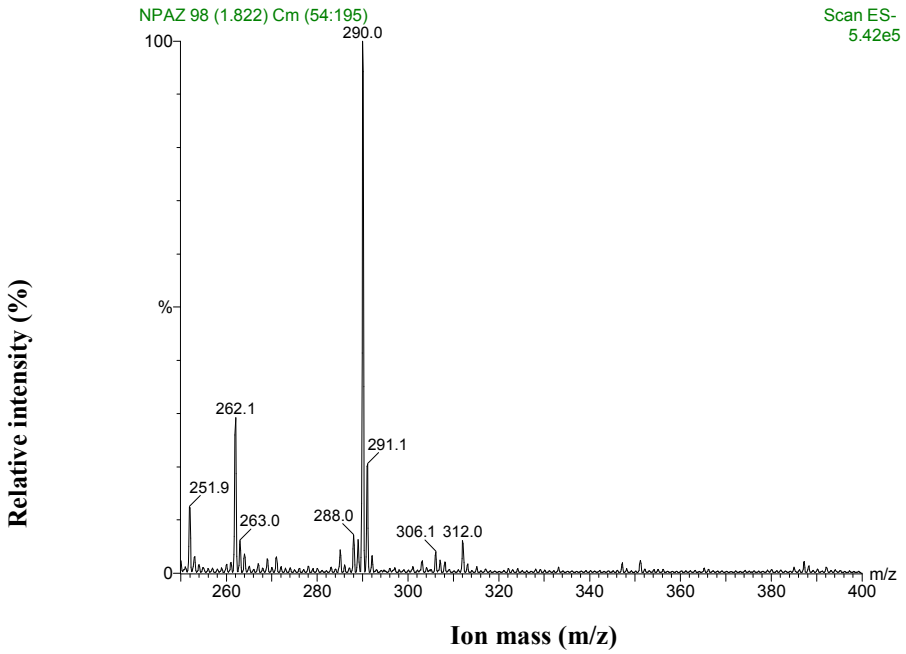

Figure 2.21. Mass spectra of 1-N-naphthylphthalamic acid synthesised using the methods of Thomson et al., (1973).

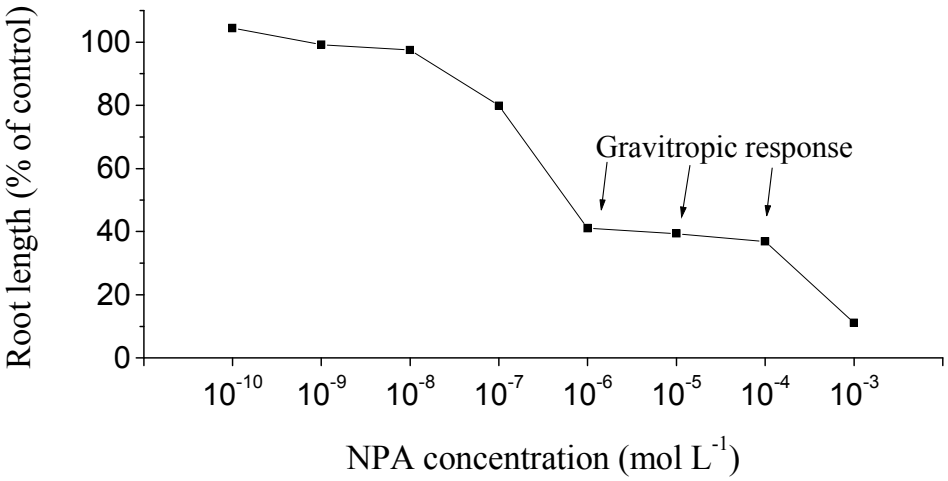

Figure 2.22. Effect of different concentrations of 1-N-naphthylphthalamic acid (NPA) on the mean length of lettuce roots expressed as a percentage of mean root length for control seedlings (grown in distilled water only). After radical emergence from the seed coat, seeds were placed into the test solutions and grown for 72 hr.

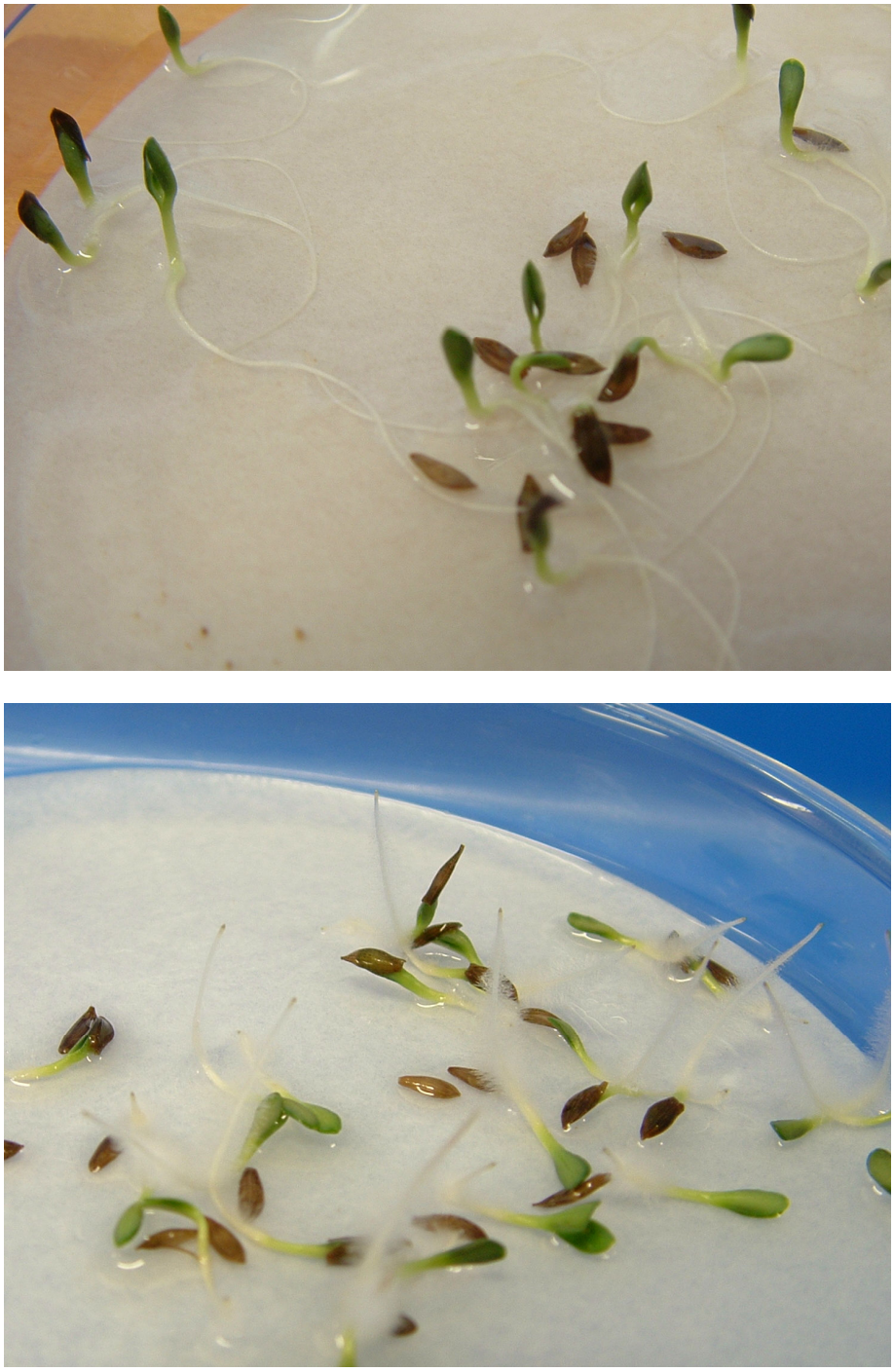

Figure 2.23. (top) Lettuce seedlings germinated in water (control) showing normal root development, (below) lettuce seeds germinated in 10^{-6} mol L^{-1} of 1-N-naphthylphthalamic acid showing roots growing upwards because of loss of gravitropism due to probable inhibition of auxin transport.

2.6.3 Determination of a suitable physiological concentration of applied 1-N-naphthylphthalamic acid and 2,3,5,-Triiodobenzoic acid to reduce the growth of apple scions

The auxin transport inhibitor 'NPA' is used as a selective herbicide to control broadleaf weeds in horticultural crops including peanuts and cucurbits. Therefore, NPA at high concentrations may be detrimental to tree growth; hence a suitable physiological concentration that could be applied to trees was determined. A comparison was also made between the effectiveness of 2,3,5,-Triiodobenzoic acid (TIBA, Sigma, USA) and NPA synthesised in our laboratory. 'Royal Gala' scions were grafted onto rootstocks of M.9 and MM.106 as described in Section 2.2. In mid November 2005, tape was removed from the graft union and a 30 mm length of epidermis (15 mm each of the rootstock and scion) was removed by carefully scraping with a sharp scalpel. NPA or TIBA was then immediately applied in 1 mL of hydrated lanolin at concentrations of 0 (control) 1, 5 and 25 mg per tree. Graft unions were wrapped with tinfoil to keep the lanolin in place and to maintain the activity of NPA and TIBA for as long as possible.

Two days after the application of NPA or TIBA, leaves showed epinasty that increased in severity with concentration (Figure 2.24). Epinasty then slowly disappeared after 10 to 14 days. Similarly, Grochowska et al., (1994) reported shoot epinasty and increased ethylene evolution after blocking basipetal IAA transport of 'Antonovka' apple seedlings with TIBA applied at 0.5 mg per seedling. In their study, basipetal transport of ^{14}C-IAA was reduced by half compared with the control trees seven days after applying TIBA (Grochowska et al., 1994). In this study, 25 mg of NPA or TIBA reduced the photosynthetic rates of the scions compared with the control (Figure 2.25). As for shoot epinasty, reductions in photosynthesis were transient, with photosynthetic rates, particularly for 5 mg of NPA or TIBA, recovering to near that of the control at day nine. After approximately 14 days, axillary buds on the rootstock began to break below the site of NPA (Figure 2.26) or TIBA application. These axillary shoots would continue to extend if left to grow.

Scion growth began to slow 18 days after NPA application for both rootstocks when compared with their respective untreated controls (Figure 2.27). By day 70, growth of the scion had fully ceased on NPA-treated rootstocks of M.9 and MM.106 because every concentration of NPA caused the SAM on the primary shoot to fully terminate.

For MM.106 at day 70, the mean length of the primary shoot was similar for 1 mg and 25 mg of NPA (Figure 2.27). However, there was a tendency for the primary shoot to resume extension growth after 70 days when the MM.106 rootstock was treated with either 1 mg or 5 mg of NPA, which caused slightly longer shoots at 120 days for these concentrations when compared with 25 mg of NPA (Figure 2.27).

Although TIBA produced very similar reductions in shoot growth to that of NPA, TIBA resulted in toxicity to the phloem tissue, with necrosis evident in a small proportion of trees (data not shown). This was not observed for NPA, with phloem tissue appearing very healthy, even at the highest concentration of 25 mg. Therefore, NPA was selected as the compound of choice as it appeared that the symptoms observed (particularly Figures 2.24, 2.26, and 2.27) were due to the blocking of basipetal auxin transport rather than toxicity. NPA (5 mg/tree) was selected as a suitable concentration with a repeat application strategy used to overcome the tendency of scions on the more vigorous MM.106 to regrow.

Figure 2.24. Epinasty of 'Royal Gala' apple scions developed within two days of applying a single application of 1-N-naphthylphthalamic acid (NPA) or 2,3,5,-Triiodobenzoic acid (TIBA) to the graft union of MM.106 rootstocks. From left to right, trees were treated with 0, 1, 5 and 25 mg of NPA in lanolin.

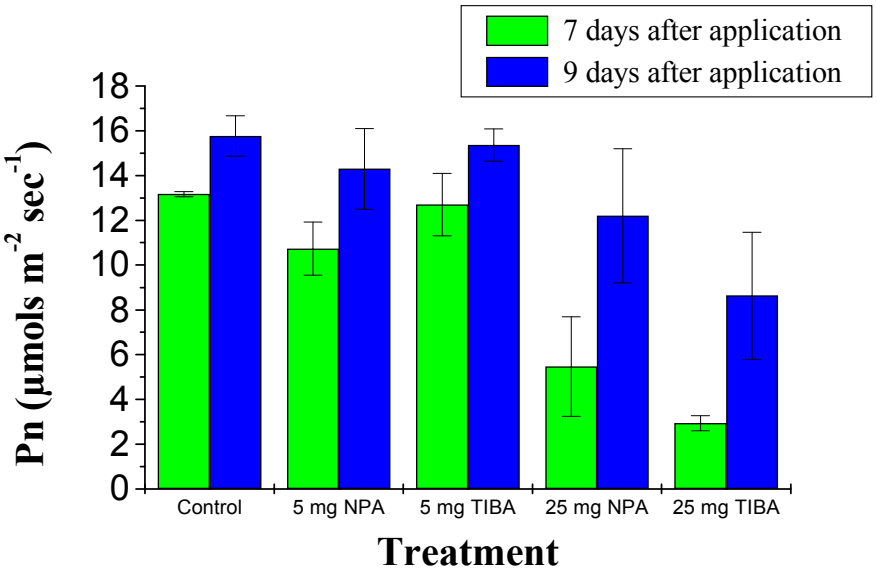

Figure 2.25. Mean photosynthetic rate (Pn) of 'Royal Gala' apple scions seven and nine days after application of 1-N-naphthylphthalamic acid (NPA) or 2,3,5,-Triiodobenzoic acid (TIBA) to the graft union of MM.106 rootstocks. A single leaf was measured per scion (n=3 trees). Vertical bars represent the SEM.

Figure 2.26. Axillary branching on the rootstock stem 28 days after a single application of 1-N-naphthylphthalamic acid was applied to the graft union. The first visible signs of axillary bud outgrowth were seen after 10 to 14 days.

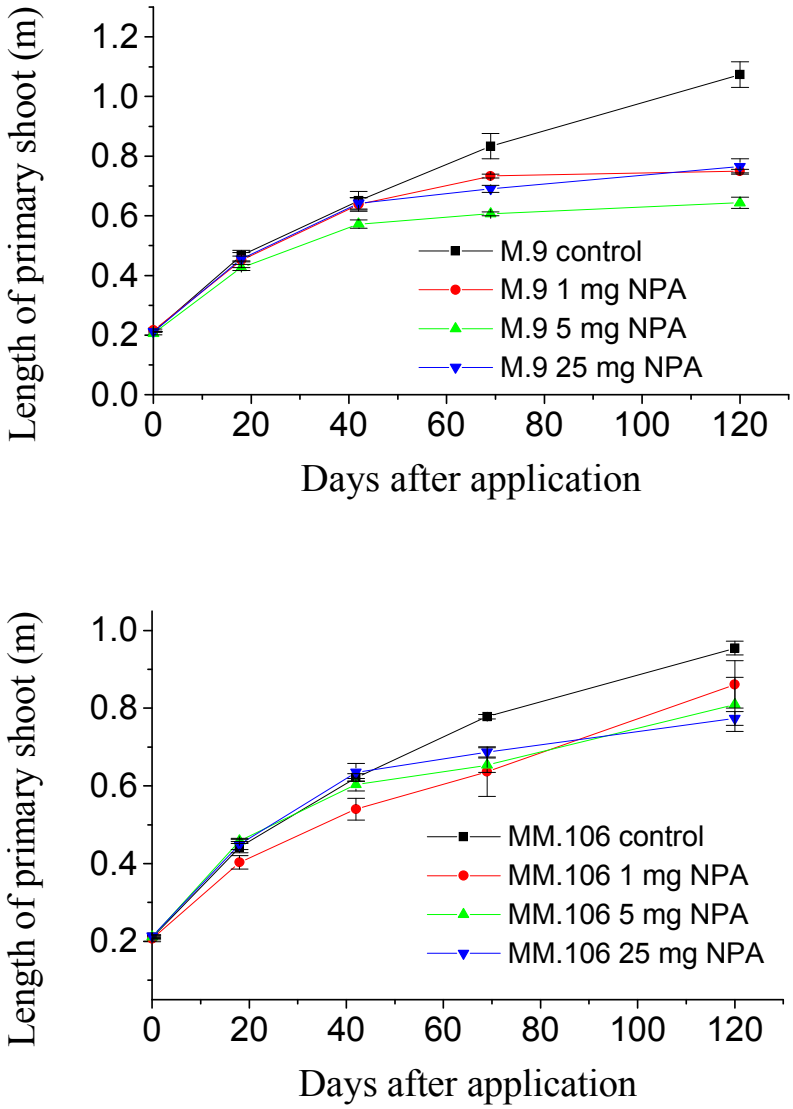

Figure 2.27. Mean cumulative length of the primary shoot of 'Royal Gala' apple scions in response to a single application of 1-N-naphthylphthalamic acid (NPA) applied to the graft union of M.9 (top) and MM.106 (bottom) rootstocks. Vertical bars represent the SEM, n=3.

2.7 Measurement of scion growth and architecture

2.7.1 Measurement of vegetative shoot growth

The measurement of vegetative growth of the primary shoot (Figure 2.29) was conducted at approximately monthly intervals throughout each experimental growing season. The length of the primary shoot was measured with a ruler, with the measurement beginning at the shoot base and ending at the first unfurled leaf at the

shoot apex. As well, the number of nodes was counted including any basal nodes. Prior to winter dormancy, the final length and node number of the primary, secondary and tertiary shoots (Figure 2.29) was measured when extension growth of the scion had completely ceased.

2.7.1.1 Shoot termination

During summer and early autumn, the number of primary and secondary vegetative shoots (Figure 2.29) were counted for each scion and each shoot apical meristem (SAM) on a primary or secondary shoot was visually assessed to determine if it was actively growing or had terminated (Figure 2.28). The proportion (%) of a shoot type that had terminated was calculated for each scion by dividing the number of terminated SAMs per shoot type by the total number of that shoot type per scion x 100.

Figure 2.28. Example of a shoot apical meristem on the primary shoot that had fully terminated (left) or that was actively extending (right).

2.7.1.2 Internode length

The internode length of a shoot was calculated by dividing the length of a shoot by the number of nodes.

2.7.1.3 Shoot cross-sectional area (SCA)

The diameters (D) of primary and secondary vegetative shoots were measured 20 mm from their base and shoot cross-sectional areas (SCA) were calculated as $\pi D^2/4$.

2.7.1.4 Proportion (%) of axillary bud break on the primary and secondary shoots

The proportion (%) of axillary buds on the primary shoot of each scion that formed secondary spurs, secondary shoots or secondary axes (i.e., total of secondary spurs plus secondary shoots) was calculated by dividing the total number of a shoot type (secondary spur or shoot) or types (secondary spurs plus shoots) per primary shoot by the total number of nodes on that primary shoot x 100. The proportion (%) of axillary buds on the secondary shoots of each scion that formed tertiary spurs, tertiary shoots or tertiary axes (i.e., tertiary spurs plus tertiary shoots) was calculated by dividing the total number of a shoot type (tertiary spur or shoot) or types (tertiary spurs plus shoots) per scion by the total node number of the secondary shoots on that scion x 100.

2.7.1.5 Angles of elevation of the secondary shoots

The angle of elevation for each secondary shoot was measured with a protractor as an acute angle with 0 and 90^{o} elevation representing a shoot that was horizontal with the ground or vertical with the primary shoot, respectively.

2.7.1.6 Coding of scion architecture at growth cessation

Scions were measured using a coding system described by Costes et al., (1997). This method provided a systematic way to describe different orders of shoot axes, their positions, number and type of growth units, and attributes including shoot length, diameter, node number and angle (Figure 2.29 and Table 2.4). The structures of 1-year-old scions were recorded by order of shoot axes (primary axis or shoot, secondary axes and tertiary axes, Figure 2.29). Within an order of axis, shoots could be composed of a single growth unit formed by one flush of growth, or more than one growth unit where the SAM temporarily slowed or ceased growing before resuming into a second flush of growth. To identify an axis with more than one growth unit, the morphological marker of a ring of bud scale scars, and/or, compressed internodes was used (Seleznyova et al., 2003; Figure 2.29). An axis was further distinguished as either a spur (minimal internode extension < 25 mm long) (Boyes, 1922; Seleznyova et al., 2003) or a vegetative shoot (≥ 25 mm with internode extension) (Seleznyova et al., 2003). In this thesis, secondary spurs and secondary shoots (Figure 2.29) are collectively called secondary axes, and this term excludes trace spurs (Figure 2.29 and Table 2.4) that sometimes formed in response to exogenous BAP. Tertiary spurs and shoots (Figure

2.29) are collectively called tertiary axes. For consistency, the primary axis (Figure 2.29) will be referred to as the primary shoot in subsequent chapters.

Code files (Table 2.4) were produced for each scion by counting the number of nodes beginning from the base of the primary shoot (Figure 2.29). As secondary axes were encountered, they were recorded for their nodal position on the primary shoot. For secondary shoots, the number of growth units and structural attributes (length, node number, shoot angles and diameters) were recorded. Where tertiary axes arose from a secondary shoot, the growth unit type, position and attributes (length and node number of tertiary shoots) were recorded before proceeding to count the next node on the primary shoot.

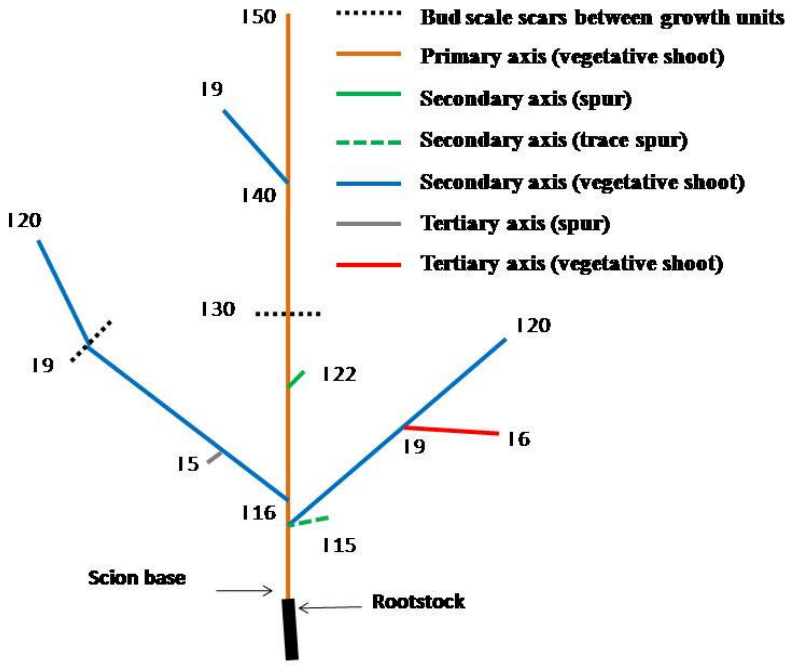

Figure 2.29. Example of possible axes and types of growth units measured on 'Royal Gala' apple scions at the end of the first year from grafting. Primary, secondary and tertiary vegetative shoots are colour-coded brown, blue and red, respectively. 'I' and its index correspond to the node at which an axis or growth unit formed, and/or, ended. Secondary spurs and shoots (i.e., excluding trace spurs) are collectively called secondary axes, whereas tertiary spurs and shoots are collectively called tertiary axes.

Table 2.4. Code file for Figure 2.29. Primary, secondary and tertiary axes are abbreviated as PA, SA and TA, respectively. Their index corresponds to the growth unit number. Sp (spur) differentiates spurs from vegetative shoots. A node along the primary shoot containing a secondary shoot may also contain a trace spur arising from basilary buds borne in the axils of bud scales (trace spur or t sp, see I 15 below and Figure 2.29).

Primary axis		Secondary axes		Tertiary axes	Shoot attributes			
	node		node		Node number	Length (mm)	Angle (°)	Diameter (mm)
PA 1								
	I 15							
		+SA 1			20	250	40	10
			I 9					
				+TA 1	6	40	-	-
		+SA t sp			-	-	-	-
	I 16	+SA 1						
			I 5					
				+TA sp	-	-	-	-
		SA 1 >	I 9 >		9	120	30	10
		SA 2 >	I 10		11	120	-	-
	I 22							
		+SA sp			-	-	-	-
PA 1 >	I 30 >				30	400	-	25
PA 2	I 31				20	200	-	-
	I 40							
		+SA 1			9	110	40	4
	150							

+ denotes a branch and > denotes that one growth unit or node is succeeded by another.

2.8 Statistical analysis

Data were manually entered in Microsoft excel (Microsoft Corporation, Redmond, USA) and then analysed using the SAS system (Version 9.1, Cary, NC, USA) for Windows. Data were checked in SAS for homogeneity of variance and for a normal distribution. When data were not homogenous or normally distributed, the raw data were subjected to an appropriate transformation before analysis of variance (ANOVA) using the general linear model procedure (proc GLM) of SAS. The ANOVA test

statistic F was used to test the null hypothesis that all treatment means were the same. If this hypothesis was rejected due to a high F-value, an alternative hypothesis was accepted that treatment means were different. The experimental designs were either completely randomised (Chapters 3, 5 and 6) or a completely randomised block design (Chapter 4).

Experiments in Chapters 3, 4 and 5 had a factorial arrangement of treatments. Where an *F*-ratio of a significant main effect was much greater than that of a significant interaction involving that main effect, both the main effect and interaction were reported for interpretation. In contrast, only interaction data were reported for interpretation when an *F*-ratio of a main effect was similar or smaller than the *F*-ratio of an interaction involving that main effect. For significant interactions, preplanned comparisons of some treatment means were made using least square means (lsmeans tests) with Tukey's adjustment. Unless otherwise stated, comparisons between interaction means were made at $P \leq 0.05$. Mean separations for the main effects were made using the Tukey's test at $P=0.05$. In Chapter 6, mean separation tests were conducted using the LSD test at $P=0.05$.

3. Architectural responses of 'Royal Gala' apple scions to the influences of rootstock, root-restriction and benzylaminopurine

3.1 Introduction

Recent studies have reported that the first observable change imposed on the scion by a dwarfing (M.9) compared with a semi-vigorous apple rootstock (MM.106) was increased flowering in the spring of year two from grafting of the composite tree (Seleznyova et al., 2005, 2007, 2008). Compared with MM.106, the greater incidence of flowering along the primary shoot of the scion on M.9 increased the proportion of floral axillary annual shoots that formed, and reduced the number of annual shoots that developed extension growth units, particularly annual axillary shoots comprised of two growth units (Seleznyova et al., 2005, 2008). In addition, the vigour of annual axillary vegetative shoots was reduced for the scion on M.9 in the second year of growth when compared with MM.106 (Seleznyova et al., 2008).

In contrast to the above studies, M.9 was reported to reduce the length of the primary shoot in the first year of growth after propagation when compared with more vigorous rootstocks (Rao and Berry, 1940; Cannon, 1941). For example, the final mean length of the primary shoot was reduced by M.9 because of slower cumulative growth from approximately midsummer onwards (Rao and Berry, 1940). More recently, Costes et al., (2001) also reported M.9 reduced the mean length and node number of the 'Rome Beauty' primary shoot that grew following the heading back of the one-year-old scion to a single bud. In that study, there was also a trend that M.9 reduced the mean number of secondary axes on the 'Starkrimson' scion when compared with the same cultivar on M.7. Similarly, there were trends that dwarfing apple rootstocks reduced the mean number of secondary shoots that formed on different scion cultivars in their first year of growth in a tree nursery (Jaumien et al., 1993; Volz et al., 1994).

Differences within the literature concerning the year in which dwarfing of the scion was first expressed may result from different propagation methods, growing environments and differences in the experimental tree material used (see Chapter 1). All of these factors have the potential to mask subtle changes in vegetative growth that may occur within the first year after grafting. Nevertheless, the increased incidence of scion flowering in year two (Seleznyova et al., 2005, 2007, 2008) must result from a physiological change induced by the dwarfing rootstock in year one, particularly because floral evocation of apple buds occurs during the previous year (Buban and Faust, 1982; Pratt, 1988; Forshey and Elfving, 1989). Year one, therefore, would appear to be the critical year of development when the first physiological changes, as modified by the dwarfing rootstock, occur in the scion.

The first two objectives of this chapter were to determine the time and year from grafting when rootstock-induced dwarfing of the scion first resulted, and how scion growth and architecture was modified by rootstocks of different vigour. The experimental tree material used was carefully prepared to be of high-uniformity (Figure 2.4) so that subtle differences in growth could be detected.

Determining when and how scion growth and architecture is first modified by the dwarfing apple rootstock may also provide important insights into which endogenous hormone(s) are causal. The endogenous control of scion vigour by dwarfing apple rootstocks is most convincingly explained by the relationship between IAA and cytokinin. The stem of dwarfing apple rootstocks reduced the basipetal transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj et al., 1997). In addition, their bark may contain greater concentrations of compounds including IAA oxidase and phenols, which may degrade IAA, therefore reducing the amount transported to the root system (Lockard and Schneider, 1981). At the root, IAA is required for the initiation of lateral roots (Jones and Hatfield, 1976; Delargy and Wright, 1979), whereas root tips are important sites for cytokinin biosynthesis (Davies, 1995; Nordstrom et al., 2004). Reduced IAA transport to the root may therefore decrease root-synthesised cytokinin by reducing the number of root tips, or perhaps act by reducing cytokinin biosynthesis directly (Lockard and Schneider, 1981).

Root-produced cytokinins are transported within the xylem vasculature of apple trees (Jones, 1964, 1973). Their role in shoots of many plant species includes bud break (Williams and Stahly, 1968; Cook et al., 2001), the formation and activity of meristems (Williams and Stahly, 1968; Werner et al., 2003), light signalling (Rashotte et al., 2005), flowering induction (Haberer and Kieber, 2002) and carbon allocation among sinks (Richards and Rowe, 1977; Fetene and Beck, 1993). For apple, Kamboj et al., (1999) reported that the 'Fiesta' scion grafted onto M.9 had a lower concentration of endogenous cytokinin in the xylem sap than the scion grafted onto MM.106.

Jones (1973) showed that exogenous cytokinin stimulated the outgrowth of isolated apple shoots *in vitro,* and subsequently it was postulated that increased endogenous concentrations of cytokinin in the xylem sap might explain the increased rates of shoot extension growth for the scion propagated on vigorous rootstocks (Kamboj et al., 1999). However, it has long been known that exogenous cytokinin does not increase the mean shoot length of primary (Wertheim and Estabrooks, 1994) or secondary shoots (Forshey, 1982; Elfving, 1984, 1985; Cody et al., 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988) of young apple trees. Furthermore, the cytokinin benzylaminopurine (BAP) applied to young apple scions primarily stimulated axillary buds along the primary shoot to break and form secondary shoots (Williams and Stahly, 1968; Kender and Carpenter, 1972; Forshey, 1982; Elfving, 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988; Volz et al., 1994; Wertheim and Estabrooks, 1994). In contrast, in a tree nursery there were trends that M.9 reduced the mean number of secondary shoots that formed on different scion cultivars in their first year of growth (Jaumien et al., 1993; Volz et al., 1994). Thus, given that exogenous cytokinin stimulates secondary shoot formation of apple scions, reduced branching of the scion grafted onto the dwarfing rootstock may result from a reduced root supply of cytokinin. Other vigour reducing practices like root restriction may also reduce cytokinin export from the root because exogenous cytokinin supplied to the shoot partially overcame reductions in vigour of root-restricted peach (Richards and Rowe, 1977).

Further objectives of this chapter were to elucidate similarities or differences in vigour reductions imposed on the scion by the dwarfing rootstock and root restriction, and whether the cytokinin BAP applied to the scion could reverse rootstock-induced or root

restriction-induced changes in shoot growth and architecture. Interactions between rootstock, root restriction and BAP were also of particular interest to provide information on what other factors may be causal in vigour control.

3.2 Materials and methods

3.2.1 Site and establishment of experimental tree material

The experiment was conducted during the 2004–2005 growing season at the Fruit Crops Unit, Massey University, Palmerston North. 'Royal Gala' scions were cleft grafted at a height of 350 mm onto rooted stools of M.9, MM.106 and M.793. Winter grafting for M.9 and MM.106 occurred in mid August, 2004. Trees on the M.793 rootstock were grafted exactly one month later due to a delay in obtaining this rootstock from the nursery. Each scion was debudded to a single shoot in October and rootstocks were transplanted into 8 L and 45 L black polythene bags in early November. Planting height was standardised for each rootstock to leave 150 mm of rootstock stem above the growing medium. Following transplanting, the potted trees were moved to a standing out row within the orchard. In-row tree spacing was 1.5 m between trees and a guard tree was positioned at the ends of the row.

3.2.2 Growing medium and irrigation

The growing medium used was previously described in Section 2.2. In this experiment, the medium also contained 300 g per 100 L of 14-month slow release fertiliser (N:15, P:4, K:7.5, Mg:1.8) (Osmocote, Scotts, USA). Supplementary liquid fertiliser (N:10, P:13, K:17, Mg:1.2) (Peters Professional, Scotts, USA) with trace elements was applied by hand (2 g L^{-1} per tree) at 14-day intervals. The irrigation system consisted of a 19 mm polytube line placed under the tree row. Adjacent to each polythene growing bag, a single pressure compensator was fitted into the 19 mm polytube line. For the 8 L and 45 L bags, 2 L hr^{-1} and 4 L hr^{-1} pressure compensators, respectively, were used. To each compensator, a 1.5 m length of flexible PVC line (3 mm internal diameter) was connected and a stake drip emitter was attached to the end of the PVC line. A single stake drip emitter was placed into the medium near the centre of each polythene growing bag. To maintain the medium near field capacity (0.30 m^3 m^{-3} ± 0.01 m^3 m^{-3}), irrigation was scheduled daily for 1 hr at dawn and dusk using an automated irrigation controller (Hunter, Smart Valve Controller, USA). To monitor the effectiveness of the irrigation schedule, volumetric water content (θ) of the medium was measured at midday throughout the growing season using Time Domain Reflectometry (TDR)

(MiniTrase, 6050X3K1, SoilMoisture Equipment, USA). Because of differences in the height of the polythene bags, 250 and 400 mm TDR probes were used for the determination of θ for the 8 L and 45 L root volumes, respectively.

3.2.3 Application of the cytokinin benzylaminopurine

Half the scions for each rootstock and root volume were sprayed with BAP to the point of run off using a 15 L knapsack sprayer. The first application of BAP for M.9 and MM.106 occurred on 11/11/2004, which coincided with the primary shoot having attained approximately 15 nodes. The first two applications of BAP (500 mg L^{-1} Cylex® + 0.5 mL L^{-1} Tween 20) occurred 14-days apart and were followed by six applications of BAP (250 mg L^{-1} Cylex® + 0.5 mL L^{-1} Tween 20) timed 15 to 20 days apart over the remainder of the growing season. For M.793, the application strategy for BAP was the same as that for M.9 and MM.106, although because of the delay in grafting (Section 3.2.1), the first BAP application commenced approximately one month later on 1/12/2004. Adjacent trees were covered during the application of BAP to prevent contamination from spray drift.

3.2.4 Measurements of scion growth

Scion growth was measured throughout the growing season as previously described (Section 2.7). The length and node number of the primary shoot was measured monthly from November, 2004. For secondary shoots, termination was assessed once on 11/3/05. In late May 2005 (before leaf fall), scions were stripped of leaves for the determination of leaf areas using a leaf area meter (Li-3100, Li-Cor Inc., USA). Following deleafing, final scion growth was measured in June, 2005 (see Section 2.7.1.6).

3.2.5 Statistical analysis

The experiment was a completely randomised design with a factorial arrangement of treatments (3x2x2). There were three rootstocks (M.9, MM.106 and M.793), two treatments of BAP (± BAP) and two root volumes (either 8 L or 45 L). Each treatment was replicated eight times. Data were analysed using the GLM procedure of SAS. Mean separations for the main effects were made using the Tukey's test at P=0.05. Where an

F-ratio of a significant main effect was much greater than that of a significant interaction involving that main effect, both the main effect and interaction were reported for interpretation. In contrast, only interaction data were reported for interpretation when an *F*-ratio of a main effect was similar or smaller than the *F*-ratio of an interaction involving that main effect. For significant interactions, preplanned comparisons of some treatment means were made using least square means (lsmeans tests) with Tukey’s adjustment. Unless otherwise stated, comparisons between interaction means were made at $P \leq 0.05$.

3.3 Results

3.3.1 Irrigation scheduling

Irrigation in this experiment maintained θ close to field capacity (0.30 ± 0.01 m^3 m^{-3}) over the growing season and, on the dates measured, the maximum deviation below field capacity was never more than 0.03 m^3 m^{-3} (Appendix 1).

3.3.2 Seasonal extension growth of the primary shoot for the main effects of rootstock, benzylaminopurine and root volume

After March, M.9 significantly reduced the mean cumulative length and node number of the primary shoot compared with MM.106 and M.793 (Figure 3.1A, B). In contrast, the primary shoot on MM.106 and M.793 grew more between March to May (Figures 3.1A, B; 3.2A, B). Despite M.793 being grafted one month later, the mean length and node number of the primary shoot were statistically similar to MM.106 from March onwards (Figure 3.1A, B).

The 8 L compared with the 45 L root volume also significantly reduced the mean cumulative length and node number of the primary shoot after March (Figure 3.1E, F), although reduced growth imposed by the 8 L root volume (Figures 3.1E, F; 3.2E, F) occurred earlier and over a different interval in the growing season than that imposed by M.9 (Figures 3.1A, B; 3.2A, B). Exogenous BAP significantly reduced the mean cumulative node number of the primary shoot from February onwards compared with untreated scions (Figure 3.1D). However, the mean cumulative length was not significantly different over the growing season for scions treated with or without BAP (Figure 3.1C). Hence, BAP significantly increased the mean internode length of the primary shoot (Table 3.3).

3.3.2.1 Treatment effects on termination of the shoot apical meristem on the primary shoot

For each treatment, the formation of the primary shoot was a result of a season-long flush of growth by the shoot apical meristem (SAM). For all treatments, 100% of

primary shoots were actively growing on the 15/3/05 (data not shown). However, a proportion of primary shoots had fully terminated on the 11/4/05, or approximately one month later. Therefore, termination of the primary shoot first began at some point in time between the growth measurements conducted on 15/3/05 and 11/4/05. Hence, treatment differences in the growth of the primary shoot up until the 15/3/05 (Figures 3.1 and 3.2) were not due to differences in the proportions of terminating SAMs.

Data for termination of the primary shoot could not be appropriately transformed for ANOVA. However, the mean proportion (%) of primary shoots that had terminated on the 11/4/05 for the main effect of rootstock was 53, 35 and 20% for M.9, MM.106 and M.793, respectively. For the BAP main effect, 51 and 23% of primary shoots had terminated with or without BAP, respectively, whilst 43 and 31% of primary shoots had terminated for trees grown in 8 L or 45 L root volumes, respectively.

3.3.3 Final leaf area of the primary shoot for the main effects of rootstock, benzylaminopurine and root volume

For M.9 and the 8 L root volume, reduced node number of the primary shoot significantly decreased its final leaf area (Tables 3.1 and 3.2, respectively). Reductions in leaf area of the primary shoot by M.9 and the 8 L root volume also resulted from a small but significant reduction in the mean area per leaf (Tables 3.1 and 3.2, respectively). In contrast, the application of BAP reduced the leaf area of the primary shoot by almost half, which occurred from a significantly decreased mean area per leaf and, to a lesser extent, reduced leaf number resulting from very small increases in internode length (Table 3.3).

3.3.4 Final extension growth of the primary shoot

3.3.4.1 Shoot length and node number

The final measurement of growth was conducted when scions were fully dormant in June, 2005. The primary shoot of all scions consisted of a single growth unit (data not shown); therefore the formation of the primary shoot was a result of a season-long growth flush by the SAM.

The M.9 rootstock significantly decreased the final mean length and node number of the primary shoot compared with MM.106 and M.793 (Table 3.1). Similarly, 8 L compared with 45 L root volumes significantly decreased the final mean length and node number of the primary shoot (Table 3.2). Rootstock type and root volumes did not significantly reduce internode length (Tables 3.1 and 3.2, respectively). Therefore, the decreased final mean length of the primary shoot imposed by M.9 and the 8 L root volume was attributable to fewer neoformed nodes resulting from differences in node production (Section 3.3.2) before shoot termination first began between 15/3/05 and 11/4/05, combined with a greater proportion of SAMs terminating early after the 15/3/05 (Section 3.3.2.1).

In addition to the highly significant main effects, the rootstock x root volume interactions were significant in June for the final mean length and node number of the primary shoot (Table 3.8). In contrast to MM.106, the 8 L root volume did not significantly reduce the final mean length and node number of the primary shoot for M.793 or M.9 (Table 3.8). For the 8 L root volume, the primary shoot on MM.106 had a similar final mean length and node number to that of M.9 grown in the 45 L root volume (Table 3.8). The M.793 rootstock grown in the 8 L root volume also had a statistically similar mean length of the primary shoot to M.9 grown in the 45 L root volume, however the mean node number of the primary shoot for M.793 grown in the 8 L root volume was significantly greater than that of M.9 grown in the 45 L root volume (Table 3.8). When grown in the 45 L root volume, the M.9 rootstock significantly decreased the mean length and node number of the primary shoot compared with MM.106, but not M.793.

Applications of BAP significantly reduced the final mean node number of the primary shoot compared with untreated scions, but without significantly (P=0.25) decreasing the mean length of the primary shoot (Table 3.3). Reduced node number of the primary shoot for BAP-treated scions resulted from significantly reduced rates of node production by the SAM in December and February when compared with untreated scions (Figure 3.2D). Notably, these reductions in the rate of node production for BAP-treated scions occurred before termination of the primary shoot had begun (see Section 3.2.2.1). Furthermore, they contributed to a significant increase in the mean internode

length of the primary shoot when compared with untreated scions, however these differences were very small (Table 3.3).

The rootstock x BAP interaction for the final node number of the primary shoot approached significance (P=0.07) by June, 2005 (Figure 3.3). Without BAP, M.9 reduced the mean node number of the primary shoot compared with MM.106 and, to a lesser extent, M.793 (Figure 3.3). In response to BAP, the mean node number of the primary shoot was decreased more markedly as rootstock vigour decreased. The M.793 rootstock produced a similar final node number on the primary shoot regardless of BAP treatment (Figure 3.3). Although the rootstock x BAP interaction for the final mean length of the primary shoot was not significant (P=0.45, data not shown), BAP reduced the final length of the primary shoot on M.9, MM.106 and M.793 by 60, 40 and 20 mm, respectively, when compared with the untreated primary shoot on the same rootstock type. This meant that the rootstock x BAP interaction for internode length was not significant (P=0.65, data not shown). Despite this, BAP increased the mean internode length of the primary shoot on M.9, MM.106 and M.793 by 2.0, 1.5 and 1.4 mm, respectively, when compared with the untreated primary shoot on the same rootstock type.

3.3.4.2 Final shoot cross-sectional area (SCA) of the primary shoot

Rootstock, root volume and BAP significantly affected the final mean SCA of the primary shoot (Tables 3.1, 3.2 and 3.3). However, interpretation of these main effects required consideration of interactions present in the data. The rootstock x BAP (data not shown) and the rootstock x root volume x BAP interactions were significant at P=0.03 and P=0.10, respectively. Although not highly significant, the latter interaction is presented for interpretation of the rootstock x BAP interaction and to compare some three-way treatment means (Figure 3.4).

Without BAP, rootstocks did not greatly differ in the mean SCA of the primary shoot when grown in 8 L or 45 L root volumes (Figure 3.4). Therefore, the significantly greater SCA of MM.106 (main effect in Table 3.1) resulted from its greater response to BAP when grown in the 45 L root volume (Figure 3.4). In contrast, SCA was similar

amongst rootstocks grown in the 8 L root volume when BAP was applied to the scion. For the 45 L root volume, BAP decreased mean SCA for M.9 and M.793 and increased SCA for MM.106 when compared with scions without BAP. For scions treated with BAP, 8 L compared with 45 L root volumes reduced SCA markedly more for MM.106 (Figure 3.4).

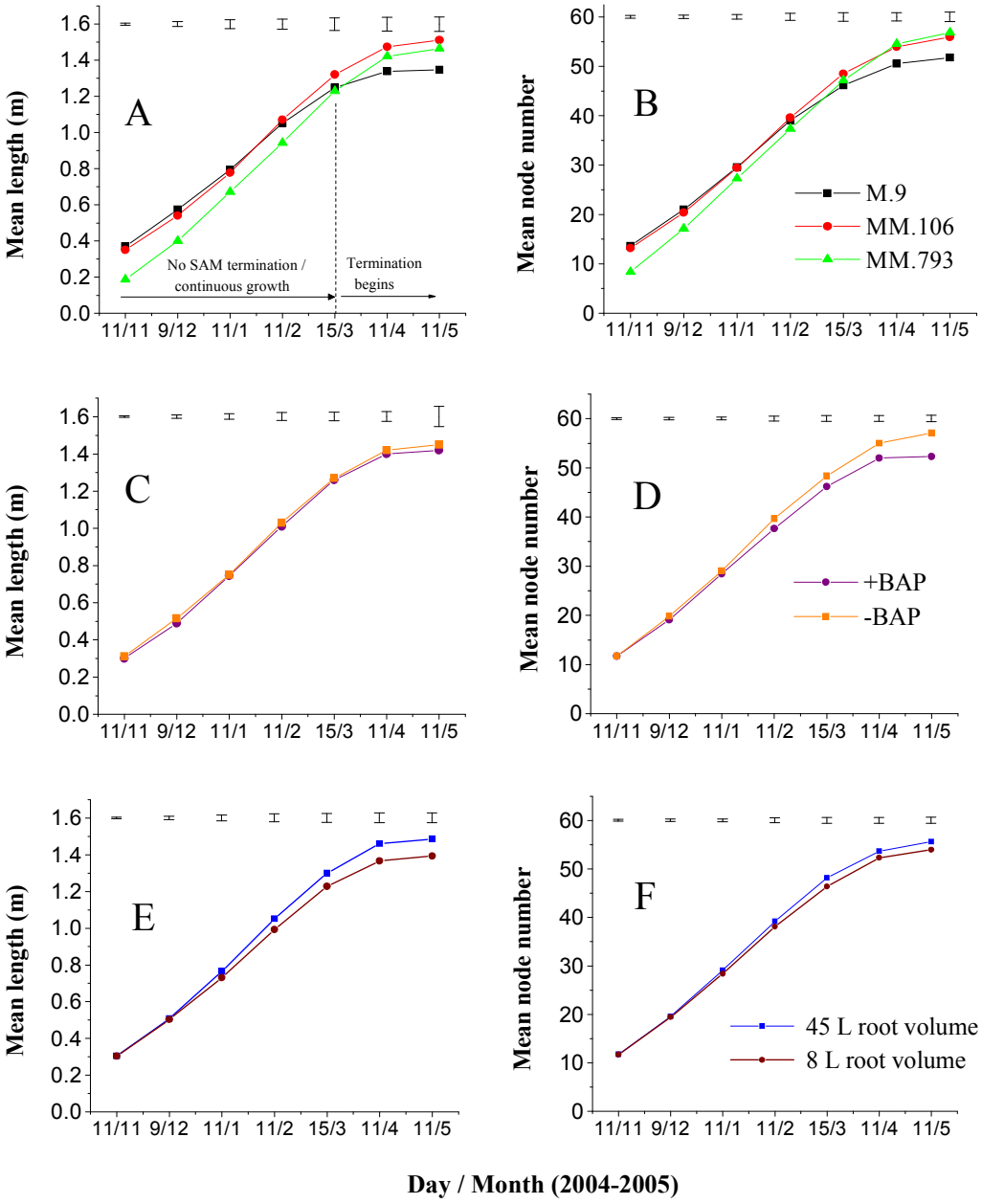

Figure 3.1. Main effect of rootstock (A and B), ± exogenous benzylaminopurine (BAP) (C and D) and root volumes (E and F) on the mean cumulative length (left) and node number (right) of 'Royal Gala' primary shoots during their first year of growth after grafting. Vertical bars represent the minimum significant difference (MSD) at *P*=0.05 using the Tukey's test. The vertical dotted line along the x-axis of 'A' denotes the time after which shoot termination first began for all treatments. Data for the main effects of rootstock, BAP or root volume are averaged over BAP and root volume, rootstock and root volume or rootstock and BAP, respectively.

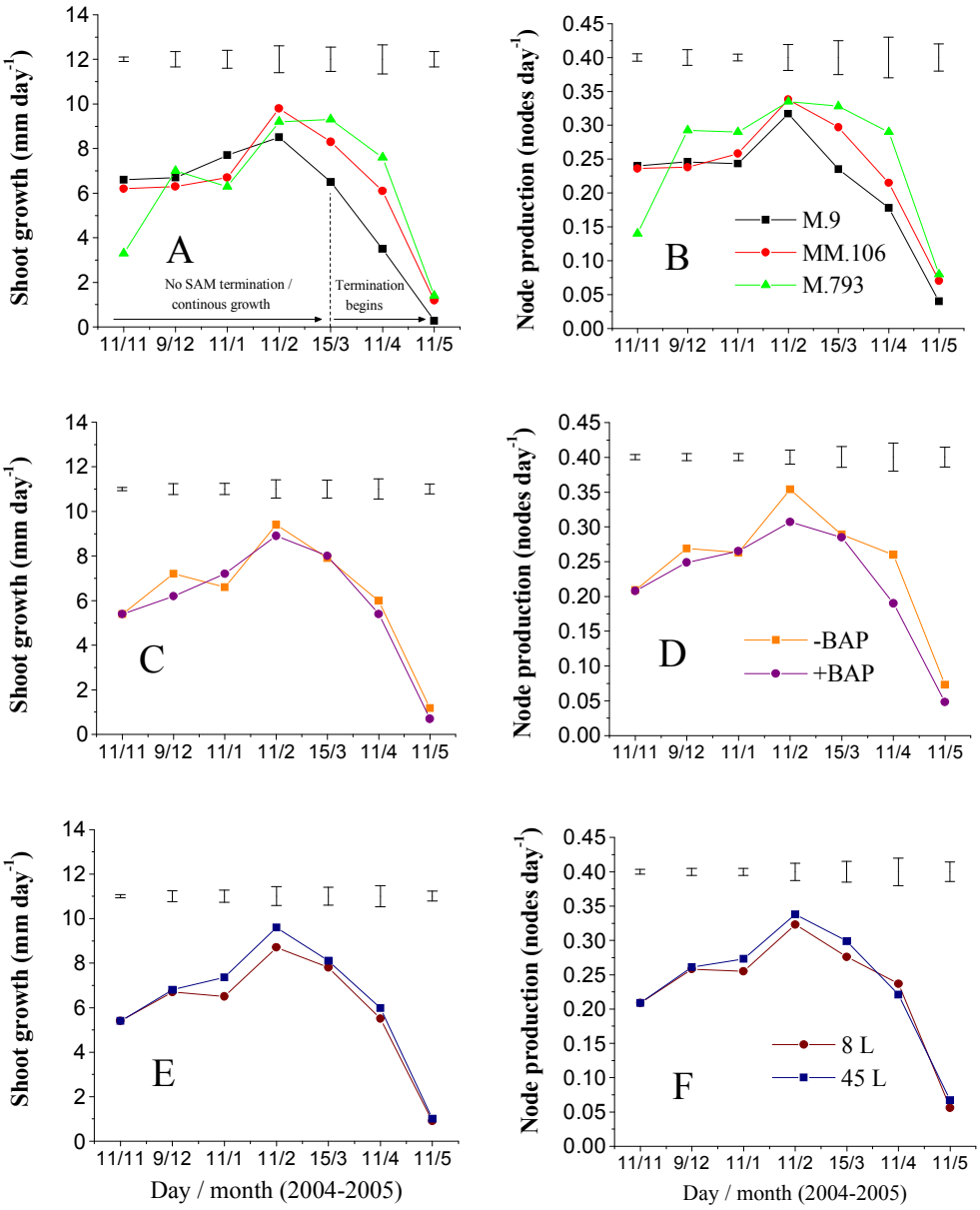

Figure 3.2. Main effect of rootstock (A and B), ± exogenous benzylaminopurine (BAP) (C and D) and root volumes (E and F) on the daily mean growth rate (left) and rate of node production (right) of 'Royal Gala' primary shoots during their first year of growth after grafting. Vertical bars represent the MSD at *P*=0.05 using the Tukey's test. The vertical dotted line along the x-axis of 'A' denotes the time after which shoot termination first began for all treatments. Data for the main effects of rootstock, BAP or root volume are averaged over BAP and root volume, rootstock and root volume or rootstock and BAP, respectively.

Table 3.1. Main effect of rootstocks on the growth attributes of the primary shoot, secondary shoots and total growth of 'Royal Gala' apple scions at the end of their first season of growth (June, 2005) after grafting.

Main effect	**A**	**B**	**A + B**
Rootstock	**Primary shoot**	**Secondary shoots**	**Total per scion**
	Length (m)		
M.9	1.35 a ***	1.72 (11.46 a)***	3.07 (17.04 a)***
MM.106	1.51 b	2.31 (13.93 b)	3.82 (19.06 b)
M.793	1.46 b	2.28 (13.92 b)	3.74 (18.93 b)
	Node number		
M.9	53 a ***	106 [v]	159 [v]
MM.106	57 b	124	181
M.793	58 b	119	177
	Leaf area (m^2)		
M.9	0.31 a ***	0.22 [v]	0.53 a ***
MM.106	0.37 b	0.30	0.67 b
M.793	0.37 b	0.26	0.63 b
	Leaf area (m^2) / leaf		
M.9	0.0058 (0.024 a) *	0.0021 [y]	-
MM.106	0.0064 (0.025 b)	0.0024	-
M.793	0.0063 (0.025 b)	0.0022	-
	Internode length (mm)		
M.9	25.5 [ns]	17.5 a ***	-
MM.106	26.5	21.9 b	-
M.793	25.4	21.0 b	-
	Shoot cross-sectional area (mm^2)		
M.9	194 a **	-	-
MM.106	213 b	-	-
M.793	186 a	-	-

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single growth attribute only, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. Data without and within parenthesis are raw or transformed means, respectively.

[v] *F*-ratio of rootstock x root volume interaction similar to main effect (see Table 3.8 for interaction data).

[y] data could not be appropriately transformed for ANOVA.

Table 3.2. Main effect of root volumes on the growth attributes of the primary shoot, secondary shoots and total growth of 'Royal Gala' apple scions at the end of their first season of growth (June, 2005) after grafting.

Main effect	**A**	**B**	**A + B**
Root volume	**Primary shoot**	**Secondary shoots**	**Total per scion**
	Length (m)		
8 L	1.40 a **	1.50 (10.71 a)***	2.90 (16.73 a)***
45 L	1.49 b	2.70 (15.49 b)	4.19 (20.05 b)
	Node number		
8 L	54 a ***	87 (8.08 a)***	141 (11.50 a)***
45 L	57 b	146 (11.27 b)	203 (13.77 b)
	Leaf area (m^2)		
8 L	0.33 a ***	0.19 a ***	0.52 a ***
45 L	0.37 b	0.33 b	0.70 b
	Leaf area (m^2) / leaf		
8 L	0.0061 (0.024 a)**	0.0022 [y]	-
45 L	0.0064 (0.025 b)	0.0022	-
	Internode length (mm)		
8 L	25.9 [ns]	19.1 [ns]	-
45 L	26.0	20.6	-
	Shoot cross-sectional area (mm^2)		
8 L	182 a ***	-	-
45 L	213 b	-	-

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single growth attribute only, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. [y] data could not be appropriately transformed for ANOVA. Data without and within parenthesis are raw or transformed means, respectively.

Table 3.3. Main effect of exogenous benzylaminopurine (BAP) on the growth attributes of the primary shoot, secondary shoots and total scion growth of 'Royal Gala' apple scions at the end of their first season of growth (June, 2005) after grafting.

Main effect	A	B	A + B
BAP	Primary shoot	Secondary shoots	Total per scion
	Length (m)		
- BAP	1.46 ^{ns}	1.00 (8.63 a)***	2.46 (15.36 a) ***
+ BAP	1.42	3.26 (17.83 b)	4.68 (21.48 b)
	Node number		
- BAP	58 a ***	42 (5.71 a)***	100 (9.67 a)***
+ BAP	53 b	195 (13.86 b)	248 (15.67 b)
	Leaf area (m^2)		
- BAP	0.45 a ***	0.15 a ***	0.60 ^{ns}
+ BAP	0.25 b	0.38 b	0.63
	Leaf area (m^2) / leaf		
- BAP	0.0078 (0.027 a) ***	0.0036 ^{y}	-
+ BAP	0.0047 (0.021 b)	0.0019	-
	Internode length (mm)		
- BAP	25.1 a ***	21.6 a ***	-
+ BAP	26.8 b	17.3 b	-
	Shoot cross- sectional area		
- BAP	207 a ***	-	-
+ BAP	187 b	-	-

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single growth attribute only, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. ^{y} data could not be appropriately transformed for ANOVA. Data without and within parenthesis are raw or transformed means, respectively.

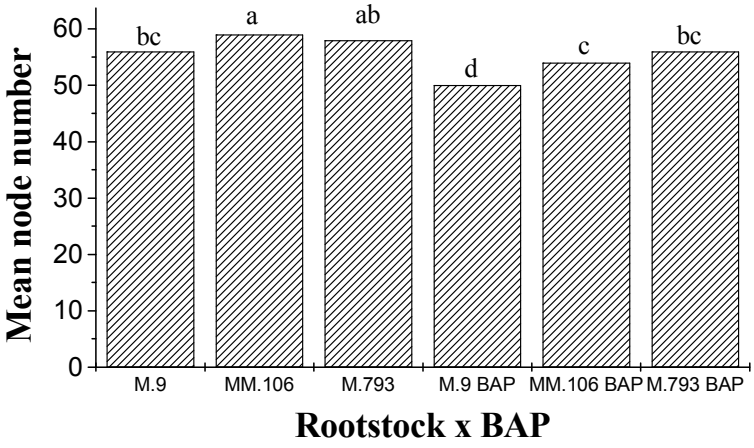

Figure 3.3. Effect of ± exogenous benzylaminopurine (BAP) on the final mean node number of the primary shoot of 'Royal Gala' apple scions on rootstocks of M.9, MM.106 and M.793 at growth cessation in June, 2005. Means sharing the same letter are not significantly different at $P \leq 0.07$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over root volume treatments.

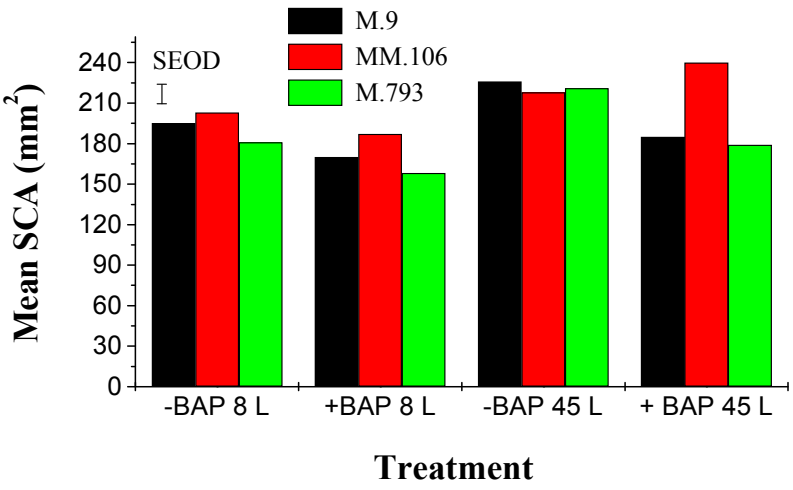

Figure 3.4. Rootstock x root volume x benzylaminopurine (BAP) interaction (P=0.10) on the final mean shoot cross-sectional area (SCA) of 'Royal Gala' primary shoots at growth cessation in June, 2005.

3.3.5 Treatment effects on the formation of secondary axes on the primary shoot and tertiary spurs on the secondary shoots

The different possible types of secondary and tertiary axes that may form on the newly grafted apple scion were described in Chapter 2 (Section 2.7, Figure 2.29). The first application of BAP occurred on the 11/11/2004 for M.9 and MM.106 and on the 1/12/2004 for M.793. These dates coincided with the primary shoot attaining approximately 15 nodes for the scion growing on each rootstock type. For all rootstocks, the axillary buds along the primary shoot began to break 14-days after the first BAP application leading to the formation of significantly more secondary shoots compared with the untreated scion (Figure 3.5). Natural formation of secondary shoots did not begin until late January for all rootstocks, before which, scions consisted of a single primary shoot (Figure 3.5, - BAP).

- BAP **+ BAP**

Figure 3.5. Effect of November and December applications of benzylaminopurine (BAP) on the number of secondary shoots formed by early January 2005 (right, + BAP) for 'Royal Gala' apple scions that were grafted onto M.9 during August, 2004. Natural feathering of the scion without BAP (left, - BAP) did not begin until late January.

3.3.5.1 Treatment effects on scion axillary bud break and the number of secondary and tertiary axes formed per scion

For rootstocks, data for the mean proportion (%) of axillary bud break on the primary shoot or secondary shoots and the mean number of different shoot types that formed on these axes are presented as rootstock x BAP interactions (Tables 3.5 and 3.6) because the *F*-ratios of rootstock x BAP interactions were typically similar or larger than the main effects of rootstock. In contrast, the *F*-ratios of BAP main effects were much greater than those of the rootstock x BAP interactions, therefore both the main effects of BAP (Table 3.4) and its interactions with rootstocks are presented (Tables 3.5 and 3.6).

Without BAP, the mean total number of axillary axes formed per scion, defined as the final total number of secondary spurs, trace spurs, secondary shoots and tertiary spurs, was not significantly affected by rootstock type (Table 3.6). However, exogenous BAP stimulated the formation of a significantly greater mean total number of axillary axes per scion on M.9 (Table 3.6). For M.9, BAP also increased the mean proportion of nodes on the primary shoot that contained a trace spur (Table 3.5) and it increased their mean number formed per scion (Table 3.6) when compared with MM.106 and M.793. Similarly, the mean proportion (%) of axillary buds on the secondary shoots that formed a tertiary spur (Table 3.5) and the mean number of tertiary spurs formed per scion (Table 3.6) was greater for the BAP-treated scion on M.9 or MM.106 than M.793. Although not significant, M.9 had nearly half the mean number of secondary shoots compared with MM.106 and M.793 when the scion was untreated. With BAP, however, M.9 produced a comparable mean number of secondary shoots to both MM.106 and M.793, whilst M.793 produced significantly more secondary shoots than MM.106 (Table 3.6).

Table 3.4. Main effect of benzylaminopurine (BAP) on the mean proportion (%) of axillary buds on the primary shoot that formed a trace spur, secondary spur or secondary shoot, the mean proportion (%) of axillary buds on the secondary shoots that formed a tertiary spur and the mean number of secondary, tertiary and total axillary axes that formed on 'Royal Gala' apple scions. Trees were measured at the end of their first year of growth (June, 2005) from grafting.

Treatment	Trace spurs	Secondary spurs	Secondary shoots	Tertiary spurs	Total per scion
		Mean proportion (%) of axillary bud break			
- BAP	0.00 [y]	2.36 a ***	5.23 a ***	0.00 [y]	-
+ BAP	3.80	4.27 b	27.14 b	2.86	-
		Mean number of axis type(s) per scion			
- BAP	0.00	1.37 a **	3.08 a ***	0.00	4.50 a ***
+ BAP	1.96	2.24 b	14.33 b	5.46	24.00 b

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. For a single attribute, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. [y] data could not be appropriately transformed for ANOVA.

Table 3.5. Effect of ± exogenous benzylaminopurine (BAP) on the mean proportion (%) of axillary buds on the primary shoot that formed a trace spur, secondary spur or secondary shoot and the mean proportion (%) of axillary buds on the secondary shoots that formed a tertiary spur for 'Royal Gala' apple scions grafted onto rootstocks of M.9, MM.106 and M.793. Trees were measured at the end of their first year of growth (June, 2005) from grafting.

Rootstock	BAP	Trace spurs	Secondary spurs	Secondary shoots	Tertiary spurs
M.9	- BAP	0.00 [y]	3.66 [ns]	3.60 c ***	0.00 [y]
M.9	+ BAP	7.59	4.30	30.47 a	3.58
MM.106	- BAP	0.00	1.33	6.12 c	0.00
MM.106	+ BAP	1.54	4.21	23.70 b	3.48
M.793	- BAP	0.00	2.11	5.98 c	0.00
M.793	+ BAP	1.80	4.47	27.25 ab	1.52

ns, *,**, *** non significant or significant interaction at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a column, means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). [y] data could not be appropriately transformed for ANOVA. Data are averaged over root volumes.

Table 3.6. Effect of ± exogenous benzylaminopurine (BAP) on the mean number of trace spurs, secondary spurs and secondary shoots formed on the primary shoot, the mean number of tertiary spurs formed on secondary shoots and the total number of axillary axes formed on 'Royal Gala' apple scions grafted onto rootstocks of M.9, MM.106 and M.793. Trees were measured at the end of their first year of growth (June, 2005) from grafting.

		Mean number of axillary axes per scion				
Rootstock	BAP	Trace spurs	Secondary spurs	Secondary shoots	Tertiary spurs	Total per scion
M.9	- BAP	0.00 [y]	2.06 [ns]	2.00 c **	0.00 [y]	4.06 c *
M.9	+ BAP	3.75	2.06	14.88 ab	6.88	27.56 a
MM.106	- BAP	0.00	0.81	3.75 c	0.00	4.56 c
MM.106	+ BAP	0.88	2.25	12.97 b	6.30	22.41 b
M.793	- BAP	0.00	1.25	3.50 c	0.00	4.75 c
M.793	+ BAP	1.00	2.52	15.22 a	3.16	21.91 b

ns, *,**, *** non significant or significant interaction at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a column, means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). [y] data could not be appropriately transformed for ANOVA. Data are averaged over root volumes.

For the main effect of root volume, the 8 L root volume significantly reduced the proportion (%) of axillary buds on the primary shoot that formed a secondary shoot when compared with trees grown in the 45 L root volume (data not shown). This was reflected in similar differences between root volumes for the mean number of secondary shoots and the mean total number of axillary axes formed per scion (Table 3.7). In addition to these main effects, the only significant rootstock x root volume interactions were for the proportion (%) of axillary buds on the primary shoot that formed a secondary shoot (data not shown) and the mean number of secondary shoots formed per scion (P=0.003, Figure 3.6). When grown in the 45 L root volume, M.9 produced significantly fewer secondary shoots than M.793. The 8 L compared with the 45 L root volume did not significantly reduce the mean number of secondary shoots formed for the scion on M.9. However, it significantly decreased the mean number of secondary shoots formed for the scion on MM.106 and M.793. When grown in the 8 L root volume, rootstocks of MM.106 and M.793 did not increase the mean number of secondary shoots formed per scion compared with M.9 grown in either the 8 L or the 45 L root volume (Figure 3.6).

Table 3.7. Main effect of root volume on the mean number of trace spurs, secondary spurs, secondary shoots, tertiary spurs and their total on 'Royal Gala' apple scions at growth cessation in June, 2005.

Root volume	Trace spurs	Secondary spurs	Secondary shoots	Tertiary spurs	Total Axes
8 L	0.68 [y]	1.92 [ns]	7.09 a ***	1.97 [y]	11.68 a ***
45 L	1.22	1.67	10.00 b	3.37	16.26 b

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a column, means sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. [y] data could not be appropriately transformed for ANOVA.

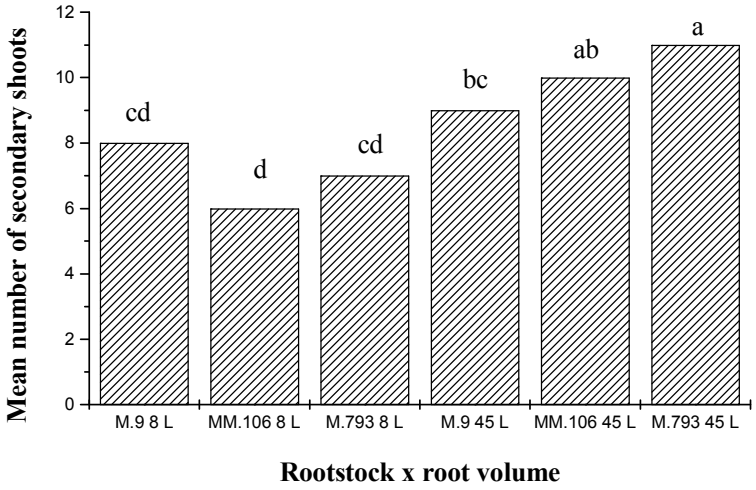

Figure 3.6. Effect of root volumes and rootstock type on the mean number of secondary shoots on 'Royal Gala' apple scions at the end of their first growing season (June, 2005) from grafting. Means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over benzylaminopurine treatments.

For the mean number of secondary shoots per scion, the two-way interaction means for rootstock x BAP (Table 3.6) include both 8 L and 45 L root volumes. Conversely, the rootstock x root volume means include plus or minus BAP treatments (Figure 3.6). Hence, interpretation of the significant rootstock x BAP interaction must consider the effect of the significant rootstock x root volume interaction and vice versa. To understand these interactions, the rootstock x root volume interactions were analysed

separately for data that was separated into ± BAP. Similarly, the rootstock x BAP interaction was analysed separately for data separated into ± 8 L and 45 L root volumes.

Excluding the influence of BAP, the rootstock x root volume interaction for the mean number of secondary shoots per scion was also significant (P=0.006) (Figure 3.7A, B). The M.9 rootstock grown in the 45 L root volume significantly reduced the mean number of secondary shoots formed per scion compared with MM.106 and M.793 (Figure 3.7B). Thus, the mean number of secondary shoots was non-significant between M.9 and MM.106 grown in the 45 L root volume (Figure 3.6) because of the response of M.9 to BAP when grown in this same root volume (Figure 3.7D). Compared with the 45 L root volume, the 8 L root volume significantly decreased the mean number of secondary shoots formed per scion for MM.106 and M.793 only (Figures 3.6 and 3.7A, B).

When grown in the 45 L root volume, MM.106 and M.793 tended to form more secondary shoots than spurs when compared with M.9 (Figure 3.7B). However, restricting the root volume of MM.106 and M.793 to 8 L resulted in the formation of almost equal numbers of secondary shoots and spurs, which was also a characteristic of M.9 grown in either root volume (Figure 3.7A, B).

For the 45 L root volume only, the rootstock x BAP interaction was significant for the mean number of secondary shoots formed per scion (P=0.02, Figure 3.7B, D). Without exogenous BAP, M.9 significantly reduced the mean number of secondary shoots formed, but produced a similar mean number to MM.106 and M.793 in response to BAP (compare Figure 3.7B and D). Therefore, the mean number of secondary shoots was not different amongst rootstocks without BAP (i.e., Table 3.6) because of the influence of the rootstock x root volume interaction, particularly as the mean number of secondary shoots was not greatly reduced by M.9 grown in the 8 L root volume (Figure 3.7A, B).

Trace spurs and tertiary spurs formed only in response to BAP (Figure 3.7) and the rootstock x root volume interaction was significant (P=0.02) for the mean number of tertiary spurs formed per scion (Figure 3.7C, D). When grown in the 45 L root volume,

M.9 produced significantly more tertiary spurs than M.793 (Figure 3.7D), however the mean number of tertiary spurs per scion was statistically similar amongst rootstocks grown in the 8 L root volume (Figure 3.7C). Hence, the 8 L root volume significantly decreased the mean number of tertiary spurs for M.9 only. For the 45 L root volume, the BAP-treated scion on M.9 formed a greater mean total number of axillary axes per scion because more tertiary spurs and, to a lesser extent, trace spurs formed (Figure 3.7D).

3.3.5.1.1 Root volume and BAP interactions

The root volume x BAP interactions were not significant for the mean number of secondary spurs (P=0.50, data not shown), secondary shoots (P=0.92, data not shown) and the total number of axillary axes formed per scion (P=0.11) (Figure 3.8). There were, however, trends that BAP partly reversed the decreased formation of axillary axes imposed on the scion by the 8 L root volume. For example, the mean total number of axillary axes formed per scion was halved by the 8 L root volume when BAP was not applied to the scion (Figure 3.8). However, BAP-treated trees grown in the 8 L root volume produced more than half the mean total number of axillary axes of that produced by BAP-treated trees grown in the 45 L root volume (Figure 3.8). This trend was also evident for the mean number of secondary shoots formed per scion, particularly for MM.106 and M.793 (Figure 3.7).

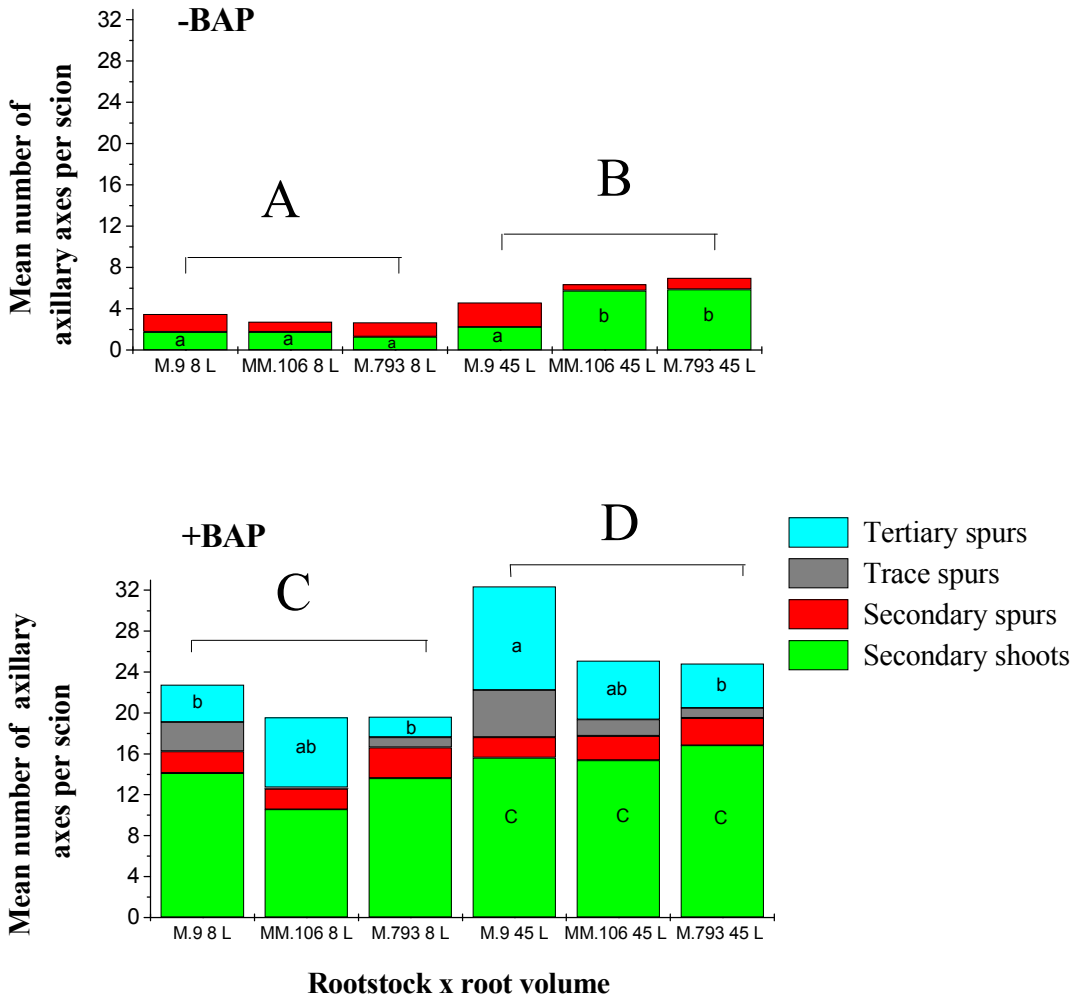

Figure 3.7. Mean spur and shoot numbers formed on 'Royal Gala' apple scions by June 2005 as modified by rootstock x root volume interactions plus (C and D) or minus (A and B) exogenous benzylaminopurine (BAP) and rootstock x BAP interactions plus (A and C) or minus (B and D) root restriction. The rootstock x root volume interactions plus or minus BAP were significant only for tertiary spurs (*P*=0.02) and secondary shoots (*P*=0.006), respectively (compare means of C and D or A and B for tertiary spurs or secondary shoots, respectively). Rootstock x BAP interactions were significant only for secondary shoots of the 45 L root volume (*P*=0.02, compare means of secondary shoots between B and D only). Within a single interaction, means sharing the same letter are not significantly different at *P*≤0.05 (lsmeans tests with Tukey's adjustment, SAS).

