STUDIES IN THE PROMOTION OF PRECOCITY IN 'DOYENNE DU COMICE' PEAR.

A thesis
submitted in partial fulfilment
of the requirements for
the degree
of
Master of Horticultural Science
at
Massey University
New Zealand

By
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Massey University
1993

Abstract of a thesis submitted in partial fulfilment of the requirement for the Degree of Master of Horticultural Science

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Flower evocation is the process, generally hormonally controlled, that occurs before the vegetative bud apex changes from the differentiation of leaves to the differentiation of floral structures, and at that stage the bud has begun the reproductive cycle.

Four treatments were chosen that had been shown in the literature to stimulate flower evocation and differentiation in pipfruit, and were applied in an experiment on 'Doyenne du Comice' pear trees. These treatments were applied to experimental potted trees during the 1990 - 1991 growing season, and consisted of the application of one of the following: nitrogen fertiliser in the form of ammonium sulphate, subtoxic levels of simazine herbicide, plant growth regulator paclobutrazol or a period of regulated deficit irrigation (R.D.I.). A Control treatment was also monitored

The flower clusters were monitored in the spring of 1991 and all treatments had increased flower clusters per centimetre of wood compared to the Control, however only the Paclobutrazol and the R.D.I. treatments increased flower clusters significantly ($P < 0.01$). Trunk diameter and shoot extension growth were both reduced although only the former was significantly reduced by the Paclobutrazol treatment ($P < 0.01$).

The total free nitrogen levels were monitored in the leaves and the buds of the experimental 'Doyenne du Comice' at various harvest dates during the season, which included assessment of ammonium, nitrate, arginine and total amino acids. There were no clear seasonal trends among the treatments in the levels of any individual nitrogenous components or the total free nitrogen levels.

The R.D.I. treatment reduced the photosynthetic rate during its application period to a maximum significance of $P < 0.01$ just prior to the reinstatement of full irrigation. The water deficit imposed significantly reduced the xylem water potential for a period of 50 days although no statistically significant differences in water content of the growing medium was demonstrated.

Examination of the bud apex with a Scanning Electron Microscope (SEM) during the development of floral structures was made during the season. This linked with a defoliation study done on spur buds on mature trees in an orchard near Wanganui which showed that defoliation before 4.12.90 significantly reduced bloom and defoliation after 11.2.91 had no effect. Evocation occurred between 14.12.90 and 8.1.91 as shown by the SEM. Fruitlet retention was also significantly affected by defoliation and the presence or absence of the bourse shoot.

The ability of the spur bud to produce flowers depends on its position in the canopy of a mature 'Doyenne du Comice' tree and a separate study showed that positions in the tree that had low PPFD had reduced flower numbers. A further study showed tying branch angle down from the vertical during winter is beneficial in terms of increased flower formation and reduced vegetative growth.

ACKNOWLEDGEMENTS

To my supervisors, Dr David Wood and Dr Stephen Lawes, for the much appreciated opportunity to study for this masterate in fruit production and also for the helpful assistance and guidance you provided in order to make this project successful. Often relevant references and appropriate comments really helped to focus my direction at the most critical periods.

To my Family and Friends, who helped by encouraging me to persist when all I wanted to do was to give up. Thankyou for all your positive comments and moral support (congratulations Phil.!).

To my Lord and Saviour, Jesus Christ; it sure has been a pleasure serving you. Your creation is full of wonderful and 'marvellous things'. I'm greatful for this opportunity to explore what you planned so long ago. There is no doubt that there is more to it than meets the eye.

To all the Postgraduates and the staff members in the Plant Science department who helped by contributing useful suggestions and problem solving techniques. I would like to especially thank Sigit Sudjatmiko for helping me get to grip's with statistics and graphic's. Thanks also to Dr Hugo Varela-Alverez (Statistics), Lance Curry (Soils) and Cherry Gordon (Library) for your help.

Thanks also to Dr Brent Clothier of Hort+Research who generously allowed me the use of equipment for data collection purposes. Thanks also to Doug Hopcroft of Hort+Research who did such a great job of the SEM (Scanning Electron Microscope) photographs and prints.

To Massey University, for allowing staff the opportunities to study part time where it would be extremely difficult to do so otherwise. Thankyou.

To Massey University Agricultural and Research Fund (M.U.A.R.F). Without financial assistance, a project like this would be impossible.

To Mr 'Timber' Woods of Wanganui and Mr Blair Glasgow of Hawkes Bay, thank you for graciously allowing me to 'process' your trees and for the time you gave to collect data for me.

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GLOSSARY OF ABBREVIATIONS

ANOVA	Analysis of variance
AOA	Aminooxyacetic acid
^{14}C	Radioactive carbon 14
Cultar^{r}	Paclobutrazol
DNA	Deoxyribonucleic acid
EPP	Effective pollination period
GA_3	Gibberellin A_3, gibberellic acid
PUT	Putrescine
PPFD	Photosynthetic photon flux density
RDI	Regulated deficit irrigation
SEM	Scanning electron microscope
YO	Year old

CHAPTER 1
General Introduction

The European pear (*Pyrus communis*) has long been recognised as an important horticultural crop in New Zealand, and throughout the world. Consistent harvestable crop yield is an important criterion for selecting a potential cultivar for orcharding. 'Doyenne du Comice' has a reputation as a high quality dessert pear. However a considerable drawback of this variety is that it starts bearing relatively late and also produces low yields as a young tree (Jaumien, 1968). Combined with these physiological hindrances has been the poor return per kilogram achieved for New Zealand pears on the local and world market during the last ten years. Because of these problems, growers and potential growers have been discouraged from establishing any significant land area for producing 'Doyenne du Comice'. The 1990 - 1991 season saw a considerable increase in the return per kilogram to growers for this and other quality cultivars on the New Zealand domestic market.

Flowering plants can be divided broadly into two groups - those which initiate flowers in response to specific stimuli such as photoperiod or vernalization, and those whose flowering is not clearly linked to a single environmental cue. Most work on flower initiation has concentrated upon plants in the first group where the flowering stimuli, once identified, can be reliably and repeatedly presented. Few woody plants fall into this group and they have thus been relatively neglected in flowering research (Jackson and Sweet, 1972).

Flowering in temperate woody plants has the following general but not exclusive characteristics.

1. There is normally a distinct rest period between evocation and anthesis.
2. There is usually a juvenile stage during which the plants will not produce flowers. At the completion of this phase the plant enters the adult phase when flowering occurs as a seasonal phenomenon, and the onset of flowering does not normally lead to senescence and/or death of the plant, as it does in many

herbaceous plants.

3. The number of sequential steps involved in woody plant flower initiation is apparently greater than in many herbaceous annuals, where one promotive factor such as day-length may predictably and repeatedly induce flowering, (Jackson and Sweet, 1972).

The physiological problem of increasing precocity, i.e. promoting flowering and obtaining a yield early in the plants life, has two aspects. Firstly, the ability of the tree to produce flowers from otherwise vegetative buds and secondly; the ability of these flowers to retain the fruitlets once satisfactory pollination of the flowers has been achieved. It is the former of these two considerations that forms the main focus of this study.

The study was prompted by a conversation in 1988 with a member of the research personnel (Ms. Stella Macleod) of the New Zealand Apple and Pear Marketing Board (N.Z.A.P.M.B.), that despite the use of Quince BA29 rootstock, New Zealand growers were reporting delayed cropping of young Comice trees relative to other pear cultivars.

Objectives of this study.

The thrust of this research was to select treatments that had been reported in the literature to increase precocity in pipfruit and apply them to three year old 'Doyenne du Comice' potted trees on BA29 rootstock for the period of one growing season only. In order to discover the effect of the applied treatments, related parameters should be monitored throughout the growing season.

A number of other factors that influence flower evocation and early cropping in pipfruit, such as canopy light levels, spur defoliation, cross pollination and branch angle were examined in associated research.

CHAPTER 2

Literature Review - Studies in the promotion of precocity in 'Doyenne du Comice' pear.

2.1: Flower evocation.

Flower evocation is the process that occurs before the vegetative bud apex changes from the initiation of leaves to the initiation of floral structures, and at that stage the bud has begun the reproductive cycle, (Fulford, 1966a).

It is well known that most woody plants are unable to flower until they attain a stage or condition known as 'ripeness to flower'. Prior to this stage the plant is said to be in a juvenile condition, and the change to the adult or mature condition is known as "phase change". Experimental work to categorize the basis of juvenility and maturation in woody plants has looked at different aspects of the problem. Longman and Wareing (1959), examined the problem of whether phase change (which occurs at an age and size characteristic of a given species) is dependant on a plant having passed through a certain number of seasonal growth cycles, or, alternatively, to it having reached a certain size and morphological complexity. They concluded that neither is it necessary for a plant to pass through a given number of seasonal growth cycles, nor is size the primary factor determining the stage at which phase change occurs. Rather, phase change occurs after meristematic initials have passed through a certain minimum number of mitotic generations since the last syngamy: that is, it is correlated with, but not determined by, the attainment of a certain size. McLaughlin and Greene (1991), reported that the spur bud initiates a certain number of appendages before the bud will become fruitful. The number of appendages required varies among the varieties of apples, but is generally around 20.

Although treatments which stimulate flowering in mature plants are generally not effective prior to the phase change from

juvenile to mature, certain treatments can sometimes stimulate precocious flowering in juvenile plants. However it may be argued that if a plant has the ability to flower then it must be mature, or at least towards the end of its juvenile phase. Notably these are different forms of shock treatment, such as girdling, excessively heavy fertilizing, drought, freezing, root pruning and heat treatment (Chandler, 1957).

Fruit trees generally bear no crop or very light crops for some years after planting. However, as most are propagated vegetatively using scion wood from mature trees, and as flower initiation is reduced but seldom absent, this cannot strictly be considered a problem of juvenility. Nevertheless, distinctive differences do exist between young and mature fruit trees. Shoots on young trees grow for a longer seasonal period and such shoots contain more leaves and longer internodes. As the trees mature, the period of shoot growth in each season is reduced and flower buds are produced in greater abundance (Jackson and Sweet, 1972). Different fruit trees come into full bearing at different ages; apples and pears, for example, take longer than peaches or apricots. Generally, in vegetatively propagated trees, factors which promote precocious flowering are those which increase the level of flower initiation in mature trees: those which delay flowering inhibit flower initiation in mature trees (Chandler, 1957).

In many fruit trees, photoperiod does not affect flowering. In apples Gorter (1965), found initiation in long days was greater than in shorter days; but since flowering was not eliminated in short days this was probably a response to an overall change in the quantity of light. Wareing (1968), agreed that most deciduous fruit trees are insensitive to daylength, but observed that the seasonal vegetative growth of many non-tropical species of forest trees is under strong photoperiodic control. In particular, photoperiod appears to control the time of cessation of shoot elongation. This cessation is closely related to the time of flower initiation in conifers (Goo, 1968).

In many trees flower-bud initiation tends to be reduced when the tree carries a heavy fruit crop; consequently the next year's crop is light and a cycle of biennial bearing may be induced. Heavy thinning of one part of a tree may lead to that part becoming out of phase with the rest of the tree in its years of bearing; and inhibition of flower initiation by fruits is greater when fruits are close to the buds. Davis (1957), suggesting that the effect may be localised in its action. Chan and Cain (1967), showed that 65 percent of the inhibition of initiation in apple trees occurred within three weeks after blossoming. This is two to three months before initiation could be seen to have occurred.

Jackson and Sweet (1972), recorded two plausible explanations of biennial bearing. First, that competition for metabolites in a tree with a heavy crop leads to a reduction in the supply of nutrients to developing buds and to the restriction of flower formation, (Childers, 1961). Second, that developing fruits produce hormones, which, by altering the hormone balance, interfere with flower bud initiation, (Fulford, 1962; Luckwill, 1964). Chan and Cain (1967), showed that in an apple cultivar capable of carrying seeded and non-seeded apples there was considerably more inhibition to flower initiation on fruiting spurs that had seeded fruits. Seeds are the major sources of hormones (Crane, 1964) and as they constitute a relatively small proportion of the total volume of the fruit and so might not greatly increase the competitive nature of the fruit for metabolites, there is some evidence for the second theory.

Other studies have looked at the relationship between leaf area and flower production. The importance of leaf area is shown by the observation that buds in the axils of small mature leaves on long shoots of apples give rise to weak spurs and, subsequently, reduced flowering (Heinicke, 1967). Leaf removal will reduce flower bud initiation in pears (Aldrich and Work, 1934), generally the effect is restricted to the bud in the axil of the removed leaf.

The effect of leaf removal on flower initiation could be due to a loss of photosynthates or to a change in the hormone balance. To examine this Minnis (1970), treated photosynthesizing leaves with $^{14}Co_2$ and counted radioactivity in buds and leaves elsewhere on the plant. The results showed that ^{14}C moved throughout the plant and accumulated in higher concentrations in the buds than in the leaves. The bud in the axil of the labelled leaf generally recorded the highest radioactivity, although (especially when treated leaves were immature) other buds sometimes had similar or higher counts. Buds lacking a subtending leaf accumulated ^{14}C from treated leaves elsewhere on the shoot as readily as did buds with leaves intact. Thus the possibilities that the bud relies on the subtending leaf as its sole source of carbohydrates, or that the leaf is essential to allow the bud to attract metabolites from elsewhere in the plant, seem unlikely. Perhaps the more likely explanation for the role of the subtending leaf lies in its contribution to the hormone balance at the site of evocation.

2.2: Dwarfing rootstocks and their influence on precocity.

The current requirements of a rootstock for pears are usually for a dwarfing effect together with induced higher productivity in the scion. Parry (1970), extended the observations of Hatton (1935), and showed that the characteristic dwarfing effect of quinces upon pear scions was even greater on Quince C than on Quince A rootstock. However both resulted in earlier and heavier cropping than seedling pear rootstocks.

Flower formation in Citrus species is promoted by drought or low temperature, followed by restoration of climatic conditions favourable to growth (Monselise and Halevy, 1964; Monselise and Goren, 1969; Monselise, 1978, 1985; Southwick and Davenport, 1986). The action of these abiotic factors, together with those of cultural practices that promote flowering in Citrus species i.e. girdling (Monselise, 1985) graft incompatibilities resulting in weak vascular connections with the rootstocks (Mosse, 1962)

confining of root systems in small pots (Furr *et. al.* 1947) and root pruning (Monselise and Halevy, 1964; Monselise, 1985) support the idea that cessation of root growth is an essential prerequisite to flowering in Citrus. This would appear to have similarities with the cessation of shoot growth being necessary in pipfruit in order for the bud meristem to be able to respond to the conditions that may cause flower evocation (Chandler, 1957).

2.3: Influence of fertilizer applications on flower evocation.

For many years the theory of the carbon/nitrogen (C/N) ratio, first proposed by Kraus (1925), was used to explain the basis of flower initiation in fruit trees and some other plants. The theory suggested that if the C/N ratio in the plant was moderately low (i.e. N being high) then vegetative growth was favoured, whilst a high C/N ratio (i.e. N being low) favoured flower production. While this has received some experimental support, it is difficult to reconcile with the now considerable data reporting a marked stimulus to flowering by N fertilizers. Thus, N applications have been shown to stimulate flower production in apples (Delap, 1967).

Several reports suggest a role for phosphorus in flowering. Anthony and Clarke (1932), found flowering in apple was increased by P applications although no relationship between the level of applied P and flowering was found. In peach, P applications have increased flower formation whereas N was found to reduce it (Hirai, 1961). Feucht (1966), found a positive correlation between flowering potential and P content in apples and pears trees. The function of mineral nutrients other than N and P is less well documented. In apple and pear reduced flower initiation has been found under copper deficiency (Wallace, 1961).

Several workers have increased flower initiation in apple trees in response to N applied during the summer growth cycle. Delap

(1967), obtained increased axillary flower initiation from increased applications of nitrate for 3 months in spring, summer or autumn to trees previously supplied with 1 mmol/l nitrate nitrogen. Williams (1963), obtained increased flower initiation in field trees of low nitrogen status in response to fertilizer nitrogen, provided extension growth had ceased prior to treatment. Hill-Cottingham (1963), confirmed that a flowering response to nitrogen applied in midsummer was dependent on a reduced extension growth rate at the time of application which in turn was associated with a low nitrogen status at the commencement of growth.

Both Delap (1967), and Ludders and Bunemann (1970), obtained a flowering response to urea applied as a foliar spray after the cessation of shoot extension in trees grown with 2 mmol/l nitrate. Delap (1967), did not obtain the flowering response in trees grown with 6 mmol/l nitrate, despite a similar level in leaf nitrogen content.

Workers in the past have achieved conflicting results when attempting to manipulate flowering by nutrient supply. Edwards (1986), found that under a controlled nutrient supply, flower initiation in the axillary meristems of maiden apple trees was significantly greater when nitrogen was supplied as ammonium ions than when supplied as nitrate ions. This response was found following even brief exposure to ammonium ions during an otherwise continuous nitrate supply. As little as 24 hours was sufficient.

Other workers have failed to produce a flowering response to the ammonium ion, including Martin (1970), with apples and Jackson (1970), with apricots.

The nitrogenous constituents of apple trees have been studied extensively, with particular reference to seasonal variations and the effects of added fertilizer (Oland, 1954; Hill-Cottingham and

Cooper, 1970). There is general agreement that at all times and in all tissues, except possibly the leaves, most of the soluble N is present as arginine or asparagine. Both these compounds are thought to act as reserve forms of N for use during periods of rapid growth (Cooper *et. al.* 1976).

Hill-Cottingham and Williams (1967), studied the effect of time of application of fertilizer nitrogen on the growth, flower development and fruit set of maiden apple trees. Application of nitrogen was carried out in spring, summer or autumn. They found that flowers were initiated in late July or early August (Northern Hemisphere) on all trees except those given spring nitrogen, in which flower primordia were not visible until September. The development of flower buds was accelerated during September on summer nitrogen trees compared with those with no nitrogen application. Summer and autumn nitrogen trees were 4-5 days in advance of the others in flowering. Summer nitrogen trees had large flowers and large green primary leaves, whereas those on autumn nitrogen trees were smaller and the leaves were initially pale, though turning dark green during blossoming.

Grasmanis and Leeper (1965), recorded higher concentrations of asparagine and arginine in apple trees supplied with ammonium nitrogen, compared with trees continuously supplied with nitrate. Grasmanis and Leeper (1967), speculated that the concentration of other important molecules may also be altered as a consequence of ammonium application, and that such changes may be causally associated with ammonium-induced flower initiation.

Tromp and Ovaa (1976), also found that nitrogen treatments at different times of the season led to marked differences in the percentage composition of the amino-nitrogen fraction of root and xylem sap of apple trees.

Grasmanis and Edwards (1974), concluded that exposure of apple trees to the ammonium ion, apart from any nutritional effect,

triggered the synthesis of molecules which promoted the process of flower initiation in axillary apices already present at the time of exposure, as well as in apices formed subsequently, which led to an increase in bloom.

Demonstration of a change in nitrogen metabolism coinciding with flower initiation in Citrus was shown by Lovatt *et. al.* (1988). Changes in the leaf ammonium content were monitored during cold-temperature induced floral induction in Citrus. These workers found that the number of flowers and the ammonium content of leaves increased significantly with an increased period at low temperature. Lemon trees 'Frost Lisbon' were subjected to water-deficit stress of increasing severity. The intensity of flowering and the leaf ammonium content increased with the severity of the stress.

2.4: The acquisition and tolerance of ammonium by plants.

Plants live in an environment where nitrates are the primary form of soluble nitrogen available for their nutrition; consequently, they have little tolerance for high levels of ammonium nitrogen in their root environment. Ammonium ions are readily absorbed by plant roots, but they must not be absorbed more rapidly than they can be utilised in the cells; otherwise, toxic reactions occur, (Maynard and Barker, 1969; Ajayi *et. al.* 1970). The ammonium intake by a plant must be carefully regulated, for the tolerance range of plants to ammonium nitrogen is quite narrow and is dependent upon the presence of nitrate in the medium (McElhannon and Mills, 1977). Theoretically, ammonium-nitrogen should be used more efficiently in the plant. In the soil, ammonium is less subject to leaching and to denitrification losses than is nitrate.

The ammonium and nitrate uptake by plants shows a wide diurnal variation. Ammonium and nitrate uptake are greater in light than in darkness and uptake increases with increased light intensity, (Van Egmond, 1978). The decline in ammonium and nitrate uptake

in the darkness is apparently due to the depletion of carbohydrate in the roots, for the assimilation of ammonium has a high energy requirement (Reisenauer, 1978).

Absorption and utilisation of ammonium-nitrogen are affected by carbohydrate supply and plant age, (Street and Sheat, 1958). Plants well supplied with carbohydrates are better able to utilise ammonium-nitrogen than are energy-starved plants. Young plants with active photosynthetic mechanisms may be more able to maintain carbohydrate levels better, and therefore be more tolerant of high ammonium levels than older plants which are declining in photosynthetic capacity; however older plants with adequate carbohydrate reserves may be quite tolerant of ammonium nutrition, particularly if they have large leaf areas.

Ketoacids, such as alpha-ketoglutarate, are essential for the initial complexing of ammonium by roots (McKee, 1962; Hewitt, 1970). Corn plants (Barker and Bradfield, 1963) rich in carbohydrates are able to supply the necessary ketoacids for the assimilation of ammonium-nitrogen into amides (asparagine and glutamine) and amino acids (aspartic acid, glutamic acid and arginine). Corn plants grown with ammonium-nitrogen nutrition accumulated larger amounts of amides than those grown on nitrate-nitrogen nutrition, and the predominant amide was asparagine (Barker and Bradfield, 1963). Also, Corn and Barley plants receiving ammonium-nitrogen have been shown to have higher ratios of aspartate:glutamate than those receiving nitrate-nitrogen (Barker and Bradfield, 1963; Ritchter *et. al.* 1975).

The tolerance of plants to external ammonium-nitrogen supply and to the internal accumulation of ammonium-nitrogen is low, whereas the tolerance for nitrate is high. Plants will accumulate nitrate and transport it throughout the plant with few toxic effects. On the other hand, ammonium accumulation in plants cannot be tolerated, and its translocation to shoots is especially deleterious (Barker *et. al.* 1966b; Putritch and Barker, 1967). Ammonium assimilation into amides within the roots appears to be

a detoxification mechanism for plants to survive on high levels of ammonium nutrition (Barker *et. al.* 1966a,b; Maynard and Barker, 1969).

2.5: The arginine biosynthesis pathway.

O'Neal and Naylor (1972), and Jacques and Sand Sung (1981), suggested that in vascular plants, the activities of the orotic acid pathway for the *de novo* biosynthesis of arginine and pyrimidine nucleotides are regulated in a coordinated manner by carbamoylphosphate synthetase, the only enzyme in vascular plants known to synthesize carbamoylphosphate. The activity of the arginine pathway and the formation of the products of the pathway were influenced by changes in the level of ornithine, ammonia, and phosphorus available to the plant tissue.

It is well documented in the literature that in plants arginine is the precursor of polyamines, e.g. putrescine, spermidine and spermine, which accumulate in plant tissue in response to stress (Galston, 1984). Recently these polyamines have been shown to be correlated with cell division and morphogenesis in many plant systems, and with flower initiation in apple (Edwards, 1986).

2.6: The role of polyamines.

Polyamines, which occur ubiquitously in plant tissues, have recently been shown to function in growth and differentiation processes (Bagni *et. al.* 1982, Altman *et. al.* 1983, Smith, 1985) as well as in plant response to stress conditions (Altman *et. al.* 1982). It has also been found that apple fruitlets and young leaves can synthesize polyamines and translocate them via the peduncle, (Altman *et al.* 1983).

The presence and abundance of putrescine and spermidine in xylem and phloem exudates indicate that polyamines may be translocated in plants. This long-distance translocation further supports the

hypothesis that polyamines have a regulatory role in plant growth and response to stress, (Friedman *et. al.* 1982).

Aminooxyacetic acid (AOA) and the polyamines putrescine (PUT) and spermidine applied at anthesis did not increase fruit set in 'Doyenne du Comice' pear, but PUT and AOA extended ovule longevity. AOA increased endogenous putrescine levels in the ovary 12-16 days after anthesis. The increase in the putrescine levels was related to an extended ovule longevity, suggesting an important role of putrescine. Endogenous levels of spermidine and spermine were less affected by the AOA than was the putrescine level. The success of PUT and AOA in extending ovule longevity suggests the possible use of PUT to increase fruit set under low EPP (Effective Pollination Period) conditions. However other factors besides EPP appear to play an important role in 'Comice' fruit set (Crisosto *et. al.* 1985). While the polyamines have been associated with improved set, they have not been associated with increased flower initiation.

Polyamine content was examined when various plant growth retardants, including paclobutrazol, were applied to apple seedlings. Polyamine content was found to increase when paclobutrazol was applied to apple seedlings, (Wang and Faust, 1986). It has been reported that polyamine content is often at its highest concentration when cell division is most active, (Mukhopadhyay *et. al.* 1983). The polyamines spermidine and spermine are synthesized from decarboxylated S-adenosylmethionine and putrescine. Therefore, putrescine plays a role in regulating spermidine and spermine levels.

Arginine is an important precursor of the polyamines putrescine, spermidine and spermine. These have recently been shown to be correlated with cell division and morphogenesis in many plant systems. Infusion of polyamines into trees supplied with nitrate caused them to flower as profusely as trees exposed to ammonium ions (Edwards, 1986).

2.7: Arginine and precursors and their relationship to the application of ammonium, and to flower evocation.

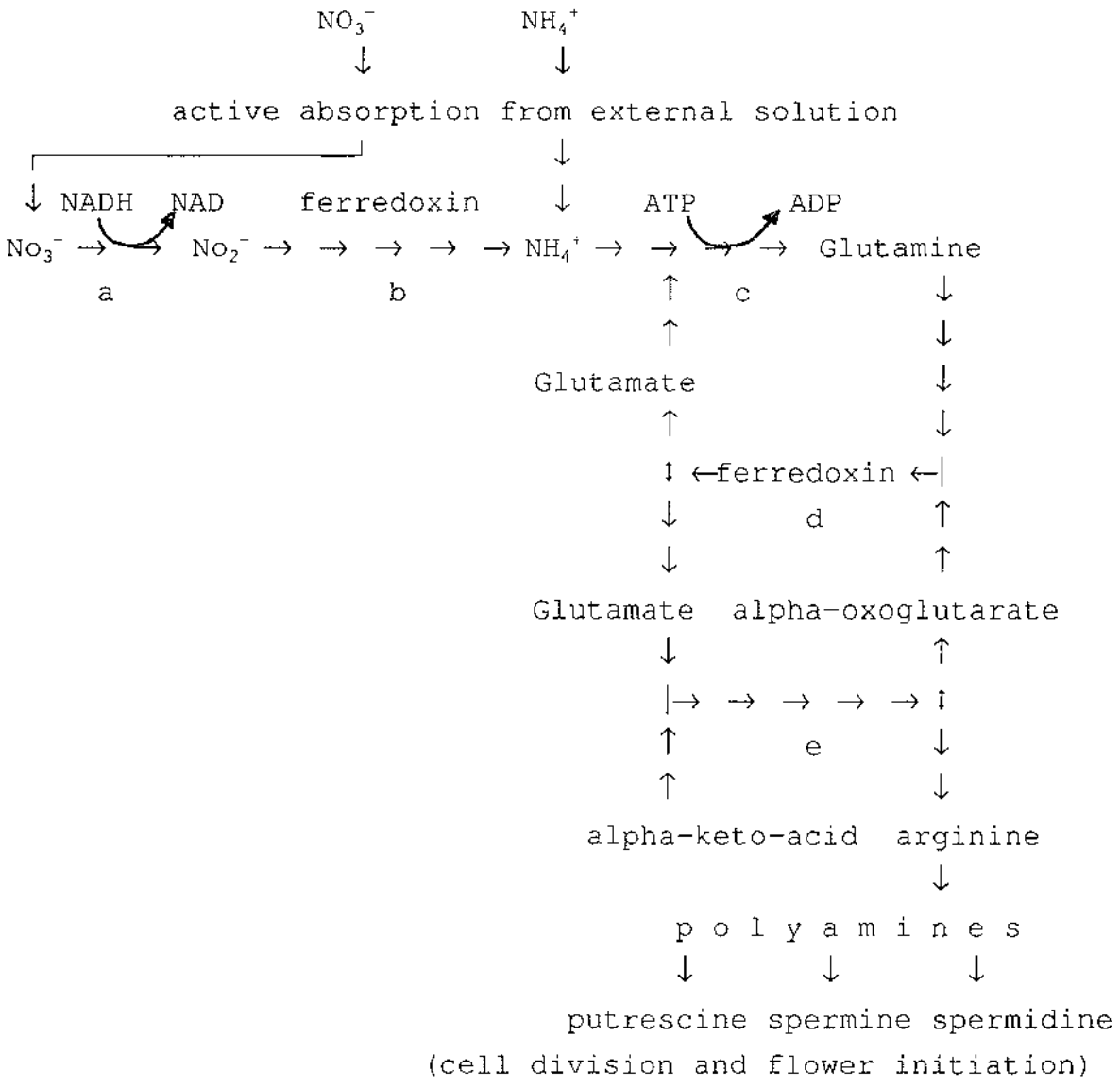
When the ammonium ion was applied to young apple trees Grasmanis and Leeper (1967), found that flowering was significantly enhanced by the ammonium ion. An investigation with different forms of nitrogen (ammonium and nitrate) found that in all cases ammonium caused the initiation of a much higher proportion of flower buds. When ammonium was supplied throughout the season, alone as ammonium sulphate or with nitrate as ammonium nitrate, vegetative growth was less than with nitrate alone. But when the trees were given nitrate only, except for an interval of two months (December-January, Southern Hemisphere) in which ammonium nitrate was supplied, the merits of both forms of nitrogen were combined.

