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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

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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 (*DWI*) 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 (\pm gibberellins (GA₄₊₇), \pm benzylaminopurine (BAP)) or an auxin transport inhibitor (\pm 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., \geq 25 mm but with \leq 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 GA₄₊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 GA₄₊₇ (compare C and E or G and I for M.9 or NPA, respectively).

Sequential applications of BAP followed by GA₄₊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 GA₄₊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 GA₄₊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 GA₄₊₇-treated scion on M.9 was still markedly smaller than the BAP x GA₄₊₇-treated scion on M.793 or ‘Royal Gala’ (Figure E). Similarly, BAP x GA₄₊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 GA₄₊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 GA₄₊₇-treated scion on the same rootstock type that was not treated with NPA (compare Figures E and I). Hence, BAP x GA₄₊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 GA₄₊₇-treated scion on M.9 was smaller than the BAP x GA₄₊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 GA₁₉ 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.

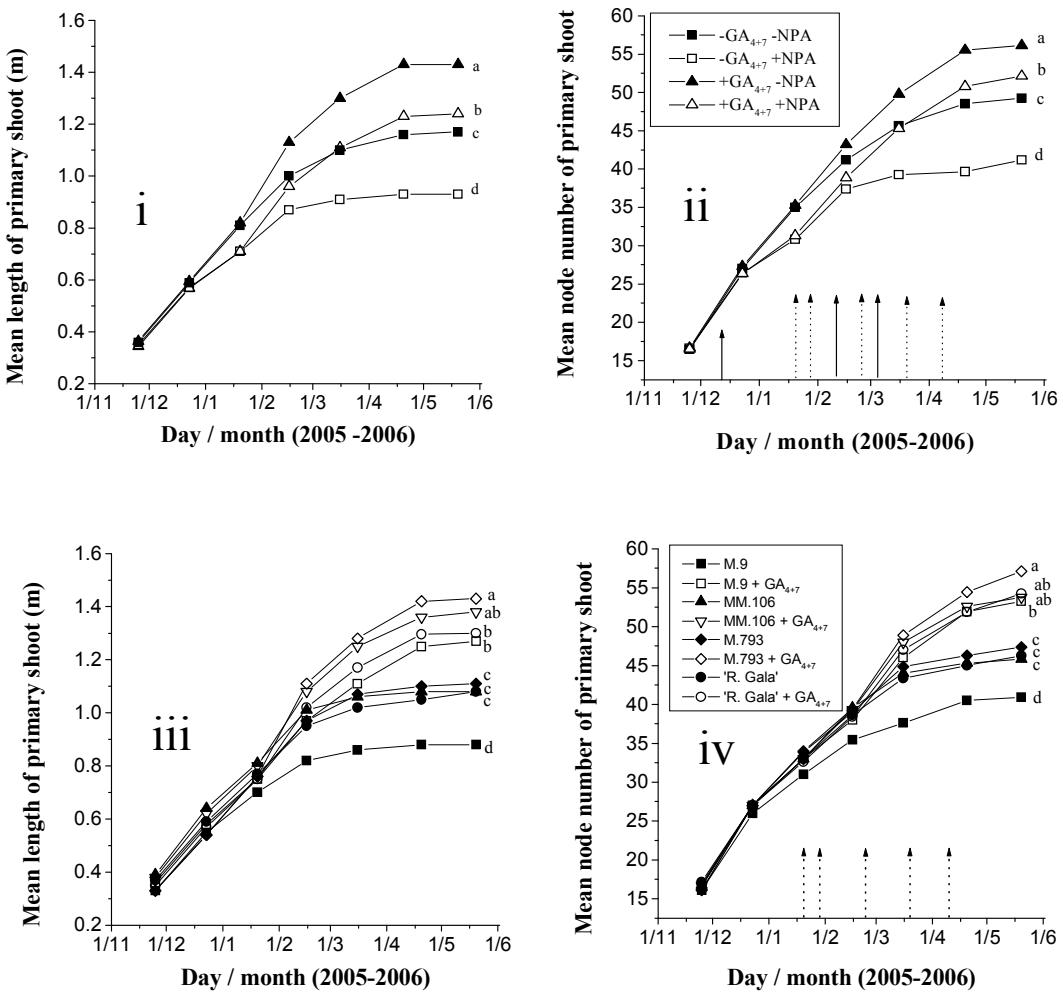


Figure A. Gibberellin (GA₄₊₇) x 1-N-naphthylphthalamic acid (NPA) (i and ii) and rootstock x GA₄₊₇ interactions (iii and iv) for the mean cumulative length and node number of 'Royal Gala' primary shoots during their first growing season from grafting. Arrows on ii or iv with a dotted line denotes the timing of GA₄₊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.