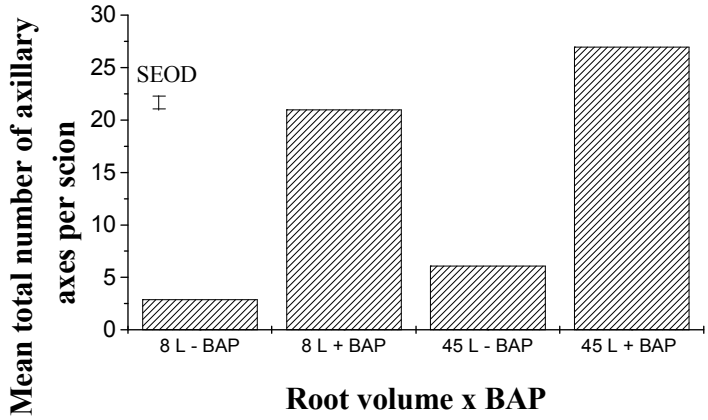

Figure 3.8. Effect of 8 L and 45 L root volumes and ± exogenous benzylaminopurine (BAP) on the mean total number of axillary axes on 'Royal Gala' apple scions at the end of their first year of growth after grafting. The mean total number of axillary axes is the sum of trace spurs, secondary spurs, secondary shoots and tertiary spurs formed per scion. Data are averaged over rootstocks.

3.3.5.2 Nodal position of secondary shoots along the primary shoot

Two distinct zones of branching occurred along the primary shoot of scions treated with or without BAP. For scions treated with BAP, secondary shoots mostly formed between nodes 1 to 25 (see Figure 3.9A where the majority of data points for shoot angles are positioned between nodes 1 to 25 along the x-axis). This was a result of early BAP applications stimulating the outgrowth of these axillary buds. Notably, few secondary shoots developed between nodes 26 to 50 (i.e., formed from January onwards, see Figure 3.1B) indicating that BAP applied after January was not effective in promoting the outgrowth of axillary buds. In contrast, scions without BAP began to form secondary axes from late January onwards and more secondary shoots developed at higher nodal positions on the primary shoot, again with two distinct zones of branching occurring between nodes 1 to 25 and 26 to 50 (see Figure 3.9B where the majority of data points for shoot angles are positioned between nodes 1 to 25 and 26 to 50 along the x-axis). Regardless of root volume, each rootstock type had approximately 25 and 75% of its total number of secondary shoots positioned between nodes 1 to 25 and 26 to 50, respectively, when the scion was untreated (data not shown).

3.3.5.3 Angle of elevation of the secondary shoots

The main effects of rootstock and root volume did not significantly affect the mean angle of elevation of the secondary shoots (P=0.14 and P=0.13, respectively; data not shown). Exogenous BAP increased the angle of elevation of secondary shoots at comparable nodal positions along the primary shoot when compared with untreated scions (compare Figures 3.9A and B), which resulted in a significantly greater (P<0.0001) mean angle of elevation of the secondary shoots on BAP-treated scions when compared with untreated scions (43 and 34^{o}, respectively). The rootstock x BAP interaction was also significant (P=0.0001) (Figure 3.10).

Without BAP, the mean angle of elevation of the secondary shoots for the scion on M.9 was similar to MM.106 and M.793, however the scion on MM.106 had a significantly greater mean angle of shoot elevation than the scion on M.793. For each rootstock type, BAP increased the mean angle of elevation of the secondary shoots when compared with the untreated scion on the same rootstock, however this increase was not significant for MM.106 (Figure 3.10). In response to BAP, there was a trend that the mean angle of elevation of the secondary shoots increased with increasing vigour of the rootstock and the scion on M.793 had a significantly greater mean angle of shoot elevation than the scion on M.9 or MM.106 (Figure 3.10). The greater mean angle of elevation of the secondary shoots for the BAP-treated scion on M.793 (i.e., Figure 3.10) resulted from a reduced number of secondary shoots positioned on the primary shoot between nodes 1 to 10, and a greater number of secondary shoots positioned between nodes 20 to 25, which had greater angles of elevation compared with shoots at lower nodal positions (Figure 3.9A). Regardless of BAP treatment to the scion, rootstocks had little effect on shoot angles at any position along the primary shoot (Figure 3.9A, B).

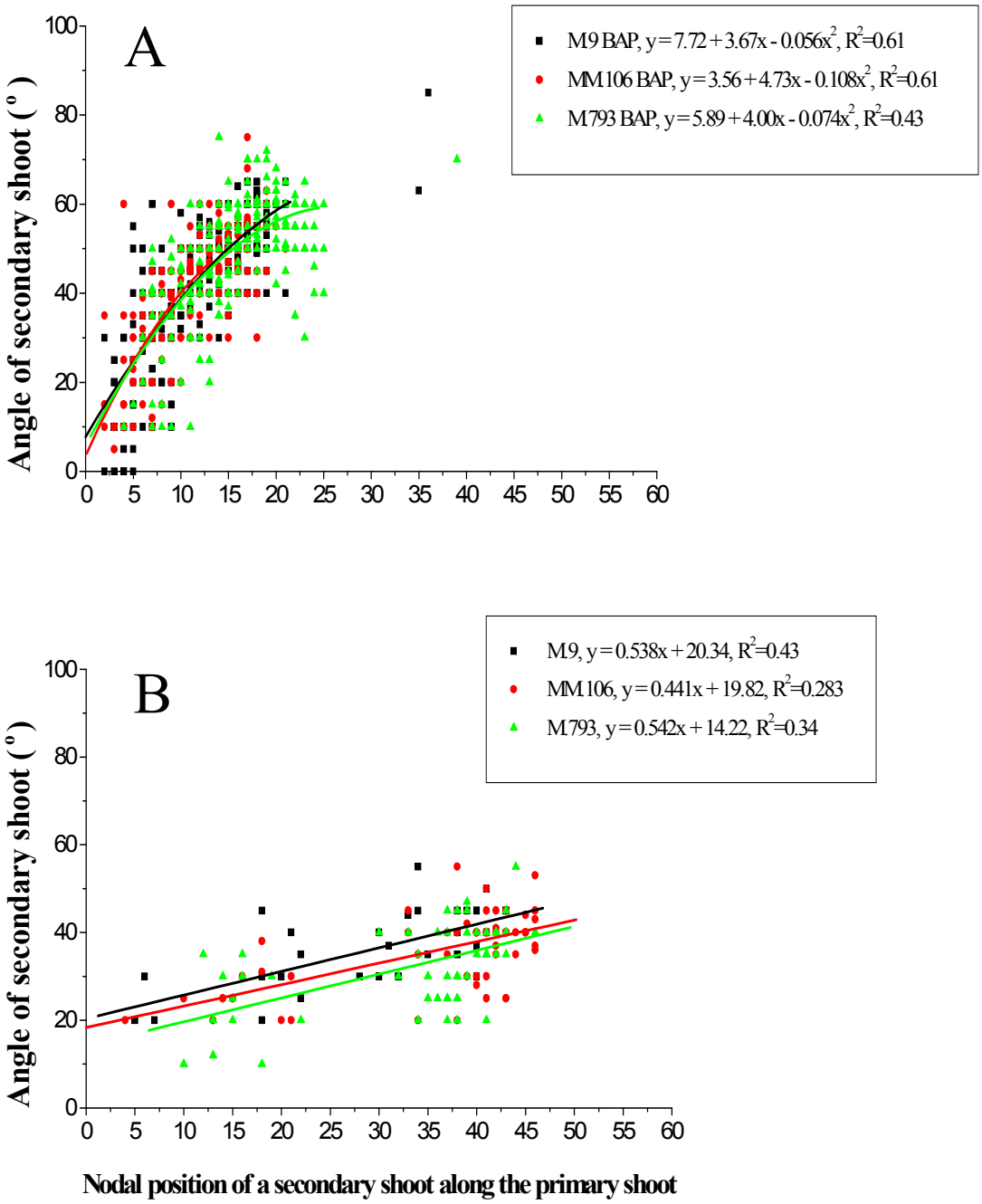

Figure 3.9. Effect of rootstock type and plus (A) or minus (B) benzylaminopurine (BAP) applied to 'Royal Gala' apple scions on the angle of elevation for secondary shoots relative to their nodal position on the primary shoot. Angles of elevation were measured as acute angles with 0 and 90° elevation representing a shoot that was horizontal with the ground or vertical like the primary shoot, respectively.

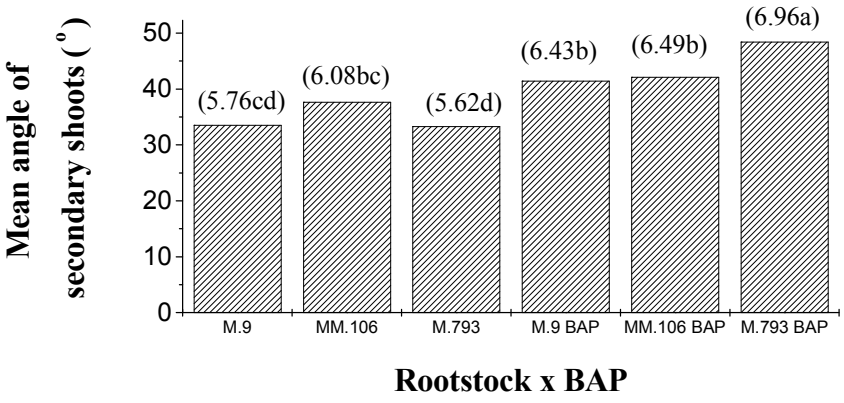

Figure 3.10. Mean angle of elevation for secondary shoots formed along the primary shoot in response to ± benzylaminopurine (BAP) applied to 'Royal Gala' apple scions grafted onto rootstocks of M.9, MM.106 and M.793. Means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Means in parenthesis are transformed. Data are averaged over root volume treatments.

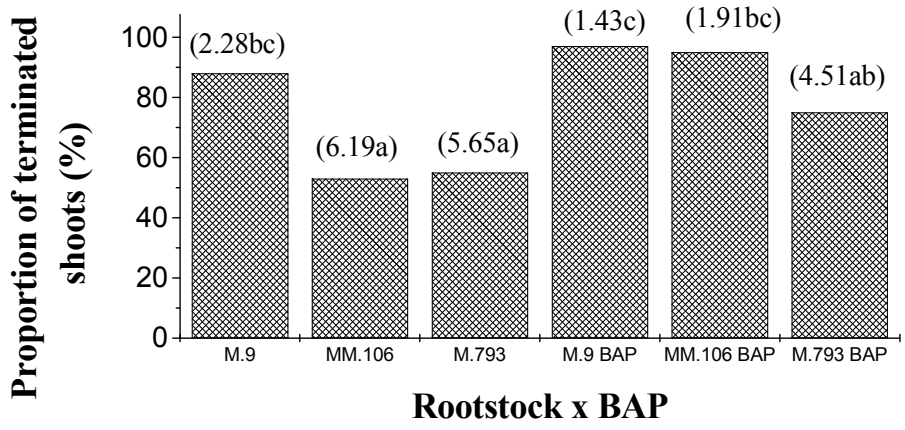

Figure 3.11. Effect of ± benzylaminopurine (BAP) applied to the scion and rootstock type on the mean proportion (%) of terminated secondary shoots on 'Royal Gala' apple scions in March, 2005. Means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Means in parenthesis are transformed. Data are averaged over root volume treatments.

3.3.5.4 Treatment effects on termination of the secondary shoots

The proportion (%) of secondary shoots that had terminated was measured once in early March, 2005. The main effects of rootstock and BAP were highly significant ($P<0.0001$ and $P<0.0001$, respectively), however, root volume was less so ($P=0.02$). For rootstocks, 92, 73 and 65% of secondary shoots had terminated for M.9, MM.106 and M.793, respectively. In addition, M.9 significantly increased the proportion (%) of secondary shoots that had terminated compared with MM.106 and M.793, whereas the latter two rootstocks did not significantly differ from each other (data not shown). For scions treated with or without BAP, 90 and 65% of secondary shoots had terminated, respectively, whilst 80 and 72% of secondary shoots had terminated for trees grown in 8 L and 45 L root volumes, respectively.

In addition to significant main effects, the rootstock x BAP interaction was significant ($P=0.04$) for the mean proportion (%) of secondary shoots that had terminated during March (Figure 3.11). Without BAP, a greater proportion (%) of secondary shoots had terminated for M.9 than MM.106 and M.793 (Figure 3.11). For MM.106 only, BAP significantly increased termination of the secondary shoots when compared with untreated scions. Rootstocks of M.9 and MM.106 did not differ in the mean proportion of terminated secondary shoots when the scion was treated with BAP. For scions treated either with or without BAP, a significantly greater proportion (%) of secondary shoots had terminated for M.9 than M.793. Notably, BAP did not significantly increase the proportion (%) of terminated secondary shoots for the scion on M.9 because secondary shoots had mostly ceased to grow by March (Figure 3.11).

At growth cessation in June 2005, the morphological marker of a ring of bud scale scars, and/or, compressed internodes (Chapter 2, Section 2.7.1.6) was used to identify what proportion of secondary shoots were formed from a bicyclic pattern of growth. Data could not be appropriately transformed for ANOVA, however there was a trend that the main effect of BAP increased the mean proportion (%) of secondary shoots per scion with two growth units when compared with untreated scions (18 and 1%, respectively). In addition, this effect of BAP depended on rootstock type (Figure 3.12). Without BAP, very few secondary shoots developed two growth units and rootstocks

did not greatly affect the proportion of secondary shoots with two growth units (Figure 3.12). Compared with untreated scions, BAP increased the proportion of secondary shoots with two growth units markedly more as rootstock vigour increased (Figure 3.12). For the main effect of root restriction, the mean proportion (%) of secondary shoots with two growth units was 10% for both the 8 L and 45 L root volumes. Hence, root restriction had no effect on the proportion of shoots with two growth units.

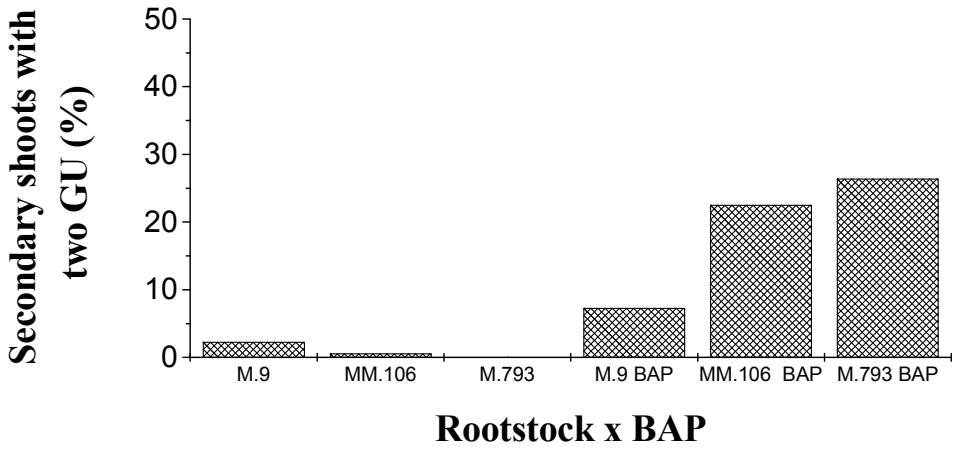

Figure 3.12. Effect of rootstock type and ± benzylaminopurine (BAP) applied to the scion on the mean proportion (%) of secondary shoots on 'Royal Gala' apple scions that had formed from two growth units (GU). At growth cessation in June 2005, the morphological marker of a ring of bud scale scars, and/or, compressed internodes was used to identify which shoots had temporarily ceased growing at some point in the growing season.

3.3.6 Leaf area of the secondary shoots

For both M.9 and the 8 L root volume, reduced mean total node number of the secondary shoots decreased the mean total leaf area of these shoots when compared with MM.106 and M.793 or the 45 L root volume, respectively (see main effects in Tables 3.1 and 3.2, respectively). Exogenous BAP reduced the mean area per leaf on the secondary shoots (Table 3.3), however it increased the mean total leaf area of the secondary shoots by increasing the mean number of secondary shoots formed per scion (Table 3.4), which greatly increased the total number of nodes (Table 3.3) and leaves that formed when compared with the secondary shoots on untreated scions. Notably, the

mean total leaf area per scion was not significantly affected by the application of BAP, but the total leaf area of the secondary shoots for BAP treated scions was greater than that developed on the primary shoot (Table 3.3).

In addition to the above main effects, the rootstock x root volume interaction was significant for the mean total leaf area of the secondary shoots (P=0.01) and the mean total leaf area per scion (P=0.02) (Table 3.8). When grown in the 45 L root volume, M.9 reduced the mean total leaf area of the secondary shoots compared with MM.106 and M.793, although means for M.9 and M.793 were not significantly different (Table 3.8). For MM.106 and M.793, the 8 L root volume significantly reduced the total leaf area of the secondary shoots and the total leaf area per scion and these mean values were comparable to that of M.9 grown in the 45 L root volume (Table 3.8). Notably, the 8 L root volume did not significantly reduce the total leaf area of the secondary shoots or the total leaf area per scion for M.9 (Table 3.8). These interactions for the mean total leaf area of the secondary shoots were caused by similar rootstock x root volume interactions for the mean total node number of the secondary shoots and the mean number of secondary shoots formed per scion. These interactions are subsequently reported.

3.3.7 Final growth measurements of the secondary shoots

3.3.7.1 Final mean total length and node number of the secondary shoots for rootstock and root volume

In addition to highly significant main effects (Tables 3.1 and 3.2), the rootstock x root volume interaction was significant for the mean total node number (P=0.04) and approached significance for the mean total length (P=0.06) of the secondary shoots at growth cessation (Table 3.8). When grown in the 45 L root volume, M.9 reduced the mean total length and node number of the secondary shoots compared with MM.106 and M.793 (Table 3.8). For each rootstock, the 8 L compared with the 45 L root volume tended to reduce the mean total length and node number of secondary shoots, although these reductions in growth imposed by the 8 L root volume were less evident for M.9 than MM.106 and M.793. Restricting the root volume of MM.106 and M.793 to 8 L produced a statistically comparable mean total length and node number of the secondary

shoots to M.9 grown in the 45 L root volume (Table 3.8). For MM.106 and M.793, large decreases in the mean total shoot length and total node number per scion (i.e., primary plus secondary shoots) were caused by the 8 L root volume reducing the mean total length and node number of the secondary shoots more than that for the primary shoot (Table 3.8).

Restricting the root volume of MM.106 and M.793 to 8 L generally produced a comparable mean total shoot length and node number per scion to M.9 grown in either the 8 L or 45 L root volume, although MM.106 grown in the 8 L root volume produced significantly fewer nodes per scion than M.9 grown in the 45 L root volume. The M.9 rootstock grown in the 45 L root volume significantly decreased the mean total length and node number per scion compared with rootstocks of MM.106 and M.793 grown in the same root volume.

Table 3.8. Rootstock x root volume interactions for the final growth attributes of the primary shoot, secondary shoots and their total for 'Royal Gala' apple scions at the end of their first year of growth (June, 2005) from grafting.

		A	B	A+B
Rootstock	**Root volume**	**Primary shoot**	**Secondary shoots**	**Total**
		Mean shoot length (m)		
M.9	8 L	1.30 c *	1.43 (10.11 b) [x]	2.73 (16.10 b) *
M.9	45 L	1.40 bc	1.99 (12.81 b)	3.39 (17.98 b)
MM.106	8 L	1.43 b	1.42 (10.62 b)	2.85 (16.84 b)
MM.106	45 L	1.59 a	3.12 (17.03 a)	4.72 (21.42 a)
M.793	8 L	1.47 ab	1.64 (11.41 b)	3.11 (17.24 b)
M.793	45 L	1.47 ab	3.01 (16.08 a)	4.48 (21.24 a)
		Mean node number		
M.9	8 L	51 d **	91 (8.10 b) *	142 (11.47 c) *
M.9	45 L	54 cd	121 (9.96 b)	175 (12.79 b)
MM.106	8 L	55 bc	77 (8.01 b)	132 (11.36 c)
MM.106	45 L	59 a	168 (12.25 a)	227 (14.46 a)
M.793	8 L	58 ab	92 (8.43 b)	150 (11.85 bc)
M.793	45 L	57 abc	150 (12.15 a)	207 (14.31 a)
		Mean leaf area (m^2)		
M.9	8 L	2.93 [ns]	1.89 c **	4.82 b *
M.9	45 L	3.29	2.41 bc	5.70 b
MM.106	8 L	3.50	1.90 c	5.40 b
MM.106	45 L	3.87	4.10 a	7.96 a
M.793	8 L	3.54	1.86 c	5.40 b
M.793	45 L	3.73	3.48 ab	7.21 a

ns [x], *,**, *** non significant or significant interaction at $P \leq 0.06$, 0.05, 0.01 and 0.001, respectively. For a single attribute, means within a column sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data without and within parenthesis are raw or transformed means, respectively. [x] mean separation at $P \leq 0.06$. Data are averaged over benzylaminopurine treatments.

Reductions in the mean total length of the secondary shoots caused by M.9, and/or, by the 8 L root volume (Table 3.8) could have resulted from decreased mean length of the secondary shoots, fewer secondary shoots per scion, or both. Similarly, decreased mean total node number of the secondary shoots (Table 3.8) could have resulted from a reduction in mean node number of the secondary shoots, differences in internode length, or fewer secondary shoots per scion. The rootstock x root volume interaction approached significance (P=0.09) for the mean length of the secondary shoots, was significant for the mean node number of the secondary shoots (P=0.04) (Table 3.9) and

the mean number of secondary shoots formed per scion (P=0.003) (Figure 3.6). Consideration of these interactions revealed that rootstocks differed in the way that mean total length and node number of the secondary shoots was reduced by the 8 L volume.

Table 3.9. Effect of rootstocks grown in 8 L or 45 L root volumes on the mean length, node number and internode length of secondary shoots on 'Royal Gala' apple scions at growth cessation in June, 2005.

Root volume	Node number	Length (mm)	Internode length (mm)
M.9 8 L	11 (3.24 b) *	182 [z]	16 [ns]
M.9 45 L	14 (3.78 ab)	292	21
MM.106 8 L	12 (3.53 ab)	252	21
MM.106 45 L	15 (3.86 a)	307	21
M.793 8 L	15 (3.89 a)	325	22
M.793 45 L	14 (3.72 ab)	307	22

ns, [z] *,**, *** non significant or significant interaction at P=0.09, $P \leq 0.05$, 0.01 and 0.001, respectively. Means within a column sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data without and within parenthesis are raw or transformed means, respectively.

For M.9, decreased mean total length of the secondary shoots caused by the 8 L root volume (Table 3.8) mostly resulted from reduced mean shoot length (Table 3.9) rather than large decreases in shoot number (Figure 3.6). Decreased mean length of secondary shoots for M.9 grown in the 8 L root volume (Table 3.9) resulted from a greater proportion of short secondary shoots forming compared with the 45 L root volume, particularly shoots 0.2 m or shorter and with 10 or fewer nodes (Figure 3.13A, B). In contrast, reduced mean total length of the secondary shoots for M.9 grown in the 45 L root volume (Table 3.8) resulted mostly from fewer secondary shoots forming compared with MM.106 and M.793 (see Figure 3.6 and Table 3.9). For MM.106, the 8 L root volume almost halved the mean number of secondary shoots formed compared with the 45 L root volume (Figure 3.6) and reduced the mean length of the secondary shoots (Table 3.9) by decreasing the formation of long shoots compared with the 45 L root volume, especially those greater than 0.5 m or with more than 20 nodes (Figure 3.13C, D). As noted for the primary shoot on M.793 (Table 3.8), the 8 L compared with the 45 L root volume did not affect the mean length of secondary shoots (Table 3.9).

Therefore, reductions in the mean total length of the secondary shoots for M.793 grown in the 8 L root volume (Table 3.8) resulted from fewer secondary shoots (Figure 3.6).

The mean length, node number and internode length of the secondary shoots was similar amongst rootstock types when grown in the 45 L root volume (Table 3.9). For M.9 and MM.106 only, 8 L compared with 45 L root volumes tended to decrease the mean node number and length of the secondary shoots (Table 3.9). This resulted partly from the 8 L root volume increasing the proportion of secondary shoots with less than 20 nodes (Figure 3.13B, D), hence decreasing the proportion of long shoots produced by both rootstocks when compared with the 45 L root volume. For all rootstocks, internode length of the secondary shoots increased with shoot length (see Figure 3.13A, C, E where internode lengths can be compared for different length shoots from the regression line on each graph by dividing a y-value by its corresponding x-value). The relationship between node number and the length of secondary shoots was also very similar amongst all treatments, and shoots with the same node number were of similar length (Figure 3.13A, C, E), hence they had very similar internode lengths. Therefore, reduced mean internode length of the secondary shoots for M.9 grown in the 8 L root volume (Table 3.9) resulted from its node number distribution where proportionally more shoots with less than 10 nodes developed (Figure 3.13B). This contributed to the significant main effect where M.9 reduced the mean internode length of the secondary shoots compared with MM.106 and M.793 (Table 3.1).

The first 9 to 10 nodes of an annual shoot are preformed within the overwintering one-year-old vegetative bud (Pratt, 1990). However, for newly grafted apple scions studied in this experiment, it was unknown at what time during the growing season that a newly formed axillary bud had developed 9 to 10 primordia (see Chapter 1, Section 1.5.3). Assuming that the first 10 nodes were preformed in each axillary bud on the primary shoot, the mean number of neoformed nodes per secondary shoot was similar for rootstocks grown in the 45 L root volume, namely, 4, 5 and 4 for M.9, MM.106 and M.793, respectively (Table 3.9). Therefore, M.9 grown in the 45 L root volume reduced the mean total node number and leaf area of the secondary shoots (Table 3.8) by mostly decreasing the mean number of shoots formed (Figure 3.6) rather than significantly decreasing the mean number of neoformed nodes per shoot (Table 3.9). In contrast, M.9

and MM.106 grown in the 8 L root volume produced an average of only 1 and 2 neoformed nodes per secondary shoot, respectively (Table 3.9). Therefore, the reduction in the mean total node number and leaf area of the secondary shoots when M.9 or MM.106 were grown in the 8 L volume (Table 3.8) was a result of fewer neoformed nodes per shoot and the respective effect of M.9 and MM.106 on the number of secondary shoots that formed when these rootstocks were grown in the 8 L root volume (Figure 3.6). For M.793, the 8 L root volume did not reduce the mean node number of the secondary shoots compared with the 45 L root volume (Table 3.9). Therefore, reductions in the mean total node number and leaf area of the secondary shoots for M.793 grown in the 8 L root volume (Table 3.8) resulted from fewer shoots per scion compared with the 45 L root volume (Figure 3.6).

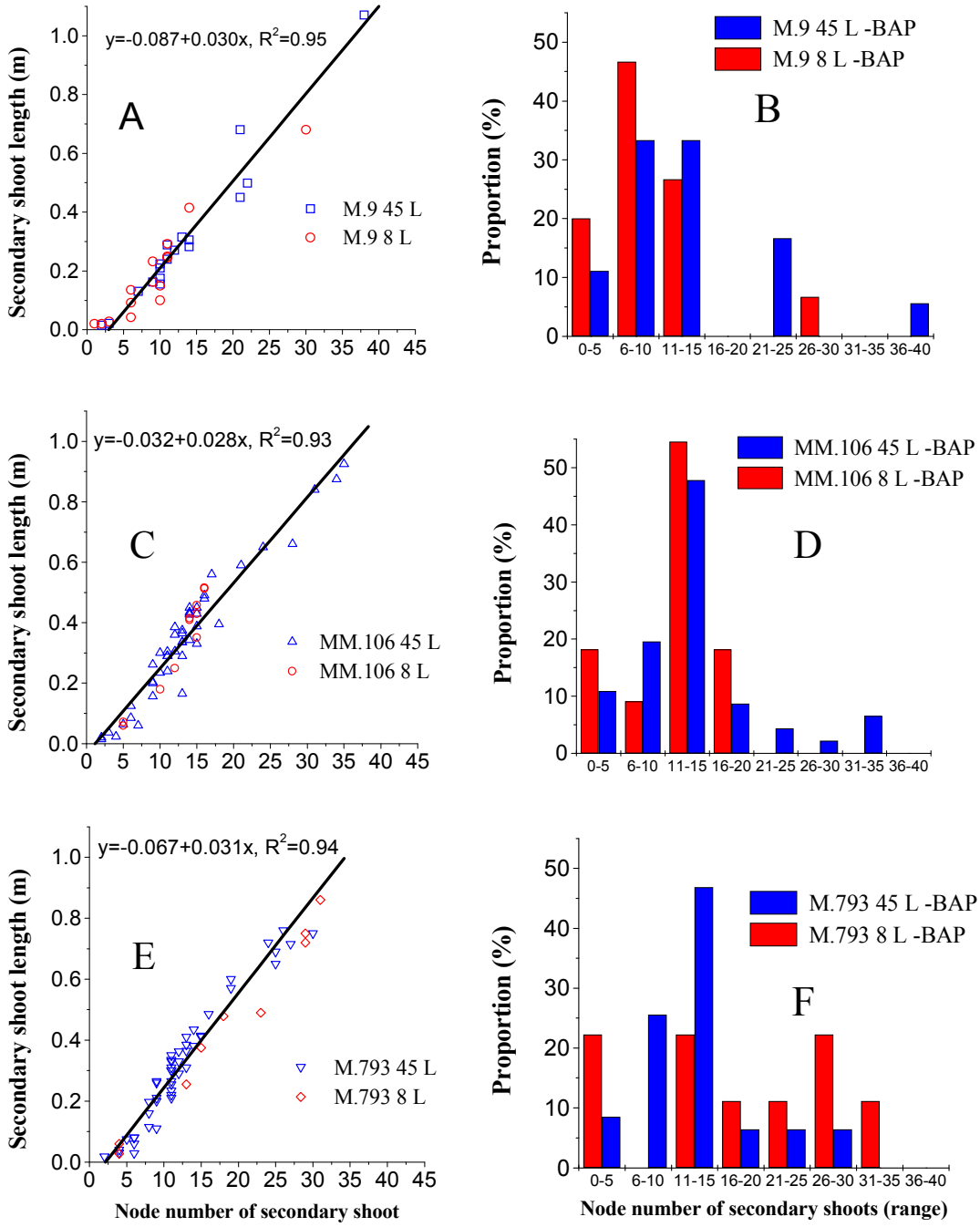

Figure 3.13. Effect of rootstock type and root volumes on the relationship between secondary shoot length and node number (A, C and E) for 'Royal Gala' apple scions without benzylaminopurine (BAP). B, D and F represent the node number distributions of the total secondary shoot population for each treatment.

3.3.7.2 Effect of BAP on the mean total length and node number of the secondary shoots

In contrast with the results for the primary shoot, the application of BAP significantly increased the mean total length and node number of the secondary shoots (Table 3.3). For the BAP main effect, the mean node number of the secondary shoots was 13 and 14 nodes (main effect, *P*=0.81) for scions treated with or without BAP, respectively, whereas the mean length was 226 and 302 mm (main effect, *P*=0.003), respectively. Therefore, secondary shoots on BAP-treated scions had a significantly decreased mean internode length compared with untreated scions (Table 3.3). Hence, BAP did not increase the mean total length and node number of the secondary shoots (Table 3.3) by promoting longer shoots with more neoformed nodes. Rather, BAP stimulated significantly more axillary buds to break on the primary shoot, therefore, increasing the number of secondary shoots formed per scion (Table 3.4).

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Despite BAP stimulating a similar number of secondary shoots per scion for M.9 compared with MM.106 and M.793 (Table 3.6), the rootstock x BAP interactions for the mean total length and node number of the secondary shoots were not significant (data not shown). This was a result of M.9 producing a greater proportion of shorter shoots with 11 to 15 nodes and a lower proportion of medium and long shoots with 16 to 20 and 26 to 30 nodes when its scion was treated with BAP and compared with the same scion treatment for MM.106 and M.793 (Figure 3.14B, D, F). In addition, the rootstock x BAP interactions for the final mean total length and node number per scion were not significant (*P*=0.52 and *P*=0.80, respectively; data not shown). For trees grown in the 45 L root volume and treated with BAP, the final mean total shoot length per scion was 3.8, 5.9 and 5.7 m, whilst the mean total node number was 256, 315 and 299 for rootstocks of M.9, MM.106 and M.793, respectively. Hence, total shoot growth of the BAP-treated scion on M.9 was still markedly less than that for rootstocks of greater vigour.

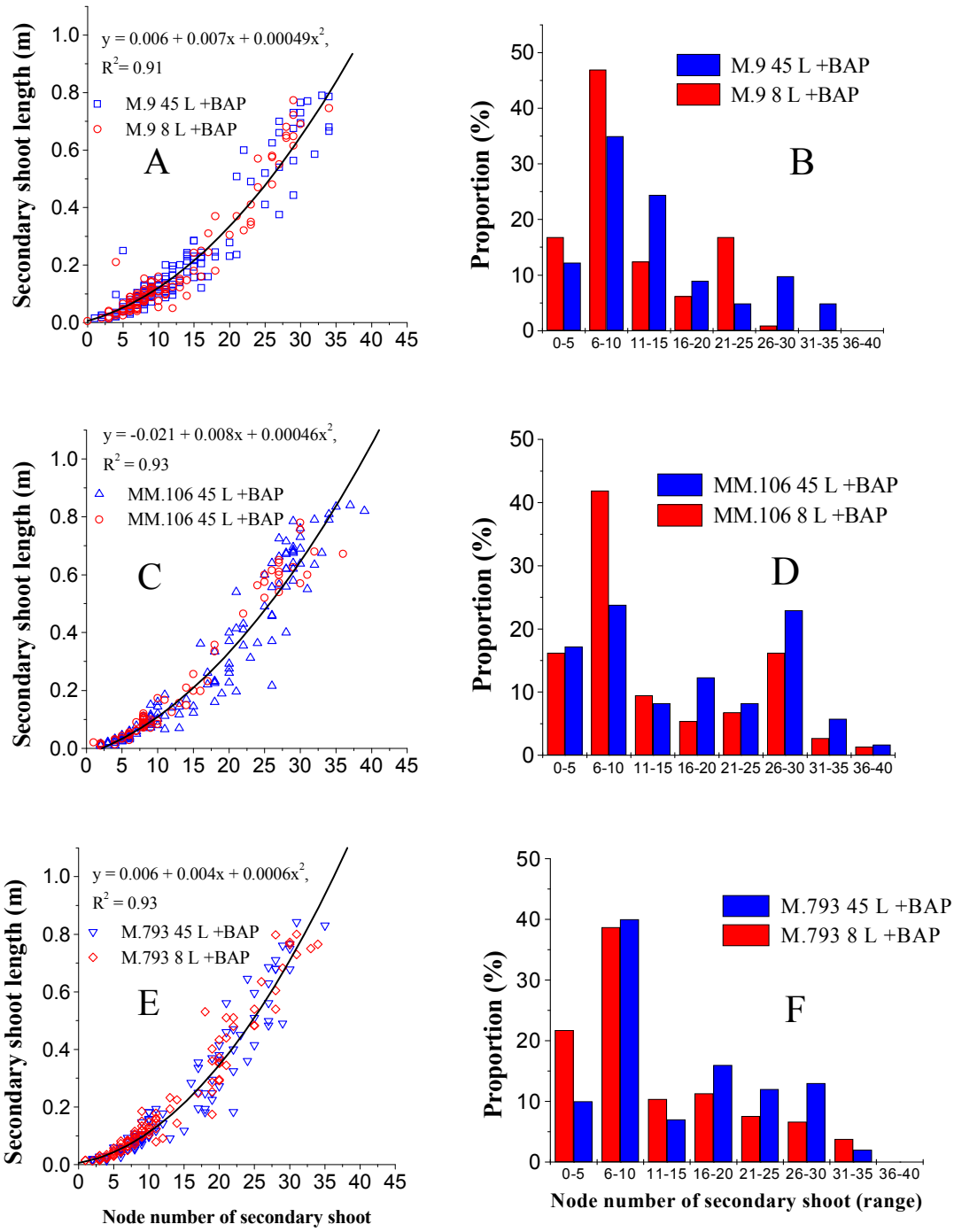


Figure 3.14. Effect of rootstock type and root volumes on the relationship between secondary shoot length and node number for 'Royal Gala' apple scions treated with benzylaminopurine (BAP) (A, C and E). B, D and F represent the node number distributions of the total secondary shoot population for each treatment.

3.4. Discussion

3.4.1 Effectiveness of irrigation scheduling

The field capacity of the medium (0.30 ± 0.01 m^3 m^{-3}) was similar to Mills (1996) (approximately 0.32 ± 0.01 m^3 m^{-3}) who used a similar growing medium. For glasshouse grown 'Braeburn' subjected to deficit irrigation, a volumetric water content (θ) reduction from 0.30 m^3 m^{-3} to 0.20 m^3 m^{-3} was required to reduce extension growth of the scion (Mills, 1996). The lowest θ recorded in this experiment was 0.27 m^3 m^{-3} (Appendix 1), therefore reductions in vigour by treatments were unlikely to be a result of reduced θ.

3.4.2 Effect of rootstock type on vegetative growth

An important objective of this chapter was to determine when and how rootstock-induced dwarfing of scion occurred after grafting of the composite tree.

3.4.2.1 Growth of the primary shoot

The primary shoot of 'Royal Gala' apple scions began growth in the spring from a single vegetative bud. According to Pratt (1990), the first 9 to 10 nodes produced are preformed within the vegetative bud while subsequent nodes produced throughout the growing season are neoformed by the SAM.

Dwarfing of the primary shoot by M.9 occurred within the first year of growth from grafting (Figure 3.1A, B; Table 3.1). For the scion on M.9, the SAM on the primary shoot had decreased rates of growth between 11/2/05 and 15/3/05 (Figure 3.2A, B). Notably, these decreases in SAM growth rate preceded the first onset of shoot termination, which began at some point in time between 15/3/05 and 11/4/05. On the 11/4/05, shoot termination had begun for all treatments and the M.9 rootstock had a greater proportion of primary shoots that had completely terminated growth compared with rootstocks of MM.106 and M.793 (Section 3.3.2.1). Collectively, slower growth rates of the SAM on the primary shoot between 11/2/05 to 15/3/05, followed by greater proportions of terminating primary shoots after the 15/3/05 onwards, significantly

decreased the final mean length and node number of the primary shoot on M.9 when compared with MM.106 and M.793 (Table 3.1). Because rootstock type did not affect mean internode length of the primary shoot, decreased mean length of the primary shoot on M.9 resulted from fewer neoformed nodes (Table 3.1).

Other studies have also reported similar effects of M.9 on the cumulative extension growth (Rao and Berry, 1940) and final mean length (Rao and Berry, 1940; Cannon, 1941) of the primary shoot within the first year of growth after propagation of the composite tree. However, some studies have reported that the mean length and node number of the primary shoot was similar for 'Royal Gala' scions at the end of their first year of growth after grafting onto M.9 or MM.106 (Seleznyova et al., 2005, 2008). As found in this chapter, M.9 was also reported to instil earlier SAM termination of the scion for composite trees in their second year of growth from propagation, and this contributed to decreased mean shoot length of the scion on M.9 compared with that on M.16 (Avery, 1969).

3.4.2.2 Secondary shoots

Throughout the season, neoformed nodes were produced along the primary shoot by the activity of the SAM. Once formed, an axillary bud either remained latent or grew out to form a secondary axis during the same growing season. After initiation, a secondary axis may grow out for a short time to form a secondary spur (< 25 mm) or shoot (≥ 25 mm).

Without exogenous BAP, rootstocks did not greatly affect the relationship between the nodal position of the secondary shoots and the angle of elevation at which they grew (Figure 3.9B). There was a trend, however, that the mean total number of secondary axes produced on the primary shoot increased with increasing vigour of the rootstock (Figure 3.7B). The scion on M.9 produced more spurs, significantly fewer secondary shoots (Figure 3.7B) and had a greater proportion of terminated secondary shoots in March (Figure 3.11). Collectively, these changes by M.9 reduced total growth of the secondary shoots compared with MM.106 and M.793 (Table 3.1). However, the earlier termination of secondary shoots caused by M.9 resulted in very small or non-significant

reductions in their mean length and node number (Table 3.9), probably because growth of the secondary shoots was very slow for rootstocks of MM.106 and M.793 in March, and/or, secondary shoots for these rootstocks terminated growth shortly after those on M.9.

Avery (1969) also reported that M.9 caused earlier termination of SAMs on the scion compared with a vigorous rootstock, hence reducing the length of annual shoots produced. Similarly, M.9 increased the proportion of short versus long shoots on the scion in its first (Costes et al., 2001) or second year of growth (Seleznyova et al., 2005, 2008). In a tree nursery, there were trends that M.9 reduced the mean number of secondary shoots formed on the 'Gala' scion during its first year of growth (Volz et al., 1994). However, some have found that M.9 did not affect the proportion of axillary buds on the primary shoot of the scion that formed an axillary structure in the first year of growth, primarily because very few secondary shoots formed regardless of rootstock type (Selezynova et al., 2007, 2008). In results similar with this Chapter, Avery (1969) reported that the scion was smaller on M.9 partly because of fewer growing points.

3.4.3 Effects of root volume on vegetative growth of the scion

3.4.3.1 Similarities and differences between M.9 and root restriction

Further objectives of this chapter were to understand similarities in vigour modifications induced by both the dwarfing rootstock and the small (8 L) root volume.

When grown in the 45 L root volume, rootstocks of MM.106 and M.793 produced significantly more total shoot growth per scion than M.9 (Table 3.8). However, restricting the root volume of MM.106 and M.793 to 8 L tended to result in very similar total shoot growth per scion when compared with M.9 grown in the 45 L root volume. Notably, the 8 L root volume reduced total shoot growth per scion less markedly for M.9 (Table 3.8). The greater reductions in total shoot growth when MM.106 and M.793 were grown in the 8 L root volume may reflect greater root confinement for these rootstocks, possibly because they had larger root systems. Conversely, a relatively small reduction in total shoot growth per scion when M.9 was grown in the 8 L root volume may indicate a smaller root system. Indeed, two-year-old composite trees of M.4

budded onto M.9 and M.12 rootstocks had small and large root systems, respectively (Vyvyan, 1955).

Smaller root systems of dwarfing apple rootstocks are thought to develop because the rootstock stem reduces polar IAA transport from scion to root, therefore, decreasing IAA available for growth of the root system (Lockard and Schneider, 1981). Experimentally, the bark of the dwarfing rootstock metabolised more IAA (Martin and Stahly, 1967; Gur and Samish, 1968) and the rootstock stem reduced polar transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj et al., 1997). However, it remains largely untested experimentally whether reduced basipetal transport of IAA by the stem of the dwarfing apple rootstock reduces the size of the root system. Results of Vyvyan (1955) would indicate this is highly probable because a vigorous M.12 root system was smaller or larger when budded with a scion of M.9 or M.12, respectively.

Although restricting the root systems of MM.106 and M.793 to 8 L imposed very similar total scion growth to M.9 grown in the 45 L root volume (Table 3.8), the way in which scion architecture was modified differed greatly. The mean length of the secondary shoots increased with increasing rootstock vigour when trees were grown in the 8 L root volume (Table 3.9). For M.793, root volume did not affect the mean length or node number of the secondary shoots, therefore decreased total growth of the secondary shoots by the 8 L root volume (Table 3.8) was due to fewer secondary shoots. Thus, confining the root volume of M.793 to 8 L resulted in similar architectural modifications to that of M.9 grown in the 45 L root volume, that is, fewer secondary shoots. This may indicate that the number of secondary shoots produced on the scion depended on the size of the root system and possibly the number of active root apices as potential sites for cytokinin biosynthesis. When grown in the 45 L root volume, M.9 may have produced fewer secondary shoots (Figure 3.7B) because of a smaller root system and, hence, fewer active root apices for cytokinin biosynthesis when compared with root systems of MM.106 and M.793.

For M.793, 8 L compared with 45 L root volumes did not affect the mean length and node number of the secondary shoots, and the mean length and node number of the secondary shoots was greater for these treatments than for M.9 grown in the 8 L root

volume (Table 3.9). Given that the root system of M.9 may have been less confined within the 8 L root volume, this result strongly indicates that other factors were modified by the 8 L root volume, and these contributed to the reduced mean length and node number of the secondary shoots produced by M.9 when grown in the 8 L root volume. Root temperature of the growing medium can modify extension growth of apple shoots (Carlson, 1965). Thus, in an above-ground growing system used in this experiment, it would be reasonable to expect a higher temperature of the growing medium within 8 L compared with 45 L growing bags. In addition, the growth of the M.9 root system was reduced markedly more at root zone temperatures above 25^{o}C than some rootstock genotypes of greater vigour (Gur et al., 1976). Therefore, M.9 grown in the 8 L root volume may have resulted in more marked reductions in the mean length and node number of secondary shoots than M.793 grown in the same root volume because M.9 roots were potentially more sensitive to elevated temperature of the growing medium.

3.4.4 Effects of exogenous cytokinin on vegetative growth of the scion

An objective of this chapter was to understand what role endogenous cytokinin may have in the development of scion dwarfing as induced by M.9 and root restriction. The cytokinin BAP was applied to scions repeatedly over the growing season to determine what major aspects of growth and architecture could be modified.

3.4.4.1 Interactions with rootstocks

Root-produced cytokinins may control the growth of SAMs because cytokinins extracted from the xylem sap of apple initiated and maintained cell division of tobacco pith (Jones, 1964) and promoted shoot growth in the absence of roots (Jones, 1973). In this chapter, however, BAP did not increase the mean length or node number of the primary shoot compared with untreated scions (Figure 3.1C, D), and caused greater proportions of primary shoots to terminate after the 15/3/05 (see section 3.3.2.1). Similar to these findings, the BAP-treated 'Red Boskoop' scion growing on M.9 had developed a primary shoot of similar length to untreated scions by the end of their first season of growth, however BAP significantly increased the mean number of secondary shoots that formed on the primary shoot (Wertheim and Estabrooks, 1994).

The predominant effect of BAP was to stimulate axillary bud break along the primary shoot, subsequently, promoting the outgrowth of more secondary axes and tertiary spurs (Figure 3.7B, D). Without BAP, there was a strong trend that M.9 reduced the proportion of axillary buds that broke on the primary shoot, and therefore the number of secondary axes that formed compared with MM.106 and M.793 (Figure 3.7B). Decreased axillary bud break on the primary shoot caused by M.9 was reversed using exogenous BAP, which resulted in the formation of a similar number of secondary axes on the primary shoot to MM.106 and M.793 (Figure 3.7D). This interaction indicates that a putative role of endogenous cytokinin in the development of rootstock-induced dwarfing of the scion may involve stimulating the initial outgrowth of axillary buds on the primary shoot. However, the development of fewer secondary axes for M.9 was probably not due to cytokinin action alone. For example, reduced amounts of cytokinin within the xylem sap may result because the dwarfing rootstock reduces polar auxin transport from scion to root, in turn, decreasing root growth and cytokinin biosynthesis (Lockard and Schneider, 1981).

Given that BAP stimulated the outgrowth of significantly more axillary axes (i.e., total of secondary axes, trace spurs and tertiary spurs) per scion on M.9 (Table 3.6), reduced concentrations of endogenous cytokinin within the xylem sap of the scion grafted onto M.9 (Kamboj et al., 1999) should have resulted in a scion phenotype with fewer growing points. Rather, Kamboj et al., (1999) postulated that increased concentrations of cytokinin in the xylem sap might explain the increased rate at which scions grafted onto vigorous rootstocks grew. Indeed, root-produced cytokinins may control the growth of SAMs because cytokinins applied to apple shoots promoted shoot extension growth in the absence of roots (Jones, 1973). In this Chapter, however, exogenous BAP decreased the mean length of secondary shoots, did not stimulate more neoformed nodes per secondary shoot (Section 3.3.7.2), increased bicyclic type growth (Figure 3.12) and promoted the earlier termination of secondary shoots in March (Figure 3.11). These results may indicate that other endogenous hormones were also required for sustained growth of the secondary shoots after axillary bud outgrowth on the primary shoot was initiated by BAP.

Similar to the results of this chapter, numerous studies have reported that BAP did not increase the mean length of secondary shoots compared with untreated control trees (Forshey, 1982; Elfving, 1984, 1985; Cody et al., 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988). However, split applications of BAP followed by GA_{4+7} tended to result in longer secondary shoots than BAP treatment alone (Popenoe and Barritt, 1988). Similarly, gibberellins applied to shoots (Sironval et al., 1962; Marcelle, 1963; Martin, 1967; Luckwill and Silva, 1979; Tromp, 1982; Steffens et al., 1985; Popenoe and Barritt, 1988) or injected into the xylem vasculature (Robitaille and Carlson, 1971, 1976) increased shoot extension growth of apple scions. In contrast, inhibitors of gibberellin biosynthesis applied to apple roots (Steffens et al., 1985) or shoots (Rademacher et al., 2006; Ramirez et al., 2006) decreased shoot extension growth (Steffens et al., 1985; Ramirez et al., 2006), and this effect for the root treatment was reversed with gibberellin applied to the shoot (Steffens et al., 1985). These effects of exogenous gibberellin on shoot growth might indicate that the growth of secondary shoots on the BAP-treated scion, particularly for M.9, was limited by endogenous gibberellin.

Endogenous gibberellins are transported within the xylem sap of apple trees (Jones and Lacey, 1968; Ibrahim and Dana, 1971; Motosugi et al., 1996) indicating that the root may produce and supply gibberellins to the scion (Jones and Lacey, 1968). Dwarfing compared with vigorous rootstocks decreased endogenous concentrations of gibberellins within the root (Yadava and Lockard, 1977), xylem sap (Ibrahim and Dana, 1971) and leaves or shoots (Yadava and Lockard, 1977). Given that exogenous gibberellins stimulated meristematic activity (Sironval et al., 1962; Marcelle, 1963; Martin, 1967; Robitaille and Carlson, 1971, 1976; Luckwill and Silva, 1979; Tromp, 1982; Steffens et al., 1985; Popenoe and Barritt, 1988) and dwarfing rootstocks decreased gibberellin supply to the shoot (Ibrahim and Dana, 1971; Yadava and Lockard, 1977), it would be reasonable to hypothesise that decreased meristematic activity of the scion grafted onto M.9 (Figures 3.1A, B; 3.2A, B; 3.11) resulted from a reduced root supply of gibberellin after February. This hypothesis, however, may also depend on interactions with IAA, particularly because the literature for apple indicates that polar IAA transport from scion to root is also important for SAM activity.

The importance of 'normal' polar auxin transport from shoot to root was demonstrated using TIBA to restrict the basipetal transport of IAA (Grochowska et al., 1994). The application of TIBA to the stems of 'Antonovka' apple seedlings reduced the basipetal transport of ^{14}C-IAA and caused the eventual termination of the SAM on the primary shoot (Grochowska et al., 1994). In a similar manner, the bark of the dwarfing rootstock metabolised more IAA (Martin and Stahly, 1967; Gur and Samish, 1968) and the rootstock stem reduced polar transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj et al., 1997), particularly as shoot growth slowed late in the season (Kamboj et al., 1997). Lee and Looney (1977) also reported that TIBA applied to apple shoots reduced extension growth, which was partly reversed with exogenous gibberellin. Therefore, IAA and gibberellin may have interacted to reduce shoot growth of the 'Royal Gala' scion grafted onto M.9 (Figures 3.1A, B; 3.2A, B; 3.11). The growth of the primary and secondary shoots may have slowed earlier for M.9 because the basipetal transport of IAA was decreased in the rootstock stem, thereby reducing the amount of IAA reaching the root system, thus decreasing the synthesis of root-produced gibberellin and its consequent transport to the scion where it was required for SAM activity. Conversely, a reduced supply of root-produced gibberellin transported to the SAMs may have decreased shoot growth, IAA synthesis and, therefore, IAA transported to the root system.

In addition to increasing secondary shoot formation, exogenous BAP increased the mean angle of elevation at which secondary shoots grew out from the primary shoot when compared with untreated scions (Figure 3.10). For M.793, BAP increased the mean angle at which secondary shoots grew out from the primary shoot when compared with the BAP-treated scion on M.9 or MM.106 (Figure 3.10). This occurred because the BAP-treated scion on M.793 developed more secondary shoots at higher nodal positions on the primary shoot than M.9 and MM.106, and higher nodal positions produced steeper shoot angles than lower nodal positions (Figure 3.9A).

The formation of more secondary shoots at higher nodal positions for M.793 may have resulted from differences in the timing of the first BAP application, which occurred in November for M.9 and MM.106 and in December for M.793. The stimulation of axillary bud outgrowth by BAP during these months resulted in the majority of

secondary shoots forming between nodes 1 to 25, particularly because at these times upper nodes (i.e., ≥ 26 nodes) had not yet formed and BAP typically did not stimulate the formation of secondary shoots after January. In contrast, axillary bud outgrowth on the primary shoot first occurred in late January for untreated scions, and resulted in the formation of two distinct zones of branching between nodes 1 to 25 and 26 to 50. Scions without BAP formed more secondary shoots between nodes 26 to 50 compared with BAP-treated scions. Tromp (1996) also reported that when axillary bud outgrowth occurred later in the season it resulted in branches being formed at higher nodal positions on the primary shoot.

3.4.4.2 Interactions with root volume

The root volume x BAP interaction for the mean total number of axillary axes formed per scion was not highly significant (Figure 3.8). Therefore, BAP did not fully reverse reductions in total axillary bud outgrowth on the scion caused by the 8 L root volume, which was in contrast to the effect of BAP on the mean total number of axillary axes formed when applied to the scion on M.9 (Table 3.6). In the above ground growing system used in this experiment, it would be reasonable to expect higher soil temperatures within 8 L compared with 45 L root volumes. High root temperatures were reported to affect natural cytokinin production by the root (Skogerbo and Mage, 1992), which may be an important requirement for axillary bud outgrowth on the primary shoot (Tromp, 1996). Such effects of root temperature on endogenous cytokinin production, however, should have been alleviated by repeated applications of BAP given to the scion over the growing season. Therefore, this result suggests that other factors were also modified by the 8 L root volume, and these were probably different from those changed by the dwarfing apple rootstock.

3.5 Summary

Dwarfing of the scion by M.9 occurred within the first year of growth from grafting. It was, therefore, expressed as a vegetative change. The primary shoot on M.9 had a slower rate of growth between 11/2/05 to 15/3/05, which preceded shoot termination that first began between 15/3/05 and 11/4/05. On the 11/4/05, M.9 had a greater proportion of terminated primary shoots compared with scions on MM.106 and M.793. Collectively, slower growth rates between 11/2/05 to 15/3/05, followed by a greater proportion of terminating meristems after the 15/3/05 onwards, resulted in a primary shoot on M.9 that was shorter at growth cessation because of fewer neoformed nodes. The M.9 rootstock also reduced the proportion of axillary buds on the primary shoot that broke to form secondary axes. Of the secondary axes formed, a lower proportion then extended to form secondary shoots that were ≥ 25 mm. In March, M.9 had caused a greater proportion of secondary shoots to terminate. Collectively, these effects of M.9 significantly reduced total shoot growth of the scion compared with MM.106 and M.793.

Restricting the root systems of MM.106 and M.793 to 8 L resulted in similar total shoot growth of the scion compared with that of M.9 grown in the 45 L root volume. Contrastingly, the 8 L root volume caused much smaller reductions in total shoot growth of the scion for M.9 than MM.106 and M.793. These results may indicate that the root system of M.9 was less confined within the 8 L root volume. Therefore, reduced total shoot growth of the scion caused by M.9 when grown in the 45 L root volume may have resulted from a smaller root system compared with rootstocks of MM.106 and M.793 grown in the same root volume. Interestingly, rootstocks differed in the way growth was reduced by the 8 L root volume. The 8 L root volume reduced the mean number of secondary shoots per scion more for MM.106 and M.793 than for M.9. Conversely, the 8 L root volume reduced the mean length of secondary shoots more as the vigour of the rootstock decreased. These results indicate that the size of the root system may affect the ability of the scion to form secondary axes. However, factor(s) other than the size of the root system were also modified by the 8 L root volume, particularly because the mean length of secondary shoots was reduced by M.9

> MM.106 > M.793. Furthermore, exogenous BAP did not fully reverse decreased axillary bud outgrowth on the scion caused by the 8 L root volume.

The primary effect of BAP applied to the scion was to increase axillary bud break on the primary shoot, therefore resulting in the formation of considerably more secondary axes per scion. The M.9 rootstock decreased the formation of secondary axes on the primary shoot, particularly the number of secondary shoots, and their formation was reinstated with exogenous BAP. This results may suggest that the role of endogenous cytokinins in the development of rootstock-induced dwarfing of the scion involves the regulation of axillary bud break and the formation of secondary axes. Other endogenous hormones may also control the growth of apple SAMs because endogenous cytokinin did not promote more nodes along an average primary or secondary shoot, but rather caused earlier termination of the SAM for each of these shoot types. Consideration of the literature for apple indicated that decreased formation of secondary axes of the scion growing on M.9 may involve interactions between cytokinin and IAA, whereas earlier slowing or termination of shoot extension growth may involve interactions between IAA and gibberellin.

4. Effect of rootstock and root restriction on scion architecture and the first occurrence of flowering for field-grown ‘Royal Gala’ apple trees

4.1 Introduction

In a recent field study, the primary shoot of ‘Royal Gala’ scions had developed a similar final mean length and node number by the end of the first season of growth from grafting regardless of whether the scion was grown on a dwarfing (M.9) or a semi-vigorous rootstock (MM.106) (Seleznyova et al., 2007, 2008). The first observable difference imposed on the scion by M.9 compared with MM.106 was increased flowering along the primary shoot in the spring of the second year after grafting (Seleznyova et al., 2005, 2007, 2008). In contrast, earlier field research showed that M.9 decreased the final mean length of the primary shoot by the end of the first year of growth from grafting when compared with rootstock(s) of greater vigour (Rao and Berry, 1940; Cannon, 1941). Therefore, the latter field studies indicate that the dwarfing apple rootstock may also reduce scion vigour during the first season of growth after grafting.

Given that M.9 decreased (Rao and Berry, 1940; Cannon, 1941) or did not affect growth of the primary shoot (Seleznyova et al., 2005, 2007, 2008) during the first year from grafting, the first objective of this chapter was to elucidate how scion architecture of field-grown ‘Royal Gala’ apple trees was first modified by rootstocks of M.9 and MM.106. Further objectives were to compare similarities or differences in scion growth and architecture between the dwarfing apple rootstock and root restriction, and whether modifications to growth imposed by these treatments were similar to those previously reported in Chapter 3.

4.2 Materials and methods

4.2.1 Experimental site

The experiment was conducted during the 2004–2005 growing season at the Fruit Crops Unit, Massey University, Palmerston North. The soil was Manawatu fine sandy loam and had no prior history of pipfruit plantings. Manawatu fine sandy loam has an upper profile of 500 mm of fine sandy loam, underlain by 400 mm of fine sand, with gravelly coarse sand below 900 mm. In November 2004, the soil was ploughed and then power harrowed twice to provide a fine tilth. Tree positions were marked and holes for planting were drilled with a tractor-mounted post hole borer.

4.2.2 Tree establishment and planting

Scions of 'Royal Gala' were cleft grafted at a height of 350 mm onto rooted stools of M.9 and MM.106. Grafted trees were established as previously described (Chapter 2, Section 2.2). On the 6/12/04, composite trees of 'Royal Gala' on M.9 and MM.106 were planted into the field at a spacing of 3 m between rows and 0.9 m within rows. Planting height was standardised for each treatment to leave 150 mm of rootstock stem above the soil.

4.2.2.1 Root control bags

For root restriction treatments, trees were planted into 10 L root control bags made of semi-permeable geotextile polyfelt that was seamed with non-perishable nylon thread (Agrisorb Ltd, Auckland, New Zealand). The semi-permeable characteristic of polyfelt enables fine roots to permeate through the bag fabric. Restriction of the root system then occurs by physical girdling of the roots as they undergo secondary thickening. Each root bag was filled with field soil before placing the bag and tree into its final position in the ground. Immediately after planting, trees were thoroughly watered by hand to minimise transplanting stress.

4.2.3 Irrigation and cultural management

Under-row trickle irrigation was installed on the day of planting. The irrigation system consisted of 19 mm polytube laid under the tree rows (Figure 4.1). A single compensating drip emitter (4 L hr^{-1}) was placed 100 mm from the trunk directly in the root zone of each tree with or without root restriction. In addition to seasonal rainfall, supplementary irrigation was scheduled when required either by manual or automated control (Hunter, Smart Valve Controller, USA) to maintain soil volumetric water content (θ) close to field capacity (0.30 m^3 m^{-3}). To check the effectiveness of irrigation, measurements of soil θ were taken in the drip zone of each emitter to a depth of 300 mm using TDR at regular intervals over the growing season. Rows between trees were grassed and a 1 m bare herbicide strip was maintained beneath the trees for the duration of the experiment to minimise weed competition (Figure 4.1).

4.2.4 Measurements of scion growth

Scion growth was measured throughout the growing season as previously described (Chapter 2, Section 2.7). The length and node number of the primary shoot were measured at monthly intervals. On the 5/3/05, the lengths of the secondary shoots and the number that had terminated per scion were measured. In mid May 2005 (before leaf fall), scions were stripped of leaves and leaf area per scion was determined using a leaf area meter (Li-3100, Li-Cor Inc., USA). Data for leaf area represents a sub-sample of half the replicate trees of each treatment and block. Following deleafing, scion architecture was measured as described in Chapter 2 (Section 2.7.1.6). During spring in the second year of growth, bud break of the scions began on the 15/9/05. On the 29/9/05, the number of buds breaking per scion was counted. Axillary buds and terminal buds that were floral were counted and their nodal positions on the primary and secondary shoots were recorded at full bloom, which occurred on the 24/10/05.

4.2.5 Statistical analysis

The experiment was a randomised block design with a factorial arrangement of treatments (2x2), which included two rootstocks (M.9 and MM.106) and two root

restriction treatments (± root restriction). There were four blocks that each contained 12 trees per treatment. Data were analysed using the GLM procedure of SAS. Interpretation of main effects and interactions were similar to that previously described in Chapter 3 (Section 3.2.5).

Figure 4.1. Example of under-row trickle irrigation and the growing system used for experimental apple trees of 'Royal Gala' on M.9 or MM.106 rootstocks grown with or without root restriction. A bare herbicide strip was maintained beneath the trees to prevent competition from weeds and the sward. Photo was taken during autumn (late April, 2005) when trees were nearing the end of their first growing season from grafting.

4.3 Results

4.3.1 Irrigation scheduling

Irrigation maintained soil θ close to field capacity over the growing season and, on the dates measured, the maximum deviation below field capacity was 0.030 m^3 m^{-3} (Appendix 2).

4.3.2 Seasonal growth of the primary shoot

Seasonal differences in the mean cumulative length and node number of the primary shoot were significant only for the main effects of rootstock and root restriction. During December, the 'Royal Gala' primary shoot on M.9 was significantly longer and had produced more nodes than that on MM.106 (Figure 4.2A, B). This resulted from greater rates of growth on M.9 between November and December (Figure 4.3A, B); a period when 100% of the primary shoots were actively growing for all treatments (as denoted on Figure 4.3A). However, the primary shoot on MM.106 was significantly longer and had more nodes than M.9 from March onwards (Figure 4.2A, B). In responses similar to M.9, root restriction significantly reduced the mean cumulative length and node number of the primary shoot from March onwards (Figure 4.2C, D).

4.3.2.1 Treatment effects on termination of the shoot apical meristem on the primary shoot

Primary shoots were formed from either a season-long flush of growth by the shoot apical meristem (SAM) or by two growth flushes caused when growth temporarily ceased before resuming at some point in the season. Primary shoots formed from two growth flushes were identifiable by the presence of bud scale scars, and/or, compressed internodes at some position along the primary shoot. The two distinct flushes of growth are referred to as growth units.

The proportion (%) of primary shoots that formed two growth units was not significantly affected by root restriction (main effect P=0.17), however there was a

trend that root restriction increased the proportion of shoots with two growth units compared with unrestricted trees (32 and 22%, respectively). In contrast, rootstocks significantly affected (main effect P=0.0003) the proportion of primary shoots with two growth units (47 and 7% for M.9 and MM.106, respectively, MSD=16%). The rootstock x root restriction interaction was not significant (P=0.56) (data not shown).

The nodal positions at which the first growth unit ended varied between 22 to 31 or 21 to 31 nodes for the primary shoot on M.9 or MM.106, respectively (data not shown). The mean node number of growth unit one was 26 and 25 for M.9 and MM.106, respectively (data not shown). Thus, the mean nodal position along the primary shoot at which the first growth unit ended was very similar between rootstocks. At planting on the 6/12/04, mean node number of the primary shoot was 20 and 19 for M.9 and MM.106, respectively. Consideration of the number of nodes formed at planting and the mean number of nodes developed for growth unit one would indicate that temporary cessation of SAM growth mostly occurred for both rootstocks between the measurements conducted on the 6/12/04 and the 21/1/05 (see Figure 4.2B).

Between the 6/12/04 and the 21/1/05, increased proportions of terminated primary shoots on M.9 only partly explained its reduced growth rates (i.e., Figure 4.3A, B). For example, the daily mean growth rate of primary shoots with one growth unit (i.e., those presumably still growing) was 0.48 and 0.53 mm day^{-1} for M.9 and MM.106, respectively (main effect P=0.02, MSD=0.04 mm day^{-1}). Similarly, the mean rate of node production for primary shoots with one growth unit was 0.23 and 0.26 nodes day^{-1} for M.9 and MM.106, respectively (main effect P=0.03, MSD=0.02 nodes day^{-1}).

On the 22/2/05, 100% of primary shoots were actively growing for each treatment (data not shown). However on the 22/3/05, 8% and 3% of primary shoots on M.9 had terminated growth with or without root restriction, respectively, whereas 7% and 3% of primary shoots on MM.106 had terminated growth with or without root restriction, respectively. On the 22/4/05, rootstocks significantly affected (main effect P=0.008) the proportion (%) of primary shoots that had terminated (70 and 31% for M.9 and MM.106, respectively, MSD=18%). In contrast, root restriction did not significantly

affect (main effect *P*=0.70) termination (data not shown). However, the rootstock x root restriction interaction approached significance (*P*=0.12) (Figure 4.4), and there were trends that root restriction increased the proportion of primary shoots that had terminated only for the scion on the MM.106 rootstock (Figure 4.4).

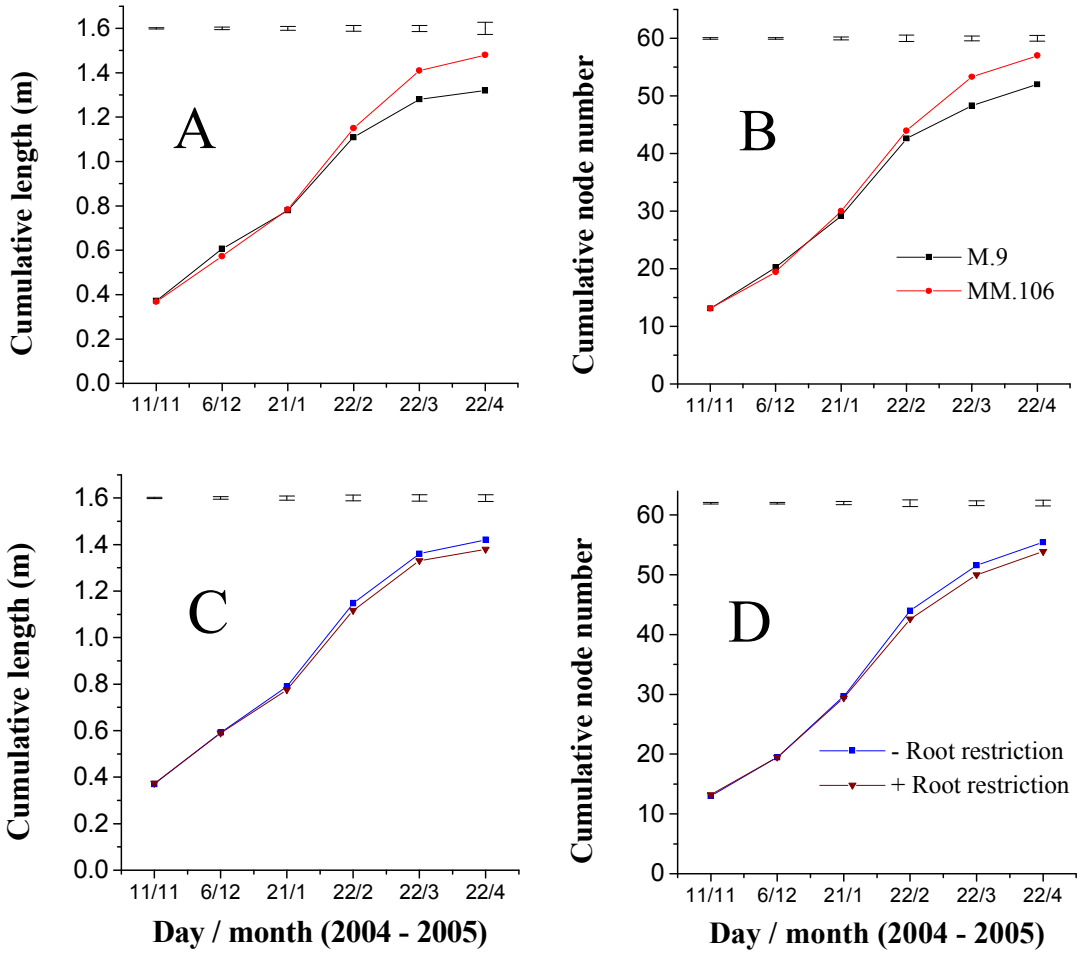


Figure 4.2. Main effects of rootstocks (A and B) and ± root restriction (C and D) on the mean cumulative length (left) and node number (right) of 'Royal Gala' primary shoots during their first year of growth after grafting. Vertical bars are the minimum significant difference (MSD) at *P*=0.05 (Tukey's test). Data for rootstock or root restriction main effects are averaged over root restriction or rootstock treatments, respectively.

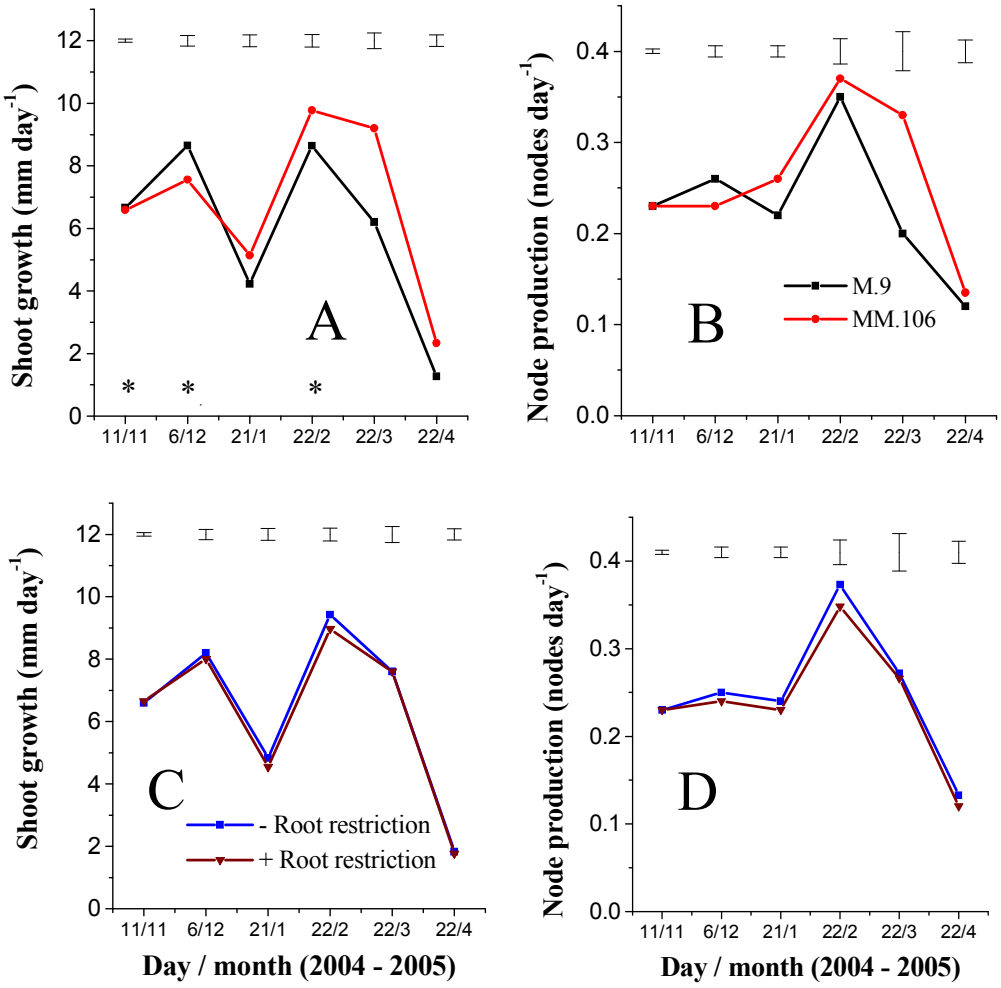


Figure 4.3. Seasonal patterns of rootstocks (A and B) and ± root restriction (C and D) effects on the daily mean rate of growth (left) and node production (right) by the shoot apical meristem on the primary shoot of 'Royal Gala' apple scions during their first year of growth after grafting. Vertical bars are the MSD at *P*=0.05 (Tukey's test). Data for rootstock or root restriction main effects are averaged over root restriction or rootstock treatments, respectively. On graph A, * denotes measurement dates where 100% of shoot apical meristems were actively growing for all treatments within the experiment.

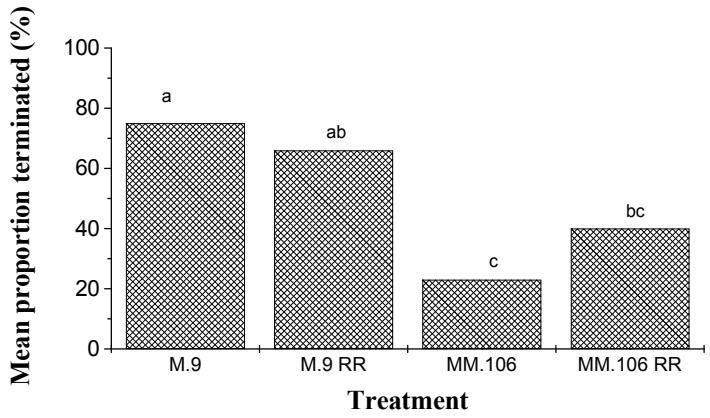


Figure 4.4. Rootstock x root restriction interaction (*P*=0.12) for the mean proportion (%) of shoot apical meristems (SAMs) that had terminated on 'Royal Gala' primary shoots in April (22/4/05). Means sharing the same letter are not significantly different at *P*≤0.12 (lsmeans tests with Tukey's adjustment, SAS).

4.3.3 Final leaf area of the primary shoot

Despite the 'Royal Gala' primary shoot on M.9 having significantly fewer nodes than MM.106, rootstocks did not significantly differ (*P*=0.92) in the final leaf area of the primary shoot (Table 4.1). This resulted from the primary shoot on M.9 having a significantly greater mean area per leaf than the primary shoot on MM.106 (Table 4.1). In contrast to M.9, root restriction significantly reduced the final leaf area of the primary shoot compared with unrestricted trees, which resulted from a decreased mean area per leaf and from fewer neoformed nodes (Table 4.1) and leaves. The rootstock x root volume interactions were not significant for the final mean leaf area of the primary shoot (*P*=0.62) and for the mean area per leaf (*P*=0.84).

4.3.4 Final growth measurements of the primary shoot

4.3.4.1 Length and node number

The final measurements of scion growth were conducted when scions were fully dormant in late June, 2005. Reduced cumulative growth of the primary shoot on M.9 after January (Figure 4.2A, B) resulted in a primary shoot with a significantly decreased

final mean length and node number when compared with the scion on MM.106 (Table 4.1 and see Figure 4.5). Similarly, root restriction reduced the cumulative growth of the primary shoot after January (Figure 4.2C, D), resulting in a primary shoot with a significantly decreased final mean length and node number compared with the primary shoot of trees grown without root restriction (Table 4.1). Rootstock type or root restriction treatment did not significantly affect the mean internode length of the primary shoot (P=0.25 and P=0.90, respectively) (Table 4.1). The rootstock x root restriction interactions were not significant for the final mean length (P=0.74), node number (P=0.64) and internode length (P=0.99) of the primary shoot (data not shown).

4.3.4.2 Final shoot cross-sectional area (SCA) of the primary shoot

The final SCA of the primary shoot was significantly reduced by M.9 compared with MM.106 (Table 4.1). There was also a trend that root restriction reduced mean SCA of the primary shoot compared with unrestricted root systems (Table 4.1).

Figure 4.5. Effect of field-grown M.9 (right) and MM.106 (left) rootstocks on the growth and architecture of 'Royal Gala' apple scions nearing the end of their first season of growth (May, 2005) from grafting.

Table 4.1. Main effects of rootstock and root restriction on growth attributes of the primary shoot, secondary shoots and total growth of 'Royal Gala' apple scions at the end of their first season of growth (June, 2005) after grafting.