Edwards (1986), as previously mentioned, found flower initiation in the axillary meristems of maiden apple trees was significantly greater when nitrogen was supplied as ammonium ions than when it was supplied as nitrate ions. The response occurred following even brief exposure to ammonium ions during an otherwise continuous nitrate supply, with as little as 24 hours exposure being sufficient. Arginine was the major amino acid in the stems subtending the axillary buds. Its concentration increased following exposure to ammonium ions and continued to rise over the following week whether or not the nitrogen source was changed back to nitrate. With a continuous nitrate supply the level of arginine was lower and declined over the period of a week. Other amino acids showed similar differences. It seemed that flower initiation in apple follows a slow natural increase or a sudden experimental upsurge in the level of reduced nitrogen of which arginine is the major component (Edwards, 1986).

The accumulation of arginine in the leaves of four citrus rootstocks undergoing phosphorus deficiency was shown by Rabe and Lovatt (1986). Ammonium content of the leaves increased as P deficiency progressed. During P deficiency *de novo* arginine

biosynthesis in rough lemon increased 10-fold. When P-deficient leaves were immersed in an ammonium solution for 3 hours they accumulated ammonium and there was a 4-fold increase in the incorporation of $NaH^{-14}CO_3$ into arginine. Arginine accumulation was not due to decreased catabolism or increased protein degradation. The data are consistent with the hypothesis that increased *de novo* arginine biosynthesis in leaves during P deficiency is in response to an increased ammonium content of the leaves.

Figure 2.1: A schematic representation of nitrogen assimilation by plants. The enzyme systems are: a, nitrate reductase; b, nitrite reductase; c, glutamine synthetase; d, glutamate synthase; e, transaminase (Haynes, 1978; Lovatt *et. al.* 1988).



2.8: Simazine and nitrogen metabolism.

In apple trees low levels of simazine, a triazine herbicide, had the same effect as ammonium application, giving increased leaf arginine level and flower initiation (Edwards, 1986). Simazine is known to increase the effectiveness of the nitrate reductase enzyme and should therefore increase the level of reduced nitrate in the tissues.

Several investigators have reported increases in a number of nitrogen components in plants following triazine herbicide treatment. Simazine and atrazine increased the protein content of maize (Gast and Grob, 1960) , and simazine increased nitrogen uptake in maize (DeVries, 1963; Ries and Gast, 1965) and the protein content of apple leaf tissue (Solecka *et. al.* 1969). The total nitrogen, protein nitrogen and total soluble nitrogen levels of apple seedlings were increased by simazine (Lin and Anderson, 1967) which was not due to an increase in nitrogen absorption. In other studies also subtoxic levels of the triazine herbicides have increased growth and nitrogen content (Ries *et. al.* 1963; Tweedy and Ries, 1967; Schweizer and Ries, 1969). It appears that such increases result from the stimulation of activity of nitrate reductase by subtoxic levels of simazine application.

In Poland, Ploszynski and his co-workers examined the phytotoxic action of triazine compounds on crops. Pre-emergence treatment of flax, beet and buckwheat resulted in increased free amino acid and amide content. The accumulation of asparagine, glutamine and basic amino acids was attributed to the inhibition of photosynthesis, with the subsequent decline in sugars leading to the formation of excess NH_2 groups which combined with glutamic and aspartic acids to form the corresponding amides (Ploszynski and Zurawski, 1971).

Pre-emergence application of simazine to pot-grown peas

resulted in growth inhibition or death. At high herbicide concentrations, total nitrogen and free amino acid levels of the leaves increased, while chlorophyll, sugar, certain organic acids and protein levels declined. At low rates, yields increased slightly and the content of chlorophyll, sugar, certain organic acids, total nitrogen and protein nitrogen increased (Ploszynski and Zurawski, 1971).

Pre-emergence treatment of spring rye with simazine and atrazine reduced saccharide and chlorophyll contents, increased total nitrogen, nitrate nitrogen, amino acids, phosphorus and potassium contents and slightly increased the percentage content of protein nitrogen Ploszynski and Rola (1974).

2.9: The influence of water stress on flower evocation and on plant constituents.

Mitchell *et. al.* (1984) in Victoria, Australia showed that the vigour of intensive peach and pear orchards could be successfully managed using regulated deficit irrigation (R.D.I.). Water deficit was imposed during the early part of the growing season to restrict shoot growth. At the beginning of the final rapid fruit growth phase full irrigation was resumed and, by harvest, the fruits were similar to, or larger than, those from continuously fully irrigated trees. The imposition of a water stress during the period of slow fruit growth and rapid shoot and frame growth greatly reduced peach vegetative growth without loss of fruit size or yield (Irving, 1987). The stages of rapid vegetative and fruit growth of pears is similarly out of phase, (Mitchell *et. al.* 1982). Blossom density on both peach and pear was increased in the following spring by the RDI treatment.

The role of drought, low temperature, and inhibitors of GA biosynthesis in promoting flowering may be due to their more general effect of overall inhibition of growth (Iwasaki *et. al.*

1959). This would cause a temporary inhibition of mitosis followed by rapid cell division conducive to flower bud formation (Stebbins, 1965; Bernier *et. al.* 1970) once the inhibiting factor was removed, as suggested by Goren in 1978 (Monselise, 1985).

Chandler (1957), suggested the inception of flowering in lateral buds of young extension shoots in apple depends upon their release from correlative inhibition by the shoot tip.

Four observations suggest that the phenomenon of ammonium accumulation during plant stress is connected to the induction of flowering in Citrus and other evergreen tree crops, (Lovatt *et. al.* 1988):

1. Plants growing successfully in a stressful environment often flower prior to death from the stress;
2. In Citrus and other evergreen tree crops, flowering is promoted by low temperature or water-deficit stress;
3. The degree of flowering in citrus is directly proportional to the severity or duration of the stress which results in accumulation of ammonium (Southwick and Davenport, 1986);
4. Nitrogen applied at the moment of stress removal enhances the degree of flowering (Monselise, 1985).

The findings of Lovatt *et. al.* (1988) demonstrate that the accumulation of ammonium is an early stress-linked event increasing floral initiation in Citrus species, with both low temperature and water-deficit stress. Thus a drought stress period can reduce growth and stimulate flower initiation and this is a result of induced changes in amino acid and ammonium levels.

Recent evidence suggests that low water potentials in plants are not, in themselves, deleterious to normal growth and metabolism provided that high turgor values are maintained throughout a period of water stress (Sharp and Davies, 1979; Hall, 1981). Maintenance of turgor during drought is thought to be achieved by the process of osmotic adjustment of cells, whereby the osmotic potential of cells becomes more negative, resulting

principally from the accumulation of a diverse range of small molecules in response to stress-directed changes in metabolism (Turner and Jones, 1980). Several studies indicate that free amino acids contribute substantially to the process of osmotic adjustment (Munns *et. al.* 1979, Jones *et. al.* 1980). In particular, the development of exceptionally high concentrations of proline has been observed in many plant species in response to drought (Palfi *et. al.* 1974).

The effects of water stress on the accumulation of free amino acids in the shoots and roots of three northern conifers were described by Cyr *et. al.* (1990). As the water stress increased, soluble amino nitrogen concentrations increased to 150-200% of control values, with moderate to large increases of several free amino acids in both shoots and roots. This work showed that Hydroxyproline was particularly abundant in the roots of all three conifer species, which may have reflected the prevalence of that amino acid in the proteins of the primary cell walls (Wilson and Fry, 1986) of the young cells. The three species of conifers showed marked differences in their ability to withstand drought, but levels of free amino acids in the roots and shoots did not show any similar significant trends. For example white spruce was the most drought resistant of the three species, but increments in free amino nitrogen were relatively small throughout the test.

However, Cyr *et. al.* (1990) could not discount the accumulation of amino acids during drought as a protective response. In addition to what may be a relatively minor contribution to the process of osmotic adjustment in some conifers and other plant species, individual amino acids may serve to stabilize specific proteins during stress, e.g., proline and glycine have been shown to protect enzymes from heat denaturation (Paleg *et. al.* 1981, Goh *et. al.* 1988), thus suggesting the possibility of a similar role during drought.

2.10: Increasing precocity with growth regulators.

Growth regulators have been used with some success on young apple trees to increase precocity. Chemicals which reduce shoot growth such as daminozide (Veinbrants, 1972; Luckwill, 1978) when applied up to six weeks after petal-fall, have increased fruit bud initiation and yield. Paclobutrazol, another growth retardant, has been shown to increase yield of young apple trees (Williams 1983b; Quinlan and Richardson, 1984). Volz (1986), applied Paclobutrazol, (Cultar^{r}), to apple 3 weeks after petal fall. The effects on fruit bud induction were varied, but paclobutrazol generally enhanced it. Post petal fall paclobutrazol increased the numbers of fruit buds in the year following treatment of mature apple trees (Tukey, 1983). Some workers have concluded that axillary flowers are a result of a reduction in shoot growth induced by growth regulator treatment (Luckwill, 1970) although there was considerable disagreement as to the relationship between shoot growth and fruit bud formation.

Browning *et. al.* (1992) found that irrespective of whether paclobutrazol was applied by shoot tip or basal treatment, the chemical required to promote flower initiation appeared to be much smaller than those which inhibit shoot growth. Small amounts of the chemical also appeared to be freely mobile in both the acropetal and basipetal directions. Thus, after localised shoot tip treatment, small, but apparently physiologically active, amounts of chemical migrated throughout the shoot, where they persisted for a period of at least several months. This resulted in a greatly increased number of axillary floral nodes. Thus any promotion of floral initiation mediated by effects on the apical dominance mechanism were not separated from any direct effects of paclobutrazol reaching the axillary buds.

Volz (1986), showed a highly significant, positive, relationship between shoot growth and spur fruit bud formation, indicating that there were more spur buds where shoots grew

longer, or were more numerous. This would appear to rule out inhibitive competition by shoot growth on fruit bud formation and, conversely, suggest that enhanced shoot growth may be beneficial to flower production. The lack of correlation between shoot growth and axillary fruit bud formation agrees with the findings of Tromp (1972), who concluded that shoot growth did not significantly influence fruit bud formation.

Luckwill (1970), also concluded that flower initiation is not always associated with a retardation of shoot growth. It is widely accepted that gibberellins can adversely influence fruit bud formation in fruit crops. Inhibition of flowering has been correlated with the presence of seeds, (Chan and Cain, 1967; Porter and Strang, 1978), and also with fruit diffusates rich in gibberellins (Hoad, 1978; Ebert and Bangerth, 1981). Exogenously-supplied GA_3 reduces flowering in many fruit crops (Luckwill, 1970). Tromp (1972), and Knight (1980), demonstrated that in apple, some gibberellins may be more inhibitory than others. Hedden and Graebe (1985), reported the inhibition of gibberellin biosynthesis by paclobutrazol.

2.11: Paclobutrazol (Cultar^r): Mode of action.

Several members of the class of chemicals known as triazoles inhibit growth of a wide range of plants and have gained attention for use in apple production systems (Curry and Williams, 1983; Greene and Murray, 1983; Quinlan and Richardson, 1984; Stinchcombe *et. al.* 1984). The active members of this chemical class are considered to inhibit growth by blocking or antagonizing biosynthesis of the gibberellins (Graebe, 1982).

The triazoles, among other chemicals types such as ancymidol (Coolbaugh and Hamilton, 1976; Coolbaugh *et. al.* 1978) inhibit the sequential oxidation of ent-kaurere to ent-kauenoic acid which most likely is directly related to the degree of growth inhibition which results. The pattern of growth inhibition resulting from the application of these biologically active

triazoles to plants are most likely due to lack of available gibberellin. The varied morphological changes resulting from treatment are accompanied by many physiological and biochemical changes.

Cell division in plants takes place in apical meristems and plant growth occurs by expansion and elongation of these cells. Therefore, the changes in cell wall carbohydrate constituents are an important part of plant growth (Cleland, 1977). The presence of hormones is required for cell elongation, and promotion of extension-growth by auxin is mediated by cell loosening and changes in the cell wall chemistry (Brummell and Hall, 1985).

Gibberellins are physiologically defined by their ability to induce shoot elongation. Applied gibberellin reversed the inhibition of shoot growth caused by light in dwarf peas Kende (1963).

Paclobutrazol caused reduction in extension shoot and fruiting spur growth on apple seedlings (Steffens *et. al.* 1985). As a result, shoots on paclobutrazol treated trees assumed a rosette-like appearance.

The plant growth regulator, paclobutrazol [1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol)], has been found to interfere with gibberellin biosynthesis in plants and has been found to be an effective growth retardant on apple (Curry and Williams, 1983), (Quinlan, 1984) and pear (Raese, 1983), (Dheim and Browning, 1988)

Applications of GA_3 to paclobutrazol inhibited plants can temporarily counteract part of the growth inhibition, and also some of the associated physiological and biochemical changes (Curry and Williams, 1983; Greene and Murray, 1983; Quinlan and Richardson, 1984; Steffens *et. al.* 1985; Wang *et. al.* 1985).

2.12: Biochemical changes associated with paclobutrazol application.

In general, paclobutrazol has the greatest effect on tissues which are rapidly growing and developing at the time of treatment or shortly thereafter. Nonstructural carbohydrate content in apple shoot and spur leaves (Steffens *et. al.* 1985) and stems, leaves and roots (Steffens and Wang, 1986) was increased by paclobutrazol.

Relative chlorophyll content on a leaf area basis was higher in leaves of paclobutrazol-treated seedlings (Steffens *et. al.* 1983; Wang *et. al.* 1985), especially in leaves which developed after paclobutrazol treatment. Paclobutrazol treatment increased the soluble protein in leaves of apple seedlings and alterations in protein content closely paralleled alterations in chlorophyll content (Wang *et. al.* 1985). Foliar applications of GA_3 reversed the effect of paclobutrazol on leaf protein and leaf chlorophyll.

Carbon dioxide evolution in the dark by shoots of paclobutrazol-treated seedlings was reduced on a unit weight basis compared with the controls (Steffens *et. al.* 1983). This effect could be seen a few days after treatment and the differences continued for an 18-day experimental period. It appears that energy requirements of treated shoots in the dark are less than for untreated shoots which would be expected for less metabolically active plants. A lower rate of respiration in the dark, would utilize less carbohydrate, and may partially account for the increased carbohydrate in treated plants. Carbohydrate levels are a factor limiting flower formation (Goldshmidt and Glomb, 1982; Goldshmidt *et. al.* 1985)

In leaves of seedlings grown in nutrient solution containing paclobutrazol, significant increases were found for N, P, K, Ca, Mg, Mn, Cu, Zn, and S compared with controls (Wang *et. al.* 1985). On the other hand, treatment of orchard-grown 'Spartan' trees by

Steffens *et. al.* (1985) resulted in only minor increases in Mg and Ca of shoot leaves but no change in fruiting spur leaves. Paclobutrazol dosages, cultivar differences and environmental factors may all influence the nutrient content of treated leaves and fruit.

Sansavini *et. al.* (1986), found that foliar paclobutrazol sprays enhanced apple fruit bud differentiation, and yield in the second year. Internode length was shorter and treated leaves were smaller, with higher dry matter, chlorophyll, N, Ca and Mg contents.

Concentrations of the polyamines putrescine and spermidine in apple leaves were reduced by paclobutrazol treatment on trees under water stress and were negatively correlated with duration of paclobutrazol treatment (Wang and Steffens, 1985). Water use and transpiration were reduced by paclobutrazol treatment of M23 apple trees (Atkinson *et. al.* 1987). Wang *et. al.* (1986) reported that paclobutrazol altered the composition of apple cell wall polysaccharides and inhibited shoot extension in the year of treatment. Paclobutrazol treatment increased rhamnose and galacturonic acid but decreased cellulose. The ratio of xylem to phloem was also reduced by paclobutrazol.

Dheim and Browning (1988), suggested the inhibition of flower initiation by strong apical dominance was through the active shoot meristem slowing node production within the buds below a threshold rate required for floral initiation. Thus reducing shoot apical activity by paclobutrazol might reduce apical dominance, indirectly increasing the plastochron in vegetative axillary and spur buds (where plastochron is the number of days between primordia initiation). Luckwill (1968), and Dheim and Browning (1988), also suggested direct inhibition of floral evocation by gibberellin exported from the shoot meristem. The production of such gibberellin would be inhibited by paclobutrazol.

2.13: Flower evocation.

Flowers, like leaves, are derived from a meristem and in the past there has been considerable controversy as to whether the meristematic activity leading to flower initiation differs qualitatively from that leading to leaf initiation. This uncertainty followed largely from the French concept (Nougarede, 1965) of distinct zones of reproductive and vegetative development in different regions of the meristem. This interpretation is not now generally held, and it is widely accepted that there is no essential structural difference between the organisation of a meristem producing only leaves and one producing flowers except that the first is indeterminate and the second is determinate in its growth.

Buban (1981), using apple trees, found that preceding the appearance of morphologically distinguishable flower primordia a distinct change takes place in the zonation of the apex within the bud. Evocation occurs in apices which still show vegetative structures and cytochemical dissimilarities are evident between apices that have been induced or inhibited for flower evocation. Evocation is a physiological change in the meristem which may be hormone induced, with no morphological change. Apices in which flower evocation is inhibited have relatively high levels of DNA only at the place of leaf primordia initiation. There is an increased level of DNA both in the central meristem and apical axil zone of the apices of the buds under flower inductive conditions (Buban, 1981).

2.14: Morphological studies of flower bud formation.

Fulford (1966a), and Dheim and Browning (1988), found that flowers were not initiated in buds which had fewer than about 20 vegetative nodes. Dheim and Browning (1988), found the morphological development of 'Comice' buds conformed to the

previously reported pattern for pears. Several workers have examined reproductive bud differentiation stained sections of apple buds under the light microscope (Bijhouwer, 1924; Fulford, 1966a; Abbott, 1970). More recently the scanning electron microscope (SEM) has been used to study flower development in many different crops.

The previously vegetative apical meristem of many buds synchronously became domed (first visible sign of flower differentiation), after which the first outer flower primordia appeared. Bergh (1985), found in apple, that the shoot apex had a smooth conical appearance enclosed by bud scales in December (Southern Hemisphere). Flattening (doming) marked the change from vegetative to reproductive phase occurred during the first week of January. Sepal primordia of the terminal and lateral flowers formed in the second week of January.

In woody plants prior to expansion developing flowers are normally enclosed in a series of scales and bracts, the whole being called a floral bud (Jackson and Sweet, 1977). A bract is a small scale like leaf and a sepal is a division of the calyx of a flower.

The formation of flowers can be regarded as the final stage of development of the terminal bud of the apple spur, when it is commonly referred to as a 'fruit bud'. It usually occurs in late summer when the bud has completed much of its development, and is generally associated with the cessation of shoot growth and leaf expansion (Fulford, 1966a).

2.15: Other aspects of flower production.

Although it has been suggested that an adequate leaf area is necessary for flower initiation (Davis, 1957), no clear quantitative relationship has been established. In many instances interpretation of such data is complicated by the presence of developing fruit on the trees, which in some varieties of apples

may have a marked effect upon the initiation of flowers. Since flower formation in terminal buds of shoots may be three weeks later than in spurs, and much later in axillary buds on extension shoots, it is evident that compared with internal factors, the effect of the external environment upon fruit-bud formation is very small (Davis, 1957).

It has been shown that the formation of the spur bud follows a characteristic pattern in which the activity of the apical meristem, and the subsequent development of the primordia it produces are controlled by self regulating mechanisms (Fulford, 1965). This pattern could be modified by an experimental treatment such as defoliation, and it was found that the differences in development which were induced in this way could affect the ability of the buds to form flowers (Fulford, 1965).

The effect of the foliage is limited by changes associated with its ageing, and with the formation of bud-scales (Fulford, 1966a). The latter results in an increasing separation of the foliage from the apical region of the bud, in terms of number of nodes, which may alter the conditions under which the apical region of the bud develops.

The importance of leaf area is shown by the observation that buds in the axils of small mature leaves in long shoots of apples give rise to weak spurs and subsequently, reduced flowering (Heinicke, 1967). In terms of the whole tree, Fulford (1966b), with apples, found no evidence for the requirement of a critical leaf area for flower initiation in apple.

Fulford (1965), found no relationship between development of foliage and the rate of increase in the total number of leaf primordia in the bud, upon which the change to fruit-bud formation seems to depend, and it is to be expected therefore that there should be no evidence of a critical leaf area for fruit-bud formation. In general, however, spurs with fewer leaves are less likely to flower, and such spurs may be affected by

correlative inhibition by other parts of the tree, this could cause bud-scales to form sooner, thus reducing the number of leaves which can expand below the bud, as well as affecting the development of the bud itself.

In apples, production of leaves gives way to the production of protective bud scales which are followed by leaf primordia and then bracts. It is in the axils of these bracts and the youngest of the leaf primordia preceding them, as well as at the apex of the bud that flower primordia are formed; and it is believed that only after the appearance of bracts in the bud does the apex undergo any real change to the flowering condition. (Fulford, 1966b).

The young developing leaves are known to be rich sources of growth substances whereas the budscales are seen as being hormonally inert. The bud scales remain active physiologically and increase in both size and weight. In doing so, they provide a protective covering to the bud, but also act as a buffer against the resumption of growth. In storing nutrients, scales provide a reserve upon which the early growth of the bud can be sustained (Abbott, 1970).

The sepals of flowers are formed in the same phyllotactic sequence as the bracts, but at a much faster rate. If the rate of production of primordia by the apical meristem can be related to the extent to which it is inhibited by primordia adjacent to it, then the faster rate of production of sepals, would be due to a reduction of this inhibition of the apical meristem to a very low level, i.e. to the formation of a sufficient number of bracts.

The formation of flower parts appeared to be closely related to bract formation. With terminal flowering plants the development of a terminal bud begins as soon as production of leaf primordia ceases. The relatively constant number of leaf primordia in a bud when it changes to reproductive development implies that the

change in development leading to bract formation may be due to the leaf primordia themselves.

The terminal meristem, by virtue of its central position, is affected by several primordia below it, the axillary meristem is affected most by the primordium which subtends it and, according to Wardlaw (1952), partly by the primordia above. The lower axillary meristems in the bud are more affected by leaf primordia than those nearer the apex, and this may account for their failure to form flowers.

Fulford (1966b), found no direct evidence to support the production of a specific flower-inducing substance in the foliage, as was thought to be the case in herbaceous plants. However he pointed out that the onset of flower production can be related to the pattern of bud development, which in turn stems from events earlier in the season that are themselves unconcerned with reproductive development. A progressive sequence of development can be seen in these buds, for the production of bud-scales leads to formation of 'reduced' leaf primordia which in turn enables bracts to form; these lead to the development of sepals and finally the specialised organs of the flower. Thus the change to reproductive development seems more to be due to factors within the bud than to those imposed upon it from without.

Fulford (1966b), suggested that the meristem will always form a flower, unless it is prevented from doing so, and that the 'vegetative phase' of development is the period during which successive obstacles to flowering are formed and overcome as the meristem grows.

2.16: The role of light and leaves in the process of flower formation and retention of the fruitlets.

Jackson and Palmer (1977), subjected Cox's Orange Pippin apple trees to varying levels of shade so as to receive 37, 25, or 11% of full daylight during the post-blossom growing season and their growth was compared with control trees. It was found that shading had a direct and a residual effect. The residual effect was found to be that on heavily shaded trees in the subsequent year the fruit bud and hence fruit number was reduced, thereby allowing assimilate to be channelled into shoot growth. The direct effect of shade increased leaf K and Mg concentrations, while not affecting those of other elements. In general, the effects of shading these orchard trees, although pronounced, appeared to have been less severe than those obtained by Maggs (1960), who shaded potted rootstocks and found a reduction in total dry matter increment to 12% at 76% shade.

Many aspects of apple tree growth and fruit development are influenced by the degree of exposure of the trees or parts of trees to sunlight (Jackson, 1975a,b). Differences in fruit tree canopy form due to pruning and training methods can substantially modify light transmission to the various regions of the canopy (Ferree, 1980; Ferree and Hall, 1980; Porpiglia and Barden, 1981; Kappel *et. al.* 1983; Rom *et. al.* 1984).

Tustin *et. al.* (1988) found that transmission of photosynthetic photon flux density (PPFD) measured in mid season under clear sky conditions varied markedly through the tree canopy. Significant main effects for tier level, canopy position, and fruiting lateral orientation were observed. Across all lateral orientations and canopy positions, transmission of PPFD decreased from the upper to the basal tier of the canopy. The reduction in PPFD penetration to the outer canopy was almost twice that for the inner canopy. PPFD penetration to fruiting laterals increased as lateral orientation became more upright. Significant

attenuation of PPFD can occur through very localised regions of the canopy and that this is a function of an interaction between canopy position and fruiting lateral orientation. As a result, variable gradients of PPFD penetration can occur irregularly within the canopy, although following the general trend for attenuation from the periphery to deep canopy positions.

When Fulford (1970), chemically defoliated apple trees in early summer a second flush of growth occurred and delayed the formation of the winter bud. It was found that the likelihood of this becoming a fruit bud was reduced the longer the delay before the bud formed. In spur buds that entered the winter season in a vegetative state no further development seemed to occur until bud burst in the following spring, unlike axillary buds which may become fruit buds in early or late winter Zeller (1960).

Ferree and Palmer (1982), found that spur leaf or bourse shoot removal would reduce the carbohydrate availability to the young fruitlets, reducing yields compared to the controls. Their work showed that these effects could be very localised on individual spurs within a tree. In the initial stages of growth fruits are very dependant on the leaves on their own spurs rather than being able to receive carbohydrates from elsewhere in the tree. The presence of the bourse shoot was detrimental to early set, but resulted in higher final yield (Ferree, 1982).

Llewelyn (1968), found that during the period of 7 to 30 days from the time of treatment, defoliations of individual spurs increased the rate of fruit drop. After this period these rates of drop became equal to those of untreated trees and the relative differences in crop numbers established by this time persisted until harvest. Llewelyn (1968), also found that the defoliations had their greatest effect when applied early in the season. The leaves most vital for the retention of the fruitlets were those on the same spur as the fruitlets.

2.17: Branch angle and its influence on flowering.