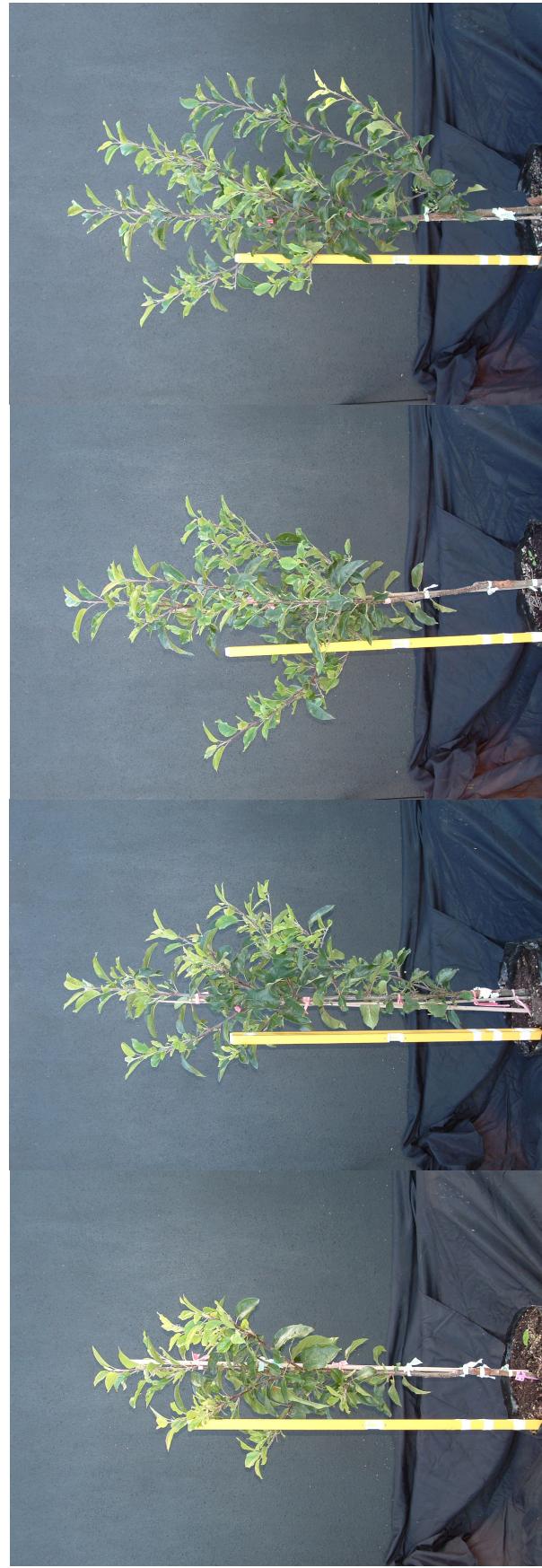


Figure C. Effect of benzylaminopurine (BAP) applied repeatedly to 'Royal Gala' apple scions on scion architecture by the end of the first growing season after grafting the scion onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (left to right, respectively). Yellow rule is 1 m. **Decreased branching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

