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The effect of a natural plant extract and synthetic plant growth regulators on growth, quality and endogenous hormones of Actinidia chinensis and Actinidia deliciosa fruit

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Abstract

Kiwifruit are of huge economic importance for New Zealand representing 29 percent of total horticultural exports. Fruit size is the biggest determinant of what consumers are willing to pay, and there is also a positive relationship between consumer preference for flavour and percentage dry matter. The two main cultivars exported from New Zealand are *Actinidia chinensis* 'Hort 16A' (gold kiwifruit) and *A. deliciosa* 'Hayward' (green kiwifruit). Under current commercial practice the only product allowed for use on kiwifruit to increase fruit size in New Zealand is Benefit®. Benefit® has been shown to induce different results when applied to *A. chinensis* and *A. deliciosa*, whereas synthetic plant growth regulators such as the cytokinin-like substance N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) have been found to promote similar increases in fresh weight of fruit in both cultivars. Final fruit size is determined by both cell division and cell enlargement. It was been shown that fresh weight can be increased in both of the major *Actinidia* cultivars even though their physiology differs. Hormonal control of fruit size in relation to cell division and cell enlargement phases of fruit growth was studied in both *A. chinensis* and *A. deliciosa*.

CPPU was applied to both cultivars in a growth response experiment where fruit were collected throughout the growing season. The objective of this experiment was to create growth curves, to compare and contrast the effect on *A. chinensis* and *A. deliciosa*, and to provide material for hormone analysis. Application of CPPU was found to significantly increase the fresh weight of both *A. chinensis* and *A. deliciosa* fruit (46.98 and 31.34 g increases

respectively), and alter the ratio of inner and outer pericarps of *A. chinensis* fruit. CPPU and Benefit® were applied individually and together to both cultivars. It was found that only *A. chinesis* fruit were affected by the application of Benefit®; fresh weight was increased by 26.38 g, and percentage dry matter was significantly reduced. There was a statistically significant ($p < 0.05$) interaction between CPPU and Benefit® when applied to *A. chinensis*. 3,5,6-trichloro-2-pyridyloxyacetic acid (3,5,6-TPA) was applied to *A. deliciosa* on two application dates at three concentrations and was found to decrease fresh weight of fruit, but significantly increase percentage dry matter regardless of application date or concentration. Lastly CPPU and 1-naphthalene acetic acid (NAA) were applied to *A. deliciosa* at two application dates and in all combinations. Application date affected the response to both a low concentration of CPPU and NAA. A synergistic interaction was observed when CPPU was applied early plus NAA late (CPPU early (4.53 g increase) plus NAA late (13.29 g) < CPPU early plus NAA late (33.85 g).

Finally endogenous hormone content was studied. Methods were developed and tested for the simultaneous analysis of both indole-3-acetic acid (IAA) and cytokinins. Freeze dried fruit were purified using Waters Sep-pak® cartridges and Oasis® columns then IAA was quantified by high pressure liquid chromatography. Preliminary results indicate a correlation between application of CPPU and endogenous IAA, high concentrations of IAA correlated well with periods of rapid fruit growth particularly for CPPU treated fruit.

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Chapter One

1. General Introduction

1.1. Overview and rationale for thesis:

New Zealand is a world leader in kiwifruit production, in terms of fruit quality and innovation. More kiwifruit are exported from New Zealand than any other fruit or vegetable crop; kiwifruit represents 29 percent of New Zealand's horticultural exports, and wine is a close second at 26 percent (Anonymous, 2007). Exports of kiwifruit were worth $765 million free on board in the year ending June 2007; this figure is more than double that for 1997 (Anonymous, 2007). As of June 2007, New Zealand had 13,250 ha planted in kiwifruit, which is an increase of 12 percent since 2002 (Anonymous, 2007). New Zealand kiwifruit are marketed and exported by ZESPRI International Limited (Mount Maunganui, New Zealand) (Zespri).