Bending of branches has commonly been practised to reduce shoot extension and promote flowering (Wareing and Nasr, 1958). Increased flowering has not been consistently found in studies utilizing bending. Tromp (1967), showed that the enhanced flower bud formation on horizontal apple branches was due to a direct effect of branch orientation and was not related to the associated growth reduction. In contrast, enhancement in flowering has been attributed to reduced shoot growth in bent branches, (Luckwill, 1970). Hamzakheyl *et. al.* clarified this relationship where he found that generally on newly planted apple trees during the first growing season shoot growth was reduced proportional to the degree of bending towards the horizontal. Branches trained at 30^{0} from the vertical the first season and then 60^{0} the second season had the greatest reduction in shoot growth and had more flowers. Trees with branchs trained to the horizontal produced the greatest number of water shoots and the least flowering.

In apples there is some evidence that varieties may vary in the effect on flowering of training the individual branches horizontally. Tromp (1967), discovered that horizontally-grown apple trees have more fruit buds, grow less, and terminate their growth sooner than vertical trees, but Poll (1966), found that growth of non-vertical trees was the same as vertical trees, although apical dominance was reduced and flowering was increased. In contrast, Mullins (1965), observed no difference in the total number of flower buds formed in horizontal apple trees. Mullins (1967), suggests that the effect of growing trees or branches horizontally is to modify the pattern of vegetative growth. When this response results in early growth cessation, flowering is promoted; but if upright laterals are formed no overall effect may be noticed.

CHAPTER 3.

General Methods and Materials.

3.1: Introduction.

It has been established that a range of conditions that limit vegetative vigour of perennial fruit trees will promote flower evocation. These treatments include chemical growth retardant application, dwarfing rootstocks and water stress. A comprehensive comparison of such treatments on 'Doyenne du Comice' has not been carried out in New Zealand. There is also limited information on the relationship of internal physiological changes to the induction of flower bud development in European pear.

An experiment was undertaken at Palmerston North in 1990 - 1991 to examine the effect of five treatments, including a control, on the flowering precocity of three year old 'Doyenne du Comice' pear trees. The main objective of the study was to evaluate alternative strategies for the promotion of flowering in young trees. The study involved monitoring of plant growth parameters, including measurement of the soluble nitrogenous plant constituents in both the leaves and the buds, and shoot growth characteristics. Morphological changes in spur buds were followed as the meristem changed from a vegetative state to a floral state during the summer.

Some investigations were also carried out on older trees of 'Doyenne du Comice' in commercial orchards in the Turakina Valley, near Wanganui and in Hawkes Bay to investigate various aspects of flowering. These included recording the effects of defoliation of spurs, and of tying down of branches, and the effect of light levels on flowering. Blossom periods of 'Doyenne du Comice' and appropriate cross pollinators were also monitored.

3.2: The Palmerston North experiment - The trees

The 'Doyenne du Comice' trees were obtained in winter 1989 at an age of 2 years from budding. They had been budded onto Quince BA29 rootstock and were planted in the winter of 1989 into 40 litre plastic containers. The growing medium was a 60 : 40, peat : sand mix. The 8-9 month fertiliser mix consisted of 300 grams Dolomite, 90 grams Micromax trace elements, 200 grams 8-9 month release Osmocote and 100 grams 3 month release Osmocote per 100 litres. The trees were allowed to establish in these containers for one year, situated in the Massey University Fruit Crops Unit.

For the 1990-1991 season, when the trees were 3 years of age, fertiliser was applied in the same proportions as the previous year. The 8-9 month fertiliser mix for each container consisted of 120 grams Dolomite, 36 grams Micromax trace elements, 80 grams 8-9 month release Osmocote and 40 grams 3 month Osmocote which was applied to all treatments except the Ammonium Sulphate treatment in September 1990. The Ammonium Sulphate treatment received 120 grams Dolomite, 36 grams Micromax, 18 grams Potassium Chloride and 23 grams Superphosphate. The fertiliser was applied evenly and scuffed into the surface of the medium by hand. Ammonium Sulphate was applied to this treatment during the growing season on 9 occasions, where the rate of application was 6 mg/l in solution at 3 litres per tree. This was calculated to give a similar level of nitrogen to that in the osmocote applied to the other treatments.

Irrigation for the plants was on a time clock mechanism that applied through compensating emitters into each pot at a rate of 4 litres/hour from the Massey water supply. A plastic barrier was placed over the top of the pots to reduce surface evaporation and eliminate rain. Irrigation was calculated based on simultaneous water requirements of 3 year Nashi trees in drainage Lysimeters also located at Massey University Fruit Crops Unit. Mean weekly

ET per tree varied from 3.3 to 10.7 litres/ tree per day and information was received by personal communication with Horst Caspari and his results are published by Chalmers *et. al.* (1992). No allowance was made for difference in crop coefficients as these were considered negligible. Irrigation was controlled by electronic controller supplied water via solenoids.

Prior to the 1990 - 1991 experimental season branches arising from the main stem were trained to 60 degrees above the horizontal by tying them to supporting wires during the winter of 1990. Some trees had produced a few flowers, and these were removed except for those on five spare trees. The growth of these fruits was monitored to indicate when R.D.I treatment in the experiment should be terminated.

3.3: The experimental design.

The trees were arranged in a randomised block design (RBD) in eight blocks each containing 1 replicate tree of each of the treatments. The RBD design is one of the most widely used experimental designs in agricultural research. The design is especially suited for field experiments where the number of treatments is not large and the experiment area has a predictable productivity gradient. In this case each block contained trees of similar trunk diameter.

Five treatments were applied as follows:
1. Control, 2. Ammonium Sulphate fertiliser, 3. Simazine, 4. Paclobutrazol, 5. Regulated Deficit Irrigation (R.D.I.).

1. Control:

Full irrigation all season with standard fertiliser and no other manipulation or chemical treatment.

2. Ammonium sulphate treatment: - (generally referred to in the text as Ammonium treatment).

The nitrogen needs of the plants were met by the application of ammonium sulphate fertilizer. Ammonium sulphate solution was applied to each container at a concentration of 6 mg/l at 3 litres per tree, which saturated the media in the pots. Irrigation was withheld from this treatment for 24 hours before the fertiliser treatment was applied, so that the media would retain the ammonium sulphate enriched water. The applications of ammonium fertiliser began on 24.11.90, and finished on 19.1.91. Applications were made once every week, (a total of 9).

3. Simazine treatment:

In a liquid culture experiment Edwards, (1986) reported that simazine was applied at a concentration of 1 ppm. However due to absorption by the organic content in the media in this study, a proportion of the simazine applied was likely to be unavailable to the plants. A simazine bioassay (Chapter 4) showed that at a rate of 10ppm (active ingredient) seedling growth in similar medium was be depressed and this rate was used in the experiment. Simazine was applied to the plants once every three weeks, with 5 applications from 24.11.90 to 19.1.91. At each treatment date, 3 litres of 10ppm suspension was applied which was enough to saturate the media in the pots. Irrigation was withheld from this treatment for 24 hours prior to the application, so that the media would retain the simazine suspension.

4. Paclobutrazol (Cultar, PP333) treatment:

Paclobutrazol was applied as a foliar spray to run off by knapsack sprayer at a rate of 500ppm active ingredient and was normally applied early in the morning. Paclobutrazol was applied 4 times, once every 3 weeks, from 2.11.90 to 10.1.91.

5. Regulated Deficit Irrigation (R.D.I.) treatment.

The period of water stress began on 18.11.90, applying irrigation at a rate of 25% of the water application that the control received. As the day length increased during the summer, the amount of irrigation increased, but this treatment always received 25% that of the control. The period of R.D.I. was completed on 13.1.91, when full irrigation was resumed.

3.4: Fruit growth.

On five spare containerised trees that were treated like control plants during this trial, fruit were being carried and the ten fruit monitored were chosen on these trees. The sole purpose of monitoring fruit growth was to guide R.D.I. irrigation application. Fruit growth was monitored from 26.11.90 to 19.1.91 at weekly intervals. Fruit volume measurements were made using a water displacement technique where the volume was measured in millilitres.

The rate of increase in fruit volume slowly increased until 12.1.91 (Figure 3.1) when fruit volume increased markedly indicating the commencement of the exponential growth rate. At this time, as suggested by Mitchell *et. al.* (1984), full irrigation was reinstated to the R.D.I. treatment trees.

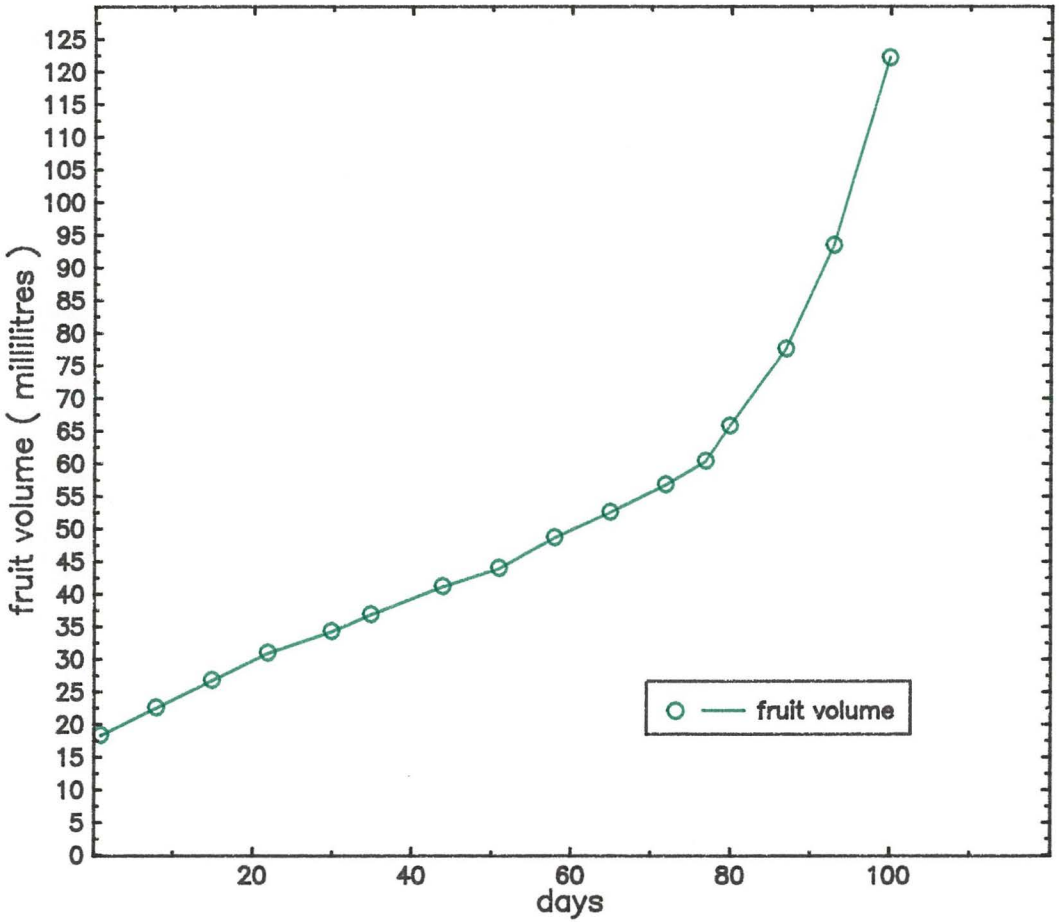


Figure 3.1 Mean Fruit volume growth of 'Doyenne du Comice' from Full Bloom. Exponential growth began day 77 (12.1.91), where day 1 = 26.10.90.

3.5: Climate.

The Climate in the area of the experiment is recorded at Grasslands Division, DSIR, Palmerston North, within a few hundred metres of the site. The following characteristics were recorded from October 1990 to September 1991.

a. Rainfall with a total of 810.2 mm, of which approximately 50% fell during the winter period, April to September. The number of rainy days per month were about the same throughout the year and the monthly average for January and August 1991 record the lowest and the highest average respectively.

b. Daily mean temperatures of 12.9 and 11.2 °C were recorded for the wet and dry bulb thermometer respectively. Monthly mean maximum and mean minimum dry bulb air temperatures, 17.2 and 7.1°C registered in the months of February and August respectively. Monthly mean maximum and mean minimum wet bulb air temperatures, 14.9 and 6.2°C registered again in the months of February and August respectively.

c. Sunshine hours recorded a total of 1648.8hrs with a monthly mean of 137.8, and a mean maximum and mean minimum of 205.1 and 67.3 for January and July respectively.

3.6: Wanganui studies.

Three studies were carried out on 'Doyenne du Comice' trees aged 4 and 7 years growing in the Turakina Valley. 1. The influence of spur bud defoliation at two dates in summer, on bloom. 2. the effect of defoliation and the influence of the bourse shoot on fruit set. 3. The influence of branch angle on flowering.
Monitoring of flowering dates and periods on cultivars 'Doyenne du Comice', 'Buerre Bosc' and 'Packhams Triumph' were also carried out.

3.7: Hawkes Bay study.

Light is an important influence in determining the amount of carbohydrate that is synthesized by the leaves. The spur buds require this carbohydrate in order to initiate flowers. This study examined the influence of varying light levels on the flowering of spur buds within the canopy.

3.8: Statistical procedure.

All data were analyzed via SAS (Statistical Analysis System) GLM (General Linear Model) procedure (SAS Institute, 1985).

Data from each experiment was analyzed for variance (ANOVA), means and standard errors. Mean comparisons were made by the t test unless stated otherwise, when usually Duncan's multiple range test was used.

CHAPTER 4

Simazine Bioassay - Growth of rye grass and radish at different rates of simazine application.

4.1: Introduction.

Edwards (1986), found simazine increased flowering when applied at 1ppm in nutrient culture to pipfruit. In the present study the movement of simazine through the growing medium was investigated to ensure it would be available to the tree roots and not made unavailable by organic matter in the medium. A wide range of rates of simazine were tried and applied to cores of the peat : sand medium and its movement through the profile assessed by its effect on seedling plant growth.

4.2: Methods and Materials.

A range of concentrations of simazine were prepared by first mixing a stock suspension of 5000ppm active ingredient, and then by sequential dilution suspensions of 1000, 100 and 10ppm. Sections of plastic tubing (400 by 50mm) were filled with firmed moist medium mix of 60% Peat : 40% sand, with fertiliser mix, as used for the experimental 'Doyenne du Comice' trees.

One Litre of each simazine suspension solution was applied to each of 2 labelled tubes. One tube for each simazine application was flushed immediately with one litre of clean water to flush the simazine into the medium profile.

After the cores had drained, the tubes were placed horizontal, and split in half longitudinally, and a row of seeds was sown densely down the centre of each profile. Rye grass seed, and radish seed, were sown independently in each of the two separate core sections.

The tubes were then placed in a glasshouse for 11 days. Seedling growth, was then assessed by measuring the plant height at 10cm intervals down each full length profile.

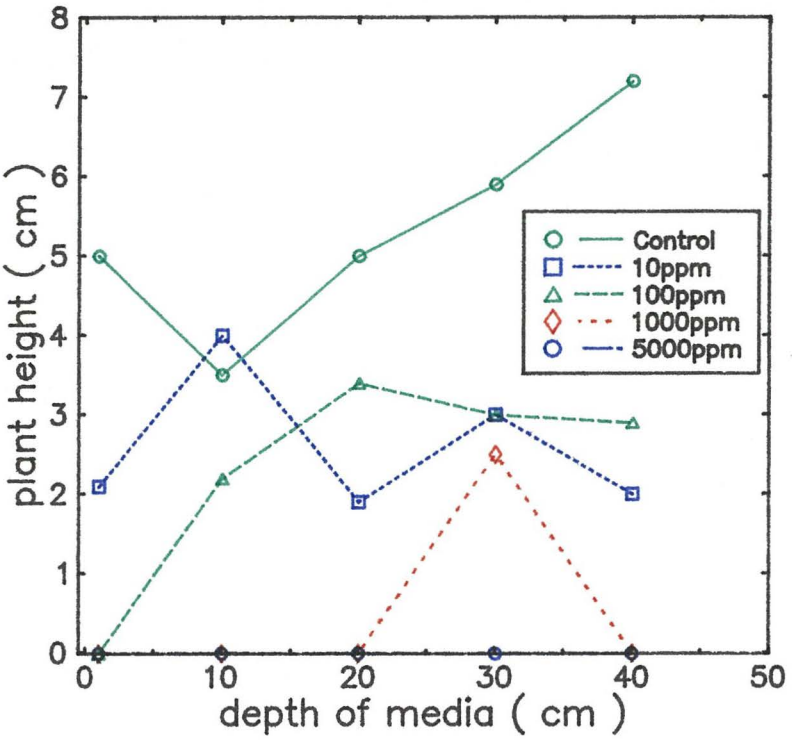


Figure 4.1 Simazine bioassay – Effect on radish plant height of varying rates of simazine down the profile (No Flush).

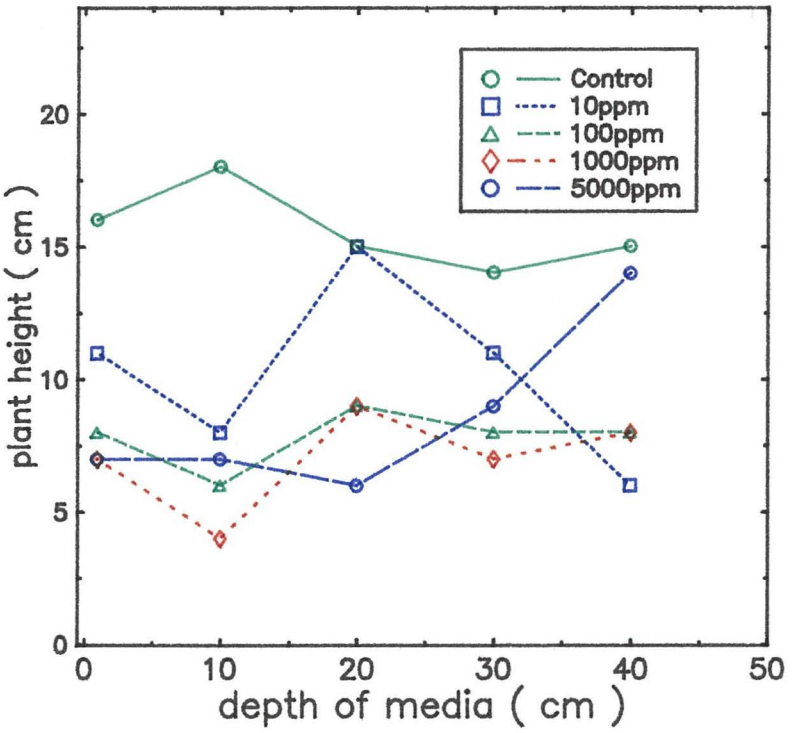


Figure 4.2 Simazine bioassay – Effect on rye grass plant height of varying rates of simazine down the profile (No Flush).

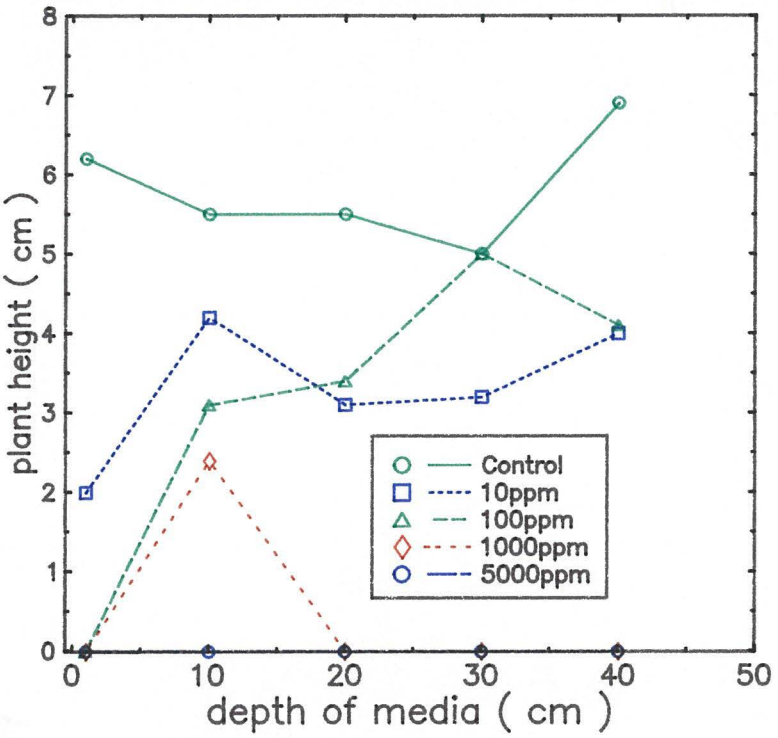


Figure 4.3 Simazine bioassay – Effect on radish plant height of varying rates of simazine down the profile (Flushed with water).

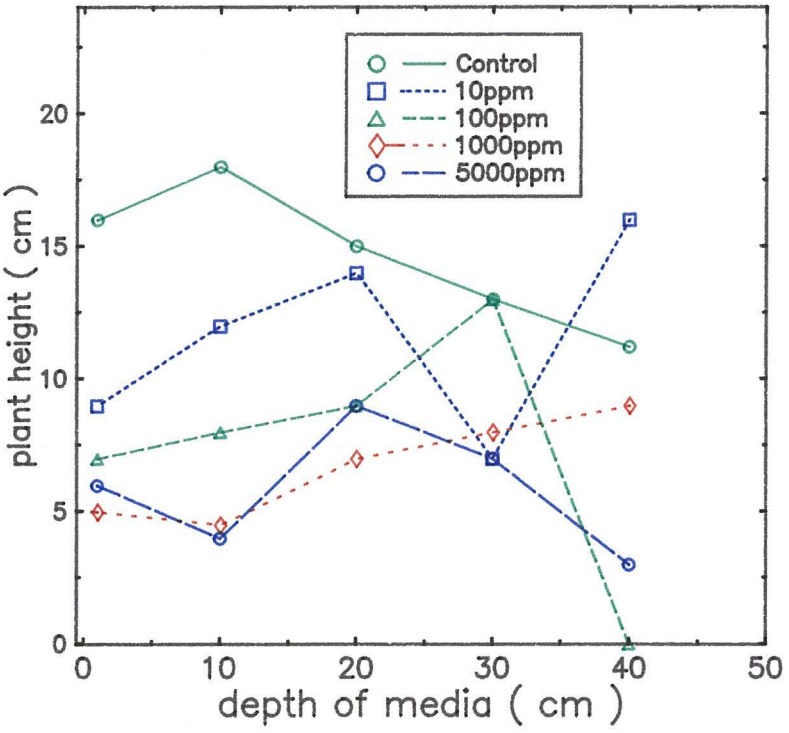


Figure 4.4 Simazine bioassay – Effect on rye grass plant height of varying rates of simazine down the profile (Flushed with water).

4.3: Results.

Radish seedling height was reduced relative to the Control by all concentrations of Simazine, with the reduction increasing with concentration, and no growth at 5000ppm (the normal orchard herbicide application rate) see Figure 4.1 and 4.3. Whilst there was evidence of greater reduction at the surface, it appeared that some simazine had penetrated the medium to full depth of 40 cms.

Rye grass seedling height was reduced by all concentrations, but this species appeared more resistant to the effects of simazine than radish, with little increase in effect over 100ppm, and growth did occur at 5000ppm, see Figure 4.2 and 4.4. There was little evidence, except with some of the higher concentrations in the rye grass bioassay, that flushing had moved the simazine further down the profile.

4.4: Discussion.

When simazine was washed into the surface of the peat-sand medium, and a 50cm core tested for herbicide residue by bioassay it was found a rate of 10ppm reduced growth by an average of about 50% for the radish, and with some variability, somewhat less for the rye grass. Growth reduction occurred through the whole profile and it was not, at 10ppm, increased by flushing. This suggested that at this rate simazine was adequately mobile in the profile. Hence it was considered that roots of the pear would be exposed to simazine following the surface application of a rate of 10ppm and this concentration was applied to the potted 'Doyenne du Comice' trees in the main experiment.

CHAPTER 5

Developmental morphology of floral buds in 'Doyenne du Comice' pear trees.

5.1: Introduction

A study was made using the scanning electron microscope of spur bud development of 'Doyenne du Comice' pear trees around the expected period of flower evocation and the early stages of differentiation. The objectives were to observe the morphological changes in the vegetative spur bud o a floral bud and compare to bud development between different treatments.

5.2: Materials and methods.

Plant material was collected from 3 year-old experimental 'Doyenne du Comice' at the Massey University Orchard and 7 year-old 'Doyenne du Comice' in Wanganui. Spur buds were collected from 'Doyenne du Comice' trees at Massey on the 19.11.90, 14.12.90, 8.1.91, and 11.2.91, and from Wanganui on 11.2.91.

Spur buds on short shoots less than 10 cm in length were chosen for dissection. The selected buds were those thought most likely to become floral, and were generally plump and of large size.

Buds were harvested, placed in distilled water to reduce desiccation, and taken for immediate dissection under an Olympus (20 times magnification) dissecting microscope. Bud scales were removed one by one until the bud apex was revealed, at which point they were immediately placed into Karnovsky's fix solution for at least 24 hours. The buds were then stored at 4°C.

After washing in 3 changes of phosphate buffer (0.1M; pH 7.0), at 30 minutes per change, the buds were dehydrated in an ethanol series (20% steps, 30 minutes per step from 20% to a maximum of 95%). They were then stored in 70% EtOH at 4°C. Specimens were mounted and dried by critical point drying and finally coated

with gold/palladium in a Magnetron Coater. The bud apices were viewed and photographed with an accelerating voltage of 8kV on a JEOL JSM 35C scanning electron microscope. Three buds were examined from each treatment on each of the sampling dates.

5.3: Results : Development of the spur floral bud.

The first examinations using buds collected on 19.11.90 of the spur bud apical meristems of 'Doyenne du Comice' revealed bud scales (see 1 in Plates 5.1 - 5.5) being initiated from the apex in a spiral sequence. Bud scales appeared typically rounded at the top and folding down to enclose the subsequent scale. Doming of the apex was not visible in any buds of the 5 treatments at 19.11.90.

Buds collected on 14.12.90, which was the second examination, revealed bud scale and leaf development in all treatments (see 1 and 2 Plates 5.6 - 5.10). The bud apex had flattened in the Control (see 3 in Plates 5.11 and 5.12) and the Simazine treatment (Plate 5.14) collected on 8.1.91., which shows that evocation has occurred. The apex was not visible in the other three treatments on 8.1.91 due to the leaves, which had not been removed adequately enough to expose the apex (Plates 5.13, 5.15 and 5.16).

Because of the difficulty experienced with buds collected on 8.1.91 of removing leaves to reveal the apex, a longitudinal section was made down the axis of the buds collected at the fourth harvest (11.2.91). This revealed the differentiating flowers amongst the bracts (4), where (5) is the terminal flower, and (6) lateral flowers. The Paclobutrazol treatment specimens were not suitable for mounting due to damage in preparation and no Plates were taken.

Buds harvested from mature trees in Wanganui on 11.2.91 also proved difficult to dissect out, (Plate 5.22) due to the plant tissue being soft. Flower bud development was well advanced at

this date showing terminal and lateral flowers and bracts and was generally at a similar stage of development to the experimental trees in Palmerston North.

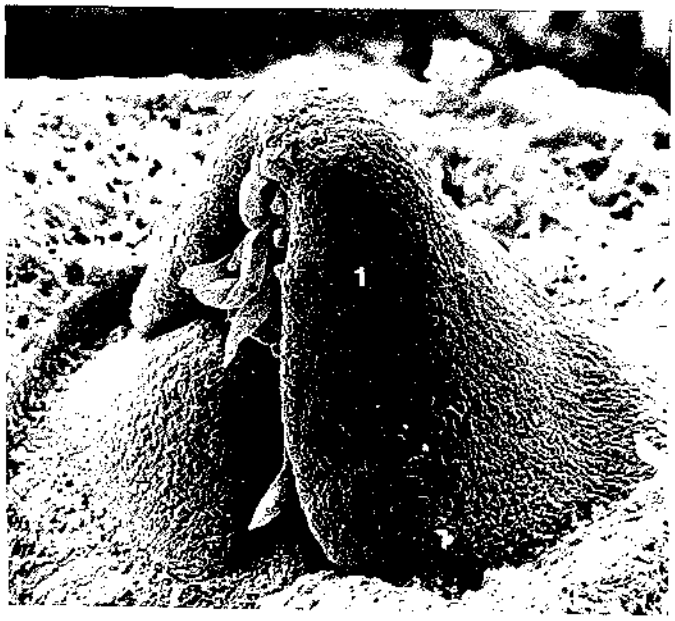


Plate 5.1 Control treatment 19.11.90 bud scales(1) (mag. * 270).