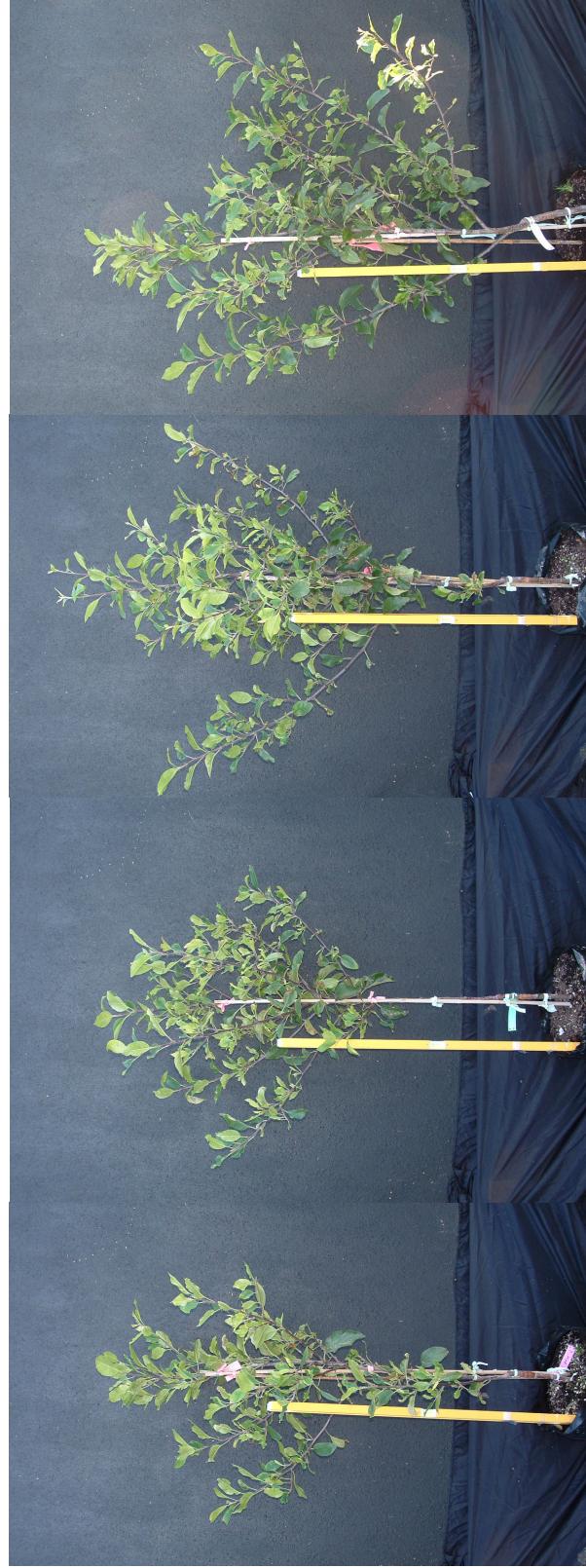


Figure E. Effect of sequential applications of BAP followed by GA₄₊₇ on scion architecture of 'Royal Gala' apple scions by the end of the first growing season after grafting the scion onto rootstocks of M.9, MM.106, M.793 and 'Royal Gala' (left to right, respectively). Yellow rule is 1 m. Applying cytokinin 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 GA₄₊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.

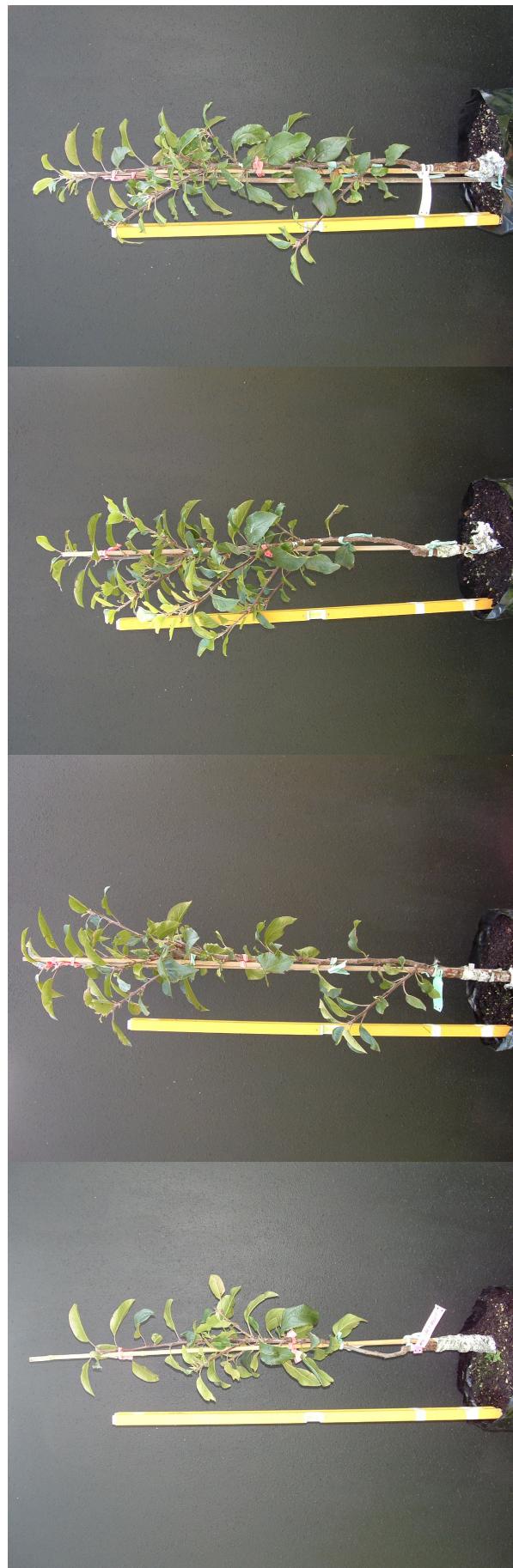


Figure G. Effect of 1-N-naphthylphthalamic acid (NPA) applied to M.9, MM.106, M.793 and 'Royal Gala' rootstocks (left to right, respectively) plus exogenous benzylaminopurine (BAP) applied to the scion on the architecture of 'Royal Gala' apple scions by the end of their first growing season from grafting. 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

| | |
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| 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 |
| [² H 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 |
| [² H ₅]IAA | Pentodeuterium indole-3-acetic acid |
| IAA-Me | Indole-3-acetic acid methyl ester |
| [² H ₅]IAA-MeTMSi | Pentodeuterium indole-3-acetic acid methyl ester trimethylsilyl ether |
| IAA-MeTMSi | Indole-3-acetic acid methyl ester trimethylsilyl ether |
| 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 |