Fruit size is an important factor in kiwifruit quality. This is because it is the main determinant of what consumers will pay and what growers are paid. Another major quality factor is the percentage dry matter, as there is a positive correlation between percentage dry matter and flavour (Burdon *et al.*, 2004). This has, in recent times, become of more importance to the grower as Zespri have increased the proportion of what growers are paid, based on the percentage dry matter. Therefore anything that can increase either the size of

fruit or percentage dry matter, without adversely affecting the other, or crop load or return bloom is of interest to both growers and Zespri.

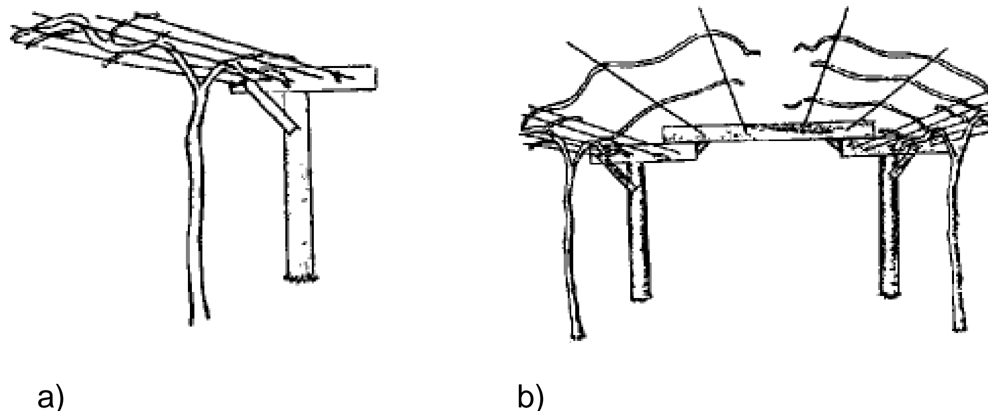
Commercially grown kiwifruit belong to the genus *Actinidia*. Within this genus three species *A. chinensis*, *A. deliciosa*, and *A. arguta* are commercially used in New Zealand. This literature review will be restricted to the two main cultivars that are currently grown in and exported from New Zealand. Those cultivars are *A. chinensis* 'Hort16A' (gold kiwifruit) and *A. deliciosa* 'Hayward' (green kiwifruit), and unless otherwise stated these are the cultivars being discussed. *Actinida deliciosa* plants were first introduced to New Zealand from China early in the 20th century, however, the 'Hayward' cultivar was not the major cultivar grown until the late 1960s when exports developed and consumers showed a preference for the 'Hayward' fruit. By the mid 1970's 'Hayward' was the only cultivar exported and soon became the prevalent cultivar being planted (Sale & Lyford, 1990). The cultivar 'Hort16A' was developed in New Zealand and commercially released in 1995; it is sold commercially as ZESPRI™ GOLD Kiwifruit. The first significant export of the fruit was from New Zealand to Japan in 1998 (Patterson *et al*., 2003).

Major factors affecting fruit size of both *A. chinensis* and *A. deliciosa* fruit are seed number (Hopping, 1976a), crop load, and photo-assimilate accumulation via light interception. These aspects can be optimised through pollination, both natural and artificial means, fruit thinning, and both summer and winter pruning to optimise the canopy. Under current commercial practice in New Zealand *A. chinensis* is sprayed with the natural plant extract Benefit® to

improve fruit size. However, no plant growth regulators, natural or otherwise, are applied under current commercial practice, to *A. deliciosa* except, to a limited extent, Benefit®.

1.2. Kiwifruit physiology and vine management:

The kiwifruit is a highly vigorous, deciduous plant which grows in the habit of a liana. In its natural habitat of well-lit forest margins it clambers over other plant life (Davison, 1990). When it is grown commercially it must be trained over support structures to be in any way manageable. The management of swiftly growing shoots is a constant battle for growers. When vines were first grown commercially they were supported by a T-bar trellis system (Figure 1.1. a); this has since been refined to a pergola structure (Figure 1.1. b) where the majority of the vine is suspended approximately 1.8m above the ground, giving growers and orchard workers the ability to work under the vine and making management of vegetative growth and fruit easier.



a) b)

Figure 1.1. A kiwifruit vine trained over a t-bar trellis system (a) and a pergola trellis (b) (Himelrick & Powell, 1998).