Main effect	A Primary shoot	B Secondary shoots	A + B Total per scion
Rootstock			
		Length (m)	
M.9	1.32 a ***	1.53 a ***	2.85 a ***
MM.106	1.49 b	2.32 b	3.81 b
		Node number	
M.9	54 a ***	70 a ***	124 a ***
MM.106	60 b	93 b	153 b
		Leaf area (m^2)	
M.9	0.30 ^{ns}	0.20 a ***	0.50 a ***
MM.106	0.30	0.27 b	0.57 b
		Leaf area (m^2) / leaf	
M.9	0.0055 a **	0.0029 a *	-
MM.106	0.0049 b	0.0027 b	-
		Internode length (mm)	
M.9	24.46 ^{ns}	19.55 a ***	-
MM.106	24.72	22.32 b	-
		Shoot cross-sectional area (SCA) (mm^2)	
M.9	176 a ***	-	-
MM.106	213 b	-	-
Root restriction (RR)			
		Length (m)	
+RR	1.39 a *	1.83 a ^{x}	3.22 a ^{y}
- RR	1.43 b	2.02 a	3.45 a
		Node number	
+RR	56 a **	80 ^{ns}	136 ^{ns}
- RR	58 b	84	142
		Leaf area (m^2)	
+RR	0.28 a **	0.23 ^{ns}	0.51 a ^{z}
- RR	0.31 b	0.25	0.56 a
		Leaf area (m^2) / leaf	
+RR	0.0050 a *	0.0027 a *	-
- RR	0.0054 b	0.0029 b	-
		Internode length (mm)	
+RR	24.61 ^{ns}	20.66 ^{ns}	-
- RR	24.58	21.17	-
		Shoot cross-sectional area (SCA) (mm^2)	
+RR	189 a ^{w}	-	-
- RR	200 a	-	-

ns, w, x, y, z, *, **, *** non significant or significant at P=0.07, P=0.14, P=0.09, P=0.06, $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single main effect and growth attribute only, means within a column sharing the same letter are not significantly different using the Tukey's test at P=0.05.

4.3.5 Seasonal growth of the secondary shoots

4.3.5.1 Formation of secondary shoots

A proportion of axillary buds on the primary shoot began to break from the 28/1/05 onwards, which coincided with a second flush of rapid growth by the primary shoot after the temporary termination of the SAM, and/or, slowing of growth that occurred predominantly between the 6/12/04 and 21/1/05 (Section 4.3.2.1).

4.3.5.2 Treatment effects on the number of secondary axes formed on the primary shoot

Compared with the MM.106 rootstock, M.9 significantly increased the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes (i.e., secondary spurs plus shoots) and the mean number of secondary axes formed per scion (Table 4.2). However, rootstocks did not significantly differ in the mean number of secondary shoots formed (Table 4.2). In responses similar to M.9, root restriction significantly increased the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes and the mean number of secondary axes formed on the primary shoot when compared to trees grown without root restriction (Table 4.2).

Increased mean number of secondary axes for the scion on M.9 or root-restricted trees (Table 4.2) occurred irrespective of the growth unit number on the primary shoot. For example, the mean number of secondary axes produced on scions with one growth unit was 13, 11, 13 and 11 for M.9, MM.106, plus root restriction and minus root restriction, respectively. For scions with two growths units, the mean number of secondary axes was 11, 9, 11 and 9 for M.9, MM.106, plus root restriction and minus root restriction, respectively. When compared with the same treatment, scions with two growth units tended to have fewer secondary axes than scions with one growth unit.

Table 4.2. Main effects of rootstock and root restriction on the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes and the mean number of secondary axes that had formed on 'Royal Gala' apple scions by the end of their first year of growth after grafting.

	Secondary axis type		
	A	B	A + B
Main effect	**Spur**	**Shoot**	**Total secondary axes**
	Mean proportion (%) of axillary bud break		
Rootstock			
M.9	7.55 (2.68 a) ***	14.73 (3.78 a) *	22.28 (4.69 a) ***
MM.106	4.62 (2.13 b)	12.54 (3.48 b)	17.17 (4.11 b)
Root restriction			
+	6.61 (2.50 a) [w]	14.44 (3.74 a) [x]	21.06 (4.55 a)*
-	5.56 (2.31 a)	12.82 (3.53 a)	18.39 (4.26 b)
	Mean number of secondary axes		
Rootstock			
M.9	4.06 a ***	7.88 [ns]	11.95 a **
MM.106	2.78 b	7.56	10.34 b
Root restriction			
+	3.64 a [y]	8.14 a [z]	11.77 a *
-	3.21 a	7.31 a	10.52 b

Proportion (%) of axillary bud break was calculated as the total number of an axis type or types on the primary shoot divided by the total number of nodes on that primary shoot x 100. ns, w, x, y, z, *,**, *** non significant or significant at P=0.12, P=0.14, P=0.18, P=0.11, $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single growth attribute and main effect only, means within a column sharing the same letter are not significantly different using the Tukey's test at P=0.05. Means without and within parenthesis are raw or transformed means, respectively.

4.3.5.3 Position of secondary shoots along the primary shoot

Secondary shoots developed along the primary shoot as a zone of branching between nodes 4 to 48 (Figure 4.6A, B and see Figure 4.5). With or without root restriction, M.9 had a tri-modal distribution of secondary shoots along the primary shoot (Figure 4.6A). Compared with MM.106, M.9 increased the proportion of nodes occupied with secondary shoots for nodal positions between 4 to 12 and 13 to 26. In contrast to M.9, MM.106 had a skewed distribution with a greater proportion of nodes forming secondary shoots between nodal positions 30 to 44 (Figure 4.6B). Thus, the intensity of

branching was greater at higher nodal positions for MM.106 than for M.9. Root restriction of M.9 resulted in very similar distributions of secondary shoots along the primary shoot compared with unrestricted trees (Figure 4.6A). For MM.106, root restriction treatments also resulted in similar distributions to each other, but slightly greater proportions of nodes formed secondary shoots between nodal positions 26 to 38 when root systems of MM.106 were restricted (Figure 4.6B).

4.3.5.4 Angle of elevation of secondary shoots

Angles of elevation at which secondary shoots grew out from the primary shoot were measured as acute angles with 0 and 90^{o} elevation representing a shoot that was horizontal with the ground or vertical like the primary shoot, respectively. Data could not be appropriately transformed for ANOVA. For the rootstock main effect, the mean angle of elevation was 30 and 27^{o} for M.9 and MM.106, respectively. For the root restriction main effect, the mean angle of elevation was 28 and 29^{o} for trees grown with or without root restriction, respectively. Angles of elevation for individual secondary shoots correlated poorly to nodal positions along the primary shoot, hence a slightly greater mean angle of elevation for the M.9 rootstock could not be attributed to differences in shoot position on the primary shoot (data not shown).

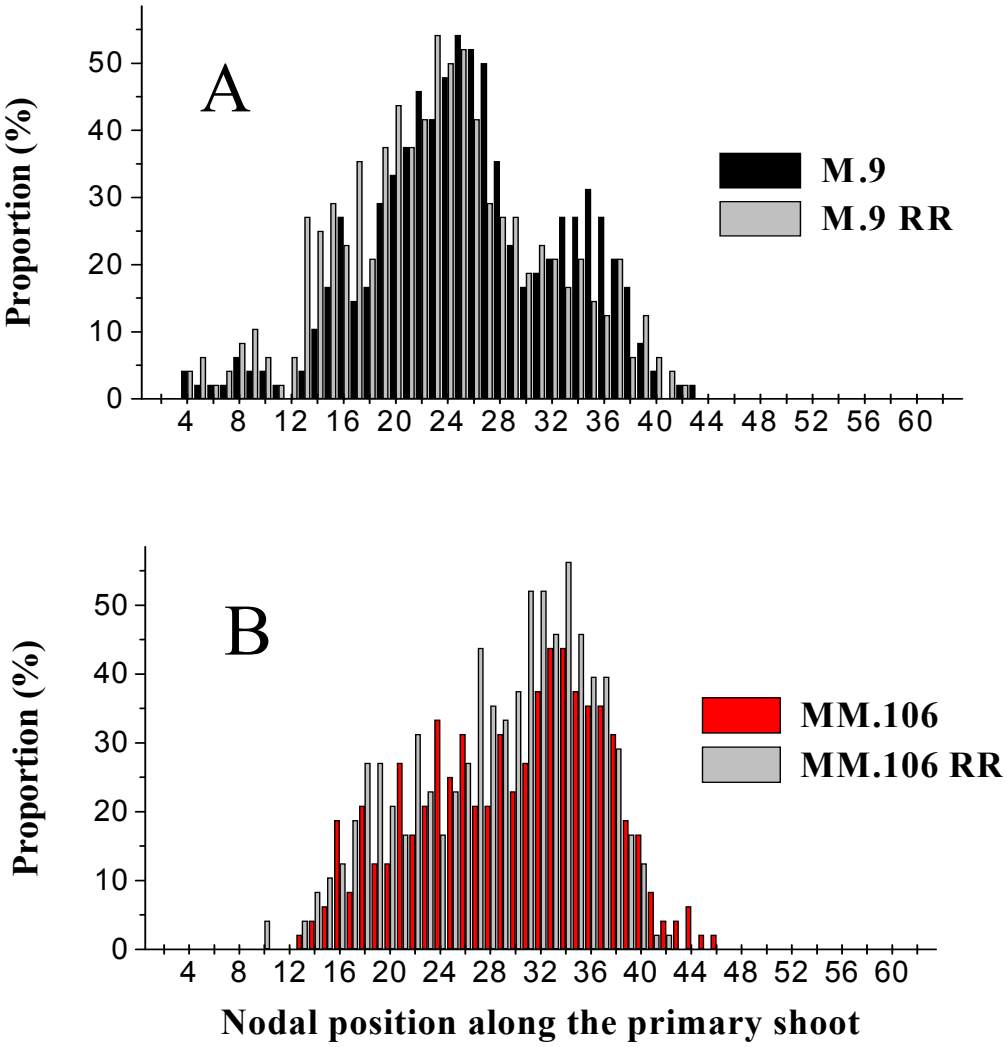


Figure 4.6. Effect of rootstocks grown with or without root restriction (RR) on the distribution of secondary shoots formed along the primary shoot of 'Royal Gala' apple scions. Proportion (%) was calculated as the total number of secondary shoots formed at a given nodal position on the primary shoot divided by the total number of nodes at each position (between nodes 1 and 46 there were 48 nodes per position arising from 48 scions per treatment).

4.3.5.5 Treatment effects on the mean length and termination of secondary shoots in March, 2005

On the 5/3/05, the lengths of the secondary shoots formed on each scion were measured and the total number of secondary shoots that were either growing or had terminated were counted. The mean length of the secondary shoots was 205 and 241 mm (main effect $P<0.0001$) for M.9 and MM.106, respectively. For plus or minus root restriction, the mean length of the secondary shoots was 210 and 241 mm (main effect $P<0.0001$), respectively. Rootstocks also significantly differed (main effect $P<0.0001$) in the proportion of secondary shoots that had terminated. The proportion (%) of terminated secondary shoots for M.9 and MM.106 was 78 and 25%, respectively. In contrast, root restriction did not significantly affect the proportion of secondary shoots that had terminated (main effect $P=0.14$), although root restriction caused 6% more terminated secondary shoots than unrestricted root systems (data not shown).

In addition to main effects, the rootstock x root restriction interaction was significant ($P=0.02$) for the mean length of the secondary shoots (Figure 4.7A) but not for the mean proportion (%) of secondary shoots that had terminated ($P=0.24$) (Figure 4.7B). Root restriction of MM.106 significantly reduced the mean length of the secondary shoots (Figure 4.7A) and there was a trend that it increased the proportion of secondary shoots that had terminated growth compared with unrestricted MM.106 (Figure 4.7B). In contrast, root restriction of M.9 did not affect the mean length of the secondary shoots or the proportion (%) that had terminated (Figure 4.7A, B). Root restriction of MM.106 also caused a similar mean length of the secondary shoots to that produced by M.9 grown with or without root restriction (Figure 4.7A). However, root restricted MM.106 still had proportionally fewer secondary shoots that were terminated in March compared with M.9 (Figure 4.7B). Consequently, secondary shoots for root restricted MM.106 had a significantly greater final mean length and node number by June than M.9 grown with or without root restriction (Figure 4.8A).

4.3.5.6 Final total leaf area of the secondary shoots

The final mean total leaf area of the secondary shoots was significantly reduced by M.9 compared with MM.106 (Table 4.1). Because rootstocks did not differ in the leaf area of the primary shoot, decreased mean total leaf area of the secondary shoots contributed solely to the reduced mean total leaf area per scion on M.9 when compared with MM.106 (Table 4.1). Total leaf area of the secondary shoots was reduced by M.9 because of significantly fewer nodes rather than a decreased mean area per leaf (Table 4.1).

Root restriction did not significantly (main effect P=0.42) reduce the mean total leaf area of the secondary shoots, although there was a trend that their total leaf area was reduced slightly compared with unrestricted root systems (Table 4.1). Collectively, reductions in leaf area of both the primary and secondary shoots resulted in a significantly decreased (at P=0.06) mean total leaf area per scion for root restricted trees (Table 4.1). In contrast to M.9, root restriction decreased the mean total leaf area per scion by reducing the mean area per leaf on the primary and secondary shoots. Although not significant, root restriction reduced the mean total number of nodes produced per scion compared with unrestricted root systems, which also contributed to its reduced mean total leaf area per scion (Table 4.1).

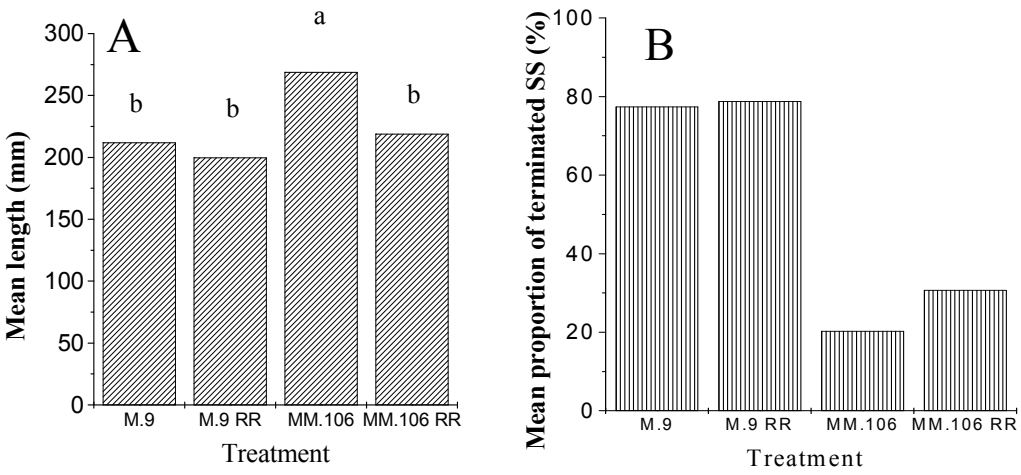


Figure 4.7. Rootstock x root restriction (RR) interactions for the mean length (A, *P*=0.02) and mean proportion (%) of secondary shoots (SS) that had terminated (B, *P*=0.24) on 'Royal Gala' apple scions in early March, 2005. For graph A, means sharing the same letter are not significantly different at *P*≤0.05 (lsmeans tests with Tukey's adjustment, SAS).

4.3.6 Final measurements of extension growth for the secondary shoots

The percentage of secondary shoots that had two growth units was 1 and 2.6% of the total shoot populations of M.9 and MM.106, respectively. For plus or minus root restriction, the percentage was 1.8 and 1.8%, respectively. Therefore, the formation of secondary shoots for all treatments was predominantly from a single flush of growth (i.e., one growth unit).

In addition to the reduced mean length and node number of the primary shoot on M.9, the mean total length and node number of the secondary shoots was significantly reduced by M.9 compared with MM.106 (Table 4.1). Collectively, these reductions in the growth of both the primary and secondary shoots significantly decreased the mean total shoot length and node number of the scion on M.9 compared with MM.106 (Table 4.1 and see Figure 4.5). There were also trends that root restriction reduced the mean total length and node number of the secondary shoots, although differences were small and not highly significant (Table 4.1). Small reductions in the mean total length and node number of the secondary shoots by root restriction meant that the final differences

in the mean total shoot length and node number per scion only approached significance (P=0.09) or were not significant (P=0.23), respectively, between root restriction treatments (Table 4.1).

Reduced mean total length and node number of the secondary shoots for the scion on M.9 and the root restriction treatment (Table 4.1) were not a result of fewer secondary shoots per scion (Table 4.2). Instead, these reductions in growth were caused by decreased mean length and node number of the secondary shoots. Significant rootstock x root volume interactions for the final mean length (P=0.02) and node number (P=0.05) of the secondary shoots resulted from root restriction reducing the mean length and node number of the secondary shoots for MM.106 only (Figure 4.8A, B). Regardless of root restriction treatment, M.9 significantly decreased the mean length and node number of secondary shoots compared with MM.106 (Figure 4.8A, B).

In contrast to the primary shoot, M.9 significantly reduced the mean internode length of the secondary shoots compared with MM.106 (Table 4.1). The rootstock x root volume interaction for the mean internode length of the secondary shoots (Figure 4.8C) was also significant (P=0.02) as root restriction decreased the mean internode length of secondary shoots for MM.106 only. Interpretation of mean internode length of the secondary shoots (Figure 4.8C) requires consideration of correlations between shoot length and node number (Figure 4.9A, C) and the node number distribution of each treatment (Figure 4.9B, D). All treatments showed an almost identical relationship between the node number and the length of their secondary shoots (Figure 4.9A, C). Thus, regardless of treatment secondary shoots with the same node number had a very similar shoot length and, therefore, internode length (comparison of internode lengths can be made from the regression lines of Figure 4.9A and C by dividing a y-axis value with its corresponding x-axis value). However, internode length of the secondary shoots increased with shoot length for each treatment. Therefore, decreased mean internode length of the secondary shoots for M.9 (i.e., Figure 4.8C) resulted from its node number distribution whereby proportionally more short shoots with 10 or less nodes formed compared with MM.106 (Figure 4.9B, D). Similar to M.9, root restriction of MM.106 reduced the mean internode length of the secondary shoots compared with unrestricted

MM.106 (Figure 4.8C) by increasing the proportion of short secondary shoots with 10 or less nodes, and by reducing the proportion of very long secondary shoots with more than 20 nodes (Figure 4.9D).

Earlier shoot termination of secondary shoots for M.9 in March (Figure 4.7B) resulted in very little additional shoot extension growth between March and June (compare Figures 4.7A and 4.8A). Therefore, the final mean length and node number of the secondary shoots was reduced by M.9 when compared with MM.106 (Figures 4.8A, B). In contrast, very few secondary shoots had terminated for MM.106 in March (Figure 4.7B) and mean shoot length increased between March and June (compare Figures 4.7A and 4.8A), presumably because a greater proportion of SAMs were actively growing late into the season.

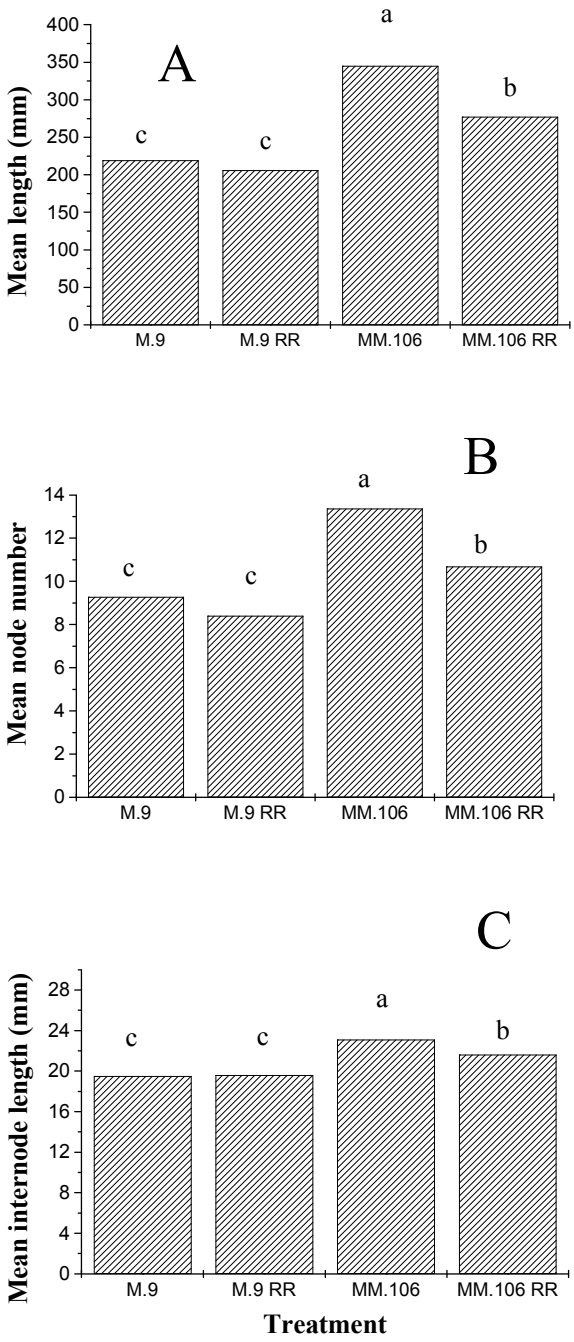


Figure 4.8. Rootstock x root restriction (RR) interactions for the final mean length (A), node number (B) and internode length (C) of secondary shoots on 'Royal Gala' apple scions at the end of their first growing season (June, 2005) from grafting. For a single interaction only, means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS).

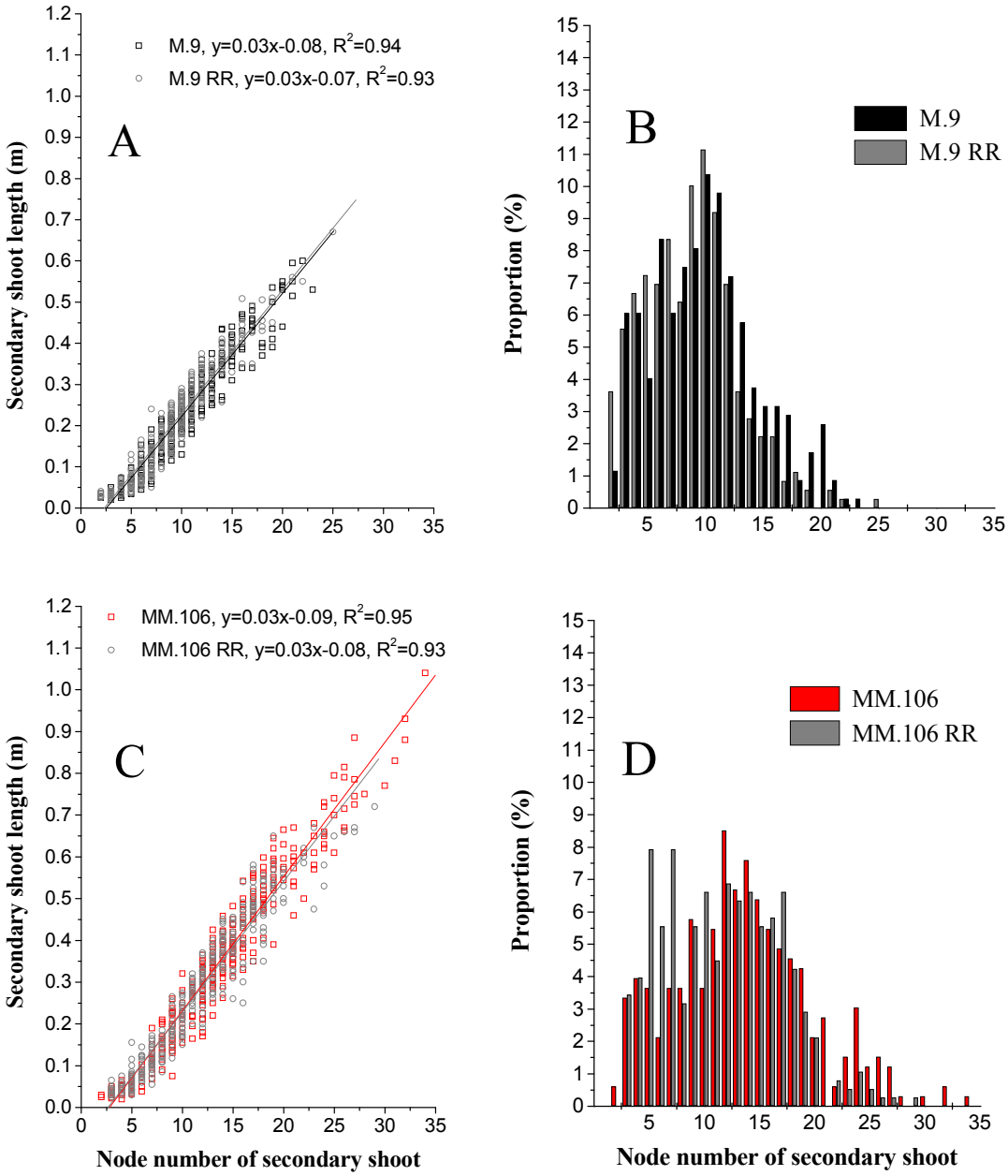


Figure 4.9. Effect of rootstock and root restriction (RR) on the relationship between secondary shoot length and node number (A and C) for 'Royal Gala' apple scions at the end of their first season of growth from grafting. B and D represent the node number distribution of the secondary shoots. Data represents the total population of secondary shoots for each treatment.

4.3.7 Treatment effects on the first occurrence of flowering in the spring of year two (2005)

4.3.7.1 Treatment differences in bud release from dormancy

In the second spring from grafting, buds on the scion first began to break on the 15/9/05. On the 29/09/05, the total number of buds that had broken per scion was recorded and expressed as a proportion to the total bud number per scion (bud break (%); Figure 4.10). The total bud number per scion is defined as the total number of nodes and terminal bud(s) on the primary and secondary shoots. The rootstock x root restriction interaction was significant (P=0.01) with M.9 increasing scion bud break (%) compared with MM.106, whereas root restriction significantly increased bud break (%) for MM.106 only (Figure 4.10). On the 5/10/05, buds that had previously broken (Figure 4.10) were identified as emerging flower clusters (Figure 4.11). For all treatments, vegetative buds first emerged much later than flower buds, namely from the 10/10/05 onwards. Because flower buds emerged before vegetative buds, data for bud break (%) recorded on the 29/09/05 (Figure 4.10) probably reflected treatment differences in the number of flower buds formed per scion (Figure 4.11 and see Section 4.3.7.2) rather than differences in the stage of dormancy release.

4.3.7.2 Comparison of rootstock and root restriction effects on flowering of the scion

Flower bud numbers were counted at full bloom, which occurred on the 24/10/05. Less than 3% of apical buds on the primary shoots formed a flower cluster, hence flower data for the apical bud and axillary buds (i.e., nodes without a secondary spur or shoot) on the primary shoot were pooled. Total flower bud numbers per scion for M.9 were twice that of MM.106 because significantly more flower clusters formed on the primary shoot, axillary buds on the secondary shoots, apical buds on secondary shoots and, to a lesser extent, secondary spurs on the primary shoot (Table 4.3). The M.9 rootstock also had proportionally more floral buds than MM.106 when the numbers of flower clusters per bud type or types on each scion were expressed as a ratio to the number of that bud type or types on that scion (Table 4.3). For both rootstocks, the total number of flower clusters produced on the secondary axes (i.e., total of secondary spurs plus shoots) was

greater than that produced on the axillary buds and apical bud of the primary shoot (Table 4.3). Fewer flower clusters formed on the primary shoot because each rootstock type produced many secondary axes (Table 4.2). In contrast to M.9, root restriction significantly increased the mean number of flower clusters formed only for the apical bud positions on the secondary shoots (Table 4.3).

In addition to significant main effects (Table 4.3), the rootstock x root restriction interactions neared significance for the mean number of flower clusters per scion formed at an apical bud position on the secondary shoots (P=0.07) and for the mean total number of flower clusters formed per scion (P=0.06) (i.e., the total height of stacked columns in Figure 4.12A). Similar interactions resulted when flower cluster numbers formed at an apical bud position on the secondary shoots (P=0.06) and the total number of flower clusters formed per scion (P=0.24) were expressed as a ratio to the total number of buds per scion (Figure 4.12B). Root restriction of MM.106 increased the mean total number of flower clusters per scion compared with unrestricted MM.106 root systems (Figure 4.12A) primarily by increasing the proportion of apical buds on the secondary shoots that were floral and, to a lesser extent, axillary buds on the secondary the shoots that flowered (Figure 4.12B). However, this effect of root restricting MM.106 caused very small increases in the mean total number of flower clusters per scion when compared with MM.106 grown without root restriction (Figure 4.12A). In contrast, root restriction of M.9 did not affect the mean total number of flower clusters formed per scion (Figure 4.12A). Treatments that promoted earlier termination of secondary shoots in March (Figure 4.7B) also caused more apical buds on the secondary shoots to form a flower cluster in the following spring (Figure 4.12A).

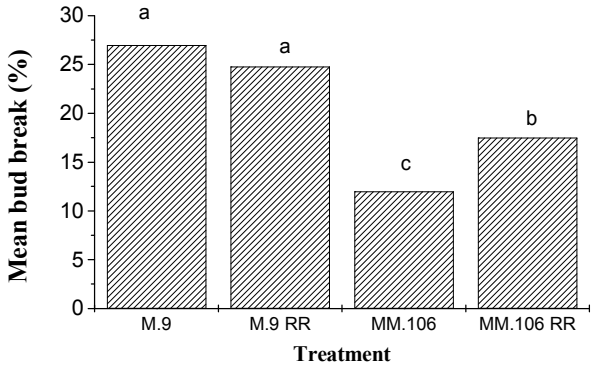


Figure 4.10. Rootstock x root restriction (RR) interaction for mean bud break (%) on 'Royal Gala' apple scions during early spring in year two (29/9/05) of growth from grafting. Bud break (%) was calculated as the total number of buds that had broken per scion divided by the total number of buds per scion x 100. Means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS).

Figure 4.11. Effect of M.9 (left) and MM.106 (right) rootstocks on the number of buds broken on 'Royal Gala' apple scions in the early spring of year two (5/10/05) after grafting. As flower buds broke before vegetative buds, the scion on M.9 had a greater number of buds broken in early spring.

Table 4.3. Main effects of rootstock and root restriction on the first occurrence of flowering for different bud types on 'Royal Gala' apple scions at full bloom (24/10/05). Mean proportion (%) of bud type(s) differentiated into floral buds was calculated by dividing the total number of flower clusters per bud type or types on each scion by the total number of that bud type or types on that scion x 100.

	Floral bud type(s) and positions					
Main effect	**Apical bud and axillary buds on primary shoot**	**Secondary spurs on on primary shoot**	**Axillary buds on secondary shoots**	**Apical buds on secondary shoots**	**Total for secondary axes**	**Total per scion**
Rootstock		*Mean number of flower clusters per scion*				
M.9	13.45 (3.64 a) ***	3.52 (1.90 a) ***	19.43 (4.29 a) ***	7.67 (2.78 a) ***	30.62 a ***	44.10 (6.63 a) ***
MM.106	4.40 (1.85 b)	2.16 (1.56 b)	10.75 (2.99 b)	4.98 (2.15 b)	17.89 b	22.29 (4.44 b)
Root restriction						
+	9.23 (2.77) ^{ns}	3.07 (1.78) ^{ns}	15.32 (3.65) ^{ns}	7.03 (2.60 a) *	25.42 ^{ns}	34.65 (5.64) ^{ns}
-	8.60 (2.71)	2.65 (1.69)	14.85 (3.62)	5.61 (2.33 b)	23.11	31.71 (5.42)
Rootstock		*Mean proportion (%) of bud type(s) that were floral per scion*				
M.9	24.34 (4.79 a) ***	81.12 a *	27.60 (5.18 a) ***	92.54 (1.80 a) ***	37.17 a ***	32.31 (5.67 a) ***
MM.106	7.23 (3.15 b)	71.78 b	10.92 (3.03 b)	58.75 (5.61 b)	16.99 b	13.59 (3.54 b)
Root restriction						
+	15.43 (4.04) ^{ns}	79.17 ^{ns}	19.98 (4.15) ^{ns}	80.40 (3.25 a) **	28.28 a *	23.71 (4.70) ^{ns}
-	16.14 (4.30)	74.74	18.66 (4.04)	71.62 (4.19 b)	25.77 b	22.17 (4.51)

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single main effect and growth attribute only, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. Data without and within parenthesis are raw or transformed means, respectively.

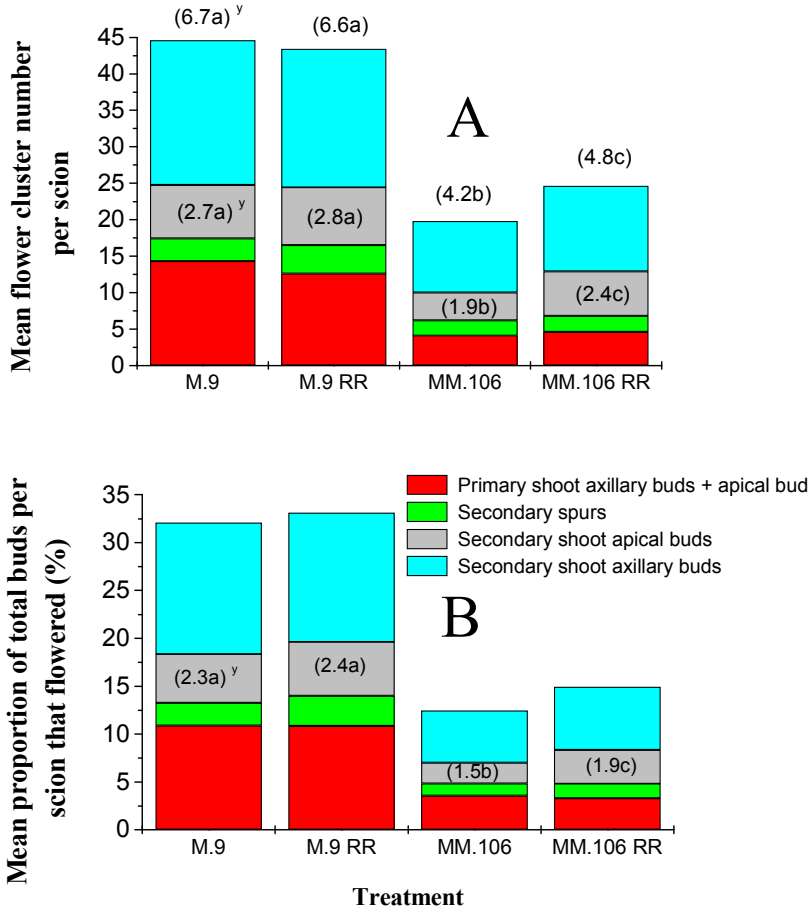


Figure 4.12. Rootstock x root restriction (RR) interactions for the mean total number of flower clusters (A) and the mean proportion (%) of total buds per scion that were floral (B) as divided into component bud types formed on 'Royal Gala' apple trees. The total height of the columns represent the interactions for the mean total flower cluster number per scion (A, *P*=0.06) and the mean proportion (%) of total buds per scion that were floral (B, *P*=0.24). For A and B, the grey portion of chart columns represent the rootstock x root restriction interaction for the mean flower cluster number per scion formed on apical buds of the secondary shoots (A, *P*=0.07) or the mean proportion (%) of apical buds on the secondary shoots that flowered relative to the total bud number per scion (B, *P*=0.06), respectively. Within a single graph and interaction only, transformed means (in parenthesis) sharing the same letter across a single row are not significantly different (lsmeans tests, SAS). [y] mean separation at *P*≤0.07.

4.4 Discussion

An objective of this experiment was to understand whether the first changes in scion growth for field-grown M.9 involved decreased vegetative growth in year one or increased flowering in year two when compared with the scion on MM.106. Further objectives of Chapter 4 were to understand similarities in vigour modifications imposed by the dwarfing apple rootstock and root restriction, and whether modifications imposed by these treatments were similar to those reported in Chapter 3.

4.4.1 Effects of rootstocks on vegetative growth

4.4.1.1 Primary shoot

The final mean length and node number of the 'Royal Gala' primary shoot on field-grown M.9 was reduced in the first year of growth from grafting compared with the scion on MM.106 (Table 4.1, Figures 4.2A, B and 4.5). The primary shoot on M.9 initially grew more quickly than that on MM.106 between 11/11/04 and 6/12/04 (Figure 4.3A, B). However, a greater proportion of primary shoots temporarily slowed or terminated growth between 6/12/05 and 21/1/05 for M.9. After the 22/3/05, greater proportions of primary shoots had terminated growth for M.9 compared with MM.106, particularly during April. The mean internode length of the primary shoot was unaffected by rootstock type despite these seasonal differences in growth (Table 4.1). Hence, the primary shoot on M.9 was shorter because of fewer neoformed nodes.

In Chapter 3, the final mean length of the primary shoot was also reduced on M.9 because of fewer neoformed nodes (Chapter 3, Table 3.1). However, between experiments there were differences in the way the primary shoot on M.9 grew throughout the season. The most notable difference for field-grown M.9 was the bicyclic growth of the primary shoot between 6/12/04 and 21/1/05, which did not occur in Chapter 3. Avery (1969) also reported that M.9 caused the temporary growth cessation of the 'Worcester Pearmain' scion during summer, whereas the scion on M.16 grew with minimal interruption, which was similar to the effect that MM.106 had on the growth of the primary shoot in the present study.

The temporary slowing or termination of primary shoot growth between 6/12/04 and 21/1/05 for both rootstocks occurred shortly after transplanting of the experimental tree material into the field on the 6/12/04. This might indicate that bicyclic growth of the primary shoots for both rootstocks was caused by transplanting. Given that trees were watered immediately after planting, it may also indicate that roots of M.9 were more sensitive to any disturbance, as indicated by a greater proportion of primary shoots that had temporarily terminated growth for M.9. Temporary termination of shoot extension growth would not be an unreasonable consequence following transplanting because a shift in growth priority from shoot to root would be required for re-establishment or repair of the root system. Tubbs (1977) has previously highlighted that the transplanting of tree material into the orchard may confound the growth of the scion, therefore making growth comparisons difficult for studies comparing the effects of rootstocks on scion vigour in the early life of the tree.

4.4.1.2 Secondary shoots

Field-grown M.9 had formed significantly more secondary axes (i.e., total of secondary spurs plus shoots) than MM.106 by growth cessation (Table 4.2). This resulted because the scion on M.9 developed more spurs, but not secondary shoots, than MM.106. Notably, this result differs from Chapter 3 where M.9 produced significantly fewer secondary shoots than MM.106. Compared with vigorous rootstocks, there were trends that the number of secondary shoots formed on the primary shoot of composite apple trees was decreased (Jaumien et al., 1993; Volz et al., 1994) or was not affected (Costes et al., 2001) by M.9 in the first year of growth. Thus, reports do differ concerning how M.9 affects the initial formation of secondary shoots on the primary shoot. However, M.9 is consistently reported to increase the proportion of short axillary shoots that form on the scion in its first (Costes et al., 2001) or second year of growth (Selezynova et al., 2005; Selezynova et al., 2008), thereby decreasing the proportion of long shoots that form compared with more vigorous rootstocks. Similar results were found for the scion on M.9 in both Chapters 3 and 4.

The reason(s) why the scion on M.9 formed more secondary axes than MM.106 in this experiment, but not in Chapter 3, may relate to differences in growth behaviour of the

SAM on the primary shoot between the experiments. In Chapter 3, all tree material was transplanted at the beginning of November and primary shoots developed from a single season-long growth flush. In addition, the scion on M.9 developed fewer secondary shoots than the scion on MM.106. In Chapter 4, the transplanting of trees into the field occurred in early December and growth for a proportion of primary shoots temporarily slowed, and/or, fully terminated for a period between December and January. From late January onwards, rapid growth of the primary shoot resumed and this coincided with the first occurrence of axillary bud outgrowth or the formation of secondary axes on the primary shoot. For 'Worcester Pearmain' trees in their second year of growth, Avery (1969) also reported that the temporary slowing, and/or, termination of shoot growth during summer was increased by M.9 compared with M.16, and resulted in 'lammas' type growth on the scion upon its regrowth.

Although speculative, more pronounced bicyclic growth of the primary shoot for M.9 between December and January may have caused a greater loss of apical dominance, and hence, increased the number of secondary axes formed compared with MM.106. In a similar manner, stem decapitation removes the IAA source **(**Thimman and Skoog, 1934**)** and transiently increases the synthesis and transport of cytokinin within the xylem sap (Bangerth et al., 2000) resulting in the loss of apical dominance and subsequent axillary bud outgrowth. However, measurements of endogenous plant hormones would be required to confirm this, particularly ratios of IAA:cytokinin and their transport. Nevertheless, the different effects of the M.9 rootstock on branch formation between Chapters 3 and 4 suggests that the dwarfing response may be plastic and depend on how the SAM on the primary shoot grows in response to environment or cultural practices.

The scion on M.9 may form an increased number of secondary axes when the seasonal growth of the primary shoot is bicyclic, which was possibly caused in this experiment by the transplanting of tree material into the field in December. In contrast, the scion on M.9 may form fewer secondary axes when the primary shoot develops from a single season-long flush of growth (i.e., Chapter 3). Scion growth and architecture on a dwarfing apple rootstock may also be modified differently between growing regions or by methods of tree establishment. In Hawke's Bay New Zealand, composite trees of

'Royal Gala' newly bench-grafted onto M.9 and MM.106 and planted into the field in early spring, thus reducing root disturbance, formed very few secondary axes in the first year of growth (Selezynova et al., 2008). In addition, 'Royal Gala' primary shoots on these rootstocks did not differ in their final mean length and node number, possibly because the primary shoot grew from a single season-long growth flush (Selezynova et al., 2007). Hence, variations in the growing environment, and/or, the cultural methods by which 'Royal Gala' trees on M.9 and MM.106 are grown, may greatly modify scion architecture by the end of the first year of growth from grafting.

The M.9 rootstock did not significantly reduce the mean number of secondary shoots formed per scion compared with MM.106 (Table 4.2). Thus, reduced mean total length and node number of the secondary shoots for M.9 (Table 4.1) was solely the result of decreased mean shoot length and node number of the secondary shoots (Figure 4.8A, B). Significantly decreased mean shoot length and node number of the secondary shoots imposed by M.9 resulted from their earlier termination in March, whereas the majority of secondary shoots continued to grow for MM.106 (Figure 4.7B). Similar trends were found in the study reported in Chapter 3, where M.9 also increased the proportion of secondary shoots that had terminated by March when compared with the scion on MM.106, and this caused a slightly shorter final mean shoot length and node number of the secondary shoots for M.9 than for MM.106 (Chapter 3, Table 3.9). For older trees, other studies have also reported that M.9 decreased the mean length of annual shoots because of earlier shoot termination when compared with more vigorous rootstocks (Swarbrick, 1929; Colby, 1935; Avery, 1969).

4.4.2 Effects of root restriction on vegetative growth

Further objectives of Chapter 4 were to understand similarities in vigour modifications imposed by the dwarfing apple rootstock and root restriction, and whether modifications imposed by root restriction were similar to those reported in Chapter 3.

4.4.2.1 Similarities and differences between M.9 and root restriction on the growth of the primary shoot

In responses similar to the dwarfing rootstock, root restriction reduced the final mean length, node number and SCA of the primary shoot compared with unrestricted root systems (Table 4.1). In Chapter 3, M.9 and root restriction also decreased the final mean length and node number of the primary shoot without affecting its mean internode length (Tables 3.1 and 3.2). In April, the proportion (%) of primary shoots that had terminated growth was increased by M.9 or root restriction treatments in both experiments, however increased shoot termination caused by root restriction was typically small, which was in contrast to M.9. In both chapters, root restriction reduced the mean total leaf area of the primary shoot because of fewer nodes and a reduced mean area per leaf. A similar result occurred for M.9 in Chapter 3, but not in Chapter 4, where despite having fewer neoformed nodes than MM.106, the primary shoot on M.9 had a similar final mean total leaf area to MM.106 because of an increased mean area per leaf (Table 4.1).

Avery (1969) reported that composite trees of 'Worcester Pearmain' on M.9 had increased leaf size in their second season of growth compared with the same scion on the M.16 rootstock. In contrast, root restriction reduced the size of leaves for field-grown apple (Webster et al., 2000) and for other crops including peach (Rieger and Marra, 1994), tomato (Hurley and Rowarth, 1999) and pepper (Ismail and Davies, 1998). For apple, Atkinson et al., (2000) reported that root restriction of M.9 and MM.106 rootstocks also reduced the final fruit diameter of 'Queen Cox' compared with non-restricted root systems of these same rootstocks. In addition, differences in fruit growth imposed by trees grown with root restriction did not appear to result from water deficit because this treatment was fully irrigated and had similar diurnal leaf water potentials to fully irrigated control trees grown without root restriction. Composite trees on M.9 and MM.106 grown with irrigation and without root restriction also had similar diurnal leaf water potentials, however fruit diameter was increased by M.9 compared with MM.106 (Atkinson et al., 2000). In responses similar to root restriction, root pruning decreased scion vigour and fruit size of apple (Ferree, 1992; Ferree et al., 1992). Collectively, the different effects on the size of leaves (Webster et al., 2000;

Table 4.1) and fruit (Atkinson et al., 2000; Ferree, 1992; Ferree et al., 1992) may indicate some fundamental physiological mechanisms causing reduced scion vigour are different for M.9 compared with root restriction and root pruning treatments.

4.4.2.2 Similarities and differences between M.9 and root restriction on growth of the secondary shoots

The M.9 rootstock significantly decreased the mean total length and node number of the secondary shoots compared with MM.106, and there were trends that root restriction also decreased the mean total length and node number of the secondary shoots compared to trees grown without root restriction (Table 4.1). Reductions in the mean total shoot length and node number of the secondary shoots by M.9 and root restriction (Table 4.1) were the result of very similar causes. Both the M.9 rootstock and root restriction did not decrease the mean number of secondary shoots formed compared with the MM.106 rootstock or unrestricted root systems, respectively (Table 4.2). However, the M.9 rootstock and root restriction of MM.106 decreased the final mean shoot length, node number and internode length of the secondary shoots (Figure 4.8A, B, C).

Interpretation of mean internode length of the secondary shoots (i.e., Figure 4.8C) requires consideration of correlations between shoot length and node number (Figure 4.9A, C) and the node number distribution of each treatment (Figure 4.9B, D). All treatments showed an almost identical relationship between the node number and the length of their secondary shoots (Figure 4.9A, C). Thus, regardless of treatment secondary shoots with the same node number had a very similar shoot length and, therefore, internode length (comparison of internode lengths can be made from the regression lines of Figure 4.9A and C by dividing a y-axis value by its corresponding x-axis value). However, internode length increased with shoot length for each treatment. Therefore, the mean internode length of the secondary shoots was reduced by both M.9 and root restriction of MM.106 because these treatments changed the node number distribution by forming greater proportions of short shoots with 10 or less nodes, whilst decreasing the proportion of long secondary shoots that developed, particularly very long shoots with 20 or more nodes (Figure 4.9B, D). Similarly, Selezynova et al.,

(2003) reported that the correlation between shoot length and node number of 'Royal Gala' annual shoots was similar regardless of whether the scion was grown on M.9 or MM.106. However, the M.9 rootstock decreased the mean internode length by changing the node number distribution (Selezynova et al., 2003).

In Chapter 3, the rootstock x root restriction interactions were typically significant because root restricted MM.106 decreased the mean total length and node number of the secondary shoots more severely than root restricted M.9, primarily because the mean number of secondary shoots formed per scion was reduced more markedly for root restricted MM.106 than for root restricted M.9. In Chapter 4, root restriction did not decrease the mean number of secondary shoots formed per scion (Table 4.2). In addition, root restriction of M.9 did not reduce the mean length and node number of the secondary shoots, which was different from Chapter 3, where root restriction of M.9 decreased the mean length and node number of the secondary shoots. For MM.106 in both chapters, root restriction decreased the mean length and node number of the secondary shoots, particularly by reducing the proportion of long secondary shoots that had developed 20 or more nodes (Figures 3.13D and 4.9D). Webster et al., (2000) also reported that root restriction of field-grown apple trees reduced the mean length of annual shoots, although unlike the results of Chapter 4, significant reductions in the number of annual shoots also occurred.

In Chapter 3, root restriction of MM.106 decreased shoot growth of the scion more than root restriction of M.9, possibly because the larger root system of MM.106 was more confined within the 8 L root volume. In Chapter 4, root restriction of MM.106 decreased total shoot growth of the scion less markedly than in Chapter 3, and this may have resulted from differences in the size of the root bags used and the method by which they restricted the growth of the root system. The volumes of the root bags used in Chapters 3 and 4 were 8 and 10 L, respectively. Therefore, the larger root volume used in Chapter 4 would result in less root confinement and perhaps decrease scion vigour only moderately. The polyfelt root bags used in Chapter 4 were also semi-permeable allowing fine roots to pass through the bag into the surrounding soil. Upon secondary thickening, these roots are girdled by the root bag, thereby reducing the growth and size of the root system. Arguably, less marked reductions in scion growth of root restricted

MM.106 in Chapter 4 may indicate that the amount of secondary thickening by the MM.106 root system was insufficient to impose large restriction effects on root and therefore scion growth. This was in contrast to Chapter 3, where root restriction was imposed using polythene bags to physically confine the whole root system within the 8 L soil volume. Clearly, the effects of root restriction in this instance would be greater because no fine roots could escape beyond the bag. Webster et al., (2003) reported that the use of semi-permeable fabric root bags resulted in less severe restrictions to root growth than less-permeable fabric root bags with smaller pore size (i.e., Webster et al., 2000), and reductions in scion vigour were moderate when root bags with increased pore size were used (Webster et al., 2003).

Despite the aforementioned differences in the degree of vigour reduction imposed by root restriction between the experiments, the studies in both Chapters 3 and 4 found that root restriction of M.9 decreased scion vigour less severely than root restriction of MM.106. Smaller reductions in scion vigour for root restricted M.9 than for MM.106 were also reported by Webster et al., (2000). Collectively, this result and those of Chapters 3 and 4 may support the hypothesis that the root system of M.9 is smaller and, therefore, was less confined by root restriction, at least in the early life of the tree when total root biomass is relatively small compared with the final expected size. Given that IAA is important for the initiation (Jones and Hatfield, 1976; Delargy and Wright, 1979) and possibly the growth of apple roots (Lockard and Schneider, 1981), information on root growth for rootstocks of different vigour during the first year after grafting may provide further insight into the probable hormonal signal(s) that initially cause scion dwarfing on M.9.

4.4.3 Effect of rootstocks on the first occurrence of flowering on 'Royal Gala' scions

Recent field studies have reported that the first observable difference imposed on the scion by a dwarfing compared with a more vigorous rootstock was increased flowering in the spring of year two (Seleznyova et al., 2005, 2007, 2008). Therefore, a further objective of this experiment was to elucidate whether the first changes in scion growth for field-grown trees grafted onto a dwarfing rootstock involved decreased vegetative growth in year one or increased flowering in year two.

The data of Chapters 3 and 4 have shown that the first measurable changes in scion growth on M.9 resulted from decreased vegetative development in the first season from grafting. In addition, these changes preceded the first occurrence of flowering on the scion in the spring of year two. Others have also reported that M.9 reduced vegetative growth during the first year after grafting (Rao and Berry, 1940; Cannon, 1941). The second notable difference imposed on the scion by M.9 was increased bud break during the spring of year two from grafting (on the 29/09/05, see Figure 4.10). This probably resulted because floral buds broke earlier than vegetative buds, and the scion on M.9 had more floral buds compared with MM.106. The increased floral precocity of the scion on M.9 was the third observable change imposed on the scion by the dwarfing apple rootstock.

Collectively, the results of Chapters 3 and 4 are different from other studies where increased flowering was the first observable difference in scion growth between a dwarfing and a semi-vigorous rootstock (Seleznyova et al., 2005, 2007, 2008). Nevertheless, the results of Chapter 4 confirm that the dwarfing apple rootstock increases the proportion of buds that are floral during the spring of year two (Hirst and Ferree, 1995; Seleznyova et al., 2005, 2007, 2008). Increased flowering of the scion is typically followed by measurable reductions in vegetative growth in year two, even when the scion was deflowered in early spring (Seleznyova et al., 2005, 2008).

Seleznyova et al., (2007) hypothesised that increased flowering of the scion on M.9 in the second year of growth from grafting may not be the first cause of scion dwarfing; rather it may be a developmental event that occurs together with the physiological processes regulating dwarfing. Under the experimental conditions of the Manawatu, the results of Chapter 4 clearly indicated that increased floral precocity of the scion on M.9 was not the first cause of scion dwarfing, particularly because flowering occurred after a measurable reduction in vigour had already been imposed on the scion by M.9 in year one. Interestingly, these results are different from Seleznyova et al., (2005, 2007, 2008) who found that M.9 did not reduce scion vigour in the first year of growth after grafting, but increased flowering and decreased scion vigour in year two. Consideration of these differing results may indicate that the first expression of rootstock-induced dwarfing of the scion can be a plastic response, possibly influenced by different growing environments. In some environments, such as the Manawatu, the physiological

expression of scion dwarfing on M.9 is measurably visible in the first year of growth and precedes the first occurrence of flowering on the scion in the spring of year two.

4.4.4 Similarities between rootstock and root restriction on the first occurrence of scion flowering

In responses similar to the M.9 rootstock, root restriction of MM.106 increased the total number of flowers formed on the scion (Figure 4.12). This increase in flowering by root restriction was very small compared with that imposed on the scion by M.9, and resulted primarily because increased numbers of apical buds on the secondary shoots were floral (Figure 4.12A). Swarbrick (1929) reported shoots that terminated earlier were more likely to be floral in the following spring than shoots that terminated later in the growing season. This may suggest that root-restricted MM.106 increased the number of floral apical buds on the secondary shoots (Figure 4.12A) because a greater proportion of secondary shoots had terminated in March compared with the scion on unrestricted MM.106 root systems (Figure 4.7B). Similarly, M.9 increased the proportion of secondary shoots that had terminated in March compared with MM.106 (Figure 4.7B) and increased the mean number of flowers that formed at apical bud positions on the secondary shoots (Figure 4.12A).

Myers (1992) also reported that restriction of apple roots increased the number of flowers that formed per scion when compared with unrestricted root systems. However, differences in flower number per scion did not become significant until the third year of growth (Myers, 1992). In Chapter 4, root restriction applied during the first growing season caused relatively small increases in flower number in the spring of year two compared with the unrestricted controls. The results of Myers (1992) would indicate that root restriction of MM.106 may have increased flower numbers markedly more in the spring of year three.

4.5 Summary

The final mean length, node number and SCA of the 'Royal Gala' primary shoot on field-grown M.9 was reduced in the first year of growth from grafting compared with the scion on MM.106. The primary shoot on M.9 initially grew more quickly than that on MM.106 between 11/11/04 and 6/12/04. However, a greater proportion of primary shoots terminated temporarily for M.9 between early December and late January, possibly as a consequence of transplanting trees into the field. After the 22/3/05, a greater proportion of primary shoots terminated for M.9 compared with MM.106, particularly during April. The mean internode length of the primary shoot was unaffected by rootstock type despite these seasonal differences in growth. Hence, the primary shoot on M.9 was shorter because of fewer neoformed nodes.

From late January, some axillary buds along the primary shoot broke to form secondary axes. The scion on M.9 formed more secondary spurs (< 25 mm) but a similar number of secondary shoots (≥ 25 mm) than the scion on MM.106. By March, M.9 had caused the majority of secondary shoots to fully terminate, which contributed to their decreased mean shoot length, node number and internode length when compared with the scion on MM.106. Regardless of treatment, secondary shoots with the same node number had a very similar shoot length and, therefore, internode length. However, internode length increased with shoot length for each treatment. Therefore, the mean internode length of the secondary shoots was reduced by both M.9 and root restriction of MM.106 because these treatments changed the node number distribution by forming greater proportions of short shoots with 10 or less nodes, whilst decreasing the proportion of long secondary shoots that developed, particularly very long shoots with 20 or more nodes.

Collectively, these effects of M.9 on the growth of the primary and secondary shoots significantly reduced the mean total length and node number of the scion by the end of the first growing season from grafting when compared with the scion on MM.106. These reductions in scion vigour on M.9 preceded the first occurrence of scion flowering in the spring of year two. On the 29/09/05, or in early spring of year two, the scion on M.9 had a greater proportion of buds breaking compared with the scion on MM.106. This probably resulted because floral buds broke earlier than vegetative buds,

and the scion on M.9 had more floral buds compared with that on MM.106. Treatments that caused earlier termination of secondary shoots in the first year of growth, such as M.9 and root restriction of MM.106, also caused more apical buds on the secondary shoots to flower in the spring of year two. The scion on M.9 had proportional more buds that were floral compared with MM.106 for every bud type or position on the scion. Some similar trends occurred for the scion on root restricted MM.106, although unlike M.9, root restriction did not greatly increase flowering at most positions on the scion when compared with trees grown without root restriction.

In both Chapters 3 and 4, M.9 had reduced total scion growth compared with MM.106 by the end of the first year of growth after grafting. However, the scion on M.9 formed significantly fewer or similar numbers of secondary shoots compared with the scion on MM.106 in Chapters 3 and 4, respectively. These contrasting results may relate to differences in the growth of the SAM on the primary shoot, which exhibited either continuous or bicyclic growth on M.9 in Chapters 3 or 4, respectively. These results and others within the literature indicate that the initial early expression of dwarfing scion architecture may be plastic and dependent on the growth behaviour of the primary shoot. The bicyclic growth of the primary shoot in Chapter 4 suggests that cultural practices, such as the transplanting of trees, can greatly modify scion architecture. Nevertheless, in both chapters measurable dwarfing occurred for the scion on M.9 in the first year of growth after grafting.

Root restriction reduced the mean node number, length and SCA of the primary shoot. For secondary shoots, there were trends that root restriction reduced their mean total length and node number, however these effects were relatively small. Reduced total growth of the secondary shoots occurred because root restriction of MM.106 reduced the mean length and node number of the secondary shoots, but not the mean number of secondary shoots formed per scion. An important difference between M.9 and root restriction was that root restriction reduced the mean area per leaf on the primary shoot, whereas M.9 increased leaf size compared with MM.106.

A major difference in the effect of root restriction between Chapters 3 and 4 was that root restriction of field-grown trees on MM.106 did not reduce the mean number of

secondary shoots formed per scion. Very small reductions in scion vigour for root restricted MM.106 in the field may reflect the larger root bags used, and possibly the mechanism by which they restricted the growth of the root system (i.e., root girdling versus physical confinement). Nevertheless, data from both chapters agree that root restriction affected many growth attributes markedly less for the scion on M.9 than MM.106. This may support the hypothesis that the root system of M.9 was less confined by root restriction because it was smaller overall.

5. Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot/root/shoot signalling sequences by auxin, gibberellin and cytokinin

5.1 Introduction

In Chapter 3, it was shown that M.9 decreased the final mean length and node number of the primary shoot and reduced the mean number of secondary axes that formed compared with the 'Royal Gala' scion on MM.106 and M.793. However in Chapter 4, field-grown M.9 increased the mean number of secondary axes that formed compared with the 'Royal Gala' scion on MM.106, and this may have been partly caused by bicyclic growth of the primary shoot soon after transplanting of the tree material into the field. Therefore, objective one was to elucidate whether reduced formation of secondary axes on the scion was an important way in which rootstock-induced dwarfing of the scion occurred on M.9 in the first year of growth after grafting. A self-rooted 'Royal Gala' rootstock grafted with a 'Royal Gala' scion was introduced as the rootstock control to determine how the 'Royal Gala' scion grew naturally when grown on its own root system, and how architecture of the 'Royal Gala' scion was modified by grafting onto three different size-controlling rootstocks (M.9, MM.106 and M.793).

In Chapter 3, reduced formation of secondary axes caused by the M.9 rootstock, particularly the formation of secondary shoots, was reversed with exogenous BAP (Figure 3.7B, D). This response might indicate that the role of endogenous cytokinin in the development of rootstock-induced dwarfing of the scion involves stimulating the initial outgrowth of axillary buds or meristems. However, the development of fewer secondary axes for the scion on M.9 was probably not due to cytokinin action alone. For example, Lockard and Schneider (1981) hypothesised that reduced concentrations of cytokinin in the xylem sap were caused by the stem tissue of the dwarfing rootstock reducing polar auxin transport from scion to root, thereby decreasing root growth, consequent cytokinin biosynthesis and the transport of cytokinins from root to scion.

Therefore, IAA and cytokinin may interact to control the formation of secondary axes on the primary shoot. Thus, objective two used exogenous growth regulators to elucidate whether shoot/root/shoot signalling of IAA and cytokinin may interact to regulate differences in the formation of secondary axes on the 'Royal Gala' scion grafted onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala'. To restrict basipetal auxin transport from scion to root, NPA was applied to the rootstock stem of half the trees on each rootstock type, whilst BAP was applied to half the scions on each rootstock type treated with or without NPA to determine the main effects or interactions on scion branching.

Kamboj et al., (1999) hypothesised that increased concentrations of cytokinins in the xylem sap of apple scions grafted onto vigorous rootstocks may partly explain their higher rates of growth compared with scions grafted onto dwarfing rootstocks. Indeed, root-produced cytokinins may control the growth of SAMs because cytokinins extracted from the xylem sap of apple trees promoted extension growth of shoots grown without roots *in vitro* (Jones, 1973). However, BAP applied to the scion of newly grafted 'Royal Gala' apple trees did not increase extension growth of the primary shoot compared with untreated trees (Figure 3.1C, D). In addition, secondary shoots of 'Royal Gala' on the M.9 rootstock terminated growth much earlier than for rootstocks of MM.106 and M.793, and this effect was not reversed with exogenous BAP (Figure 3.11). Hence, total growth of the BAP-treated scion on M.9 was reduced compared with the BAP-treated scion on MM.106 and M.793. Evaluation of the literature (Chapter 3, Section 3.4.4) indicated that the earlier termination of secondary shoots for the scion on M.9 might have resulted because endogenous concentrations of root-produced gibberellins were limiting growth of the SAMs. Therefore, objective three was to elucidate whether applying BAP to induce branching of the scion on M.9, followed by applications of GA_{4+7}, kept a greater proportion of SAMs actively growing, and whether the combined effects of exogenous cytokinin and gibberellin prevented the development of scion dwarfing on M.9 in the first year of growth from grafting.

In addition to an endogenous supply of gibberellin, the sustained growth of an apple SAM may require an IAA signal moving basipetally from scion to root. For example, the inhibition of polar auxin transport from shoot to root of apple seedlings caused the

eventual termination of the SAM on the primary shoot (Grochowska et al., 1994). This could indicate that the earlier slowing of scion growth, and/or, shoot termination for M.9 (Figures 3.1A, B and 3.11) may have resulted from decreased basipetal transport of IAA within the rootstock stem to the root system, which in turn decreased the amount of root-produced gibberellin transported in the xylem sap to the SAMs of the scion. Conversely, reduced amounts of root-produced gibberellin transported to the scion may have decreased the activity of the SAMs, consequent IAA synthesis by shoot apices and, therefore, IAA transported basipetally to the root system.

Given the above hypothesis, objective four was to elucidate whether reduced basipetal transport of IAA from scion to root was important for meristematic activity, and whether the basipetal transport of IAA interacted with gibberellin to control this. For the scion on M.9, gibberellin treatments were of particular interest to determine whether SAMs could be prevented from slowing, and/or, terminating growth early. In contrast, objective five was to elucidate whether applying NPA to the stem tissue of vigorous rootstocks, to restrict their basipetal IAA transport, decreased scion vigour, and whether modifications in scion architecture were similar to those imposed on the scion by M.9 during the first year of growth from grafting. The effects of the dwarfing rootstock and NPA treatment of the rootstock stem on the final growth of the root system were also compared (objective six) because it remains untested experimentally whether reduced polar auxin transport from scion to root actually decreases root growth and root mass of apple trees.

5.2 Materials and methods

5.2.1 Site preparation and management of tree material

The experiment was conducted during the 2005–2006 growing season at the Fruit Crops Unit, Massey University, Palmerston North. A 'Royal Gala' scion was cleft grafted at a height of 350 mm onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (self-rooted, vigorous rootstock control). Dormant grafting occurred on the 1/9/05 and the root systems of newly grafted trees were bedded into moist sawdust until the first signs of scion bud movement, which occurred on the 20/9/05. Trees were then planted into 50 L black polythene bags. The growing medium and fertiliser regime was previously described in Chapter 3 (Section 3.2.2). Planting height was standardised for each rootstock to leave 150 mm of rootstock stem above the surface of the growing medium. Each scion was debudded to a single shoot during October and thereafter scions received no pruning. After initially establishing in a tunnel house and then hardening off outside, trees were moved to a standing out row within the orchard in early December. In-row tree spacing was 1 m between trees and a guard tree was positioned at the ends of the row. The irrigation system was also similar to that described in Chapter 3 (Section 3.2.2); however 4 L hr^{-1} mini sprinklers were used in this experiment. In addition to rainfall, irrigation was scheduled daily for 1 hour at dawn and dusk using an automated time controller (Hunter, Smart Valve Controller, USA) to maintain the volumetric water content (θ) of the growing medium close to field capacity (0.30 m^3 m^{-3} ± 0.01 m^3 m^{-3}). Measurements of medium θ were taken at regular intervals over the growing season using TDR. Medium θ was measured 200 mm from the rootstock stem to a depth of 400 mm.

5.2.2 Application of plant growth regulators

The synthesis of the auxin transport inhibitor 'NPA' and its application to the rootstock stem was described in Chapter 2 (Section 2.6). NPA was applied to the rootstock stem of half the experimental trees on each rootstock type, whilst the rootstock stem of the remainder were treated with lanolin only. The first, second and third applications of NPA occurred on the 12/12/05, 10/2/06 and the 3/3/06, respectively. Shortly after the first NPA application, BAP (500 mg L^{-1} BAPSoL™, Gro-Chem, New Zealand, Ltd)

was sprayed (see Section 3.2.3) onto half the scions on each rootstock treated with or without NPA on 23/12/05 and 30/12/05 to promote axillary bud outgrowth. Following the formation of axillary shoots, GA_{4+7} (500 mg L^{-1} Gib 47™, Gro-Chem, New Zealand, Ltd) was spayed onto half the scions on each rootstock/BAP/NPA combination on the 20/1/06 and again on the 27/1/06 to maintain shoot outgrowth. A further three sprays of either BAP or GA_{4+7} were then applied sequentially over the remainder of the growing season on 18/2/06, 10/3/06, 28/3/06 or on 25/2/06, 17/3/06, 7/4/06, respectively. Adjacent trees were covered during the application of BAP or GA_{4+7} to prevent contamination from spray drift. Suckers that developed on the rootstock below the site of NPA application were removed over the growing season as required.

5.2.3 Measurements of tree growth

The length and node number of the primary shoot was measured monthly during the growing season (see Section 2.7.1) and the final measurement of scion growth was conducted at growth cessation in late May, 2006 as previously described (Chapter 2, Section 2.7). Termination of the primary and secondary shoots was assessed in February, March and April, 2006. In June 2006, the root system of each tree was washed to remove the growing medium. Roots were excised from the rootstock stem and the total length of each root system was measured using a root length scanner (Commonwealth Aircraft Corp. Ltd., Melbourne, Australia). Roots were then oven dried at 80^{o}C for 21 days to a constant mass and their dry weights recorded on a four-decimal place balance (METTLER AE200, Switzerland) within 30 sec of removal from the oven.

5.2.4 Statistical analysis

The experiment was a completely randomised design with a factorial arrangement of treatments. Composite ‘Royal Gala’ trees were grafted onto four rootstocks (M.9, MM.106, M.793 and ‘Royal Gala’) and trees on each rootstock type were treated with two treatments of BAP (± BAP), or GA_{4+7} (± GA_{4+7}) or NPA (± NPA) and in all possible combinations. Each treatment was replicated four times. Data for the main effects and interactions were analysed using the GLM procedure of SAS as previously described (Chapter 3, Section 3.2.5).

5.3 Results

The final effects of all treatment combinations on scion architecture are presented pictorially in Figures 5.27 and 5.28. The quantitative data are reported from Section 5.3.2 onwards.

5.3.1 Irrigation scheduling

Irrigation in this experiment maintained medium θ close to field capacity (0.30 ± 0.01 m^3 m^{-3}) over the growing season and, on the dates measured, the maximum deviation below field capacity was never more than 0.035 m^3 m^{-3} (Appendix 3).

5.3.2 Growth of the primary shoot

5.3.2.1 Main effects of rootstock, BAP, GA_{4+7} and NPA on the mean cumulative length and node number of the primary shoot

Each main effect of rootstock, GA_{4+7} and NPA significantly affected (P<0.0001) the final mean length and node number of the primary shoot in May, 2006. These main effects were much more significant than interactions in the data, and therefore, are briefly reported (Figure 5.1). The main effect of BAP was highly significant for the final mean node number of the primary shoot (P<0.0001), but the main effect for the final mean length of the primary shoot was less significant (P=0.04, Figure 5.1C) than the rootstock x BAP x GA_{4+7} interaction (P=0.02, see Figure 5.4).

For rootstocks, the 'Royal Gala' primary shoot on M.9 was significantly shorter and had fewer nodes than the primary shoot on MM.106, M.793 and 'Royal Gala' from mid February 2006 onwards (Figure 5.1A, B). In May, the primary shoot on 'Royal Gala' was significantly shorter than the primary shoot on M.793, however the mean node number was not significantly different between these rootstocks (Figure 5.1A, B and Table 5.3). The mean node number of the primary shoot on MM.106 was significantly decreased compared with M.793 (Table 5.3). Exogenous BAP was first applied on the 23/12/05 and initially did not affect the mean node number of the primary shoot (Figure 5.1D). However, cumulative node number of the primary shoot was significantly reduced by BAP from mid April onwards when compared with untreated scions (Figure 5.1D). In contrast, GA_{4+7} was first applied on the 20/1/06 and stimulated significant

increases in the mean cumulative length and node number of the primary shoot from mid February onwards when compared with untreated scions (Figure 5.1E, F). Following the first application of NPA to the rootstock stem on the 12/12/05, the mean cumulative length and node number of the primary shoot was significantly reduced from mid January onwards when compared with untreated rootstocks (Figure 5.1G, H).

5.3.2.2 Interactions between NPA and GA_{4+7} on the extension growth of the primary shoot

Reduced mean cumulative length and node number of the primary shoot in response to NPA treatment of the rootstock stem (Figure 5.1G, H) depended on whether exogenous GA_{4+7} was applied to the scion (Figure 5.2). At growth cessation in May 2006, the NPA x GA_{4+7} interaction was highly significant ($P<0.001$) for the final mean node number of the primary shoot (Figure 5.2B). A very similar interaction occurred for the final mean length of the primary shoot, although this interaction was less significant ($P=0.11$) (Figure 5.2A). By May, treatments of GA_{4+7} or NPA had increased or decreased, respectively, the final mean node number and length of the primary shoot compared with untreated trees (Figure 5.2). However, GA_{4+7} applied to the scion growing on NPA-treated rootstocks increased the final mean length and node number of the primary shoot by May when compared with trees that were untreated (i.e., - GA_{4+7} - NPA) or treated with NPA alone (i.e., - GA_{4+7} + NPA) (Figure 5.2A, B). Decreased cumulative growth of the primary shoot in response to NPA (Figure 5.2A, B) resulted from a greater proportion of apical meristems fully terminating, and/or, slowing in their growth 14 to 21 days after NPA treatment. However, this effect of NPA was reversed with exogenous GA_{4+7}, which greatly reduced the proportion of primary shoots that slowed or fully terminated growth during the growing season (see Section 5.3.2.6).

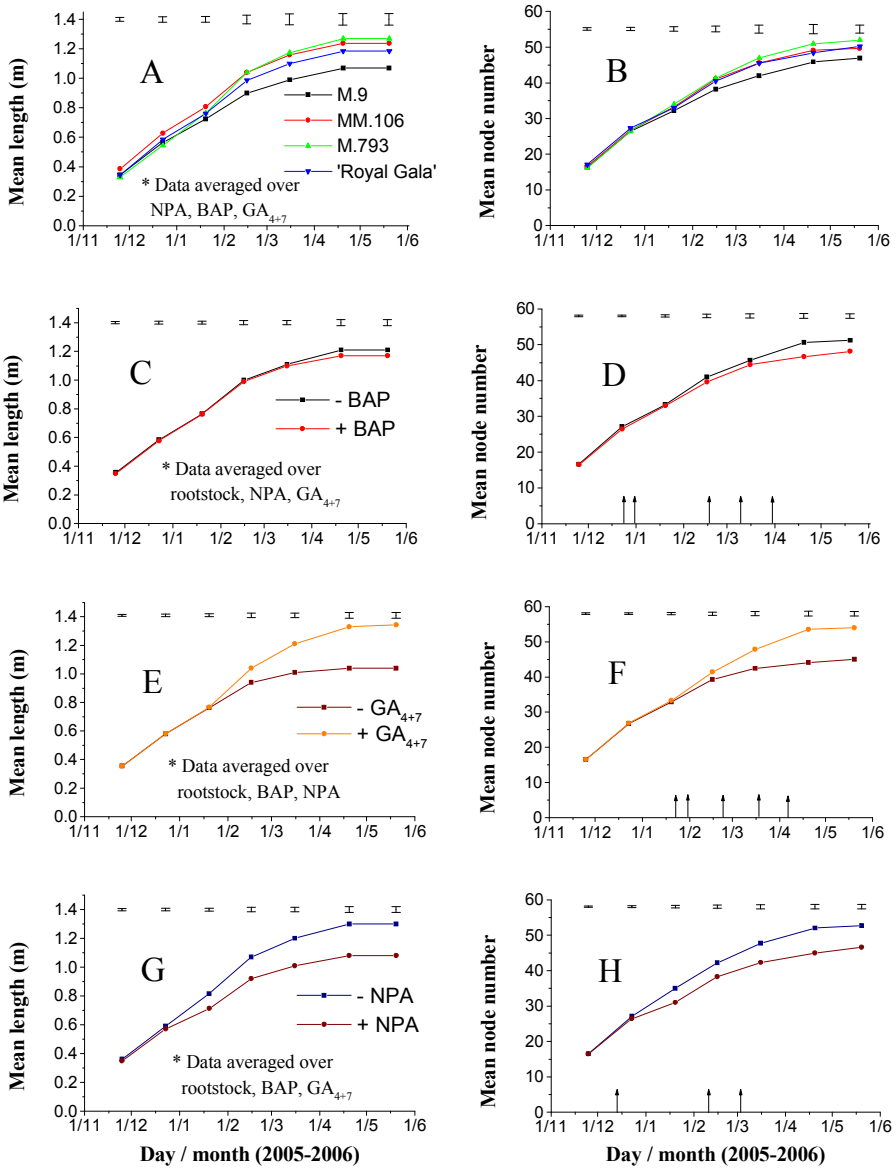


Figure 5.1. Main effects of rootstocks (A and B), ± exogenous benzylaminopurine (BAP) (C and D) or gibberellin (GA_{4+7}) applied to the scion (E and F) and ± 1-N-naphthylphthalamic acid (NPA) (G and H) applied to the rootstock stem on the mean cumulative length and node number for primary shoots of 'Royal Gala' apple scions during their first year of growth from grafting. Vertical bars denote the minimum significant difference (MSD) at *P*=0.05 using the Tukey's test. Arrows on D, F and H denote the timing of application for each growth regulator.

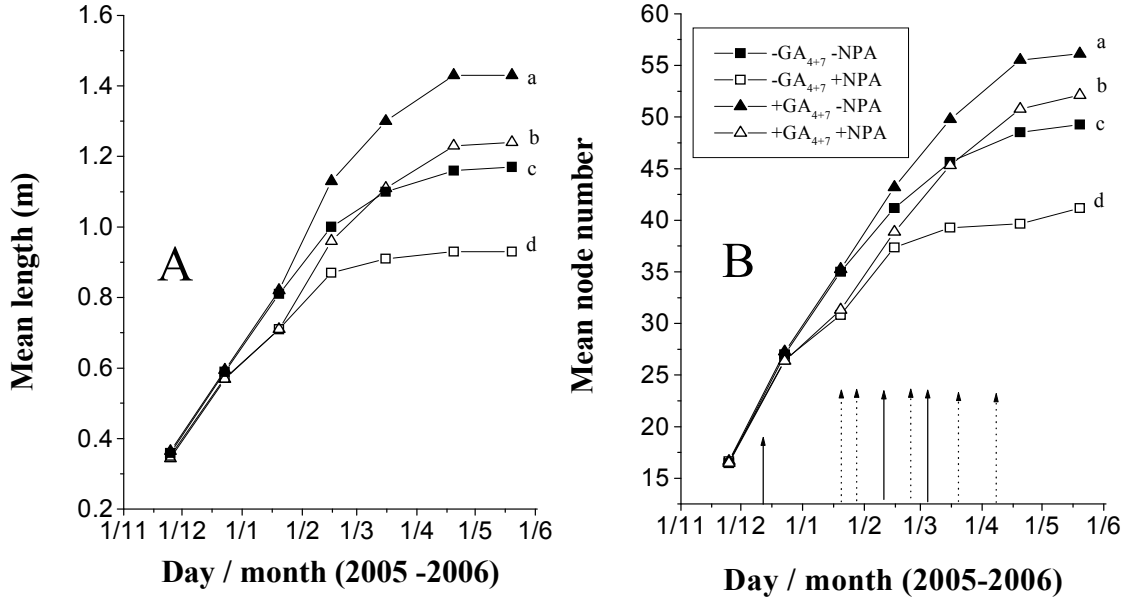


Figure 5.2. Interactions between gibberellin (GA_{4+7}) applied to the scion and 1-N-naphthylphthalamic acid (NPA) applied to the rootstock stem on the mean cumulative length (A) and node number (B) for primary shoots of 'Royal Gala' apple scions during their first growing season after grafting. Arrows on 'B' with a solid or dotted line denote the timing of NPA or GA_{4+7}, respectively. On a single graph, means sharing the same letter are not significantly different. On the 20/5/06, mean separation is at $P \leq 0.11$ or $P \leq 0.05$ for graph A or B, respectively (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over BAP and rootstock treatments.