Plate 5.2 Ammonium treatment 19.11.90 bud scales(1) (mag. * 290).

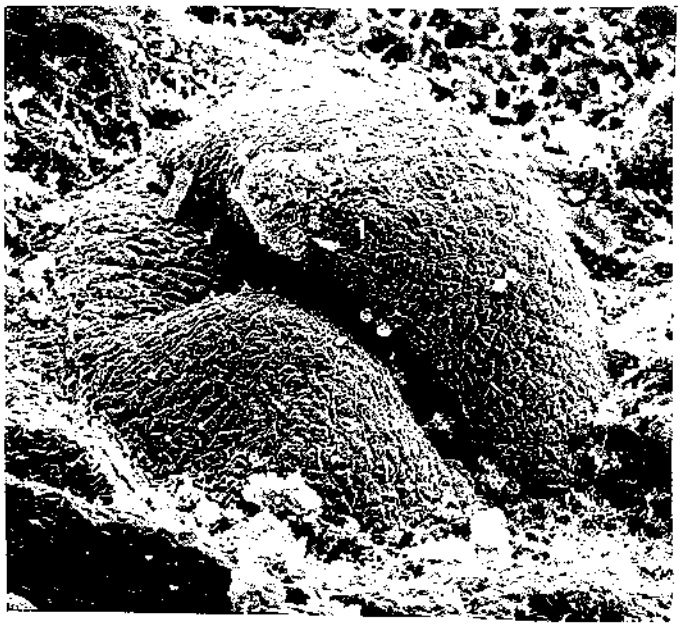


Plate 5.3 Simazine treatment 19.11.90 bud scales(1) (mag. * 370).

Plate 5.4 Paclobutrazol treatment 19.11.90 bud scales(1) (mag. * 330).

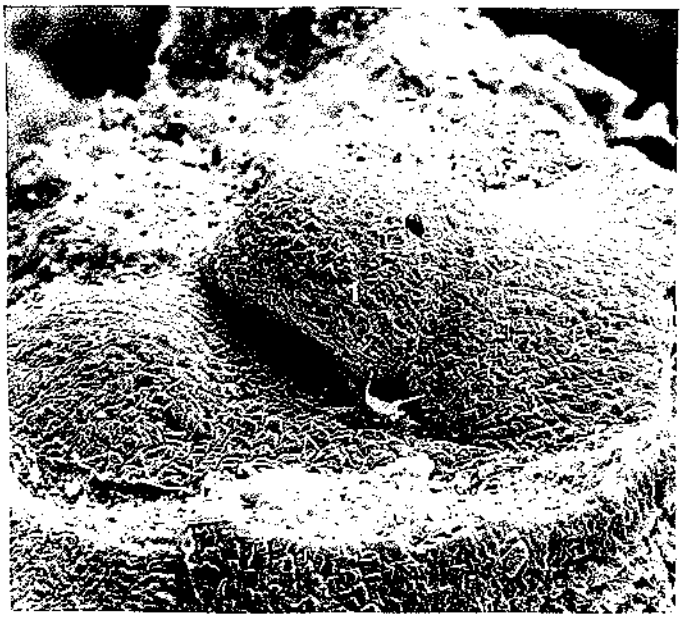


Plate 5.5 R.D.I treatment
19.11.90 bud scales(1)
(mag. * 360).

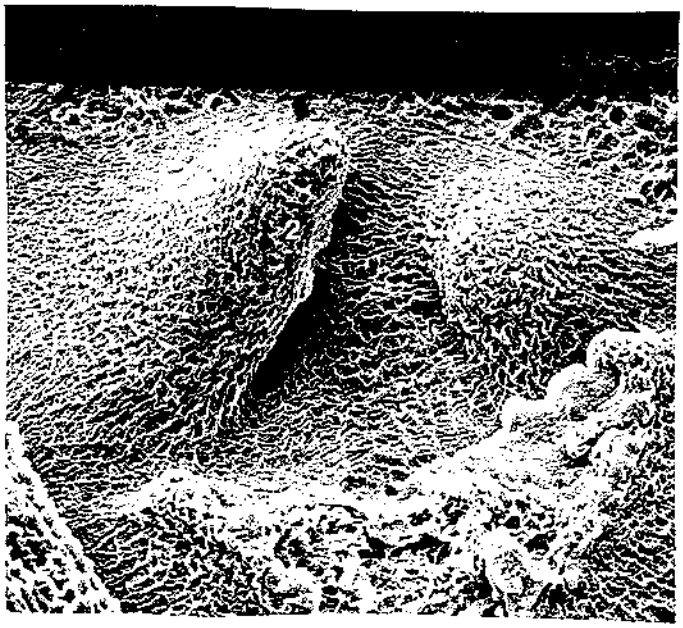


Plate 5.6 Control treatment
14.12.90 leaves(2)
(mag. * 350).

Plate 5.7 Ammonium treatment
14.12.90 leaves(2)
(mag. * 170).

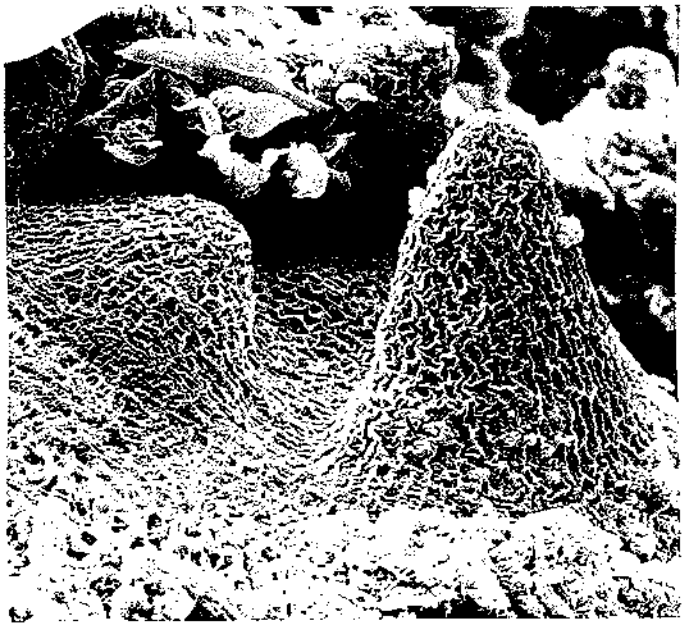


Plate 5.8 Simazine treatment
14.12.90 leaves(2)
(mag. * 400).

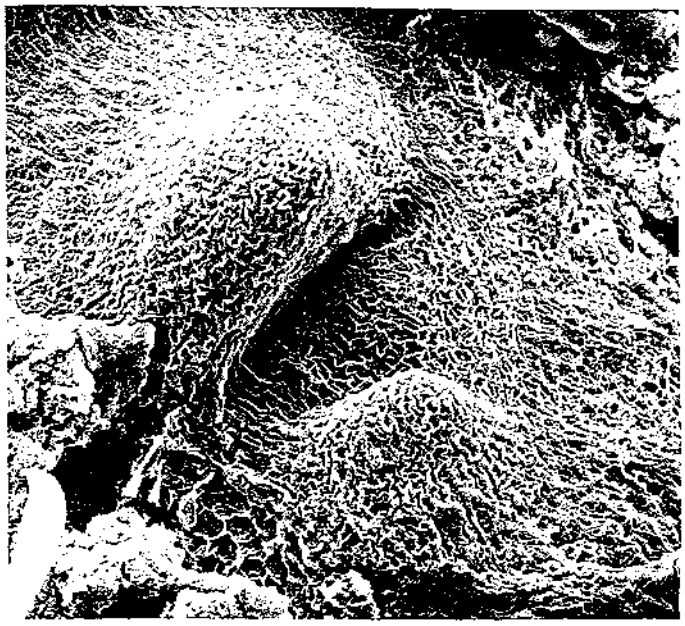


Plate 5.9 Paclobutrazol treatment
14.12.90 leaves(2)
(mag. * 360).

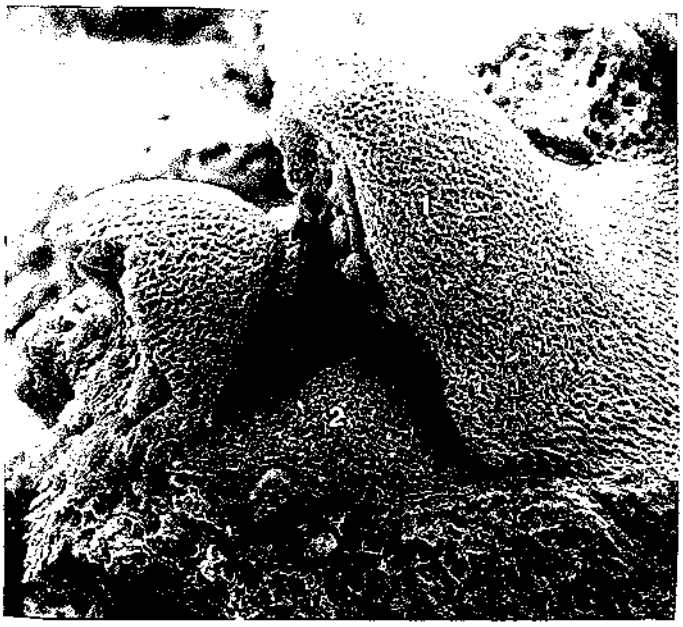


Plate 5.10 R.D.I treatment
14.12.90 bud scales(1)
leaves(2) (mag. * 270).

Plate 5.11 Control treatment
8.1.91 leaves(2)
domed apex(3) (mag. * 150).

Plate 5.12 Control treatment
8.1.91 top view of 5.11
(mag. * 181).

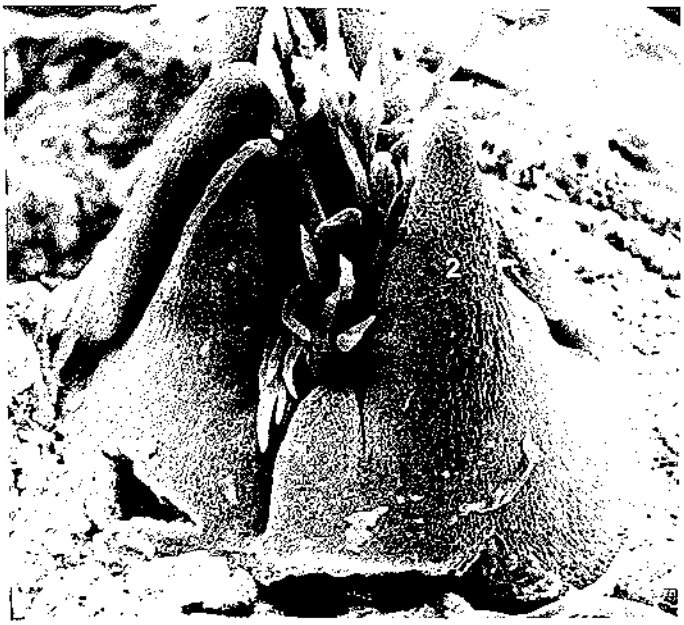


Plate 5.13 Ammonium treatment
8.1.91 leaves(2)
(mag. * 139).

Plate 5.14 Simazine treatment
8.1.91 leaves(2)
(mag. * 310).

Plate 5.15 Paclobutrazol
treatment leaves(2)
8.1.91 (mag. * 87).

Plate 5.16 R.D.I. treatment
8.1.91 leaves(2)
(mag. * 95).

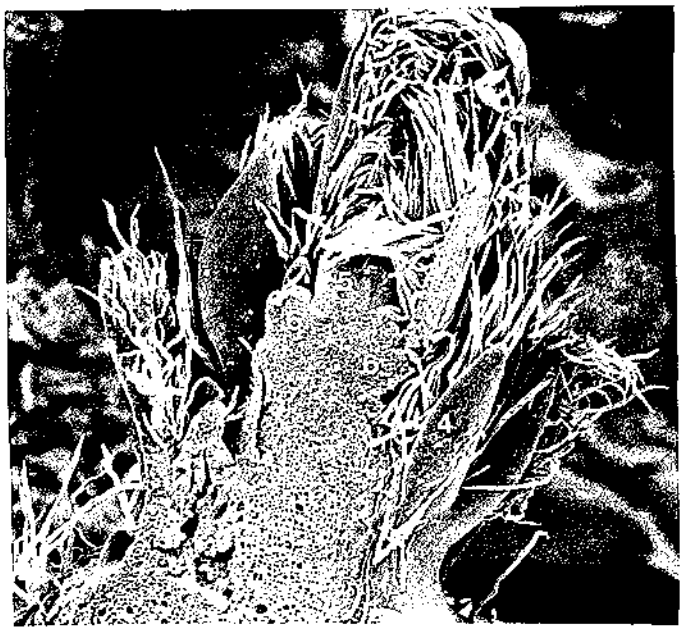


Plate 5.17 Control treatment terminal flower(5) lateral flower(6) 11.2.91 bract(4) (mag. * 40).

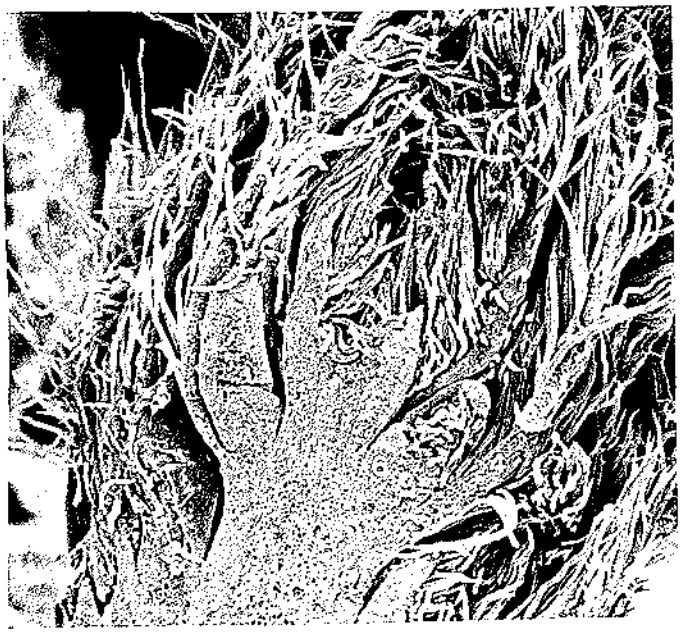


Plate 5.18 Ammonium treatment 11.2.91 bract(4) lateral flower(6) (mag. * 52).

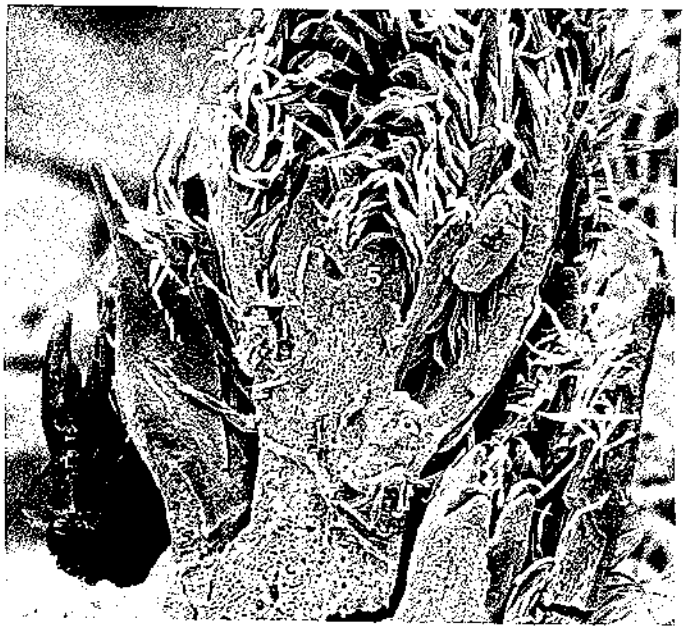


Plate 5.19 Simazine treatment terminal(5) and lateral flower(6) bract(4) 11.2.91 (mag. * 54).

Plate 5.20 R.D.I treatment terminal flower(5) and bract(4) 11.2.91 (mag. * 52).

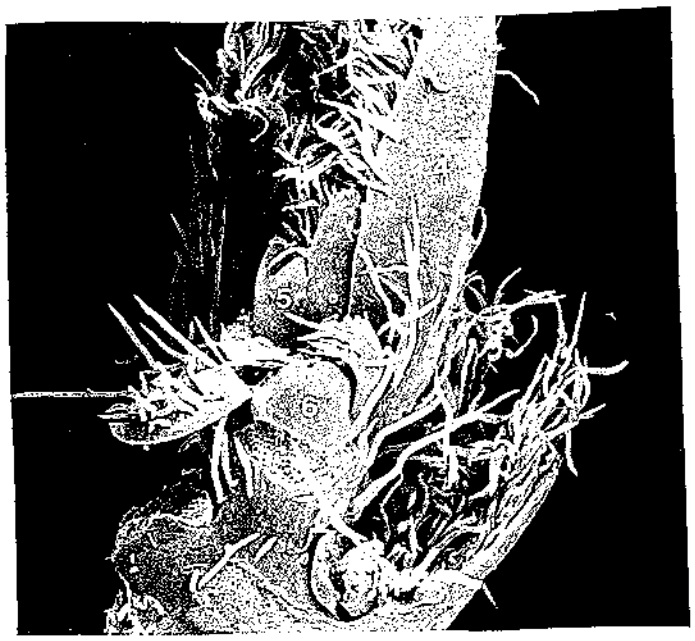


Plate 5.21 From 7 year old 'Doyenne du Comice' from Mr Woods orchard at Wanganui, terminal(5) and lateral flowers(6) 11.2.91 (mag. * 47).

5.4: Discussion

The sequence of differentiation at the apex begins with bud scales development followed by transitional leaves, true leaves (giving with transitional leaves 8-9 in all) and then bracts (3-4). The small bud in the axil of the true leaf is vegetative, while all the others are flower buds McLaughlin and Greene (1991).

The findings of Bergh (1985), who examined the spur buds of apple trees on 3 occasions at similar dates to those in the experimental trees found bud scale development was well advanced on 11 December (in South Africa), which was his first date of examination. The apex had flattened out by 7 January, his second examination. On his third examination at 15.1.91 he found that the terminal flower had begun to differentiate showing sepal primordia, and lateral flowers were beginning to differentiate.

The results obtained with 'Doyenne du Comice' spur buds are consistent with the literature with respect to the morphological development in pipfruit, although the development was about 2 weeks behind Bergh (1985). The number of leaves initiated were not counted in this study and so the first sign of floral differentiation was the doming of the apex. The apex became domed in Control and the Simazine treatments before 8.1.91, and this dome flattened out after 8.1.91, as reported by Dheim (1988), and Bergh (1985). The flattening of the apex is possibly due to the start of flower primordia forming on the edge of the dome. The Ammonium, Paclobutrazol and the R.D.I. treatments all show well advanced leaf development.

Leaves differed in appearance from bud scales, the leaves being more pointed than the budscales and appeared notched at either side, (see 2 in Plates 5.7, 5.11 and 5.12). As leaves developed, so too did the associated bud hairs which surround the developing leaf and they were abundant at the last harvest (see Plate 5.15). Numerous leaves were present on 8.1.91 and flower differentiation was well advanced on 11.2.91.

Flowering development progressed in similar stages for all treatments throughout the period of monitoring with the exception of the Ammonium treatment which was slightly earlier in developing leaves than other treatments. Terminal flowers were well developed by 11.2.91 in all treatments, but could not be seen in the Paclobutrazol treatment, which had no result.

The development of the buds taken from 7 year old trees at Wanganui was at a similar stage to the experimental tree buds harvested on 11.2.91, showing developing lateral flowers within the protection of bracts. Thus the flower development process appears not to be greatly affected by the age of the tree.

5.5: Conclusion.

The photographs taken of morphological changes that occur at the bud apex show flower structures forming in the spur bud. The changes occurred in an ordered and sequential manner, and at times consistent with other Southern Hemisphere observations of pipfruit.

Summary of developmental stages at the apex of spur buds observed in 'Doyenne du Comice':-

Differentiation of bud scales and leaves	between 19.11.90 and 14.12.90
Apex became domed (evocation)	early January between 14.12.90 and 8.1.91
Differentiation of terminal flower	between 8.1.91 and 11.2.91
Lateral flowers and sepals	11.2.91 onwards.

CHAPTER 6

Assessment of amino acids, ammonium and nitrate in samples of leaves and buds, of the experimental 'Doyenne du Comice' pear trees.

6.1: Introduction.

The role of the amino acid arginine in the formation of flowers has been reviewed. In this study the levels of free arginine and total free amino acids were monitored in the leaves and spur buds of the potted experimental trees. The levels of free ammonium and nitrate in the leaves were also monitored. Ammonium is the precursor for arginine, and ammonium and nitrate were the alternative forms of available nitrogen supplied to the trees.

6.2: Methods and materials.

Analyses were performed mainly on spur leaves to limit the number of spur buds harvested from the small trees. Limitations to volume of freeze drying and handling necessitated the selection of as few leaves as practicable with minimal variability. Two leaves were harvested per tree from eight blocks giving a total of sixteen leaves collected per treatment for these analyses. The leaves chosen for sampling were recently matured spur leaves. From the sample of sixteen leaves only 1 subsample was taken for analysis due to the time consuming procedure of extraction.

Two harvests of spur buds were also carried out during the season to monitor the levels of amino acids at the point of most significance - the spur bud, where flowers would be developed. These harvests were carried out on 11.12.90 and 15.1.91. The leaf harvests were carried out on five occasions between 27.11.90 and 15.1.91.

Leaves and buds were cut from the trees and placed immediately into liquid air. These leaves were then held in cold storage (-4°C) until placed on the freeze drier, usually within 1 hour. The buds were treated in the same way as the leaves. For a minimum of 24 hours the leaves and buds were dried and then stored in the freezer, for not longer then 2 weeks. Leaves and buds were then pulverized by pestle and mortar to a fine powder.

6.2.1: Assessment of Amino Acids.

A 50 mg sample of the powder was weighed directly into a 50 ml centrifuge tube. Non-protein amino acids were extracted from the leaf and bud material by a modified method of Labanauskas and Handy (1971). A standard of 100 nanomoles BCM amino acid solution, which is a solution containing a known concentration of a wide range of amino acids, was also taken through the following recovery and analysis procedure to determine percentage recovery of the amino acids.

A mixture of 10 mls of water, chloroform ($CHCl_3$), and methanol (CH_3OH), (7 : 8.7 : 17.3 v/v/v) was added to the sample, mixed thoroughly, and allowed to stand for 30 min. Then 3 mls of water was added, stirred and the mixture allowed to stand for 5 min. An additional 2 mls of chloroform was stirred into the mixture which after 5 more minutes, was centrifuged at 3000 rpm. for 10 min. The water layer was then transferred to a flask. Then a further 4 mls of water was added to the chloroform layer in the centrifuge tube, stirred then centrifuged for 10 minutes at 3000 rpm. The water layer was then transferred to the flask containing the initial water removed from the sample.

For each sample, an 8 cm bed of Dowex resin 50 W-X 4 (50-100 mesh) was gravity-packed with water in a 1 x 40 cm column, a piece of cotton wool being used to retain the resin. The aqueous mixture was then poured through the resin, followed by a rinse of 10 mls water.

and the accumulated effluent discarded. A scintillation vial was then placed under the column, and the amino acids eluted from the column into the vial with six mls of 25% diluted 7 mol/l NH_4OH (ammonium hydroxide) followed by a 10 ml water wash.

The vials were removed from the column, the solution frozen and dried on a lyophilizer. The residue was dissolved in 2 ml of loading buffer (0.2 M Na citrate) and adjusted to pH 2.2, filtered into a vial and capped ready for analysis. The amino acid analysis was carried out on a Pharmacia 4150 alpha plus column, through an ion exchange resin.

6.2.2: Assessment of Ammonium and Nitrate.

The method of extraction and determination of ammonium and nitrate nitrogen was similar to that outlined by Rabe and Lovatt (1986). Following extraction with KCl, free ammonium (measured as the combined free NH_3-NH_4^+ pool) and nitrate were quantified spectrophometrically on a Technicon AutoAnalyser.

Each 200 mg (dry weight) sample of the powder prepared as above was measured into a 50 ml tube and 50 ml of 1M KCl was added and rotated for one hour to allow the ammonium and nitrate to be released. The sample was then filtered through Whatman 42 grade filter paper which had been selected for its very low content of ammonium. The absorbance of the sample at wavelength 590 nanometers was determined and compared with a standard. Only leaf samples were analyzed for ammonium and nitrate as there was not enough bud dry weight sample left from the amino acids analysis.

6.3: Results.

6.3.1: Free arginine and total free amino acids in leaves and buds.

Results are presented in Tables 6.1 - 6.6. Total free amino acids included up to sixteen other amino acids. Free arginine was of particular interest but was not always detected in the samples. The percentage recovery figures of the standard mixture of amino acids through the analysis process showed that an average over 3 runs of 93.6% of the arginine, and an average of total free amino acid of 95.6%.

Table 6.1. Influence of the treatments applied on leaf free arginine concentration (µg/g) of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
27.11.90	0	0	0	0	0
11.12.90	103.0	7.2	3.1	69.5	22.3
18.12.90	0	4.2	6.5	5.4	1.7
3. 1.91	0	89.5	1.9	4.4	6.9
15. 1.91	4.0	6.0	83.0	5.3	4.4

Table 6.2 Influence of the treatments applied on total free amino acids in the leaves (µg/g) of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
27.11.90	1688.5	3136.1	1119.8	1324.9	1220.0
11.12.90	1533.4	1659.6	725.8	1096.7	985.0
18.12.90	8103.3	14063.0	7201.8	9849.0	11479.3
3. 1.91	1940.5	1105.1	483.5	1382.5	838.7
15. 1.91	595.2	751.9	718.9	506.1	757.0

Table 6.3 Leaf free arginine expressed as percentage of total free amino acids in the leaves.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
27.11.90	0	0	0	0	0
11.12.90	6.7	0.4	0.4	6.3	2.3
18.12.90	0	0	0	0	0
3. 1.91	0	8.1	0.4	0.3	0.8
15. 1.91	0.7	0.10	11.5	1.0	0.6

Table 6.4 Influence of the treatments applied on bud free arginine concentration (µg/g) of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
11.12.90	206.0	181.0	91.3	161.0	70.9
15. 1.91	4626.9	4178.9	1417.0	1275.5	2819.6

Table 6.5 Influence of the treatments applied on bud free total amino acids concentration (µg/g) of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
11.12.90	4833.4	4156.0	3604.7	1439.8	2052.9
15. 1.91	15925.0	12399.7	4235.5	4152.5	7532.1

Table 6.6 Bud free arginine expressed as percentage of total free amino acid in the buds.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
11.12.90	4.3	4.4	2.5	11.1	3.4
15. 1.91	29.1	33.7	33.5	30.7	37.4

Arginine was not found in the leaves on 27.11.90 but was present at the other 4 sampling dates (Table 6.1). Leaf arginine levels varied considerably with both treatments, and sampling date. In the control plants a peak level was found on 11.12.90, however levels dropped off after this date. The Ammonium treatment showed a peak level in arginine on 3.1.91, where ammonium treatments had begun on 24.11.90, some 41 days earlier. The Ammonium treatment arginine level fell to previous low levels after 15.1.91.

The Simazine treatment showed a large rise in arginine on 15.1.91, which was about 53 days after treatments were applied for the first time. The Paclobutrazol treatment showed a large rise in arginine on 11.12.90, which was 9 days after first Paclobutrazol application, after which the level dropped. The R.D.I. treatment showed a moderate rise in arginine level on 11.12.90, which was 7 days after the R.D.I. treatment began, and again the level fell

after this date. The Paclobutrazol and the R.D.I. treatments, reached peak levels at the same sampling date as the Control, whilst both Ammonium and Simazine treatments delayed the peaks.