Key differences between the vines of the two major kiwifruit cultivars are the timing of bud break and therefore shoot growth and flowering. Bud break of *A. chinensis* occurs 3-4 weeks earlier than *A. deliciosa* (Patterson *et al*., 2003). Good pollination is important for both cultivars, however seed numbers required for *A. chinensis* fruit to reach the same size as *A. deliciosa* fruit are lower (Patterson *et al.,* 2003). The relationship between seed number and fruit size is not as direct in *A. chinensis* fruit as it is in *A. deliciosa* fruit.

1.2.1. Vine management:

The female vines are pruned once in winter and again in summer. In the pergola system the winter pruning removes old or non-bearing canes and replaces them with low-vigour bearing canes. Generally self terminating (Figure 1.2.) canes that have produced in the previous season are tied down, provided that these are of a suitable size and in a suitable position within the canopy. These canes are preferred to vigorous non-self terminated canes or vigorous water shoots that did not bear fruit in the previous season. The new canes are laid down to give ~1 cane every 30cm (Figure 1.3.). The number of canes tied down in winter determines the density of the canopy and the crop load; if more canes are tied down the canopy will be dense and require more intensive summer pruning and there will be a heavier crop load though the fruit are likely to be slightly smaller. Alternatively if fewer canes are tied down the canopy will be more open, however, summer pruning will still be required and the crop load will be lighter with slightly larger fruit.

Figure 1.2. A self terminated cane

Figure 1.3. Before and after pruning of *A. deliciosa* on a pergola trellis

Before summer pruning is started canes are generally NAA-gel (1-naphthalene acetic acid) pruned and/or pinched in early October. The ends of non-terminated canes are either pinched to crush the apical meristem or cut and NAA-gel applied. NAA-gel contains a synthetic auxin which maintains apical dominance thereby inhibiting auxiliary buds from developing; both methods are temporary.

Summer pruning is necessary to keep the canopy from becoming too dense. Unnecessary vegetation such as water shoots that grow from the main leaders and do not produce fruit are cut back and tangles are removed. Tangles form when non-terminated shoots grow and wind around each other.

Kiwifruit vines are known for their high vigour, so summer pruning is necessary to prevent the canopy becoming too dense. A vine that has been pruned well in summer is also easier to prune in the winter. Other advantages of a well pruned canopy are: greater bee activity at pollination, as bees to not like to fly through a dense canopy, a lower incidence of disease such as on vine botrytis and sclerotinia, and finally maintenance of the optimum leaf area index (LAI). It is often necessary to summer prune several times in the growing season. Vines should be pruned to an LAI of about 3.5 so that about 95% of light is absorbed by the vine and only 5% filters through, in this way light interception is optimised (Figure 1.4.). It has been shown that shading of vines, from within canopy shading or external sources such as shelter belts, can cause losses in vine productivity and affect fruit quality due to an increase in misshapen fruit. It was found that it is leaf exposure as opposed to direct fruit exposure that is important (Biasi *et al.*, 1995).

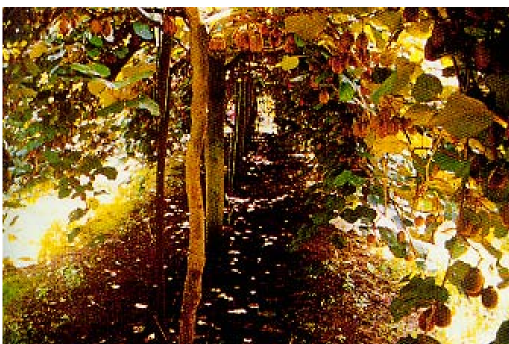


Figure 1.4. View at midday of an *A. deliciosa* vine trained on a T-bar system with an overall leaf area index of about 3. Note the amount of speckled light penetrating the canopy and reaching the ground (Buwalda *et al.*, 1992).