5.3.2.3 Interactions between rootstock and GA_{4+7} on the extension growth of the primary shoot

For the main effect of rootstock, the final mean node number and length of the primary shoot (Figure 5.1A, B) depended on whether GA_{4+7} was applied to the scion (Figure 5.3A, B). At growth cessation in May 2006, the rootstock x GA_{4+7} interaction was significant for the final node number (P=0.003) and length (P=0.03) of the primary shoot (Figure 5.3A, B). Similar to NPA treatment of the rootstock stem (Figure 5.2), the untreated scion on the M.9 rootstock developed fewer neoformed nodes on the primary shoot when compared with the untreated scion on rootstocks of MM.106, M.793 and 'Royal Gala' (Figure 5.3B). However, very similar cumulative growth resulted for the

primary shoot on M.9 and 'Royal Gala' rootstocks when GA_{4+7} was applied to the scion (Figure 5.3A, B). At growth cessation in May 2006, the primary shoot on M.9 had a comparable mean node number to MM.106 and 'Royal Gala' rootstocks, but significantly fewer nodes than the primary shoot on M.793 (Figure 5.3B). A very similar rootstock x GA_{4+7} interaction occurred for the cumulative length of the primary shoot, although for GA_{4+7}-treated trees, the primary shoot on M.9 and 'Royal Gala' was slightly shorter at growth cessation than that on MM.106 and M.793 (Figure 5.3A).

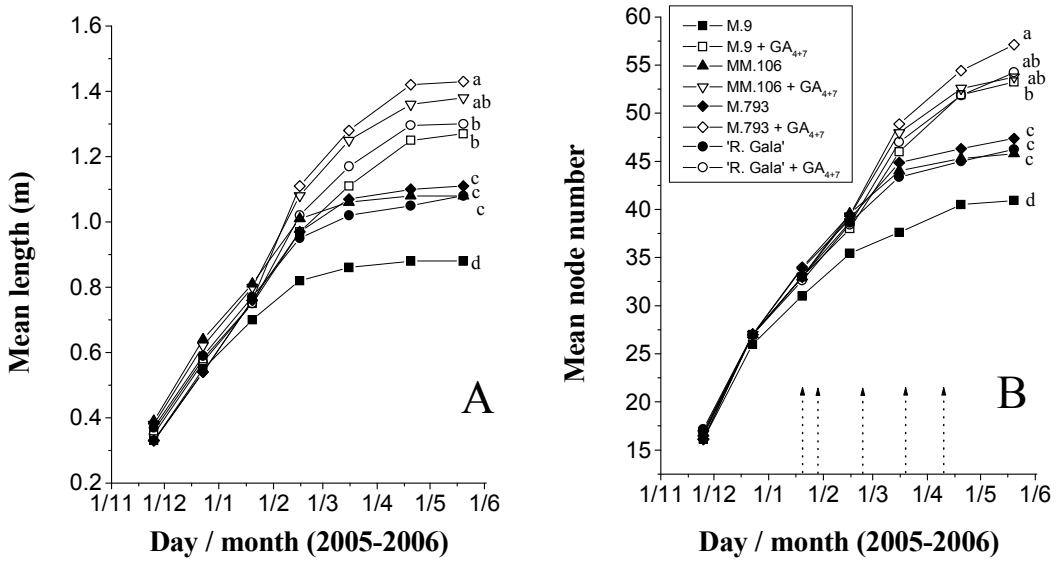


Figure 5.3. Rootstock x gibberellin (GA_{4+7}) interactions on the mean cumulative length (A) and node number (B) for primary shoots of 'Royal Gala' apple scions during their first growing season after grafting. Arrows on 'B' denote the timing of GA_{4+7}. On the 20/5/06, means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over BAP and NPA treatments.

5.3.2.4 Rootstock x BAP x GA_{4+7} interactions on the mean cumulative length and node number of the primary shoot

The rootstock x BAP x GA_{4+7} interactions for the final mean length and node number of the primary shoot were significant at growth cessation in May, 2006 (P=0.02 and P=0.02, respectively) (Figure 5.4). The final mean length and node number of the primary shoot on M.9 was similar to MM.106, M.793 and 'Royal Gala' only with treatments of BAP x GA_{4+7} (Figure 5.4D, H). When compared with untreated trees (i.e.,

Figure 5.4A, E), exogenous BAP decreased the final mean length and node number of the primary shoot only for the scion on the M.9 rootstock (Figure 5.4C, G), which resulted from much earlier termination of the primary shoot from mid February onwards for the BAP-treated scion on M.9 (Table 5.1). With only GA_{4+7} treatment, the final mean length and node number of the primary shoot on M.9 and 'Royal Gala' rootstocks was reduced compared with MM.106 and M.793 (Figure 5.4B, F). However, these factorial means (i.e., Figure 5.4B, F) are comprised of - NPA - BAP + GA_{4+7} and + NPA - BAP + GA_{4+7} treatments.

Examination of the four-way means (see Figure 5.5A, B) indicated that reductions in the final mean length and node number of the primary shoot on M.9 and 'Royal Gala' rootstocks (i.e., Figure 5.4B, F) resulted predominantly from the + NPA - BAP + GA_{4+7} treatment, which decreased the final mean length and node number markedly more for M.9 and 'Royal Gala' than for MM.106 and M.793 (Figure 5.5A, B). However, the rootstock x NPA x GA_{4+7} interaction was not significant for the final mean length (P=0.41) or node number (P=0.20) of the primary shoot (data not shown). For the - NPA - BAP + GA_{4+7} treatment, the final mean node number of the primary shoot was very similar amongst rootstocks (Figure 5.5B), although the primary shoot on M.9 had a slightly shorter final mean shoot (Figure 5.5A) and mean internode length (Figure 5.5C) compared with M.M.106, M.793 and 'Royal Gala'. However, the BAP x GA_{4+7} treatment promoted a primary shoot with a similar final mean length and node number regardless of rootstock type (i.e., Figure 5.4D, H). For the four-way treatment means, the BAP x GA_{4+7} treatment also resulted in a similar final mean length, node number and internode length of the primary shoot on each rootstock type, irrespective of NPA treatment to the rootstock stem (Figure 5.5A, B, C).

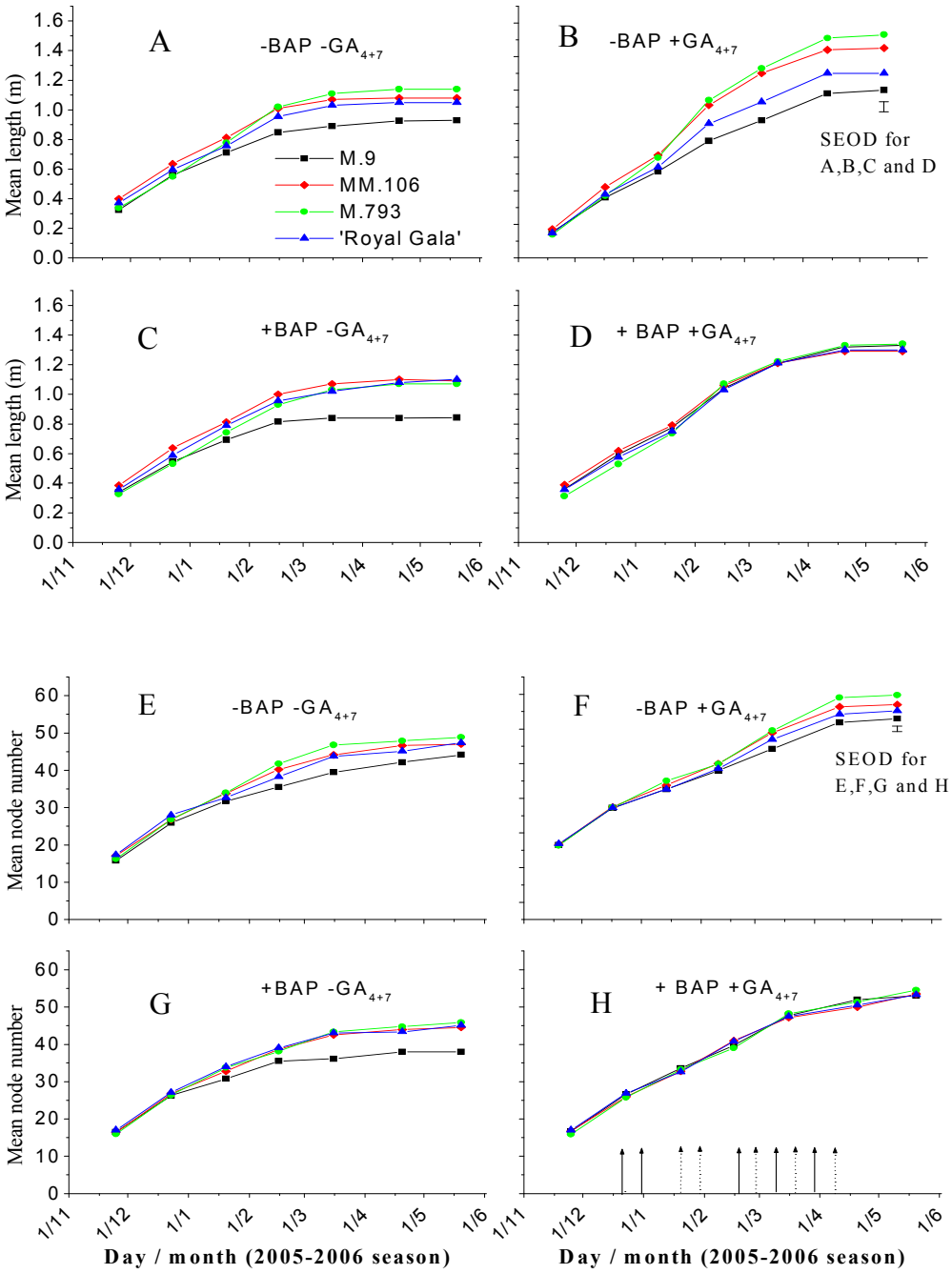


Figure 5.4. Rootstock x benzylaminopurine (BAP) x gibberellin (GA_{4+7}) interactions on the mean cumulative length (A, B, C and D) and node number (E, F, G and H) for primary shoots of 'Royal Gala' apple scions during their first year of growth after grafting. Arrows with a solid or dotted line on H denote the timings of exogenous BAP or GA_{4+7}, respectively. Data are averaged over NPA treatments.

5.3.2.5 Effect of rootstock type and treatment combinations of GA_{4+7}, BAP and NPA on the mean internode length and the relationship between the final length and node number of the primary shoot

The main effects of rootstock, BAP, GA_{4+7} and NPA significantly affected the final mean internode length of the primary shoot (P=0.0004, P=0.004, P<0.0001 and P<0.0001, respectively). For the main effect of rootstock, the final mean internode length of the primary shoot was 23, 25, 24 and 23 mm (MSD=1.2 mm) for M.9, MM.106, M.793 and 'Royal Gala', respectively. For scions with or without BAP, mean internode length of the primary shoot was 24 and 23 mm (MSD=0.7 mm), respectively, whereas for scions treated with or without GA_{4+7} the mean internode length was 25 and 23 mm (MSD=0.7 mm), respectively. For the rootstock treated with or without NPA, the final mean internode length of the primary shoot was 23 and 25 mm (MSD=0.14 mm), respectively. Changes in mean internode length by the main effects, however, were very small.

In addition to the main effects, the four-way rootstock x NPA x BAP x GA_{4+7} interaction was significant (P=0.02) for the final mean internode length of the primary shoot (Figure 5.5C). For untreated trees, the M.9 rootstock decreased the mean length, node number and internode length of the primary shoot compared with MM.106, M.793 and the 'Royal Gala' rootstock control (Figure 5.5A, B, C) With NPA, however, the mean internode length of the primary shoot was decreased only for the scion on MM.106, M.793 and 'Royal Gala' when compared with untreated trees on the same rootstock type (Figure 5.5C). For the untreated scion, rootstocks did not differ in the mean internode length of the primary shoot when NPA was applied to the rootstock stem (Figure 5.5C).

Applications of BAP to the scion on M.9 increased internode length when compared with untreated trees on M.9 (Figure 5.5C). In a similar manner to the BAP-treated scion on M.9 (i.e., - NPA + BAP - GA_{4+7}), NPA x BAP treatment of trees on MM.106, M.793 and 'Royal Gala' rootstocks increased the mean internode length of the primary shoot when compared with the untreated primary shoot on these same NPA-treated rootstocks (Figure 5.5C). In contrast, the NPA x BAP treatment did not affect the mean internode length of the primary shoot on M.9 when compared with the untreated scion on NPA-

treated M.9 (Figure 5.5C). With NPA x BAP treatment, the primary shoot on M.9 had a decreased mean internode length compared with the primary shoot on MM.106, M.793 and 'Royal Gala' (Figure 5.5C).

Exogenous GA_{4+7} increased the mean internode length of the primary shoot on each rootstock type when compared with untreated trees on the same rootstock (Figure 5.5C). However, the mean length of the primary shoot was reduced slightly for the GA_{4+7}-treated scion on M.9 compared with the GA_{4+7}-treated scion on MM.106, M.793 and 'Royal Gala', whilst the mean node number was not (Figure 5.5A, B). Hence, the mean internode length of the primary shoot was decreased slightly for the GA_{4+7}-treated scion on M.9 compared with MM.106, M.793 and 'Royal Gala' (Figure 5.5C). With or without NPA, treatment of the scion with BAP x GA_{4+7} resulted in a very similar final mean shoot length, node number and internode length of the primary shoot on each rootstock type (Figure 5.5A, B, C).

Each rootstock type had a very similar linear relationship between the final length and node number of the primary shoot when the data for all treatments were plotted (Figure 5.6).

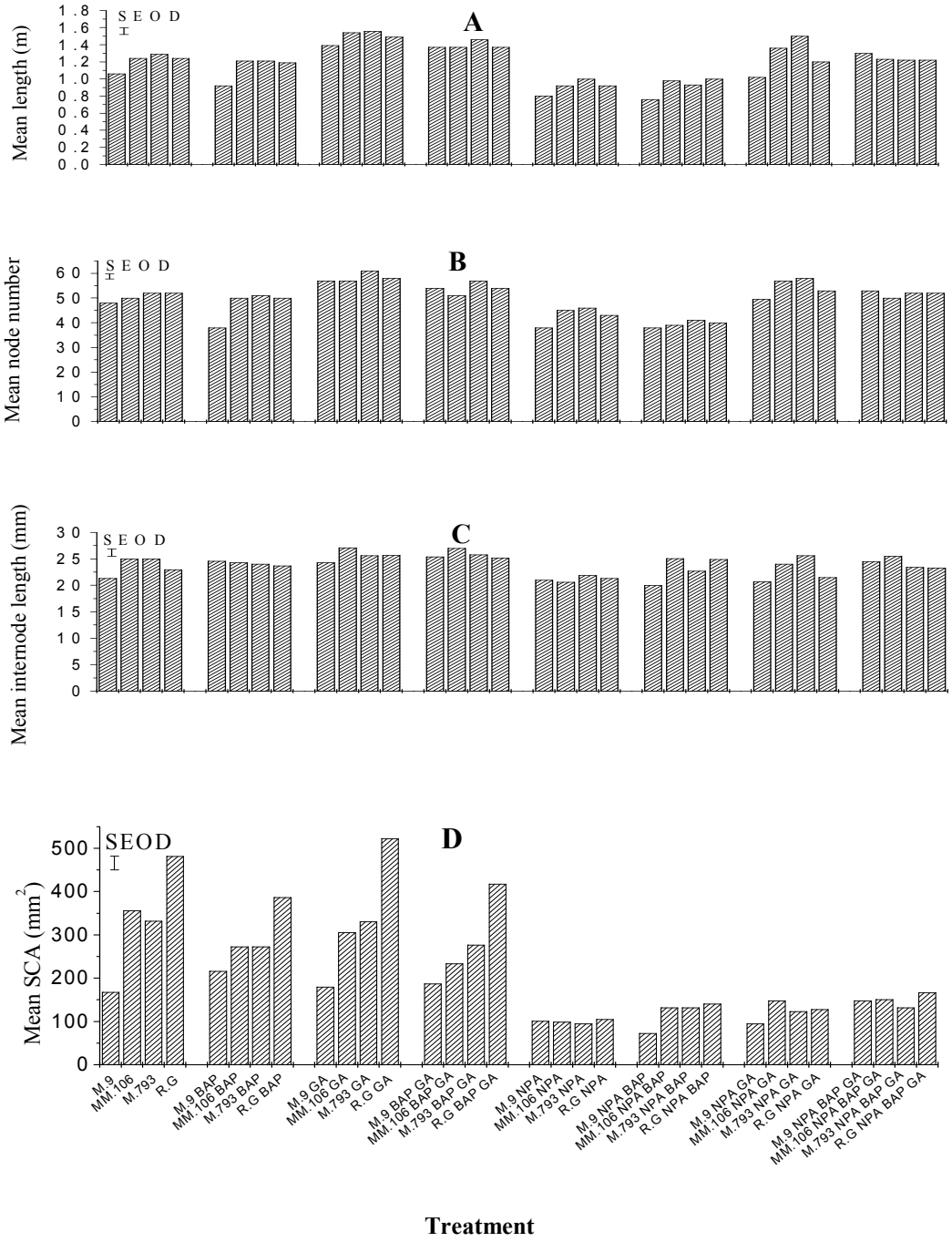


Figure 5.5. Effect of apple rootstocks (M.9, MM.106, M.793 and 'Royal Gala' (R.G)), ± benzylaminopurine (BAP), ± gibberellin (GA) and ± 1-N-naphthylphthalamic acid (NPA) treatments on the final mean length (A), node number (B), internode length (C) and shoot cross-sectional area (SCA) (D) for primary shoots of 'Royal Gala' at the end of their first growing season after grafting.

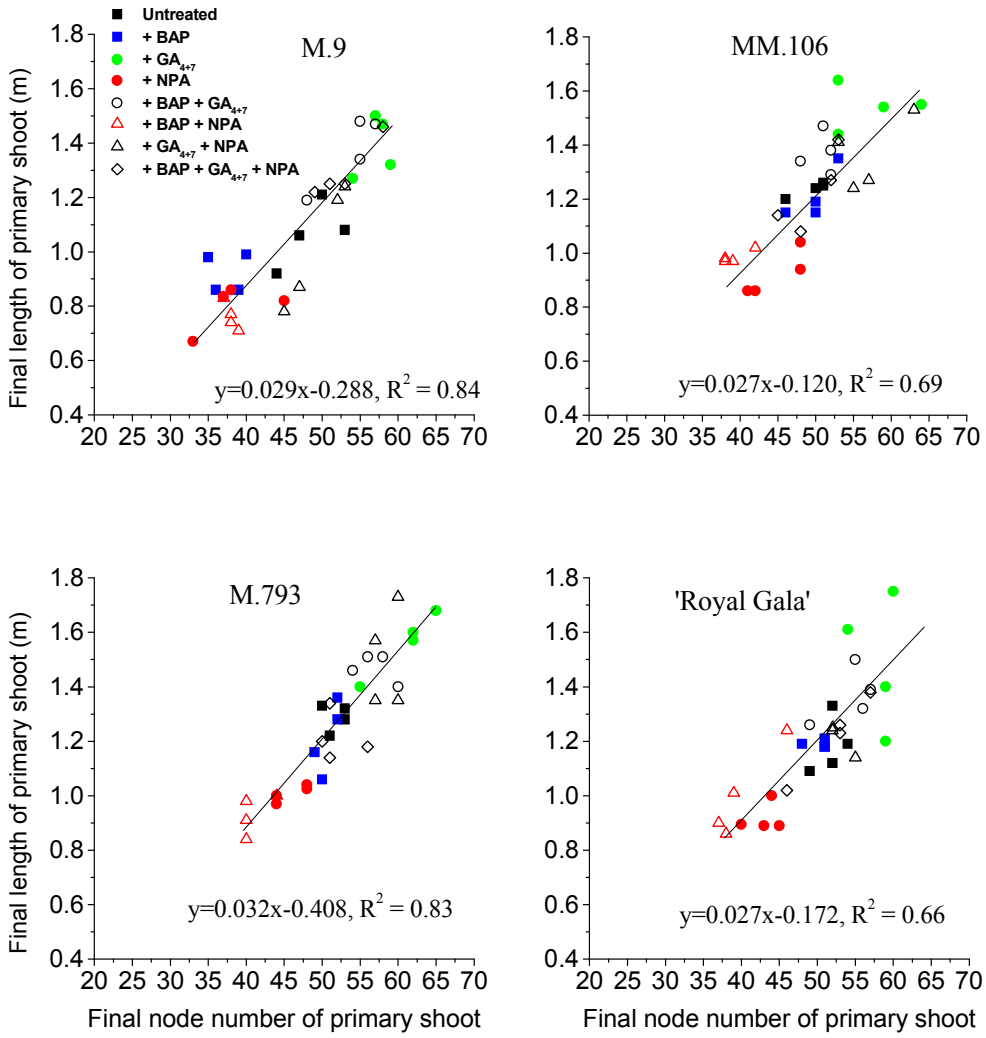


Figure 5.6. Effect of rootstock, benzylaminopurine (BAP), gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) treatments on the relationship between the final length and node number of 'Royal Gala' primary shoots at the end of their first growing season after grafting.

5.3.2.6 Effect of rootstock, NPA, BAP, and GA_{4+7} on termination of the shoot apical meristem (SAM) on the primary shoot

The activity of the SAM on the primary shoot was visually assessed during shoot measurements conducted in February, March and April, 2006. The number of fully terminated apical meristems (Figure 5.7C) was counted for both the primary and secondary shoots and the proportion (%) of terminated shoots for each shoot type was calculated by dividing the number of terminated SAMs by the number of that shoot type

per scion x 100. Data for termination of the primary or secondary shoots could not be appropriately transformed for ANOVA. However, the four-way means are presented to highlight important treatment effects on SAM activity (Table 5.1).

Applications one, two, and three of NPA to the rootstock stem occurred on the 12/12/05, 10/2/06 and the 3/3/06, respectively. Following each application of NPA, young leaves near the tip of the primary shoot exhibited transient epinasty (Figure 5.7B). This was followed by the slowing of growth, and/or, complete termination of the apical meristem after 14 to 21 days (Figure 5.7C). After the first application of NPA, an apical meristem on the primary shoot that had fully terminated typically remained resting for approximately 14 days. Termination of primary shoots on the 16/2/06, or nearly one week after NPA application two, was 100%, 100%, 75% and 75% for M.9, MM.106, M.793 and 'Royal Gala' rootstocks, respectively, which was much greater than the termination of primary shoots for untreated trees on the same rootstock type (compare - BAP - GA_{4+7} + NPA and - BAP - GA_{4+7} - NPA; Table 5.1). Assessment of termination of the primary shoot on the 16/3/06 (Table 5.1) occurred approximately two weeks after the third application of NPA. By the 16/3/06, termination of the primary shoot had decreased for M.793 and, to a lesser extent M.9, as some SAMs began to regrow before fully terminating by April (Table 5.1). Treatments of NPA resulted in a primary shoot with more growth units compared with untreated trees (Figure 5.8A).

Similar to NPA treatment of the rootstock stem, untreated trees on M.9 had increased termination of the primary shoot in February (Table 5.1). However, termination of the primary shoot had decreased in March for untreated trees on M.9, which resulted from previously terminated apical meristems resuming growth. Bicyclic growth of the primary shoot on M.9 was in contrast to the primary shoot on MM.106, M.793 and 'Royal Gala', which developed from a continuous season-long growth flush and was comprised of a single growth unit (Figure 5.8A). For secondary shoots, M.9 and MM.106 imposed greater termination than M.793 and 'Royal Gala' in February and March (compare rootstocks - BAP - GA_{4+7} - NPA; Table 5.1). The M.793 rootstock had a greater proportion of secondary shoots that regrew between February and March (Table 5.1), hence it had a greater proportion of secondary shoots with two growth units when compared with M.9, MM.106 and 'Royal Gala' (i.e., - BAP - GA_{4+7} - NPA; Figure 5.8B).

Similar to NPA, exogenous BAP promoted greater termination of the primary shoot in February when compared with trees on the same rootstock that were untreated (compare - BAP - GA_{4+7} - NPA and + BAP - GA_{4+7} - NPA; Table 5.1). With the exception of M.9, a proportion of primary shoots on BAP-treated scions resumed growth in March before ceasing growth fully by April (Table 5.1). Hence, the BAP-treated scion on rootstocks of MM.106, M.793 and 'Royal Gala' had a greater proportion of primary shoots with two growth units when compared with the untreated primary shoot on these same rootstocks (Figure 5.8A). For secondary shoots, exogenous BAP caused fewer SAMs to terminate in February for rootstocks of M.9, MM.106, M.793 and 'Royal Gala' when compared with untreated trees on these same rootstocks (Table 5.1). In March, shoot termination of the secondary shoots on BAP-treated scions then decreased, presumably as growth resumed (Table 5.1). For scions treated with BAP, the proportion of secondary shoots with two growth units increased with increasing rootstock vigour (Figure 5.8B).

In contrast to BAP or NPA treatment, exogenous GA_{4+7} prevented the termination of the apical meristem on the primary shoot in February and March (Table 5.1). In April, the apical meristem on the primary shoot, even for M.9, had not fully terminated despite shoot extension having mostly ceased (Figure 5.1E, F). With GA_{4+7}, the primary shoot formed solely from a single flush of growth and therefore was comprised of a single growth unit (Figure 5.8A). Exogenous GA_{4+7} also reduced the termination of the secondary shoots for M.9, MM.106, M.793 and 'Royal Gala' during February, March and April when compared with untreated trees on these same rootstocks (Table 5.1). In February, these reductions in termination (%) of the secondary shoots stimulated by GA_{4+7} were more marked for M.9 and MM.106 than for M.793 and 'Royal Gala' rootstocks when each rootstock type was compared with trees on the same rootstock that were untreated (Table 5.1).

5.3.2.7 Effect of rootstock, NPA, BAP and GA_{4+7} on the final shoot cross-sectional area (SCA) of the primary shoot

The main effects of rootstock, NPA and BAP significantly affected the final SCA of the primary shoot ($P<0.0001$, $P<0.0001$ and $P=0.04$, respectively), whereas gibberellin did not ($P=0.11$) (data not shown). For the main effect of rootstock, the mean SCA of the

primary shoot was 146, 212, 212 and 294 mm^2 (MSD=26 mm^2) for M.9, MM.106, M.793 and 'Royal Gala' respectively (data not shown). Similar to the M.9 rootstock, applications of NPA to the rootstock stem significantly decreased the mean SCA of the primary shoot compared with untreated rootstocks (124 and 309 mm^2, respectively; MSD= 14 mm^2). Applications of BAP also reduced the mean SCA of the primary shoot compared with untreated scions (209 and 224 mm^2, respectively; MSD=14 mm^2), but these reductions imposed by BAP were small in comparison to M.9 and the NPA treatment.

In addition to the above main effects, the rootstock x NPA interaction was significant for the final mean SCA of the primary shoot (P<0.0001; data not shown). Rootstocks did not significantly differ in the final mean SCA of the primary shoot when NPA was applied to the rootstock stem and, compared with untreated trees on the same rootstock type, reduced mean SCA caused by NPA was more marked for the scion on NPA-treated MM.106, M.793 and 'Royal Gala' than for NPA-treated M.9 (two-way data not shown, but see Figure 5.5D).

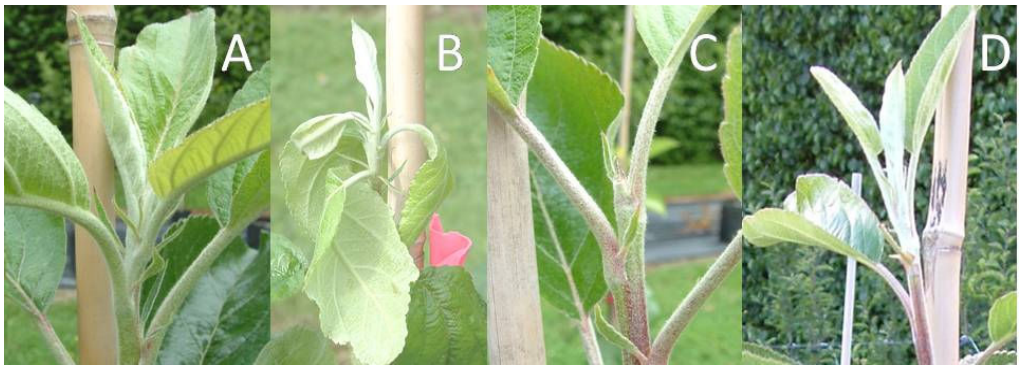


Figure 5.7. Effect of 1-N-naphthylphthalamic acid (NPA) applied to the rootstock stem on activity of the apical meristem on the primary shoot of 'Royal Gala' apple scions. Photo 'A' shows the apex of the primary shoot actively growing before NPA application one (12/12/05), B shows transient epinasty developed 2 to 3 days after NPA treatment, C shows the termination of growth 14 to 21 days after NPA application one and D shows growth resumption of the shoot apex approximately 28 to 35 days after application one of NPA.

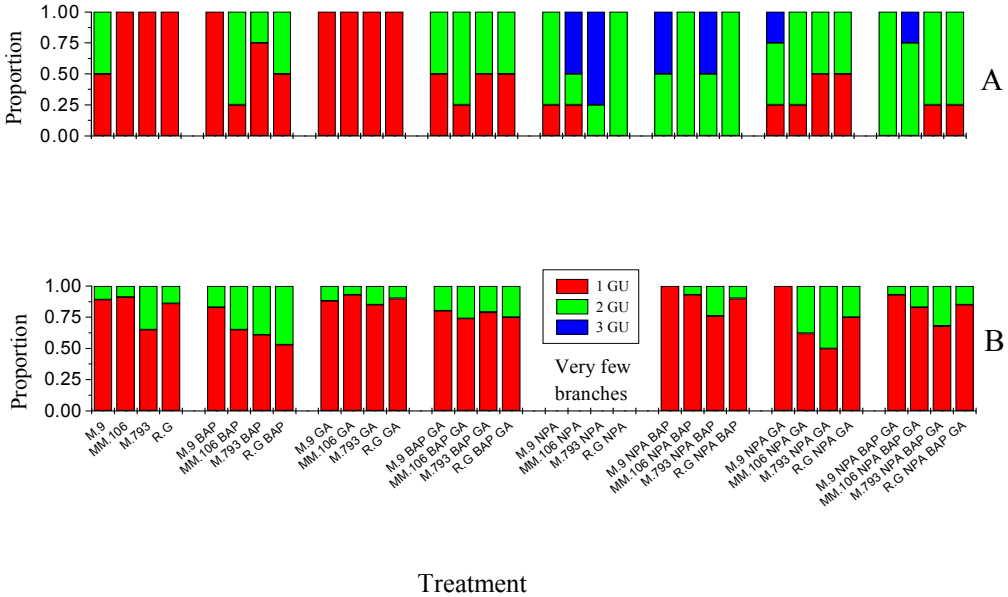


Figure 5.8. Effect of rootstocks, ± benzylaminopurine (BAP), ± gibberellin (GA) and ± 1-N-naphthylphthalamic acid (NPA) on the proportion of 'Royal Gala' primary (A) and secondary shoots (B) that were comprised of one, two or three growth units (GU) at the end of the first season of growth after grafting. Growth units were identified by the presence of bud scale scars, and/or, compressed internodes along each shoot type.

Table 5.1. Effect of rootstock, ± benzylaminopurine (BAP), ± gibberellin (GA_{4+7}) and ± 1-N-naphthylphthalamic acid (NPA) treatments on the mean proportion (%) of primary and secondary shoots that were fully terminated in February, March and April for 'Royal Gala' apple scions during their first growing season after grafting.

				Shoot termination (%) for shoot type and month					
				Primary			Secondary		
Rootstock	BAP	GA_{4+7}	NPA	16/2/06	16/3/06	20/4/06	16/2/06	16/3/06	20/4/06
M.9	-	-	-	50	0	100	89	100	100
MM.106	-	-	-	0	50	100	82	91	100
M.793	-	-	-	0	0	100	69	50	100
'R.Gala'	-	-	-	0	0	75	48	48	100
M.9	+	-	-	75	100	100	85	70	100
MM.106	+	-	-	75	0	100	69	38	100
M.793	+	-	-	50	25	100	54	21	100
'R.Gala'	+	-	-	25	25	100	37	29	100
M.9	-	+	-	0	0	0	16	36	68
MM.106	-	+	-	0	0	0	23	23	80
M.793	-	+	-	0	0	0	22	29	37
'R.Gala'	-	+	-	0	0	0	26	39	45
M.9	-	-	+	100	75	100	NA	NA	NA
MM.106	-	-	+	100	100	100	NA	NA	NA
M.793	-	-	+	75	25	100	NA	NA	NA
'R.Gala'	-	-	+	75	75	100	NA	NA	NA
M.9	+	+	-	0	25	0	33	54	89
MM.106	+	+	-	0	25	0	24	43	91
M.793	+	+	-	0	0	0	35	39	83
'R.Gala'	+	+	-	0	50	0	24	50	90
M.9	+	-	+	100	75	100	92	92	92
MM.106	+	-	+	75	75	100	96	71	100
M.793	+	-	+	100	50	100	81	35	92
'R.Gala'	+	-	+	100	75	100	97	76	100
M.9	-	+	+	0	25	0	NA	NA	NA
MM.106	-	+	+	0	0	0	NA	NA	NA
M.793	-	+	+	0	25	0	NA	NA	NA
'R.Gala'	-	+	+	0	0	0	NA	NA	NA
M.9	+	+	+	25	25	50	20	59	96
MM.106	+	+	+	0	0	100	36	43	96
M.793	+	+	+	0	50	100	40	80	98
'R.Gala'	+	+	+	0	50	75	48	62	87

5.3.3 Treatment effects on the formation of secondary and tertiary axes

The different possible types of secondary and tertiary axes that may form on the newly grafted apple scion were described in Chapter 2 (Section 2.7, Figure 2.29). The first application of BAP occurred on the 23/12/2005. As found in Chapter 3, the axillary buds on the primary shoot began to break approximately 14-days after the first BAP application. In contrast, natural formation of secondary shoots for the 'Royal Gala' scion on each rootstock type was first observed from the 17/12/2005, or one week before the first BAP application. Hence in Chapter 5, natural formation of secondary shoots on the primary shoot occurred approximately five weeks earlier in the growing season than on trees described in Chapter 3 (i.e., in the previous experimental growing season). In addition, the first application of BAP in Chapter 5 coincided very closely with the first major flush of natural branching.

5.3.3.1 Treatment effects on the formation of secondary spurs on the primary shoot

The final number of each axis type was counted at complete growth cessation in late May, 2006. Compared with untreated trees, the main effect of BAP significantly increased (P<0.0001) the proportion (%) of axillary buds on the primary shoot that broke to form a secondary spur and the mean number of secondary spurs that formed (Table 5.2). In contrast, the main effects of rootstock and NPA were not significant (Table 5.2). The main effects of GA_{4+7} (Table 5.2) were less significant than the BAP x GA_{4+7} interactions for the mean proportion (%) of axillary buds on the primary shoot that formed a secondary spur (P<0.0001, data not shown) and the mean number of secondary spurs formed per scion (P<0.0001, Figure 5.9A). Compared with untreated scions, BAP stimulated the greatest number of secondary spurs when applied alone, whereas applications of BAP x GA_{4+7} resulted in only very small increases in mean spur number compared with untreated trees (Figure 5.9A). Exogenous GA_{4+7} significantly increased the mean spur number compared with untreated scions only when applied sequentially after BAP (Figure 5.9A).

The NPA x BAP interaction was also significant for the mean proportion (%) of axillary buds on the primary shoot that formed a secondary spur (P=0.0003, data not shown) and the mean number of secondary spurs formed per scion (P=0.002, Figure 5.9B). Without BAP, NPA applications to the rootstock stem significantly reduced the mean number of

secondary spurs formed on the primary shoot when compared with untreated trees (Figure 5.9B). However, this effect of NPA treatment was reversed when BAP was applied to the scion (Figure 5.9B). Although not significant, the NPA x BAP treatment tended to increase the mean number of secondary spurs that formed compared with trees treated with BAP alone (Figure 5.9B).

The four-way rootstock x NPA x BAP x GA_{4+7} interaction was also significant for the mean proportion of axillary buds on the primary shoot that formed a secondary spur (P=0.05) and approached significance for the mean number of secondary spurs formed (P=0.06) (see data for secondary spurs in Figure 5.13A, B). For untreated trees, the mean number of secondary spurs decreased with increasing rootstock vigour, and this rootstock effect was greater when BAP was applied to the scion (Figure 5.13B). However, rootstocks produced a comparable mean number of secondary spurs when the scion was treated with BAP x GA_{4+7}, and this occurred regardless of whether NPA was applied to the rootstock stem (Figure 5.13B). Similar to trees on M.9 treated with BAP only, NPA x BAP-treated trees on MM.106, M.793 and 'Royal Gala' produced an increased number of secondary spurs when compared with untreated trees on the same rootstock type (Figure 5.13B).

Table 5.2. Main effects of rootstock, benzylaminopurine (BAP), gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) on the mean proportion (%) of axillary buds on the primary shoot that broke to form an axillary structure (spur or shoot) and the mean number of shoot types on 'Royal Gala' apple scions by the end of their first season of growth after grafting.

	Axis type on					
	Primary shoot			Secondary shoots		
Main effect	Secondary spurs	Secondary shoots	Total on primary shoot	Tertiary spurs	Tertiary shoots	Total axes per scion
Rootstock	*Proportion (%) of axillary bud break on primary shoot*					
M.9	6.30 (2.08) ^{ns}	14.77 a ***	21.07 ^{ns}			
MM.106	5.33 (1.83)	17.92 b	23.25			
M.793	3.70 (1.59)	19.76 b	23.46			
'Royal Gala'	4.40 (1.72)	18.48 b	22.88			
BAP						
-	2.63 (1.16 a) ***	7.00 a ***	9.63 a ***			
+	7.24 (2.45 b)	28.48 b	35.72 b			
GA_{4+7}						
-	6.62 (2.02) ** ^{x}	15.17 a ***	21.79 ^{ns}			
+	3.24 (1.58)	20.29 b	23.53			
NPA						
-	4.89 (1.93) ^{ns}	23.16 a ***	28.05 a ***			
+	4.98 (1.68)	12.29 b	17.27 b			
Rootstock	*Mean number of shoot type(s)*					
M.9	2.81 (1.67) ^{ns}	7.31 a ***	10.12 a *	4.69 ^{y}	7.06 ^{y}	21.87 (4.00 a) ***
MM.106	2.50 (1.58)	8.84 b	11.34 ab	5.63	9.06	26.03 (4.47 b)
M.793	1.87 (1.44)	10.31 b	12.18 b	4.94	11.47	28.59 (4.68 b)
'Royal Gala'	2.09 (1.50)	9.28 b	11.37 ab	4.09	12.25	27.71 (4.57 b)
BAP						
-	1.42 (1.26 a) ***	3.79 a ***	5.21 a ***	0.13 ^{y}	0.47	5.81 (2.22 a) ***
+	3.22 (1.84 b)	14.08 b	17.30 b	9.55	19.45	46.30 (6.64 b)
GA_{4+7}						
-	2.88 (1.66) * ^{x}	6.98 a ***	9.86 a ***	5.21^{y}	6.28	21.35 (3.88 a) ***
+	1.77 (1.44)	10.89 b	12.66 b	4.45	13.64	30.75 (4.97 b)
NPA						
-	2.48 (1.62) ^{ns}	12.08 a ***	14.56 a ***	5.75 ^{y}	15.90 ^{y}	36.21 (5.51 a) ***
+	2.16 (1.47)	5.80 b	7.96 b	3.92	4.01	15.89 (3.36 b)

Proportion (%) for secondary spurs, shoots and secondary axes was calculated as the total number of these shoot type(s) per scion divided by the total node number on the primary shoot x 100. ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single growth attribute and main effect only, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. Data without and within parenthesis are raw or transformed means, respectively. ^{x} denotes that the F-ratio of a significant main effect is similar or smaller than interaction(s) involving that main effect. ^{y} data unable to be appropriately transformed for ANOVA.

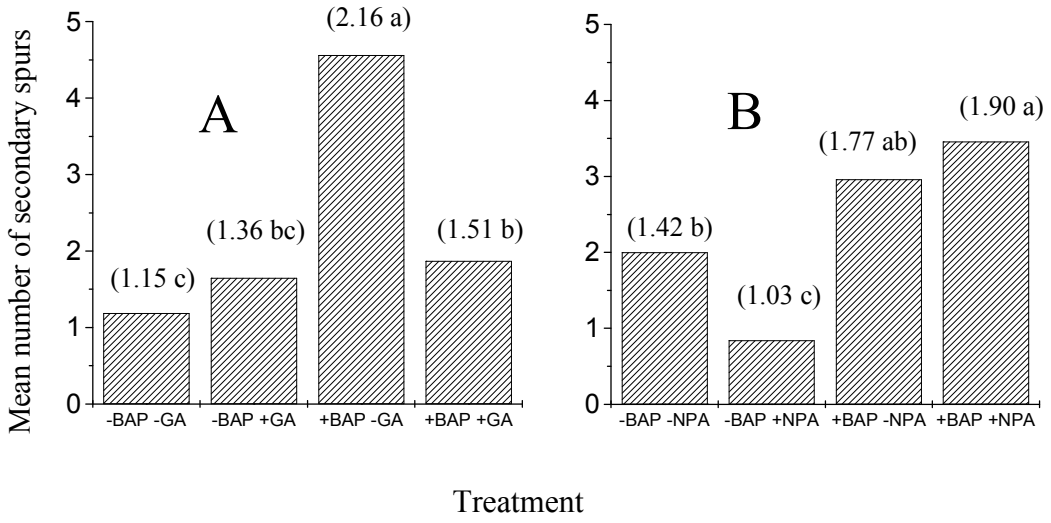


Figure 5.9. Interactions between benzylaminopurine (BAP) and gibberellin (GA_{4+7} (GA)) applied to 'Royal Gala' apple scions (A) or applications of BAP applied to the scion and 1-N-naphthylphthalamic acid (NPA) applied to the rootstock stem (B) on the mean number of secondary spurs formed on the primary shoot. For a single graph, means in parenthesis sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over rootstocks and NPA or rootstocks and GA_{4+7} for graph A or B, respectively.

5.3.3.2 Treatment effects on the formation of secondary shoots on the primary shoot

The main effects of rootstock, BAP, GA_{4+7} and NPA significantly ($P<0.0001$) affected the proportion of axillary buds on the primary shoot that broke to form a secondary shoot and the mean number of secondary shoots that formed per scion (Table 5.2). Applications of BAP and, to a lesser extent, GA_{4+7} increased the proportion of axillary buds on the primary shoot that formed a secondary shoot and the mean number of secondary shoots that formed per scion. In contrast, the M.9 rootstock or NPA significantly decreased the formation of secondary shoots (Table 5.2).

The NPA x BAP x GA_{4+7} interactions were significant for the mean proportion (%) of axillary buds on the primary shoot that formed a secondary shoot ($P=0.02$) and the mean number of secondary shoots formed per scion ($P=0.0006$) (Figure 5.10A and B,

respectively). Without NPA, exogenous BAP or GA_{4+7} significantly increased the proportion (%) of axillary buds that formed a secondary shoot and the mean number of secondary shoots that formed per scion when compared with the untreated trees (Figure 5.10A, B). However, exogenous BAP promoted a significantly greater proportion of axillary buds to break and the formation of significantly more secondary shoots than GA_{4+7} (Figure 5.10A, B). In contrast, NPA significantly decreased the mean proportion (%) of axillary buds on the primary shoot that formed a secondary shoot and the mean number of secondary shoots that formed per scion compared with trees that were untreated (Figure 5.10A, B).

Without NPA, there were trends that BAP x GA_{4+7} increased the mean proportion (%) of axillary buds that formed a secondary shoot and the mean number of secondary shoots that formed when compared with scions treated with BAP only. However, secondary shoot formation in response to BAP x GA_{4+7} was significantly greater than the BAP treatment only when NPA was applied to the rootstock stem (Figure 5.10A, B). The NPA x GA_{4+7} treatment did not significantly increase the formation of secondary shoots compared with untreated trees or trees treated with NPA alone (Figure 5.10A, B). In contrast, the NPA x BAP treatment significantly increased the mean proportion (%) of axillary buds on the primary shoot that formed a secondary shoot and the mean number of secondary shoots formed when compared with untreated scions growing on NPA-treated rootstocks. Despite this, the mean number of secondary shoots formed in response to the NPA x BAP treatment was not statistically different from untreated trees (Figure 5.10B). Thus, BAP did not significantly increase the mean number of secondary shoots compared with the untreated trees when it was applied to scions growing on NPA-treated rootstocks (Figure 5.10B).

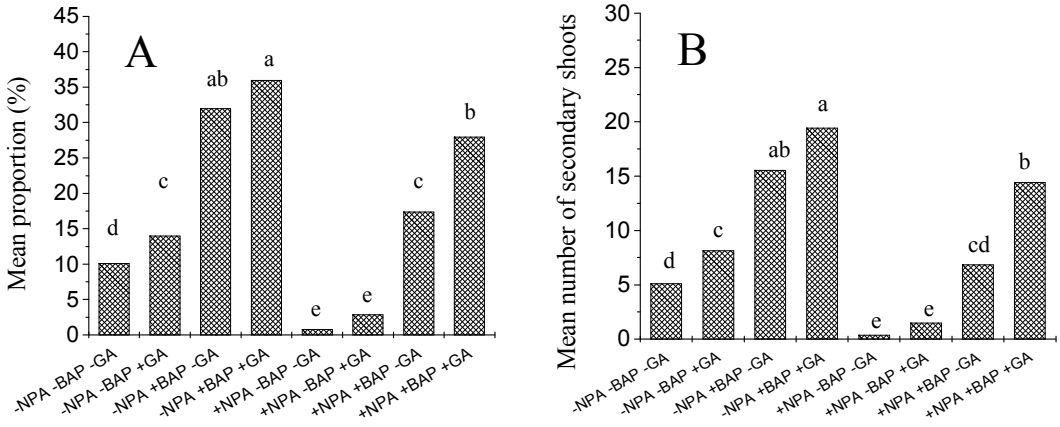


Figure 5.10. 1-N-naphthylphthalamic acid (NPA) x benzylaminopurine (BAP) x gibberellin (GA_{4+7} (GA)) interactions for the mean proportion (%) of axillary buds on the primary shoot that formed a secondary shoot (A) and the mean number of secondary shoots formed per 'Royal Gala' scion (B). For a single graph, means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over rootstocks.

The three-way rootstock x BAP x GA_{4+7} interaction was also significant for the mean number of secondary shoots formed on the primary shoot (P=0.04), but less so for the proportion (%) of axillary buds that formed a secondary shoot (P=0.20) (data not shown). The four-way treatment means are presented to show how the rootstock x BAP x GA_{4+7} interaction for the secondary shoots arose, and to compare some treatment means of particular interest, especially the effect of rootstock type on the mean number of secondary shoots formed per scion (Figure 5.11A, B).

For trees that were untreated, the scion on M.9 had approximately a third fewer secondary shoots than MM.106, M.793 and the 'Royal Gala' rootstock control (Figure 5.11B). Using unrestricted t-tests, the M.9 rootstock significantly decreased the mean number of secondary shoots formed compared with the scion on MM.106, M.793 and 'Royal Gala' at P=0.05, 0.01 and 0.02, respectively (data not shown). Although reductions in the mean number of secondary shoots imposed by M.9 may appear small (Figure 5.11B), they had a pronounced effect on the final size and architecture of the scion (see untreated trees in Figure 5.27).

Exogenous GA_{4+7} increased the mean number of secondary shoots per scion for each rootstock type, although increases in secondary shoot number when compared with the untreated trees were more marked for M.9 and M.793 than for MM.106 and 'Royal Gala' (Figure 5.11B). Interestingly, the scion on M.9 treated with GA_{4+7} (i.e., - NPA - BAP + GA_{4+7}) developed a very similar mean number of secondary shoots compared with the scion on MM.106, M.793 and 'Royal Gala' that was untreated (i.e., - NPA - BAP - GA_{4+7}) (Figure 5.11B). For each rootstock type, exogenous BAP increased the mean proportion (%) of axillary buds on the primary shoot that formed a secondary shoot and the mean number of secondary shoots formed per scion. However, the BAP-treated scion on M.9 had a reduced proportion (%) of axillary buds that formed a secondary shoot and therefore fewer secondary shoots when compared with the BAP-treated scion on MM.106, M.793 and 'Royal Gala' (Figure 5.11A, B). Notably, the scion on M.9 treated with BAP x GA_{4+7} developed a very similar final mean number of secondary shoots compared with the BAP x GA_{4+7}-treated scion on M.793 and 'Royal Gala' (Figure 5.11B).

The rootstock x NPA and the rootstock x BAP x NPA interactions approached significance (P=0.14 and P=0.12, respectively) for the mean number of secondary shoots formed per scion (data not shown, but see four-way means in Figure 5.11B). Applications of NPA to the rootstock stem almost completely inhibited secondary shoot formation, and this effect of NPA was more marked for the scion on MM.106, M.793 and 'Royal Gala' rootstocks than on M.9 when compared with untreated trees on these same rootstocks (Figure 5.11B). The NPA x BAP treatment increased the mean number of secondary shoots formed per scion for each rootstock type when compared with trees on the same rootstock that were treated with only NPA or that were untreated (Figure 5.11B). However, the NPA x BAP treatment promoted more secondary shoots for the scion on MM.106, M.793 and 'Royal Gala' than on M.9. In contrast, application of BAP x GA_{4+7} to the scion on NPA-treated M.9 markedly increased the mean number of secondary shoots formed compared with untreated trees on M.9, and resulted in a very similar mean number of secondary shoots compared with trees on MM.106, M.793 and 'Royal Gala' treated with NPA x BAP x GA_{4+7} (Figure 5.11B). For M.9 only, the NPA x BAP x GA_{4+7} treatment resulted in a greater mean number of secondary shoots compared with trees on M.9 treated with BAP alone.

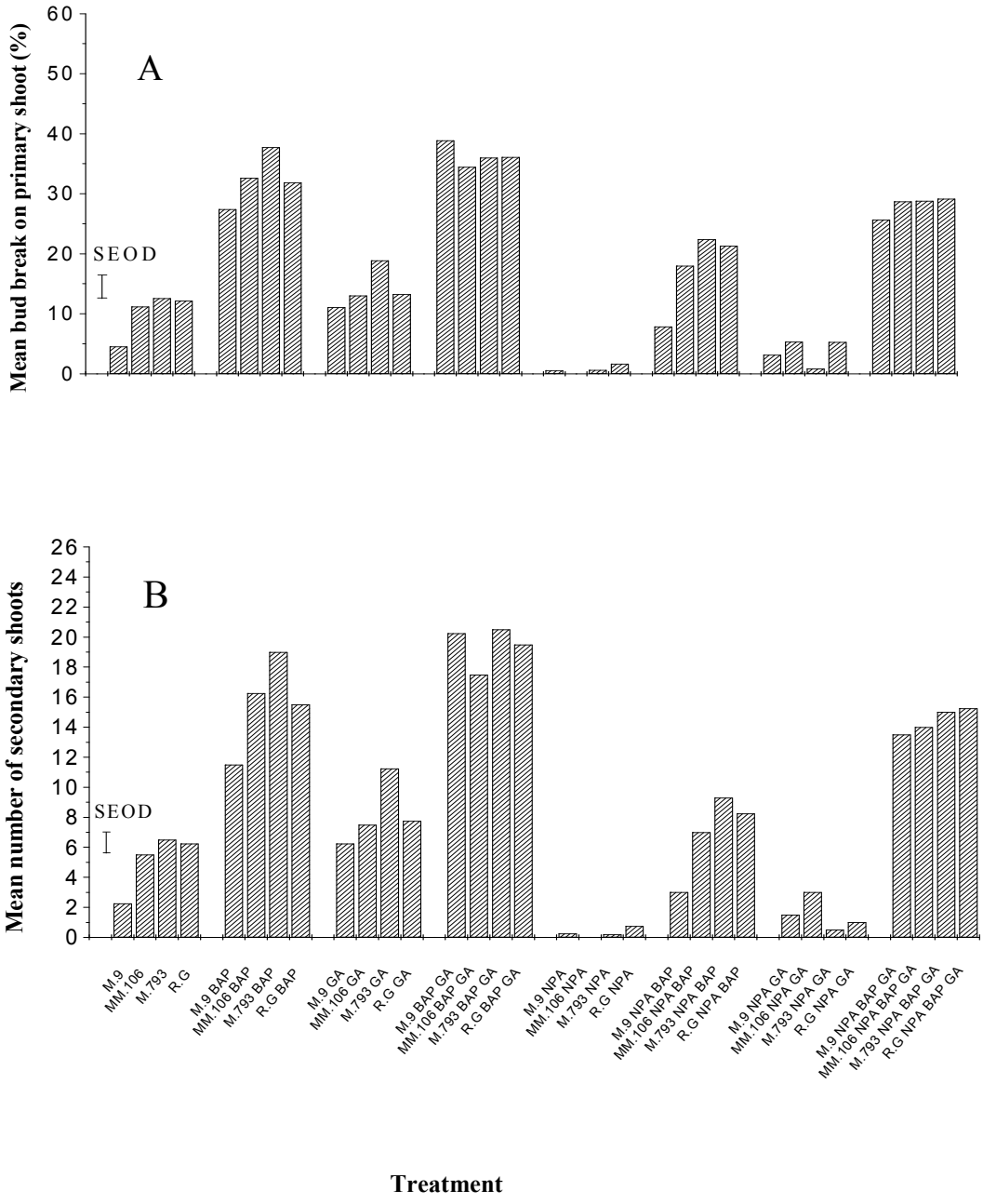


Figure 5.11. Effect of rootstocks and treatment combinations of benzylaminopurine (BAP), gibberellin (GA_{4+7} (GA)) and 1-N-naphthylphthalamic acid (NPA) on the mean proportion (%) of axillary buds on the primary shoot that formed a secondary shoot (A) and the mean number of secondary shoots (B) on 'Royal Gala' apple scions at the end of their first growing season from grafting.

5.3.3.3 Treatment effects on the total number of secondary axes formed on the primary shoot

The main effects of BAP and NPA significantly increased or decreased, respectively, the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes (i.e., total of spurs plus shoots) and the mean number of secondary axes that were formed per scion (Table 5.2). Exogenous gibberellin also increased the mean number of secondary axes formed on the primary shoot compared with untreated trees, although when the number of secondary axes was expressed as a proportion (%) to the number of nodes formed on the primary shoot, the main effect of gibberellin was not significant (i.e., plus gibberellin formed proportionally more secondary shoots but proportionally fewer secondary spurs, Table 5.2).

Although differences were large between treatment combinations, the NPA x BAP x GA_{4+7} interaction was not highly significant (P=0.16) for the proportion (%) of axillary buds on the primary shoot that formed secondary axes (Figure 5.12A), but was significant (P=0.002) for the mean number of secondary axes that formed on the primary shoot (Figure 5.12B). With NPA, scions that were untreated or treated with BAP, GA_{4+7} or BAP x GA_{4+7} produced significantly fewer secondary axes when compared with the same treatment applied to the scion on untreated rootstocks (Figure 5.12B). Nevertheless, BAP or BAP x GA_{4+7} applied to the scion growing on NPA-treated rootstocks significantly increased the mean number of secondary axes that formed per scion when compared with the untreated scion growing on rootstocks treated with or without NPA (Figure 5.12B). Compared with exogenous BAP, the BAP x GA_{4+7} treatment increased the mean number of secondary axes formed per scion, although differences were significant only when NPA was applied to the rootstock stem (Figure 5.12B). Compared with the untreated trees, GA_{4+7} significantly increased the mean number of secondary axes formed per scion only when the rootstock stem was untreated (Figure 5.12B).

Without NPA, exogenous BAP promoted significantly more secondary axes than GA_{4+7}, and a similar difference occurred between these treatments for the scion on NPA-treated rootstocks (Figure 5.12B). In addition, BAP tended to increase the mean number of secondary spurs that formed compared with the untreated trees, and this effect was more

marked when NPA was applied to the rootstock stem (Figure 5.12B). Interestingly, treatments of NPA x BAP or NPA x BAP x GA_{4+7} caused a very similar proportion (%) of axillary buds on the primary shoot to break and form secondary axes (Figure 5.12A). However, these treatments significantly differed in the mean number of secondary axes that formed per scion (Figure 5.12B). Notably, the NPA x BAP x GA_{4+7} treatment also had a greater final mean number of nodes on the primary shoot than the NPA x BAP treatment (Figure 5.5B). In addition, trends existed within the raw data of these treatments for the number of secondary axes formed per scion to increase with increasing node number of the primary shoot (data not shown).

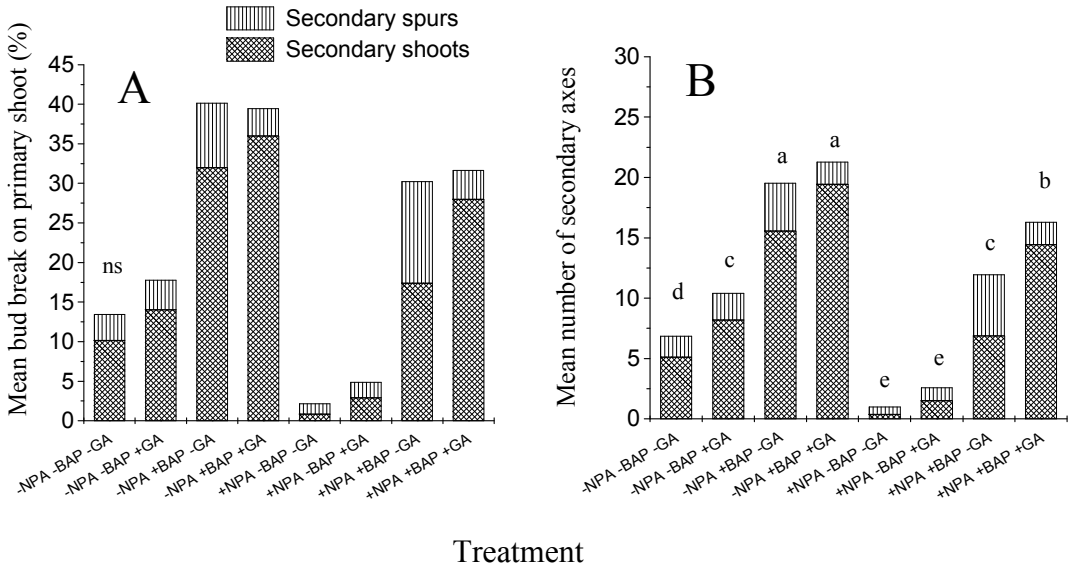


Figure 5.12. 1-N-naphthylphthalamic acid (NPA) x benzylaminopurine (BAP) x gibberellin (GA_{4+7} (GA)) interactions for the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes (spurs + secondary shoots) (A) and the mean number of secondary axes formed per 'Royal Gala' scion (B). The interaction is for the total height of the columns and A and B were significant at *P*=0.16 and *P*=0.002, respectively. Within graph B, means sharing the same letter are not significantly different at *P*≤0.05 (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over rootstocks.

The main effect of rootstock was not significant for the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes (*P*=0.43). However, the mean number of secondary axes formed on the primary shoot was significantly affected by rootstock type (*P*=0.02) (Table 5.2). The four-way rootstock x NPA x BAP x GA_{4+7}

interactions approached significance for the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes (P=0.08) and for the mean number of secondary axes formed per scion (P=0.10). Although not highly significant, these data are presented for interpretation of trends that are of physiological importance, particularly comparisons of how rootstocks affected the initial formation of secondary axes differently in response to treatment combinations of BAP, GA_{4+7} and NPA (Figure 5.13A, B).

For untreated trees on M.9, the proportion (%) of axillary buds on the primary shoot that formed secondary axes and the mean number of secondary axes that formed per scion was reduced by approximately one-third when compared with the scion on MM.106, M.793 and the 'Royal Gala' rootstock control (Figure 5.13A, B). The decreased mean number of secondary axes for the scion on M.9 was the result of decreased formation of secondary shoots rather than secondary spurs (Figure 5.13B).

With BAP (i.e., - NPA + BAP - GA), a similar mean proportion (%) of axillary buds on the primary shoot formed secondary axes for the scion on M.9, MM.106 and M.793, but the primary shoot on M.9 had a slightly greater proportion (%) of axillary buds that formed secondary axes than 'Royal Gala' (Figure 5.13A). In contrast, the BAP-treated scion on M.9 produced a similar mean number of secondary axes to 'Royal Gala', but fewer secondary axes than MM.106 and M.793 (Figure 5.13B). Slight differences in the rootstock x BAP interaction for M.9 between Figure 5.12A and B resulted partly from the BAP-treated primary shoot on M.9 developing slightly longer internodes (Figure 5.5C) and forming fewer neoformed nodes (Figure 5.5B), hence the formation of secondary axes was greater for M.9 than for 'Royal Gala' when the number of secondary axes on BAP-treated trees was expressed as a proportion (%) to the number of nodes formed on the primary shoot (i.e., Figure 5.13A). With BAP x GA_{4+7}, however, the primary shoot on M.9 developed a similar mean number of nodes (Figure 5.5B), internode length (Figure 5.5C) and mean number of secondary axes compared with the BAP x GA_{4+7}-treated scion on M.793 and 'Royal Gala' (Figure 5.13B).

Despite BAP reducing the mean node number of the primary shoot on M.9 (Figure 5.5B), it promoted a comparable mean number of secondary axes to the BAP-treated

scion on 'Royal Gala', but the BAP-treated scion on M.9 had an increased mean number of secondary spurs (Figure 5.13B). For the NPA x BAP treatment, the scion on MM.106 and 'Royal Gala' also formed markedly more secondary spurs than trees on these same rootstocks that were treated with BAP only (Figure 5.13B). With NPA, the untreated scion on each rootstock type developed very few secondary axes when compared with untreated trees on the same rootstock (Figure 5.13B).

Treatment of the scion with BAP x GA_{4+7}, particularly on M.9, decreased the mean number of secondary spurs and increased the mean number of secondary shoots that formed when compared with the BAP-treated scion (compare M.9 - NPA + BAP - GA_{4+7} and M.9 - NPA + BAP + GA_{4+7}, Figure 5.13B). A very similar result occurred for NPA-treated rootstocks whereby more secondary shoots developed, particularly for M.9, with treatments of BAP x GA_{4+7} than with BAP (Figure 5.13B).

5.3.3.4 Treatment effects on the formation of tertiary spurs on the secondary shoots

The data sets for tertiary spur or shoot numbers could not be appropriately transformed to meet the assumptions of ANOVA because tertiary axes formed predominantly in response to exogenous BAP, hence almost half of the data set contained means equal to zero (see Figure 5.13C). Data for tertiary spurs was able to be analysed using ANOVA by removing treatments without BAP. For BAP-treated scions, this enabled interactions among rootstock, GA_{4+7} and NPA to be tested.

Rootstock type or gibberellin treatment did not significantly affect the mean number of tertiary spurs formed on the BAP-treated scion (data not shown). However, the rootstock x NPA interaction was significant for the mean number of tertiary spurs formed per scion (P=0.01, Figure 5.14A). Without NPA, rootstock type did not significantly affect the mean number of tertiary spurs formed on the BAP-treated scion, although there was a trend for the mean number of tertiary spurs to decrease with increasing rootstock vigour (Figure 5.14A). NPA treatment of the rootstock stem significantly decreased the mean number of tertiary spurs only for the BAP-treated scion on the M.9 rootstock (Figure 5.14A).

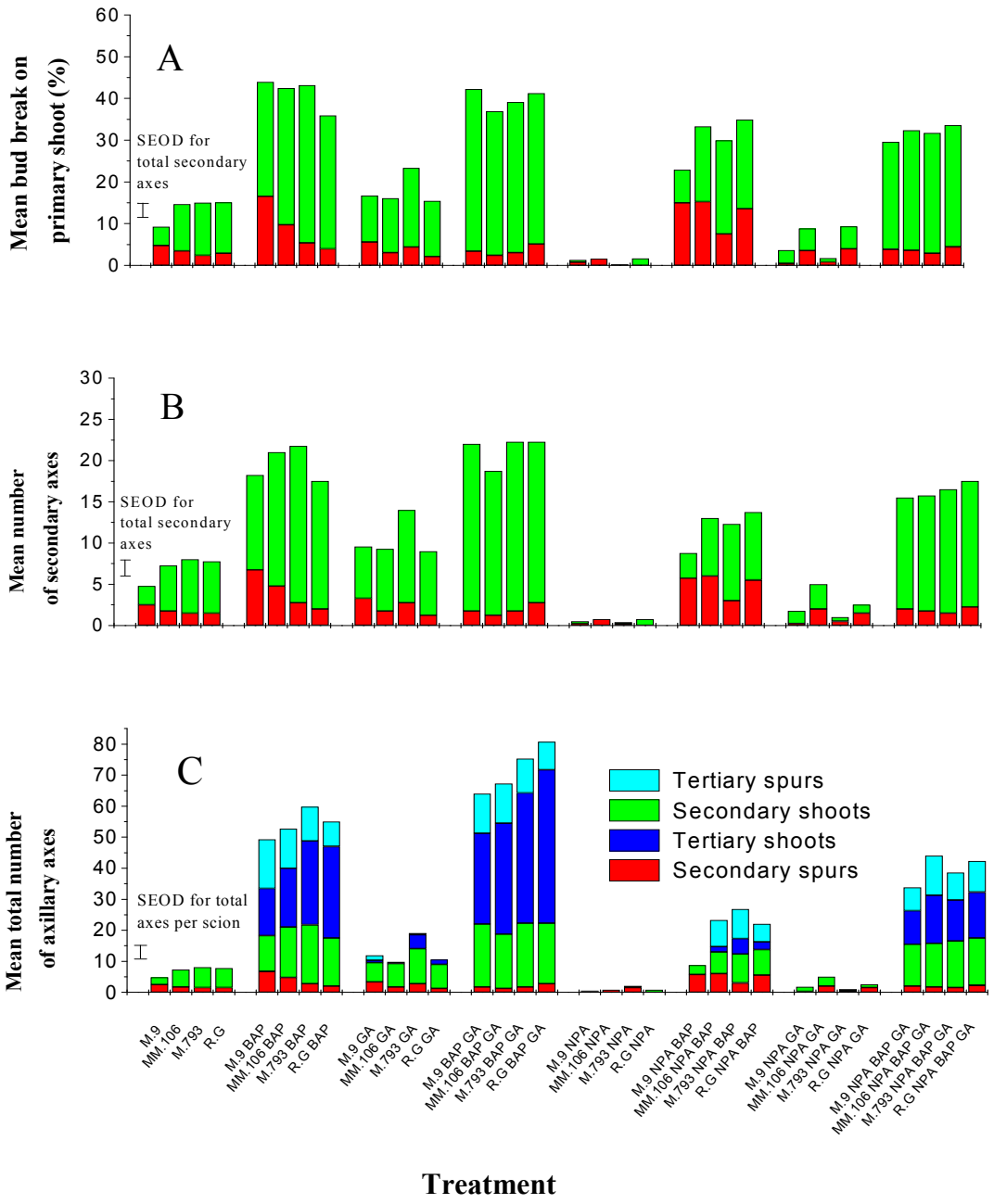


Figure 5.13. Effect of rootstock type and treatment combinations of benzylaminopurine (BAP) gibberellin (GA_{4+7} (GA)) and 1-N-naphthylphthalamic acid (NPA) on the mean proportion (%) of axillary buds on the primary shoot that formed secondary axes (A), the mean number of secondary axes (B) and the mean total number of axillary axes formed per scion (C) shown as the component parts comprising of tertiary spurs, secondary shoots, tertiary shoots or secondary spurs. The SEOD on A, B and C are for the total height of the columns.

5.3.3.5 Treatment effects on the formation of tertiary shoots on the secondary shoots

As for tertiary spurs, the formation of tertiary shoots was predominantly a result of exogenous BAP (see Figure 5.13C). Even with the removal of treatments without BAP, data could not be satisfactorily transformed for ANOVA.

For the scion treated with BAP, the main effect of NPA was to decrease the mean number of tertiary shoots formed per scion. With or without NPA, the mean number of tertiary shoots formed per scion was 8 and 31, respectively. In contrast to the main effect of NPA, GA_{4+7} applied to the BAP-treated scion increased the mean number of tertiary shoots that formed when compared with the scion treated with BAP alone (26 and 13 tertiary shoots per scion, respectively). For the rootstock main effect, the mean number of tertiary shoots formed on BAP-treated scions was 7, 9, 11 and 12 for M.9, MM.106, M.793 and 'Royal Gala', respectively. Hence, the mean number of tertiary shoots increased with increasing rootstock vigour.

There were also trends of a rootstock x NPA interaction (Figure 5.14B) whereby rootstocks did not greatly differ in the mean number of tertiary shoots formed when NPA was applied to the rootstock stem (Figure 5.14B). Without NPA, the mean number of tertiary shoots on the BAP-treated scion increased with increasing rootstock vigour (Figure 5.14B), which was in contrast to the mean number of tertiary spurs that decreased with increasing rootstock vigour (Figure 5.14A).

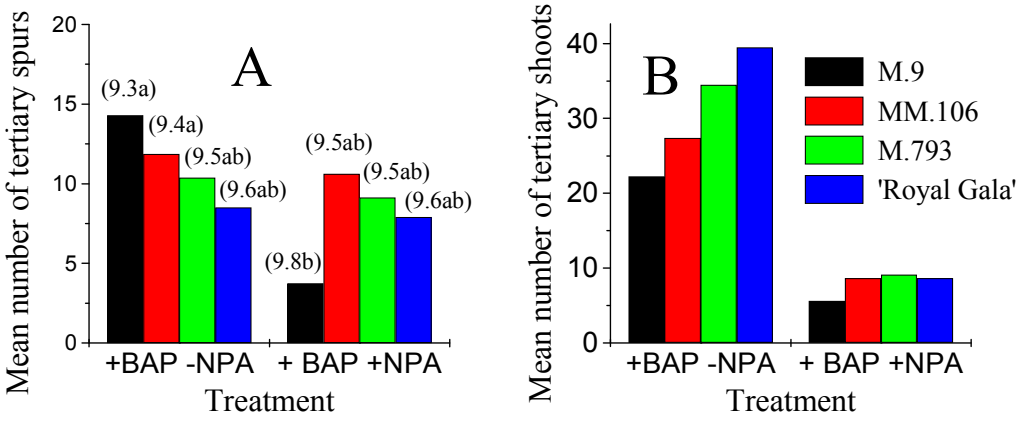


Figure 5.14. Rootstock x 1-N-naphthylphthalamic acid (NPA) interaction for the mean number of tertiary spurs (A) and shoots (B) formed on secondary shoots of 'Royal Gala' apple scions treated with benzylaminopurine (BAP). Transformed means in parenthesis on 'A' sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over gibberellin treatments and excludes data for the minus BAP treatment. Data for 'B' could not be transformed appropriately for ANOVA.

5.3.3.6 Treatment effects on the total number of axillary axes formed per scion

The main effects of rootstock, BAP, GA_{4+7} and NPA significantly affected ($P<0.0001$) the mean total number of axillary axes that formed per scion (i.e., secondary spurs and shoots plus tertiary spurs and shoots) (Table 5.2). For the main effect of rootstock, M.9 significantly decreased the mean total number of axillary axes produced per scion compared with rootstocks of MM.106, M.793 and 'Royal Gala', but means were not statistically different amongst the latter three rootstocks (Table 5.2). Exogenous BAP or GA_{4+7} significantly increased the mean total number of axillary axes formed compared with untreated scions, whereas NPA significantly decreased the mean total number of axillary axes compared with untreated rootstocks (Table 5.2). Interpretation of the BAP, GA_{4+7} and NPA main effects also depended on interactions among these treatments ($P=0.02$) (Figure 5.15).

Compared with untreated trees (i.e., - NPA - BAP - GA_{4+7}), exogenous GA_{4+7} did not significantly increase the mean total number of axillary axes formed per scion when NPA was applied to the rootstock stem (Figure 5.15). With or without NPA, exogenous

BAP stimulated a significantly greater mean total number of axillary axes per scion compared with untreated trees, although the effect of BAP was markedly less when NPA was used (Figure 5.15). Without NPA, the mean total number of axillary axes that formed per scion was significantly greater for the BAP x GA_{4+7} treatment when compared with the scion treated with BAP alone or trees that were untreated (Figure 5.15). Similarly, the mean total number of axillary axes formed per scion was significantly greater for the NPA x BAP x GA_{4+7} treatment compared with the NPA x BAP treatment or the untreated trees (Figure 5.15). Notably, the NPA x BAP x GA_{4+7} treatment significantly reduced the mean total number of axillary axes that formed per scion when compared with trees treated with BAP x GA_{4+7} only (Figure 5.15). Compared with the untreated trees, NPA significantly decreased the mean total number of axillary axes that formed per scion only when BAP was not used (Figure 5.15).

Without NPA, GA_{4+7} promoted small but significant increases in the mean total number of axillary axes when compared with the untreated trees, and this resulted primarily from the formation of more secondary and tertiary shoots (Figure 5.15). Interestingly, the BAP x GA_{4+7} treatment increased the mean total number of axillary axes that formed per scion compared with the BAP treatment by stimulating the formation of more tertiary shoots and, to a lesser extent, secondary shoots (Figure 5.15). With NPA, the BAP x GA_{4+7} treatment also increased the formation of tertiary shoots and secondary shoots when compared with the BAP treatment (Figure 5.15). Reductions in the mean total number of axillary axes for the NPA x BAP or the NPA x BAP x GA_{4+7} treatment resulted predominantly from fewer secondary and tertiary shoots when compared with the BAP or the BAP x GA_{4+7} treatment, respectively (Figure 5.15).

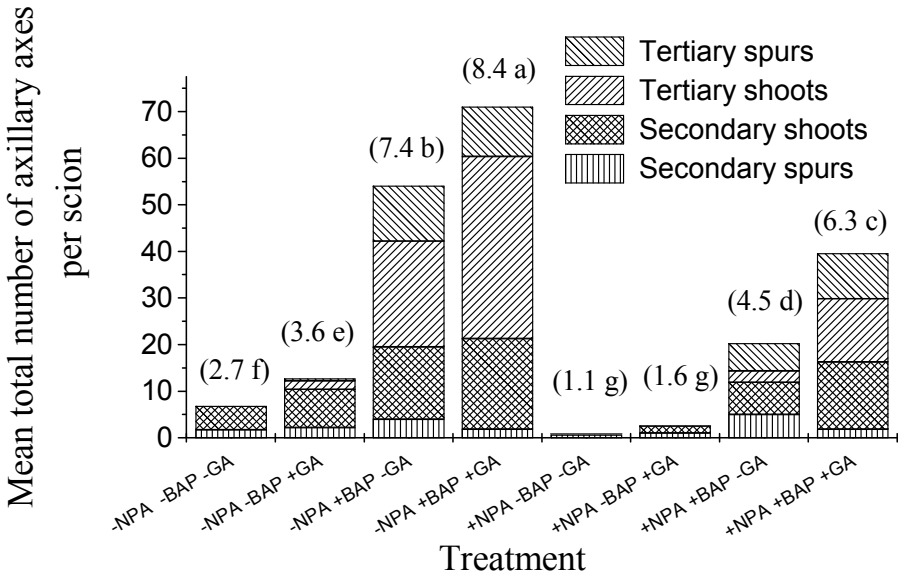


Figure 5.15. 1-N-naphthylphthalamic acid (NPA) x benzylaminopurine (BAP) x gibberellin (GA_{4+7} (GA)) interaction for the mean total number of axillary axes per 'Royal Gala' scion at the end of the first year of growth after grafting. The interaction for the mean total number of axillary axes formed per scion is equal to the total height of the columns. Means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over rootstocks.

Unlike in Chapter 3, the rootstock x BAP interaction was not significant for the mean total number of axillary axes formed per scion (P=0.17, data not shown). Notably, the BAP-treated scion on M.9 did not produce more axillary axes than the BAP-treated scion on MM.106, M.793 or 'Royal Gala' (see four-way means of Figure 5.13C). The rootstock x NPA x BAP x GA_{4+7} interaction for the mean total number of axillary axes produced per scion was not significant (P=0.15, Figure 5.13C). However, it is presented for the interpretation of important biological trends, particularly to show how each treatment affected the total number of axillary axes formed per scion, and how treatments modified the composition of different shoot types (Figure 5.13C).