Leaf total free amino acids (Table 6.2) in all treatments generally varied considerably between dates during the season, however between treatments there was generally not as much variation. After 27.11.90 the total amino acid levels in all treatments generally dropped before rising to a peak on 18.12.90. After this date levels in all treatment generally dropped. Generally the peaks in total amino acids did not correspond to the peaks in arginine and on 18.12.90 when all treatments showed the highest levels of total amino acids, there were no peaks recorded in any treatment for arginine.

Table 6.3, arginine as a percentage of total amino acids in the leaves, shows exactly the same peaks as Table 6.1. Perhaps the most notable observation from this method of expressing the results is that the absolute peaks of arginine are still peaks relative to total amino acids.

Table 6.4 shows arginine levels in the buds showed higher levels than the leaves especially on 15.1.91 sampling date. On both occasions the control levels were the highest. The levels of bud total free amino acids increased between 11.12.90 and 15.1.91 for all treatments (Table 6.5) as did bud arginine (Table 6.4) and percentage bud arginine (Table 6.6).

Bud samples on 11.12.90 showed the highest percentage arginine in the Paclobutrazol treatment. Other treatments showed percentage arginine values at about 1/3 that of the Paclobutrazol treatment, (Table 6.6). On 15.1.91, the highest recorded percentage bud arginine was the R.D.I. treatment, with other treatments recording similar levels.

6.3.2: Free ammonium and nitrate in the leaves.

Results are shown in Tables 6.7 - 6.8 and Figures 6.1 and 6.2.

Table 6.7 Influence of the treatments applied on free ammonium levels in the leaves (µg/g) of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
27.11.90	111.3	138.8	100.0	98.1	106.3
11.12.90	121.3	131.3	93.8	98.1	100.0
18.12.90	93.8	123.8	111.3	106.3	106.3
3. 1.91	125.0	100.0	104.4	100.0	100.0
15. 1.91	98.8	109.4	101.5	112.5	113.8

Table 6.8 Influence of the treatments applied on free nitrate levels in the leaves (µg/g) of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
27.11.90	11.9	5.6	10.0	8.1	8.1
11.12.90	0.6	0.6	5.6	5.6	5.6
18.12.90	8.1	0.6	0.6	8.1	4.9
3. 1.91	0.6	4.9	4.9	3.8	3.8
15. 1.91	3.8	6.9	5.9	5.6	5.6

Tables 6.7 and 6.8 and Figures 6.1 and 6.2 show that the Ammonium treatment in December contained a higher level of ammonium and a lower level of nitrate compared to the Control but in January these differences were less clear. The other treatments showed similar

and relatively uniform levels of ammonium.

Generally the levels of nitrate recorded were low compared with the ammonium levels and showed more variability between sampling dates. Although the nitrate levels were lower (by a factor of between 10 and 100) than the ammonium levels, in the Control when one is high the other is low.

Table 6.9 Influence of the treatments applied on percentage of dry weight of total free nitrogen in leaves of the experimental 'Doyenne du Comice' trees.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I
27.11.90	0.2	0.3	0.1	0.1	0.1
11.12.90	0.2	0.2	0.1	0.1	0.1
18.12.90	0.8	1.4	0.7	1.0	1.2
3. 1.91	0.2	0.1	0.1	0.2	0.1
15. 1.91	0.1	0.1	0.1	0.1	0.1

Table 6.9 shows the combined totals of the free amino acids and ammonium and nitrate, expressed as a percentage of dry weight. All treatments and Control generally show similar figures at each date and all show a peak on 18.12.90.

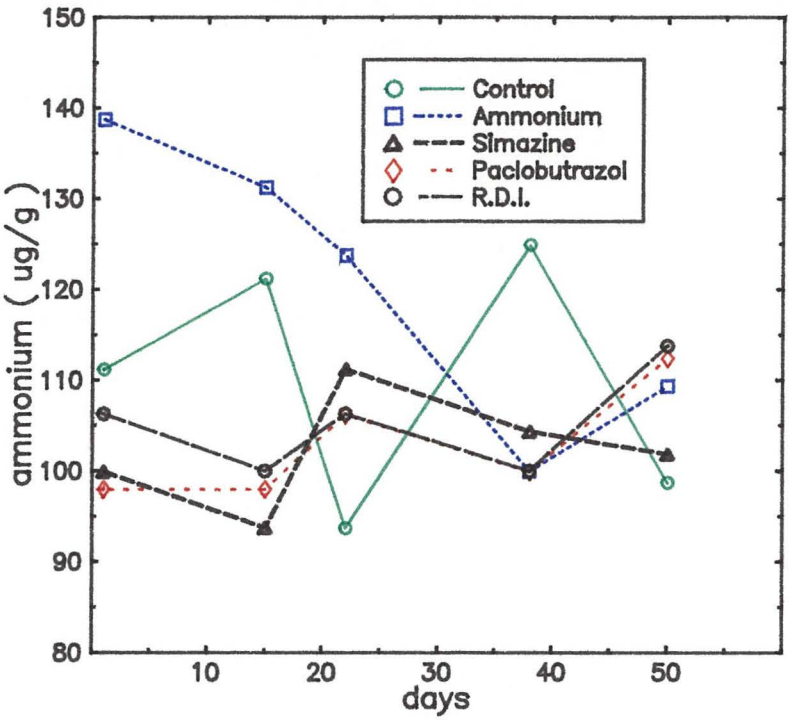


Figure 6.1 Influence of the treatments applied on ammonium levels in the leaves of the experimental 'Doyenne du Comice' trees. (day 1 = 27.11.90).

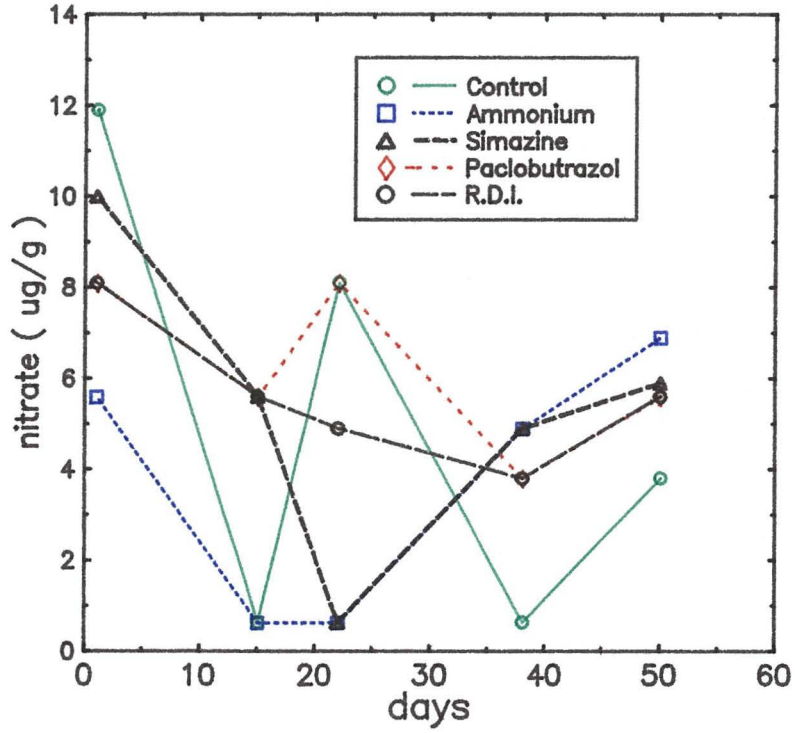


Figure 6.2 Influence of the treatments applied on the nitrate levels in the leaves of the experimental 'Doyenne du Comice' trees. (Day 1 = 27.11.90).

6.4: Discussion.

6.4.1: Arginine and total amino acid content.

No literature concerning research done on the free nitrogen levels in pear trees or any other pipfruit has been found. This is because pipfruit trees are not used as a source of amino acids by biochemists and associated disciplines. Harada *et. al.* (1968) found levels of total free nitrogen in tomato leaf to be of the same order as the results presented in this study. In plants that had been given 15ppm ammonium for 28 days, 0.63% total free nitrogen was recovered. In plants that were given 15ppm nitrate for 28 days, 0.34% total free nitrogen was recovered (total hydrolysed nitrogen was 3.0% for 15ppm ammonium treatment and 3.82% for the 15ppm nitrate treatment)

Total nitrogen by hydrolysis using the Kjeldahl method of extraction was not used in this study because it does not reveal the treatment effects on the arginine content. Measurement of free nitrogen should reveal if the treatments succeeded in raising the level of ammonium and therefore arginine in the plant. Because ammonium and arginine are part of the pool of nitrogenous forms in plants (where nucleic acid and protein make up the vast majority of total hydrolysed nitrogen), then a rise in their levels would not contribute much to the total free nitrogen and hence total hydrolysed nitrogen status of the plant. The proportion of free nitrogen in its various forms as compared to total nitrogen in the plant is small because the process of conversion from its free state to more stable proteins is rapid and therefore the pool of free nitrogen can vary very quickly.

Until 18.12.90. the Ammonium treatment recorded a total free nitrogen level greater than the Control. Generally the total free nitrogen in the Ammonium treatment was higher than the Control, and the other three treatments were generally a little lower.

The percentage recovery of Arginine through this extraction procedure was very good as shown by the recovery from the standard BCM mixture, with 93.6% arginine being recovered and 95.6% total amino acid recovery. Assuming the amino acids levels were not altered by initial leaf and bud the preparation, the levels recorded for leaf and bud amino acids should closely reflect the concentrations present in the plant.

A loss of 3 - 4% through the extraction procedure shows that the results presented do not reflect exactly the levels of total free amino acids and arginine. The variability of the results is unlikely to arise solely from the extraction procedure as the standard BCM amino acid mixture entered the procedure at the point of addition of the first extraction solutions. However some variability in the data may have been caused by delays between harvesting of the plant material and its subsequent placement into liquid air, even though the samples were quickly frozen for storage. As the method of extraction was very time consuming there was no replication of samples analyzed, and so no statistical analysis.

Another reason for the variable nature of the results may lie in natural processes within the plant tissue. Only free amino acid content was measured, and conversion to more bound forms in proteins may cause the levels in the plant to vary rapidly. The variability in the data may reflect the plant's changing requirements for nitrogenous substrate, where the free forms of nitrogen act as a reserve for plant use. As the plant changes from a vegetative to a floral state the requirement for nitrogenous substrate is likely to change and the data may be reflecting this variability.

Low levels of free arginine relative to total free amino acids were extracted from both leaves and buds of the 'Doyenne du Comice'

during the period of sampling. However higher levels of arginine were found in the buds than in the leaves. The levels of arginine in the buds on the two sampling dates did not show any correlation to arginine levels in the leaves on the same dates (Table 6.1 and 6.4).

In seeking literature referring to arginine levels in pipfruit leaves, the closest found concerned citrus which was studied by Lovatt and Cheng (1984). They obtained arginine from the leaves of citrus by the same method as arginine was extracted from the 'Doyenne du Comice' trees. Levels of arginine ranged between 62 and 88 µg/g for four varieties of the citrus where phosphorus was not deficient. The levels increased to between 178 and 308 µg/g when phosphorus was deficient. The levels of arginine in 'Doyenne du Comice' by comparison to citrus were generally low in the leaves, except for the peaks which occurred occasionally in each of the treatments, when the latter were comparable.

Whilst the highest level of arginine in leaves was recorded on 11.12.90 in the Control, the other treatments generally increased the level of leaf arginine by admittedly small amounts at all sampling dates after this date. The 11.12.90 was 17 days after the Ammonium and Simazine treatments began, 9 days after the Paclobutrazol treatment began and 24 days after the R.D.I. treatment began.

The Ammonium treatment gave higher levels of total amino acids in the leaves (except on 3.1.91) than the Control, and indeed than most other treatments. In leaves however there was no clear trend over the period of sampling. In the buds, levels of both arginine and total amino acids were higher than in the leaves at the same dates, and as a percentage of total amino acids arginine was higher in buds than in leaves. In all the treatments the bud arginine levels rose considerably on the later sampling date, the reverse of what happened in the leaves. It is difficult to see any association

between treatment and levels of arginine or total amino acids in the buds.

During December the arginine in the buds was around 4 percent of total amino acids and rose to around 30 percent in mid January. O'Neal and Taylor (1972), found that the *de novo* arginine biosynthesis pathway was slow in vascular plants. Important changes relating to flower differentiation occur in January, as shown by the scanning electron microscope pictures in Chapter 5, so the presence of arginine may become more important. Polyamines, which accumulate as a result of the presence of arginine, appear to be important constituents influencing the change to floral initiation, as found by Edwards (1986).

6.4.2: Ammonium and Nitrate.

The consistency of the ammonium data relative to the varying arginine levels shows that there is generally not any correlation between these either with the treatments or dates of sampling (Table 6.1, 6.7).

Overall the ammonium levels are relatively consistent as compared to the arginine and total amino acids levels. Initially the ammonium levels in the Ammonium treatment were high compared to the Control and other treatments. The highest recorded level occurred at the start of the sampling period on 17.11.90, which was 3 days after the first ammonium application. In January the levels dropped off to more 'typical' levels (Table 6.7). The Control ammonium levels fluctuated throughout the sampling dates whereas the other treatments showed less variation. The Simazine, Paclobutrazol and R.D.I. treatment showed similar trends to each other during the sampling period.

The ammonium levels in the leaves were higher than the nitrate levels throughout all the treatments. The levels of ammonium in the

leaves were very low in comparison with other crops such as citrus recording levels of between 500 - 700 µg/g, Lovatt *et. al.* (1988). The highest level of ammonium (138.8 µg/g) was recorded in the ammonium treatment on 27.11.90, and these readings generally decreased throughout the period of sampling. The water stress treatment did not increase the levels of leaf or bud ammonium dramatically in contrast to the effects as reported by Lovatt *et. al.* (1988) in citrus.

Lovatt *et. al.* (1988) found that ammonium levels in citrus leaves were variable when water stress treatment was applied. They recovered 519 µg/g dry weight ammonium from the leaves of the control. Following water stress of -3 MPa for 20 days, then 40 days of -20 MPa they recovered 728 µg/g dry weight. This exceeds the values obtained from the Control 'Doyenne du Comice' by about 400 µg/g, though the sampling procedure was similar. From the water stress treatment of the citrus were recovered about 600 µg/g more ammonium than from the water stress treatment of the 'Doyenne du Comice'. The R.D.I. treatment on the 'Doyenne du Comice' did not show the same increase in leaf ammonium as did the water stress treatment on citrus.

Leaf nitrate levels were more variable than the leaf ammonium levels through the period of sampling, however the Ammonium treatment generally recorded low levels of nitrate until January, especially when its levels of ammonium were high. The levels of nitrate generally decreased through the period of sampling for all treatments, but levels increased in all treatments at the final sampling, where all treatments had been imposed for at least 4 weeks.

The low levels of nitrate could be linked to the activity of nitrate reductase and the fact that plants cannot tolerate high levels of unreduced NO_3^- in their leaves Maynard and Barker (1969).

6.5: Conclusion

There appeared to be no clear treatment effects on leaf or bud arginine, or on leaf ammonium and nitrate. No definite association of any nitrogen constituent with flower initiation was demonstrated. The results for total free nitrogen are of the same order as found in other the Tomato leaf as reported in the literature, but tend to be slightly lower. The variability in the results during the season displayed by many of the forms of measured nitrogen is not easily explained, although it may reflect the plants changing requirements for nitrogen substrate during the developmental change from vegetative to floral differentiation.

CHAPTER 7

Analysis of the responses to the Regulated Deficit Irrigation treatment.

7.1: Introduction:

During the 1990-1991 growing season, the influence of R.D.I. (Regulated Deficit Irrigation) treatment on xylem water potential was monitored. This was to quantify the degree of internal plant water deficit that the R.D.I. treatment imposed on the plants.

Changes in the total water content of each container of 'Doyenne du Comice' trees in the fully irrigated Control and the R.D.I treatment were monitored during the summer of 1990 - 1991, by the Time Domain Reflectometer (T.D.R.). This was deemed the most suitable apparatus to accurately measure moisture changes in the confined volume of growing medium. The T.D.R. produces an electrical pulse and measures the resistance to its propagation through the medium. The greater the resistance the lower the moisture content in the medium between and around the probes.

Fruit growth measurements on similar fruiting trees were used to determine when to discontinue R.D.I. and resume full irrigation. Pear fruit volume increases initially at a slow rate and after about six weeks growth increases exponentially. In an R.D.I regime irrigation is restricted during the first few weeks of the initial spring shoot growth period, and full irrigation is reinstated from the start of a rapid increase in fruit growth. In this study shoot and fruit growth measurements were used as a guide to the application of the R.D.I. programme, as reported by Mitchell *et. al.* (1984).

7.2: Methods and materials.

7.2.1: Water content in the pots.

The 30 cm T.D.R. probes were placed vertically in the sand:peat medium in the containers, Two probes 5 cm apart were used per container to indicate the moisture content (% v/v) of the whole profile.

Measurements were made weekly from 17.11.90 to 17.1.91. In addition, a comparison of moisture variation across the medium was made to examine the lateral spread of water from the emitter. The lateral spread reading were taken in 3 replicates both the R.D.I. and the Control treatments.

7.2.2: Water potential.

Tree response to reduced irrigation was assessed by weekly determination of the xylem water potential with a Scholander pressure chamber. Measurements were taken on 9 occasions from 17.11.90 to 19.1.91 during the duration of the R.D.I. treatment on plants in the control and the R.D.I. treatments.

Measurements were made on 2 leaves per plant at dawn (5.00-6.00am) and also at solar noon (12.00-1.00pm) on eight replicates of the R.D.I. and the Control treatments. Plant xylem water potential measurements were made on fully developed leaves from spurs upto 5cm long. A small plastic bag was placed over a leaf and the petiole cut with a sharp razor blade. The leaf was then immediately transferred to the Scholander pressure chamber, and xylem water potential (in Bars) determined with a moist paper tissue in the chamber.

No readings were taken if the leaves had received a shower of rain in the previous 2 hours or the leaves were excessively covered with dew so that water was running off them.

In the Control treatment (full irrigation) water was applied daily to the surface of each pot by emitter until some drainage loss occurred from the bottom of the profile. The amount applied varied according to weather conditions and monitoring of any drainage loss following irrigation was carried out every 3 days through the season. In the R.D.I. treatment irrigation was reduced to the potted trees beginning on 17.11.90. The water application to the R.D.I. trees was reduced by 75% compared to the Control application. R.D.I. continued for 57 days, until 13.1.91.

7.3: Results:

7.3.1: Water content in the pots.

Mean values for water content by date and treatment are shown in Figure 7.1. Water content decreased in both treatments as growth proceeded but was always lower in R.D.I. In the water stressed treatment, reduced water content (drying) occurred immediately R.D.I. started, and water content was usually 60 - 70% of the Control during the next 50 days to 10.1.91., but replication was not sufficient for this difference to be shown significant.

The data from the comparison of moisture variation across the medium revealed that there were only minor differences in the water content across the containers.

7.3.2: Water potential.

Water potential measurements within a treatment were averaged and treatment differences for the dawn and the noon measurements were tested by ANOVA.

Table 7.1 Mean values for dawn leaf water potential (bars) at weekly intervals from 17.11.90 - 19.1.91 by treatment.

Date	R.D.I.	Control	Significance
17.11.90	-1.78	-2.10	n.s.
24.11.90	-4.20	-3.09	*
8.12.90	-7.34	-4.53	*
15.12.90	-7.65	-4.21	*
22.12.90	-5.05	-3.89	**
5. 1.91	-9.93	-4.80	*
12. 1.91	-9.48	-5.11	*
13. 1.91	-7.58	-5.05	**
19. 1.91	-3.70	-3.19	n.s.

where n.s. = not significant (P > 0.05)
* = P < 0.05
** = P < 0.01

The dawn water potential was significantly lower than the Control in R.D.I. treatment shown by the different letter groupings except at the first and last dates when R.D.I. was started and stopped. (Table 7.1). This shows that xylem water potential was reduced predawn as a result of applying less water.

Table 7.2 Mean values for noon leaf water potential (bars) 17.11.90 - 19.1.91 at weekly intervals by treatment.

Date	R.D.I.	Control	Significance
17.11.90	-5.5	-5.7	n.s.
24.11.90	-10.1	-6.7	*
8.12.90	-10.9	-6.3	**
15.12.90	-12.4	-7.4	*
22.12.90	-12.6	-8.5	**
5. 1.91	-22.2	-11.0	**
12. 1.91	-12.8	-8.2	**
13. 1.91	-18.8	-11.4	n.s.
19. 1.91	-11.2	-11.1	n.s.

where n.s. = not significantly different ($P > 0.05$)

* = $P < 0.05$

** = $P < 0.01$

The noon water potential was significantly lower for R.D.I. shown by the different letter groupings except at the start and the end of the R.D.I. period (Table 7.2).

The water potential was lower in the R.D.I. treatment at noon than in the Control treatment throughout the sampling period, reaching the lowest water potential on day 58 (Figure 7.2), 5.1.91, (49 days after R.D.I. began). The dawn water potentials for the R.D.I. were generally only a little lower than the Control. The noon water potentials R.D.I. treatment were considerably below those of the Control trees.

Both the dawn and the noon water potential measurements showed significant treatment differences during the period of sampling. The water potential recovered with full irrigation on January 13th and within 24 hours the noon water potential showed insignificant differences compared to the Control.

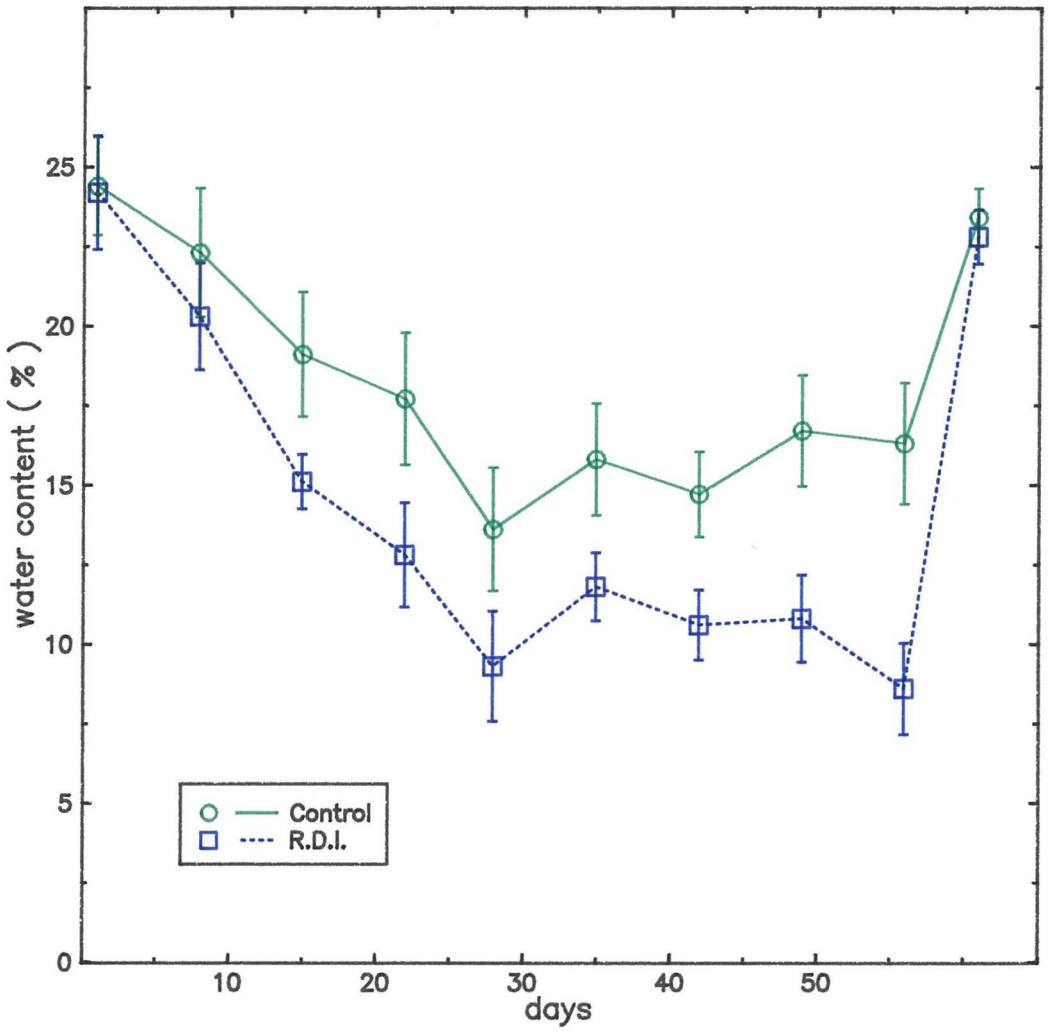


Figure 7.1 Media water content in containers of 'Doyenne du Comice' trees of Control and R.D.I. treatments, where day 1 and start of R.D.I. = 17.11.90, (1990–1991). Vertical lines represent standard error of the mean at each data point.

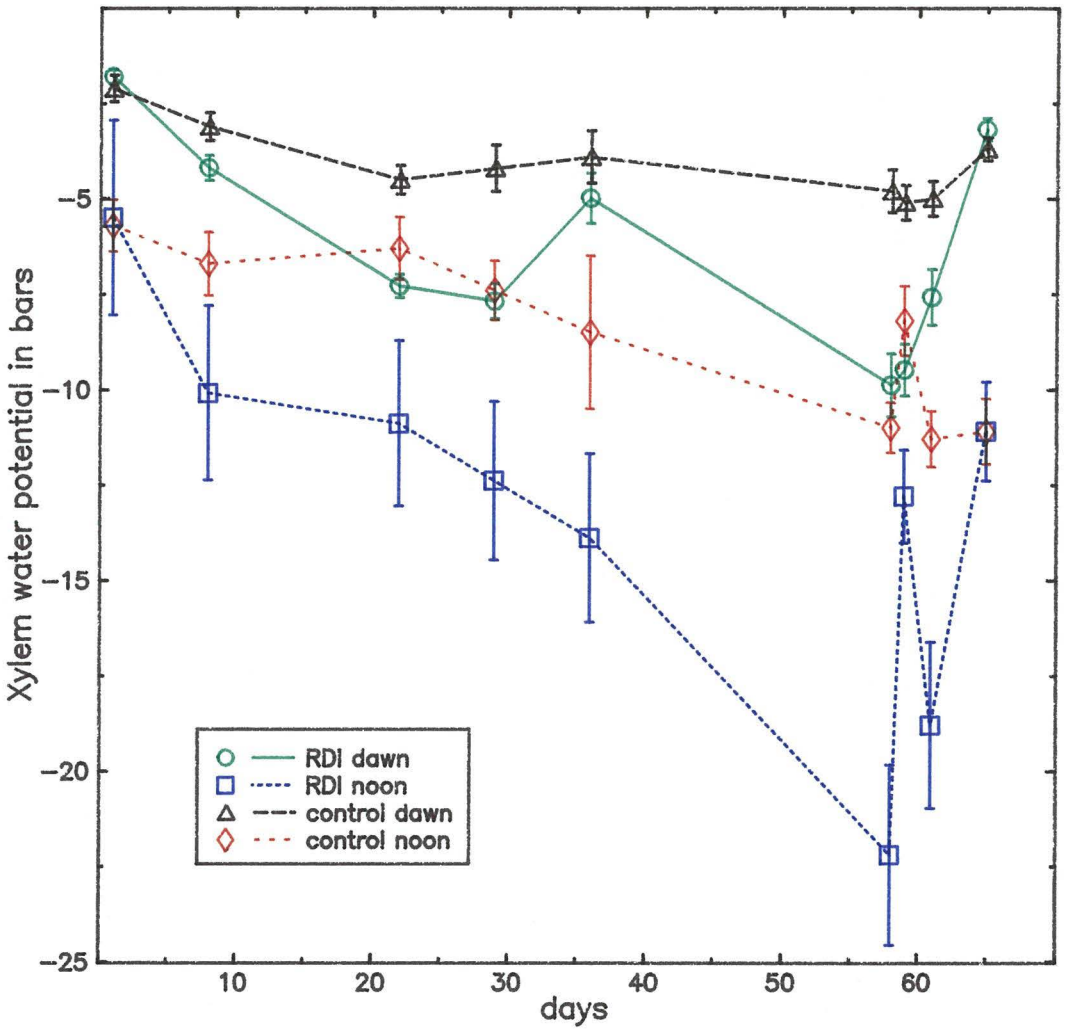


Figure 7.2 'Doyenne du Comice' Xylem Water Potential for Control and R.D.I. treatments at dawn and noon, where day 1 and start of R.D.I. = 17.11.90, (1990–1991). Vertical lines represent standard errors of the mean at each data point.