1.2.2. Flowering and fruit:

It is necessary to determine an optimum crop load. In doing this a compromise needs to be made between fruit number and fruit size. A vine

with a high crop load generally produces small fruit and a vine with a low crop load will generally produce large fruit. A high crop load has also been shown to lead to a reduced flower initiation in the following season. The fruit are thinned from the beginning of December to March, they are thinned not just to obtain the optimum crop load but also to remove rejects. Reject fruit are removed first to reduce the crop load. Reject fruit are those that are not of the desired shape (caused by developmental deformities, genetic abnormalities or poor pollination), small fruit, and frost, wind, sun, or insect damaged fruit.

Timing of anthesis has also been found to affect fruit size of *A. deliciosa*. It was observed that fruit formed during an early period of anthesis were significantly larger than fruit formed during a late period approximately 10 days after the first. Average fruit weights were 100 g and 86 g respectively (Cruz-Castillo, *et al*., 1991).

Actinidia fruit have been shown to follow a double sigmoid growth curve (Figure 1.5. a) which can be split into three stages (Figure 1.6.) (Hopping, 1976a). Stage I was described as period in which fresh fruit weight and volume increased rapidly (0-58 days after flowering); stage II was the phase of slow growth; following this was another period of rapid growth until maturity, stage III. Fruit growth in stage I was then divided into two, the first part being due to cell division in both the inner (IP) and outer pericarp (OP) and the central core, the second part was said to be predominantly due to cell enlargement in all of these tissues (Figure 1.5. a, 1.5. c). Cell division was found to end after 23 and 33 days in the OP and IP respectively, but

continued in the core until 110 days after flowering. No division of stages of growth was determined for dry weight only fresh weight. This growth pattern was established using *A. deliciosa* 'Monty', a previous study by Pratt & Reid (1974) using the 'Bruno' kiwifruit cultivar found that those fruit followed a triple sigmoidal growth curve, and it has been suggested that 'Monty' may follow the same growth curve but that the sampling interval was too wide to detect it (Hopping, 1976a). A potential hypothesis behind the perhaps inconsistent appearance of a triple sigmoidal growth curve is that growing conditions such as rain during the final period of growth may induce a small increase in fresh fruit weight and thus cause the appearance of a third period of rapid growth (Woolley personal communication). *Actinidia deliciosa* 'Hayward' has been shown to follow the same double sigmoidal growth curve observed by Hopping (1976a) (Woolley *et al.*, 1991).