For untreated trees (i.e., - NPA - BAP - GA_{4+7}), there was a trend that M.9 almost halved the mean total number of axillary axes formed compared with the scion on MM.106, M.793 or the 'Royal Gala' rootstock control (Figure 5.13C). The M.9

rootstock decreased the mean total number of axillary axes per scion by reducing the formation of secondary shoots when compared with the 'Royal Gala' rootstock control. Notably, the scion on M.9 produced equal proportions of secondary shoots and spurs, whereas the more vigorous rootstocks produced greater proportions of secondary shoots than spurs (Figure 5.13C).

The application of BAP or, to a lesser extent, GA_{4+7} increased the mean total number of axillary axes that formed for each rootstock type when compared with trees on the same rootstock that were untreated (Figure 5.13C). With BAP, fewer secondary and tertiary shoots formed for M.9, and hence, the mean total number of axillary axes produced per scion was reduced compared with the BAP-treated scion on MM.106, M.793 and 'Royal Gala'. With BAP x GA_{4+7}, the mean total number of axillary axes produced per scion increased with increasing rootstock vigour, which was predominantly a result of differences in the mean number of tertiary shoots that formed per scion (Figure 5.13C). For M.9, no tertiary axes developed in response to the NPA x BAP treatment, hence M.9 reduced the mean total number of axillary axes that formed per scion compared with MM.106, M.793 and 'Royal Gala' with the same treatment (Figure 5.13C). However, this effect for NPA x BAP-treated M.9 was partly reversed with NPA x BAP x GA_{4+7} treatment that produced a more comparable mean total number of axillary axes per scion to NPA x BAP x GA_{4+7}-treated trees on MM.106, M.793 and 'Royal Gala' (Figure 5.13C).

Similar to untreated trees on M.9, NPA reduced the mean total number of axillary axes that formed per scion for each rootstock type, primarily by reducing the mean number of secondary shoots that formed (Figure 5.13C).

5.3.4 Final growth measurements of the scion

5.3.4.1 Main effects for the final mean length and node number of the primary and secondary shoots

The main effects of rootstock, BAP, GA_{4+7} and NPA on the final mean length and node number of the primary shoot were previously described in Section 5.3.2. The main effects of rootstock, BAP, GA_{4+7} and NPA were highly significant (P<0.0001) for the

final mean total length and node number of the secondary shoots (Table 5.3). The M.9 rootstock significantly decreased the mean total length and node number of the secondary shoots compared with MM.106, M.793 and 'Royal Gala' (Table 5.3). The final mean length (m) of the secondary shoots for the rootstock main effect was 0.21, 0.27, 0.24 and 0.32 for M.9, MM.106, M.793 and 'Royal Gala', respectively, whereas the final mean node number was 11, 13, 12 and 15 nodes, respectively. Thus, decreased mean total length and node number of the secondary shoots for M.9 (main effect, Table 5.3) resulted partly from small reductions in both the mean length and node number of the secondary shoots. In addition, fewer secondary shoots developed per scion on M.9 when compared with MM.106, M.793 and 'Royal Gala' (main effect, Table 5.2). In a similar manner to the M.9 rootstock, the main effect of NPA significantly decreased the mean total length and node number of the secondary shoots compared with untreated trees (Table 5.3). NPA reduced mean total growth of the secondary shoots by decreasing the mean number of secondary shoots that formed per scion (main effect, Table 5.2) and by decreasing both the mean length (0.15 and 0.37 m for + or - NPA, respectively) and node number of the secondary shoots (9 and 16 nodes, respectively).

In contrast to NPA, the main effects of BAP or GA_{4+7} significantly increased the mean total length and node number of the secondary shoots compared with untreated scions (Table 5.3). However, these treatments differed in the way total growth of the secondary shoots was modified. Exogenous BAP or GA_{4+7} both increased the mean number of secondary shoots that formed per scion when compared with untreated trees (main effect, Table 5.2). However, BAP promoted the formation of significantly more secondary shoots than GA_{4+7} (Figure 5.10B). Exogenous GA_{4+7} also increased the mean total length of the secondary shoots (Table 5.3) by increasing both the final mean length and node number of the secondary shoots. With or without GA_{4+7}, the final mean length of the secondary shoots was 0.31 and 0.22 m, respectively, and the final mean node number was 15 and 11 nodes, respectively. In contrast to GA_{4+7}, BAP reduced both the final mean length (0.22 and 0.31 m for + or - BAP, respectively) and node number (11 and 15 nodes, respectively) of the secondary shoots compared with untreated trees.

Table 5.3. Main effects of rootstock, benzylaminopurine (BAP), gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) on the mean total growth of the primary shoot, secondary shoots, tertiary shoots and the total growth of 'Royal Gala' apple scions at the end of their first growing season after grafting.

Main effect	A Primary shoot	B Secondary shoots	C Tertiary shoots	A + B + C Total per scion
		Length (m)		
Rootstock				
M.9	1.08 a ***	1.66 (1.35 a) ***	0.37 [y]	3.11 (1.64 a) ***
MM.106	1.23 bc	2.52 (1.64 b)	0.49	4.24 (1.97 b)
M.793	1.27 c	3.01 (1.73 b)	0.83	5.11 (2.11 c)
'Royal Gala'	1.18 b	2.90 (1.72 b)	0.91	4.99 (2.10 c)
BAP				
-	1.22 a *	1.69 (1.35 a) ***	0.10	3.01 (1.62 a) ***
+	1.17 b	3.35 (1.89 b)	1.20	5.71 (2.29 b)
GA_{4+7}				
-	1.04 a ***	1.68 (1.38 a) ***	0.41	3.13 (1.66 a) ***
+	1.36 b	3.36 (1.85 b)	0.89	5.61 (2.25 b)
NPA				
-	1.30 a ***	4.00 (2.06 a) ***	1.12	6.42 (2.46 a) ***
+	1.08 b	1.03 (1.16 b)	0.18	2.29 (1.45 b)
		Node number		
Rootstock				
M.9	47 a ***	90 (8.01 a) ***	33 [y]	170 (12.34 a) ***
MM.106	50 b	121 (9.94 b)	41	212 (13.74 b)
M.793	52 c	142 (10.49 b)	62	256 (14.91 c)
'Royal Gala'	50 bc	144 (10.52 b)	65	259 (14.99 c)
BAP				
-	52 a ***	71 (6.85 a) ***	4	127 (10.78 a) ***
+	48 b	174 (12.64 b)	96	318 (17.16 b)
GA_{4+7}				
-	45 a ***	86 (7.97 a) ***	34	165 (11.95 a) ***
+	55 b	159 (11.52 b)	66	280 (16.17 b)
NPA				
-	53 a ***	181 (13.03 a) ***	84	318 (17.21 a) ***
+	47 b	62 (6.46 b)	16	125 (10.73 b)

ns, *, **, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single growth attribute and main effect only, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. [y] data unable to be appropriately transformed for ANOVA. Data without and within parenthesis are raw or transformed means, respectively.

5.3.4.2 NPA x BAP x GA_{4+7} interactions on the final mean total length and node number of the secondary shoots

The NPA x BAP x GA_{4+7} interactions were significant for the mean total length (P=0.001) and node number (P=0.007) of the secondary shoots (Figure 5.16A, B). Exogenous BAP or GA_{4+7} stimulated significantly more total growth of the secondary shoots compared with the untreated trees only when NPA was not applied (Figure 5.16A, B). Without NPA, the BAP x GA_{4+7} treatment promoted significantly more total growth of the secondary shoots compared with trees that were untreated or treated with BAP or GA_{4+7} only (Figure 5.16A, B). The mean total length of the secondary shoots was significantly reduced by the NPA, NPA x BAP and the NPA x GA_{4+7} treatments when compared with the untreated trees (Figure 5.16A). Similar results occurred for the mean total number of nodes produced on the secondary shoots, although the NPA x BAP treatment was not statistically different from the untreated trees (Figure 5.16B). The mean total length and node number of secondary shoots on NPA x BAP x GA_{4+7}-treated trees was significantly greater than that on untreated trees (Figure 5.16A, B). The NPA x BAP x GA_{4+7} treatment had a similar mean total node number of the secondary shoots compared with scions treated with BAP or GA_{4+7} alone that were growing on untreated rootstocks (Figure 5.16B). However, this did not occur for the mean total length of the secondary shoots when comparing these same treatments (Figure 5.16A).

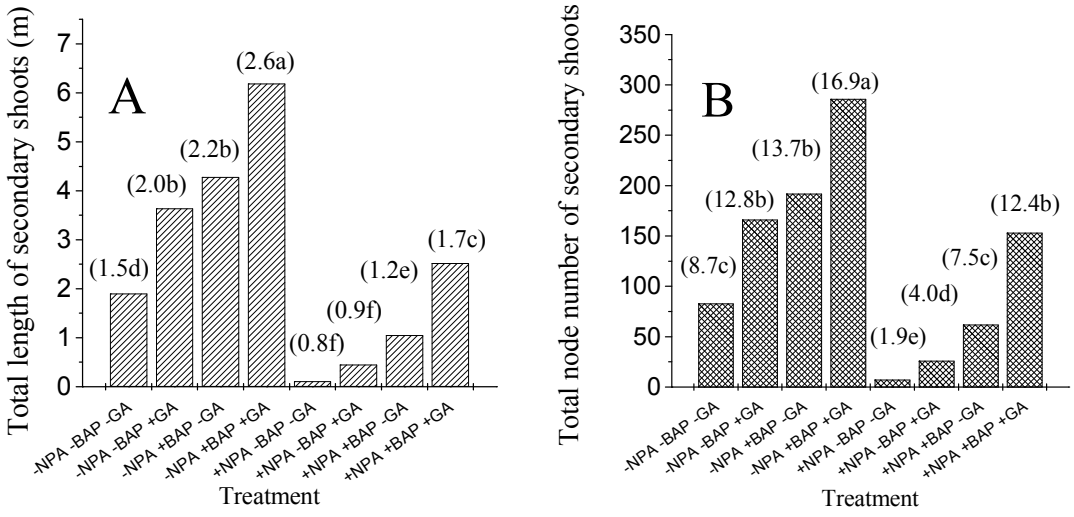


Figure 5.16. 1-N-naphthylphthalamic acid (NPA) x benzylaminopurine (BAP) x gibberellin (GA_{4+7} (GA)) interactions for the mean total length (A) and node number (B) of 'Royal Gala' secondary shoots at the end of the first year of growth after grafting of composite 'Royal Gala' apple trees. Transformed means in parenthesis sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over rootstocks.

5.3.4.3 Rootstock x BAP x GA_{4+7} and rootstock x NPA x BAP interactions on the mean total length and node number of the secondary shoots

In addition to the main effect of rootstock on the final mean total growth of the secondary shoots (Table 5.3), the rootstock x BAP x NPA interactions were significant for the final mean total length (P=0.002) and node number (P=0.009) of the secondary shoots (Figure 5.17A, B). In addition to these data (i.e., Figure 5.17A, B), four-way means are presented to elucidate how alterations in the total growth of the secondary shoots occurred (Figures 5.11B, 5.19 and 5.22).

Without BAP, NPA significantly decreased the mean total length and node number of the secondary shoots for each rootstock type when compared with untreated trees on the same rootstock (Figure 5.17A, B). Notably, rootstocks did not significantly differ in the mean total length and node number of the secondary shoots when trees were treated with NPA alone. (Figure 5.17A, B). For each rootstock type, NPA reduced the mean total length and node number of the secondary shoots compared with untreated trees on

the same rootstock by decreasing the mean number, length and node number of the secondary shoots (Figures 5.11B and 5.22A, B). Reduced mean length of the secondary shoots for NPA-treated rootstocks resulted partly from compressed internodes compared with untreated trees (see Figure 5.20A and C where internode lengths can be compared from the regression line of pooled data for each treatment by dividing a y-value by its corresponding value of x). When compared with untreated trees, NPA treatment of M.9 reduced the mean total length and node number of the secondary shoots less markedly compared with NPA-treated MM.106, M.793 and 'Royal Gala' (Figure 5.17A, B), primarily because very few secondary shoots developed for untreated trees on M.9 (Figure 5.11B).

Compared with trees treated with only NPA, the NPA x BAP treatment significantly increased the total growth of the secondary shoots for each rootstock type, although this increase in growth was much smaller for M.9, particularly when compared with the M.793 rootstock (Figure 5.17A, B and see Figure 5.19A, B). For M.9 treated with NPA x BAP, total growth of the secondary shoots was reduced (Figure 5.19A, B) compared with MM.106, M.793 and 'Royal Gala' because fewer secondary shoots formed (Figure 5.11B) and the mean length and node number of the secondary shoots was reduced (Figure 5.22).

Without NPA, BAP increased the mean total length of the secondary shoots for M.9, MM.106, M.793 and 'Royal Gala' by 2.27, 1.21, 1.68 and 2.14 m, respectively, when compared with untreated trees on the same rootstock type (Figure 5.17A). Hence, the final mean total length of the secondary shoots was similar for the BAP-treated scion on M.9 or MM.106, but the BAP-treated scion on M.9 still had a significantly decreased mean total length of the secondary shoots than the BAP-treated scion on M.793 or 'Royal Gala' (Figure 5.17A and see Figure 5.19A). Very similar trends also occurred for the mean total node number of the secondary shoots (Figure 5.17B). Notably, these three way means (i.e., rootstock - NPA + BAP, Figure 5.17A, B) include scions that were treated with BAP or BAP x GA_{4+7}. Examination of the four-way treatment means indicated that the BAP x GA_{4+7} treatment primarily caused the greater increase in the mean total length of the secondary shoots on M.9 rather than the BAP treatment (Figure 5.19A, B). However, the rootstock x BAP x GA_{4+7} interactions only approached

significance for the final mean total length (P=0.06) and node number (P=0.11) of the secondary shoots (Figure 5.18A, B).

Without BAP, there were trends that GA_{4+7} increased the mean total length and node number of the secondary shoots for each rootstock type when compared with the untreated trees on the same rootstock (Figure 5.18A, B). For the four-way means, gibberellin increased the mean total length and node number of the secondary shoots (Figure 5.19) by increasing their mean number (Figure 5.11B), length and node number (Figure 5.22A, B) for each rootstock type. In contrast, BAP increased the mean number of secondary shoots per scion markedly more than GA_{4+7} (Figure 5.11B), but decreased the mean length and node number of the secondary shoots when compared with the GA_{4+7} treatment or untreated trees (Figure 5.22A, B). Therefore, BAP increased the mean total length of the secondary shoots for each rootstock type by increasing secondary shoot number.

Exogenous BAP reduced the mean length and node number of the secondary shoots by increasing the proportion of short shoots that formed 6 to 10 nodes, whereas GA_{4+7} increased the mean length and node number of the secondary shoots by increasing the proportions of very long shoots that formed, particularly with 26 or more nodes (Figure 5.21A, B, D). Compared with untreated trees, exogenous GA_{4+7} also increased the mean length of the secondary shoots for each rootstock type by increasing internode length very slightly, whereas BAP tended to decrease it (see Figure 5.20A, B and D where internode lengths can be compared from the regression line of pooled data on each graph by dividing a y-value by its corresponding value of x).

Compared with untreated trees, GA_{4+7} significantly increased the mean total length of the secondary shoots for M.9, however GA_{4+7} did not significantly increase growth for ‘Royal Gala’ (Figure 5.18A). For the four-way means (i.e., Figure 5.19), exogenous GA_{4+7} increased mean total growth of the secondary shoots for M.9 by increasing their mean number, length and node number, whereas GA_{4+7} applied to the scion on ‘Royal Gala’ mainly increased the mean length and node number of the secondary shoots (Figures 5.11B and 5.22A, B). For M.9, exogenous GA_{4+7} did not affect the proportion of secondary shoots with 10 or fewer nodes compared with untreated trees on M.9, however it greatly increased the proportion of shoots that formed 16 to 35 nodes (Figure

5.21A, B). Compared with the GA_{4+7}-treated scion on 'Royal Gala', the GA_{4+7}-treated scion on M.9 formed greater proportions of secondary shoots with 10 or fewer nodes and reduced proportions of shoots with 11-15, 21-25 and 31-55 nodes (Figure 5.21B). Hence, the mean length and node number the secondary shoots for the GA_{4+7}-treated scion on M.9 was reduced compared with the GA_{4+7}-treated scion on 'Royal Gala' (Figure 5.22A, B).

Applications of BAP x GA_{4+7} produced a markedly greater increase in the final mean total length and node number of the secondary shoots for the scion on M.9 than on MM.106, particularly when compared with the scion on the same rootstock type treated with only BAP or GA_{4+7} (Figure 5.18A, B). For the four-way treatment means, BAP x GA_{4+7} promoted greater total growth of the secondary shoots than BAP when applied to the scion on M.9 (Figure 5.19) because it stimulated the formation of more secondary shoots (Figure 5.11B) with increased mean length and node number (Figure 5.22). Increased mean length and node number of the secondary shoots for the BAP x GA_{4+7} treated scion on M.9 was primarily the result of this treatment decreasing the formation of short secondary shoots with 6 to 10 nodes when compared with the BAP-treated scion on M.9 (Figure 5.21D, F). With BAP x GA_{4+7}, the final mean total length and node number of the secondary shoots was similar between M.9 and the more vigorous MM.106 rootstock (Figures 5.18 and 5.19). The M.9 rootstock developed very similar total growth of the secondary shoots compared with MM.106 because the BAP x GA_{4+7} treatment stimulated a slightly greater final mean number of secondary shoots for M.9 (Figure 5.11B) rather than increasing the mean length and node number of the secondary shoots (Figure 5.22A, B).

Despite the BAP x GA_{4+7}-treated scion on M.9 having a very similar final mean number of secondary shoots to the scion on M.793 and 'Royal Gala' (Figure 5.11B), the mean total length and node number of the secondary shoots was reduced by M.9 (Figures 5.18, 5.19 and 5.27) because increased proportions of short secondary shoots formed with 10 or less nodes, and because fewer very long secondary shoots formed with more than 31 nodes (Figure 5.21F). Hence, the mean length and node number of the secondary shoots was reduced for the BAP x GA_{4+7}-treated scion on M.9 (Figure 5.22). Interestingly, the BAP, GA_{4+7} or BAP x GA_{4+7}-treated scion had secondary shoots with slightly decreased, increased or very similar internode lengths, respectively, when

compared with secondary shoots on the untreated trees (see Figure 5.20A, B, D and F where internode lengths can be compared from the regression line of pooled data by dividing a y-value by its corresponding value of x).

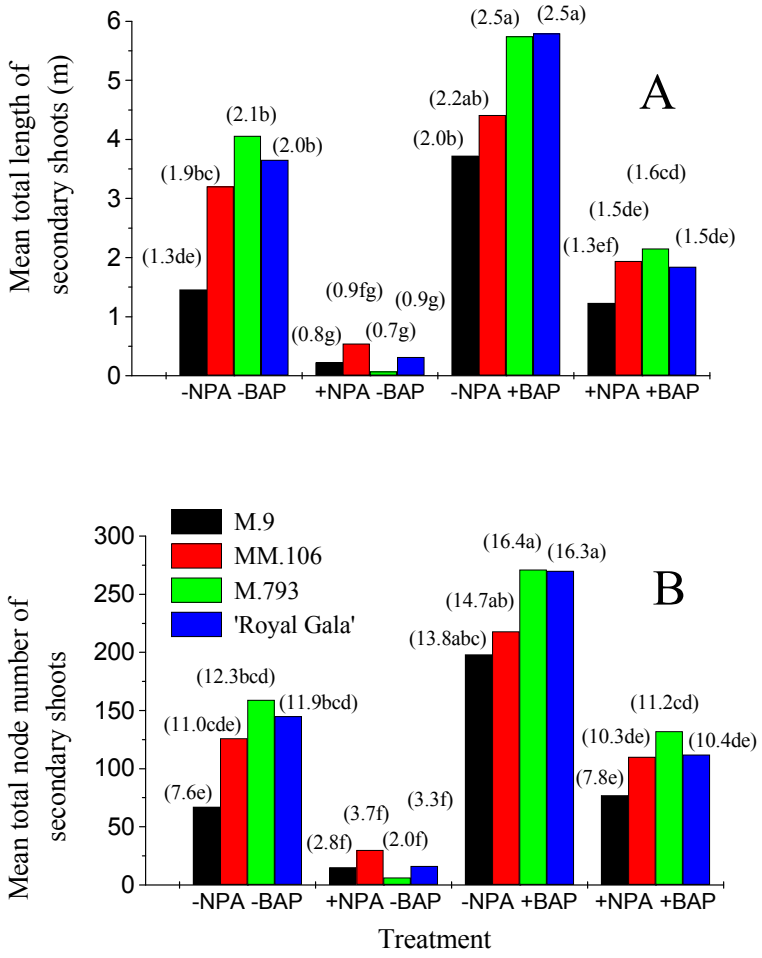


Figure 5.17. Rootstock x 1-N-naphthylphthalamic acid (NPA) x benzylaminopurine (BAP) interaction for the mean total length (A) and node number (B) of secondary shoots on 'Royal Gala' apple scions at the end of their first year of growth after grafting. On a single graph, transformed means in parenthesis sharing the same letter are not significantly different at *P*≤0.05 (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over gibberellin treatments.

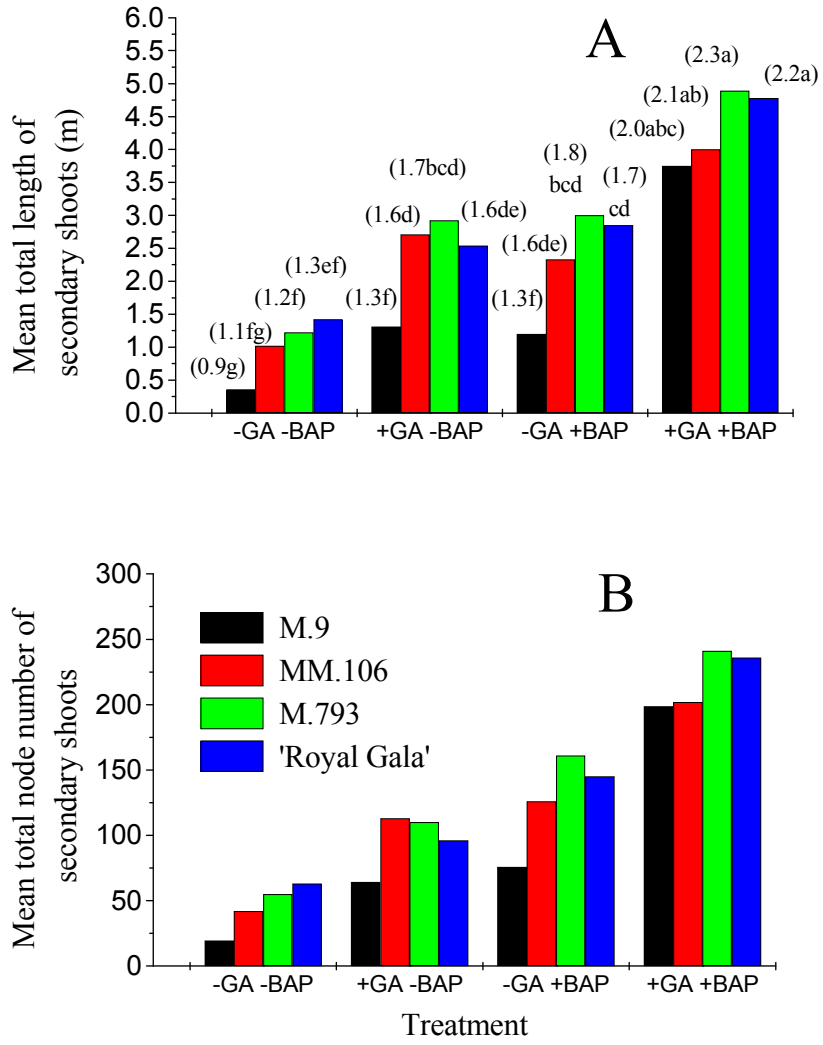


Figure 5.18. Rootstock x benzylaminopurine (BAP) x gibberellin (GA) interaction for the mean total length (A, *P*=0.06) and node number (B, *P*=0.11) of secondary shoots on 'Royal Gala' apple scions at the end of their first year of growth after grafting. On graph A, transformed means in parenthesis sharing the same letter are not significantly different at *P*≤0.06 (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over NPA treatments.

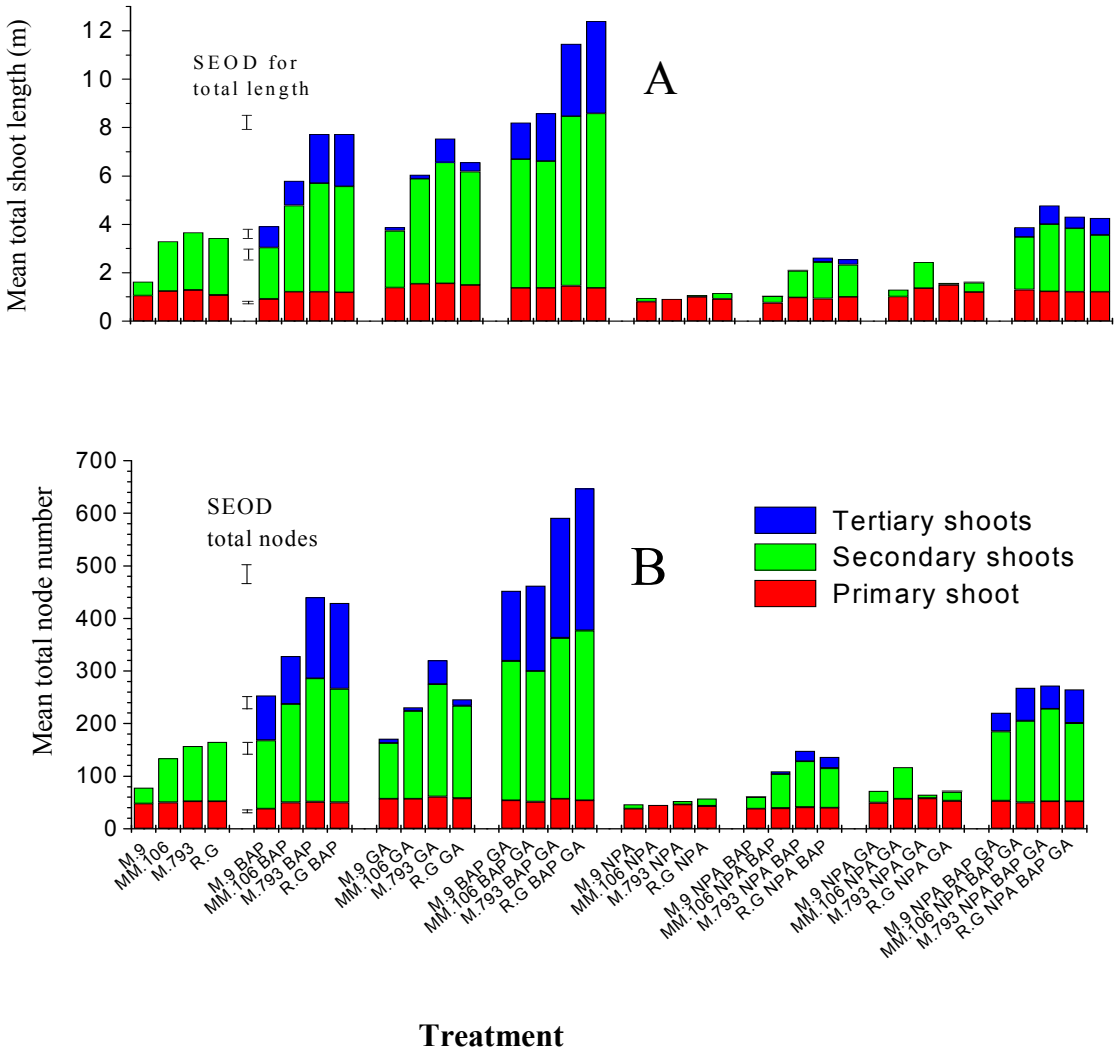


Figure 5.19. Effect of rootstock, benzylaminopurine (BAP), gibberellin (GA_{4+7} (GA)) and 1-N-naphthylphthalamic acid (NPA) treatments on the mean total length (A) and node number (B) of 'Royal Gala' apple scions at the end of their first year of growth from grafting. Total shoot length (A) or node number per scion (B) is shown as the component parts comprising of tertiary shoots, secondary shoots, primary shoot and is equivalent to the total height of a column. Error bars adjacent M.9 plus BAP are the SEOD for that shoot type calculated from the raw data.

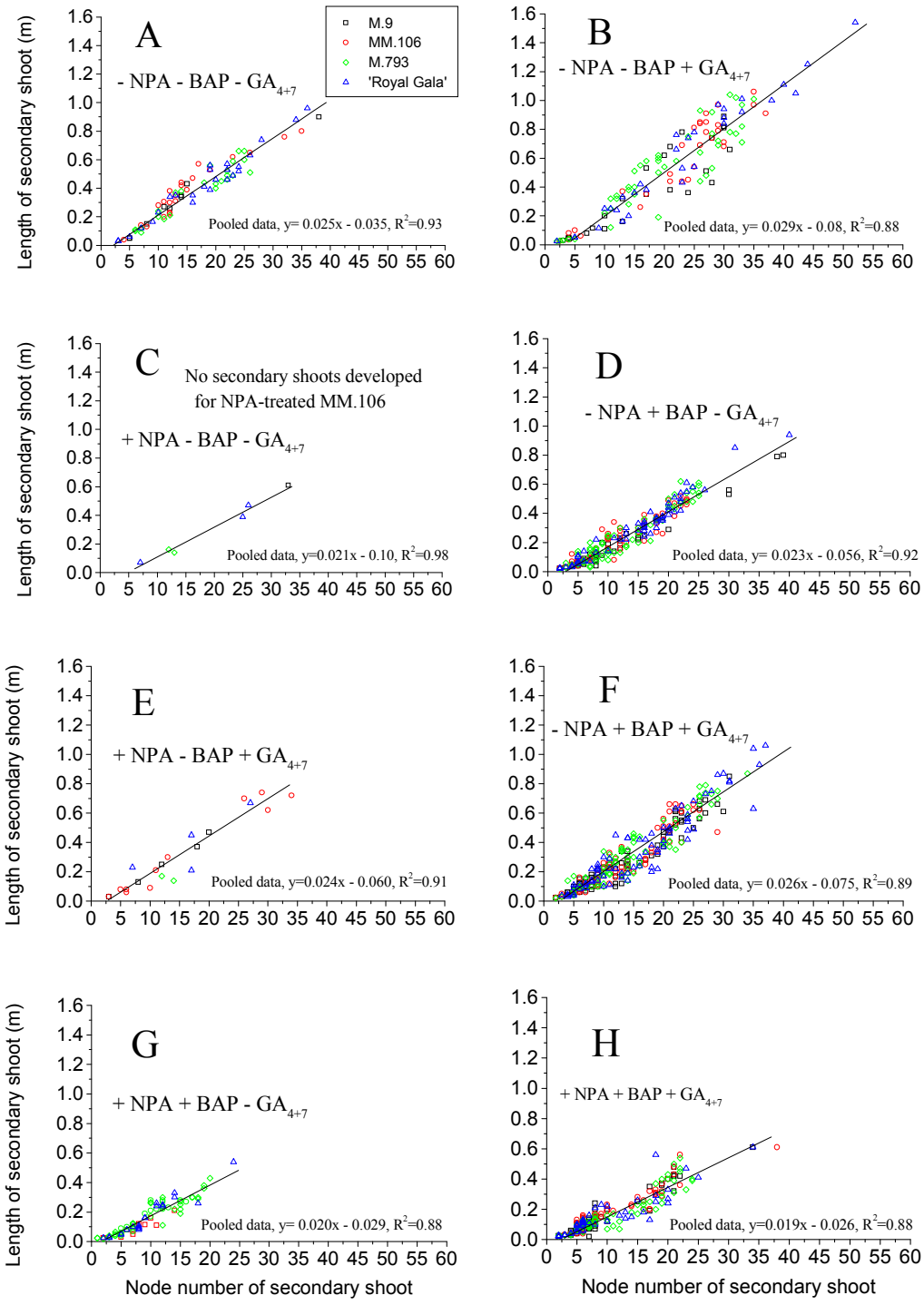


Figure 5.20. Effect of rootstock type, benzylaminopurine (BAP), gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) treatments on the relationship between the final length and node number of secondary shoots on 'Royal Gala' apple scions at the end of their first year of growth from grafting.

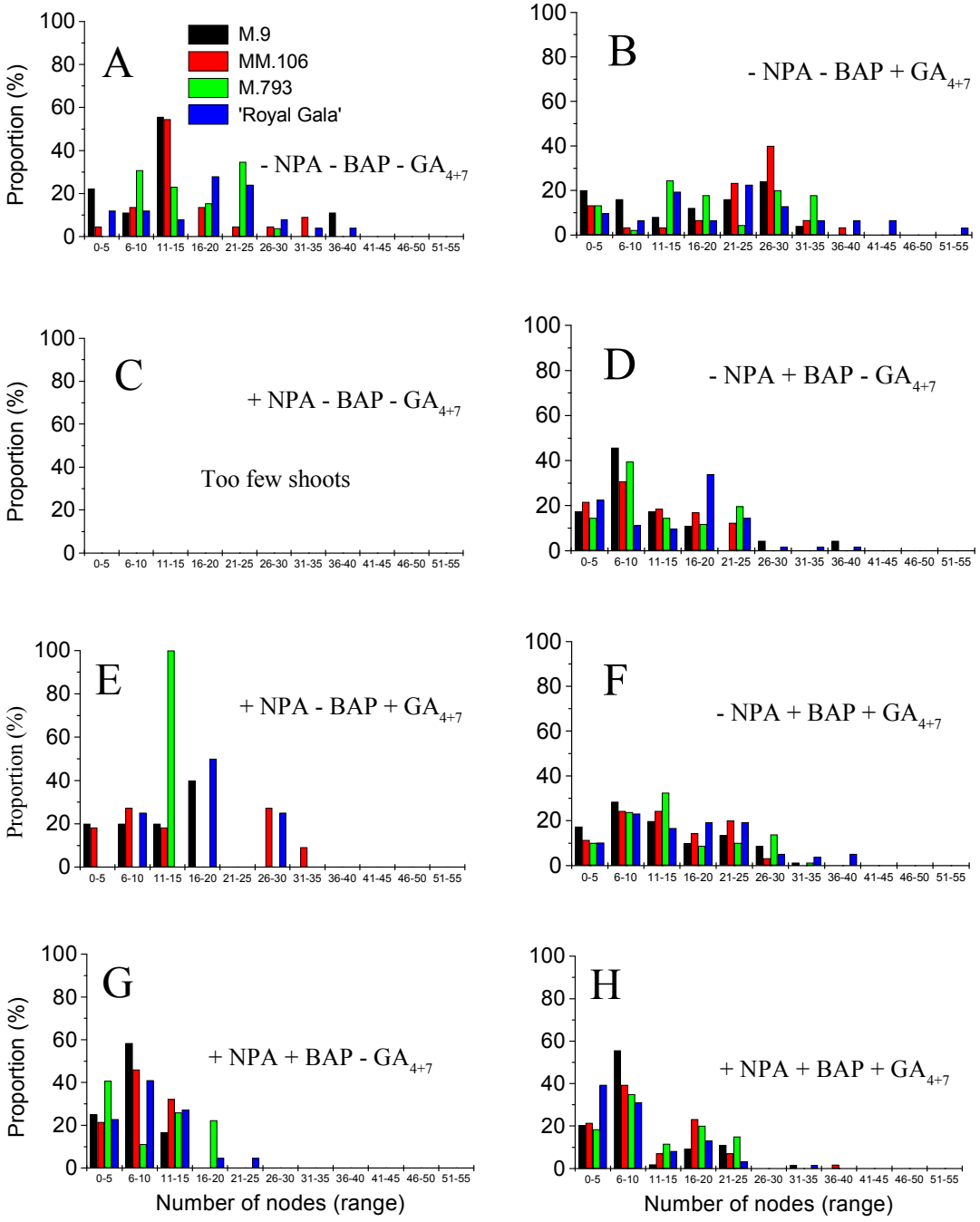


Figure 5.21. Effect of rootstock type, benzylaminopurine (BAP), gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) treatments on the node number distributions of secondary shoots that had formed on the primary shoot of 'Royal Gala' apple scions by the end of their first year of growth from grafting. Node number distributions are of the total shoot population for each treatment.

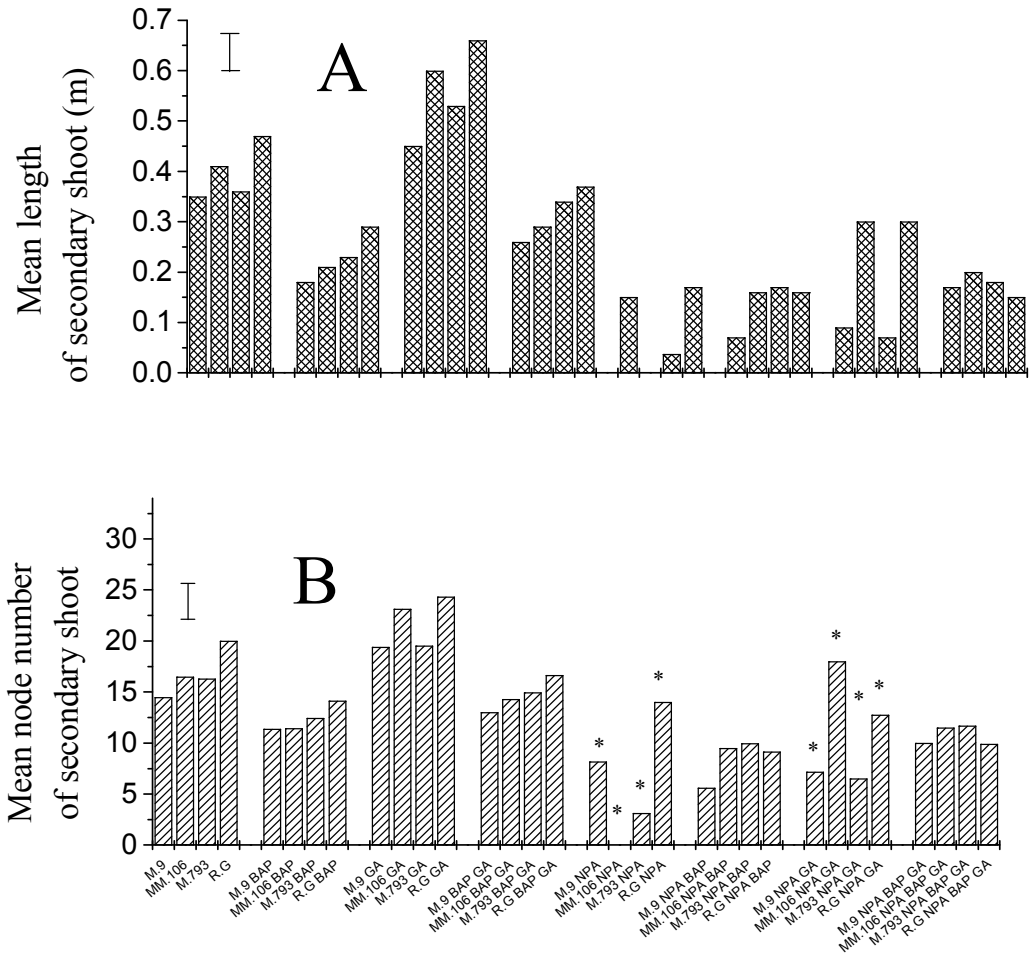


Figure 5.22. Effect of rootstock type, benzylaminopurine (BAP), gibberellin (GA_{4+7} (GA)) and ± 1-N-naphthylphthalamic acid (NPA) treatments on the mean length (A) and node number (B) of 'Royal Gala' secondary shoots at the end of the first year of growth from grafting. Error bars are the SEOD. * on graph B denotes very few shoots per treatment mean in graph A or B.

5.3.4.4 Final length and node number of tertiary shoots

The data sets for the total length and node number of the tertiary shoots could not be appropriately transformed for ANOVA because tertiary axes formed predominantly in response to exogenous BAP, hence almost half of the data set contained means equal to zero. Data were able to be analysed using ANOVA by removing treatments without

BAP. For BAP-treated scions, this enabled the main effects of rootstock, GA_{4+7}, NPA and their interactions to be tested for statistical significance.

With BAP treatment, the main effects of rootstock, GA_{4+7} and NPA significantly ($P<0.0001$) affected the mean total length and node number of the tertiary shoots formed on the secondary shoots (Table 5.4). For rootstocks, M.9 significantly decreased the mean total length and node number of tertiary shoots compared with M.793 and 'Royal Gala' only (Table 5.4). There was also a trend for the mean total length and node number of the tertiary shoots to increase with increasing vigour of the rootstock, which resulted because the mean number of tertiary shoots that formed in response to BAP also increased with rootstock vigour (Figure 5.13C). With BAP treatment, the application of GA_{4+7} to the scion or NPA to the rootstock stem increased or decreased, respectively, the mean total length and node number of the tertiary shoots compared with trees treated with BAP alone (Table 5.4). Decreased mean total length and node number of the tertiary shoots for NPA-treated rootstocks (Table 5.4) resulted from NPA decreasing the number of secondary shoots, and hence, the consequent number of tertiary shoots able to be formed in response to BAP (Figure 5.13C).

With BAP treatment, the rootstock x NPA interaction was significant ($P=0.001$) for the mean total length of the tertiary shoots (Figure 5.23A). A similar rootstock x NPA interaction occurred for the mean total node number of the tertiary shoots (Figure 5.23B), although this was less significant ($P=0.16$). For each rootstock type, the mean total length and node number of the tertiary shoots was similar when NPA was applied to the rootstock stem (Figure 5.23A, B). Treatments of NPA reduced the mean total length and node number of the tertiary shoots markedly more for the BAP-treated scion on M.793 or 'Royal Gala' than on M.9 or MM.106 when compared with the BAP-treated scion on the same untreated rootstock type (Figure 5.23A, B).

Table 5.4. Main effects of rootstock, gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) on the mean total length and node number of tertiary shoots that formed on secondary shoots of 'Royal Gala' apple scions in response to exogenous benzylaminopurine (BAP).

Main effect (+ BAP data)	Total length of tertiary shoots (m)	Total node number of tertiary shoots
Rootstock		
M.9	0.71 (1.06 a) ***	64 (6.88 a) ***
MM.106	0.96 (1.16 ab)	81 (8.07 ab)
M.793	1.42 (1.31 bc)	112 (9.58 bc)
'Royal Gala'	1.72 (1.40 c)	130 (10.26 c)
GA_{4+7}		
-	0.82 (1.09 a) ***	68 (6.90 a) ***
+	1.58 (1.37 b)	125 (10.46 b)
NPA		
-	2.05 (1.56 a) ***	161 (12.37 a) ***
+	0.35 (0.91 b)	32 (4.99 b)

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single main effect, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. Data without and within parenthesis are raw or transformed means, respectively.

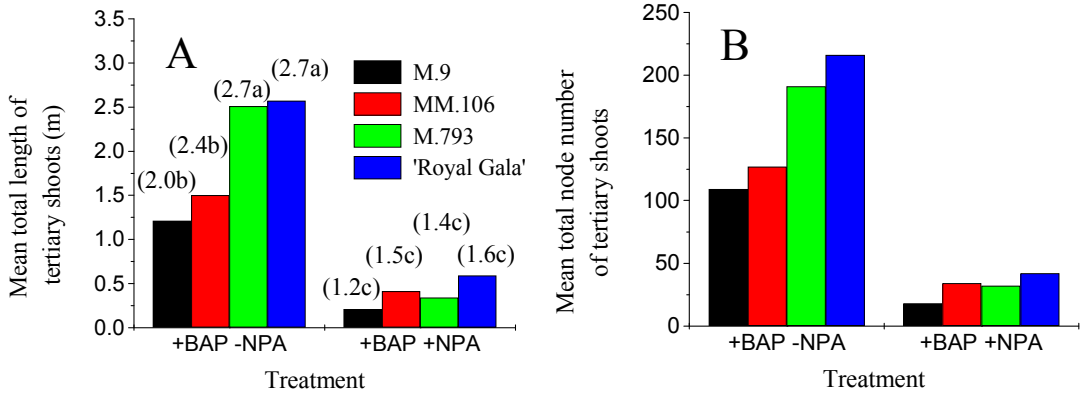


Figure 5.23. Rootstock x 1-N-naphthylphthalamic acid (NPA) interactions on the mean total length (A, $P=0.001$) and node number (B, $P=0.16$) of tertiary shoots formed on secondary shoots of 'Royal Gala' apple scions in response to exogenous benzylaminopurine (BAP). For graph A, means sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over gibberellin treatments.

5.3.4.5 Mean total shoot length and node number per scion

The final mean total shoot length and node number per scion was significantly (P<0.0001) affected by the main effects of rootstock, BAP, GA_{4+7} and NPA (Table 5.3). The M.9 rootstock significantly decreased the mean total shoot length and node number of the scion compared with MM.106, M.793 and 'Royal Gala'. Rootstocks of M.793 and 'Royal Gala' produced a statistically similar mean total shoot length and node number per scion, but a significantly greater mean total shoot length and node number per scion compared with either M.9 or MM.106 (Table 5.3). Exogenous BAP or GA_{4+7} significantly increased the mean total shoot length and node number compared with untreated scions (Table 5.3). In contrast, NPA treatment of the rootstock stem significantly decreased the mean total shoot length and node number per scion compared with untreated rootstocks (Table 5.3).

The NPA x BAP x GA_{4+7} interactions were significant for the mean total shoot length (P=0.002) and node number (P=0.001) per scion (Figure 5.24A, B). As for the main effects (Table 5.3), BAP or GA_{4+7} treatment of the scion stimulated significantly more total growth than untreated trees (Figure 5.24A, B). However, BAP x GA_{4+7} promoted significantly more total shoot growth per scion compared with BAP or GA_{4+7} applied alone or compared with the untreated trees (Figure 5.24A, B). Exogenous BAP or GA_{4+7} did not significantly increase the mean total shoot length or node number of the scion compared with the untreated trees when applied to the scion growing on NPA-treated rootstocks (Figure 5.24A, B). However, NPA x BAP x GA_{4+7} promoted a significantly greater mean total shoot length and node number per scion when compared with the untreated trees or trees that were treated with NPA only (Figure 5.24A, B).

Without NPA, the application of BAP or GA_{4+7} to the scion resulted in a similar mean total shoot length per scion (Figure 5.24A, B), but the means by which these hormone treatments increased total shoot length differed. Exogenous BAP primarily increased mean total shoot length compared with the untreated trees by promoting the formation of much more secondary and tertiary shoots (see Figures 5.15 and 5.27), whereas GA_{4+7} predominantly stimulated shoot extension growth of the primary and secondary shoots (Figures 5.4, 5.22 and 5.27) whilst promoting very small increases in secondary and tertiary shoot number when compared with BAP (Figures 5.15 and 5.27). For the final

mean total number of nodes formed per scion, the BAP-treated scion growing on untreated rootstocks formed significantly more nodes than the scion treated with GA_{4+7} (Figure 5.24B), which was different from the data for the mean total shoot length per scion (Figure 5.24A). This resulted mostly from exogenous BAP stimulating the formation of more tertiary shoots (see Figure 5.15) that were shorter on average and had reduced internode length compared with similar length shoots on the GA_{4+7}-treated scion (data not shown).

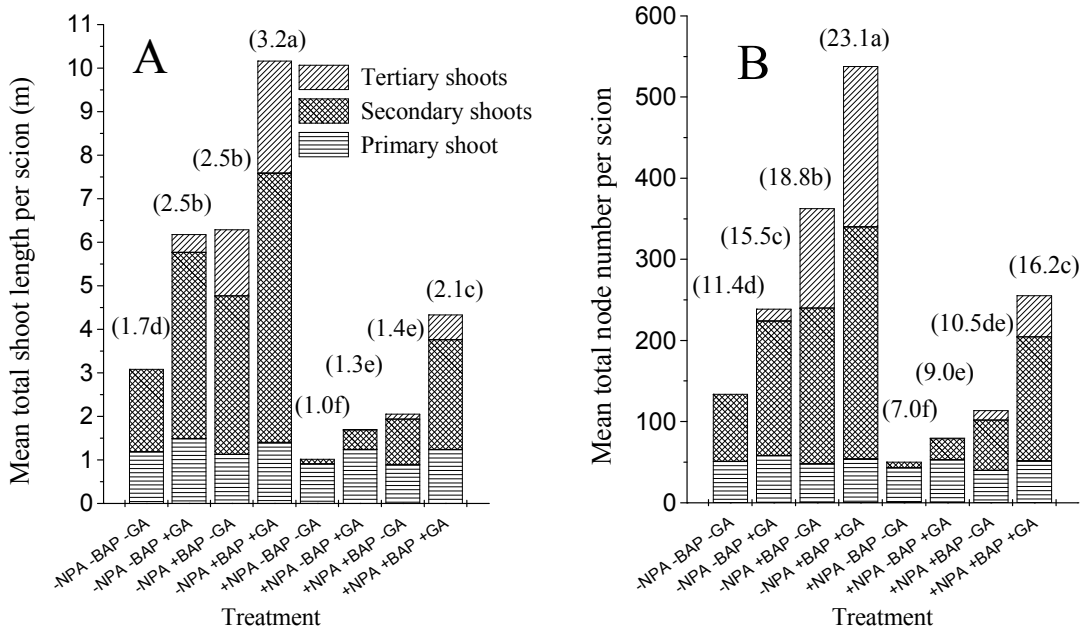


Figure 5.24. 1-N-naphthylphthalamic acid (NPA) x benzylaminopurine (BAP) x gibberellin (GA_{4+7} (GA)) interactions for the mean total shoot length (A) and node number (B) formed per 'Royal Gala' scion. The total shoot length or node number per scion is equivalent to the total height of the columns of A and B, respectively. For a single graph, transformed means in parenthesis sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over rootstocks.

The effect of rootstock type on mean total growth of the scion (Table 5.3) depended on whether NPA was applied to the rootstock stem as the rootstock x NPA interactions were significant for the final mean total shoot length ($P<0.001$) and node number ($P=0.005$) formed per scion (Figure 5.25). Without NPA, rootstocks of MM.106, M.793 and 'Royal Gala' produced significantly more total scion growth than M.9 (Figure

5.25). For each rootstock type, NPA significantly reduced the mean total shoot length and node number per scion when compared with trees on the same rootstock type that were untreated (Figure 5.25A, B). However, these reductions in total scion growth were more marked for M.793 and 'Royal Gala', primarily because the scion on these rootstocks developed significantly more total shoot growth than both MM.106 and M.9 when NPA was not used (Figure 5.25). With NPA, total shoot growth of the scion on MM.106, M.793 and 'Royal Gala' did not significantly differ, but these rootstocks produced significantly more total shoot growth per scion than M.9 (Figure 5.25).

For the four-way means (i.e., Figure 5.19), the M.9 rootstock decreased the mean total length and node number of the scion compared with the 'Royal Gala' rootstock control by reducing the mean length and node number of the primary shoot (Figure 5.5A, B), and by decreasing the mean total length and node number of the secondary shoots (Figures 5.19A, B and 5.27). Reduced total growth of the secondary shoots resulted from M.9 reducing the mean number of secondary shoots formed per scion compared with the 'Royal Gala' rootstock control (Figures 5.13B and 5.27). In addition, a greater proportion of secondary shoots were shorter for M.9, and typically formed 15 or fewer nodes (Figure 5.21A). Similarly, NPA applied to MM.106, M.793 and 'Royal Gala' rootstocks reduced the mean total shoot length and node number per scion (Figure 5.19A, B). These reductions in total scion growth also occurred because NPA reduced the mean length and node number of the primary shoot (Figure 5.5A, B) and dramatically reduced the mean number of secondary shoots that formed per scion (Figures 5.13B, 5.27 and 5.28), which was very similar to the effects imposed on the scion by the M.9 rootstock.

The rootstock x BAP x GA_{4+7} interaction for the final mean total shoot length and node number per scion was significant (P=0.02) or approached significance (P=0.12), respectively (Figure 5.26A, B and see Figure 5.19). Exogenous BAP or GA_{4+7} significantly increased the mean total shoot length per scion on each rootstock type when compared with untreated trees on the same rootstock (Figure 5.26A). However, the GA_{4+7}-treated scion on M.9 developed significantly less total shoot length than the GA_{4+7} treated scion on MM.106, M.793 and 'Royal Gala' (Figure 5.26A). Reduced total shoot growth for the GA_{4+7}-treated scion on M.9 (Figure 5.26A, B) resulted predominantly from fewer secondary shoots (Figure 5.11B), the formation of greater

proportions of secondary shoots with 10 or fewer nodes and from reduced proportions of long shoots with 31 to 55 nodes compared with the GA_{4+7}-treated scion on MM.106, M.793 and 'Royal Gala' (Figure 5.21B).

The BAP-treated scion on M.9 also had a greatly reduced mean total shoot length and node number compared with the BAP-treated scion on MM.106, M.793 and 'Royal Gala' (Figure 5.26A, B), which resulted from the BAP-treated scion on M.9 forming fewer secondary and tertiary shoots in response to BAP (Figure 5.13C). For the secondary shoots that formed for M.9, a greater proportion of the shoot population was comprised of short shoots with 6 to 10 nodes, whilst reduced proportions of long shoots with 16 to 25 nodes formed compared with the BAP-treated scion on MM.106, M.793 and 'Royal Gala' (Figure 5.21D). With BAP x GA_{4+7}, the scion on M.9 and MM.106 had a statistically similar final mean total shoot length, but total shoot length of the scion on M.793 and 'Royal Gala' was significantly greater than M.9 (Figure 5.26A) predominantly because fewer tertiary shoots formed for M.9 (Figure 5.13C) and, to a lesser extent, the mean length and node number of secondary (Figure 5.22) and tertiary shoots (data not shown) was reduced for the BAP x GA_{4+7}-treated scion on M.9.

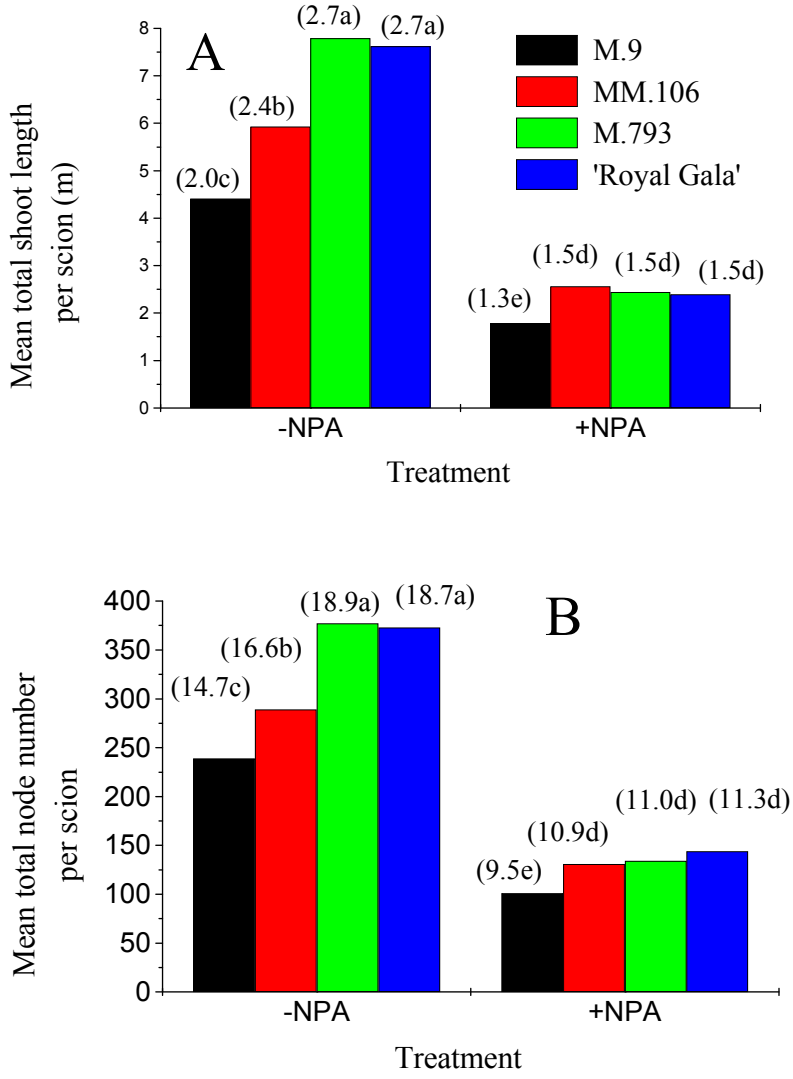


Figure 5.25. Rootstock x 1-N-naphthylphthalamic acid (NPA) interactions on the final mean total length (A) and node number (B) formed on 'Royal Gala' apple scions at the end of their first year of growth from grafting. For a single graph, means within parenthesis sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over BAP and gibberellin treatments.

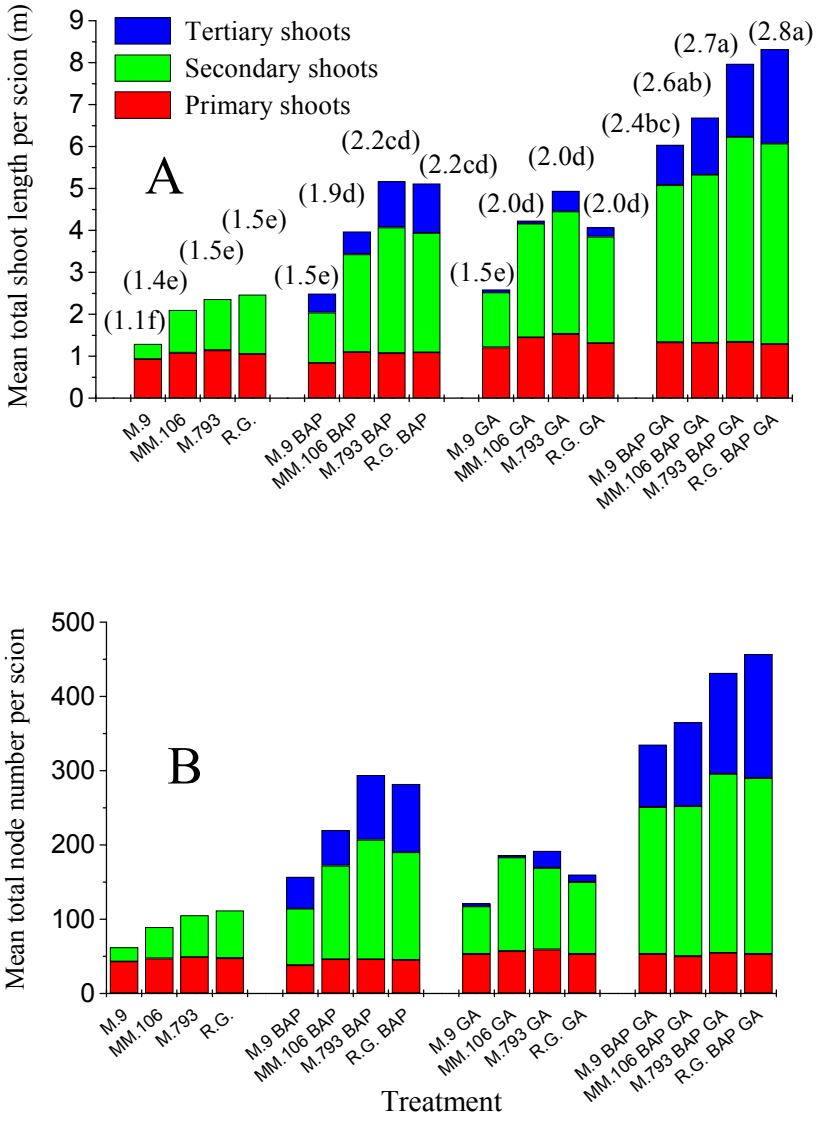


Figure 5.26. Rootstock x benzylaminopurine (BAP) x gibberellin (GA) interactions for the mean total length (A, *P*=0.02) and node number (B, *P*=0.12) of 'Royal Gala' apple scions. The interaction for the total length or node number per scion is equivalent to the total height of the columns on A or B, respectively. On graph A, means within parenthesis sharing the same letter are not significantly different at *P*≤0.05 (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over NPA treatments.

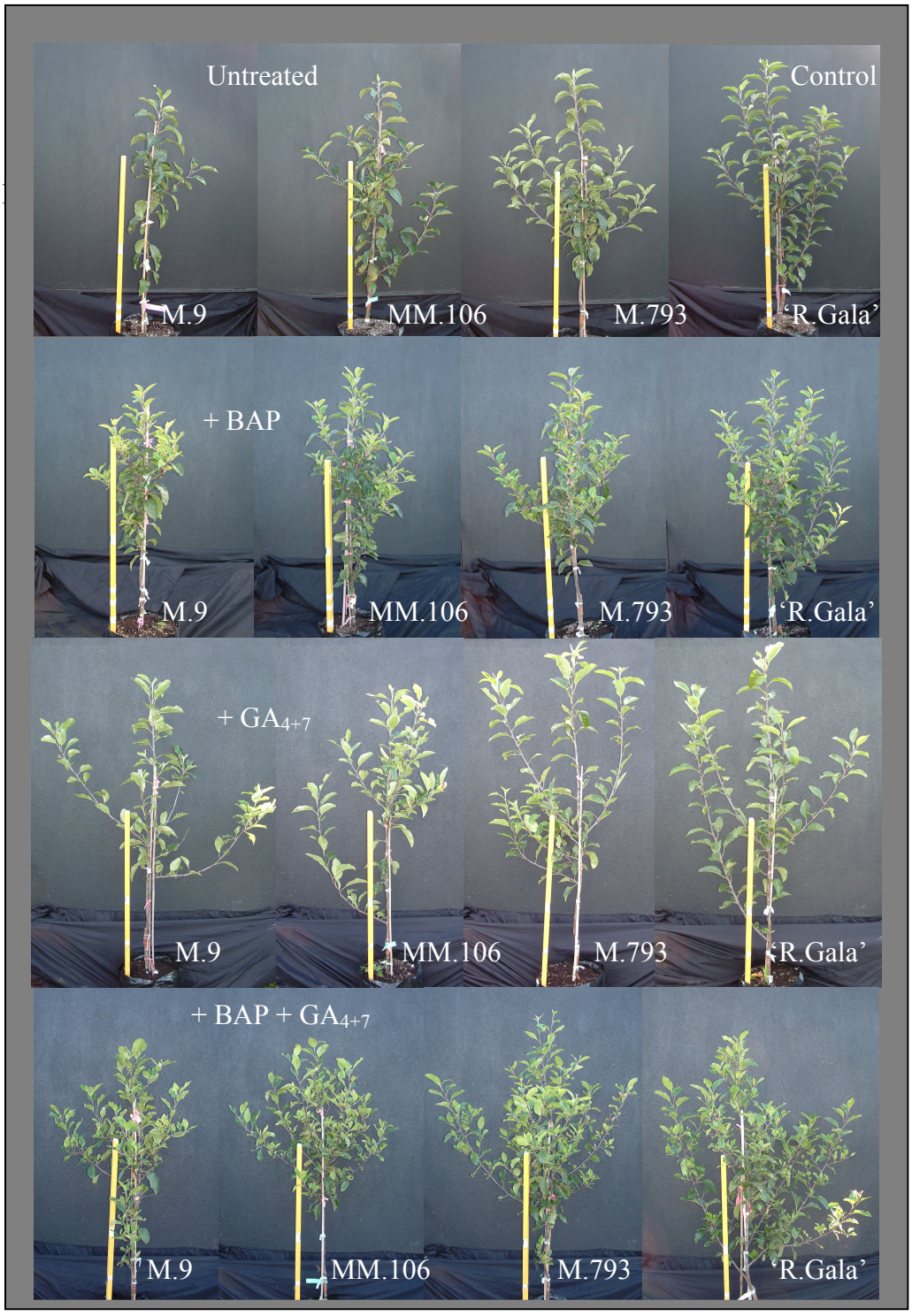


Figure 5.27. Effect of rootstocks (minus NPA), benzylaminopurine (BAP) and gibberellin (GA_{4+7}) treatments on the architecture of 'Royal Gala' apple scions at the end of their first year of growth (April, 2006) from grafting. Yellow rule is 1 m.

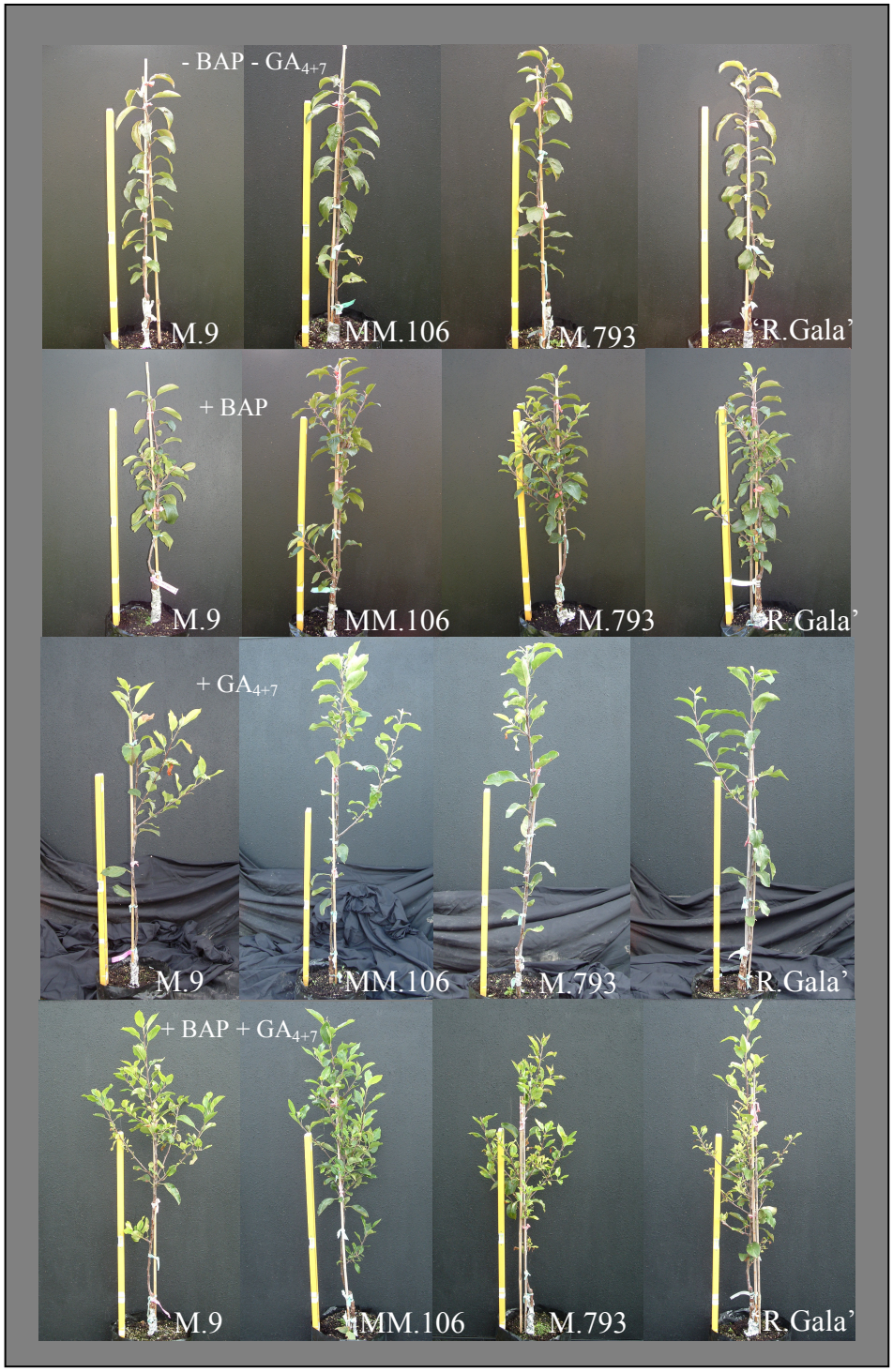


Figure 5.28. Effect of rootstocks (plus NPA), benzylaminopurine (BAP) and gibberellin (GA_{4+7}) treatments on the architecture of 'Royal Gala' apple scions at the end of their first year of growth (April, 2006) from grafting. Yellow rule is 1 m.

5.3.5 Final dry mass and length of the root system

The main effects of rootstock, GA_{4+7} and NPA significantly affected the final mean dry mass and length of the root system (Table 5.5). For rootstocks, M.9 significantly reduced the mean dry mass and length of the root system compared with MM.106, M.793 and 'Royal Gala', whereas 'Royal Gala' had a significantly smaller final root dry mass, but not root length, compared with MM.106 and M.793 (Table 5.5). Exogenous GA_{4+7} reduced both the final mean dry mass and length of the root system compared with untreated trees, whilst BAP significantly decreased mean root dry mass compared with untreated trees, but not mean total root length (Table 5.5). The application of NPA to the rootstock stem significantly decreased the final mean root dry mass and length when compared with untreated rootstocks, and these decreases were greater than those decreases imposed by BAP or GA_{4+7} (Table 5.5).

Table 5.5. Main effects of rootstock, benzylaminopurine (BAP), gibberellin (GA_{4+7}) and 1-N-naphthylphthalamic acid (NPA) on the final mean dry mass and length of root systems on rootstocks of M.9, MM.106, M.793 and 'Royal Gala' at the end of the first growing season from grafting of composite 'Royal Gala' apple trees.

Main effect	Total root dry mass (g)	Total root length (m)
Rootstock		
M.9	30.33 (0.20 a) ***	271 (15.8 a) ***
MM.106	66.83 (0.14 b)	417 (19.5 b)
M.793	74.37 (0.14 b)	452 (20.1 b)
'Royal Gala'	53.51 (0.16 c)	410 (18.9 b)
BAP		
-	61.21 (0.16 a) **	412 (18.9) ns
+	51.31 (0.17 b)	363 (18.2)
GA_{4+7}		
-	73.79 (0.14 a) ***	448 (20.1 a) ***
+	38.72 (0.18 b)	327 (17.0 b)
NPA		
-	84.39 (0.12 a) ***	602 (24.2 a) ***
+	28.13 (0.20 b)	173 (12.9 b)

ns, *,**, *** non significant or significant at $P \leq 0.05$, 0.01 and 0.001, respectively. Within a single main effect, means within a column sharing the same letter are not significantly different using the Tukey's test at $P=0.05$. Data without and within parenthesis are raw or transformed means, respectively.

In addition to the main effects, the rootstock x NPA interactions were highly significant for the final mean dry mass (P=0.0009) and root length (P=0.0005). The application of NPA to each rootstock type resulted in a significant decrease in mean root dry mass and root length when compared with the same rootstock without NPA (Figure 5.29). However, NPA caused markedly smaller reductions in mean root dry mass and length for M.9, particularly because without NPA, the mean dry mass and root length of M.9 was much smaller than MM.106, M.793 and 'Royal Gala'. The mean dry mass of the M.9 and 'Royal Gala' root system did not significantly differ when NPA was applied to the rootstock stem (Figure 5.29A and also see four-way means in Figure 5.30), whereas the mean length of the MM.106, M.793 and 'Royal Gala' root systems were similar to M.9 only when NPA was applied to the rootstock stem (Figure 5.29B). Without NPA, decreased mean dry mass of the root system for the 'Royal Gala' rootstock (Figure 5.29A) resulted from more dry mass being partitioned into the rootstock stem than the root system; hence the mean total dry mass of the rootstock (i.e., rootstock stem plus roots) did not significantly differ between M.793 and 'Royal Gala' (data not shown).

5.3.5.1 Effect of the BAP x GA_{4+7} treatment on final root dry mass and length

For each rootstock type, exogenous BAP x GA_{4+7} significantly increased the total shoot growth of the scion when compared with untreated trees on the same rootstock (Figures 5.26A and 5.27). Despite BAP x GA_{4+7} stimulating large increases in scion growth, it decreased the final mean dry mass and length of each rootstock's root system when compared with untreated trees on the same rootstock (Figure 5.30A, B). Although the BAP x GA_{4+7}-treated scion on M.9 produced very similar total shoot growth to the BAP x GA_{4+7}-treated scion on MM.106 (Figure 5.26A), the final growth of the M.9 root system was not increased (Figure 5.30A, B). Similar to the BAP x GA_{4+7}-treated scion on M.9, BAP x GA_{4+7} increased total scion growth on NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' compared with the untreated scion on these same NPA-treated rootstocks (see Figure 5.19A, B). However, these increases in total shoot growth by NPA x BAP x GA_{4+7} decreased the total dry mass of the MM.106, M.793 and 'Royal Gala' root system when compared with trees on the same rootstock type treated with only NPA (Figure 5.30B).

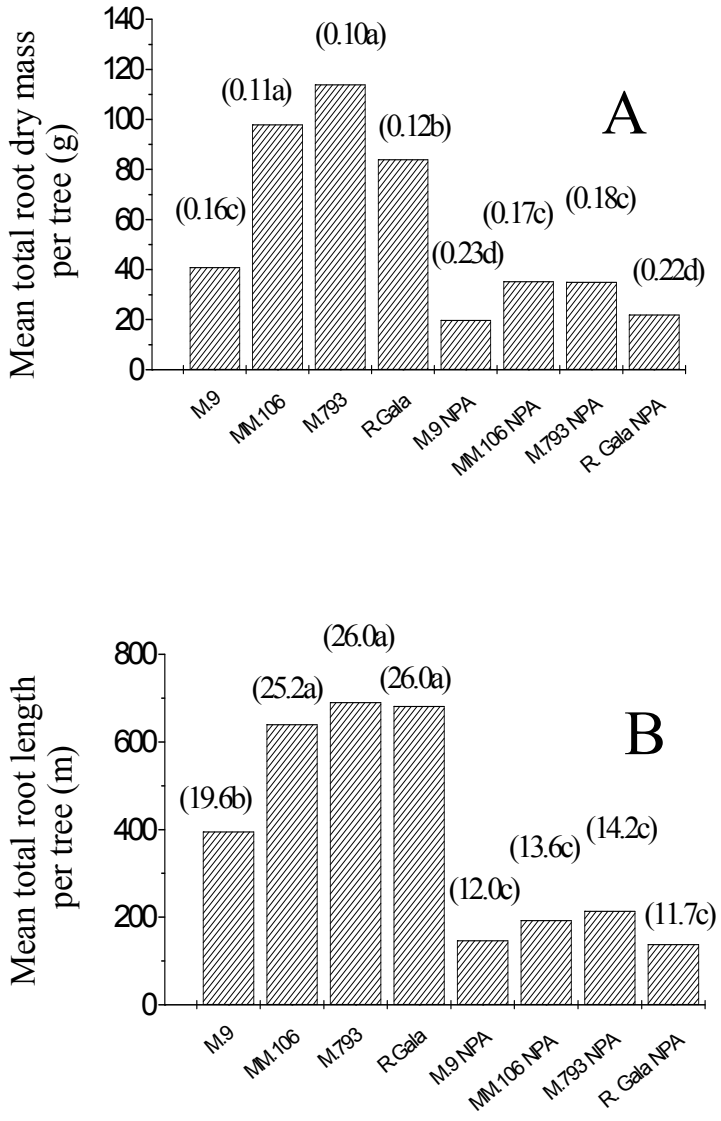


Figure 5.29. Rootstock x 1-N-naphthylphthalamic acid (NPA) interactions on the final mean total dry mass (A) and length (B) of the root system for M.9, MM.106, M.793 and 'Royal Gala' rootstocks grafted with 'Royal Gala' apple scions. Root growth was measured at the end of the first growing season from grafting. For a single interaction only, means within parenthesis sharing the same letter are not significantly different at $P \leq 0.05$ (lsmeans tests with Tukey's adjustment, SAS). Data are averaged over BAP and gibberellin treatments.