7.4: Discussion.

The container medium water contents of the R.D.I. and Control treatments were similar until the period of water stress began. The water contents of both the Control and R.D.I treatments did not show any significant differences during the period of measurement. Regardless of the statistics the values show a trend, and the water potential records of the trees indicate there was a difference in water availability between the R.D.I. and the Control treatments. The tree water potentials reflected the medium percentage water content. The Control plants received daily irrigation until drainage occurred, however the medium dried out considerably by upto 40% and the control leaf water potential was correlated with this drying. This caused Control tree water potential to fall to -11 bars at noon, but they largely recovered overnight.

Significant differences between the Control and the R.D.I. treatments were shown at $P < 0.05$ and $P < 0.01$ for leaf water potential at dawn and noon. The differences were greater at noon than at dawn. The seasonal water potential pattern in the Control showed a similar trend to the R.D.I. treatment, except the water potential values were lower by between 5 and 11 bars. There is no recorded explanation for the decrease in stress from 5.1.91 to 12.1.91, but cloudy weather may have contributed to a lower daily water loss. The recovery of the R.D.I.trees from the water stress was apparent by noon the day after full irrigation was resumed.

According to Mitchell *et. al.* (1984) and Irving and Drost (1987) water stress in the R.D.I. period reduces shoot growth and increases bloom in the following year, without a reduction in fruit growth. Shoot growth extension in the R.D.I. treatment (Chapter 8) was limited from 24.11.90 at which date this treatment's leaf water potential was significantly lower than the irrigated Control trees. The levels of water deficit induced here

were similar to those effective in reducing vigour in the work of Irving and Drost (1987), where a moderate water stress was developed of between 17 and 23 bars in apples.

7.5: Conclusion

The water deficit imposed by the R.D.I. treatment significantly reduced the 'Doyenne du Comice' xylem/leaf water potential for a period of 50 days in early summer, although no statistically significant difference in water content of the growing medium was demonstrated. The R.D.I. reduced both dawn and noon water potential to a minimum of 9.9 and 22 bars respectively.

CHAPTER 8

The influence of the treatments on shoot extension and trunk diameter growth of 'Doyenne du Comice' during the 1990-1991 season.

8.1: Introduction.

Shoot extension was monitored through the season to examine the influence of the treatments on plant growth. Trunk diameter measurements were carried out to monitor the growth of all the trees before and during the 1990-1991 growing season. This reflected the total amount of growth the trees made during the season. It has been shown by Westwood and Roberts (1970), that on lightly pruned apple trees, trunk cross sectional area shows a linear relationship with total above ground weight and indicates the potential bearing area.

8.2: Methods and materials.

8.2.1: Shoot extension.

On trees of all treatments in 4 blocks, five current season shoots per tree were monitored allowing 20 shoots per treatment. Shoots were selected in similar positions in the mid canopy region. Shoot length was measured weekly from 24.10.90 to 19.1.91 when all shoot growth had terminated.

8.2.2: Trunk diameter.

Trunk diameter was measured on all trees using a Mitutoyo digital calliper, at a preset mark on the trunk 20 cm above the bud union. Measurements were made at right angles to the row on each occasion to ensure the same position was measured. A total of 5 readings were taken between 18.8.90, and flowering on 20.9.91.

8.3: Results.

8.3.1: Shoot extension.

New shoots on trees in all treatments showed a rapid growth phase at the beginning of the season which was followed by the slowing of growth and finally the cessation of growth. The data presented in Figure 8.1 shows the shoot length extension. Mean values for shoot extension by treatment showed that there were no significant differences between the five treatments at $P < 0.05$ by Duncan's multiple range test. However, the trends will be commented on. Control shoots continued rapid growth longest during the season and gained the longest average length compared with the other treatments. The Control ceased growth after 15.12.90.

The Ammonium treatment had a slightly suppressed rate of growth, but continued growing for a longer period than the Controls, so that mean shoot length closely approached that of the Control. Ammonium treatment began on 24.11.90, and was applied at weekly intervals.

Both the Simazine and Paclobutrazol treatments reduced both the rate of extension in the growth phase, and the length of the period of growth compared to the Control. The Simazine treatment (applied once a month) began on 24.11.90, but even before this was applied the mean rate of shoot extension was slightly lower than that of the Control. After the start of applications of Simazine, growth was not markedly further suppressed, and ceased on about 15.12.90, at about the same time as the Control.

Paclobutrazol treatment virtually stopped growth after 23.11.90 (day 27), but continued to grow very slowly after this date. The first Paclobutrazol spray was applied on 2.11.90 before any of the other treatments, and shoot growth slowed down 21 days later a few days before the second spray. This and subsequent sprays effectively stopped any further extension during the season.

The Paclobutrazol treated shoots made the smallest increase in

length of all treatments during the season.

The R.D.I. treatment began on 17.11.90 and shoot extension stopped from 23.11.90. This treatment had the most immediate effect on shoot growth extension as the growth ceased 6 days after water application was reduced.

8.3.2: Trunk diameter.

Table 8.1. Mean values per treatment for trunk diameter (mm) at dates of measurement.

Date	Cont.	Ammon.	Simaz.	Paclo.	R.D.I.	Sign.
22.8.90	23.1a	24.6a	23.7a	23.1a	24.3a	n.s.
24.11.90	27.5a	28.6a	27.9a	26.0a	28.1a	n.s.
14. 1.91	31.0a	33.0a	31.3a	28.9a	30.6a	n.s.
25. 6.91	36.0a	37.7a	34.6ab	31.2b	35.2ab	*
20. 9.91	36.3a	37.8a	34.8ab	31.4b	35.3ab	*

where n.s. = not significant ($P > 0.05$)
* = $P < 0.05$

Means within a row not followed by the same letter are significantly different by Duncan's multiple range test.

Table 8.2 Mean increase in trunk diameter (mm) of treatments between each measurement date.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I
22.8.90- 24.11.90	4.4a ±0.66	4.0a ±0.53	4.2a ±0.21	2.9a ±0.38	3.8a ±0.42
24.11.90 -14.1.91	3.5a ±0.63	4.4a ±0.62	3.4a ±0.48	2.9a ±0.20	2.5a ±0.33
14.1.91- 25.6.91	5.0a ±0.59	4.7a ±0.60	3.3a ±0.06	2.3b ±0.07	4.6a ±0.06
25.6.91- 20.9.91	0.3a ±0.14	0.1a ±0.13	0.2a ±0.06	0.2a ±0.07	0.1a ±0.06
Total	13.2a ±0.60	13.2a ±1.10	11.1a ±0.51	8.3b ±1.35	11.0a ±0.98

Means within a row not followed by the same letter are significantly different $P < 0.01$, by Duncan's multiple range test. Standard errors follow the significance grouping on the second line.

The trunk diameter means displayed in Table 8.1 show the Paclobutrazol treatment reduced trunk diameter significantly ($P < 0.01$) at 25.6.91, the end of the season. No other clear trends are apparent in Table 8.1. Table 8.2 showing the mean increase in the intervals between measurement showed a significant difference ($P < 0.01$) for the Paclobutrazol treatment for the period 14.1.91 - 25.6.91 and also a significant difference ($P < 0.01$) for the total difference in trunk growth.

The total increase in trunk diameter shows that the Ammonium treatment and the Control treatment made the most trunk diameter growth. The Simazine treatment, applied monthly from 24.11.90, suppressed the total rate of trunk increase by a similar amount as the R.D.I. treatment. The R.D.I. applied from 17.11.90,

reduced the rate in the period 24.11.90 to 14.1.91, see also Figure 8.2, but this was not significant. Figure 8.2 suggests that after R.D.I. was stopped on 12.1.91 the trunk diameter growth rate recovered.

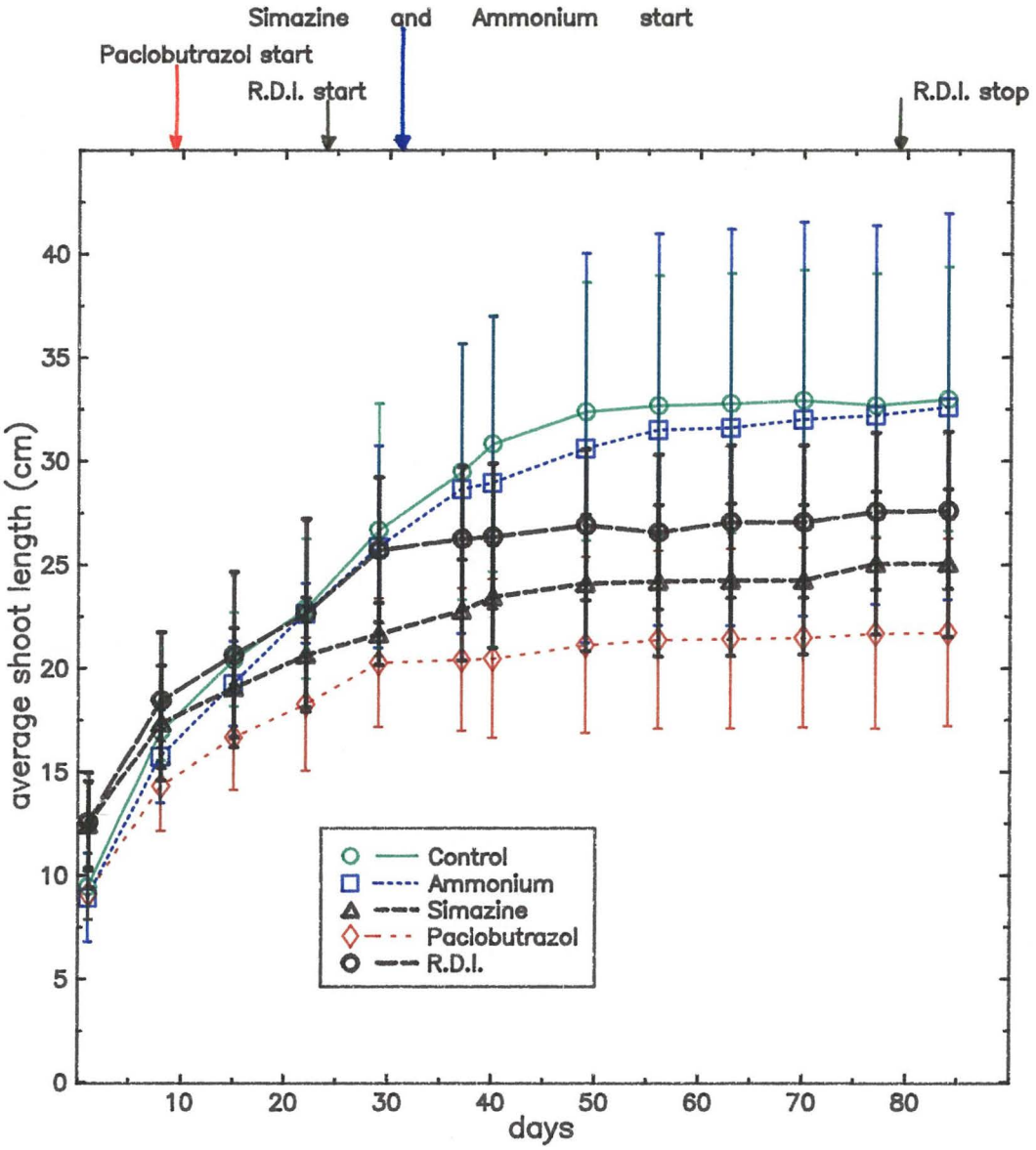


Figure 8.1 Shoot length extension for Control, Ammonium, Simazine, Paclobutrazol and R.D.I. treatments on 'Doyenne du Comice', where day 1 = 24.10.90 (1990–1991), vertical lines at each data point represent standard error of each mean.

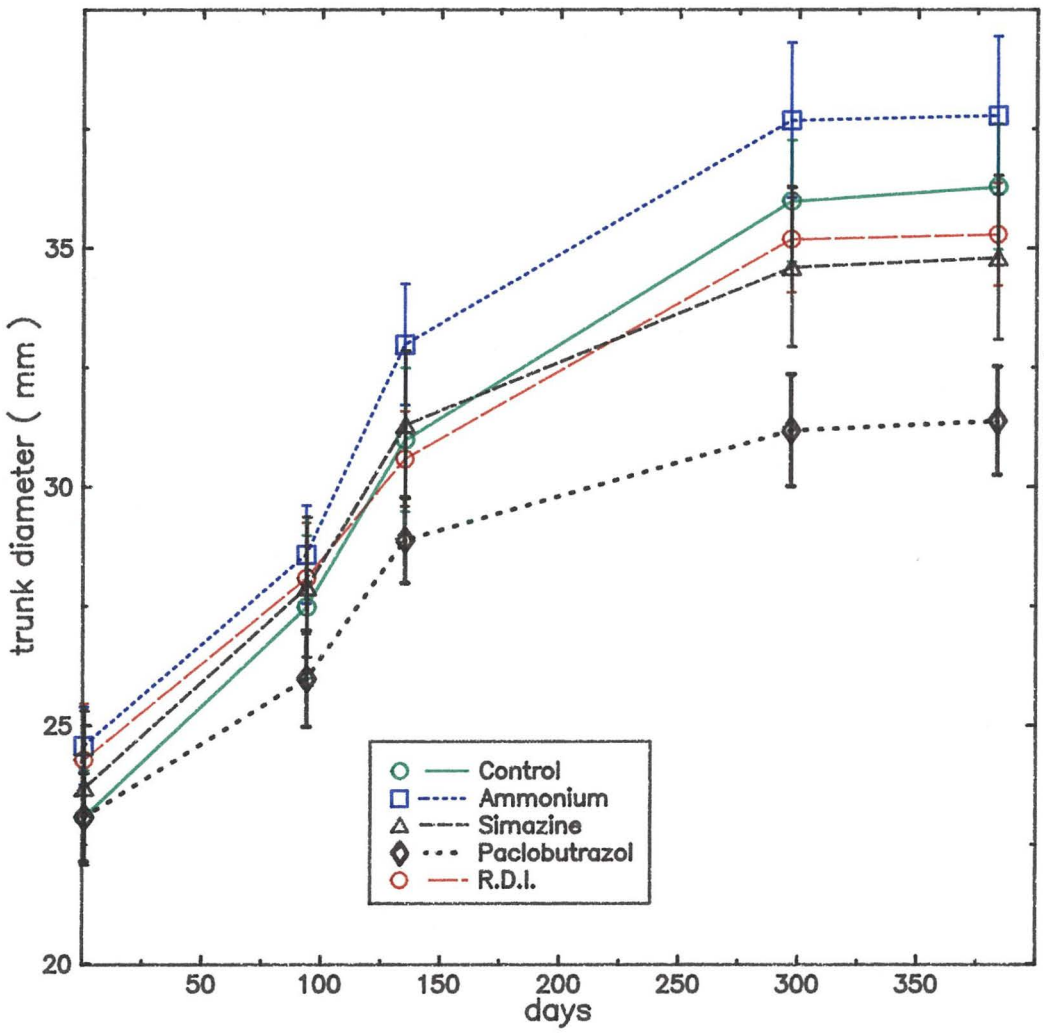


Figure 8.2 Trunk diameter growth for Control, Ammonium, Simazine, Paclobutrazol and R.D.I. treatments, (1990 – 1991), day 1 = 22.8.90, vertical lines at each data point represents the standard error of the mean.

8.4: Discussion.

8.4.1: Shoot extension.

It was intended that the Ammonium treatment should give an equivalent amount of nitrogen to the nitrate of the Control treatment. Whilst there is a hint of growth suppression from 1.12.90., 7 days after this treatment started, this was recovered by persistent slow growth after the Control had stopped, giving a final shoot growth suppression of only 1% and suggesting that the total nitrogen supplied to the two was indeed similar.

The Simazine treatment showed a reduction in shoot extension growth by 31% and obtained the second shortest total average extension growth. It would appear likely that this reduction was a herbicide growth suppression, similar in nature to those obtained in the bioassay.

The effect of a lowered water potential in the R.D.I. treatment, was to reduce the shoot growth extension, but not significantly. The reduction of 19% was not as large as expected, as other workers have achieved greater shoot reduction. Irving and Drost (1987), achieved a shoot growth reduction of 37% on apple trees where the stress period and degree of stress was similar to that imposed on the experimental 'Doyenne du Comice'. The shoot extension period was very short compared to the other treatments and cessation of rapid growth occurred after 23.11.90.

Paclobutrazol showed a reduction of 52% in growth although the difference from the Control was not significant. This treatment obtained the lowest total shoot extension due to the effect of the applied growth inhibitor.

8.4.2: Trunk diameter.

Of all the treatments, only Paclobutrazol showed a significant ($P < 0.01$) decrease in the increase in trunk diameter.

CHAPTER 9

Effect of the treatments on the photosynthetic rate and related parameters.

9.1: Introduction.

The effect of each treatment was examined with respect to changes in the photosynthetic rate of the spur leaves that contribute directly to the accumulation of carbohydrates in the spur bud that would potentially become floral.

9.2: Methods and materials.

At intervals through the 1990-1991 growing season, the photosynthetic rate of leaves on the trees in each treatment was measured. This was carried out using a Licor 6200 portable photosynthesis console. Two spur leaves exposed to full light were chosen on each tree and a reading was taken over 30 seconds. Eight replicate trees (with a total of 16 leaves) per treatment were measured. Measurements were taken between 12.00noon and 1.00pm.

9.3: Results.

Figure 9.1 and Table 9.1 present the mean photosynthetic rates ($\mu molCO_2/sq.m/sec$) for each treatment on the dates of measurement. Figure 9.2 shows the mean P.P.F.D. (Photosynthetic Photon Flux Density), (μmolPhoton/sq.cm/sec) available at the time the photosynthetic rate was measured.

The only treatment which reduced photosynthesis significantly was the R.D.I. treatment, at $P < 0.05$ on 8.12.90 and 22.12.90 and $P < 0.01$ on 12.1.91, on the day before full irrigation was reinstated. Slight recovery was recorded on 13.1.91, and virtually full recovery by the last date of measurement, on 19.1.91.

Table 9.1 Mean values for Photosynthesis ($\mu molCO_2/sq.m/sec$) for different dates by treatment.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
8.12.90	10.0a ±1.06	9.4a ±1.12	9.2a ±0.94	10.6a ±1.52	5.7b ±0.76
15.12.90	8.8a ±0.87	8.6a ±0.77	7.3a ±0.54	7.6a ±1.08	6.4a ±1.06
22.12.90	16.4a ±2.27	16.2a ±0.86	12.8ab ±0.79	14.6ab ±0.9	11.0b ±1.81
12. 1.91	9.4a ±1.6	8.7a ±1.33	8.5a ±0.85	6.5a ±0.89	2.5b ±0.83
13. 1.91	11.0a ±1.2	NA	NA	NA	4.3b ±1.46
19. 1.91	11.2a ±0.94	11.1a ±0.73	11.6a ±0.71	10.7a ±0.7	10.2a ±1.09

NA = not available

Means within a row not followed by the same letter are significantly different, at $P < 0.05$ on 8.12.90, 13.1.91 and $P < 0.01$ on 12.1.91, by Duncan's multiple range test. Standard errors follow the significant grouping on the second line.

The mean PPFD at each photosynthesis measurement is shown in Figure 9.2, where the highest PPFD for any measurement date was recorded on 22.12.90. This corresponded to the highest photosynthetic rate. Table 9.2 shows that there were no significant differences in PPFD received by treatments on any date of measurement.

Table 9.2 Photosynthetic Photon Flux Density (P.P.F.D.), (µmolphoton/sq.m/sec) light energy available for different treatments by date.

Date	Control	Ammonium	Simazine	Paclo.	R.D.I.
8.12.90	351.0a ±34.0	407.5a ±35.6	345.4a ±29.0	371.9a ±51.1	402.0a ±56.9
15.12.90	403.5a ±50.0	425.7a ±45.4	341.1a ±80.4	555.5a ±177.9	778.0a ±229.4
22.12.90	2169.9a ±63.0	2174.9a ±40.7	2188.8a ±28.1	2033.9a ±84.4	1990.5a ±160.9
12. 1.91	1256.0a ±267.5	1206.2a ±238.3	1333.9a ±215.6	1265.1a ±290.0	1125.2a ±232.6
13. 1.91	1891.1a ±170.5	NA	NA	NA	2076.2a ±106.7
19. 1.91	640.2a ±36.2	715.2a ±58.5	676.5a ±43.9	611.1a ±51.6	616.2a ±29.0

Means within a row not followed by the same letter are significantly different, by Duncan's multiple range test. Standard errors follow the significant grouping on the second line.

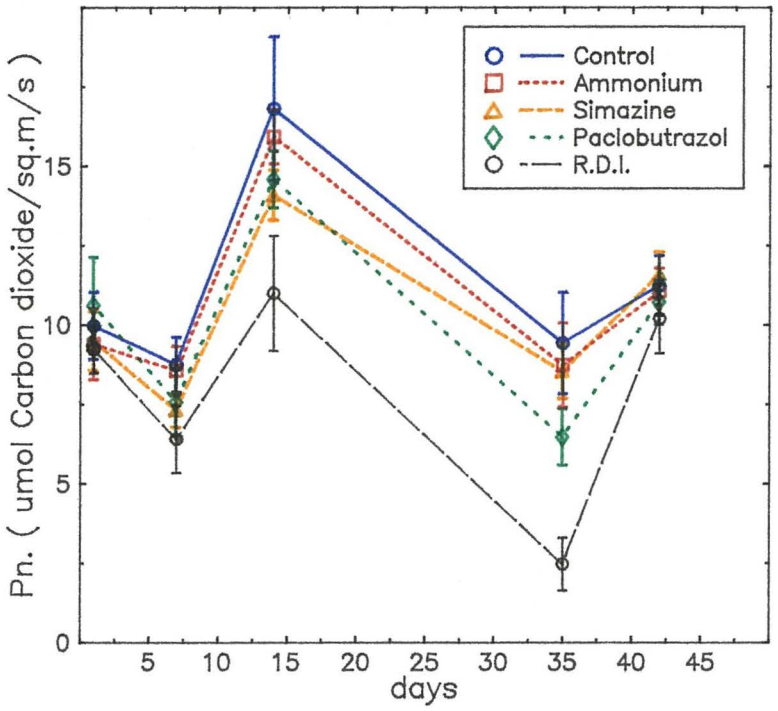


Figure 9.1 Photosynthetic rates for Control, Ammonium, Simazine, Paclobutrazol and R.D.I. treatments, where vertical lines at each data point represent the standard error of the mean, Day 1 = 8.12.90, other dates as shown in Table 9.1.

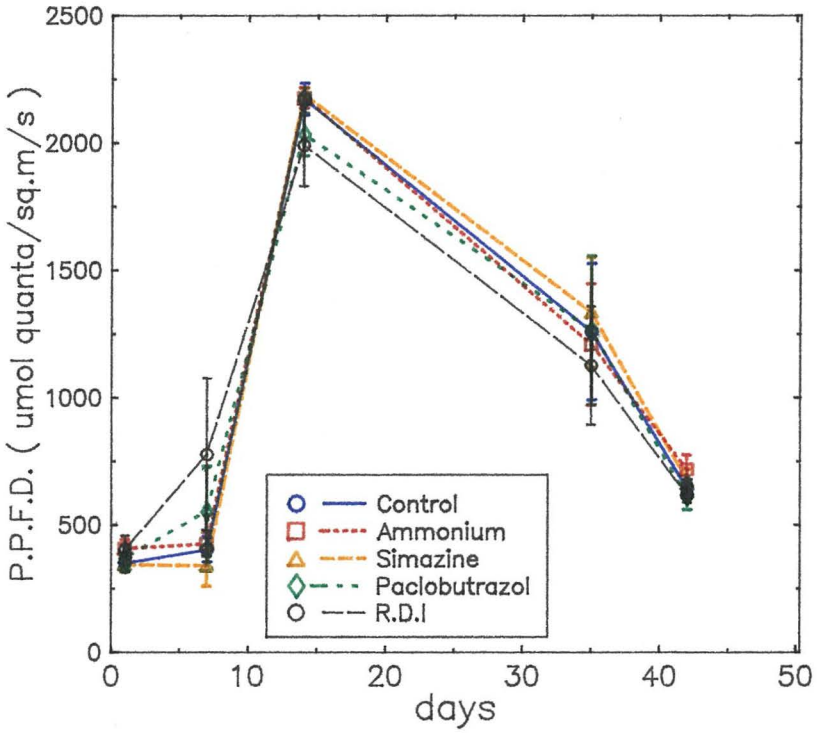


Figure 9.2 P.P.F.D. (Photosynthetic Photon Flux Density) light energy available (umol quanta/sq.m/s) for Control, Ammonium, Simazine, Paclobutrazol and R.D.I. treatments, where vertical lines at each data point represents the standard error of the mean. day 1 = 8.12.90, other dates shown in Table 9.1.

9.4: Discussion

The internal water deficit of plants is controlled by the rate of water uptake by the roots and the rate of transpiration (Kozlowsky, 1986). As a water deficit develops, physiological processes are altered and subsequently growth and yield are reduced (Hagen *et. al.* 1967).

Durand (1990), found that the photosynthetic rate of apples in orchard conditions under R.D.I. was reduced on 27.11.88 53 days after full bloom, where a similar water stress regime had been imposed.

The only treatment in the experimental 'Doyenne du Comice' trees to show a significant reduction in the photosynthetic rate was the R.D.I. treatment. Regulating the supply of water to the plant controls the rate at which the plant can carry out photosynthesis. The effect was at its greatest on the 12.1.91 which showed a significant reduction in photosynthesis at $P < 0.01$. The removal of water stress allowed a complete recovery in the photosynthetic rate by 19.1.91.

It is of interest to note that the trend to slight shoot and trunk growth suppression by the Simazine treatment (Chapter 8) is barely apparent here in the Simazine photosynthetic rate. And in agreement with other reports Steffens and Wang (1984), the strong growth reducing effects of Paclobutrazol are not reflected by any real reduction in photosynthetic rate.

CHAPTER 10

Flower numbers from the experimental 'Doyenne du Comice' trees following the application of treatments during the previous growing season.

10.1: Introduction.

The effect of the treatments on flowering was determined by counting flower clusters on each tree on 17.9.91.

10.2: Methods and Materials.

Flower clusters were counted on each of the trees after bud burst but before blossom opening. The cluster numbers were then corrected for total shoot length by calculating clusters per centimetre shoot length. Shoot length was recorded on each tree in July 1991.

10.3: Results

Table 10.1 Mean values for flower clusters, total shoot length, clusters per cm of shoot and clusters as a percentage of Control by treatment (1991).

	Control	Ammon.	Simazine	Paclo.	R.D.I.
Clusters	128.3a ±36.9	164.9a ±24.3	152.4a ±31.1	267.8b ±16.1	163.6a ±24.0
Total shoot length(cm)	471.4ab ±34.6	498.3a ±42.0	455.6ab ±21.9	393.9c ±16.6	437.6ab ±30.2
Shoot length as % of Control	100	106	97	84	93
Clust. per cm shoot	0.27a ±0.07	0.30ab ±0.07	0.34ab ±0.06	0.68c ±0.03	0.38b ±0.06
Clust.as % of Control	100	111	126	252	141

Means within a row not followed by the same letter are significantly different, $P < 0.05$ for R.D.I. clusters per cm shoot length and $P < 0.01$ for Paclobutrazol clusters and clusters per cm shoot length, by Duncan's multiple range test (standard errors follow the significance grouping on the second line).

All treatments appear to have increased clusters per tree, although only Paclobutrazol treatment shows a significant increase ($P < 0.01$). However when flower clusters are expressed as clusters per cm shoot length, both the Paclobutrazol (at $P < 0.01$) and R.D.I. ($P < 0.05$) treatments are significantly different from the Control. The increase in flowering of the Simazine treatment did not reach $P < 0.05$, (actually $P < 0.81$). Whilst least successful, it did not appear that the Ammonium treatment had reduced flowering.