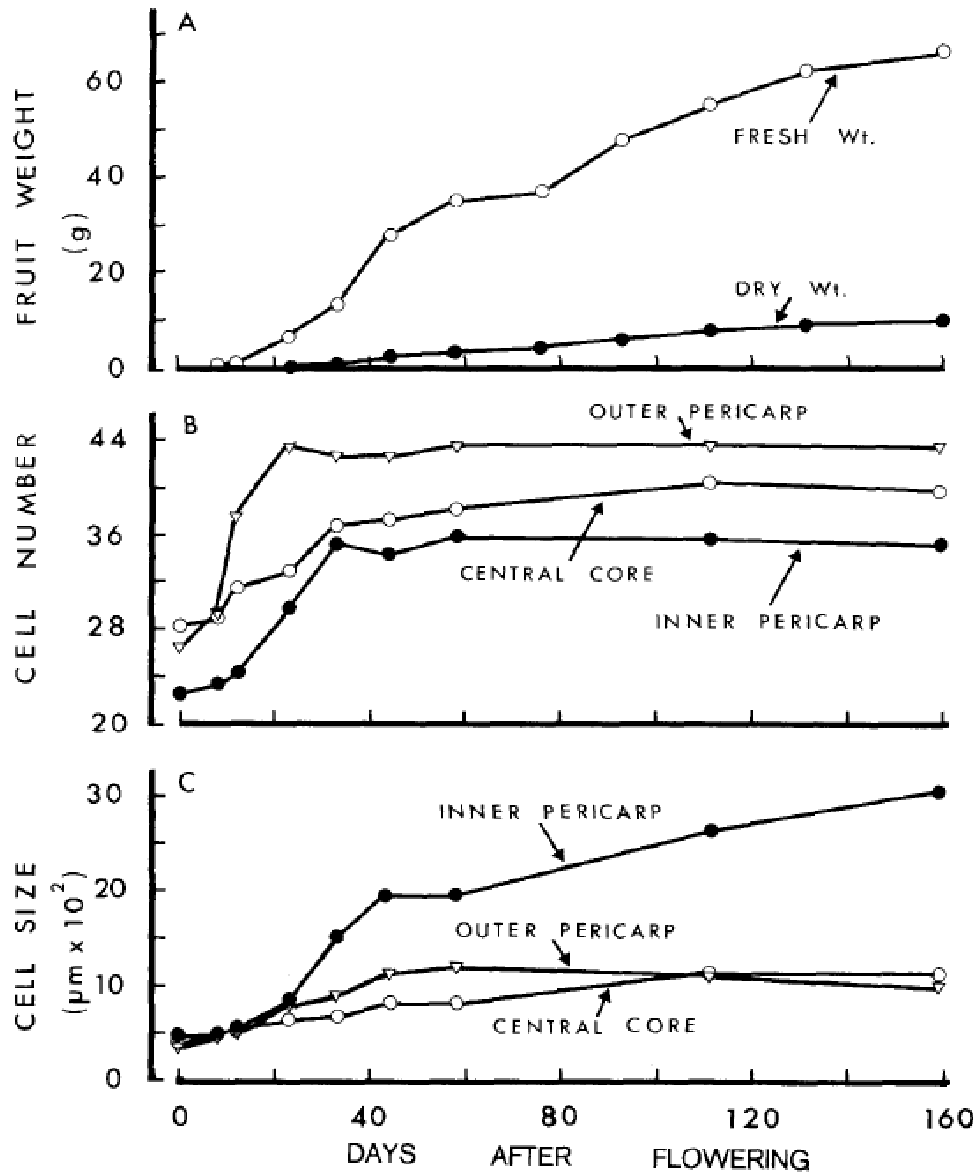


Figure 1.5. Fruit development of *Actinidia deliciosa* ‘Monty’. A, cumulative increases in fresh and dry weight of fruit; B, cell number of the inner and outer pericarps and core; C, mean cell size of the inner and outer pericarp and core (Hopping, 1976a).

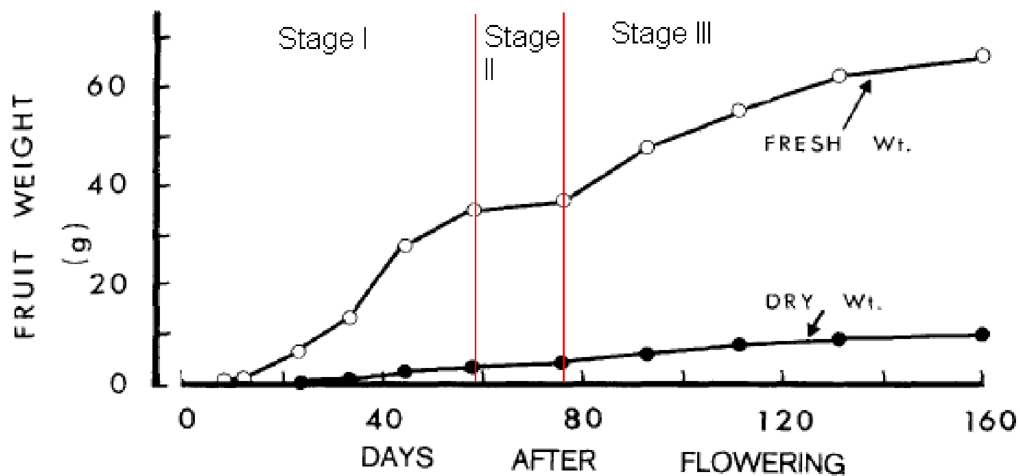


Figure 1.6. Fruit development of *Actinidia deliciosa* 'Monty', cumulative increases in fresh and dry weight of fruit (Hopping, 1976a). Showing the three stages of growth; stage I: rapid growth due to first predominant cell division then cell expansion; stage II: a phase of slow growth; and stage III rapid growth until maturity due predominantly to cell expansion.