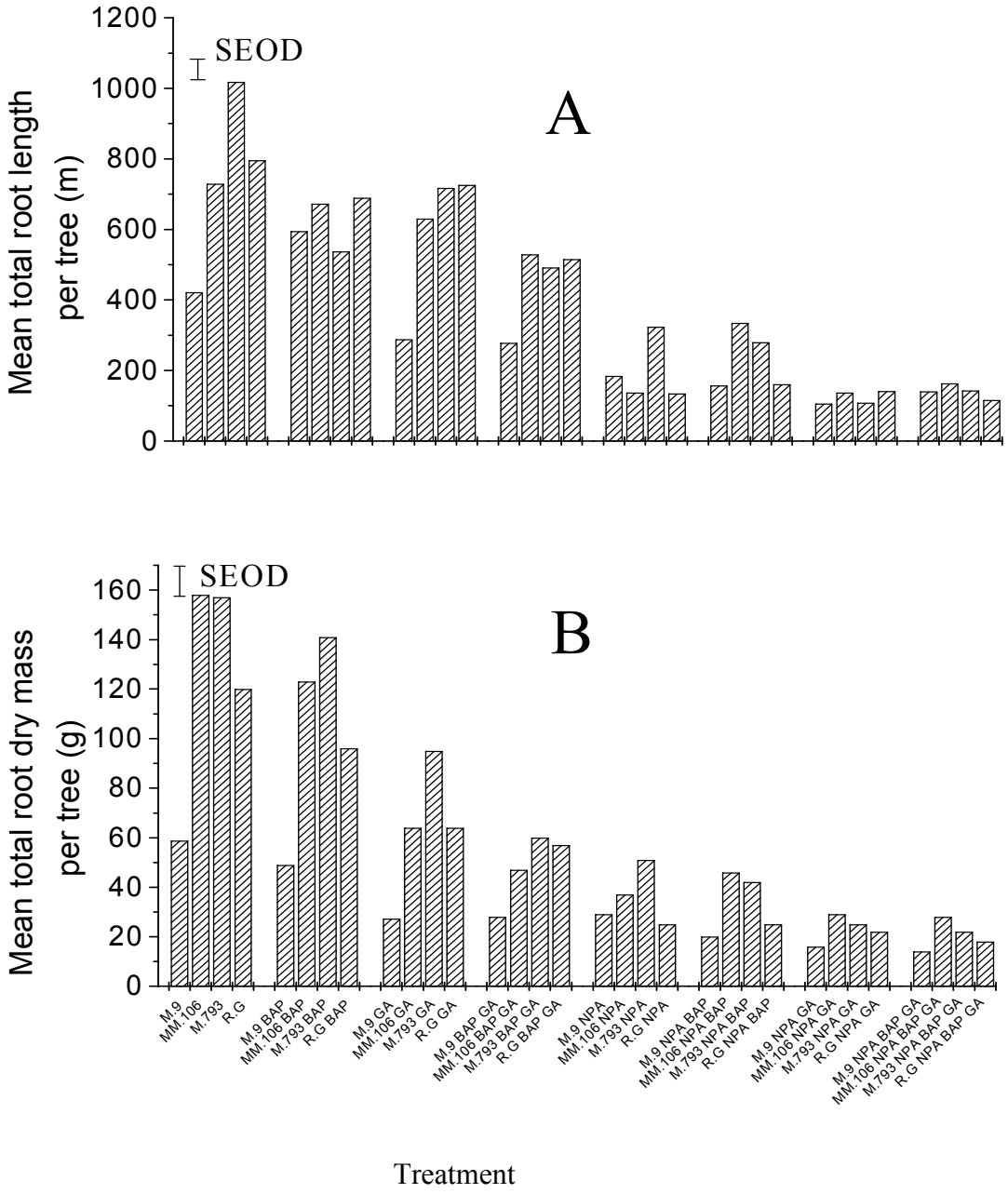


Figure 5.30. Effect of rootstock, benzylaminopurine (BAP), gibberellin (GA_{4+7} (GA)) and ± 1-N-naphthylphthalamic acid (NPA) treatments on the mean total length (A) and dry mass of the root system for composite 'Royal Gala' apple trees at the end of their first growing season from grafting. Error bars are the SEOD of the raw data.

5.4 Discussion

5.4.1 Effect of rootstocks on scion vigour and architecture

In Chapters 3 and 4, the M.9 rootstock significantly decreased total growth of the scion by the end of the first growing season after grafting when compared with rootstocks of greater vigour (Tables 3.1 and 4.1). However, between the experiments there were notable differences in how total growth of the scion on M.9 was decreased. In Chapter 3, M.9 decreased the mean number of secondary axes formed per scion compared with MM.106, whereas in the same growing season, field-grown 'Royal Gala' trees on M.9 produced more secondary axes per scion than MM.106 (i.e., Chapter 4). Therefore, objective one determined whether reduced formation of secondary axes was an important way in which the dwarfing rootstock initially decreased scion vigour during the first year from grafting.

The M.9 rootstock decreased the mean number of secondary axes that formed per scion when compared with rootstocks of MM.106, M.793 and the 'Royal Gala' control, particularly the mean number of secondary shoots (Table 5.2 and see Figures 5.11B and 5.13B). Although reductions in the mean number of secondary shoots for M.9 were small (Figure 5.11B), they had a pronounced effect on the final total growth (Figure 5.19) and architecture of the scion (Figure 5.27) when compared with the 'Royal Gala' rootstock control. Hence, data from Chapters 3 and 5 are similar in that M.9 decreased the mean number of secondary axes that formed per scion, and this phenomenon was important in the initial development of rootstock-induced dwarfing of the scion when compared with rootstocks of greater vigour.

In addition to M.9 reducing the mean number of secondary axes that formed per scion, total scion growth was decreased on M.9 because a greater proportion of short secondary shoots developed compared with the scion on the 'Royal Gala' rootstock control, particularly shoots with 15 or less nodes (Figure 5.21A). Similar results occurred in Chapters 3 and 4 when secondary shoot populations of 'Royal Gala' on M.9 were compared with those on rootstock(s) of greater vigour (Figures 3.13 and 4.9). Increased proportions of short secondary shoots may have formed for the scion grafted onto M.9 because a greater proportion of secondary shoots had terminated by late summer or early autumn when compared with rootstocks of greater vigour (Figures 3.11

and 4.7B; Table 5.1). In each experiment, M.9 also decreased the total growth of the scion by reducing the final mean length and node number of the primary shoot when compared with rootstocks of greater vigour (Figures 3.1A, B; 4.2A, B and 5.1A, B). The causes of reduced primary shoot growth on the M.9 rootstock for the data of Chapters 3, 4 and 5 are discussed in Chapter 7.

5.4.2 Effect of rootstock, NPA, BAP and GA_{4+7} on meristematic activity

Objective four elucidated whether endogenous IAA and gibberellin may interact to regulate growth of apple SAMs. For the M.9 rootstock, exogenous gibberellin treatments were of particular interest to determine whether SAMs could be prevented from slowing, and/or, terminating growth early.

5.4.2.1 Primary shoot

At growth cessation in May, interactions between NPA x GA_{4+7} were significant for mean node number and, to a lesser extent, mean length of the primary shoot (Figure 5.2). Compared with untreated trees (i.e., - NPA - GA_{4+7}), cumulative node production of the primary shoot was decreased shortly after the first application of NPA to the rootstock stem because the apical meristem on the primary shoot dramatically slowed in its growth, and/or, fully terminated 14 to 21 days after NPA treatment (Figure 5.7C). After a short period of rest, the SAM resumed growth, presumably as the effect of NPA lessened (Figure 5.7D). Since NPA reduces the basipetal transport of IAA, it was likely that less IAA was transported from the scion to the root system and this reduced the activity of the apical meristem that in turn decreased the final mean length and node number of the primary shoot (Figure 5.2).

In contrast to NPA treatment of the rootstock stem, GA_{4+7} applied repeatedly to the scion significantly increased the mean node number of the primary shoot by the end of the growing season when compared with untreated trees (Figure 5.2). For NPA-treated rootstocks, GA_{4+7} also promoted greater cumulative growth of the primary shoot over the growing season compared with untreated scions, thereby resulting in a primary shoot with a significantly greater final mean node number at growth cessation (Figures 5.2 and 5.28). Exogenous GA_{4+7} increased cumulative growth of the primary shoot by reactivating the growth of the SAM that had slowed, and/or, fully terminated in

response to NPA treatments (Table 5.1). Applications of GA_{4+7} to the scion growing on NPA-treated rootstocks also increased the final mean length and node number of the primary shoot compared with trees that were untreated (Figure 5.2). In responses similar to NPA treatment of the rootstock stem, the M.9 rootstock decreased the final mean length and node number of the primary shoot compared with the 'Royal Gala' rootstock (Figure 5.3). However, with GA_{4+7} the final mean length and node number of the primary shoot on M.9 and 'Royal Gala' was very similar (Figure 5.3). The ability of GA_{4+7} to increase the cumulative node number and length of the primary shoot on M.9 or on NPA-treated rootstocks was very similar (Figures 5.2 and 5.3). Hence, reduced cumulative growth of the primary shoot on M.9 may have been caused by interactions that involved the endogenous transport of IAA and gibberellin.

Endogenous gibberellins are transported within the xylem sap of apple trees (Jones and Lacey, 1968; Ibrahim and Dana, 1971; Motosugi et al., 1996) indicating that the root may produce and supply gibberellins to the scion (Jones and Lacey, 1968). Dwarfing compared with vigorous rootstocks had decreased endogenous concentrations of gibberellins within the root (Yadava and Lockard, 1977), xylem sap (Ibrahim and Dana, 1971) and leaves or shoots (Yadava and Lockard, 1977). Given that exogenous gibberellins were shown to stimulate meristematic activity of apple shoots (Sironval et al., 1962; Marcelle, 1963; Martin, 1967; Robitaille and Carlson, 1971, 1976; Luckwill and Silva, 1979; Tromp, 1982; Steffens et al., 1985; Popenoe and Barritt, 1988; Figures 5.2 and 5.3) and dwarfing apple rootstocks decreased gibberellin supplied to the shoot (Ibrahim and Dana, 1971), a reasonable hypothesis would be that decreased cumulative growth of the 'Royal Gala' primary shoot on M.9 (Figure 5.3) or on NPA-treated rootstocks (Figure 5.2) resulted from a reduced root supply of gibberellin.

In the order of most to least abundant, root-produced gibberellins in the xylem sap of apple include GA_{19}, GA_{53}, GA_{23}, GA_{44}, GA_{15}, GA_{17} and GA_{18} (Motosugi et al., 1996), which are typically regarded as biologically inactive gibberellin forms (Yamaguchi, 2008). However, gibberellin forms like GA_{19} might be converted at the shoot to GA_{20} by GA 20-oxidase and then to bioactive GA_1 by GA 3-oxidase (Yamaguchi, 2008). For example, the suppression of GA 20-oxidase in the shoot of the apple cultivar 'Greensleeves' grown on its own roots increased endogenous concentrations of GA_{19} and decreased concentrations of GA_{20} and GA_1 in the shoot apex and young leaves

(Bulley et al., 2005). Thus, inactive root-produced GA_{19} may be readily converted to bioactive GA_1 by the scion, and hence, may be an important signalling mechanism between the rootstock and scion. Furthermore, interactions between rootstock x GA_{4+7} and NPA x GA_{4+7} for the cumulative node number and length of the primary shoot may indicate that decreased basipetal transport of IAA from scion to root downregulated gibberellin biosynthesis by the root, thereby decreasing the transport of root-produced precursors of GA_1 to the scion (e.g. GA_{19}) in the xylem vasculature. This would consequently reduce node neoformation and growth of the primary shoot, which could be reversed with exogenous GA_{4+7} (Figures 5.2 and 5.3). In a similar manner to NPA treatment of the rootstock stem, shoots of M.9 terminated earlier than shoots of vigorous rootstocks, and earlier termination of M.9 shoots coincided with no basipetal transport of ^{3}H-IAA within isolated stem segments into the agar receptor (Kamboj et al., 1997).

The role of endogenous hormones within the apical and subapical regions of an apple SAM are largely unknown, particularly how hormones like IAA, gibberellin and cytokinin may interact to regulate meristematic activity. However, models of hormonal control of meristematic activity are well established for many annual species including *Arabidopsis* and pea. The formation of new nodes and internode extension for apple shoots (Pratt, 1990) and many other plant species (Sachs, 1965) is controlled by the activity of the apical and subapical regions of the SAM, respectively. For pea, both IAA and gibberellin are required for internode extension by the subapical region of the SAM. For example, removal of the shoot apex, or the basipetal IAA signal, decreased the concentration of endogenous GA_1 in subtending elongating internodes, which resulted from decreased GA 3-oxidase activity, thereby decreasing the conversion of GA_{20} into bioactive GA_1 (Ross et al., 2000). Compared with intact plants, the concentration of bioactive GA_1 and expression of GA-oxidase transcript levels in the subtending internodes was restored with exogenous IAA applied to the cut stem (Ross et al., 2000). Therefore, IAA transport out of the shoot apex may mediate internode elongation by regulating bioactive gibberellin.

Conversely, gibberellin may also be required for IAA synthesis at the shoot apex. For example, the restriction of basipetal IAA transport from scion to root decreased the neoformation of nodes (Figure 5.2) and leaves by the SAM on the primary shoot. Leaf primordia are initiated in the peripheral zone of the SAM (Carraro et al., 2006; Evert,

2006), whilst young leaves at the shoot apex are a rich source of endogenous IAA (Ljung et al., 2001), and leaves subtending the SAM may supply the peripheral zone with IAA via acropetal transport (Vogler and Kuhlemeier, 2003). Exogenous IAA applied to the peripheral zone of the SAM initiated leaf primordia in *Arabidopsis* at the site of application (Reinhardt et al., 2000), thereby suggesting the IAA signal is vital for primordia initiation. However, high concentrations of both IAA and gibberellin are considered to be prerequisites for leaf initiation in the peripheral zone of an *Arabidopsis* SAM (Took and Battey, 2003; Shani et al., 2006). Kuraishi and Muir (1962) reported that gibberellin applied to sunflower plants yielded 10 times more diffusible IAA than in untreated plants. Collectively, the above studies and the NPA x GA_{4+7} interactions (Figure 5.2) indicate that sufficient concentrations of both IAA and gibberellin are important for node neoformation and internode extension by the apical and subapical regions, respectively, of an apple SAM.

In order to promote shoot growth, bioactive gibberellin must bind to its protein receptor (GID1) to induce degradation of growth repressing nuclear DELLA proteins (Jiang and Fu, 2007; Desgagne-Penix and Sponsel, 2008; Yamaguchi, 2008). *Arabidopsis* seedlings grown in solutions of NPA showed decreased hypocotyl growth and increased accumulation of the DELLA repressor (RGA) compared with control plants (Desgagne-Penix and Sponsel, 2008). Furthermore, exogenous GA_4 decreased RGA accumulation and partially reversed decreased hypocotyl length imposed by NPA. Notably, these growth interactions between NPA x gibberellin for *Arabidopsis* are very similar to the effects of NPA x GA_{4+7} and the rootstock x GA_{4+7} interaction measured for the cumulative length of the apple primary shoot (Figures 5.2 and 5.3). Furthermore, the expression of *MdDELLAs* were much higher in summer-arrested shoot apices of 'Royal Gala' than apices that were actively growing (Foster et al., 2007).

Although speculative, mechanisms may exist whereby the stem tissue of the dwarfing rootstock begins to reduce polar auxin transport from scion to root in summer due to lower daily light integrals and decreasing temperature. Decreased basipetal transport of IAA may reduce the amount of inactive root-produced gibberellin transported in the xylem vasculature to the scion (i.e., GA_{19}), thereby limiting the biosynthesis of bioactive gibberellin at the shoot apex (GA_1). Lack of bioactive GA_1 may initially limit IAA and gibberellin controlled initiation of leaf primordia in the peripheral zone of the

SAM (Took and Battey, 2003; Shani et al., 2006), thereby decreasing the emergence of new leaves as potential sites of IAA synthesis. This may consequently decrease IAA synthesised at the shoot apex, therefore reducing the amount of IAA available for acropetal transport from young leaves to the above peripheral meristematic zone (Vogler and Kuhlemeier, 2003). Low concentrations of IAA at the shoot apex may limit GA 3-oxidase activity, thus preventing the conversion of GA_{20} to GA_1 (Ross et al., 2000), whilst low concentrations of GA_1 may prevent gibberellin mediated destruction of MdDELLAs (Foster et al., 2007). Collectively, these mechanisms may explain putative mechanisms by which the M.9 rootstock decreases meristem activity and would be worthy of further investigation.

Although the primary shoot on M.9 produced a very similar final mean number of nodes in response to exogenous GA_{4+7} when compared with MM.106, M.793 and 'Royal Gala' (Figure 5.5B), the final mean length of the primary shoot was slightly shorter for the GA_{4+7}-treated scion on M.9 (Figure 5.5A). Hence, the primary shoot on M.9 that was treated with GA_{4+7} tended to have slightly shorter internodes than the GA_{4+7}-treated scion on MM.106, M.793 and 'Royal Gala' (Figure 5.5C). In contrast to GA_{4+7}, exogenous BAP caused the primary shoot on M.9 to cease producing neoformed nodes very early in the growing season (Figure 5.4G; Table 5.1). However, the BAP-treated primary shoot on M.9 had increased internode length compared with untreated trees on M.9 (Figure 5.5C). Notably, each rootstock type produced a primary shoot that was very similar in its final mean length, node number and internode length when the scion was treated with BAP x GA_{4+7} (Figures 5.4D, H and 5.5A, B, C). Therefore, a slightly shorter primary shoot may have developed for the GA_{4+7}-treated scion on M.9 because endogenous cytokinin was lacking for internode extension, which would result from reduced activity of the subapical region of the SAM (Sachs, 1965). In contrast, fewer neoformed nodes may have developed for the BAP-treated scion on M.9 because endogenous gibberellin and IAA were limiting node formation, which occurs from the activity of the apical region of the SAM (Sachs, 1965; Pratt 1990), particularly the peripheral zone (Carraro et al., 2006; Evert, 2006). Collectively, these results indicate that shoot extension growth produced by the apple SAM (apical and subapical regions) requires a suitable balance of IAA, gibberellin and cytokinin.

5.4.2.2 Secondary shoots

In addition to the primary shoot, GA_{4+7} increased meristematic activity and growth of the secondary shoots. Without the application of GA_{4+7}, greater proportions of secondary shoots had terminated for M.9 during February and March when compared with the scion on MM.106, M.793 and the 'Royal Gala' rootstock control (Table 5.1). However, with GA_{4+7} the proportion of terminated secondary shoots was not dramatically different amongst rootstocks in February and March (Table 5.1). For M.9, GA_{4+7} stimulated greater proportions of long secondary shoots compared with the untreated scion, especially shoots with 16 to 35 nodes (Figure 5.21A, B). This was a probable consequence of GA_{4+7} increasing the proportion of SAMs that were actively growing during February, March and April (Table 5.1). The ability of exogenous GA_{4+7} to decrease the proportion of secondary shoots that terminated early for M.9 (Table 5.1), and to increase the proportion of long shoots that developed (Figure 5.21), may indicate that the scion on the M.9 rootstock was lacking endogenous gibberellin from late summer. Similar to these results, exogenous gibberellin increased the mean length of secondary shoots that developed for the 'Golden Delicious' scion on the M.9 rootstock grown in a tree nursery (Marcelle, 1963). Luckwill and Silva, (1979) also reported that exogenous GA_3 increased the proportion of annual shoots that were growing on apple scions late in the season.

For untreated trees on each rootstock type, secondary shoots of the same node number had comparable shoot lengths (Figure 5.20A), hence the internode length (i.e., length ÷ node number) of secondary shoots was not affected by rootstock type. However, secondary shoots on BAP-treated scions were shorter compared with secondary shoots with the same node number on untreated trees, hence BAP reduced internode length of the secondary shoots (see Figure 5.20A and D where internode lengths can be compared from the regression line fitted to the pooled data by dividing a y-value by its corresponding x-value). In contrast, secondary shoots on GA_{4+7}-treated scions were slightly longer compared with secondary shoots of the same node number on untreated trees or on BAP-treated scions, hence GA_{4+7} increased the internode length of the secondary shoots (see Figure 5.20A, B, D). However, secondary shoots on BAP x GA_{4+7}-treated scions were of similar length compared with secondary shoots of the same node number on untreated trees, therefore internode lengths were more similar

between these treatments (see Figure 5.20A and F). These results strongly indicate that internode extension by the subapical region of SAMs on untreated trees (Figure 5.20A) was controlled by an intricate balance of endogenous cytokinin and gibberellin.

5.4.3 Effect of treatments on the formation of secondary axes

A further objective of Chapter 5 was to determine if reduced formation of secondary axes for the scion on M.9 involved putative interactions between decreased shoot to root transport of endogenous IAA and reduced root to shoot transport of root-synthesised cytokinin (objective two). To examine this interaction experimentally, NPA was applied to the rootstock stem of half the trees on each rootstock type to restrict basipetal auxin transport from scion to root, whilst BAP was applied to half the scions on each rootstock type treated with or without NPA to elucidate the effects, and/or, interactions on branch induction.

As found in Chapter 3, the primary effect of exogenous BAP was to increase the formation of secondary axes (i.e., total of spurs plus secondary shoots) that formed on the primary shoot (Figure 5.12B). Interestingly, GA_{4+7} also stimulated significant increases in the mean number of secondary axes that formed when compared with untreated trees, particularly the mean number of secondary shoots (Figures 5.10B and 5.12B). However, BAP stimulated the formation of significantly more secondary axes than GA_{4+7} (Figures 5.10B and 5.12B). These data suggest that endogenous cytokinin is more important as a primary regulator of axillary bud outgrowth on the primary shoot than endogenous gibberellin.

With NPA treatment of the rootstock stem, probable inhibition of IAA transport from scion to root may explain the decreased number of secondary axes formed on the primary shoot (Figure 5.13B). However, the formation of secondary axes was reinstated when BAP was applied to the scion growing on NPA-treated rootstocks (Figure 5.13B). This effect of the NPA x BAP treatment may indicate that reduced basipetal transport of IAA to the root decreased the amount of root-produced cytokinin transported to the scion, which may have modified scion architecture by reducing the mean number of secondary axes that formed. In a similar manner, the stem tissue of the dwarfing rootstock decreased IAA transport (Soumelidou et al., 1994a; Kamboj et al., 1997) to

the root (Kamboj, 1996), which may have decreased cytokinin transported from root to scion (Kamboj et al., 1999).

The mechanism(s) by which reduced basipetal transport of IAA decreases the synthesis of root-produced cytokinin may involve sub-optimal concentrations of IAA decreasing root growth, and/or, directly reducing cytokinin biosynthesis by the root (Lockard and Schneider, 1981). With regard to the latter, decapitation of bean plants transiently increased cytokinin concentrations in the xylem sap, which were then decreased by applying IAA to the cut stem (Bangerth et al., 2000). Other studies have also shown that decreased transport of IAA to the root increased cytokinin biosynthesis (Currie, 1997; Nordstrom et al., 2004). Thus, these studies do not support the hypothesis that reduced transport of IAA to the root decreases cytokinin biosynthesis as proposed by Lockard and Schneider (1981).

Clearly, it would not be unreasonable to question whether the physiological responses from short-term decapitation studies (i.e., Bangerth et al., 2000) reflect those responses imposed over a longer timeframe. Indeed, the application of NPA to the decapitated stem of kiwifruit vines initially increased cytokinin concentrations in the xylem sap, but this effect was transient, with NPA-treated stems exhibiting significantly lower endogenous cytokinin concentrations than control (decapitated only) vines after 72 hours (Currie, 1997). A transient increase in root-produced cytokinin (Currie, 1997) may partly explain why axillary shoots developed on the rootstock stem soon after NPA treatment (see Chapter 2, Figure 2.26). However, increased amounts of cytokinin in the xylem sap in reponse to NPA would not necessarily reflect actual cytokinin transport from root to scion over the longer term, particularly when the effects of inhibition of IAA transport on root morphology and growth are considered.

The NPA treatment and the dwarfing rootstock both decreased the final growth of the root system (Figure 5.29). In other plant species, NPA decreased root elongation (Fujita and Syono, 1996; Reed et al., 1998; Fu and Harberd, 2003) and initiation (Reed et al., 1998). For apple, IAA is also required for root initiation (Delargy and Wright, 1979). Lateral root meristems are the major sites of cytokinin biosynthesis in *Arabidopsis* (Nordstrom et al., 2004). Thus, an important way in which the shoot-derived IAA signal may down-regulate the synthesis of cytokinin by the root could involve decreasing the

number of lateral roots, hence the number of root tips available for cytokinn biosynthesis. Over a longer period of time, such changes in root architecture may explain how decreased IAA transport by the dwarfing rootstock or the NPA treatment could have down-regulated cytokinin synthesis by the root.

5.4.3.1 Putative interactions between IAA, cytokinin and gibberellin on the formation of secondary axes

In addition to reducing cytokinin produced by the root system, inhibition of IAA transport by M.9 or the NPA treatment may have decreased the biosynthesis of root-produced gibberellin. For example, cumulative growth of the primary shoot on M.9 or NPA-treated rootstocks was significantly increased when the scion was treated with GA_{4+7} (Figures 5.2 and 5.3). Interestingly, exogenous gibberellin also promoted small increases in the mean number of secondary shoots that developed for the scion on the M.9 or the NPA-treated rootstock (Figure 5.11B). Similar to these results, exogenous gibberellin increased the formation of secondary shoots for composite apple trees on M.9 grown in a nursery (Sironval et al., 1962; Marcelle, 1963). Therefore, gibberellin may also have a role in the formation of secondary shoots.

For M.9, treatments of BAP x GA_{4+7} produced more secondary shoots than BAP alone (Figure 5.11B). In addition, the mean number of secondary shoots was very similar for the BAP x GA_{4+7}-treated scion on each rootstock type, whereas with only BAP, the scion on M.9 developed fewer secondary shoots than MM.106 and M.793 (Figure 5.11B). For M.9, decreased secondary shoot formation in response to BAP may have been caused by decreased basipetal transport of IAA from scion to root. For example, NPA treatment of M.9 further reduced the effectiveness of BAP in promoting secondary shoot formation on the primary shoot (Figure 5.11B). However, with NPA x BAP x GA_{4+7}, the scion on M.9 produced more secondary shoots than the NPA x BAP treatment, and had a similar number of secondary shoots to NPA x BAP x GA_{4+7}-treated trees on MM.106, M.793 and ‘Royal Gala’ (Figure 5.11B). Thus, interactions among IAA, cytokinin and gibberellin may explain the regulation of secondary shoot formation on the primary shoot more satisfactorily than interactions between IAA and cytokinin. During the course of the experiment, it was observed that exogenous BAP stimulated less bud outgrowth on scions whose shoots had mostly terminated growth, whereas

scions that were actively growing, such as those treated with GA_{4+7}, exhibited more bud outgrowth soon after an application of BAP. Kender and Carpenter (1972) also reported that BAP applied to the scion was less effective in breaking apical dominance of young apple trees when timed after shoot termination. Thus, gibberellin may have enhanced branch development by keeping more meristems actively growing (Table 5.1), thereby enabling subsequent applications of BAP to stimulate comparatively more bud break than when scions were treated with BAP alone (Figure 5.13C).

With or without BAP, the total number of secondary axes that formed for the scion on M.9 consisted of proportionally more secondary spurs than secondary shoots when compared with other rootstocks (Figure 5.13B). There were also trends that the number of secondary spurs decreased with increasing vigour of the rootstock, particularly for the BAP-treated scion (Figure 5.13B). Thus with BAP treatment, the scion on M.9 tended to develop fewer secondary shoots (i.e., $\geq$ 2.5 cm) than the scion treated with BAP on MM.106, M.793 and 'Royal Gala' (Figure 5.13B). However, the scion on M.9 developed a very similar mean number of secondary shoots to MM.106, M.793 and 'Royal Gala' when treated with BAP x GA_{4+7} (Figure 5.11B). This occurred because BAP x GA_{4+7} stimulated greater proportions of breaking axillary buds to extend and form secondary shoots rather than spurs, and because BAP x GA_{4+7} stimulated the formation of more secondary shoots compared with the scion on M.9 that was treated with BAP only (Figure 5.13B). These results strongly suggest that the BAP-treated scion on M.9 was receiving reduced amounts of root-produced gibberellin; hence it formed more secondary spurs and fewer secondary shoots.

5.4.4 Effect of treatments on total scion growth

5.4.4.1 Similarities between the dwarfing rootstock and the inhibition of basipetal IAA transport from scion to root using NPA

Dwarfing apple rootstocks may reduce scion vigour by decreasing the amount of IAA that is transported basipetally within the phloem and cambial cells of their stem to the root system (Lockard and Schneider, 1981; Soumelidou et al., 1994a; Kamboj et al., 1997). Grochowska et al., (1994) reported that the growth of apple shoots eventually ceased when the basipetal transport of IAA was decreased from shoot to root using TIBA applied to the root collar of apple seedlings. Thus, evidence exists that the

basipetal auxin signal is indeed a critical factor regulating shoot growth of the apple tree. However, it is largely unknown how the inhibition of IAA transport from shoot to root modifies scion architecture of the composite apple tree, and whether such alterations in growth are similar to those imposed by the dwarfing rootstock. Therefore, objective five was to elucidate whether the use of NPA to inhibit auxin transport within the stem tissue of vigorous rootstocks decreased scion vigour, and whether modifications in scion architecture were similar to those initially imposed on the scion by the dwarfing apple rootstock in the first year of growth from grafting.

Interruption of basipetal IAA transport from scion to root imposed dramatic reductions in scion vigour, particularly for the 'Royal Gala' rootstock control (Figure 5.25). More importantly, the changes in scion architecture imposed by NPA treatment of the rootstock stem were generally similar to those induced by the M.9 rootstock (Figures 5.27 and 5.28). For example, NPA and M.9 both decreased the mean number of secondary shoots that formed per scion (Figures 5.11, 5.27 and 5.28), caused greater proportions of meristems to slow, and/or, fully terminate much earlier in the growing season (Table 5.1) and reduced the final mean length, node number and SCA of the primary shoot (Figures 5.1, 5.2, 5.3 and 5.5A, B, D). Collectively, these results strongly suggest that basipetal auxin transport from scion to root is a critical process in the regulation of scion vigour. In addition, the basipetal IAA signal may also regulate growth of root system, as indicated by the significant reductions in the final mean root dry mass and length that were imposed by M.9 and the NPA treatment (Figure 5.29). Therefore, these data support the hypothesis of Lockard and Schneider (1981) who proposed that reduced transport of IAA from scion to root may decrease root growth (Figure 5.29) and scion vigour (Figures 5.19, 5.27 and 5.28).

5.4.4.2 Influence of BAP x GA_{4+7} in reversing rootstock-induced dwarfing of the scion

In Chapter 3, the mean total shoot length and node number of the 'Royal Gala' scion on M.9 was decreased compared with MM.106 and M.793 rootstocks. Total growth per scion was reduced on M.9 partly because fewer secondary shoots formed on the primary shoot (Figure 3.7). With BAP, the scion on M.9 developed a similar number of secondary shoots to MM.106 and M.793 (Figure 3.7), although the scion was still markedly smaller because secondary shoots terminated earlier for M.9 than for MM.106

or M.793 (Figure 3.11). Hence, the final growth and size of the BAP-treated scion on M.9 was reduced compared with the BAP-treated scion on MM.106 and M.793.

Evaluation of the literature (Chapter 3, Section 3.4.4) indicated that earlier termination of secondary shoots (Figure 3.11), particularly those for M.9, may have resulted because endogenous concentrations of gibberellins were limiting growth of the SAMs. Therefore, a further objective of this experiment was to apply BAP to reinstate the formation of secondary shoots for the scion on M.9 followed by sequential applications of GA_{4+7} and then BAP to try and keep the secondary shoots actively growing (objective three). As a treatment, BAP x GA_{4+7} was used to determine whether the initial scion dwarfing on M.9 could be prevented in the first year of growth from grafting by supplying the 'Royal Gala' scion with exogenous hormones that, hypothetically, the root system of M.9 could not.

Similar to Chapter 3, M.9 with or without BAP increased the proportion of secondary shoots that terminated early when compared with the same treatment on MM.106, M.793 or 'Royal Gala' rootstocks (Table 5.1). With BAP x GA_{4+7}, 52% more secondary shoots were growing in February for M.9 when compared to treatment with BAP alone (Table 5.1). Thus, BAP x GA_{4+7} kept more meristems actively growing during February than treatment with BAP alone. For the scion on M.9, GA_{4+7} was comparatively more effective at preventing termination of the primary and secondary shoots over the growing season than either BAP or the BAP x GA_{4+7} treatment (Table 5.1), thereby indicating that gibberellin rather than cytokinin was primary regulator of continuous SAM activity.

For M.9, treatment of the scion with BAP x GA_{4+7} increased total scion growth compared with the BAP-treated scion (Figure 5.19A, B). This resulted because BAP x GA_{4+7} increased the mean shoot length and node number of the primary and secondary shoots (Figures 5.5A, B and 5.22A, B) and increased the mean number of secondary and tertiary shoots that formed compared with the BAP-treated scion on M.9 (Figure 5.13C). With BAP x GA_{4+7}, total scion growth on M.9 was reduced compared with the scion on M.793 and 'Royal Gala' (Figures 5.19A, B and 5.27). Arguably, the dwarfing effect of M.9 was reversed to some extent with BAP x GA_{4+7} because the BAP x GA_{4+7}-treated scion on M.9 or MM.106 developed very similar total growth. In addition, total

scion growth of the BAP x GA_{4+7}-treated scion on M.9 was much greater than untreated trees on the 'Royal Gala' rootstock control (Figure 5.19A, B). However, the BAP x GA_{4+7}-treated scion on M.9 formed fewer tertiary shoots (Figure 5.13C) and the mean length and node number of the secondary shoots was reduced compared with the BAP x GA_{4+7}-treated scion on M.793 or 'Royal Gala'. Hence, total scion growth was decreased for the BAP x GA_{4+7}-treated scion on M.9 when compared with that on M.793 or 'Royal Gala' (Figures 5.19A, B and 5.27).

In a similar manner to M.9, BAP x GA_{4+7} applied to the scion on NPA-treated rootstocks of M.793 and 'Royal Gala' stimulated markedly less total shoot extension growth when compared with the BAP x GA_{4+7}-treated scion on the same rootstock type that was untreated (Figure 5.19A, B). Hence, BAP x GA_{4+7} could not fully reverse reductions in scion vigour whilst IAA transport from scion to root was impaired, even for the highly vigorous 'Royal Gala' rootstock. A probable reason for this was the dramatic decrease in root growth caused by NPA, and because these decreases in root growth were further compounded when GA_{4+7} and, to a lesser extent, BAP were applied to the scion (Figure 5.30).

5.4.5 Effect of rootstock and NPA on root growth

Objective six elucidated whether the inhibition of basipetal polar auxin transport from scion to root decreased root growth, particularly for vigorous apple rootstocks that had the auxin transport inhibitor 'NPA' applied to the rootstock stem. The effects of NPA on the final growth of the root system were also compared with the M.9 rootstock, which was reported to reduce the basipetal transport of IAA within its stem (Soumelidou et al., 1994a; Kamboj et al., 1997) to the roots (Kamboj, 1996), and decrease the size of the root system compared with rootstock(s) of greater vigour (Vyvyan, 1955; Abod and Webster, 1989).

Application of NPA to the rootstock stem significantly decreased the final mean dry mass and length of the root system when compared with untreated trees (Table 5.5). Similarly, the M.9 rootstock significantly reduced the total growth of the root system when compared with rootstocks of greater vigour (Figure 5.29A, B). However, the application of NPA to the rootstock stem of MM.106, M.793 and 'Royal Gala' dramatically decreased root growth, thereby resulting in a root system that typically had

an equivalent or smaller final root dry mass and length compared with the root system formed on the untreated M.9 rootstock (Figure 5.29 A, B). As hypothesised by Lockard and Schneider (1981), these results may indicate that shoot produced IAA is required for the growth of apple roots. In addition, inhibition or reduction of the IAA signal from scion to root may be an important mechanism by which M.9 decreased root growth compared with rootstocks of MM.106, M.793 and 'Royal Gala'. Furthermore, reduced size of the root system would appear to be hormonally based as the scion on M.9 or NPA-treated rootstocks did not show any symptoms of water stress or nutrient deficiencies.

Decreased total growth of the root system caused by M.9 or the NPA treatment may have resulted from reductions in average root length, and/or, the number of lateral roots formed. Auxin is required for initiation of lateral roots in many plant species including apple (Delargy and Wright, 1979) and *Arabidopsis* (Bhalerao et al., 2002). For *Arabidopsis*, the application of NPA to the root-shoot transition region decreased the basipetal transport of stem applied ^{3}H-IAA into the root, the concentration of endogenous IAA within the root and the number of lateral roots that formed on the primary root (Reed et al., 1998). In addition, shoot derived IAA promoted root elongation in maize (Pilet et al., 1979) and *Arabidopsis* (Fujita and Syono, 1996; Reed et al., 1998; Fu and Harberd, 2003). However, promotion of root elongation by IAA is highly dependent on its concentration because either low or supraoptimal concentrations of IAA stimulated or inhibited root elongation, respectively (Pilet et al., 1979). Collectively, these results indicate that decreased basipetal IAA transport within the rootstock stem of M.9 may cause sub-optimal IAA concentrations within the root that may have limited root growth by preventing both root initiation and elongation.

In *Arabidopsis*, IAA and gibberellin may interact to control root elongation. The gibberellin deficient mutant (*ga1-3)* had shorter primary roots compared with wild types. However, the mean root length was similar between these treatments when exogenous GA_3 was applied to the primary root of *ga1-3* indicating that gibberellin controlled root elongation (Fu and Harberd, 2003). However, GA_3 applied to roots of *ga1-3* did not increase root elongation when the shoot apex was removed. Therefore, shoot derived IAA may regulate root growth from a distance by modulating gibberellin controlled growth responses in the root, particularly gibberellin mediated destabilisation

of DELLA growth repressor proteins (Fu and Harberd, 2003). These results for *Arabidopsis* may also have relevance for the elongation of apple roots, particularly given that reduced IAA transport from shoot to root might decrease the biosynthesis of root-produced gibberellins (Figure 5.2), which may prevent gibberellin mediated destruction of DELLA proteins within the root, thereby reducing root elongation.

5.5 Summary

In order to clearly understand the physiological control of rootstock-induced dwarfing of the scion it is important to ascertain changes that occur in the first season of growth after grafting. Subsequent responses in year two or three after grafting could well be an effect of dwarfing rather than the initial cause.

In Chapters 3 and 4, M.9 had significantly reduced the mean total length and node number of the scion by the end of the first year of growth from grafting when compared with rootstock(s) of greater vigour. However, a major architectural difference between these studies was that M.9 reduced the mean number of secondary axes that formed per scion compared with MM.106 in Chapter 3, whereas in Chapter 4, the scion on M.9 formed more secondary axes than the scion on MM.106. Therefore, an important objective of Chapter 5 was to elucidate whether reduced formation of secondary axes was an important way in which the dwarfing rootstock initially decreased total shoot growth of the scion during the first year after grafting.

Compared with the 'Royal Gala' rootstock control, M.9 decreased the mean number of secondary axes that formed on the primary shoot, particularly the mean number of secondary shoots. Therefore, reduced formation of secondary axes was an important way in which M.9 decreased total scion growth, which further supports the previous results reported in Chapter 3. Similar to the M.9 rootstock, NPA applied to the rootstock stem of MM.106, M.793 and 'Royal Gala' decreased the formation of secondary axes, and resulted in the earlier slowing, and/or, termination of shoot meristems when compared with untreated trees on these same rootstocks. In addition, both M.9 and NPA-treatment of MM.106, M.793 and 'Royal Gala' rootstocks decreased the final mean length, node number and shoot cross-sectional area of the primary shoot. Collectively, growth changes imposed by M.9 or NPA treatment of MM.106, M.793 and 'Royal Gala' rootstocks dramatically reduced total scion growth. In addition, NPA treatment of MM.106, M.793 and 'Royal Gala' rootstocks resulted in a scion that was architecturally most similar to the untreated scion of 'Royal Gala' grafted onto M.9. At growth cessation, decreased total scion growth caused by M.9 or NPA treatments were accompanied by significantly reduced mean total dry mass and length of the root system. Similarities between the effects of M.9 and NPA on root and scion growth

indicate that reduced basipetal transport of IAA from scion to root maybe an important hormonal signal regulating scion vigour of apple trees, particularly for composite trees grafted onto a dwarfing rootstock.

For M.9 and NPA-treated rootstocks, exogenous BAP reinstated the formation of secondary axes, whereas GA_{4+7} promoted the formation of few additional secondary axes when compared with BAP. Treatment of the scion with BAP x GA_{4+7} promoted more secondary shoots than BAP for both M.9 or rootstocks treated with NPA. Without NPA, BAP x GA_{4+7} stimulated the formation of a similar mean number of secondary axes, particularly secondary shoots, regardless of rootstock type. In contrast, the BAP-treated scion on M.9 developed fewer secondary axes than the BAP-treated scion on MM.106 and M.793, but a similar number to the BAP-treated scion on 'Royal Gala'. Collectively, these results may indicate that reduced branching of the scion on M.9 was caused by reduced IAA transport from shoot to root that decreased the transport of root-produced cytokinin to the scion, therefore decreasing axillary bud outgrowth on the primary shoot. In addition, the ability of the BAP x GA_{4+7} treatment to increase the formation of secondary axes for the scion on M.9 and on NPA-treated rootstocks strongly indicates that root-produced gibberellin was also important in primary branch formation. Gibberellin may enable cytokinin to stimulate more bud outgrowth by keeping SAMs actively growing, or it may stimulate a greater proportion of outgrown axillary meristems to remain active and extend to form longer secondary shoots. Although the BAP x GA_{4+7}-treated scion on M.9 formed a similar mean number of secondary axes to the BAP x GA_{4+7}-treated scion on MM.106, M.793 and 'Royal Gala', fewer tertiary axes developed for M.9, particularly tertiary shoots.

The application of GA_{4+7} to the scion on M.9 or NPA-treated rootstocks kept a greater proportion of meristem(s) on the primary and secondary shoots actively growing. For the primary shoot, reduced cumulative node production for M.9 or NPA-treated rootstocks could be reversed with exogenous GA_{4+7}. Hence, the GA_{4+7}-treated scion on M.9 developed a primary shoot with a similar final node number compared with the GA_{4+7}-treated scion on 'Royal Gala'. In addition, the GA_{4+7}-treated scion on M.9 developed a significantly greater final mean node number compared with untreated 'Royal Gala' trees on rootstocks of M.9, MM.106, M.793 and 'Royal Gala'. Treatment

with NPA x GA_{4+7} also stimulated a significantly greater final mean node number on the primary shoot compared with trees treated with only NPA or trees that were untreated.

With exogenous GA_{4+7}, the final node number of the primary shoot was similar amongst rootstocks, however the primary shoot on M.9 had a slightly reduced mean shoot length, hence the mean internode length of the primary shoot was reduced on M.9. However, treatment of the scion with BAP x GA_{4+7} resulted in primary shoots of similar length, node number and internode length regardless of rootstock type. Collectively, the interactions of NPA x GA_{4+7} and rootstock x GA_{4+7} may indicate that cumulative node production was reduced earlier in the growing season for the primary shoot on M.9 or on NPA-treated rootstocks because of decreased IAA transport within the rootstock stem to the root system. Reduction of the basipetal IAA signal to the root may have decreased the transport of root-produced gibberellin (probably precursors of GA_1) to the scion, which decreased the synthesis of bioactive GA_1 at the shoot, thus limiting the initiation of leaf primordia in the peripheral zone of the apical meristem, presumably in conjunction with IAA. The development of slightly compressed internodes for the primary shoot of the GA_{4+7}-treated scion on M.9, and the reversal of this effect with BAP x GA_{4+7}, may indicate that cytokinins are also required for meristematic growth in the subapical meristem, possibly in conjunction with IAA and GA_{4+7}.

Treatment of the scion on M.9 with BAP x GA_{4+7} markedly increased the mean total shoot length and node number formed per scion when compared with untreated trees on M.9, MM.106, M.793 and the 'Royal Gala' rootstock control. The BAP x GA_{4+7} treatment increased total growth of the scion on M.9 by increasing the mean shoot length and node number of the primary and secondary shoots, and the mean number of secondary and tertiary shoots that formed compared with untreated trees on M.9. The scion on M.9 treated with BAP x GA_{4+7} developed very similar total growth to MM.106, but was still markedly smaller than the BAP x GA_{4+7}-treated scion on M.793 and 'Royal Gala', which predominantly resulted from the latter rootstocks forming more tertiary shoots than M.9 in response to the BAP x GA_{4+7} treatment. In a similar manner to BAP x GA_{4+7} treatment of trees on M.9, BAP x GA_{4+7} applied to the scion on NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' increased total growth of the scion by increasing the mean shoot length and node number of the primary and secondary shoots, and the mean number of secondary and tertiary shoots that formed when compared with

the untreated scion on these same NPA-treated rootstocks. However, BAP x GA_{4+7} applied to the scion on NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' stimulated markedly less total shoot extension growth when compared with the BAP x GA_{4+7}-treated scion on the same rootstock type that was not treated with NPA. Hence, BAP x GA_{4+7} could not fully reverse reductions in total scion growth whilst IAA transport from scion to root was impaired by the NPA treatment, even for the highly vigorous 'Royal Gala' rootstock.

6. Effect of rootstock type on scion architecture, root growth and transport of some endogenous hormones in newly grafted 'Royal Gala' apple trees

6.1 Introduction

Lockard and Schneider (1981) hypothesised that dwarfing apple rootstocks reduced scion vigour by decreasing the basipetal transport of IAA within the phloem and cambial cells of the rootstock stem to the roots. Sub-optimal amounts of IAA transported to the root system may decrease root growth, the synthesis of root-produced cytokinins and their transport in the xylem vasculature to the scion. In support of this hypothesis, the M.9 rootstock and NPA treatment of the rootstock stem greatly reduced scion vigour and the final dry mass of the root system (Chapter 5).

Root growth in Chapter 5 was measured at the end of the first growing season after grafting of the composite tree. Hence, it was unknown at what time during the growing season root growth of M.9 first decreased compared with the 'Royal Gala' rootstock control. Elucidating how the roots of different size-controlling rootstocks grow during the first season of growth after grafting may provide important physiological insights into rootstock-induced dwarfing of the scion. In particular, identifying the time during the growing season when root growth of M.9 is first decreased may approximately indicate when shoot to root signalling of IAA is first restricted by the rootstock stem. Therefore, the first objective of this experiment was to elucidate how root growth was modified differently on newly-grafted composite 'Royal Gala' apple trees on rootstocks of M.9, MM.106, M.793 and 'Royal Gala' during their first growing season. In addition, it was of interest to determine how each rootstock type modified the final distribution of biomass between root and scion.

In Chapter 5, both the M.9 rootstock and NPA treatment of the rootstock stem decreased the final growth of the root system, and the scion on these treatments developed very few secondary axes (see Section 5.3.3.3). However, BAP applied to the

scion on M.9 or on NPA-treated rootstocks reinstated the formation of secondary axes (see Section 5.3.3.3). Therefore, IAA and cytokinin appeared to interact to regulate the formation of secondary axes. In addition, these interactions suggest that reduced concentrations of root-produced cytokinin in the xylem sap of dwarfing apple rootstocks (Kamboj et al., 1999) would induce a scion phenotype with fewer secondary axes in the first season of growth from grafting. Therefore, further objectives were to elucidate whether rootstocks modified the formation of secondary axes, and whether differences in their formation were explained by the rate of IAA diffusing from the apex of the primary shoot, and/or, the concentration of cytokinin in its xylem sap.

Reducing the basipetal transport of IAA to the root by repeatedly applying NPA to the rootstock stem also decreased cumulative node production of the primary shoot compared with the untreated trees (Figure 5.2B). However, gibberellin applied repeatedly to the scion over the growing season fully reversed these effects of NPA on node production (Figure 5.2B). Similarly, the cumulative node number of the primary shoot was reduced by M.9, and this decrease in growth was reversed with exogenous gibberellin (Figure 5.3B). Collectively, these interactions indicate that decreased basipetal transport of IAA from scion to root of the dwarfing apple rootstock reduces the amount of root-produced gibberellin transported to the scion, which may decrease node neoformation by the SAM on the primary shoot.

As discussed in Chapter 5, apple roots mainly synthesise and transport biologically inactive precursors of GA_1, particularly GA_{19} (Motosugi et al., 1996), which might be converted by the shoot to bioactive GA_1. This is supported by evidence that the suppression of GA 20-oxidase in the shoot of the apple cultivar 'Greensleeves' grown on its own roots increased endogenous concentrations of GA_{19} and decreased concentrations of GA_{20} and GA_1 in the shoot apex (Bulley et al., 2005). Therefore, further objectives were to elucidate whether the M.9 rootstock reduced endogenous concentrations of GA_{19} and GA_{20} in the xylem sap of 'Royal Gala' apple scions during the first growing season after grafting, and whether reduced concentrations of gibberellin coincided with decreased node production, and/or, increased SAM termination of the scion growing on M.9 when compared with the 'Royal Gala' rootstock control.

6.2 Materials and methods

6.2.1 Experimental site, establishment and management of tree material

The experiment was conducted during the 2005–2006 growing season at the Fruit Crops Unit, Massey University, Palmerston North. Composite trees of 'Royal Gala' on rootstocks of M.9, MM.106, M.793 and 'Royal Gala' were propagated and grown as previously described (Chapter 5, Section 5.21). For each rootstock type, six trees were randomly selected on the 12/12/05, 9/1/06, 9/2/06, 6/3/06 and 6/4/06 for destructive harvest for measurement of the following:

6.2.2 Scion extension growth

At each harvest, the shoot length and node number of each scion was measured and the number of secondary axes were counted (see Section 2.7). Data for shoot cross-sectional area (SCA) was measured only for the primary shoot (Chapter 2, Section 2.7).

6.2.3 Leaf area

Total leaf area of the primary shoot and secondary axes were measured using a leaf area meter (Li-3100, Li-Cor Inc., USA). Leaves from secondary shoots and spurs were pooled at each harvest and, therefore, data presented are for the total leaf area of the secondary axes.

6.2.4 Diffusion of indole-3-acetic acid from the primary shoot apex

The apex of the primary shoot was excised 20 mm from the shoot tip. The diffusion of IAA from the shoot apex of each scion was measured by placing the cut basal end of the apex into a buffer solution for twenty hours (see Chapter 2, Section 2.3.2). IAA was quantified using HPLC fluorescence detection (Chapter 2, Section 2.4.6).

6.2.5 Extraction of xylem sap from the primary shoot

Xylem sap was extracted from the primary shoot and quantified for endogenous cytokinins and gibberellins as previously described (see Chapter 2). Because of the small stem diameter of the primary shoot, minute (100 to 200 μL per scion) volumes of

xylem sap were extracted on 12/12/05 and 9/1/06. Therefore, insufficient volumes were available for hormone quantification on these dates.

6.2.6 Total dry mass of stems and leaves

The primary shoot and secondary axes (spurs plus shoots) were divided into leaves and stems and oven dried at 80^{o}C for 21 days to a constant mass and their dry mass recorded on a four-decimal place balance (METTLER AE200, Switzerland) within 30 sec of removal from the oven.

6.2.7 Total length and dry mass of the root system

Root systems were washed free of growing medium and their total length and dry mass were measured as previously described (Chapter 5, Section 5.2.3).

6.2.8 Statistical analysis

The experiment was a completely randomised design with four rootstocks (M.9, MM.106, M.793 and the 'Royal Gala' rootstock control) replicated six times per harvest. For hormone analysis, two replicate trees were pooled and therefore there were only three replicates for each rootstock type and harvest date. Data were analysed by ANOVA using the GLM procedure of SAS. Mean separations were made at P=0.05 using the LSD test.

6.3 Results

6.3.1 Growth of the primary shoot

6.3.1.1 Mean cumulative length and node number

In December, the mean length and node number of the primary shoot on M.793 was significantly reduced compared with the primary shoot on MM.106, but not the primary shoot on M.9 or the 'Royal Gala' rootstock control (Figure 6.1A, D). At the final measurement of growth conducted in April, rootstocks did not significantly differ in the mean length or node number of the primary shoot (Figure 6.1A, D). However, there were trends that the mean length of the primary shoot on M.9 and M.793 was less than MM.106 and the 'Royal Gala' rootstock control (Figure 6.1A). With the exception of trees harvested in January, the mean internode length of the primary shoot was not significantly affected by rootstock type (Figure 6.2A). In January, the mean internode length of the primary shoot on the 'Royal Gala' rootstock control was significantly reduced compared with the primary shoot on M.9, MM.106 and M.793 (Figure 6.2A). Differences, however, were very small. For each rootstock type, termination of the SAM on the primary shoot first began between the measurements conducted on the 9/2/06 and 6/3/06. In March, the percentage of primary shoots that had fully terminated was 33%, 50%, 0% and 33% for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. By the 6/4/06, 100% of primary shoots had terminated on each rootstock type (data not shown).

6.3.1.2 Shoot cross-sectional area (SCA)

The mean SCA of the primary shoot on the 'Royal Gala' rootstock was significantly greater than M.9, MM.106 and M.793 by January (Figure 6.2B). However, the mean SCA of the primary shoots for MM.106 and 'Royal Gala' were not significantly different in February (Figure 6.2B). In March, statistical differences in SCA were not detected, although there were trends that mean SCA was reduced for M.9 and M.793 (Figure 6.2B). From March to April, the mean SCA of the primary shoot did not increase for M.9, hence SCA was significantly decreased compared with MM.106, M.793 and the 'Royal Gala' rootstock control in April (Figure 6.2B). The mean SCA of

the primary shoot on M.793 was also significantly reduced in April compared with MM.106 and the 'Royal Gala' rootstock control (Figure 6.2B).

6.3.2 Growth of the secondary shoots

6.3.2.1 Formation of secondary shoots and the mean number of secondary axes formed

The formation of secondary shoots was first observed from late December, 2005. During measurements in January and February, the scion on the 'Royal Gala' rootstock control had developed more secondary shoots than the scion on M.9, MM.106 and M.793 (Figure 6.3B). Therefore, the latter rootstocks, particularly M.9, appeared to delay the initial formation of secondary shoots on the primary shoot compared with the 'Royal Gala' rootstock control (Figure 6.3B). However, in March and April the mean number of secondary shoots formed per scion was not statistically different amongst rootstock types. Rootstocks did not significantly differ ($P \leq 0.05$) in the mean number of secondary spurs (Figure 6.3A) or secondary axes (i.e., spurs plus shoots, Figure 6.3C) that had formed per scion at any harvest date. However, the mean total number of secondary spurs, shoots and axes produced over the entire growing season (i.e., pooled over harvest dates) was significantly affected by rootstock type (Figure 6.3D).

Over the entire growing season, M.793 produced significantly more secondary spurs than MM.106 and 'Royal Gala', but not M.9 (Figure 6.3D). Rootstocks of M.9, MM.106 and M.793 produced significantly fewer secondary shoots than the 'Royal Gala' rootstock control. However, MM.106 and M.793 produced significantly more secondary shoots than M.9 over the entire growing season (Figure 6.3D). Notably, the mean total number of secondary axes produced over the entire growing season increased with increasing rootstock vigour, with M.793 and 'Royal Gala' producing significantly more secondary axes than M.9 and MM.106 (Figure 6.3D). As found in Chapters 3 and 5, secondary axes formed for the scion on the M.9 rootstock were comprised of almost equal proportions of spurs and shoots, whereas the more vigorous rootstocks formed proportionally more secondary shoots than spurs (Figure 6.3D).

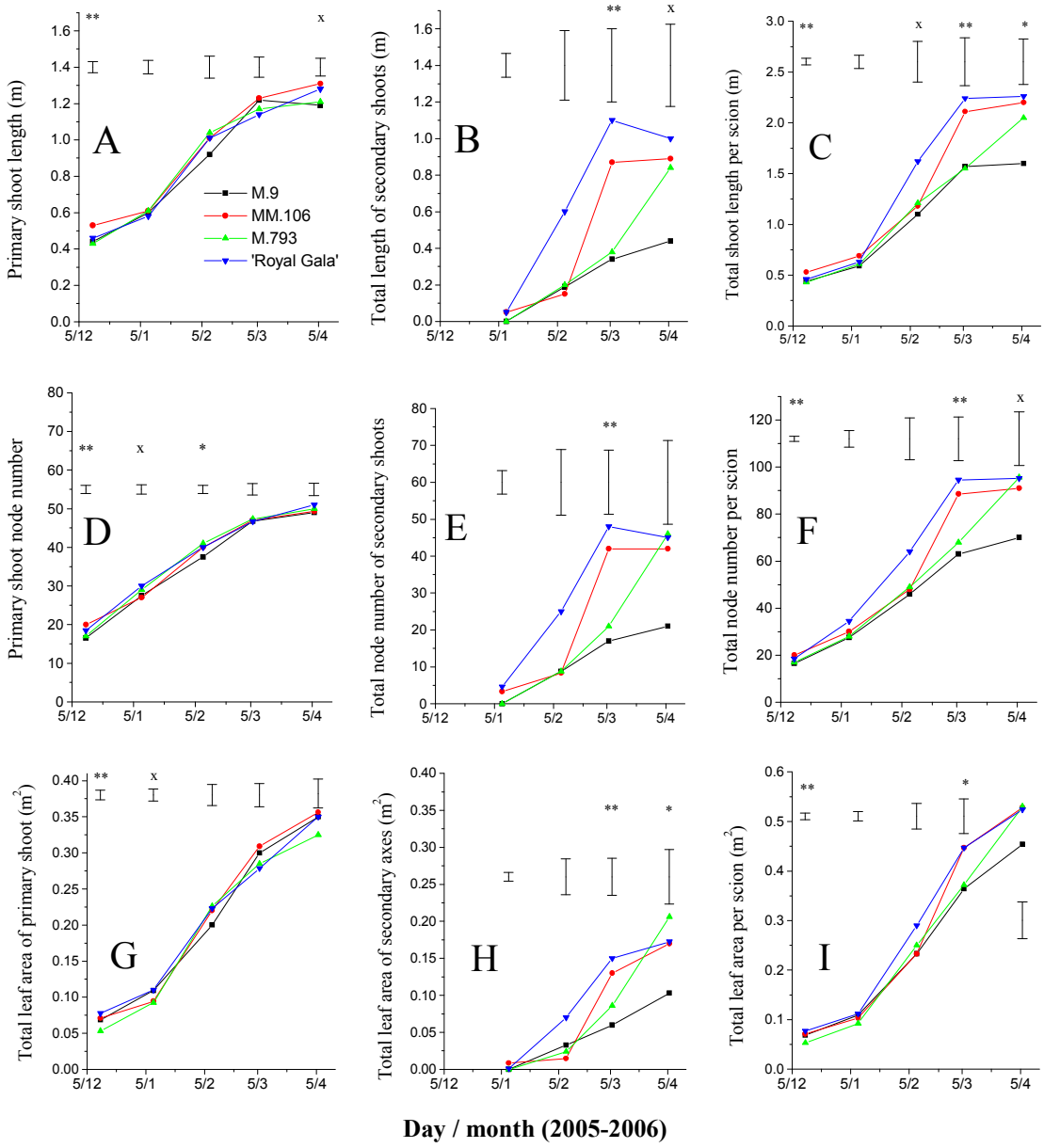


Figure 6.1. Effect of rootstock type on the mean cumulative length, node number and leaf area of the primary shoot (A, D and G), the mean total length and node number of the secondary shoots (B and E), the mean total leaf area of the secondary axes (i.e., spurs plus shoots) (H) and the mean total shoot length, node number and leaf area (C, F and I) of 'Royal Gala' apple scions during their first growing season from grafting. [x], *, **, * significant ANOVA at $P \leq 0.12$, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Bars denote the LSD at $P = 0.05$.**

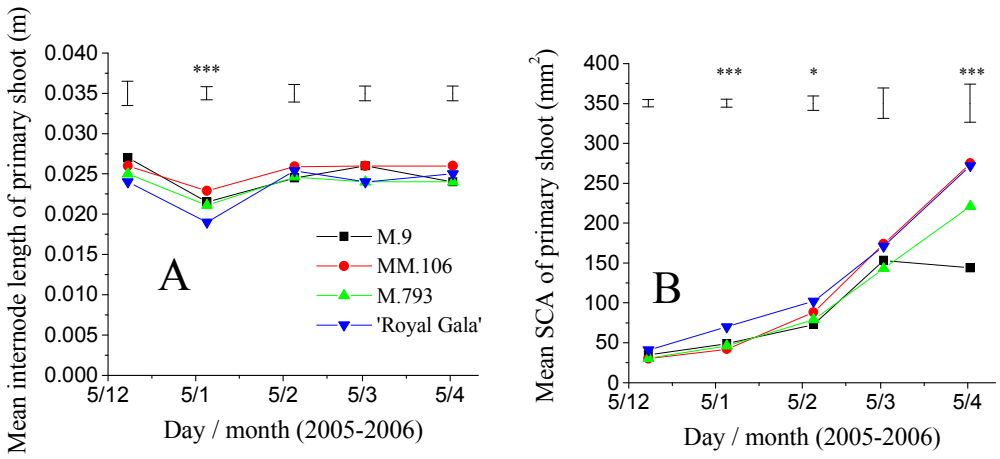


Figure 6.2. Effect of rootstock type on the mean internode length (A) and cumulative shoot cross-sectional area (SCA) (B) for primary shoots of 'Royal Gala' apple during their first growing season from grafting. *, **, * significant ANOVA at *P*≤0.05, *P*≤0.01, *P*≤0.001, respectively. Bars denote the LSD at *P*=0.05.**

6.3.2.2 Total growth of the secondary shoots

In March, M.9 and M.793 had significantly reduced the mean total length and node number of the secondary shoots compared with MM.106 and the 'Royal Gala' rootstock control (Figure 6.1B, E). Reduced total growth of the secondary shoots for M.9 and M.793 resulted partly from these rootstocks having formed fewer secondary shoots in March (Figure 6.3B). In addition, the mean length of the secondary shoots was 0.21, 0.27, 0.16 and 0.31 m (ANOVA, *P*=0.16), whereas the mean node number was 10.8, 12.3, 7.8 and 12.4 (ANOVA, *P*=0.21) for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. Therefore, total growth of the secondary shoots for M.9 and M.793 were also decreased compared with the 'Royal Gala' rootstock control because of very small reductions in the mean length and node number of the secondary shoots.

On the final growth measurement conducted on the 6/4/06, there were trends that the mean total length (*P*=0.10) and node number (*P*=0.12) of the secondary shoots were reduced by M.9 (Figure 6.1B, E), which partly resulted from the scion on M.9 forming fewer secondary shoots compared with MM.106, M.793 and the 'Royal Gala' rootstock

control (Figure 6.3B). In April, the final mean node number of the secondary shoots was 11.1, 11.3, 8.3 and 12.5 nodes (ANOVA, P=0.31), whereas the final mean shoot length was 0.22, 0.25, 0.16 and 0.32 m (ANOVA, P=0.19) for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. Hence, in April there were trends that the mean length and node number of secondary shoots was reduced by M.9 compared with 'Royal Gala', although differences were very small.

With the exception of M.793, mean total growth of the secondary shoots for each rootstock type did not greatly increase from March to April (Figure 6.1B, E). On the 6/3/06, the proportion (%) of secondary shoots that had terminated for each rootstock type was not statistically significant (P=0.68). However, 90%, 84%, 82% and 74% of secondary shoots had terminated for rootstocks of M.9, MM.106, M.793 and 'Royal Gala', respectively. On the 6/4/06, 100% of secondary shoots had terminated for each rootstock type (data not shown). Given that very little additional growth of the secondary shoots occurred from March to April for M.9, MM.106 and 'Royal Gala' (Figure 6.1B, E), secondary shoots for these rootstocks probably fully terminated very soon after the measurement of growth conducted in March. For M.793, the mean node number of the secondary shoots was 7.8 and 8.3 nodes in March and April, respectively. Hence, the mean node number of the secondary shoots did not greatly increase from March to April for the M.793 rootstock, indicating that increased total growth of the secondary shoots during this period (i.e., Figure 6.1B, E) was probably mostly attributable to an increase in the mean number of secondary shoots (Figure 6.3B).

6.3.2.3 Relationship between the length and node number of secondary shoots

To examine the relationship between the length and node number of the secondary shoots for each rootstock type, data collected in February, March and April were pooled to provide sufficient shoot numbers, particularly for M.9. Therefore, comparisons between the length and node number of the secondary shoots in this experiment included proportions of shoots that were extending, particularly during February and March, as well as data collected when all secondary shoots had fully terminated in April (Figure 6.4A, C, E and G). The relationship between the length and node number of the secondary shoots was almost identical amongst rootstocks (compare the slope of the regression line in Figure 6.4A, C, E and G). Hence, secondary shoots of the same length

had a comparable node number (Figure 6.4A, C, E and G) and, therefore, internode length was not affected by rootstock type (internode length can be compared from the fitted regression line in Figure 6.4A, C, E and G by dividing a value on the y-axis with its corresponding value on the x-axis).

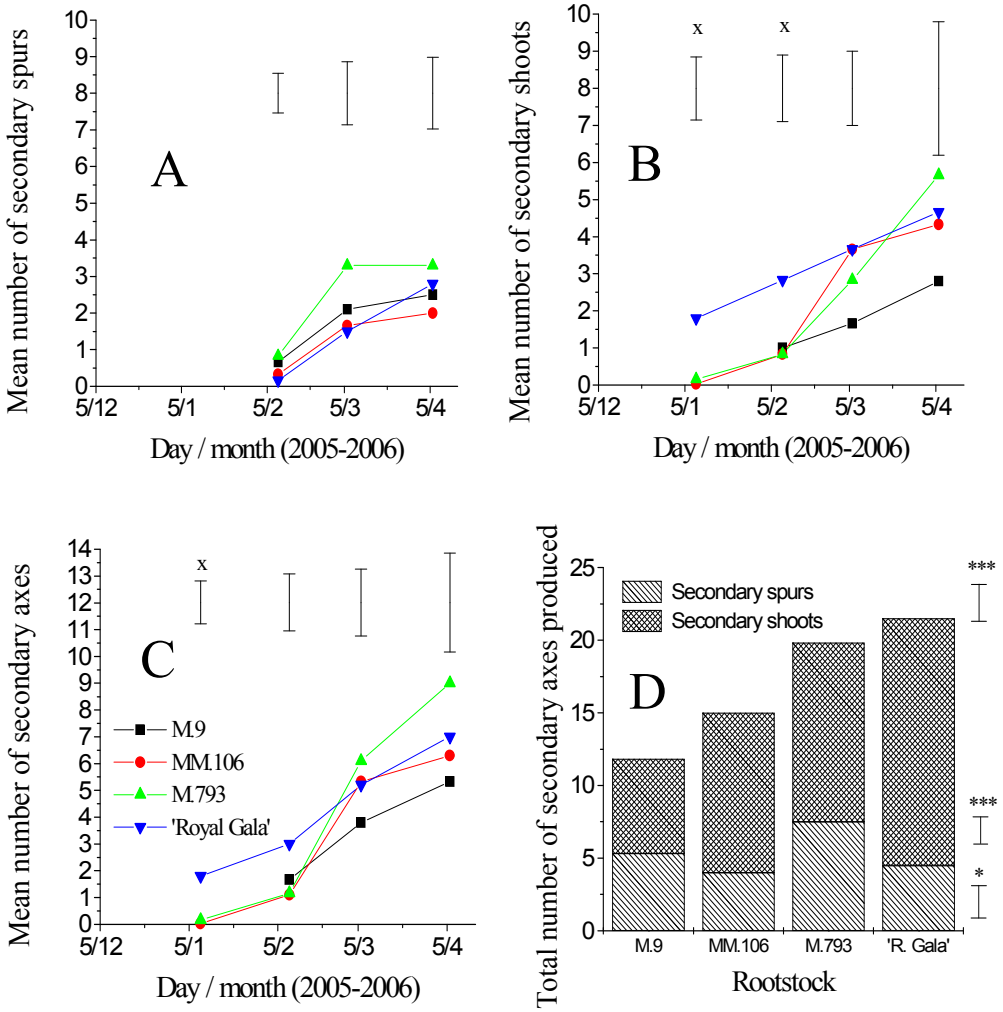


Figure 6.3. Effect of rootstock type on the mean number of secondary spurs (A), shoots (B) and secondary axes (spurs plus shoots) (C) formed on the primary shoot of 'Royal Gala' apple scions during their first growing season from grafting. D is the mean number of secondary spurs, shoots and their total produced by each rootstock type over the entire growing season (n=30 trees). [x], *, **, * significant ANOVA at *P*≤0.10, *P*≤0.05, *P*≤0.01, *P*≤0.001, respectively. Bars denote the LSD at *P*=0.05.**

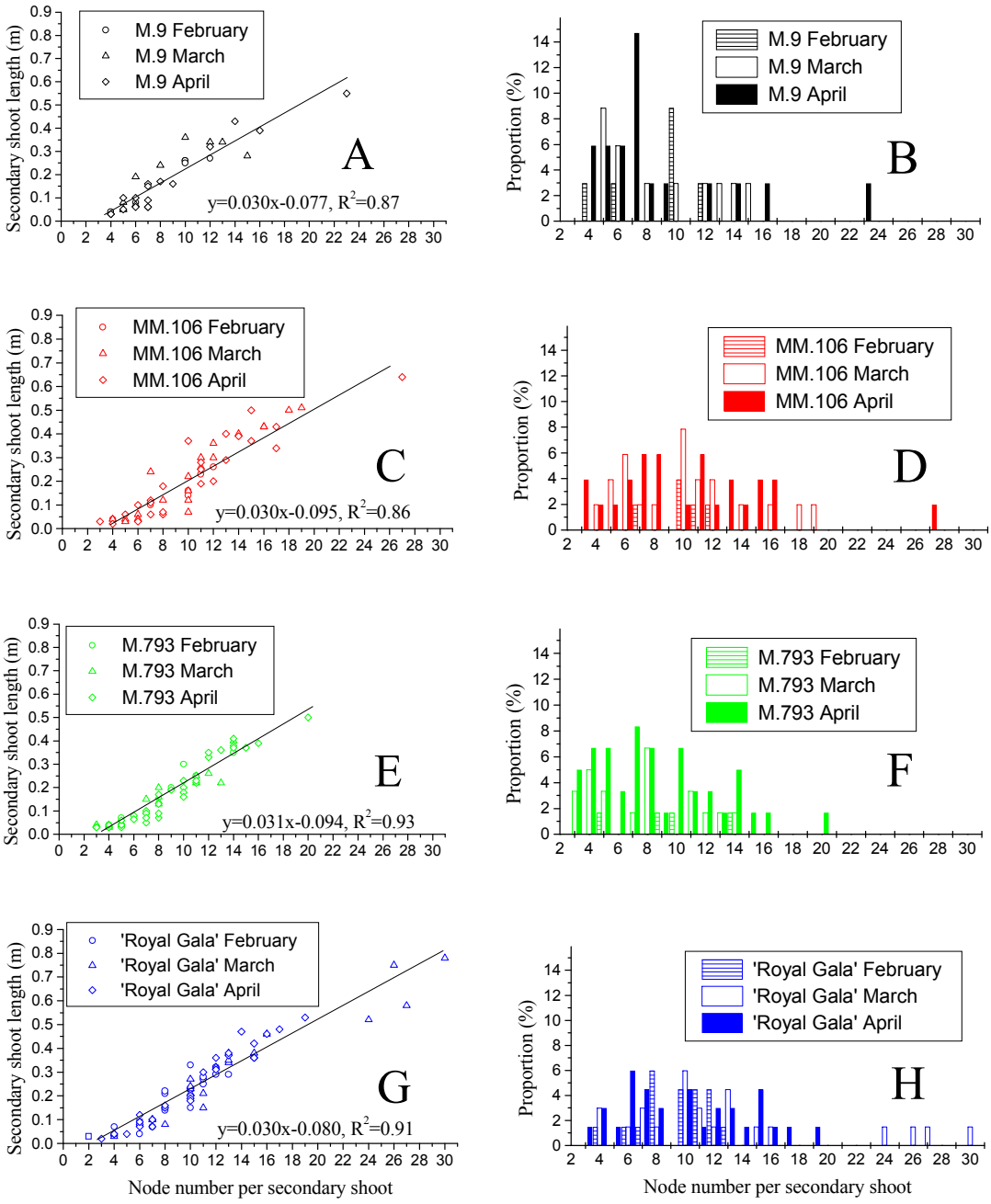


Figure 6.4. Effect of rootstock type on the relationship between the length and node number of secondary shoots on 'Royal Gala' apple scions (A, C, E and G) measured in February, March and April, 2006. B, D, F and H are the node number distributions of secondary shoots for each rootstock type and month.

6.3.3 Effect of rootstock type on the total shoot length and node number per scion

From February onwards, mean total shoot length and node number per scion was reduced by M.9 compared with the 'Royal Gala' rootstock control (Figure 6.1C, F). By the final measurement of growth conducted on 6/4/06, mean total shoot length of the scion on MM.106, M.793 and 'Royal Gala' was not significantly different, however the scion on MM.106 and 'Royal Gala' had a significantly greater mean total shoot length than M.9 (Figure 6.1C). Similarly, in April there were trends that M.9 had reduced the mean total number of nodes formed per scion compared with MM.106, M.793 and 'Royal Gala', but differences were less significant (Figure 6.1F).

In April, M.9 had reduced the mean total shoot length of the scion (Figure 6.1C) by decreasing the mean length of the primary shoot (Figure 6.1A), the mean number of secondary shoots formed (Figure 6.3B) and the mean length of those secondary shoots. Although not significant (P=0.19), the final mean length of the secondary shoots was 0.22, 0.25, 0.16 and 0.32 m for M.9, MM.106, M.793 and the 'Royal Gala' rootstock, respectively. Reduced mean shoot length for M.9 resulted from greater proportions of short secondary shoots forming compared with the 'Royal Gala' rootstock control, particularly shoots with eight or fewer nodes (see data for April in Figure 6.4B, H). In April, the final mean length of the primary (Figure 6.1A) and secondary shoots (see above) for M.9 and M.793 were not greatly different, however M.793 had formed more secondary shoots than M.9 (Figure 6.3B), which meant that the mean total shoot length of the scion on M.793 was greater, but not statistically different from M.9 (Figure 6.1C).

6.3.4 Effect of rootstock type on leaf area of the scion

6.3.4.1 Total leaf area of the primary shoot

In December, the primary shoot on M.793 had a significantly reduced mean total leaf area compared with M.9, MM.106 and the 'Royal Gala' rootstock control (Figure 6.1G). For the primary shoot on M.793, decreased mean total leaf area resulted from fewer neoformed nodes (Figure 6.1D) and leaves. In addition, the mean area per leaf on the primary shoot was 0.0042, 0.0035, 0.0031 and 0.0042 m^2 (P=0.0072, LSD=0.0007) for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively (data not

shown). Therefore, M.793 also had reduced the area of individual leaves in December. After January, mean total leaf area of the primary shoot was not significantly different amongst rootstocks (Figure 6.1G).

6.3.4.2 Total leaf area of the secondary axes

During March, mean total leaf area of the secondary axes (i.e., spurs plus shoots) was significantly reduced by M.9 compared with the scion on MM.106 and the 'Royal Gala' rootstock control, whilst the mean total leaf area of the secondary axes for M.793 was only statistically different from the 'Royal Gala' rootstock (Figure 6.1H). Reduced total leaf area of the secondary axes for M.9 and M.793 during March resulted partly from these rootstocks having fewer secondary shoots compared with the scion on the 'Royal Gala' rootstock control (Figure 6.3B), and from very small reductions in the respective mean node number of the secondary shoots for M.9 and M.793 (see Section 6.3.2.2). In April, mean total leaf area of the secondary axes was reduced by M.9 compared with MM.106, M.793 and the 'Royal Gala' rootstock control, however differences were significant only between M.9 and M.793 (Figure 6.1H).

6.3.4.3 Total leaf area per scion

In March, the scion on MM.106 and 'Royal Gala' had a significantly greater mean total leaf area per scion than M.793 and M.9 (Figure 6.1I). However, mean total leaf area per scion was not significantly different amongst rootstocks in April (P=0.13), although there was a trend that total leaf area was reduced by M.9 (Figure 6.1I). During March, reduced mean total leaf area of the scion on M.9 and M.793 resulted mostly from these rootstocks decreasing the mean total leaf area of the secondary axes compared with the 'Royal Gala' rootstock (Figure 6.1H), the causes of which were described above (Section 6.3.4.2)

6.3.5 Effect of rootstock type on the mean total length of the root system

From December to February, rootstocks did not differ in the mean total cumulative length of the root system (Figure 6.5A). However, from March to April the mean total length of the M.9 root system did not greatly increase, which was in contrast to root systems of 'Royal Gala' and, to a lesser extent, MM.106 and M.793 (Figure 6.5A). Hence, by April the mean total length of the M.9 root system was significantly reduced

compared with the 'Royal Gala' rootstock control, but not compared with MM.106 and M.793 (Figure 6.5A).

The mean specific root mass (i.e., total dry mass of the root system divided by its total length) initially decreased for each rootstock type from December to January, however it steadily increased from January onwards (Figure 6.5B). In February, mean specific root mass was significantly decreased by M.9 compared with MM.106 and M.793 only (Figure 6.5B). By April, mean specific root mass was significantly reduced by M.9 compared with MM.106, M.793 and the 'Royal Gala' rootstock control (Figure 6.5B). In contrast, mean specific root mass of M.793 was significantly increased compared with M.9, MM.106 and the 'Royal Gala' rootstock control (Figure 6.5B).