Plate 10.1 Flower clusters on a Control branch, where photograph was taken on 17.9.90.

Plate 10.2 Flower clusters on an Ammonium treatment branch, where photograph taken on 17.9.90.

Plate 10.3 Flower clusters on a Simazine treatment branch, where photograph taken on 17.9.90.

Plate 10.4 Flower clusters on a Paclobutrazol branch, where photograph taken on 17.9.90.

Plate 10.5 Flower clusters on an R.D.I. branch, where photograph taken on 17.9.90.

Plates 10.1-10.5 show the flowers borne on a typical shoot on a tree from each of the treatments. It was observed during flowering, that the Paclobutrazol treatment bloom period was extended over a longer time than the other treatments. Flowers in Plate 10.4 can be seen at a range of maturities.

10.4: Discussion.

The results show that the most effective of the treatments to stimulate flowering in 'Doyenne du Comice', was the foliar application of Paclobutrazol.

This observation is supported by Luckwill (1978), who found that application of paclobutrazol increased fruit bud production and yield of apple trees. Tukey (1983), also found that paclobutrazol at 100ppm increased fruit buds in the year following treatment in pipfruit. It was observed by Tukey (1983), that the flowering duration was extended over more days for flowers on the

paclobutrazol treatment and a similar effect was observed in the present experiment.

Regulated deficit irrigation treatment, showed a higher flower cluster count than the Control and when shoot length is taken into consideration the number of clusters per centimetre of shoot was found to be significantly higher at $P < 0.05$. Mitchell *et. al.* (1984) similarly found increased bloom profusion on 'Bartlett' pear trees.

As reviewed, several workers have increased flower evocation in apple trees in response to nitrogen applied during the summer growth cycle. Grasmanis and Edwards (1974) concluded that exposure of apple trees to the ammonium ion, apart from any nutritional effect, triggered the synthesis of molecules which promote the process of flower evocation in the axillary apices already present at the time of exposure. In the present experiment the number of clusters was greater than the Control but not significantly different, either on a per tree or per centimetre of shoot basis.

Simazine is known to increase the effectiveness of the nitrate reductase enzyme and should therefore increase the level of reduced nitrogen in the tissues. The addition of 1ppm simazine to apple plants was found by Edwards (1986) to be as effective in promoting flowering as substituting ammonium for nitrate ions during similar periods of time. In the present experiment simazine appears to have had some flower promoting effect.

Flowering of the Control plants was relatively good for 3 year old 'Doyenne du Comice' trees, which could be related to the confinement in containers. It was found by Monselise (1985) that a confined root volume increased bloom profusity in citrus plants. However it might also have been influenced by the slight water stress that developed in the Control (see Chapter 7). The 'Doyenne du Comice' trees were grown in containers because the orchard management at the time had a policy of avoiding the use

of persistent growth retardant paclobutrazol in orchard soil, where it might destroy uniformity for later plantings. The cluster number in the Control indicates that the trees have at this young age, developed 'ripeness to flower', and treatments are not breaking juvenility, but enhancing flower evocation. It is possible that paclobutrazol increases flowers most because it gives the greatest and longest reduction in vigour, as seen by trunk growth reduction (Figure 8.4).

CHAPTER 11

Bloom dates of 'Doyenne du Comice', 'Winter Nelis', and 'Buerre Bosc'.

11.1: Introduction.

Two important cross pollinators for 'Doyenne du Comice' are 'Winter Nelis' and 'Buerre Bosc'. The duration of blossom overlap of these two pollinators with 'Doyenne du Comice' should be as long as possible in order to maximize the possibility of pollination. During the 1990 blossoming period a small study was made on older orchard trees.

11.2: Methods and Materials.

In Mr Woods orchard at Turakina, near Wanganui, there were trees of these three cultivars on BA29 rootstocks. The original planting was then seven years of age, and the remainder was four years old. In the first bloom study the pollinators and the 'Doyenne du Comice' bloom was assessed for percentage open bloom on the four and seven year trees at the orchard on six occasions between 28.9.90 and 15.10.90.

In the second bloom study, measurements were made on the same dates as the first study. On the four year trees of the 'Doyenne du Comice', blossom clusters were present on one, two and three year old wood. However the seven year old 'Doyenne du Comice' trees showed evidence that a biennial cropping pattern was emerging, and virtually all blossom was carried on spurs on three year old wood. Percentage open bloom was measured on both tree ages for 'Doyenne du Comice' only.

11.3: Results

Results of the blossom overlap study are shown in Figures 11.1, 11.2 and 11.3. Figure 11.1 shows bloom study one where the

comparison of 3 varieties of 4 year old pear trees, combining bloom on different ages of wood, where day 1 = 28.9.90. The same blossoming trends are shown by the 7 year old trees (Figure 11.2), however the 4 year old 'Doyenne du Comice' trees were about 2-3 days ahead of the 7 year old trees. In both cases the pollinators provide good overlap with 'Doyenne du Comice'.

Figure 11.3 shows bloom study two where the flowering characteristics of one, two and three year old wood on 4 year old 'Doyenne du Comice' trees and 3 year old wood on 7 year old 'Doyenne du Comice' trees, where day 1 = 28.9.90. One, two and three year old wood on 4 year trees was earliest to flower. The 3 year old wood on 7 year old trees followed the 4 year old trees by about 2 - 4 days. Full bloom and 100 percent petal fall in all ages of wood was the same.

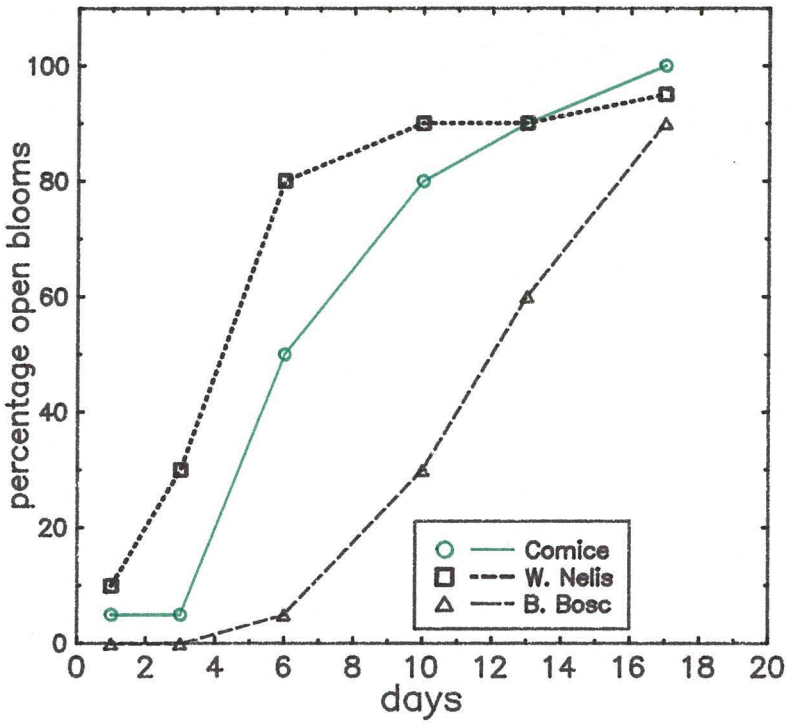


Figure 11.1 Blossoming period trends (open blooms) for 3 pears cvs. trees aged 4 years (spurs of all ages) located at Wanganui, day 1 = 28.9.90.

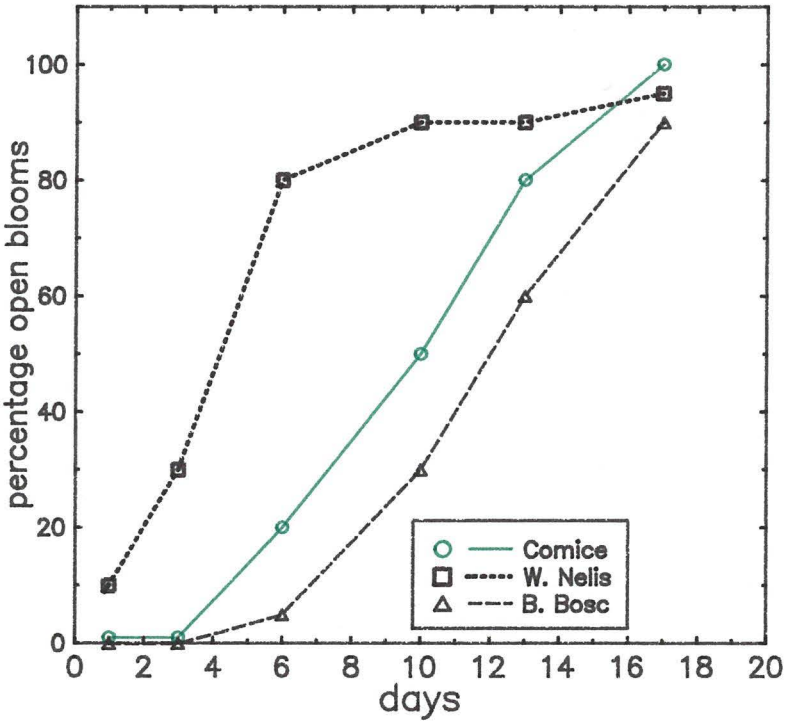


Figure 11.2 Blossoming period trends (open blooms) for three pear cvs trees aged 7 years (spurs of all ages) located at Wanganui, day 1 = 28.9.90.

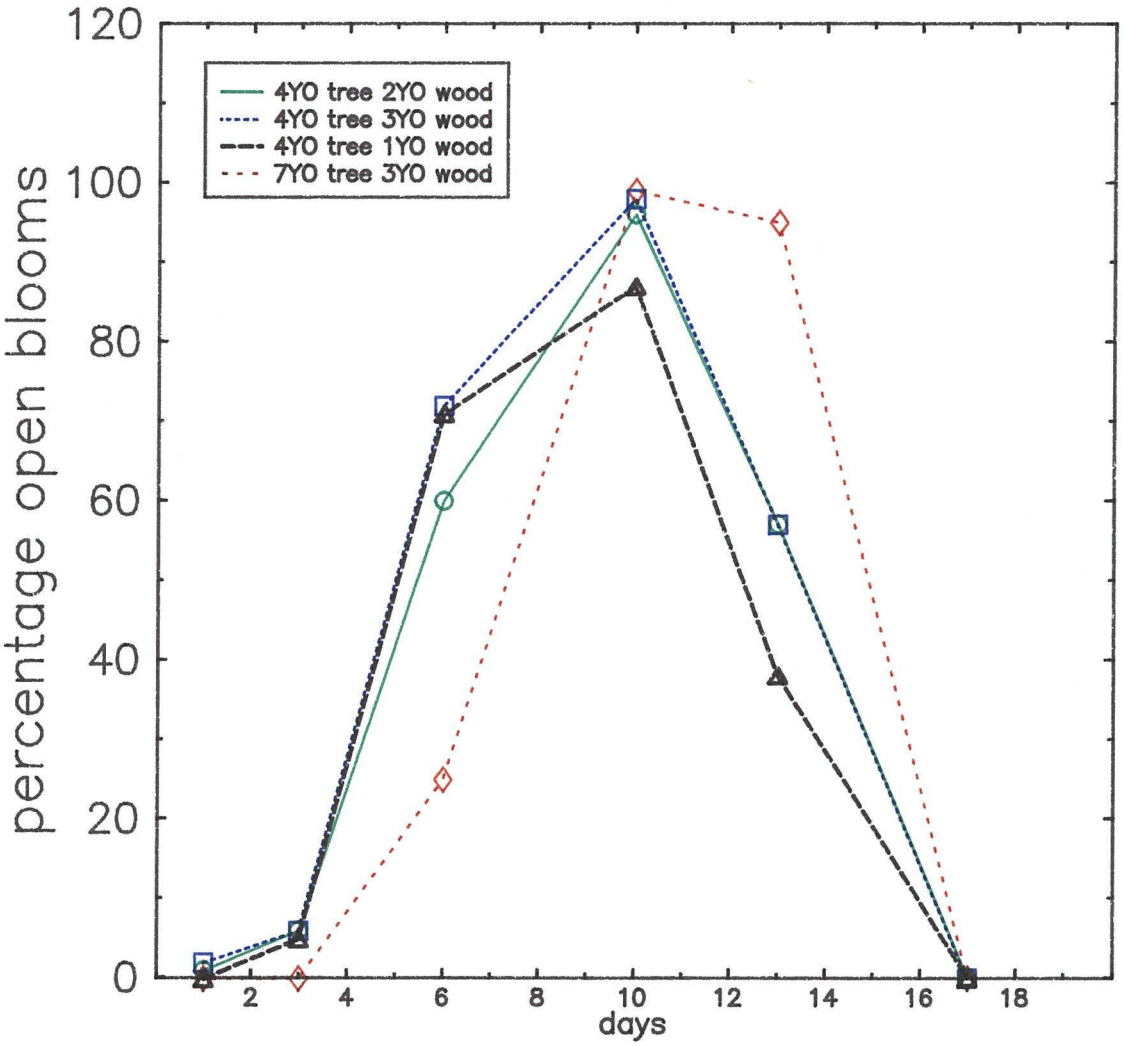


Figure 11.3 Blossoming period trends (open blooms) for different aged trees and wood on 'Doyenne du Comice' at Wanganui, day 1 = 28.9.90.

11.4: Discussion.

The flowering data presented shows the flowering period of 'Doyenne du Comice' is comfortably bracketed by the flowering of 'Winter Nelis' and 'Buerre Bosc'. This would allow good cross-pollination.

Flowering characteristics did not vary much between the differently aged wood, but 3 year wood on 7 year old trees was delayed by about 3-4 days behind all ages of wood on the 4 year old trees. This might have been related to the relatively heavy crop the older trees had borne in the previous year.

CHAPTER 12

The influence of light levels on bud formation and flowering in mature 'Doyenne du Comice'.

12.1: Introduction.

Many aspects of pipfruit tree growth are influenced by the degree of exposure of the trees or parts of trees to sunlight (Jackson, 1975a, b) and a wide range of management techniques, such as pruning, training, spacing and tree size control by rootstocks enables the light regime in the canopy to be modified at will.

This study attempted to determine the effect that light levels have on spurs within the canopy of 'Doyenne du Comice' pear trees with regard to their ability to initiate flowers. Rom (1989), reported that in pipfruit increased light penetration into the canopy improved spur and fruit development.

12.2: Methods and materials.

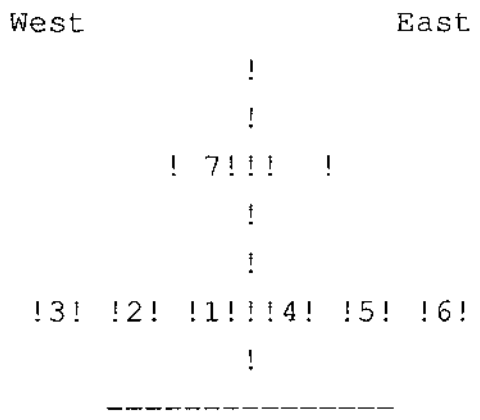
In this subsidiary study, ten mature 'Doyenne du Comice' trees in a Hawkes Bay orchard were selected. On each tree, seven positions were chosen within the canopy to represent the gradations of light levels. The trees were conical, with two tiers arising from the main trunk, and ran in rows somewhat east of north to west of south, so that the west tier was exposed to more direct sunlight than the east tier. The bottom tier was more extensively developed and carried the most dense foliage. Three positions were chosen along the lower east tier, and 3 positions on the lower west tier, along a transect at right angles to the row giving a total of 6 positions on this tier equidistant from each other. The seventh position was chosen in the upper tier close to the trunk, only one

position being chosen because this tier was not very long. Light levels were measured in March 1991 at each of the 7 positions in each tree, between 12.30pm and 1.30pm, with solar noon at 1.00pm.

The weather was fine, with a clear sky. Light measurements were carried out with a Licor portable photosynthesis console (Licor 6200) and data recorded as PPFD (Photosynthetic Photon Flux Density),(µmol/sq.m/sec). Light levels were measured at each position within the canopy and also an open sky check was made at each tree at the beginning and the end of measurements. Light levels were also calculated as percentages of the open sky PPFD.

Buds were measured in winter on 4.7.91 recording base diameter of the spur and bud diameter. Spur base diameter was measured at the base of the spur bud and bud diameter was measured across the widest part the bud. Five buds were measured at each position, giving a total of thirty five buds per tree. In the following spring a flower count was made for each bud at each position within the canopy.

The location of the seven positions on the 10 trees was as follows:-



12.3: Results.

Table 12.1. Mean values for PPFD (µmolphoton/sq.m/sec), spur base diameter, spur bud diameter and flower number per cluster, at 7 positions in each of 10 mature 'Doyenne du Comice' trees in Hawkes Bay.

	Pos. 1	Pos. 2	Pos. 3	Pos. 4
PPFD March 1991	164.3de ±135.90	472.0cd ±178.80	1863.7a ±28.90	21.8e ±1.29
Base dia.(mm) July 1991	3.4bc ±0.08	3.4bc ±0.10	3.9a ±0.08	3.2c ±0.11
Bud dia.(mm) July 1991	3.2cd ±0.10	3.5bc ±0.13	4.6a ±0.29	3.0d ±0.16
Flower no. Sept. 1991	4.6c ±0.60	5.9b ±0.37	7.0a ±0.37	6.9ab ±0.59

Table 12.1 continued

	Pos. 5	Pos. 6	Pos. 7
PPFD March 1991	334.4cde ±118.8	764.4c ±245.9	1290.7b ±208.7
Base dia.(mm) July 1991	3.5b ±0.11	3.7ab ±0.09	3.6b ±0.08
Bud dia.(mm) July 1991	3.9bc ±0.18	4.0b ±0.11	4.0b ±0.07
Flower no. Sept. 1991	5.9b ±0.66	7.0a ±0.70	6.9ab ±0.26

Means within a row not followed by the same letter are significantly different ($P < 0.01$), by Duncan's multiple range test (Standard errors follow below the significance grouping).

Results are shown in Table 12.1 and Figures 12.1 - 12.4. Table 12.1 shows that there were trends in mean light levels recorded. The outermost position (3) on the western basal tier of the tree was significantly best lighted, levels declined through positions 2 and 1 to the trunk and reached a minimum at the innermost position (4) on the eastern basal tier. The mean light levels then increased through positions 5 and 6, reaching a maximum under half that at position 3. Position 7 in the upper tier had a mean level intermediate between positions 3 and 6.

With minor variations, similar trends occurred in the means of spur base diameter and bud diameter. The mean flower number has a low range, and shows more deviation from the trends noted above. However, the outer and upper positions (3, 6 and 7) produced most flowers. A similar fall occurs in the flower data through positions 2 and 1, and with an unexplained peak at 4, a similar rise through position 5 and 6.

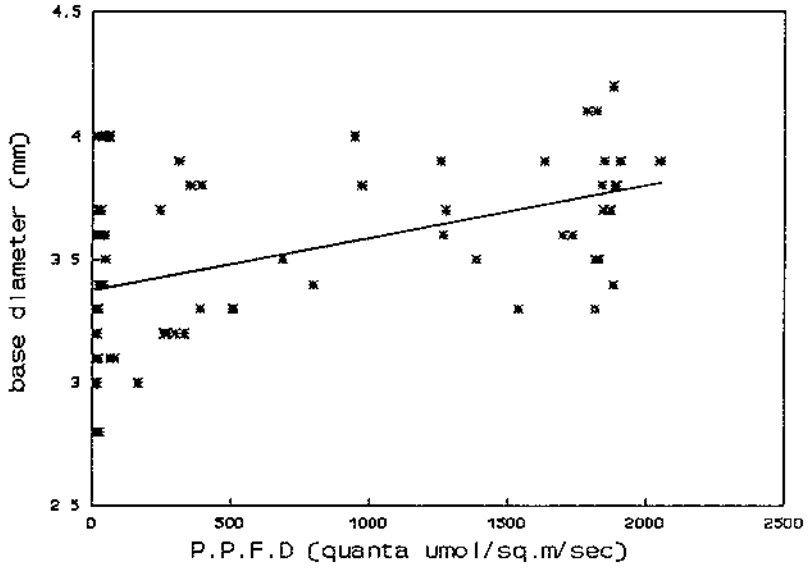


Figure 12.1 Spur base diameter (mm) as a function of PPFD (quanta µmol/sq.m/sec).

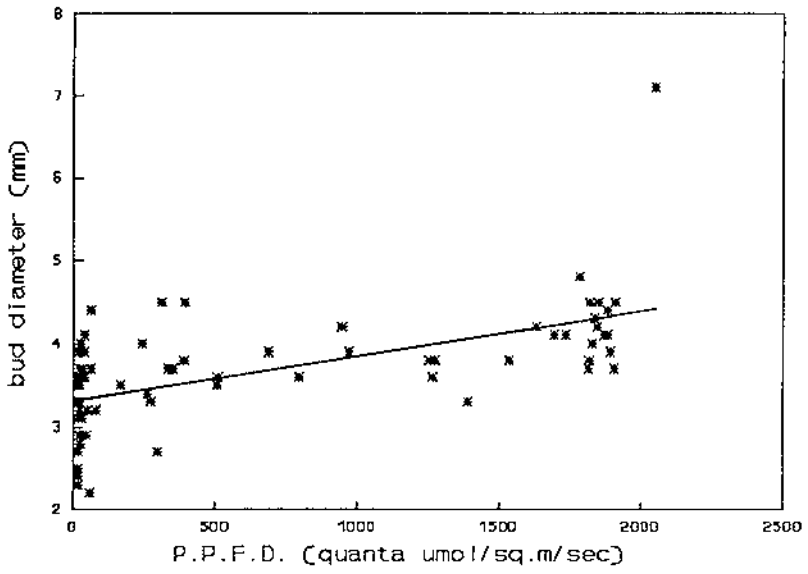


Figure 12.2 Bud diameter (mm) as a function of PPFD (quanta µmol/sq.m/sec).

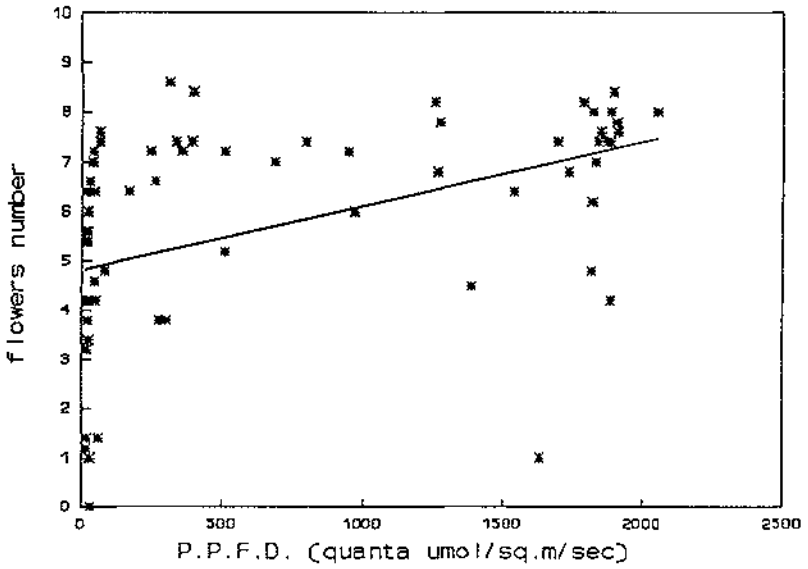


Figure 12.3 Flower number as a function of PPFD (quanta µmol/sq.m/sec).

The close relationship between mean light levels and spur base and bud diameter means is shown in Figure 12.4. Mean spur bud diameter increases at a greater rate than mean spur base diameter with increase in percentage of mean light level. The regressions between the means of spur base diameter, bud diameter and flower number at each location at which the light level was measured are shown in Figures 12.1, 12.2 and 12.3,

The equation for the line in Figure 12.1; Y = 3.87 + (0.000213 * x), where x = PPFD (quanta µmol/sq.m/s) and Y = spur base diameter (mm), shows the regression relationship between increasing PPFD and bud base diameter. R squared was 0.243 which is significant at $P < 0.01$. In Figure 12.2 the equation for the line; Y = 3.31 + (0.000545 * x) shows the regression relationship where x = PPFD (µmol/sq.m/s) and Y = bud diameter (mm). R squared was 0.353 which was significant at $P < 0.01$. Figure 12.3 shows the relationship Y = 4.82 + (0.00129 * x), where x = PPFD (µmol/sq.m/s) and Y = flower. R squared was 0.202, which was significant at $P < 0.01$.

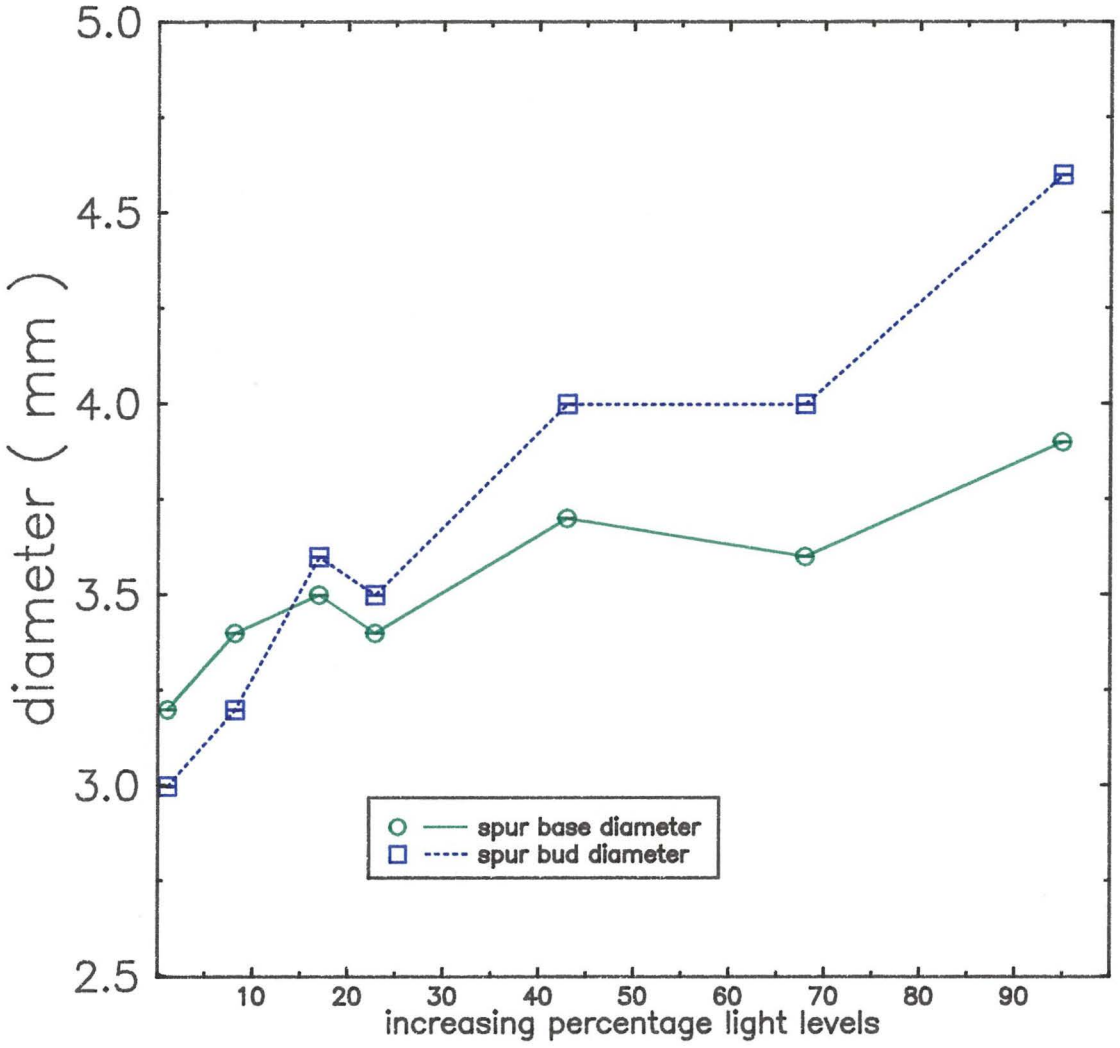


Figure 12.4 Percentage light and its effect on spur base and spur bud diameter, light measurements taken in March 1991, buds measured on 4.7.92.