1.3. Role of hormones in fruit growth:

Plant hormones are pleiotropic, that is, they play multiple roles in the regulation of plant growth and development. It appears that hormones derived from seeds play the main regulatory role in fruit development, with the exception being parthenocarpic fruit (some of which are induced by the application of exogenous hormones) (Atwell, *et al.*, 1999). The three main hormones associated with fruit growth are auxin, cytokinins and gibberellins. As a general rule it appears that auxin and gibberellins work in conjunction with one another to initiate cell division and expansion and cytokinins initiate cell division (Atwell, *et al.*, 1999). Auxin and gibberellins have been found to stimulate plant growth by increasing the extensibility of cell walls and thus allowing cell expansion (Raven, *et al.*, 1992).

1.3.1. Auxin:

Developing seeds are a source of auxin and most fruit require the auxin from seeds to grow (Raven, *et al*., 1992). For example, if the achenes are removed from a strawberry the fruit will not grow, however, if exogenous IAA is applied it will (Nitsch, 1950). This experiment fulfilled several conditions of causality as defined by Jacobs (1959), though not all. By removing the suspected source of IAA, the achenes, it fulfils the excision condition and by applying a synthetic auxin it fulfils the substitution condition. By fulfilling these conditions it can be said that the argument, that IAA is causally associated with fruit growth, is strengthened. The achenes of a strawberry are the true fruit, however, they contain one seed each and this is what contributes the IAA to the false fruit. This experiment was replicated in kiwifruit by Hopping (1976a). As the seeds of a kiwifruit fruit are internal they could not be removed as in the case of the strawberry, however, by removing all but two of the stigmatic surfaces from the flower prior to pollination the seed number could be significantly reduced. Fruit were left with less than 100 seeds. These fruit were then treated with auxin, cytokinin, gibberellin, or all combinations. Neither one hormone nor any of the combinations could restore the fruit size to that of fruit from an untreated whole flower, though treated fruit size was greater than untreated fruit from a flower with low numbers of styles. It was suggested that a hypothetical seed factor(s) is required above some level before the response to any of the hormones can be effective (Hopping, 1976a). This suggestion is supported by the finding that the strawberry

receptacle development could be maintained, in the absence of achenes, by exogenous auxin provided that the achenes were allowed to stay on the fruit for some days following pollination (Nitsch, 1950).

1.3.2. Cytokinins:

Cytokinins are primarily associated with actively dividing tissues such as seeds and fruits (Raven, *et al*., 1992). Cytokinins are suspected to be causally associated with division in the cell cycle (Jacqmard *et al*., 1994).

1.3.3. Gibberellins:

Gibberellins have been found in varying concentrations in all plant parts, the highest concentrations are usually associated with immature seeds (Raven, *et al*., 1992). Some fruits such as mandarins, almonds, and peaches have been found to be responsive to gibberellins but not auxin (Raven, *et al*., 1992). This indicates that the limiting factor in fruit growth of these plants is gibberellins, not auxin. Gibberellins are used commercially to increase the size of some varieties of table grapes (Raven, *et al*., 1992). Gibberellins have been found to promote fruit elongation of *A. deliciosa* that have been stimulated with a synthetic cytokinin (Cruz-Castillo *et al*., 1999).