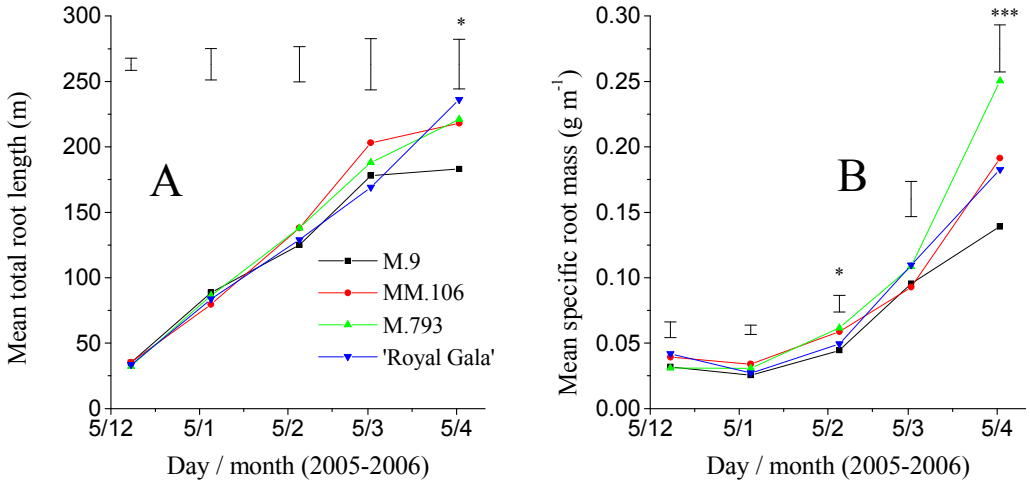


Figure 6.5. Effect of rootstock type on the mean cumulative root length (A) and mean specific root mass (B) of composite 'Royal Gala' apple trees during their first year of growth from grafting. [x], *, **, * significant ANOVA at $P \leq 0.10$, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Bars denote the LSD at $P = 0.05$.**

6.3.6 Effect of rootstock type on mean total dry mass of stems, leaves and roots

6.3.6.1 Dry mass of the primary shoot stem

In December, the dry mass of the primary shoot stem was significantly reduced on M.9 and M.793 compared with MM.106 and the 'Royal Gala' rootstock control (Figure 6.6A). Reduced dry mass of the primary shoot stem in December for both M.9 and M.793 was caused by very small decreases in mean SCA compared with the 'Royal

Gala' rootstock control (Figure 6.2B). By April, the mean dry mass of the primary shoot stem on M.9 and M.793 was significantly reduced compared with the 'Royal Gala' rootstock control (Figure 6.6A). Decreased dry mass of the primary shoot stem on M.9 and M.793 in April (Figure 6.6A) was the result of reduced mean length (Figure 6.1A) and SCA (Figure 6.2B) compared with the 'Royal Gala' rootstock control.

6.3.6.2 Dry mass of leaves on the primary shoot

In December, mean total dry mass of the leaves on the primary shoot was significantly reduced by M.793 compared with M.9, MM.106 and the 'Royal Gala' rootstock control (Figure 6.6D). Compared with MM.106 and the 'Royal Gala' rootstock control, M.793 reduced the mean total dry mass of leaves on the primary shoot in December (Figure 6.6D) by imposing small reductions in the mean node number (Figure 6.1D) and leaf number on the primary shoot, and by decreasing the area of individual leaves (see Section 6.3.4.1). Compared with M.9, M.793 reduced the mean total dry mass of leaves on the primary shoot mostly by decreasing the area of individual leaves (see Section 6.3.4.1). Rootstocks did not significantly differ (P=0.46) in specific leaf mass (i.e., dry mass of leaf per node divided by leaf area per node) in December (data not shown).

In March, mean total dry mass of leaves on the primary shoot was significantly greater for M.9 than for M.793 and 'Royal Gala' (Figure 6.6D), and this did not arise from differences in the mean number of neoformed nodes (Figure 6.1D) and thus leaves formed on the primary shoot. Rather, M.9 increased the area per leaf and increased specific leaf mass. The mean area per leaf on the primary shoot in March was 0.0065, 0.0066, 0.0060 and 0.0060 m^2 (P=0.17), whereas the mean specific leaf mass was 119, 113, 109 and 112 g m^{-2} (P=0.11) per leaf for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively.

In April, mean total dry mass of leaves on the primary shoot was similar between M.9 and the 'Royal Gala' rootstock control, whereas the primary shoot on MM.106 or M.793 had a significantly decreased mean total leaf dry mass compared with 'Royal Gala' (Figure 6.6D). For MM.106, reduced mean total dry mass of leaves on the primary shoot resulted from very small reductions in the mean node number of the primary shoot (Figure 6.1D), and from decreased specific leaf mass of individual leaves compared with the 'Royal Gala' rootstock control. For example, in April the area per

leaf was 0.0072, 0.0072, 0.0065 and 0.0069 m^2 (P=0.21) for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. Mean specific leaf mass was 118, 97, 112 and 123 g m^{-2} (P=0.058) per leaf for the primary shoot on M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. In contrast to MM.106, mean total dry mass of leaves on the primary shoot was reduced by M.793 in April (Figure 6.6D) from a combination of small but non-significant reductions in mean node number (Figure 6.1D) and leaf number, reduced area of individual leaves and decreased specific leaf mass (see above) compared with the primary shoot on the 'Royal Gala' rootstock control.

6.3.6.3 Total dry mass of the primary shoot (stem plus leaves)

Mean total dry mass of the primary shoot (i.e., stem plus leaves) was significantly reduced by M.793 compared with the 'Royal Gala' rootstock control in December (Figure 6.6G). These differences arose because M.793 significantly decreased the mean total dry mass of primary shoot stem and leaves (Figure 6.6A, D). During March, mean total dry mass of the primary shoot on M.9 and MM.106 was significantly greater than M.793 only (Figure 6.6G). Compared with M.793, M.9 and MM.106 had increased the mean total dry mass of the primary shoot in March by increasing the total dry mass of both the stem and leaves (Figure 6.6A, D). Notably, the mean SCA of the primary shoot stem was statistically similar amongst rootstocks in March (Figure 6.2B), hence M.9 may have increased the dry mass of the primary shoot stem compared with M.793 (Figure 6.6A) by mostly increasing mean length of the primary shoot (Figure 6.1A), whereas MM.106 appeared to increase both the mean length and SCA of the primary shoot (Figures 6.1A and 6.2B). In March, the primary shoot on each rootstock had a very similar mean node number (Figure 6.1D). Therefore, M.9 and MM.106 may have increased the mean total dry mass of the leaves on the primary shoot compared with M.793 (Figure 6.6D) by increasing the area per leaf and by increasing the mean specific leaf mass (see Section 6.3.6.2).

By growth cessation in April, mean total dry mass of the primary shoot was significantly decreased by M.9, MM.106 and M.793 compared with the 'Royal Gala' rootstock control (Figure 6.6G). However, rootstocks differed in the way total dry mass of the primary shoot was allocated compared with 'Royal Gala'. The M.9 rootstock

greatly decreased mean dry mass of the primary shoot stem in April (Figure 6.6A), but not the total dry mass of leaves (Figure 6.6D). In contrast, MM.106 and M.793 decreased both the mean dry mass of the primary shoot stem and leaves compared with the 'Royal Gala' rootstock control (Figure 6.6A, D). However, these reductions in mean dry mass of the primary shoot stem were markedly less than that imposed by M.9 (Figure 6.6A).

6.3.6.4 Dry mass of stems, leaves and their total for the secondary axes

The stems harvested from secondary spurs and shoots were pooled prior to drying, as were the leaves harvested from secondary spurs and shoots. Therefore, the relative contribution of each axis type to the total dry mass of stems or leaves of the secondary axes could not be determined.

During February, mean total dry mass of stems (Figure 6.6B), leaves (Figure 6.6E) and their total (Figure 6.6H) was greater for secondary axes of the 'Royal Gala' rootstock control when compared with M.9, MM.106 and M.793. For the 'Royal Gala' rootstock, increased mean total dry mass of the secondary axes in February (Figure 6.6H) probably resulted from the formation of more secondary shoots (Figure 6.3B). By March, mean total dry mass of stems (Figure 6.6B), leaves (Figure 6.6E) and their total (Figure 6.6H) was significantly greater for MM.106 and 'Royal Gala' than for M.9 and M.793. For MM.106 and 'Royal Gala', increased mean total dry mass of stem for the secondary axes (Figure 6.6B) may have resulted in March from more secondary shoots (Figure 6.3B) with increased mean length (see Section 6.3.2.2). Data for SCA of secondary axes (spurs or shoots) was not collected; therefore the contribution of SCA to the total dry mass of stems (Figure 6.6B) cannot be ascertained. Increased total dry mass of leaves for secondary axes on MM.106 and 'Royal Gala' during March (Figure 6.6E) arose from increased total node number (Figure 6.1E) and leaf number of the secondary shoots, which was a result of increased mean number of secondary shoots (Figure 6.3B) and very small increases in their mean node number (see Section 6.3.2.2).

In April, rootstocks did not significantly differ in the mean total dry mass of stems (Figure 6.6B) and leaves (Figure 6.6E) of the secondary axes, although there were trends that the mean dry mass of stems, leaves and their total were decreased by M.9 (Figure 6.6H). Compared with the 'Royal Gala' rootstock control, decreased mean total

dry mass of the secondary axes for M.9 in April may have resulted from fewer secondary shoots (Figure 6.3B) and, to a lesser extent, a very small reduction in their mean length and node number (see Section 6.3.2.2).

6.3.6.5 Total dry mass of the scion

In December, mean total dry mass of the scion was significantly reduced by M.793 compared with MM.106 and the 'Royal Gala' rootstock control (Figure 6.6I). Reduced total dry mass of the scion on M.793 was a result of decreased total dry mass of the primary shoot stem (Figure 6.6A) and leaves (Figure 6.6D). The means by which M.793 decreased the dry mass of the primary shoot stem and leaves in December were reported in Sections 6.3.6.1 and 6.3.6.2, respectively.

In January and February, mean total dry mass of the scion was not significantly different amongst rootstocks (Figure 6.6I). However, by March M.793 had significantly decreased the mean total dry mass of stems (Figure 6.6C), leaves (Figure 6.6F) and hence the mean total dry mass of the scion (Figure 6.6I) compared with MM.106 and the 'Royal Gala' rootstock control. In April, the mean total dry mass of the leaves was not significantly different amongst rootstocks (Figure 6.6F), whereas the mean total dry mass of stems was (Figure 6.6C). Hence, significant final differences in the mean total dry mass of the scion (Figure 6.6I) were predominantly caused by rootstocks modifying the mean total dry mass of stems (Figure 6.6C). In April, M.9 had significantly decreased the mean total dry mass of the scion compared with the 'Royal Gala' rootstock control by reducing the mean dry mass of the primary shoot stem (Figure 6.6A) and the mean total dry mass of stems (Figure 6.6B) and leaves (Figure 6.6E) of the secondary axes. The means by which M.9 decreased stem dry mass of the primary shoot and secondary axes in April were described in Sections 6.3.6.1 and 6.3.6.4, respectively.

From March to April, mean total dry mass of the scion (Figure 6.6I) increased by 32, 36, 55 and 59 g for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. Therefore, total dry mass gain of the scion during this late-season period increased with increasing rootstock vigour (Figure 6.6I). With the exception of M.793, these increases in total dry mass of the scion (Figure 6.6I) were not a result of large increases in total shoot length or node number (Figure 6.1C, F).

6.3.6.6 Dry mass of root, rootstock stem and rootstock (root plus rootstock stem)

From December to March, rootstocks did not statistically differ in the mean cumulative dry mass of the root system (Figure 6.6J). However, from March to April the mean dry mass of the M.9 root system did not greatly increase, which was in contrast to the more vigorous rootstocks, especially M.793 (Figure 6.6J). Therefore, in April the mean dry mass of roots was significantly less for M.9 compared with MM.106, M.793 and the 'Royal Gala' rootstock control (Figure 6.6J). In contrast, M.793 had a significantly greater final mean root dry mass than M.9, MM.106 and the 'Royal Gala' rootstock control (Figure 6.6J). By April the mean dry mass of the rootstock stem was significantly greater for the 'Royal Gala' rootstock control than for M.793 (Figure 6.6K). Hence, mean total dry mass of the rootstock (i.e., stem plus roots) was not significantly different between M.793 and 'Royal Gala' in April (Figure 6.6L). However, the final mean total dry mass for the rootstock of M.9 and MM.106 was significantly decreased compared with the rootstock of M.793 and 'Royal Gala' (Figure 6.6L). Compared with 'Royal Gala', mean total dry mass of the MM.106 rootstock was reduced in April solely because of significantly decreased total dry mass of the rootstock stem (Figure 6.6J, K, L). The mean total dry mass of the M.9 rootstock was significantly reduced in April because the final dry mass of both the roots and stem were significantly less than the 'Royal Gala' rootstock (Figure 6.6J, K, L).

6.3.6.7 Total dry mass per tree

In February, mean total dry mass per tree (i.e., rootstock plus scion) was not significantly affected by rootstock type (Figure 6.7A). However, rootstocks significantly affected mean total dry mass per tree in March (P=0.03) and April (P=0.0001) (Figure 6.7A). In March, mean total dry mass per tree was significantly reduced by M.9 and M.793, but not MM.106, when compared with the 'Royal Gala' rootstock control (Figure 6.7A). Rootstocks of M.9 and M.793 reduced the mean total dry mass per tree in March by significantly decreasing the mean dry mass of secondary axis stems (Figure 6.6B), leaves (Figure 6.6E) and rootstock stem (Figure 6.6K) compared with the 'Royal Gala' rootstock control. However, by April the mean total dry mass of trees on M.793 and the 'Royal Gala' rootstock control were not statistically

different, whereas trees on M.9 and MM.106 had gained significantly less total dry mass than the ‘Royal Gala’ rootstock (Figures 6.7A and 6.8A).

From March to April, the mean total dry mass gained per tree nearly doubled for the M.793 rootstock (Figure 6.7A), which in part resulted from greatly increased total dry mass accumulation by the secondary axes (Figure 6.6 B, E, H) and the root system (Figure 6.6J). Subsequently, M.793 had produced a significantly greater root dry mass than ‘Royal Gala’ by April (Figure 6.8A). In contrast, mean total dry mass per tree did not greatly increase for M.9 from March to April (Figure 6.7A) and this resulted from relatively small increases in dry mass gain by the root (Figure 6.6J), rootstock stem (Figure 6.6K) and the primary shoot stem (Figure 6.6A) when compared with the ‘Royal Gala’ rootstock control. Therefore, in April the M.9 rootstock had significantly decreased the final mean dry mass of root (Figure 6.6J), rootstock stem (Figure 6.6K) and the primary shoot stem (Figure 6.6A) compared with the ‘Royal Gala’ rootstock control, which greatly contributed to the significant decrease in the mean total dry mass of the tree for M.9 (Figure 6.8A). For MM.106, the total gain in dry mass per tree from March to April was also less than the ‘Royal Gala’ rootstock control (Figure 6.7A). This resulted from decreased dry mass accumulation in the primary shoot stem (Figure 6.6A), leaves (Figure 6.6D) and rootstock stem (Figure 6.6K).

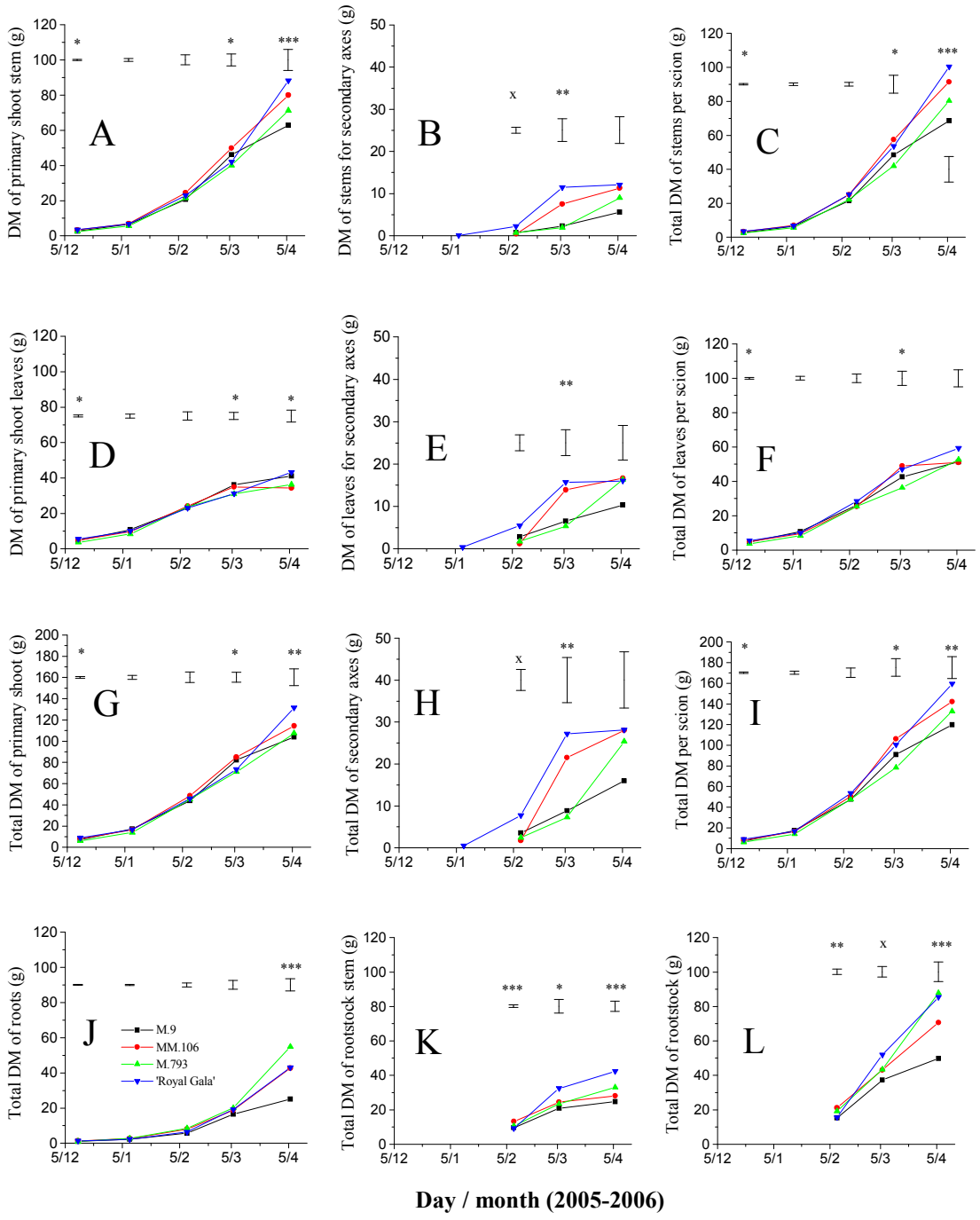


Figure 6.6. Effect of rootstock type on the mean cumulative dry mass (DM) of the scion (A to I) and rootstock (J, K and L) of composite 'Royal Gala' apple trees during their first season of growth from grafting. ^{x}, *, **, * significant ANOVA at $P \leq 0.12$, $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively. Bars denote the LSD at $P = 0.05$.**

6.3.6.8 Effect of rootstock type on the final allocation of dry mass in the tree

Although M.9 significantly decreased the final mean dry mass of the primary shoot stem compared with the 'Royal Gala' rootstock (Figure 6.8A), the proportion of total tree dry mass allocated into the primary shoot stem was very similar between M.9 and the 'Royal Gala' rootstock (Figure 6.8B). Interestingly, M.9 allocated a significantly greater proportion of total tree dry mass into the leaves on the primary shoot compared with rootstocks of MM.106, M.793 and 'Royal Gala' (Figure 6.8B). In addition, M.793 allocated a significantly greater proportion of total tree dry mass into the root than the 'Royal Gala' rootstock. Alternatively, M.9 had a significantly reduced proportion of total tree dry mass allocated into the root than MM.106, M.793 and 'Royal Gala' (Figure 6.8B).

Unlike M.9 and M.793, MM.106 did not differ from the 'Royal Gala' rootstock in the proportion of total tree dry mass allocated into the root system, however both M.9 and MM.106 had a significantly decreased proportion of dry mass allocated into the rootstock stem compared with the 'Royal Gala' rootstock, whereas M.793 did not (Figure 6.8B). A significantly greater proportion of total tree dry mass was allocated into the rootstock (stem plus roots) of M.793 than M.9, MM.106 and 'Royal Gala' (Figure 6.8B). In contrast, trees on M.9 had a significantly reduced proportion of total tree dry mass allocated into the rootstock than trees on M.793 and the 'Royal Gala' rootstock control (Figure 6.8B). Therefore, M.9 had a greater proportion of total tree dry mass allocated into the scion than M.793 and 'Royal Gala' (Figure 6.8B). Conversely, M.793 had a significantly reduced proportion of total tree dry mass allocated into the scion compared with M.9, MM.106 and the 'Royal Gala' rootstock control (Figure 6.8B).

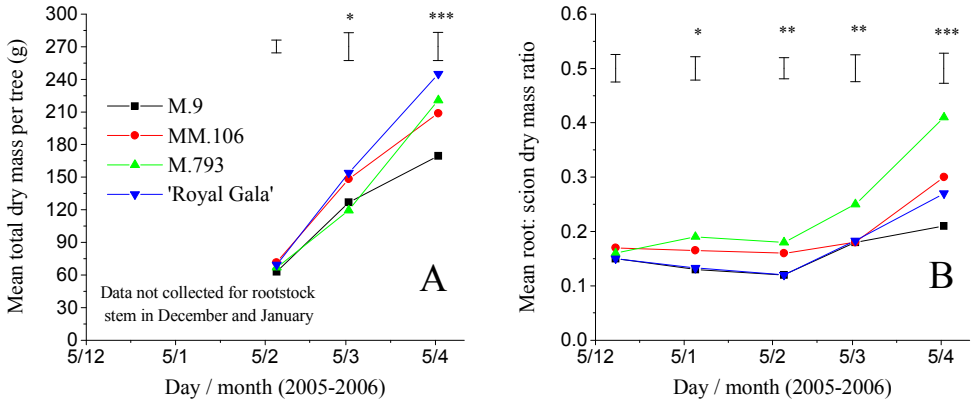


Figure 6.7. Effect of rootstock type on the mean cumulative total dry mass (A) and mean root:scion dry mass ratio (B) of 'Royal Gala' apple trees during their first season of growth from grafting. *, **, * significant ANOVA at *P*≤0.05, *P*≤0.01, *P*≤0.001, respectively. Bars denote the LSD at *P*=0.05.**

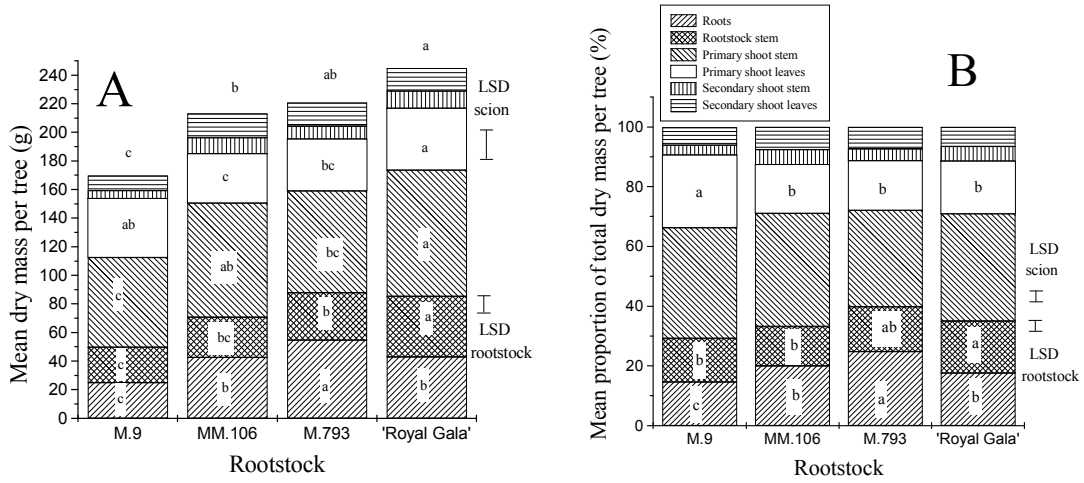


Figure 6.8. Summary of component tree parts and the mean total dry mass (A) of composite 'Royal Gala' apple trees on M.9, MM.106, M.793 and 'Royal Gala' rootstocks (control) by the end of the first year of growth (6/4/06) after grafting (1/9/05). For graph A, total dry mass per tree is equivalent to the total height of a column. Graph B denotes the mean proportion (%) of total dry mass per tree allocated into stems, leaves and roots. For a single tree part and individual graph only, means sharing the same letter across the columns are not significantly different. LSD bars on A and B are for the rootstock (root plus rootstock stem) and scion (leaves plus stems).

6.3.6.9 Allometry of root dry mass and scion dry mass (leaves plus stems)

Treatment means for root (i.e., excluding the rootstock stem) and total scion dry mass were natural log transformed and plotted to determine the allometric coefficient (i.e., the slope of fitted lines) between the root and scion of composite 'Royal Gala' trees on M.9, MM.106 and M.793 relative to the 'Royal Gala' rootstock control (Figure 6.9). The mean root:scion dry mass ratio for each rootstock type is presented in Figure 6.7B.

From December to February, the allometric coefficient for root and scion was 0.88 and 0.87 for M.9 and the 'Royal Gala' rootstock control, respectively (Figure 6.9A). Therefore, during this period of growth these rootstocks had an almost identical allometric relationship between the total dry mass of root and scion (Figure 6.9A and also see root:scion dry mass ratio in Figure 6.7B). An allometric coefficient of less than one from December to February indicated that dry mass allocation was prioritised slightly more towards scion growth than root growth for both M.9 and the 'Royal Gala' rootstock control (Figure 6.9A). Hence, these rootstocks had a decreasing, but identical, root:scion dry mass ratio during this period (Figure 6.7B). However, from February to April the allometric coefficient nearly doubled for both M.9 and the 'Royal Gala' rootstock (0.88 to 1.61 and 0.87 to 1.75 for M.9 and 'Royal Gala', respectively) indicating a switch in dry mass allocation priority from scion to root, and that dry mass allocation into the root of M.9 during this late-period of the growing season was of slightly lower priority than roots of 'Royal Gala' (Figure 6.9A).

From March to April, the root:scion dry mass ratio was greatly increased for the 'Royal Gala' rootstock control and, to a much lesser extent, M.9 (Figure 6.7B). During this period, the root:scion dry mass ratio was greatly increased by 'Royal Gala' because mean root dry mass for 'Royal Gala' doubled (Figure 6.6J), whereas total scion dry mass did not (Figure 6.6I). In contrast, gains in root and scion dry mass by M.9 were much more proportional from March to April than for 'Royal Gala', although the gain in root dry mass for M.9 was proportionally greater than its gain in scion dry mass, as indicated by a very small increase in the root:scion dry mass ratio from March to April (Figure 6.7B). Interestingly, there were trends that the final reductions in root and scion dry mass imposed by M.9 in April (Figure 6.6I, J) initially began from very small decreases in dry mass accumulation after January when compared with the 'Royal Gala'

rootstock control (from January onwards, compare natural log transformed means of root or scion dry mass for these rootstocks along the y or x-axis, respectively, of Figure 6.9A).

From December to February, the allometric coefficients for root and scion were 0.99, 1.03 and 0.87 for rootstocks of MM.106, M.793 and 'Royal Gala', respectively (Figure 6.9B, C). Thus, during this period MM.106 and M.793 were allocating dry mass without bias between root and scion, whereas the 'Royal Gala' rootstock was allocating dry mass preferentially into the scion. Therefore, the 'Royal Gala' rootstock had a significantly reduced root:scion dry mass ratio in January and February when compared with MM.106 and M.793 (Figure 6.7B). Hence, by February there were trends that MM.106 and M.793 had a slightly greater total root dry mass than the 'Royal Gala' control, but slightly decreased total dry mass of the scion (compare natural log transformed means of root or scion dry mass along the y or x-axis, respectively, of Figure 6.9B, C). Reduced total dry mass of the scion on MM.106 and M.793 in February resulted from decreased total dry mass of the stems (Figure 6.6B) and leaves (Figure 6.6E) of the secondary axes, which was possibly a result of fewer secondary shoots forming (Figure 6.3B).

From February to April, allometric coefficients for root and scion of MM.106 and 'Royal Gala' were 1.51 and 1.75, respectively (Figure 6.9B). Hence, dry mass allocation for both rootstocks was prioritised more to roots, which was in contrast to their allometric coefficient from December to February (Figure 6.9B). From February to March, the smaller allometric coefficient for MM.106 compared with the 'Royal Gala' rootstock control meant that allocation of dry mass was more strongly prioritised into the scion on the MM.106 rootstock (Figure 6.6I). This was expressed particularly in the stem (Figure 6.6A) and leaves of the primary shoot (Figure 6.6D) and in the leaves of the secondary axes (Figure 6.6E), which gained more dry mass during this period than the 'Royal Gala' rootstock control. However, increased dry mass allocation into the scion on MM.106 from February to March was at the expense of gains in dry mass by the MM.106 root system. For example, MM.106 had a slightly greater root dry mass than the 'Royal Gala' rootstock control in February, but a very similar root dry mass from March onwards (compare natural log transformed means of root dry mass for these rootstocks along the y-axis of Figure 6.9B).

Initially in December, the total dry mass of the scion and roots was reduced by M.793 compared with the ‘Royal Gala’ control (compare natural log transformed means in Figure 6.9C). However, allometric coefficients from December to February were 1.03 and 0.87 for M.793 and ‘Royal Gala’, respectively (Figure 6.9C). Consequently, the total dry mass of the M.793 root system was greater than ‘Royal Gala’ from January onwards (compare natural log transformed means of root dry mass for these rootstocks along the y-axis of Figure 6.9C). In addition, the root:scion dry mass ratio was significantly greater for M.793 than for the ‘Royal Gala’ rootstock control from January onwards (Figure 6.7B). However, increased allocation of dry mass into the roots of M.793, particularly from March to April (Figure 6.6J), reduced the total dry mass gain of the scion compared with the ‘Royal Gala’ rootstock control (Figure 6.6I). In particular, the dry mass gain by the stem (Figure 6.6A) and leaves (Figure 6.6D) of the primary shoot was reduced by M.793 from March to April.

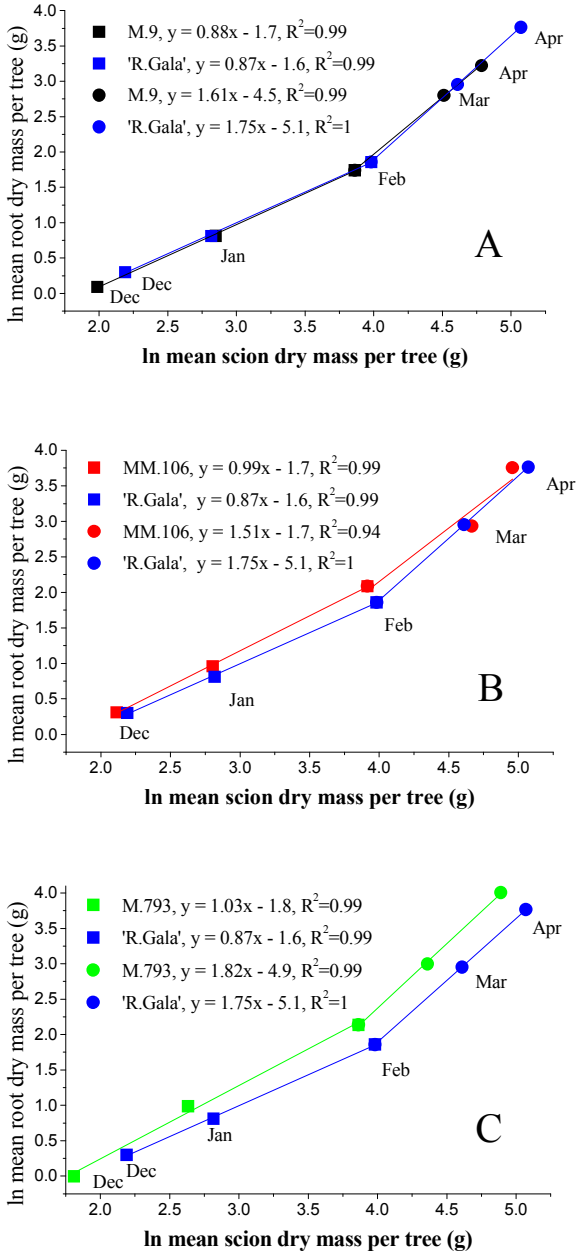


Figure 6.9. Root and scion dry mass allometry of composite 'Royal Gala' apple trees grafted onto rootstocks of M.9 (A), MM.106 (B) and M.793 (C) and compared with a 'Royal Gala' rootstock (control) during their first year of growth from grafting. An increase in slope (i.e., allometric coefficient between root and scion) from February onwards indicates a large shift in biomass allocation to root growth. Each data point is the mean of six trees and is natural log transformed. Mean root dry mass excludes the rootstock stem, while mean scion dry mass includes stems and leaves.

6.3.7 Effect of rootstock type on endogenous transport of IAA, cytokinins and gibberellins

6.3.7.1 Rate of IAA diffusion from the primary shoot apex

Throughout the growing season, statistical differences in the rate of IAA diffusion from the primary shoot apex were not detected amongst rootstocks (Figure 6.10). There were, however, trends evident in the data. The maximum rate of IAA diffusion from the primary shoot apex peaked during January for each rootstock type and, thereafter, declined (Figure 6.10). In December, the mean rate of IAA diffusion was almost identical for M.9 and the 'Royal Gala' rootstock control (Figure 6.10), however from January onwards the primary shoot apex on M.9 had reduced rates of IAA diffusion than 'Royal Gala'. From February onwards, rates of IAA diffusion were also greater for the primary shoot apex on the more vigorous MM.106 and M.793 rootstocks than on M.9 (Figure 6.10).

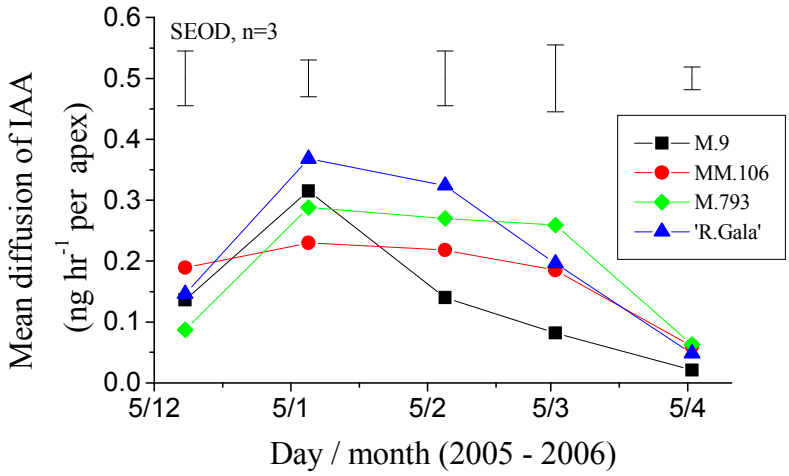


Figure 6.10. Mean concentration of endogenous indole-3-acetic acid (IAA) diffusing from the primary shoot apex of 'Royal Gala' apple scions on rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (control) during the first year of growth from grafting. For the measurement of IAA, the primary shoot apex from two scions were pooled providing three replicates per rootstock at each harvest date.

6.3.7.2 Putative relationships between the rate of IAA diffusion from the primary shoot apex and cumulative gains in root dry mass

During January, the mean rate of IAA diffusion from the primary shoot apex was reduced slightly by M.9 compared with the 'Royal Gala' rootstock control, however these rootstocks had developed an almost identical mean root dry mass (Figure 6.11A). In contrast, from January to February the mean rate of IAA diffusion from the primary shoot apex halved on the M.9 rootstock, and this reduction in IAA diffusion was concomitant with a very small decrease in dry mass gain by the M.9 root system when compared with the 'Royal Gala' rootstock (Figure 6.11A). From February to April, the mean rate of IAA diffusion from the primary shoot apex was almost halved by M.9 compared with the 'Royal Gala' rootstock control. By April, the mean dry mass of the M.9 root system was nearly half that of the 'Royal Gala' rootstock, and this difference in root dry mass largely eventuated from March to April when the rate of IAA diffusion for M.9 had declined below 0.1 ng hr^{-1} and therefore was very low (Figure 6.11A).

In similar patterns to M.9, from January to February the mean rate of IAA diffusion from the primary shoot apex was lower for MM.106 and M.793 than for the 'Royal Gala' rootstock control (Figure 6.11B, C). In contrast to M.9, the mean rate of IAA diffusion for MM.106 and M.793 did not greatly decrease from January to February (Figure 6.11A, B, C). In addition, these rootstocks had a slightly greater mean root dry mass by February compared with the 'Royal Gala' rootstock control (Figure 6.11B, C). However, the mean rate of IAA diffusion from the primary shoot apex was very similar for MM.106 and 'Royal Gala' from March to April, and this coincided with almost identical gains in root dry mass during this same period (Figure 6.11B). Somewhat differently, M.793 had greater mean rates of IAA diffusion from the primary shoot apex than the 'Royal Gala' rootstock control from March to April, which were accompanied by increased final root dry mass for M.793 when compared with 'Royal Gala' (Figure 6.11C).

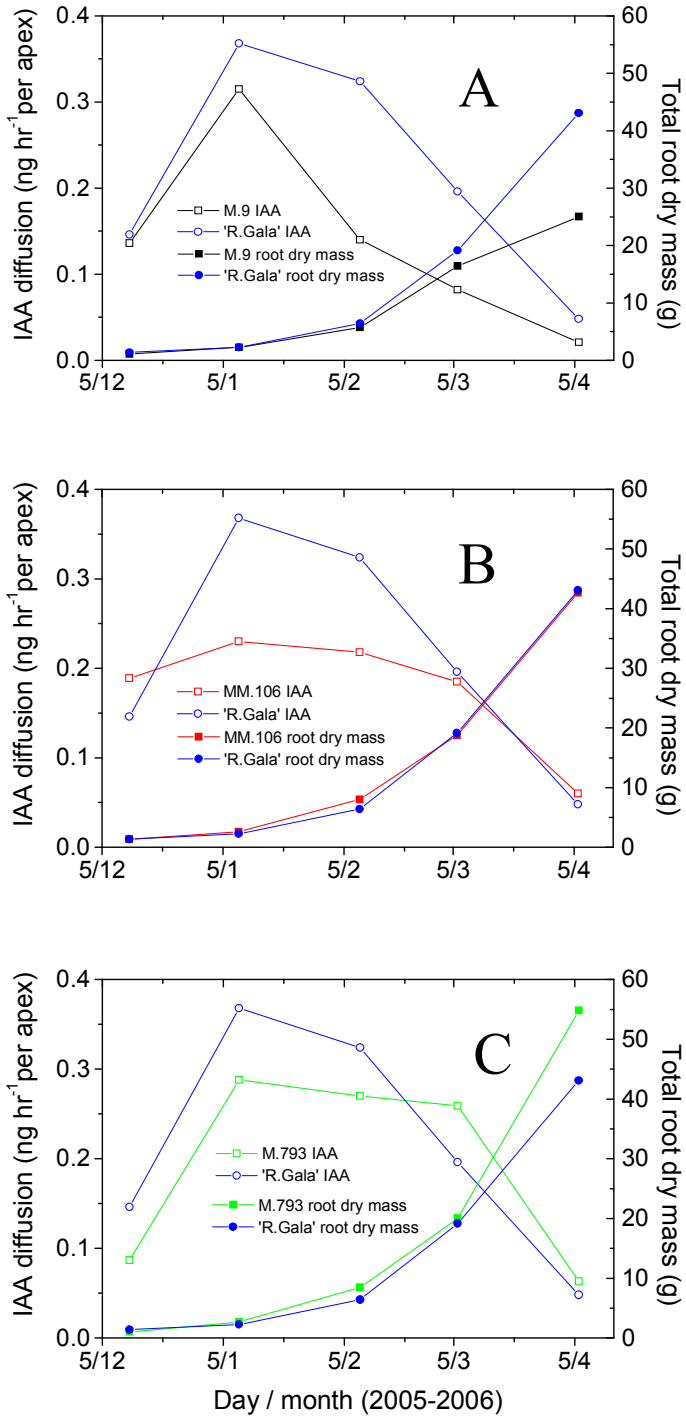


Figure 6.11. Relationships between the mean concentration of endogenous indole-3-acetic acid (IAA) diffusing from the primary shoot apex of 'Royal Gala' apple scions and the mean root dry mass of M.9 (A), MM.106 (B) and M.793 (C) rootstocks compared with the 'Royal Gala' rootstock control during the first year of growth from grafting.

6.3.7.3 Effect of rootstock type on the concentration of endogenous gibberellins in the xylem sap of the primary shoot

From December to January, the volumes of xylem sap extracted from the primary shoot were insufficient for gibberellin analysis. However, in February, March and April approximately 2, 4 and 6 mL, respectively, of sap was extracted per primary shoot, which was sufficient for gibberellin analysis. From February to April, GA_{19} and GA_{20} were present in the xylem sap for each rootstock, however concentrations of GA_{20} were very small and in many samples below the reliable limit of quantification by gas chromatography-mass spectrometry (0.01 ng mL^{-1}). Therefore, data for GA_{20} is excluded.

During February, concentration of GA_{19} in the xylem sap was not significantly affected by rootstock type (Figure 6.12A). From February to March, the mean concentration of GA_{19} in the xylem sap decreased for M.9, MM.106 and M.793, whereas it increased greatly for the 'Royal Gala' rootstock control (Figure 6.12A). Subsequently, in March the mean concentration of GA_{19} in the xylem sap was significantly reduced by M.9, MM.106 and M.793 compared with the 'Royal Gala' rootstock control (Figure 6.12A). There were also trends in March that the mean concentration of GA_{19} increased with increasing rootstock vigour (Figure 6.12A). From March to April, the concentration of GA_{19} in the xylem sap greatly decreased for the 'Royal Gala' rootstock control and, to a lesser extent, MM.106 and M.793, which was in contrast to a slight increase in the concentration of GA_{19} in the xylem sap for M.9 during the same period (Figure 6.12A). Hence, in April the xylem sap from M.9 contained significantly greater concentrations of GA_{19} than the 'Royal Gala' rootstock control, and there were trends that GA_{19} concentration decreased with increasing rootstock vigour (Figure 6.12A).

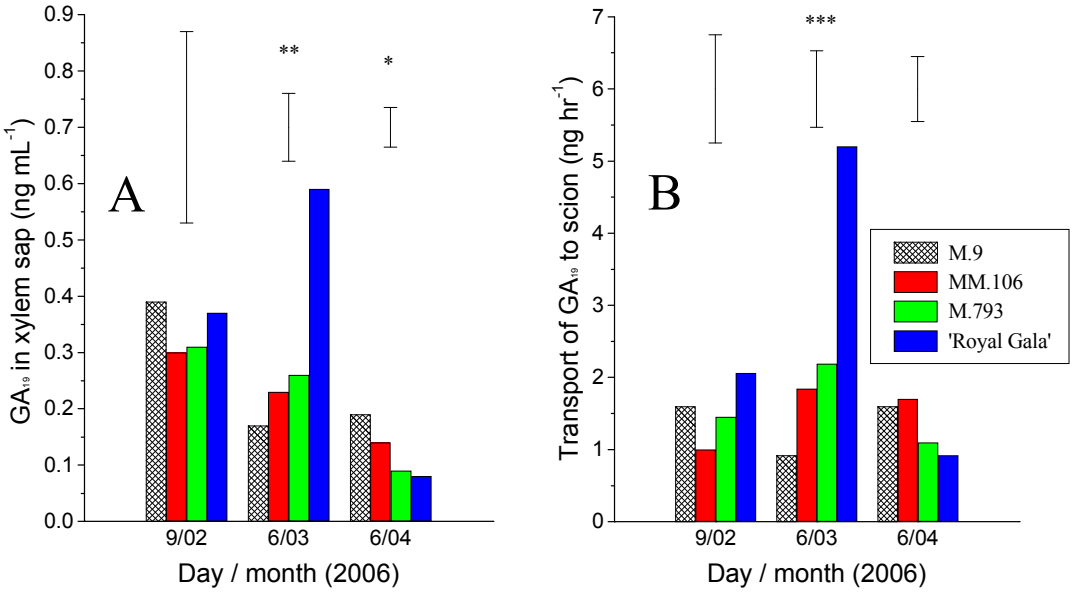


Figure 6.12. Mean concentration of endogenous gibberellin A19 (GA_{19}) in the xylem sap of 'Royal Gala' primary shoots on rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (control) during the first year of growth from grafting. Data for graph A are expressed as ng mL^{-1} of GA_{19} in the xylem sap, whereas data for graph B are the estimated mass of GA_{19} transported in the xylem sap to 'Royal Gala' primary shoots per hour as calculated for differences in leaf area and transpiration imposed on the scion by each rootstock type in February, March and April (see Section 6.3.7.4 for calculations). [x] ***, ****, ***** **significant ANOVA at** $P \leq 0.10$ $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, **respectively. Bars denote the LSD at** $P=0.05$.

6.3.7.4 Estimated mass of endogenous gibberellin and cytokinin delivered in the xylem sap to the canopy per hour (ng hr^{-1})

Although not measured in this experiment, photosynthetic measurements taken from the experimental tree material of Chapter 5 (data not shown) indicated that rootstocks also modified rates of leaf transpiration differently from February to April. In particular, transpiration tended to decrease with decreasing rootstock vigour (see Table 6.1 for mean values for each rootstock and month). Collectively, reduced canopy leaf area in March (Figure 6.1I) and decreased rates of leaf transpiration (Table 6.1) for the scion on M.9 would have reduced the rate of sap flow in the xylem compared with the scion on

the 'Royal Gala' rootstock control. Hence, it is possible that 'Royal Gala' may have transported greater amounts of hormone to the scion than indicated by data expressed as ng mL^{-1} (i.e., Figures 6.12A and 6.13A, C, E, G). Therefore, transpiration data collected in Chapter 5 (Table 6.1) and actual leaf area per tree measured in this study (Figure 6.1I) were used to estimate canopy transpiration (mL hr^{-1}) per tree (Table 6.1), thereby providing an approximation of sap flow within the xylem to the canopy for each rootstock during February, March and April. The amount of hormone transported (ng hr^{-1}) was then calculated by multiplying hormone concentration in the xylem sap (ng mL^{-1}) by canopy transpiration per tree (mL hr^{-1}) (Table 6.1).

During February, M.9 had slightly greater concentrations of GA_{19} in the xylem sap than the 'Royal Gala' rootstock control when was data were expressed as ng mL^{-1} (Figure 6.12A). However, the total mass of GA_{19} transported to the scion may have been reduced by M.9 compared with the 'Royal Gala' rootstock control (Table 6.1 and compare Figure 6.12A, B) because of decreased leaf area and reduced rates of leaf transpiration (Table 6.1). During March, M.9 may have also transported approximately half the amount of GA_{19} to the scion than MM.106 and M.793 (Figure 6.12B and Table 6.1), and differences between M.9 and M.793 may have been statistically significant (compare Figure 6.12A and B). During April, M.9 had a greater mean concentration of GA_{19} in the xylem sap than MM.106 (Figure 6.12A), however MM.106 may have transported very similar amounts of GA_{19} per unit time to M.9 (Figure 6.12B). In addition, rootstocks did not statistically differ in the mass of GA_{19} transported to the scion during April, which was in contrast to GA_{19} concentration data expressed as ng mL^{-1} (compare Figure 6.12A and B).

Table 6.1. Estimated mass of gibberellin A19 (GA_{19}) transported in the xylem sap per hour to the 'Royal Gala' primary shoot grafted onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' as calculated for actual differences in scion leaf area and probable differences in transpiration (T) imposed on the scion by each rootstock type during February, March and April.

	T (± SEM) of leaves		Leaf area per scion	T of total leaf area per scion	GA_{19} in sap	GA_{19} transported
Rootstock	(mmol H_20 m^{-2} sec^{-1})	(µL H_20 m^{-2} sec^{-1})	(m^2)	(mL H_2O hr^{-1})	(ng mL^{-1})	(ng hr^{-1})
			February			
M.9	2.65 ± 0.24*	47.70	0.233	4.01	0.39	1.56
MM.106	2.30 ± 0.21	41.40	0.233	3.48	0.30	1.04
M.793	2.83 ± 0.40	50.94	0.251	4.60	0.31	1.43
'R. Gala'	3.01 ± 0.23	54.18	0.294	5.73	0.37	2.12
			March			
M.9	2.33 ± 0.15	41.94	0.364	5.49	0.17	0.93
MM.106	2.83 ± 0.13	50.94	0.447	8.19	0.23	1.88
M.793	3.40 ± 0.09	61.20	0.372	8.19	0.26	2.13
'R. Gala'	3.20 ± 0.15	57.60	0.436	9.04	0.59	5.33
			April			
M.9	2.98 ± 0.29	53.64	0.454	8.77	0.19	1.67
MM.106	3.40 ± 0.18	61.20	0.528	11.63	0.15	1.74
M.793	3.45 ± 0.14	62.10	0.532	11.89	0.09	1.07
'R. Gala'	3.47 ± 0.14	62.46	0.524	11.78	0.08	0.94

*Data for transpiration were measured for composite trees grown under the experimental conditions of Chapter 5 (n=4) and therefore are not the actual transpiration rates for tree material in this experiment.

6.3.7.5 Effect of rootstock type on the concentration of endogenous cytokinins in the xylem sap of the primary shoot

Cytokinins were not measured in December and January because very small volumes of xylem sap extracted from the primary shoot were insufficient for hormone quantification (see Section 6.3.7.3). Therefore, data are available only for February, March and April.

In the order from greatest concentration, putative cytokinins identified within the xylem sap included zeatin riboside (ZR), isopentyladenosine (IPA), isopentyladenine (2iP), zeatin (Figure 6.13), 2iP ribotide, zeatin ribotide and a novel isopentenyl-type compound recovered as 2iP after incubation of the sap with alkaline phosphatase. However, concentrations of cytokinin ribotides and the novel isopentenyl-type

cytokinin were extremely small and well below the reliable limit of quantification by the RIA (0.1 ng). Therefore, these are not presented.

During February, rootstocks did not significantly differ in the mean concentration of Z, 2iP and IPA in the xylem sap (Figure 6.13A, C, E), however M.9 had a significantly greater concentration of ZR compared with MM.106, M.793 and the 'Royal Gala' rootstock control (Figure 6.13G). When data were expressed as ng hr^{-1}, M.9 only significantly increased (at P=0.06) amounts of ZR compared with MM.106 (Figure 6.13H). During March, rootstocks did not statistically differ in the concentrations of cytokinin in the xylem sap when data were expressed as ng mL^{-1} (Figure 6.13A, C, E, G). However, there were trends that 'Royal Gala' and, to a lesser extent, M.9 had greater concentrations of ZR than MM.106 and M.793 (Figure 6.13G). Rootstocks of M.9, MM.106 and M.793 were transporting approximately half the amount of ZR to the scion during March than the 'Royal Gala' rootstock control when data were expressed as ng hr^{-1} (Figure 6.13H).

During April, the concentration of ZR in the xylem sap of the scion increased with increasing rootstock vigour, however significant differences were not detected for the data expressed as ng mL^{-1} (Figure 6.13G). In contrast, expression of data as ng hr^{-1} of ZR resulted in statistical differences amongst rootstocks at P=0.07, with M.9 reducing the amount of ZR transported to the scion compared with the 'Royal Gala' rootstock control (Figure 6.13H). From February to April, the mean concentration of ZR in the xylem sap (Figure 6.13G) increased for each rootstock. In addition, these increases in mean ZR concentration in the xylem sap coincided with decreased mean rates of IAA diffusion from the primary shoot apex (Figure 6.14C). In contrast, concentrations of IPA or 2iP in the xylem sap tended to decrease from February to April and this coincided with decreasing rates of IAA diffusing from the primary shoot apex (Figure 6.14A, B).

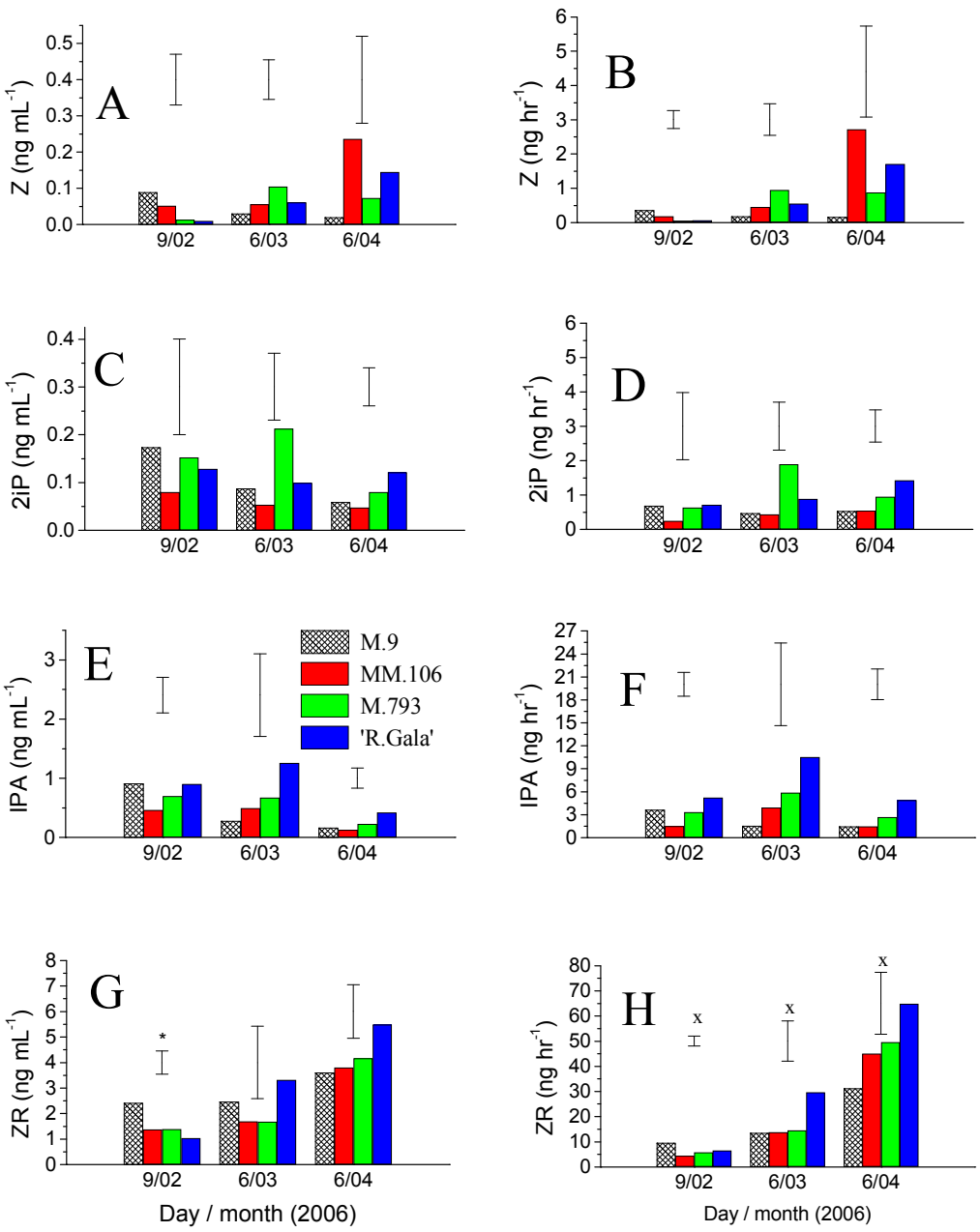


Figure 6.13. Mean concentration of zeatin (Z), zeatin riboside (ZR), isopentenyladenosine (IPA) and isopentenyladenine (2iP) in the xylem sap of 'Royal Gala' primary shoots on different apple rootstocks during the first year of growth from grafting. Data for A, C, E and G are expressed as ng mL^{-1} of cytokinin in the xylem sap, whereas data for B, D, F and H are estimated masses of cytokinins transported to the scion per hour (ng hr^{-1}) calculated for differences in scion leaf area and transpiration on each rootstock type (see Section 6.3.7.4). ^{x} *, **, * significant ANOVA at *P*≤0.10, *P*≤0.05, *P*≤0.01, *P*≤0.001, respectively. Bars denote the LSD at *P*=0.05.**

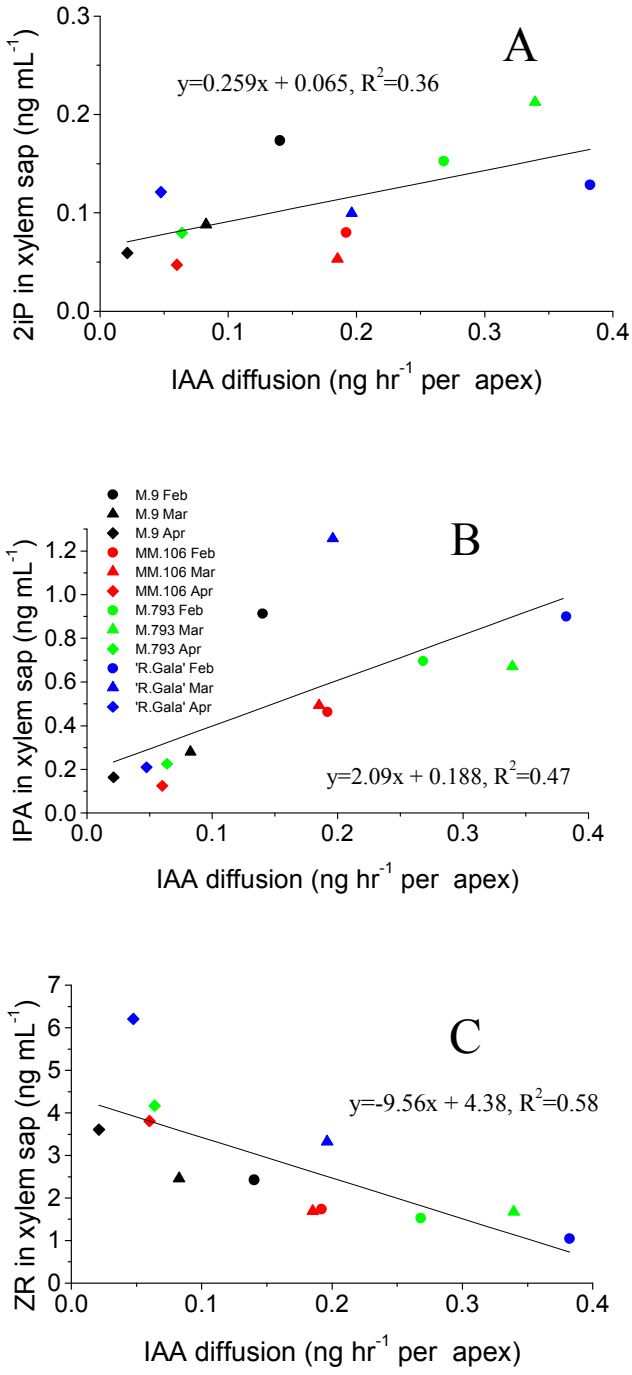


Figure 6.14. Relationship between the mean rate of indole-3-acetic acid (IAA) diffusing from the primary shoot apex and the mean concentration of isopentenyladenine (2iP) (A), isopentenyladenosine (IPA) (B) and zeatin riboside (ZR) (C) in the xylem sap of 'Royal Gala' primary shoots during their first growing season after grafting onto different size-controlling apple rootstocks.

6.3.8 Relationships between IAA, cytokinin and the formation of secondary axes

The concentrations of cytokinin were not measured in December and January because insufficient volumes of xylem sap were extracted from the primary shoot during this time of the growing season. The formation of secondary axes first began in late December onwards (Figure 6.3C), therefore it cannot be determined how rootstocks modified the concentration of endogenous cytokinins in the xylem sap prior to the initial occurrence of axillary bud outgrowth on the primary shoot.

The diffusion of IAA from the primary shoot apex increased from December to January for each rootstock type (Figure 6.10) and, during this time, no secondary axes had formed for rootstocks of M.9, MM.106 and M.793 (Figure 6.3C). From January onwards, the mean rate of IAA diffusion decreased each month irrespective of rootstock type (Figure 6.10). As the mean rate of IAA diffusion from the primary shoot apex decreased over the growing season the mean dry mass of roots (Figure 6.11) and mean concentration of ZR in the xylem sap (Figure 6.14C) increased. From February to April, increasing mean dry mass of root correlated with increasing mean concentration of ZR in the xylem sap (Figure 6.15B). In addition, the mean cumulative number of secondary axes formed on the primary shoot increased with increasing mean dry mass of the root system (Figure 6.15A) and concentrations of ZR in the xylem sap (Figure 6.15C).

In February, M.9 had a reduced rate of IAA diffusing from the primary shoot apex and had a greater concentration of ZR in the xylem sap than the 'Royal Gala' rootstock control (Figure 6.14C). However, in February the M.9 rootstock had formed fewer secondary axes compared with the 'Royal Gala' rootstock control (Figures 6.3B, C and 6.15C). From March onwards, M.9 had a reduced mean rate of IAA diffusion, root dry mass (Figure 6.11A), concentration of ZR in the xylem sap and number of secondary axes compared with the 'Royal Gala' rootstock control (Figure 6.15C). In contrast to M.9, from March onwards MM.106 had a very similar mean rate of IAA diffusion, mean root dry mass (Figure 6.11B) and number of secondary axes compared with the 'Royal Gala' rootstock control, but had a decreased concentration of ZR in the xylem sap (Figure 6.15C). From March to April, M.793 had greater rates of IAA diffusion from the primary shoot apex, gains in root dry mass (Figure 6.11C) and numbers of secondary axes compared with the 'Royal Gala' rootstock control (Figure 6.15C).

However, during this same period M.793 had a lower concentration of ZR in the xylem sap compared with the 'Royal Gala' rootstock control (Figure 6.15C).

6.3.9 Relationships between IAA and gibberellin on shoot apical meristem activity

By March, M.9 had a reduced rate of IAA diffusing from the shoot apex of the primary shoot (Figure 6.10) and a significantly decreased concentration of GA_{19} in the xylem sap compared with the 'Royal Gala' rootstock control (Figure 6.12A). However, in March M.9 and 'Royal Gala' rootstocks did not differ in the mean node number of primary shoot because cumulative node production over the growing season was very similar between these rootstocks (Figure 6.1D). The proportion of primary shoots that had terminated in March was not different between M.9 and 'Royal Gala' (see Section 6.3.1.1), even despite a significantly increased mean concentration of GA_{19} in the xylem sap of the scion on the 'Royal Gala' rootstock control (Figure 6.12A).

For secondary shoots, the mean proportion (%) of SAMs terminated in March was 90%, 84%, 82% and 74% for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively, but differences were not significant. The mean amount of GA_{19} transported to the scion was 0.9, 1.9, 2.1 and 5.3 ng hr^{-1} for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively (Figure 6.12B). Thus, during March there were trends that termination of secondary shoots increased with decreasing rootstock vigour, and rootstocks that had increased shoot termination were transporting reduced amounts of GA_{19} to the scion. However, these relationships did not exist in February (data not shown), whilst in April 100% of secondary shoots had terminated for each rootstock type (data not shown).

Unlike data for cytokinins (Figure 6.14), decreased diffusion of IAA from the primary shoot apex correlated poorly with GA_{19} concentration in the xylem sap, particularly for the 'Royal Gala' rootstock, for which rates of IAA diffusion decreased from February to March (Figure 6.10), whilst concentrations of GA_{19} in the xylem sap greatly increased during the same period (Figure 6.12A). For M.9, IAA concentration diffusing from primary shoot apex decreased from February to March (Figure 6.10), which was concomitant with decreasing GA_{19} concentration in the xylem sap over the same period (Figure 6.12A). However, as rates of IAA diffusion for M.9 further declined from March to April (Figure 6.10), the mean concentration of GA_{19} did not greatly change

(Figure 6.12A). In contrast, there were trends that reduced rates of IAA diffusion from the primary shoot apex on MM.106 and M.793 from February to April (Figure 6.10) coincided with decreasing GA_{19} concentration in the xylem sap (Figure 6.12A), although this did not occur when data for GA_{19} were expressed as ng hr^{-1} (Figure 6.12B).

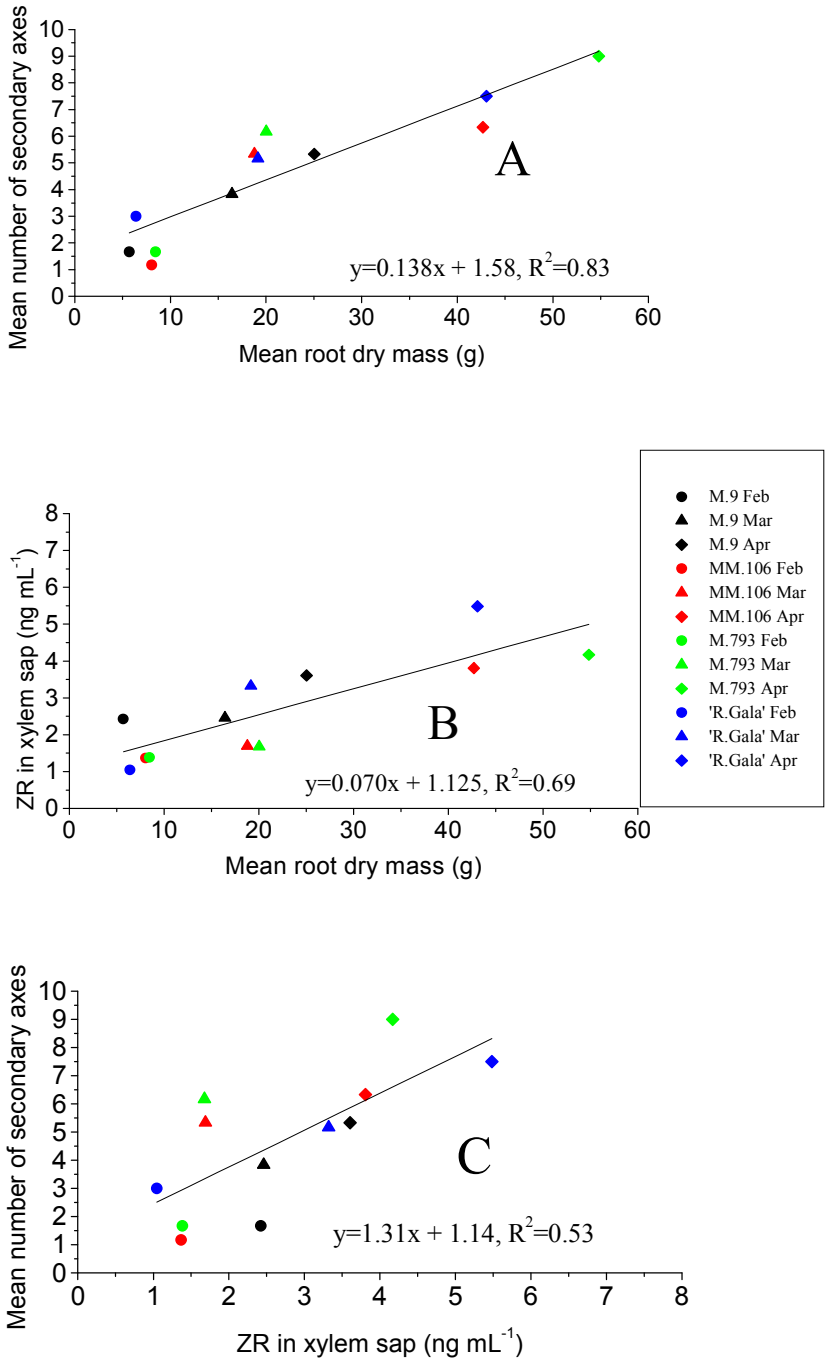


Figure 6.15. Relationship between mean dry mass of roots and the mean number of secondary axes (spurs plus shoots) per scion (A), mean dry mass of roots and the mean concentration of zeatin riboside (ZR) in the xylem sap of the primary shoot (B) and mean concentration of ZR in the xylem sap of the primary shoot and the mean number of secondary axes formed per scion (C) for composite 'Royal Gala' apple trees on different size-controlling rootstocks during the first growing season from grafting.

6.4 Discussion

6.4.1 Influence of rootstock type on growth of the scion

6.4.1.1 Primary shoot

The final mean length, node number and internode length of the primary shoot was not significantly reduced by M.9 compared with the 'Royal Gala' rootstock control (Figures 6.1A, D and 6.2A). In contrast, M.9 significantly decreased the final mean dry mass of the primary shoot stem compared with the 'Royal Gala' rootstock control, especially from March onwards (Figure 6.6A) when the SCA of the primary shoot increased greatly for 'Royal Gala', but not M.9 (Figure 6.2B). Similarly, the girth of the primary shoot stem increased in the autumn for composite trees of 'Spartan' in their second year of growth, but increases in stem girth were markedly less for trees on M.9 than on MM.106 (Abod and Webster, 1989).

An increase in girth of apple stems occurs from cambial activity producing annual increments of secondary phloem and xylem (Pratt, 1990). Notably, cambial activity in perennial trees is stimulated by IAA (Digby and Waring 1966; Lachaud et al., 1999) and from February to March the rate of IAA diffusion from the primary shoot apex was less for M.9 than for the 'Royal Gala' rootstock control (Figure 6.10). Similarly, Michalezuk (2002) reported that the concentration of IAA in the cambial sap from stems of M.9 was four-fold lower than stems of MM.106 late in the growing season. It has also been shown that dwarfing rootstocks reduced the basipetal transport of radio-labelled IAA within their stem (Soumelidou et al., 1994a; Kamboj et al., 1997). Similar to these effects of M.9, in Chapter 5 the application of NPA to the rootstock stem greatly decreased the final SCA of the primary shoot on the highly-vigorous 'Royal Gala' rootstock (Figure 5.5D). Therefore, it is possible that SCA of the primary shoot did not increase for M.9 from March onwards (Figure 6.2B) because the basipetal transport of IAA from scion to root may have largely ceased, thereby limiting cambial activity in the primary shoot stem.

6.4.1.2 Secondary shoots

As found in Chapters 3 and 5, M.9 reduced the mean total length and node number of the secondary shoots by the end of the growing season (Figure 6.1B, E), particularly because the first occurrence of axillary bud outgrowth on the primary shoot was delayed, and therefore no secondary shoots had formed by January (Figure 6.3B). The M.9 rootstock also imposed small reductions in the final mean length and node number of individual secondary shoots (see Section 6.3.2.2).

Reduced mean length of the secondary shoots for M.9 was not a result of differences in internode length of these shoots. For example, secondary shoots of the same length had an almost identical node number regardless of rootstock type (Figure 6.4A, C, E, G). Thus, the mean length of the secondary shoots was decreased slightly for the scion on M.9 because greater proportions of short shoots formed compared with the 'Royal Gala' rootstock control, particularly shoots with less than 8 nodes (Figure 6.4B, H). In addition, the proportion of secondary shoots that had terminated by March increased with decreasing rootstock vigour (see Section 6.3.2.2). Hence, small reductions in the mean length and node number of the secondary shoots for M.9 probably occurred because greater proportions of secondary shoots terminated growth earlier. The combination of fewer secondary shoots and small reductions in the mean length of both the primary and secondary shoots significantly decreased the final mean total shoot length of the scion on M.9 when compared with the 'Royal Gala' rootstock control (Figure 6.1C). Very similar decreases occurred for the mean total node number of the scion on M.9, although differences compared with 'Royal Gala' were not highly significant in April (Figure 6.1F).

6.4.1.3 Effect of rootstock type on the distribution of dry mass between root and scion

Rootstocks greatly differed in their effect on how dry mass was allocated between the root and scion over the growing season. From December to February, the allometric coefficients for root and scion were 0.88 and 0.87 for M.9 and the 'Royal Gala' rootstock control, respectively (Figure 6.9A). Therefore, these rootstocks were allocating dry mass preferentially into the growth of the scion (Figures 6.9A and 6.7B). In contrast, the allometric coefficients were 0.99 and 1.03 for MM.106 and M.793,

respectively. Hence, these rootstocks were allocating dry mass more equally between scion and root from December to February (Figures 6.9B, C and 6.7B).

In December, small reductions in dry mass of the root and scion had occurred for M.793 and, to a lesser extent, M.9 when compared with the 'Royal Gala' rootstock control (see natural log transformed means of root or scion dry mass along the y or x-axis, respectively, of Figure 6.9A and C). These initial reductions in root dry mass gain in early spring may reflect different growth patterns by the genetically different root systems. For example, a large proportion of apple roots present at transplanting will die and the root system re-establishes by initiating new roots on the rootstock stem and, to a lesser extent, from one-year-old roots present at planting (Hatton et al., 1923; Abod and Webster, 1989).

Soon after transplanting, rootstocks may also differ in the way the new root system is initially established. For example, following transplanting most of the new roots formed on M.9 regenerated from the rootstock stem while for MM.106 they grew from lateral roots present at planting (Abod and Webster, 1989). Unfortunately, detailed root architecture data were not collected in this study to elucidate the cause of reduced rates of root dry mass gain by M.9 and M.793 early season (Figure 6.9A, C). However, in future studies it would be of interest to elucidate how rootstocks initially modify root architecture in early spring. For example, the ability of M.9 to readily initiate new roots from the rootstock stem in spring (Abod and Webster, 1989), and the importance of IAA for the initiation of apple roots (Jones and Hatfield, 1976; Delargy and Wright, 1979), may indicate that the M.9 root system was not deficient in IAA, at least in the early part of the growing season. Tustin (1976) reported apple rootstock cuttings that had either high or low endogenous concentrations of an IAA-like substance during the autumn and winter produced a peak of an IAA-like substance that was associated with bud break in early spring.

From February to April, the allometric coefficient for root and scion greatly increased for each rootstock type, thereby indicating that dry mass was being allocated preferentially into growth of the roots than the scion (Figure 6.9). This increase or switch in the allometric coefficient approximately coincided with the slowing of shoot extension growth, which became very apparent for rootstocks of M.9, MM.106 and

'Royal Gala' from early March onwards (Figure 6.1C, F). Similarly, Abod and Webster (1989) reported that root growth rather than shoot extension growth predominated from late summer onwards for composite apple trees of 'Spartan' on M.9 and MM.106. Therefore, it seems likely that as shoot growth slowed in late summer, possibly because of decreasing shoot/root transport of IAA and root/shoot transport of gibberellin (see Chapter 5, Section 5.4.2.1), dry mass allocation was reprioritised from shoot growth to root growth. Indeed, Hansen and Grauslund (1973) reported that $^{14}CO_2$ absorbed by apple leaves in the autumn was predominantly recovered as ^{14}C in the root system, therefore indicating that apple roots are an important sink for plant carbon late in the growing season.

Kamboj (1996) reported that newly grafted trees of 'Fiesta' on M.9 tended to transport less ^{14}C-sorbitol from scion to root in summer than trees on MM.106 and M.111. In this study, dry mass gain of the M.9 root system slowed from March to April when compared with the 'Royal Gala' rootstock control (Figure 6.6J). However, there were trends that M.9 initially imposed very small decreases in dry mass gain of both root and scion much earlier in the growing season (Figure 6.9A). For example, mean root and scion dry mass were almost identical for M.9 and 'Royal Gala' by January, although from February onwards the mean dry mass of both root and scion were reduced by M.9 (see natural log transformed means in Figure 6.9A). Notably, the mean total length of the M.9 root system was very similar to the 'Royal Gala' rootstock control in February (Figure 6.5A). Therefore, small decreases in dry mass gain of the M.9 root system may have resulted from slightly decreased specific root mass in February when compared with the 'Royal Gala' rootstock control (Figure 6.5B).

For the scion on M.9, small reductions in dry mass gain by February (Figure 6.9A) were mainly caused by decreased formation of secondary axes compared with the 'Royal Gala' rootstock control (Figure 6.3C), thereby decreasing dry mass gain of stems (Figure 6.6B) and leaves (Figure 6.6E) of the secondary axes. Interestingly, the formation of secondary shoots had begun for the 'Royal Gala' rootstock in early January (Figure 6.3B), which was a time when the dry mass gain of the root system was almost identical for M.9 and the 'Royal Gala' rootstock control (Figure 6.9A). Thus, delayed secondary shoot formation for the scion on M.9 (Figure 6.3B) did not appear to result from reduced root dry mass (Figure 6.9A) or root length (Figure 6.5A).

Abod and Webster (1989) reported that M.9 did not greatly increase the stem girth of 'Spartan' scions in autumn when compared with MM.106. However, during the same period of the growing season total root length of M.9 increased, whereas scion extension growth did not (Abod and Webster, 1989). The aforementioned results differ from this study where the total extension growth of the root (Figure 6.5A) and scion (Figure 6.1C) did not greatly increase for M.9 from March to April. Therefore, extension growth of roots and shoots appeared to cease concurrently. Notably, this effect of M.9 was in contrast to the 'Royal Gala' rootstock control, which greatly increased total root (Figure 6.5A) but not shoot length (Figure 6.1C) from March to April.

For M.9, very little root extension growth from March to April (Figure 6.5A) may indicate that root apical meristems were not actively extending, and/or, new axillary roots were not being initiated. However, M.9 roots gained small amounts of dry mass during the same period of the growing season (Figure 6.6J). Hence, from March to April gains in root dry mass by the M.9 root system (Figure 6.6J) were probably caused mostly by secondary thickening, which appeared to increase late in the season for each rootstock type, as indicated by increasing specific root mass (Figure 6.5B). In contrast, the root system of 'Royal Gala' gained more total root length (Figure 6.5A) and dry mass (Figure 6.6J) during the same period and by April had developed a significantly increased specific root mass than the M.9 root system (Figure 6.5B). Thus, the 'Royal Gala' root system probably gained much more root dry mass from March to April because of greatly increased root extension growth and secondary thickening than roots of M.9.