2.4: Discussion.

Jackson and Palmer (1977), reported that the light levels penetrating the canopy contribute directly to the accumulation of substrate that is available for encouraging flower initiation.

The results show that spur base diameter, spur bud diameter and flowering are highly correlated with light levels in the canopy. In New Zealand, Tustin *et. al.* (1988), have shown similar relationships between light transmission and fruit weight, fruit colour and soluble solids levels in apples.

Management techniques such as pruning and training should take into account the light levels reaching the spur buds at equivalent positions in the canopy of mature 'Doyenne du Comice' trees.

CHAPTER 13

Defoliation studies on mature 'Doyenne du Comice' trees.

13A. The effect of defoliation and the influence of the bourse shoot on the fruitlet retention of spur buds of 'Doyenne du Comice'.

13.1a: Introduction.

In the early stages of growth, fruitlets are very dependent on the leaves on their own spurs rather than receiving carbohydrates from leaves elsewhere on the tree (Ferree and Palmer 1982). It is also reported that at this time the growth of the bourse shoot competes strongly with fruitlets for carbohydrate (Abbott, 1984), and so tends to reduce fruit set. Removal of cluster leaves to varying degrees and bourse shoot retention or removal early in the season was designed to determine the dependency of 'Doyenne du Comice' fruitlets on the spur and bourse shoot competition by monitoring the resulting levels of fruitlet drop.

13.2a: Methods and materials.

In Mr Wood's orchard a study was set up on 4YO 'Doyenne du Comice' trees. Four treatments were chosen to examine the effects of spur defoliation and of the bourse shoot on fruit set. The leaf area was assessed using prepared templates of known areas, that were measured on a Licor 3100 leaf area meter. A total leaf area was determined for each spur and each bourse shoot by comparison against the templates on 19.10.90, which was 9 days after full bloom. Each treatment was replicated ten times on 3 trees, in the same row.

The treatments consisted of;

Treatment 1 - the bourse shoot was removed, and all cluster leaves on the spur were retained.

Treatment 2 - the bourse shoot was not removed and all cluster

leaves on the spur were retained.

Treatment 3 - the bourse shoot was removed and the cluster leaves on the spur were reduced to approximately 50% of original leaf area.

Treatment 4 - the bourse shoot was retained and all cluster leaves on the spur were removed.

At this time, 9 days after full bloom, cluster leaves appeared to be fully expanded, but were relatively few in number, and did not constitute a large area. One, or sometimes two, bourse shoots were present on all selected spurs, varying in length from under 2 cms to 12 cms. Mostly these carried a similar number of leaves (4 - 6), although the degree of leaf area at this date, varied considerably.

Many unset fruitlets had to be removed from the spurs in setting up the study, normally leaving 3 or 4 apparently fertilised fruitlets per spur. The number of fruitlets retained expressed as mean percentages of flowers carried by the spurs was 52%, 47%, 43% and 55% for treatments 1 to 4 respectively.

After recording pre-treatment data the treatments were imposed and post treatment data recorded. Subsequent recording occurred 8 days later on 27.10.90, and 45 days later on 4.12.90.

13.3a: Results.

Table 13.1 Mean values of flower number, number of cluster and bourse shoot leaves and cluster and bourse shoot leaf area and total leaf area before treatments imposed (data gathered on 19.10.90).

	Tmt1	Tmt2	Tmt3	Tmt4
Original flower No.	6.7CB ±0.21	7.1B ±0.35	6.8C ±0.25	8.2A ±0.25
cluster leaf No.	3.2ab ±0.53	2.2b ±0.25	2.8b ±0.2	3.9a ±0.43
cluster leaf area (cm^2)	29.2ab ±5.16	20.6b ±3.62	22.3b ±2.52	35a ±4.41
bourse shoot leaf No.	5.4a ±0.67	7.5a ±0.16	5a ±0.21	5.9a ±0.16
bourse shoot leaf area (cm^2)	75.6a ±12.90	99.6a ±5.30	58.8a ±4.70	78.9a ±5.01
Total leaf area (cm^2)	104.8ab ±15.8	95.3ab ±8.1	81.1b ±4.8	109.2a ±15.8

Means within a row not followed by the same letter are significantly different where upper case letters indicate $P < 0.01$ and lower case indicate $P < 0.05$, by Duncan's multiple range test. Standard errors follow below the significance groupings.

Table 13.2 Mean values for number of cluster and bourse shoot leaves and cluster and bourse shoot leaf area and total leaf area after treatments imposed (data gathered on 19.10.90).

	Tmt1	Tmt2	Tmt3	Tmt4
cluster leaves	3.2A ±0.53	2.5B ±0.25	1.4C ±0.1	0D ±0.0
cluster leaf area (cm^2)	29.2A ±5.1	20.6B ±3.6	11.3C ±1.2	0D 0.0
bourse shoot leaves No.	0A ±0.0	5.6B ±0.16	0A ±0.0	5.4B ±0.16
bourse shoot leaf area (cm^2)	0A ±0.0	99.6B ±5.3	0A ±0.0	78.9B ±0.0
Total leaf area (cm^2)	29.2C ±5.06	120.2A ±8.33	11.3D ±1.2	78.9B ±5.04

Means within a row not followed by the same letter are significantly different, where upper case letters indicate $P < 0.01$ and lower case letters indicate $P < 0.05$, by Duncan's multiple range test (standard errors follow the significant groupings on the below).

Table 13.3 Mean numbers of fruit set, and as percentage of original number of fruitlets after applying treatments on 19.10.90 on treated spurs.

	Tmt1	Tmt2	Tmt3	Tmt4
19.10.90	3.5ab ±0.54	3.3a ±0.45	2.9a ±0.28	4.5b ±0.50
27.10.90	2.2a ±0.29 (63%)	2.4a ±0.31 (73%)	1.9ab ±0.23 (66%)	3.3b ±0.26 (73%)
4.12.90	0.4ab ±0.22 (11.4%)	0.9a ±0.31 (27.3%)	0.1b ±0.10 (3.4%)	0.3b ±0.21 (6.7%)

Means within a row not followed by the same letter are significantly different $P < 0.05$, by Duncan's multiple range test, standard errors follow the significant grouping on the second line, followed by percentage retention.

The percentage retention of fruitlets at 27.10.90, 8 days after treatment, was relatively similar in all treatments, so retention at the final recording date 4.12.90 is considered.

Treatment 1. Cluster leaves only ($29.2cm^2$), no bourse shoot and 11.4% of fruitlets retained.

Treatment 2. Cluster leaves ($20.6cm^2$), and bourse leaves ($99.6cm^2$) giving a total leaf area of $120.2cm^2$ which was retained. The bourse shoot had a mean increase in length of 152% over the period to 4.12.90. This treatment retained 27.3% of the fruitlets.

Treatment 3. Cluster leaves ($11.3cm^2$) and no bourse shoot, retained 3.4% of fruitlets.

Treatment 4. Cluster leaves removed ($0cm^2$) and bourse leaves retained ($78.9cm^2$) giving a total leaf area of $78.9cm^2$. Bourse shoot length increased by 190% over the period to 4.12.90. This treatment retained 6.7% of fruitlets.

13.4a: Discussion.

Treatment 2, effectively the natural 'Control' situation, with all cluster leaves and both the competitive and contributory effects of the bourse shoot, retained the most fruitlets (27.3%). Treatment 1, which had a somewhat larger cluster leaf area, and no competitive or contributory effects from the bourse shoot retained 11.4% of the fruitlets. Treatment 3, was essentially similar to treatment 1, however with under half the cluster leaf area, only retained 3.4% of the fruitlets. Treatment 4, with no cluster leaves, but with both competitive and contributory effects from the bourse shoot did not retain significantly more fruitlets than treatment 3, but its result (6.7% retention of fruitlets) is not in disagreement with the apparently net positive effects of the bourse shoot presence shown by treatment 2 compared with treatment 1.

Proctor and Palmer (1991), found that the development of a complete and healthy early season canopy of spur leaves, and later addition of bourse shoot leaves, is essential for fruit set, fruit growth and fruit quality. Ferree (1982), reported that in apple the fruitlets are very dependent on the leaves on their own spurs rather than being able to receive carbohydrates from elsewhere in the tree. This situation changes later in the season. Ferree (1982) found the yield response to spur leaf removal was nonlinear; a 50% reduction in spur leaf area reduced yield by 30% but a complete removal reduced yield by 80%. The presence of the bourse shoot was detrimental to early set, but resulted in higher final yield. In the present study it is probable that competition between bourse shoots and developing fruitlets occurred, but the leaf area of the bourse shoot aided retention, being able to supply carbohydrate both to its own apex and the fruitlets.

13.5a: Conclusion

Overall the treatments suggested that on 'Doyenne du Comice' a high cluster leaf area was of prime significance in fruitlet retention, but that during the period to the final recording (45 days after full bloom) the net effect of the bourse shoot presence and growth was positive on fruitlet retention.

13B. The effect of varying degrees of defoliation on the flowering potential of spur buds of 'Doyenne du Comice'.

13.1b: Introduction.

It has been shown by Fulford (1966a), that leaves are necessary to the process of flower evocation. Since spur leaves contribute most directly to this, it was reasoned that if spur leaves were removed prior to evocation it might be expected to prevent it on that spur. Conversely, if evocation had already occurred, subsequent spur defoliation might be expected to have little or no effect on evocation and subsequent flowering. To examine the importance of the spur leaves for flower production, a series of defoliation treatments were imposed on spurs on two separate dates during the 1990-1991 season.

13.2b: Methods and materials.

Four similarly sized 4YO 'Doyenne du Comice' trees in one row of Mr Woods orchard, near Wanganui, were chosen to examine the effect of defoliation of spurs during the 1990-1991 summer on the following season's flowering of the spur buds. Full bloom had occurred on 10.10.90, and defoliation was carried out either 7 weeks after full bloom on 4.12.90 or 16 weeks after full bloom on 11.2.91. Spurs randomly distributed through out the canopy were selected and labelled, and the spur leaves were removed to leave either 0, 3, 6 or 9 leaves per spur. There were 20 replicates of each treatment. Numbers of spurs flowering and of flowers produced by each spur were counted at full bloom, on 17.9.91.

13.3b: Results.

Table 13.4 Percentages of spurs which flowered, and mean number of flowers per cluster, in spring 1991, following total or partial defoliation of the spur on one of two dates in the previous season.

Date		0 leaves left	3 leaves left	6 leaves left	9 leaves left
Defol. 4.12.90	% of spurs which flowered.	15b	55a	75a	75a
	Mean number of flowers per cluster.	1.0b ±0.53	4.0a ±0.84	5.3a ±0.80	6.0a ±0.81
Defol. 11.2.91	% of spurs which flowered.	65b	65b	75b	100a
	Mean number of flowers per cluster.	5.1b ±0.86	4.5b ±0.84	5.0b ±0.68	7.8a ±0.18

Means within a row not followed by the same letter are significantly different, by Duncan's multiple range test, $P < 0.01$ for flowers on both dates and percentages on 14.12.90. $P < 0.05$ for percentages on 11.2.91 (standard errors follow the significance grouping on the second line for flowers).

Complete defoliation on 4.12.90 prevented 85% of spurs flowering, so that the percentage of clusters and the mean number of flowers were significantly lower than were produced from the partial defoliations. However there is a general trend for less severe defoliation at this date to allow increasing levels of flowering, and even 3 leaves were sufficient to allow a moderate level of flowering.

It appeared that complete defoliation on 11.2.91 was much less effective in affecting flower production, although the minimal defoliation (to 9 leaves) showed significantly more flowers than the other defoliations at this date.

13.4b: Discussion.

The number of leaves on a spur during the previous season contributes directly to the flowering potential of the spur bud. The largest contributor to plant carbohydrates in apple spur buds are the associated spur leaves of apple, Arthey and Wilkinson (1964) and Llewelyn (1966). Defoliation to varying degrees showed how important these leaves are in influencing flowering of 'Doyenne du Comice' pear.

Proctor and Palmer (1991), reported that the development of a complete and healthy early season canopy of spur leaves, and later addition of bourse leaves, is essential for return bloom Procter and Palmer (1991). They applied defoliation treatments to the spur 2, 4 and 8 weeks after full bloom and found that return bloom was dependent on the presence of bourse shoots on the spur but not on the spur leaves.

In general terms the study confirms the importance of spur leaves to flower production by the bud. At 14.12.90 total defoliation of spurs severely reduced flowering, and at 11.2.91 reductions to below 9 leaves per spur reduced flower numbers significantly.

The study and its results do not allow definite statements about whether defoliation has affected evocation or differentiation or both, and so there are different possible interpretations. However as much of the literature suggests that evocation in spur buds of pipfruits occurs early, and is more or less synchronised, the following interpretation is offered first.

The very severe reduction of flowering following complete spur

defoliation on 4.12.90 is consistent with the prevention of evocation. However less severe defoliations at this time do not appear to have prevented this in many cases. That these allowed a range of flowering of the same order as the defoliations on 11.2.91, when even total defoliation allowed many buds to flower, and it is reasonable to assume that evocation had occurred earlier, is consistent with reduced leaf number also having a quantitative influence on the differentiation process that ensued.

This interpretation suggests that evocation occurred after 4.12.90, or possibly was in process, since a few buds flowered after total defoliation at that date. However, if the significantly reduced flowering following total spur defoliation on 4.12.90 is not considered as qualitatively different from that after the lesser and later defoliations, all reductions in flowering can be considered as post-evocation reductions in flowering. So evocation may have occurred before 4.12.90.

The dissection study reported in Chapter 5 suggested that in the buds of experimental pot trees the apex became domed, the first visible sign that evocation has occurred from 14.12.90, which could support either interpretation. However, the two sets of trees were different, in maturity, in fruit bearing and in location.

13.5b: Conclusion.

Spur leaves were required for evocation of the spur bud to take place. When leaves were present the evocation process was in process or occurred after 4.12.90. Whilst the data does not exclude the possibility that some buds may undergo evocation later, the flower number data is consistent with reduced spur leaf area after evocation reducing subsequent differentiation of flowers. Generally the treatments that retained more leaves on the spur had more flowers in the following spring.

CHAPTER 14

The relationship between flowering, shoot growth and the position of the branch on mature 'Doyenne du Comice' trees.

14.1: Introduction.

The angle of the branch relative to the horizonal influences flowering. Bending of branches has commonly been practised to reduce shoot extension and promote flowering (Wareing and Nasr, 1958).

It was reasoned that the shoots from branches that were tied down would have their extension reduced, which would be shown in shorter average shoot length and possibly a reduction in shoot base diameter. Tromp (1967), attributed enhanced flower bud formation on horizontal apple branches to reduced shoot growth. It was also expected that as a result of branches being tied down that flower production would be increased.

14.2: Methods and materials.

On his 4 year old (YO) 'Doyenne du Comice' trees, Mr Woods had tied down, in July 1989, a number of branches to an approximate angle of 40 to 45 degrees from the horizontal. Some branches, however, were not tied down and remained growing more vertically, at approximately 70 or 80 degrees from the horizontal.

It was therefore possible to compare the flowering performance of tied down and not tied down branches on 19.10.91. Three tied down and three not tied down branches in similar locations on similar trees were measured and the results are presented in Table 14.1. Branches were selected that had at least 20 shoots, and any shoots after the 20th were not measured. Shoots that arose along each branch, from the trunk to the end of the branch, were recorded for shoot length, the base diameter of each shoot and the flower numbers on each shoot.

14.3: Results.

The small number of branches involved in this minor study did not permit any statistical analysis of the data which was gathered.

Table 14.1 Mean values for shoot length, shoot base diameter and flower number per shoot on tied and not tied down branches of 4 YO 'Doyenne du Comice'.

	Tied down	Not tied down
shoot length (cm)	4.9	12.1
shoot diam. (mm)	4.1	5.4
flowers/shoot	2.3	1.9

The tied down branches recorded reduced mean shoot length and shoot base diameter, and slightly increased flowering, than the not tied down branches. The flowers were produced terminally on the young shoots, with no axillary flowers in either treatment.

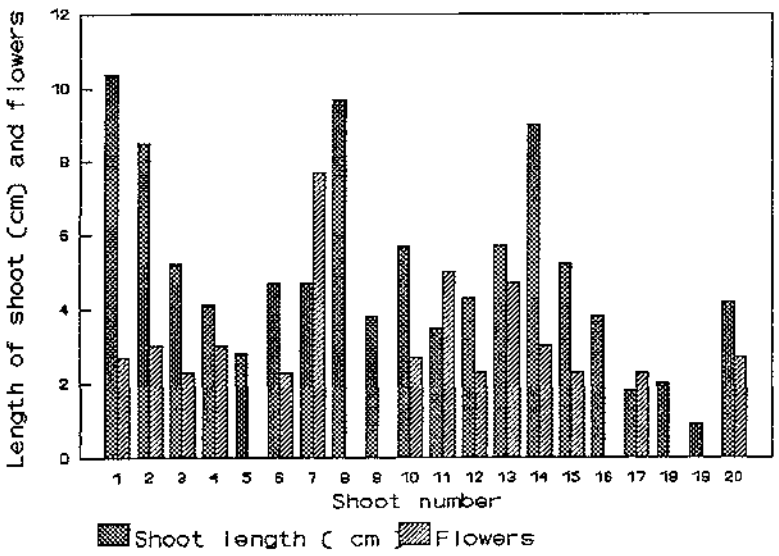


Figure 14.1 Mean shoot lengths along tied down branches and mean flower number per shoot, where shoot 1 is closest to the trunk -'Doyenne du Comice' Wanganui (1990).

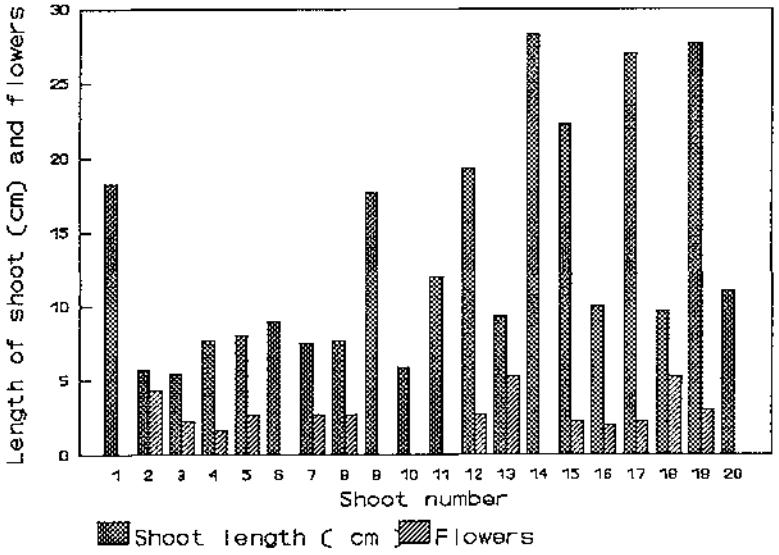


Figure 14.2 Mean shoot lengths along not tied down branches and mean flower number on each shoot, where shoot 1 is closest to the trunk - 'Doyenne du Comice' Wanganui (1990).

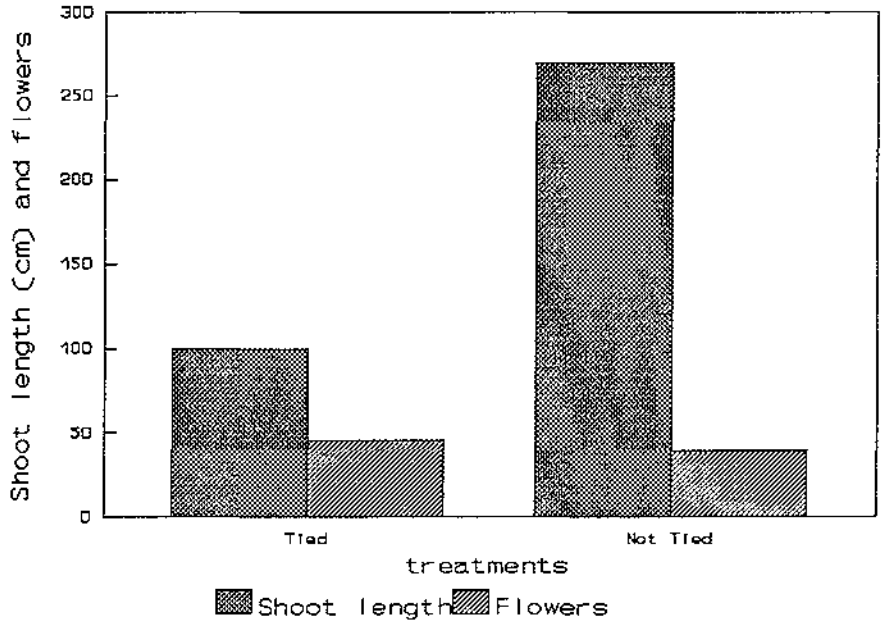


Figure 14.3 Total shoot length and flower numbers for tied and not tied down branches - 'Doyenne du Comice' Wanganui (1990).

The mean shoot length for each of the 20 shoots is shown for tied down and not tied down branches in Figures 14.1 and 14.2, and total shoot length and flower number is shown in Figure 14.3. Average shoot length is longer on the not tied down branches (12.1 cm) than the tied down branches (4.9 cm). Tying down has also reduced the number of long shoots at the distal end of the branches.

14.4: Discussion.

These results indicated that the effect of tying down branches on 'Doyenne du Comice' pear in winter was to reduce the length and the base diameter of the individual shoots that arose from it in the following season, and to give a slight increase in flower numbers per shoot in the spring following that. The practice had a positive effect on terminal flower production in 'Doyenne du Comice', and also might reduce pruning time.

CHAPTER 15

GENERAL CONCLUSIONS

15.1: Introduction.

Precocity and yield are two aspects of 'Doyenne du Comice' fruit production which are troublesome to the fruit industry. This study was set up to compare strategies for increasing bloom, to identify time of evocation and endogenous factors relating to flower development.

15.2: Factors influencing bloom.

15.2.1: Treatment effects on flowering.

The literature provides reports on various treatments that have been successful in promoting precocity in pipfruit and other fruit crops. These treatments include R.D.I., Paclobutrazol, Ammonium based fertilisers and subtoxic application of the herbicide Simazine. Flower clusters on the experimental 'Doyenne du Comice' were significantly increased ($P < 0.01$) by the Paclobutrazol treatment and for the Regulated Deficit Irrigation (R.D.I.) treatment when flower clusters were expressed per unit of branch length ($P < 0.05$). The Simazine and Ammonium treatments did increase the number of flower clusters per unit branch length but significant levels were not achieved. Paclobutrazol treated trees had a longer flowering period than the other treatment's trees.

15.2.2: Shoot and tree growth, water potential and branch angle.

Shoot growth extension in the R.D.I. treatment (Chapter 8) was limited during the period when leaf water potential was significantly lower than the irrigated Control trees.

In the R.D.I. treatment the water deficit imposed significantly reduced leaf water potential for a period of 50 days in early summer although no statistically significant differences in water

content of the growing medium were demonstrated. The R.D.I. reduced both dawn and noon water potential to a minimum of −8 and -22 bars respectively.

Tree growth was reduced significantly by the Paclobutrazol treatment as shown by the shoot and the trunk growth. The effect of the R.D.I. treatment was not as great as expected, however, shoot growth was restricted as it was by Simazine treatment, but the reductions were not significant. Ammonium treatment did not reduce growth.

A minor study on 4 year old trees suggested that tying branches down from their natural upright growth habit in winter reduced the length and the base diameter of the individual shoots that arose from them in the following season and gave a slight increase in terminal flower numbers per shoot in the following spring.

15.2.3: Photosynthetic rate, light levels and flowering.

The photosynthetic rate of the experimental 'Doyenne du Comice' was reduced significantly ($P < 0.01$) by the R.D.I. treatment but not by any other treatment.

On mature 'Doyenne du Comice' trees in the Hawkes Bay a study was made of light levels penetrating the canopy, and the effects of these. As light levels in the canopy increased, increases were recorded in spur base diameter, spur bud diameter and mean flowering.

15.2.4: Endogenous plant factors.

Monitoring of the endogenous levels of free nitrogenous compounds in the plants obtained variable results. The levels of arginine and ammonium did not show significant trends during the period of sampling in any treatment. The bud arginine levels were higher than the leaf levels in all treatments. No links could be established between the levels recorded and subsequent flowering.

15.3: Time of evocation and characteristics of flower bud development

15.3.1: Examination of spur bud apex.

Bud scales and leaves were the first structures to be shown developing in the spur bud in the experimental 'Doyenne du Comice' between 19.11.90 and 14.12.90. This did not indicate floral initiation although the onset of evocation was apparent when the apex became domed between 14.12.90 and 8.1.91. The differentiation of the terminal flower occurred between 8.1.91 and 11.2.91, and further floral structures appeared after this date including lateral flowers and bracts. A similar developmental stage was found in spur buds taken from an orchard in the Turakina Valley, near Wanganui.

15.3.2: Spur defoliation.

Defoliation at different periods during the season influenced flower production depending on when the defoliation was performed. The very severe reduction in flowering following complete spur defoliation on 14.12.90 was consistent with the prevention of evocation. However less severe defoliations at this time did not appear to have prevented evocation. Complete spur defoliations on 11.2.91 allowed many buds to flower. Whilst the data did not exclude the possibility that some buds may have

undergone evocation after 14.12.90, the flower number data was consistent with reduced spur leaf area after evocation reducing subsequent differentiation of flowers. Generally the treatments that retained more leaves on the spur had more flowers in the following spring. The Scanning Electron Microscope (SEM) pictures in Chapter 5, showed a domed apex on 8.1.91, suggesting evocation process was occurring in the spur bud of 'Doyenne du Comice'.

15.4: Factors influencing set.

15.4.1: Bloom periods and cross pollination.

Flowering periods was monitored on 'Doyenne du Comice' and cross pollinators in a separate study in an orchard near Wanganui. This study revealed that the period of flowering of 'Doyenne du Comice' was comfortably between the flowering periods of the cross pollinators and would allow good pollination. It was also found that flowering periods did not vary much on different aged wood on 'Doyenne du Comice' trees aged four years, but was slightly ahead of the trees aged seven years.

15.4.2: The influence of the bourse shoot.

Overall the treatments suggested that on 'Doyenne du Comice' a high cluster leaf area was of prime significance in fruitlet retention, but that during the period to the final recording (45 days after full bloom) the net effect of the bourse shoot presence and growth was positive on fruitlet retention.

15.5: Conclusions and recommendations

Of the experimental treatments foliar application of Paclobutrazol had the most significant influence in increasing flowering, followed by R.D.I. where low vigour was induced in the plant by reducing the water potential. The other experimental treatments were not as successful in increasing precocity in 'Doyenne du Comice'. Other factors that are important in promoting flowering in 'Doyenne du Comice' are high canopy light levels and good spur leaves. Evocation was found to occur in December.

Paclobutrazol is a very effective method of influencing precocity, and can be applied to young trees that are difficult to bring into flowering. Regulated Deficit Irrigation is practical in areas where irrigation is required in the early months of the growing season. However if the rainfall during the months of November and December is sufficient to supply adequate water, so that irrigation is not necessary, then R.D.I. is not easy to implement. Tying down of branches to between 40 to 45 degrees from the horizontal is recommended. Winter pruning should remove wood that will cause excessive shading within the canopy during the summer.

15.6: Further work.

A series of further investigations of the bud apex closely spaced during December would reveal more precisely the timing of evocation, and indicate variability between years. The potential for successful use of R.D.I. for increasing precocity of 'Doyenne du Comice' in other areas of New Zealand should be investigated.

Research into the further use of foliar applied Paclobutrazol on 'Doyenne du Comice' at varying ages from year 2 - 6 would provide useful information for crop establishment and flowering.

The relationship between root restriction and precocity could be studied by using subsurface root restriction bags. This would maintain a smaller root volume and possibly increase flowering.

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