1.4. *Effect of Benefit® on A. chinensis and A. deliciosa fruit:*

Benefit® is a commercial preparation used to increase the size of kiwifruit. It has been marketed under several names, originally Benefit® PZ, then Benefit® Gold, and now, as it is being recommended for use on both major cultivars, Benefit® Kiwi. It is currently only applied to *A. chinensis*, however, recently it has been recommended for use on both *Actinidia sp.*. Application of Benefit® to *A. chinensis* has been shown to significantly increase fresh weight of fruit (16.9 g per fruit), however, when applied to *A. deliciosa* the fresh weight was not significantly increased (2.29 g) (Woolley & Cruz-Castillo, 2006). In the experiment conducted by Woolley & Cruz-Castillo (2006) Benefit® was applied once at 20 days after full bloom (DAFB) at the commercially recommended application rate of 2.5 mlL^{-1}. The commercial recommendation for Benefit® is three applications with the first at 90-95 percent petal fall (approximately 10-15 days after full bloom), then the second and third at seven day intervals. The application of Benefit® in this experiment was likely to have achieved a better coverage of fruit and leaves than when it is applied commercially, however, given that canes were specifically targeted rather than the general vine application achieved commercially by tractor spray units. Those results indicated that the two *Actinidia* cultivars have different limiting factors. In contrast to the aforementioned results of Woolly and Cruz-Castillo (2006) Costa *et al*. (2002) found that application of Benefit® to *A. deliciosa* did significantly increase fruit weight. Benefit® was applied at 3mlL^{-1} three times, with the first application time being 15 days after full bloom and the second and third at 14 day intervals. This experimental set up was repeated over

three years with no adverse effect on return bloom and the average increase in fruit weight over the three years was 8.3 g per fruit.

Benefit® has been found to have very little cytokinin activity (D.J. Woolley, personal communication). However, results have shown that cytokinins do appear to be a limiting factor in both cultivars (Woolley and Cruz-Castillo, 2006). When the synthetic cytokinin-like compound CPPU was applied to both gold and green kiwifruit statistically significant (P=0.05) increases in weight were observed (43.2 and 47.9 g respectively). The manufacturer of Benefit® describes it as a fluid organic nitrogenous fertilizer and states that: “Benefit Kiwi® promotes cell division in the early phases of development after setting” (Valagro, 2008). They also state that Benefit Kiwi® is comprised of:

- nucleotides which stimulates [sic] cell division;
- specific amino acids which induce an acceleration of all the most important metabolic reactions (and, particularly of protein synthesis) and give cell greater resistance to stress of difference sources;
- vitamins, cofactors essential to cell metabolism. (Valagro, 2008).

It would appear that Benefit® can be used to increase the fruit weight of *A. chinensis*. However, the effect on *A. deliciosa* is inconclusive. More study is required to establish the effect on *A. deliciosa*, the mechanism behind the increase in fruit weight and, if the response of the *Actinidia sp.* does differ, what the underlying difference in the physiology between these two cultivars are. In view of its properties (Woolley, personal communication) Benefit® has been associated with known plant growth regulators particularly

gibberellins. Therefore throughout this thesis Benefit® will be referred to and treated as a natural plant growth regulator.

1.5. Effect of CPPU on *A. chinensis* and *A. deliciosa* fruit

The effect of the synthetic cytokinin-like compound CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) on both *A. chinensis* and *A. deliciosa* has been shown to be an increase in fruit weight. These results have been repeated multiple times under different conditions. Growers of kiwifruit in Japan use CPPU to improve fruit size (Patterson *et al*., 1993). Individual fruitlets are dipped by hand approximately 20 days after full bloom, this labour intensive application method is, however, not feasible in New Zealand. CPPU is also used commercially in Italy and China, however Zespri do not allow it to be used commercially on kiwifruit of any cultivar in New Zealand.

Application of CPPU on *A. deliciosa* at 5 mgL^{-1} has been shown to produce a significant increase in fresh weight (47.91 g) of fruit when compared to untreated fruit. An increase in fresh weight (43.3 g) is also seen when CPPU is applied to *A. chinensis* at 5 mgL^{-1} (Woolley & Cruz-Castillo, 2006). In contrast, in another experiment, application of 1 mgL^{-1} CPPU to *A. chinensis* only increased fresh weight of fruit by 4.4 g per fruit which was found to be a statistically insignificant increase (Woolley & Currie, 2006). The experimental procedure did differ between those two trials, application of CPPU being 5 mgL^{-1} when a response was achieved in *A. chinensis* and 1 mgL^{-1} when a response was not achieved. Therefore, it may be that the concentration of