Dry mass gain of the root system for each rootstock type during this late-season period (i.e., March to April) would have also occurred from the storage of carbohydrate reserves in the roots, particularly starch (Hansen and Grauslund, 1973). Colby (1935) reported that M.9 roots were not deficient in starch compared with rootstocks of greater vigour. However, the smaller root system of M.9 (Figures 6.5A, B and 6.6J) would decrease the total amount of carbohydrate able to be stored compared with the larger root systems formed on rootstocks of greater vigour. This may consequently decrease scion vigour in the early spring of year two after grafting. For example, increased flowering of the scion on M.9 compared with rootstocks of greater vigour (Selezynova

et al., 2007; Chapter 4, Table 4.3) and subsequent fruit set would presumably expend a greater proportion of carbohydrate remobilised from the smaller root system of M.9, therefore limiting carbohydrate available for other newly establishing sinks. In particular, decreased carbohydrate status of young apple trees during early spring was reported to reduce the initial growth of establishing shoots (Abusrewil et al., 1983).

In April, a significantly decreased specific root mass for M.9 compared with the 'Royal Gala' rootstock (Figure 6.5B) may indicate that root cross-sectional area was reduced by M.9. Similar to shoots, secondary thickening of apple roots occurs from cambial activity (Pratt, 1990) and there is strong evidence that cambial activity of roots is controlled by the synthesis and basipetal transport of IAA from shoot to root (Digby and Wangermann, 1964; Digby and Waring 1966; Lachaud et al., 1999). For example, excision of the shoot apex reduced the secondary thickening of pea roots by decreasing the number of cambium cells and their undifferentiated derivatives. However, this effect of decapitation was fully reversed with exogenous IAA applied to the cut stem (Digby and Wangermann, 1964). Similar mechanisms may exist for apple whereby the rootstock stem of M.9 decreases the basipetal transport of IAA to the root system (Kamboj, 1996), thereby decreasing cambial activity and reducing secondary thickening of the M.9 root system later in the growing season.

6.4.2 Effect of rootstock type on scion and root growth and the transport of endogenous IAA, cytokinins and gibberellins

6.4.2.1 Relationships between IAA, cytokinin and the formation of secondary axes

The M.9, MM.106 and M.793 rootstocks all delayed the first occurrence of axillary bud outgrowth or the formation of secondary shoots on the primary shoot compared with the 'Royal Gala' rootstock control, particularly from January to February (Figure 6.3B). However, from February to March the scion on MM.106 formed more secondary shoots than 'Royal Gala' resulting in a similar mean number of secondary shoots for these rootstocks by March (Figure 6.3B). In contrast, M.793 formed more secondary shoots from February onwards and hence had a slightly greater final mean number of secondary shoots than the 'Royal Gala' rootstock control (Figure 6.3B). The M.9 rootstock produced very similar cumulative gains in secondary shoot number to 'Royal Gala' from February to April, hence fewer secondary shoots for M.9 in April appeared

to result from their delayed formation before February (Figure 6.3B). Unfortunately, it could not be ascertained whether rootstocks differed in the concentration of cytokinins in the xylem sap before February because insufficient xylem sap was extracted from the scion for hormone analysis.

In the order of greatest concentration, putative cytokinins identified within the xylem sap included zeatin riboside (ZR), isopentyladenosine (IPA), isopentyladenine (2iP), zeatin (Figure 6.13), 2iP ribotide, zeatin ribotide and a novel isopentenyl-type compound recovered as 2iP after incubation of the sap with alkaline phosphatase (data not shown). A similar isopentenyl-type cytokinin was previously reported to be present in the fruit of *Actinidia deliciosa* (Woolley and Currie, 2006). For apple trees, other studies have also identified Z (Tromp and Ovaa, 1994; Kamboj et al., 1999), ZR (Jones, 1973; Young, 1989; Cutting et al., 1991; Skogerbo and Mage, 1992; Tromp and Ovaa, 1994; Kamboj et al., 1999) IPA (Skogerbo and Mage, 1992; Tromp and Ovaa, 1994), 2iP (Tromp and Ovaa, 1994) and zeatin ribotide (Jones, 1973) in the xylem sap. However, no previous literature has reported the presence of 2iP ribotide or the novel isopentenyl-type cytokinin within xylem sap of apple. Others have also reported that ZR was the predominant cytokinin form in the xylem sap of a vigorous (Tromp and Ovaa, 1994) or semi-vigorous rootstock (Kamboj et al., 1999) while, in contrast, Z was the predominant cytokinin in the xylem sap of 'Fiesta' trees on M.27 or M.9 in their second growing season from grafting (Kamboj et al., 1999).

Lockard and Schneider (1981) hypothesised that reduced basipetal transport of IAA within the rootstock stem to the root system may reduce the synthesis of root-produced cytokinins and their consequent transport to the scion. Indeed, total cytokinin concentrations (Z plus ZR) in the xylem sap of 'Fiesta' scions were reduced by M.9 compared with MM.106 in the summer of year two from grafting (Kamboj et al., 1999). Data for newly grafted trees in this study, however, indicated that the concentration of ZR in the xylem sap increased for each rootstock type from February to April, and these increases in ZR concentration correlated with decreasing rates of IAA diffusion by the primary shoot apex (Figure 6.14C). Although the rate of IAA diffusion from the primary shoot apex may not represent the amount of IAA transported basipetally within the rootstock stem to the root system, correlations between rates of IAA diffusion and cytokinin concentrations in the xylem sap (Figure 6.14) strongly suggest that basipetal

transport of IAA from scion to root regulates cytokinin biosynthesis at the root system. In addition, data from this study indicated that the reduced basipetal transport of IAA from scion to root increased concentrations of ZR and decreased concentrations of IPA and 2iP (Figure 6.14A, B, C). Therefore, the basipetal IAA signal differentially affected the different cytokinin forms.

In Chapters 3 and 5, the M.9 rootstock reduced the formation of secondary axes on the primary shoot compared with rootstocks of greater vigour (Figures 3.7 and 5.13B). The formation of secondary axes could be reinstated for the scion on M.9 by repeatedly applying BAP to the scion over the growing season (Figures 3.7 and 5.13B). Similarly, restricting the basipetal transport of IAA from scion to root using the auxin transport inhibitor 'NPA' applied to the rootstock stem also decreased the formation of secondary axes on the primary shoot, and their formation was reinstated with exogenous BAP (Figures 5.13B, 5.27 and 5.28). These results suggest that IAA and cytokinin interact to regulate the formation of secondary axes on the primary shoot. As hypothesised by Lockard and Schneider (1981), a mechanism may exist whereby M.9 reduces the basipetal transport of IAA from scion to root (Kamboj, 1996), which reduces root growth, and/or, cytokinin biosynthesis, thereby decreasing the transport of cytokinins to the scion. For M.9, the architectural consequence of decreased cytokinin transport from root to scion may be the development of a scion phenotype with fewer secondary axes by the end of the first season of growth from grafting.

Indeed, in March and April trees on M.9 had reduced rates of IAA diffusion from the primary shoot apex, reduced dry mass of roots (Figure 6.11A), decreased concentrations of ZR in the xylem sap (Figure 6.13G) and had formed fewer secondary axes (Figure 6.3C) compared with those on the 'Royal Gala' rootstock. In contrast, the scion on MM.106 and M.793 formed more secondary axes from February to March than the 'Royal Gala' rootstock control (Figure 6.3C), but this was a period when the rate of IAA diffusion from the primary shoot apex did not greatly change for MM.106 and M.793 (Figure 6.10) and when increases in the concentration of ZR in the xylem sap were less marked than that for 'Royal Gala' (Figure 6.13G).

In contrast to these results for MM.106 and M.793, Bangerth et al., (2000) reported that reduced basipetal IAA transport from the shoot apex combined with increased

concentrations of zeatin and isopentenyl-type cytokinins in the xylem sap was a perquisite for the release of axillary buds from apical dominance. Explanation of why the scion on MM.106 and M.793 formed more secondary axes from February to March cannot be explained from these data. In particular, the total amount of IAA produced by the scion and transported to the root system was not quantified. Bangerth et al., (2000) reported that exogenous cytokinin increased the rate of polar IAA transport from pea shoots by stimulating the outgrowth of additional axillary shoot apices. Thus, apple rootstocks that increased the outgrowth of axillary buds on the primary shoot (Figure 6.3C) also presumably had greatly increased potential for IAA transport from scion to root. However, rates of IAA diffusion from the apices of both the primary and secondary shoots would need to be quantified over the growing season to elucidate this. In addition, the amounts of IAA transported and metabolised by the rootstock stem would need to be ascertained so that the amount of IAA transported to the root system of each rootstock could be determined and correlated to cytokinin concentration in the xylem sap preceding the time(s) when major flushes of branching occur.

Hormones other than IAA and cytokinin may also control branching in some plant species. For example, the *rms* 1 mutant of pea has elevated concentrations of IAA in the shoot and greatly reduced concentrations of ZR in the root sap compared with wild type plants (Beveridge et al., 1997). However, *rms* 1 mutants have a highly branched phenotype compared with wild type plants (Beveridge et al., 1997). In addition, the *rms* 1 scion grafted onto wild type roots had greatly reduced branching, possibly because of the transport of a graft transmissible signal from root to scion that inhibited branch formation (Beveridge et al., 1997). Gomez-Roldan et al., (2008) has since identified this signal as a strigolactone type compound. At present, it is unknown whether strigolatones are present in the xylem sap of apple and, more importantly, whether dwarfing apple rootstocks increase the amount of strigolactones transported from root to scion when compared with rootstocks of greater vigour.

6.4.2.2 Relationships between IAA and root growth

In Chapter 5, repeated applications of the auxin transport inhibitor 'NPA' to the rootstock stem decreased root dry mass of M.9, MM.106, M.793 and 'Royal Gala' rootstocks by the end of the growing season (Figure 5.29A). Similar to NPA, the M.9

rootstock also decreased the final root dry mass compared with the 'Royal Gala' rootstock control (Figures 5.29A and 6.8A), possibly because the stem tissue of M.9 can decrease the basipetal transport of IAA (Kamboj, 1996). Therefore, an important objective of this study was to elucidate the time during the growing season when root growth of M.9 was first decreased compared with the 'Royal Gala' rootstock control. Such information may indicate the time from grafting when shoot to root signalling of IAA was first modified by M.9.

In December, a small decrease in root dry mass had occurred for M.9 compared with the 'Royal Gala' rootstock control (see natural log transformed data along the y-axis of Figure 6.9A). By January, however, the mean dry mass of roots was the same for M.9 and the 'Royal Gala' rootstock control (both 2.25 g). Therefore, from December to January the dry mass gain of the M.9 root system was greater than the 'Royal Gala' rootstock control (Figure 6.9A). Given that restricting the basipetal signal of IAA from scion to root decreased dry mass of apple roots (Figure 5.29A), greater gains in root dry mass by M.9 from December to January (Figure 6.9A) may indicate that IAA transport from scion to root was not initially limiting growth of the M.9 root system.

By February, rootstocks did not statistically differ in their mean root dry mass (Figure 6.6J), however the mean root dry mass was 5.70 and 6.40 g for M.9 and 'Royal Gala', respectively. Therefore, there were trends that from February onwards root growth was decreased by M.9 compared with the 'Royal Gala' rootstock (Figure 6.9A). In addition, this initial decrease in dry mass of M.9 roots by February was preceded by a large decrease in IAA diffusion from the primary shoot apex between January and February (0.32 to 0.14 ng hr^{-1}), whereas rates of IAA diffusion did not greatly decrease for 'Royal Gala' during the same period (0.37 to 0.33 ng hr^{-1}) (Figure 6.11A). From March to April, gains in root dry mass for M.9 were markedly less than the 'Royal Gala' rootstock control and, during this time, rates of IAA diffusion from the primary shoot apex on M.9 were approximately half that of 'Royal Gala' (Figure 6.11A). These results might indicate that root dry mass of M.9 was initially decreased in February because the basipetal transport of IAA from the apex of the primary shoot was reduced over the preceding month.

Decreased rates of IAA diffusion from the shoot apex between January and February may not reflect the actual amount of IAA transported from scion to root, particularly within the rootstock stem of M.9. However, it would seem highly probable that root growth of M.9 was decreased slightly compared with 'Royal Gala' by February because basipetal IAA transport had begun to decline. For example, Kamboj (1996) reported that newly grafted composite trees of 'Fiesta' on M.9 transported less ^{3}H to the root system in mid-summer compared with MM.106 and MM.111 when ^{3}H-IAA was applied to a mature basal leaf on the scion. Unfortunately, Kamboj (1996) did not correlate these differences in ^{3}H transport to root growth. Therefore, further research using radio-labelled IAA is required to determine whether decreased basipetal transport of IAA within the rootstock stem of M.9 precedes the initial reductions in its root growth compared with the 'Royal Gala' rootstock control. It would also be important to demonstrate that decreased basipetal transport of IAA within the rootstock stem correlates with decreased root growth of M.9, especially from March to April, when large reductions in root dry mass gain occurred for M.9 when compared with rootstocks of greater vigour (Figure 6.6J).

6.4.2.3 Relationships between IAA, GA_{19} and meristem activity

In Chapter 5, reducing the basipetal transport of IAA to the root by repeatedly applying NPA to the rootstock stem decreased the cumulative node production of the primary shoot compared with untreated trees (Figure 5.2). However, gibberellin applied repeatedly to the scion over the growing season fully reversed these effects of NPA on node production (Figure 5.2). Similarly, the cumulative node number of the primary shoot was reduced by M.9 and this decrease in growth was reversed with gibberellin applied repeatedly to the scion over the growing season (Figure 5.3). Collectively, these interactions may indicate that decreased basipetal transport of IAA from scion to root of a dwarfing apple rootstock reduces the amount of root-produced gibberellin transported to the scion, which consequently decreases node neoformation by SAMs on the scion. Therefore, further objectives of Chapter 6 were to elucidate whether the M.9 rootstock reduced the concentration of gibberellins in the xylem sap of 'Royal Gala' apple scions during the first growing season after grafting, and whether this correlated with decreased cumulative node production, and/or, increased SAM termination for the scion on M.9.

Gibberellins A19 (Figure 6.12) and A20 were present in the xylem sap, although concentrations of GA_{20} were below the reliable limit of quantification by GC-MS. This study primarily focused on GA_{19} and GA_{20} because the literature for apple indicated that these were important transport forms in apple xylem sap (Motosugi et al., 1996), which can be converted to bioactive GA_1 (Yamaguchi, 2008), presumably after their transport to the scion. Bulley et al., (2005) reported that suppression of GA 20-oxidase in apple shoots of 'Greensleeves' increased endogenous concentration of GA_{19} and decreased concentrations of GA_{20} and GA_1 in the shoot apex and young leaves, hence indicating that GA_{19} is an important precursor of GA_1 in the shoot apex.

The presence of GA_{19} in the xylem sap of apple rootstocks in this study and in the study of Motosugi et al., (1996) strongly suggests that gibberellins are synthesised by apple roots and transported to the scion in the xylem sap. In addition, gibberellin forms like GA_{19} could be important root/shoot signals because they are biologically inactive and presumably a stable transport form that may be converted to bioactive GA_1 at the site of action in the SAM. Notably, a number of other potentially important gibberellin forms have been identified in the xylem sap of apple including GA_{53}, GA_{23}, GA_{44}, GA_{15}, GA_{17} and GA_{18} (Motosugi et al., 1996). In the shoot, forms like GA_{53} and GA_{44} can be converted to GA_{19}, whereas GA_{15} has the potential to be converted to bioactive GA_4 (Yamaguchi, 2008). Therefore, further work is required to identify whether different size-controlling rootstocks modify other gibberellin forms in the xylem sap.

A recent study at East Malling reported that gibberellin concentrations were very low and below the reliable limit of detection for xylem sap collected from three-year-old 'Queen Cox' growing on M.9 and MM.106. Hence, it was concluded that gibberellins were not important in rootstock-induced dwarfing of the scion (East Malling, 2005). However, Ibrahim and Dana (1971) reported that purified exudate from the root system of 3-year-old 'Golden Delicious' on M.9 had reduced activity during gibberellin bioassay than exudate from 'Golden Delicious' on M.25. In this study, M.9 significantly decreased the mean concentration of GA_{19} in the xylem sap in March compared with the more vigorous 'Royal Gala' rootstock control (Figure 6.12A).

Despite M.9 significantly decreasing the concentration of GA_{19} in the xylem sap during March (Figure 6.12A), growth of the primary shoot was not greatly affected by

rootstock type from February to April (Figure 6.1A, D). In addition, during March the proportion of primary shoots that had fully terminated growth was 33%, 50%, 0% and 33% for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. Hence, the concentration of GA_{19} did not correlate with growth or termination of the primary shoot. However, the mean proportion (%) of terminated secondary shoots in March was 90%, 84%, 82% and 74% for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, whereas the amount of GA_{19} transported to the scion was 0.9, 1.9, 2.1 and 5.3 ng hr^{-1} for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively (Figure 6.12B). Thus, in March there were trends that termination of secondary shoots increased with decreasing rootstock vigour, and rootstocks that had increased shoot termination were transporting reduced amounts of GA_{19} to the scion. However, similar relationships did not exist during February or April.

Decreased rates of IAA diffusion from the primary shoot apex did not show consistent relationships with the concentration of GA_{19} in the xylem sap (see Section 6.3.9). These results may indicate that there is no relationship between shoot/root/shoot signalling of IAA and gibberellin, however given the rootstock x GA_{4+7} and NPA x GA_{4+7} interactions in Chapter 5, it would be premature to draw such conclusions given some limitations of this study. For example, IAA diffusing from primary shoot apex does not necessarily reflect the concentration of IAA transported basipetally within the rootstock stem to the root system. In addition, rootstocks had formed different mean numbers of secondary shoots over the growing season and, in March, different proportions of secondary shoots were actively growing. Because IAA is synthesised by shoots, particularly young leaves (Ljung et al., 2001), rootstocks that produced more secondary shoots per scion and increased the proportion of these that were producing new leaves would have presumably synthesised more IAA available for basipetal transport from scion to root. Therefore, future work will need to quantify whether differences in the seasonal formation of secondary shoots and SAM termination affect the ability of the scion to synthesise IAA. In addition, radio-labelled IAA should be used to quantify what proportion of scion-produced IAA is transported basipetally within the rootstock stem and actually reaches the root system of each rootstock type.

Concentration of detected hormones expressed as ng mL^{-1} also potentially underestimated hormone available for scion growth, particularly for vigorous

rootstocks, which may have had increased rates of sap flow in the xylem of the primary shoot from February to March because of their greater total leaf area per scion and increased rates of leaf transpiration. Hence, further work will need to consider such factors so that amounts of gibberellin transported to the scion can be more appropriately determined for different size-controlling rootstocks. In addition, full identification of gibberellin forms in the xylem sap is required to ascertain what other biologically important forms of gibberellin are present, whether rootstocks preferentially transport different forms of gibberellin and whether the composition of gibberellins changes in the xylem sap over the growing season. In addition, quantification of gibberellins in the shoot apex would provide useful information on whether the growth of SAMs is limited by root-produced gibberellin forms (i.e., GA_{19}), or if other mechanisms exist in the meristem itself. For example, reduced IAA transport out of the shoot apex may decrease gibberellin oxidase activity (i.e., GA 20 and GA 3-oxidase) in the shoot, thereby limiting the conversion of inactive gibberellin forms into bioactive GA_1 late in the growing season. In such a scenario, xylem transported gibberellins like GA_{19} may accumulate in the xylem sap and shoot apices as growth ceases, whilst bioactive GA_1 would decline in the shoot apex.

6.5 Summary

The final mean length, node number and internode length of the primary shoot was not significantly affected by rootstock type. However, M.9 and M.793 significantly decreased the final mean dry mass of the primary shoot stem compared with the 'Royal Gala' rootstock control. For M.9, decreased dry mass gain of the primary shoot stem occurred from March onwards mainly because SCA of the primary shoot did not greatly increase, which was in contrast to the 'Royal Gala' rootstock control.

There were strong trends that the M.9 rootstock reduced the mean total length of the secondary shoots, total node number of the secondary shoots and total dry mass of the secondary axes by the end of the growing season when compared with the 'Royal Gala' rootstock control. Total growth of the secondary shoots was decreased by M.9 because fewer secondary shoots formed and their mean length and node number were reduced slightly, presumably because a greater proportion of secondary shoots had terminated for M.9 by March. Regardless of rootstock type, secondary shoots of the same length had an identical node number. Hence, rootstocks did not affect internode length of individual secondary shoots.

In April, fewer secondary shoots per scion combined with very small reductions in the mean length of the primary and secondary shoots significantly decreased the final mean total shoot length of the scion growing on M.9 when compared with the 'Royal Gala' rootstock control. Very similar trends also occurred for the final mean total node number per scion, although statistical differences were not detected. The M.9 rootstock also reduced the mean total dry mass of both the primary shoot and secondary axes, which significantly decreased mean total dry mass of the scion on M.9 when compared with the 'Royal Gala' rootstock control.

In addition to decreasing total growth of the scion, M.9 significantly decreased the mean total dry mass of the rootstock (i.e., both stem and roots) compared with the 'Royal Gala' rootstock control. Collectively, decreased dry mass gain of scion and rootstock for M.9, especially from March to April, significantly reduced the final mean total dry mass per tree when compared with the 'Royal Gala' rootstock control. Mean total dry mass per tree also increased with increasing rootstock vigour in April, however

rootstocks greatly differed in the final distribution of dry mass into various component tree parts.

Compared with the 'Royal Gala' rootstock control, M.9 significantly increased the mean proportion of total tree dry mass allocated into the scion and decreased that to the rootstock (i.e., roots plus stem). In addition, these final differences in dry mass distribution mostly developed from March to April. In contrast, a significantly greater mean proportion of tree dry mass was allocated into the M.793 rootstock, particularly the root system. Hence, M.793 had allocated proportionally less dry mass into the scion than 'Royal Gala' in April. Rootstocks of 'Royal Gala' and MM.106 had allocated very similar proportions of total tree dry mass into the rootstock and scion in April. However, MM.106 reduced the proportion of total tree dry mass allocated into the rootstock stem, which was a similar to M.9.

Although decreased dry mass gain by the M.9 root system was very apparent from March to April, dry mass gain of M.9 roots was initially decreased from February onwards compared with the 'Royal Gala' rootstock. This initial decrease in dry mass gain by the M.9 root system approximately coincided with a large reduction in the basipetal transport of diffusible IAA from the shoot apex between January and February. These data may indicate the initial decrease in growth of M.9's root system resulted from decreased basipetal transport of shoot-produced IAA to the root from early summer onwards. However, further research using radio-labelled IAA would be required to demonstrate that decreased IAA transport within the rootstock stem of M.9 precedes the initial reductions in its root growth when compared with the 'Royal Gala' rootstock control. It would also be important to demonstrate that reduced IAA transport within the rootstock stem correlates with decreased root growth of M.9, especially from March to April, when root dry mass gain rapidly slowed for M.9 when compared with rootstocks of greater vigour.

Gibberellins A19 and A20 were identified in the xylem sap of each rootstock type, although concentrations of GA_{20} were below the reliable limit of quantification. In March, the concentration of GA_{19} was significantly decreased in the xylem sap of M.9 compared with the 'Royal Gala' rootstock control. However, the concentration of GA_{19} did not correlate with growth or termination of the primary shoot. For secondary shoots,

the mean proportion (%) of terminated SAMs in March was 90%, 84%, 82% and 74% for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, whereas the estimated amount of GA_{19} transported to the scion was 0.9, 1.9, 2.1 and 5.3 ng hr^{-1} for M.9, MM.106, M.793 and the 'Royal Gala' rootstock control, respectively. Thus, there were trends that termination of secondary shoots increased with decreasing rootstock vigour, and rootstocks that had increased shoot termination were transporting reduced amounts of GA_{19} to the scion. However, similar relationships were not evident in February or April.

By April, the scion on the M.9 rootstock had a decreased mean number of secondary shoots, and this appeared to result from delayed branch formation before February when compared with the 'Royal Gala' rootstock control. Unfortunately, it could not be ascertained whether rootstocks differed in the concentration of cytokinin in the xylem sap before February because insufficient xylem sap was extracted from the scion for hormone analysis. The mean concentration of ZR in the xylem sap increased for each rootstock type from February to April, and increased ZR concentration correlated with decreased rates of IAA diffusion from the primary shoot apex. In contrast, concentrations of IPA or 2iP decreased with decreasing rates of IAA diffusion. Therefore, the basipetal IAA signal appears to have differentially affected different cytokinin forms produced by the root.

From March onwards, there were trends that the M.9 rootstock decreased the amount of ZR in the xylem sap, particularly when data were expressed as ng hr^{-1} of ZR transported to the canopy. There were also trends that as mean concentration of ZR increased in the xylem sap over the growing season, the mean number of secondary axes formed per scion increased. However, relationships between ZR and IAA did not always consistently or satisfactorily explain the differences in the mean number of secondary axes formed on the primary shoot for each rootstock type and in each growing month. Further research will need to ascertain the total concentration of IAA produced by the scion (i.e., from primary and secondary shoot apices) and the proportion of this that is metabolised by the rootstock stem so that the actual amount of IAA reaching the root system of each rootstock type can be determined and then correlated to rates of cytokinin transport in the xylem sap preceding the time(s) when major flushes of branching occur on the scion.

7. General discussion and conclusions

7.1 Effect rootstock type on the initial development of dwarfing scion architecture

An important objective of this thesis was to elucidate how a dwarfing apple rootstock initially modified scion architecture compared with rootstocks of greater vigour and the precise stage of tree phenology when this occurred following propagation of the composite tree. Elucidation of this objective was essential to clearly identify processes that were the first physiological causes of rootstock-induced dwarfing of the scion from those that were subsequent developmental effects. Knowledge of the initial architectural modifications to the scion by a dwarfing apple rootstock also provided important information concerning which hormone groups were initially causal in scion dwarfing.

Within the literature, dwarfing apple rootstocks are reported to modify scion architecture in either year one or two of growth after propagation of the composite tree. In the first year of growth, initial modifications to scion architecture by a dwarfing apple rootstock may include decreased mean length (Rao and Berry, 1940; Cannon, 1941) and node number (Costes et al., 2001) of the primary shoot and the formation of fewer secondary shoots (Jaumien et al., 1993; Volz et al., 1994). However, the primary shoot of the scion may grow similarly in the first year of growth from propagation regardless of rootstock vigour (Tukey and Brase, 1941; Selezynova et al., 2008). Subsequently, reductions in scion vigour on M.9 occurred in year two of growth from grafting after increased flowering of the scion (Selezynova et al., 2008). Collectively, the above studies indicate that the first occurrence of rootstock-induced scion dwarfing can occur at different times in various locations, and differences in growing climate may have a large influence on what dwarfing growth responses are observed on the scion and their timing during early tree phenology.

Under the experimental growing climate of the Manawatu New Zealand, a consistent finding of this thesis was that M.9 significantly decreased the mean total shoot length and node number of the ‘Royal Gala’ apple scion by the end of the first year of growth from grafting (Tables 3.1, 4.1, 5.3 and Figure 6.1C). Therefore, the first occurrence of rootstock-induced dwarfing of the scion was a vegetative growth change that preceded

the first occurrence of flowering on the scion in the spring of year two. However, there were notable differences among experiments in the way total growth of the scion was reduced by M.9 within the first year from grafting. These are subsequently discussed for the component scion parts of the primary shoot and secondary axes:

7.1.1 Extension growth of the primary shoot

The cumulative growth of the primary shoot before December was generally very similar for M.9 compared with rootstocks of greater vigour (Figures 3.1A, B; 4.2A, B; 5.1A, B). Remarkably, in Chapter 4 it was shown that the cumulative growth of the primary shoot was initially greater for M.9 compared with MM.106 from November to December (Figures 4.2A, B and 4.3A, B). Therefore, at least in the early part of the growing season, 'Royal Gala' apple trees newly grafted onto M.9 may not be deficient in endogenous plant hormones, particularly IAA (see Section 7.3.1.1) and presumably gibberellin.

From December onwards, the primary shoot on M.9 grew very differently in each experiment. In Chapter 3, the primary shoot grew from a season-long growth flush before the primary shoot on M.9 exhibited a slower rate of growth between 11/2/05 to 15/3/05 (Figure 3.2A, B), which preceded shoot termination that first began between 15/3/05 and 11/4/05. On the 11/4/05, a greater proportion of primary shoots had terminated for M.9 compared with the scion on MM.106 and M.793 (see Section 3.3.2.1). Collectively, these differences, particularly earlier shoot termination, resulted in a primary shoot on M.9 that was shorter at growth cessation because of fewer neoformed nodes. Thus, mean internode length was not affected (Table 3.1).

In Chapter 4, it was shown that growth of the primary shoot could also be bicyclic, particularly on M.9, because a large proportion of primary shoots temporarily terminated for M.9 between early December and late January, possibly as a consequence of transplanting trees into the field (see Section 4.3.2.1). Primary shoots then resumed growth, however after the 22/3/05, a greater proportion of primary shoots had fully terminated for M.9 compared with the scion on MM.106, particularly during April (Figure 4.4). Collectively, these seasonal differences in scion growth on M.9 significantly decreased the mean length and node number of the primary shoot at

complete growth cessation compared with MM.106, but did not affect the mean internode length of the primary shoot (Table 4.1).

In Chapter 5, it was shown that the M.9 rootstock significantly decreased the final mean length and node number of the primary shoot compared with MM.106, M.793 and 'Royal Gala' (Figure 5.1A, B). For untreated trees (i.e., four-way means), there were trends that M.9 decreased the final mean length and node number of the primary shoot (Figure 5.5A, B) because a greater proportion of primary shoots temporarily terminated growth in mid February, although thereafter the proportion of terminated primary shoots was not reduced by M.9 when compared with rootstocks of greater vigour (Table 5.1). Unlike in Chapter 4, however, bicyclic growth of the primary shoot on M.9 during the summer (Table 5.1) was unlikely to have been the result of transplanting because, in Chapter 5, transplanting occurred prior to the outgrowth of the primary shoot in spring (see Section 5.2.1).

In contrast to Chapters 3, 4 and 5, cumulative growth of the primary shoot in Chapter 6 was very similar for each rootstock type throughout the growing season (Figure 6.1A, D). In addition, M.9 did not increase shoot termination of the primary shoot compared with rootstocks of greater vigour during any growing month (see Section 6.3.1). Hence, the final mean length and node number of the primary shoot was not significantly reduced by M.9 (Figure 6.1A, D). Similar to results in Chapters 3, 4 and 5, the final mean internode length of the primary shoot was not greatly affected by rootstock type (Figure 6.2A).

A recent study at Massey University in Palmerston North during the 2007-2008 growing season compared the growth of newly grafted 'Royal Gala' scions growing on M.9 and 'Royal Gala' rootstocks (Hernandez, 2008). In that study, measurements of scion extension growth were conducted at weekly intervals and the 'Royal Gala' primary shoot on both rootstocks grew from a season-long growth flush. At growth cessation in April, M.9 had significantly decreased the final mean length and node number of the primary shoot compared with the 'Royal Gala' rootstock control. This occurred because M.9 decreased the rate of node emergence by the primary shoot over a prolonged period from early January to late March when compared with the 'Royal Gala' rootstock control. The primary shoot on M.9 first began to terminate growth in

April, by which, significant reductions in the mean length and node number of the primary shoot had already occurred (Hernandez, 2008).

In contrast, Seleznyova et al., (2007) reported that rootstocks of M.9 and MM.106 did not affect the rate of node emergence of the 'Royal Gala' primary shoot in its first year of growth from grafting in the warmer growing region of Hawke's Bay, New Zealand. Hence, the final mean node number, length and internode length of the primary shoot was not different between these rootstocks at growth cessation (Seleznyova et al., 2007, 2008). These results are very similar for the 'Royal Gala' primary shoot growing on both M.9 and MM.106 in Chapter 6, which did not greatly differ in their final mean length, node number and internode length.

Collectively, results of this thesis and consideration of other studies indicates that the growth of the 'Royal Gala' primary shoot on the M.9 rootstock exhibits a high degree of plasticity within the first year of growth from grafting, typical of a probable rootstock genotype x environment interaction. This would suggest that comparisons between future studies could be made more meaningful if trees were grown in similar environmental conditions, such as those artificially imposed by the use of climate rooms where conditions of humidity, air temperature and irradiance could be consistently controlled. Equally, an understanding of how environmental factors contribute to the dwarfing response would potentially lead to an improved understanding of the physiological mechanisms involved and improved knowledge that could be applied to the management of these rootstocks in different climates.

7.1.2 Formation of secondary axes and extension growth of the secondary shoots

7.1.2.1 Formation of secondary axes

The formation of secondary axes on the primary shoot began in late January in the 2004-2005 growing season (Chapters 3 and 4) and in late December in the 2005-2006 season (Chapters 5 and 6). In Chapter 4 only, the scion on the M.9 rootstock produced a greater mean number of secondary axes compared with the scion on MM.106 after a greater proportion of primary shoots temporarily terminated for M.9, which in that experiment, may have been imposed by root disturbance from transplanting trees into

the field soil in early December. This result highlights that extreme care must be taken to ensure that rootstock effects on scion branching are not inadvertently modified by cultural practice.

In Chapters 3, 5, and 6, the M.9 rootstock decreased the mean number of secondary axes that formed on the primary shoot compared with rootstocks of greater vigour, particularly the number of secondary shoots (Figures 3.7B, 5.13B and 6.3). In the 2007-2008 growing season, the M.9 rootstock also reduced the formation of secondary shoots on the newly grafted 'Royal Gala' scion when compared with a 'Royal Gala' rootstock control (Hernandez, 2008). Thus, four experiments carried out over three different growing seasons in the Manawatu New Zealand have found that M.9 decreased the formation of secondary shoots during the first year of growth from grafting. In addition, this phenomenon was an important way in which total growth of the secondary shoots, and therefore total growth of the 'Royal Gala' scion, was initially reduced by M.9 compared with rootstock(s) of greater vigour.

Similarly, others have reported that the mean number of secondary shoots on scions of 'Gala' (Volz et al., 1994) and 'Cortland', 'Gloster', 'Jonagold' or 'Melrose' (Jaumien et al., 1993) were reduced by M.9 in a tree nursery by the end of the first year of growth. However, the 'Royal Gala' scion bench-grafted onto either M.9 or MM.106 and planted into the field in early spring formed very few secondary shoots on the primary shoot in the first year when grown in Hawke's Bay, New Zealand (Selezynova et al., 2008). Therefore, reduced secondary shoot formation of the 'Royal Gala' scion growing on the M.9 rootstock does not occur in all growing regions and, like the primary shoot, can exhibit great plasticity as typified by a probable rootstock genotype x environment interaction.

Understanding the environmental differences between the Manawatu and the Hawke's Bay that cause such contrasting differences in the formation of secondary axes on the scion should be a priority for further research. Knowledge of the environmental conditions that promote the formation of secondary axes for the 'Royal Gala' scion growing on M.9 would facilitate further studies where scion branching could be induced under controlled environmental conditions, and the timing of axillary bud outgrowth controlled at the discretion of the researcher. This would then enable endogenous

shoot/root/shoot signalling of IAA and cytokinins to be appropriately quantified prior to the initial occurrence of axillary bud outgrowth on the primary shoot (see Section 7.3.1.2). As found in Chapter 6, this was very difficult to achieve using potted trees grown outside as it was largely unknown if and when axillary bud outgrowth on the primary shoot would occur during the growing season. Environmental factors that affect sylleptic shoot formation can include high air humidity, soil temperature (Tromp, 1996) and air temperatures in the range of 16 to 26^{o}C (Abbas et al., 1980; Tromp, 1993), particularly if air temperatures in this range during the early growing season are interspersed by periods of low air temperatures between 0 and 10^{o}C (Abbas et al., 1980).

In this thesis, 'M.9' was used to elucidate the initial architectural modifications imposed on the scion by a dwarfing apple rootstock. Therefore, it still remains largely unknown whether other genotypes of dwarfing apple rootstocks impose similar modifications in scion branching during the first year of growth from grafting. Recently, Fazio and Robinson (2008) reported that the 'Brookfield Gala' scion budded onto the 'Geneva 16' (G.16) dwarfing apple rootstock developed many more secondary shoots by the end of the first year of growth in a tree nursery compared with the same scion budded onto M.9. Similarly, the 'Fuji' scion budded on 'Geneva 935' (G.935) or 'Cornell-Geneva 4213' (CG.4213) produced more secondary shoots than the scion budded onto more vigorous rootstocks of CG.7037 and CG.8534 (Fazio and Robinson, 2008). Therefore, not all dwarfing rootstock genotypes appear to decrease the formation of secondary shoots in the first year of growth from propagation.

Further research will need to elucidate whether the reduced formation of secondary shoots is an important way in which other dwarfing rootstock genotypes reduce total scion growth in the first year from propagation of the composite tree. This is highly important because dwarfing apple rootstocks that increase branching of the scion, such as G.16, may impose scion dwarfing by very different architectural and physiological means to M.9. In future research, however, it is recommended that trees are propagated by grafting a one-year-old scion onto a one-year-old rootstock so that initial growth of the scion develops on a root system that is not disproportionate in starting size to the scion, and the rootstock and scion are the same physiological age. The adoption of a standardised methodology by international researchers to propagate and grow

experimental tree material would allow some comparisons to be made with the architectural modifications imposed on the scion by M.9 in the first year of growth as found in this thesis, and in other important studies where M.9 imposed scion dwarfing in either year one (Hernandez, 2008) or two (Selezynova et al., 2007, 2008).

7.1.2.2 Extension growth of secondary shoots

In addition to decreasing the mean number of secondary axes that formed per scion (Figures 3.7B, 5.13B and 6.3), the M.9 rootstock decreased the final mean total length and node number of the secondary shoots by imposing very small decreases in their mean shoot length and node number (Table 3.9; Figure 4.8A, B; 5.22A, B; Section 6.3.3). However, these effects of M.9 were usually not statistically significant at $P \leq 0.05$ (Table 3.9, 5.22A, B; Section 6.3.3). Although the M.9 rootstock did not decrease the mean number of secondary shoots formed in the experiment described in Chapter 4 (Table 4.2), the total growth of the secondary shoots was reduced by M.9 because of significantly decreased mean length and node number of the secondary shoots (Figure 4.8A, B).

Nodes developed along a secondary shoot can be solely preformed within the axillary bud or formed from a combination of preformed and neoformed nodes (Pratt, 1990). Although 9 to 10 preformed primordia exist in the one-year-old overwintering vegetative bud (Pratt, 1990), it is unclear at exactly what time during the first year from propagation a newly formed axillary vegetative bud on the primary shoot has fully developed 9 to 10 primordia (see Chapter 1, Section 1.5.3). Therefore, for studies in this thesis, it was unknown whether secondary shoots with 10 or less nodes had formed solely from preformed nodes. Clarification of this would be invaluable for further studies.

In each experiment, M.9 typically formed proportionally more secondary spurs (i.e., < 25 mm) and short secondary shoots (i.e., ≥ 25 mm but with ≤ 10 nodes), which may have developed solely from preformed primordia within the vegetative axillary bud on the primary shoot, and presumably these shoot types terminated growth very soon after their initial outgrowth. The M.9 rootstock also decreased the proportion of secondary shoots with more than 10 nodes, or those that presumably grew by producing

neoformed nodes, particularly very long secondary shoots with more than 20 nodes (Figures 3.13B, D, F; 4.9B, D; 5.21A; 6.4B, H). This probably occurred for M.9 because proportionally more secondary shoots terminated their growth earlier in the season when compared with rootstocks of greater vigour, especially by February and March (Figure 3.11, 4.7B, Table 5.1, Section 6.3.2.2). For older trees, dwarfing apple rootstocks also increased the proportion of shoots that terminated growth early compared with more vigorous rootstocks (Swarbrick, 1929; Tubbs, 1951; Avery, 1969), which would decrease node neoformation (Selezynova et al., 2003), therefore explaining why increased proportions of short versus long shoots form for the scion growing on M.9 when compared with rootstock(s) of greater vigour (Avery, 1969; Costes et al., 2001; Selezynova et al., 2008).

The final length of an annual apple shoot is determined by the number of nodes and the length of its internodes, whilst internode length increases with shoot length (Selezynova et al., 2003). Hence, the major determinant of shoot length is node number, with the additive effect that internode length also increases on shoots with more nodes. For both M.9 and MM.106, annual shoots formed on 3-year-old 'Royal Gala' branches had an almost identical relationship between their node number and length (Selezynova et al., 2003). In this thesis, similar results occurred for secondary shoots of 'Royal Gala' newly grafted onto different size-controlling rootstocks (see the slopes of regression lines in Figures 3.13A, C, E; 4.9A, C; 6.4A, C, E, G). Thus, secondary shoots with the same node number had a very similar shoot length regardless of rootstock type. Therefore, the M.9 rootstock did not affect internode length of secondary shoots compared with rootstocks of greater vigour. Differences amongst rootstock types in the mean internode length of the secondary shoots in this study (Tables 3.1 and 4.1) and in others (Selezynova et al., 2003) occurred because of differences in their node number distributions (Figures 3.13B, D, F and 4.9B, D).

7.2 Effect rootstock and root-restriction on scion architecture

Objectives of Chapters 3 and 4 were to understand similarities in architectural modifications imposed on the scion by both the dwarfing apple rootstock and root-restriction.

In both Chapters 3 and 4, root restriction tended to decrease the mean total shoot length of the scion when compared with trees grown without root restriction (Tables 3.2 and 4.1), and this effect was similar to M.9 when compared with rootstock(s) of greater vigour (Tables 3.1 and 4.1). Similar to the root restriction treatment that physically limited the size of the root system, the final size of the M.9 root system was decreased by the end of the first growing season from grafting when compared with rootstocks of greater vigour (Figures 5.29A, B; 6.5A and 6.8A). Therefore, a smaller root system imposed by both treatments was reflected in decreased total shoot extension growth or size of the scion, thereby indicating that a functional-structural relationship existed between the size of the root system and the scion. In addition, this suggests that part of the dwarfing effect imposed on the scion by M.9 may be explained by the development of a smaller root system compared with rootstocks of greater vigour. The possible hormonal basis for the reduced size of the M.9 root system is discussed in Section 7.3.

7.2.1 Growth of the primary shoot

In responses similar to the dwarfing rootstock, root restriction reduced the final mean length and node number of the primary shoot without affecting the mean internode length (Tables 3.1, 3.2 and 4.1). In Chapters 3 and 4, the proportion (%) of primary shoots that had terminated growth was increased by both M.9 and root restriction treatments in April, but increased shoot termination caused by root restriction was typically small relative to M.9. In the second spring from grafting, M.9 significantly increased the number of axillary buds on the primary shoot that were floral when compared with MM.106, whereas restricted root systems did not when compared with unrestricted root systems (Table 4.3). In Chapter 3, flowering data were not collected meaning that differences between M.9 and root-restriction could not be ascertained.

In Chapters 3 and 4, root restriction reduced the leaf area of the primary shoot because of fewer and smaller leaves. A similar result occurred for M.9 in Chapter 3, but not in Chapter 4, where despite having fewer neoformed nodes than MM.106, the primary shoot on M.9 had a similar final leaf area because of larger leaves compared with MM.106 (Tables 3.1, 3.2 and 4.1). Avery (1969) also reported that the ‘Worcester Pearmain’ scion on M.9 had increased leaf size compared with the same scion on the M.16 rootstock. In contrast, root restriction reduced the size of leaves for field-grown

apple (Webster et al., 2000) and other crops including peach (Rieger and Marra, 1994), tomato (Hurley and Rowarth, 1999) and pepper (Ismail and Davies, 1998). For apple, Atkinson et al., (2000) reported that root restriction of M.9 and MM.106 rootstocks also reduced the final fruit diameter of 'Queen Cox' compared with non-restricted root systems of these same rootstocks. Collectively, the different effects on the size of leaves (Webster et al., 2000; Table 4.1) and fruit (Atkinson et al., 2000) may indicate some physiological mechanisms that reduced scion vigour were different between M.9 and root restriction.

7.2.2 Growth of the secondary shoots

In Chapter 3, the M.9 rootstock and root restriction reduced the final mean number of secondary axes formed on the primary shoot (Figure 3.7A, B). However, root restriction of MM.106 and M.793 caused much greater reductions in total growth of the secondary shoots compared with root restriction of M.9 (Table 3.8) because the mean number of secondary shoots formed per scion was reduced markedly more for root restricted MM.106 and M.793 than for M.9 (Figures 3.6 and 3.7A, B). Data from Chapters 5 and 6 (i.e., Figures 5.29A, B; 6.5A; 6.8A) indicated that these interactions in Chapter 3 (i.e., Figures 3.6 and 3.7A, B) probably resulted because the root system of MM.106 and M.793 was larger and therefore was more confined in the small (8 L) root volume than M.9.

Although M.9 and root restriction both decreased the formation of secondary axes in Chapter 3, an important difference was that decreased axillary bud outgrowth imposed by M.9 was fully reversed with exogenous BAP because the BAP-treated scion on M.9 formed more axillary axes (i.e., total of trace spurs, secondary spurs, secondary shoots and tertiary spurs) compared with the BAP-treated scion on MM.106 and M.793 (Figure 3.7B, D). This was in contrast to the root volume x BAP interaction where trees grown in the 8 L root volume and treated with BAP produced fewer axillary axes per scion compared with BAP-treated trees grown in the 45 L root volume (Figure 3.8).

For field grown trees in Chapter 4, in the fifth growing season from grafting (i.e., 2008-2009) it was observed that the scion on root restricted MM.106 was generally of similar size to the scion growing on unrestricted M.9 root systems, but it was very apparent that the scion on root restricted MM.106 had developed large areas of bare wood (i.e.,

extinct axillary buds) on many of the axillary branches positioned in well-lit areas of the canopy, and this was in contrast to the scion on M.9 that developed very little bare wood (data not shown). This observation would be worthy of further investigation and may indicate that, with time, root restriction also reduces the size of the scion through increased bud extinction. Collectively, differences in the rootstock x BAP and root restriction x BAP interactions (Figures 3.7B, D and 3.8) combined with likely differences in bud extinction on the scion as the tree ages, suggests that some physiological processes modified by M.9 and root restriction are very different.

Despite the differences described above, there were some important similarities in the way M.9 and root restriction affected growth of the secondary shoots. Both M.9 and root restriction did not affect internode length of the secondary shoots. For example, secondary shoots with the same node number were of similar length, and this relationship was the same regardless of rootstock type or root restriction treatment (Figures 3.13A, C, E and 4.9A, C). Similar to M.9, root restriction of MM.106 decreased the proportion of long secondary shoots that developed, in particular, very long secondary shoots with more than 20 nodes (Figures 3.13B, D and 4.9D). In Chapter 4, this contributed to decreased final mean length and node number of the secondary shoots for both M.9 and root restricted MM.106 (Figure 4.8A, B), presumably because greater proportions of shoots had terminated growth by March (Figure 4.7B), therefore decreasing node neoformation.

However, restriction of the M.793 root system to 8 L did not affect the mean length and node number of the secondary shoots compared with M.793 grown in the 45 L root volume (Chapter 3, Table 3.9). Rather, total growth of the secondary shoots was decreased for the scion growing on root restricted M.793 solely because fewer secondary shoots formed (Figure 3.6). Therefore, decreased extension growth of the secondary shoots was not a consistent effect of root restriction, but depended on rootstock type. Given that exogenous GA_{4+7} stimulated the extension growth of secondary shoots (Figure 5.27), the ability of M.793 grown in the 8 L root volume to produce secondary shoots of similar mean length and node number to M.793 grown in the 45 L root volume may indicate that root restriction of M.793 did not affect the synthesis of root-produced gibberellins. Analysis of endogenous gibberellins would be necessary to ascertain this.

7.3 Endogenous hormonal control of the initial modifications in scion architecture on the M.9 rootstock

Important objectives of this thesis were to elucidate the time from grafting when rootstock-induced dwarfing of the scion first occurred on M.9, and how scion architecture was initially modified compared with rootstocks of greater vigour (see Section 7.1). With this knowledge, exogenous growth regulators were used to determine the likely roles that important endogenous hormones may have in the regulation of the initial architectural modifications imposed on the scion by M.9. Collectively, this knowledge provided the justification for quantifying hormones that may cause the initial changes in scion architecture on a dwarfing apple rootstock.

7.3.1 Shoot/root/shoot signalling of IAA, cytokinin and gibberellin

7.3.1.1 Indole-3-acetic acid

Endogenous control of scion vigour by dwarfing apple rootstocks is most convincingly explained by hormonal signalling between endogenous IAA and cytokinin. Lockard and Schneider (1981) hypothesised that dwarfing apple rootstocks reduced the basipetal transport of IAA to the root system, which in turn decreased the synthesis of root-produced cytokinins transported to the scion, consequently limiting shoot extension growth of the scion. In support of this hypothesis, the stem tissue of the dwarfing apple rootstock reduced the basipetal transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj, 1996; Kamboj et al., 1997) and had a decreased concentration of endogenous IAA in its cambial sap (Michalczuk, 2002). In addition, composite trees of 'Fiesta' grafted onto M.9 tended to transport less ^{3}H to the root system compared with rootstocks of greater vigour when ^{3}H-IAA was applied to a mature basal leaf on the scion (Kamboj, 1996).

If the basipetal transport of IAA from scion to root is an important endogenous signal regulating scion vigour, as suggested by the above studies, then applying the auxin transport inhibitor 'NPA' to the rootstock stem of composite apple trees newly grafted onto vigorous rootstocks should, theoretically, impose scion dwarfing. In addition, the modifications in scion architecture on those vigorous rootstocks should be very similar to those initially imposed on the scion by M.9 in the first year of growth from grafting.

In Chapter 5, the application of NPA to the rootstock stem of MM.106, M.793 and 'Royal Gala' markedly decreased the final mean total length and node number of the scion, and the architectural modifications that caused these reductions in scion vigour were generally similar to those that occurred for untreated 'Royal Gala' trees growing on M.9 (Figures 5.27 and 5.28). In particular, NPA caused the SAM on the primary shoot to slow, and/or, terminate its growth temporarily (Section 5.3.2.6), and decreased the formation of secondary axes on the primary shoot (Figures 5.27 and 5.28). In addition, both M.9 and the NPA treatment significantly decreased the mean dry mass and mean total length of the root system at the end of the first year of growth from grafting (Figure 5.29). Therefore, NPA treatment appeared to promote very similar changes in both scion architecture and root growth to that imposed by the M.9 rootstock. This new information strengthens the findings of previous studies that showed the stem tissue of M.9 decreased the basipetal transport of radio-labelled IAA (Soumelidou et al., 1994a; Kamboj, 1996; Kamboj et al., 1997), but provided limited information on what aspects of scion architecture the basipetal IAA signal may affect, and the time from grafting of the composite tree when scion and root growth were likely to be modified on a dwarfing rootstock.

An objective of Chapter 6 was to elucidate the precise time during the first year of growth after grafting when root growth was first decreased on M.9 compared with rootstocks of greater vigour. A very small decrease in the dry mass of the M.9 root system had occurred in December when compared with the 'Royal Gala' rootstock control (Figure 6.9A). By January, however, the mean dry mass of roots was the same for M.9 and the 'Royal Gala' rootstock control (i.e., both 2.25 g). Therefore, from December to January the dry mass gain of the M.9 root system was greater than the 'Royal Gala' rootstock control (Figure 6.9A), but there were trends that from February onwards root growth was decreased by M.9 compared with the 'Royal Gala' rootstock (Figure 6.9A). In addition, this initial decrease in dry mass gain of the M.9 root system by February was preceded by a large reduction in the basipetal transport of diffusible IAA from the primary shoot apex during the preceding month (Figure 6.11A). Given that NPA decreased the final mean dry mass of apple roots (Figure 5.29A), these data may indicate that the M.9 rootstock was not sufficiently impairing IAA transport to reduce root growth prior to January but, thereafter, root growth was decreased as the basipetal transport of IAA was progressively decreased by the M.9 rootstock.

To ascertain whether IAA is the correlative signal that first decreases root growth of M.9, further studies will need to use radio-labelled IAA to demonstrate that decreased IAA transport within the rootstock stem to the M.9 root system precedes the initial reduction in its root growth during the summer. Reduced basipetal IAA transport within the rootstock stem to the root system should also be correlated with decreased root growth of M.9, especially from March to April, when both root and scion dry mass gain were greatly decreased by M.9 when compared with rootstocks of greater vigour. Although Kamboj (1996) reported that composite trees of 'Fiesta' newly grafted onto M.9 tended to transport less ^{3}H to the root system in summer than vigorous rootstocks when ^{3}H-IAA was applied to a mature basal leaf on the scion, future work should ascertain what proportion of ^{3}H reaching the M.9 root system is still associated with IAA, and how rootstocks of different vigour may metabolise ^{3}H-IAA differently over the growing season.

Interestingly, the mechanisms by which the rootstock bark of M.9 acts to decrease the basipetal transport of IAA to the root still remains largely unknown. Given that application of NPA to the stem tissue of vigorous apple rootstocks markedly decreased the growth of both the scion and the root, and the general similarity of these growth modifications to composite trees growing on M.9, it is proposed that dwarfing gene(s) that regulate rootstock-induced scion dwarfing are highly likely to control physiological processes involving the transport and metabolism of IAA, particularly within the stem and roots of the dwarfing rootstock. Putative physiological mechanisms within these tissues that modify auxin transport could include increased IAA oxidase activity (Gur and Samish, 1968), increased concentrations of growth inhibiting phenols and lower concentrations of growth promoting phenols (Martin and Stahly, 1967) that may act to enhance or suppress the oxidative decarboxylation of IAA, respectively, (Lockard and Schneider, 1981) and reduced active polar IAA transport out of the cell by efflux carrier proteins (Soumelidou et al., 1994a; Kamboj, 1996).

With regard to the latter, *pin-formed (PIN1)* proteins are putative auxin efflux carriers located on the plasma membrane at the basal end of the cell that facilitate IAA efflux from the cell in *Arabidopsis* stems (Estelle, 2001). Plants may regulate the basipetal transport of IAA by modifying the number, activity and location of efflux carriers at the plasma membrane during growth and development, and there is increasing evidence that

some of these modifications interact with environmental signals (Feraru and Friml, 2008). A similar mechanism may well occur in the bark of the M.9 rootstock and would be worthy of further investigation. The potential for the function of IAA efflux carriers, and therefore IAA transport, to be modified differently in response to growing environment may also explain why the dwarfing apple rootstock does not affect the growth of the primary shoot in the first year from grafting in some growing regions of New Zealand (i.e., Selezynova et al., 2007, 2008).

7.3.1.2 Indole-3-acetic acid and cytokinin

Lockard and Schneider (1981) hypothesised that reduced basipetal transport of IAA from scion to root decreased root growth and the consequent amount of cytokinin transported to the scion in the xylem vasculature, thereby limiting shoot extension growth of the scion. Kamboj et al., (1999) also reported that M.9 decreased the concentration of endogenous Z plus ZR in the xylem sap of the 'Fiesta' scion when compared with rootstocks of greater vigour, and that this might explain increased rates of shoot growth for the scion propagated on vigorous rootstocks. Therefore, an objective of Chapters 3 and 5 was to determine what aspects of scion dwarfing could be reversed on the M.9 rootstock by repeatedly applying BAP to the scion.

The application of BAP to 'Royal Gala' scions did not increase the mean length or node number of the primary or secondary shoots. Rather, exogenous cytokinin stimulated the initial outgrowth of axillary buds on the primary shoot, hence increasing the formation of secondary axes (i.e., secondary spurs plus shoots). These general effects of exogenous cytokinin on the growth of 'Royal Gala' apple scions were also reported for a number of different scion cultivars in other previous studies (Williams and Stahly, 1968; Kender and Carpenter, 1972; Forshey, 1982; Elfving, 1985; Miller and Eldridge, 1986; Popenoe and Barritt, 1988; Volz et al., 1994; Wertheim and Estabrooks, 1994). Collectively, these results strongly indicate that endogenous cytokinins primarily regulate the outgrowth of axillary buds, and that reduced concentrations of endogenous cytokinin in the xylem sap of the scion growing on the M.9 rootstock (Kamboj et al., 1999) would cause a scion phenotype with fewer secondary axes by the end of the first year of growth from grafting (Figure 5.27).

Indeed, an important way in which the M.9 rootstock decreased total shoot growth of the 'Royal Gala' scion compared with the 'Royal Gala' rootstock control was to reduce the formation of secondary axes on the primary shoot, and this effect of M.9 was reversible with exogenous BAP (Figure 5.27). Similarly, application of the auxin transport inhibitor 'NPA' to the rootstock stem of vigorous rootstocks decreased the formation of secondary axes on the primary shoot, and like M.9, the formation of secondary axes on the primary shoot was reinstated with exogenous BAP (Figure 5.28). Similarities between the rootstock x BAP and NPA x BAP interactions strongly indicates that shoot-derived IAA and root-produced cytokinin interact to control the initial outgrowth of axillary buds on the primary shoot of composite apple trees in their first year of growth from grafting (Figures 5.27 and 5.28).

Therefore, a shoot/root/shoot signalling mechanism may exist for dwarfing apple rootstocks whereby the rootstock stem decreases the amount of IAA transported to the root (Lockard and Schneider, 1981; Kamboj, 1996) that in turn may decrease root growth (Figure 5.29) and the consequent amount of cytokinin transported to the scion (Lockard and Schneider, 1981; Kamboj et al., 1999). The rootstock x BAP and NPA x BAP interactions add new architectural information to this hypothesis suggesting that decreased transport of cytokinin from root to scion by the M.9 rootstock in the first year of growth from grafting is likely to reduce the number of axillary buds on the primary shoot that outgrow and form secondary axes, particularly secondary shoots (Figure 5.27).

To determine the above, further research would be required to correlate decreased shoot/root/shoot transport of endogenous IAA and cytokinin with decreased formation of secondary axes on the primary shoot. As previously highlighted, to demonstrate that interactions between IAA and cytokinin are causal in axillary bud outgrowth an understanding of the environmental prerequisites that induce axillary bud break on the primary shoot should be elucidated so that the timing of branching could be manipulated in a controlled growing environment. This would enable the amount of scion-produced IAA transported to the root system and the amount of root-produced cytokinin transported to the scion to be quantified prior to the first occurrence of axillary bud outgrowth on the primary shoot.

The presence of both IPA and 2iP in the xylem sap of M.9 (see Chapter 6) implies that future physiological studies into the hormonal control of branching will need to consider these cytokinin forms, particularly because both cytokinins could be converted to bioactive Z in the scion. In addition, the presence of a novel isopentenyl-type compound (see Chapter 6) suggests there may be other potentially important cytokinin forms that are present in xylem sap of apple, but are yet to be identified. In particular, the hydroxylated derivatives of benzyladenine (topolins or aromatic cytokinins) have been identified in many plant species and the meta-topolin form has high bioactivity (Mok et al., 2005). As concluded in Chapter 6, future studies should also elucidate whether root/shoot/root signalling mechanisms regulating scion branching in apple also involves root-produced branching inhibitors, including the recently identified strigolactones (Gomez-Roldan et al., 2008).

As found in Chapter 5, gibberellins may also play a role in the branching phenomenon because exogenous GA_{4+7} increased the formation of secondary axes slightly for the scion growing on M.9 (Figure 5.13B). However, it was observed that exogenous BAP stimulated very few axillary buds to break on scions whose shoots had ceased growing; therefore it is highly probable that exogenous gibberellin increased branching indirectly by prolonging the duration for which the SAM on the primary shoot grew. Further research is required to elucidate this.

7.3.1.3 Indole-3-acetic acid and gibberellins

The inability of BAP applied repeatedly to the 'Royal Gala' scion growing on M.9 to increase the mean length or node number of both the primary or secondary shoots strongly indicates that other endogenous hormones were necessary to stimulate shoot growth. Lockard and Schneider (1981) concluded that there was little evidence to support the role of endogenous gibberellins in rootstock-induced scion dwarfing. Similarly, a recent review into the physiological causes of rootstock-induced scion dwarfing reported that gibberellins might not be important in the dwarfing mechanism (Webster, 2004). However, Luckwill and Silva (1979) reported that exogenous GA_3 stimulated node production by the apple shoot apical meristem and increased the proportion of annual shoots that were actively growing late in the season. In contrast, the dwarfing apple rootstock is widely reported to increase the proportion of SAMs on

the scion that terminate growth early (Swarbrick, 1929; Colby, 1935; Tubbs, 1951; Avery, 1969; Robitaille and Carlson, 1976).

Therefore, in Chapter 1 it was hypothesised that earlier termination of SAMs for a scion growing on a dwarfing apple rootstock may result because reduced amounts of root-produced gibberellin were transported to the scion. The literature for apple also indicated that the basipetal transport of IAA from scion to root was important for the growth of SAMs. For example, the application of the auxin transport inhibitor 'TIBA' to the root/shoot transition region of 'Antonovka' apple seedlings reduced the basipetal transport of ^{14}C-IAA and caused the eventual termination of the SAM on the primary shoot (Grochowska et al., 1994). Therefore, in Chapter 1 it was hypothesised that the decreased basipetal transport of IAA may reduce the amount of root-produced gibberellin transported to the scion, thereby causing early shoot termination of the scion growing on M.9. Subsequently, an objective of Chapter 5 was to elucidate whether reduced basipetal transport of IAA from scion to root was important for meristematic activity of apple shoots, and whether the basipetal transport of IAA interacted with gibberellin to control this. Gibberellin applied to the scion on M.9 was also of particular interest to determine whether SAMs could be prevented from terminating growth early.

Reducing the basipetal transport of IAA to the root by repeatedly applying NPA to the rootstock stem decreased cumulative node production of the primary shoot compared with untreated trees (Figure 5.2). However, gibberellin applied repeatedly to the scion over the growing season fully reversed these effects of NPA on cumulative node production (Figure 5.2). Similarly, the cumulative node number of the primary shoot was reduced by M.9, and this decrease in growth was reversed with exogenous gibberellin (Figure 5.3). In addition, GA_{4+7} markedly decreased the proportion of secondary shoots that terminated early for M.9 (Table 5.1). The rootstock x GA_{4+7} interaction for the cumulative growth of the 'Royal Gala' primary shoot reported in this thesis (Figure 5.3) was recently reconfirmed in a subsequent study using composite 'Royal Gala' apple trees newly grafted onto M.9 and a 'Royal Gala' rootstock control (Hernandez, 2008). Collectively, these data indicate that a shoot/root/shoot signalling mechanism may exist for M.9 whereby decreased basipetal transport of IAA from scion to root reduces the amount of root-produced gibberellin transported to the scion, thereby decreasing node neoformation by increasing the proportion of SAMs on the scion that

terminate growth early. These data, therefore, suggest that endogenous gibberellins are indeed very important in rootstock-induced scion dwarfing and, like cytokinin, appear to interact with the basipetal transport of IAA.

As hypothesised in Chapters 5 and 6, the stem tissue of the dwarfing rootstock may begin to reduce polar auxin transport from scion to root in summer due to lower daily light integrals and decreasing temperature. Reduced basipetal transport of IAA may consequently decrease the amount of inactive root-produced gibberellin transported to the scion in the xylem vasculature (Figure 6.12). Reduced transport of root-produced gibberellin forms, particularly important precursors to GA_1 (GA_{19}, GA_{20}, GA_{53} and GA_{44}), may initially reduce the biosynthesis of bioactive gibberellin (GA_1) at the shoot apex. Lack of bioactive GA_1 may limit IAA and gibberellin controlled initiation of leaf primordia in the peripheral zone of the SAM (Took and Battey, 2003; Shani et al., 2006), thereby decreasing the emergence of new leaves as potential sites for IAA synthesis. This may consequently decrease IAA synthesised at the shoot apex, therefore, reducing the amount of IAA available for acropetal transport from young leaves to the above peripheral meristematic zone (Vogler and Kuhlemeier, 2003). Low concentrations of IAA at the shoot apex may limit GA 3-oxidase activity, thus preventing the conversion of GA_{20} to GA_1 (Ross et al., 2000), whilst low concentrations of bioactive GA_1 may prevent gibberellin mediated destruction of MdDELLAs (Foster et al., 2007). This proposed mechanism for the endogenous hormonal control of SAM activity for composite trees growing on M.9 and other dwarfing apple rootstock genotypes would be worthy of further investigation.

It is also suggested that a balance of root-produced gibberellin (promoter) and abscisic acid (ABA) (inhibitor) may more satisfactorily explain earlier shoot termination of the scion growing on M.9. For example, the injection ABA into the xylem sap of composite trees of ‘Red Prince Delicious’ grown on M.9, M.7 and MM.111 caused the primary shoot to terminate, and composite trees on the more dwarfing rootstocks terminated shoot growth sooner after ABA treatment (Robitaille and Carlson, 1971). In contrast, injection of GA_3 into the xylem sap increased the extension growth of the primary shoot markedly more as rootstock vigour decreased (Robitaille and Carlson, 1971). Furthermore, composite apple trees on dwarfing rootstocks contained higher endogenous concentrations of ABA in the xylem sap compared with rootstocks of

greater vigour (Kamboj, 1996; East Malling, 2005). Therefore, the M.9 rootstock may reduce the synthesis of root-produced gibberellins while increasing the synthesis of root-produced ABA, hence increasing the ratio of inhibitor to promoter, which could explain earlier shoot termination for the scion growing on the dwarfing apple rootstock. It would also be of physiological interest to elucidate whether the synthesis of root-produced ABA is also regulated by the basipetal transport of IAA from scion to root.

For *Arabidopsis*, IAA and gibberellin interact to control root elongation. The gibberellin deficient mutant (*ga1-3)* had shorter primary roots than wild types, although mean root length was similar between these treatments when exogenous GA_3 was applied to the primary root of *ga1-3* indicating that gibberellin controlled root elongation (Fu and Harberd, 2003). However, GA_3 applied to roots of *ga1-3* did not increase root elongation when the shoot apex was removed. Therefore, shoot derived IAA may regulate root growth from a distance by modulating gibberellin controlled growth responses in the root, particularly gibberellin mediated destabilisation of DELLA growth repressor proteins (Fu and Harberd, 2003).

These results for *Arabidopsis* may also have relevance for the elongation of apple roots, particularly given that reduced IAA transport from shoot to root might decrease the biosynthesis of root-produced gibberellins (Figure 5.2), which could prevent gibberellin mediated destruction of DELLA proteins within the root, thereby reducing root elongation. In contrast, a reduction in the basipetal transport of IAA from scion to root may directly modify root architecture by decreasing root initiation and therefore the number of roots formed. Therefore, the role of endogenous hormones in modifying root architecture is worthy of further investigation because, as found in this thesis for scion architecture, it is highly probable that IAA may control some aspects of root architecture through interactions with other endogenous hormonal signals.

7.3.1.4 Indole-3-acetic acid as a primary coordinating signal

The probable decrease in the basipetal transport of IAA from scion to root for the M.9 rootstock or for NPA-treated rootstocks may have reduced the synthesis of root-produced gibberellin, thereby decreasing meristematic activity of the primary and secondary shoots (Table 5.1; Figures 5.27 and 5.28) and decreased the synthesis of root-

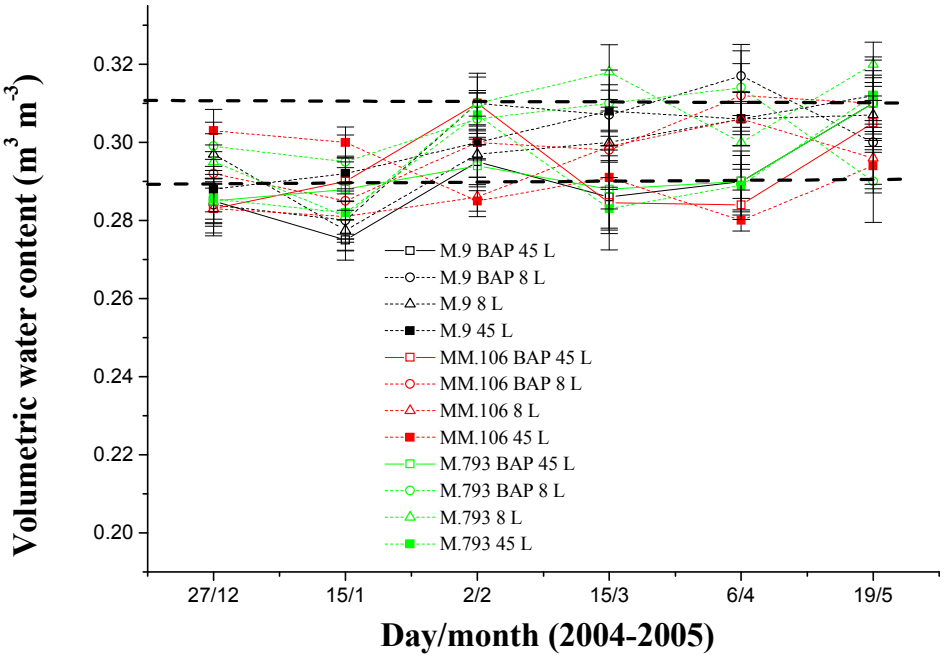
produced cytokinin, therefore reducing axillary bud outgrowth on the primary shoot (Figures 5.27 and 5.28). Hence, the basipetal IAA signal interacted with both gibberellin and cytokinin and appeared to act as the primary coordinating signal regulating scion architecture and vigour for both the NPA treatment and for M.9. This further indicates that major dwarfing gene(s) regulating rootstock-induced scion dwarfing are highly likely to control physiological processes involving the transport and metabolism of IAA within the stem and root tissue of the rootstock.

In Chapter 5, the scion on M.9 treated with both BAP and GA_{4+7} developed very similar total growth to that on MM.106, but was still markedly smaller than the BAP x GA_{4+7}-treated scion on M.793 and 'Royal Gala' (Figure 5.27). In a similar manner to BAP x GA_{4+7} treatment of trees on M.9, BAP x GA_{4+7} applied to the scion on NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' increased total growth of the scion by increasing the mean shoot length and node number of the primary and secondary shoots, and the mean number of secondary and tertiary shoots that formed compared with the untreated scion on these same NPA-treated rootstocks. However, BAP x GA_{4+7} applied to the scion on NPA-treated rootstocks of MM.106, M.793 and 'Royal Gala' stimulated markedly less total shoot extension growth when compared with the BAP x GA_{4+7}-treated scion on the same rootstock type that was not treated with NPA (Figures 5.27 and 5.28). Hence, BAP x GA_{4+7} could not fully reverse reductions in total scion growth whilst IAA transport from shoot to root was impaired by the NPA treatment and possibly M.9.

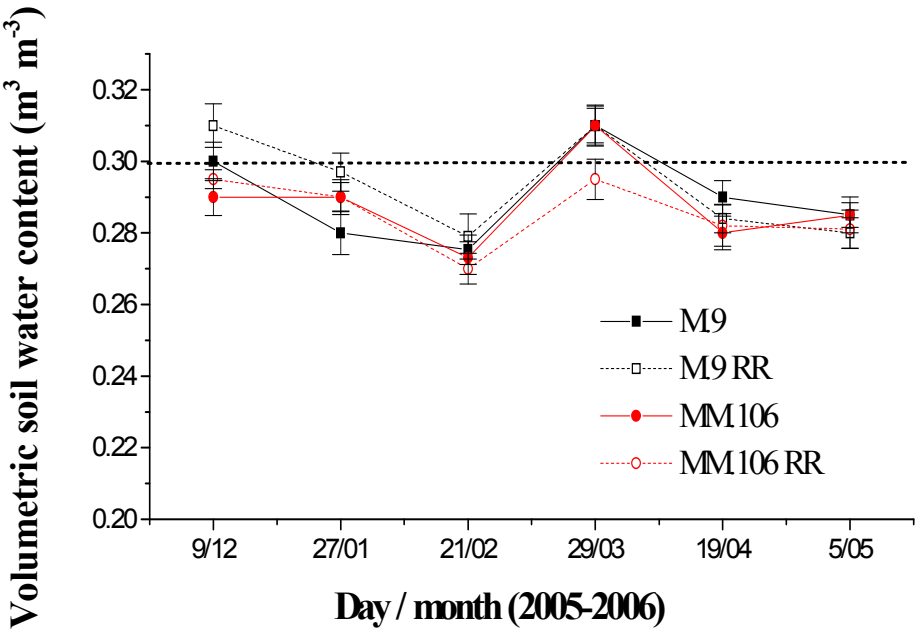
These results also demonstrate the central importance of the basipetal IAA signal in regulating rootstock-induced scion dwarfing. In future work, it would be of interest to determine whether applying exogenous IAA alone to the root system of M.9 or NPA-treated rootstocks would be sufficient to overcome dwarfing of the scion by these treatments.

8. Appendices

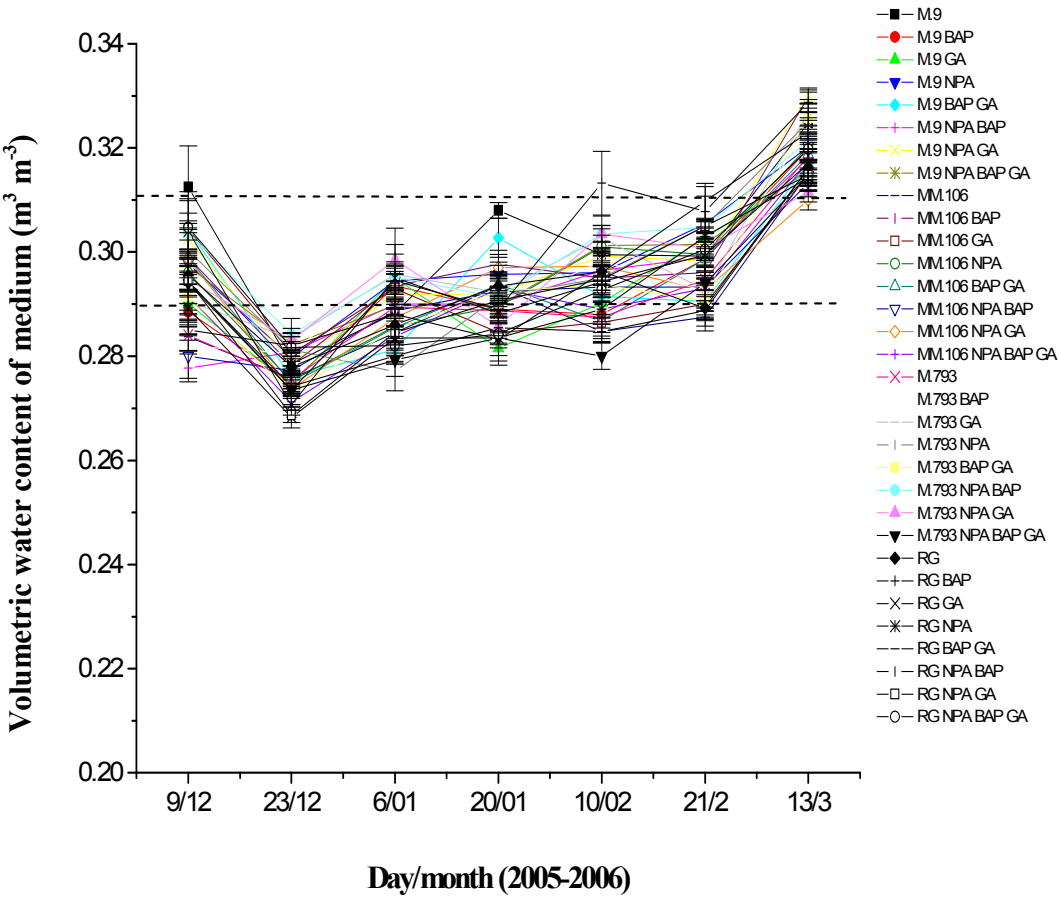
Appendix 1. The irrigation schedule maintained volumetric water content of the growing medium close to field capacity (0.30 m^3 m^{-3}) over the 2004-2005 growing season for composite 'Royal Gala' apple trees grafted onto M.9, MM.106 and M.793 rootstocks. Trees were grown in two root volumes (8 L and 45 L) and treated with or without benzylaminopurine (BAP). Horizontal dotted lines represent field capacity of the medium (0.30 ± 0.01 m^3 m^{-3}). Vertical bars are ± SEM.



Appendix 2. The irrigation schedule maintained soil volumetric water content close to field capacity (0.30 m^3 m^{-3}) over the 2004-2005 growing season for 'Royal Gala' apple trees grafted onto M.9 and MM.106 rootstocks grown with or without root restriction (RR). The horizontal dotted line indicates field capacity. Vertical bars are ± SEM.



Appendix 3. The irrigation schedule maintained volumetric water content of the growing medium close to field capacity over the growing season for composite 'Royal Gala' apple trees grafted onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (RG) and treated with or without benzylaminopurine (BAP), GA_{4+7} (GA) and 1-N-naphthylphthalamic acid (NPA). Horizontal dotted lines represent field capacity of the medium (0.30 ± 0.01 m^3 m^{-3}). Vertical bars represent ± SEM.



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