CPPU required by *A. chinensis* to generate a response is higher than 1 mgL^{-1}; application dates of the two experiments were consistent. However, in the same experiment when CPPU was applied to *A. deliciosa* (at 1 mgL^{-1}) fresh weight of fruit was increased by a significant 37.9 g per fruit which was consistent with the other results. Given that the different application rates of CPPU both generated similar results in *A. deliciosa* it may imply that *A. deliciosa* is more sensitive to CPPU or that the two cultivars have different limiting factors. Similar results were achieved by Patterson *et al.* (1993) on *A. deliciosa*, in that study application methods were also tested. CPPU was applied at 21 days after full bloom at 5 mgL^{-1} either by dipping individual fruitlets in the solution for approximately 5 seconds or by spraying canes. At final harvest the average dipped fruit was 48 g heavier and the average sprayed fruit was 36 g heavier than control fruit. This was a 44 and 33 percent weight increase respectively. In another study conducted on *A. deliciosa,* application of CPPU 24 days after anthesis increased fruit weight by 58.8 g (Iwahori *et al.*, 1988). In this study multiple application dates were tested. CPPU was applied at 3 days after anthesis, at anthesis, and 3 and 24 days after anthesis, it was applied at 40 mgL^{-1}. All treatments, with the exception of 3 days before anthesis, resulted in an increase in fruit weight. It was noted that fruits treated at or prior to anthesis showed parthenocarpic development. Similar results were achieved under similar conditions by Lewis *et al.* (1996). Intact *A. deliciosa* flowers were dipped in 40 mgL^{-1} CPPU at anthesis and fruit were, at final harvest, 17 percent heavier than control. This differs from previous results achieved by Nickel (1986); where application of CPPU at anthesis was ineffective at increasing fruit growth (Iwahori *et al.*, 1988).

Parthenocarpic fruit development was also achieved by Lewis *et al.* (1996). *Actinidia deliciosa* flowers with all styles removed, which were therefore unpollinated, were dipped in 40 mgL^{-1} CPPU at anthesis, fruit produced were of a similar size to fully pollinated controls. Lawes *et al.* (1991) also found application time and concentration of CPPU were important in determining the response of *A. deliciosa* fruit. It was found that an early application, that is application prior to 21 days after full bloom, resulted in poor fruit shape. It is suggested that multiple applications of a low concentration (5 mgL^{-1} for example) would give the most satisfactory response, that is, increased fruit size without an alteration of fruit shape. These results are consistent with those achieved by Cruz-Castillo *et al.* (1999) where *A. deliciosa* fruit were dipped in 20 mgL^{-1} CPPU 56 days after full bloom and 10 mgL^{-1} CPPU 21 days after full bloom, average fruit fresh weight was increased by 15 and 48 g respectively.

1.5.1. Fruit maturity and storage:

CPPU was found to advance fruit maturity of *A. deliciosa*, as determined by the time to reach 6.2 percent soluble solids, by approximately 1 week (Patterson *et al.*, 1993). CPPU treated fruit were softer, at harvest, than untreated fruit. Nevertheless, CPPU had no significant effect on the rate of fruit softening in storage. These results differ from those achieved by Iwahori *et al.* (1988), where it was found that fruit treated with CPPU had a significantly increased rate of softening after harvest. However, the concentration of CPPU used by Iwahori *et al.* (1988) was higher, 40 mgL^{-1} as

opposed to 5 mgL^{-1}, and storage conditions may have also differed. It was found in *A. deliciosa* 'Monty' that fruit treated with CPPU had a significantly higher total sugar content than untreated control fruit (Kurosaki & Mochizuki (1990). In another experiment to observe the effects of CPPU on ripening and storage life of *A. deliciosa* fruit, fruit soluble solids and flesh firmness were determined at harvest and followed at 0, 3, and 5 months after storage (Costa *et al.*, 1995). Fruit were sprayed with 20 mgL^{-1} CPPU 15 days after full bloom in Italy and fruit were stored in an unaltered atmospheric condition at -0.5°C. It was observed that at harvest fruit treated with CPPU had higher soluble solids (brix) 7.5 compared with 6.9 for the control fruit, and had a lower flesh firmness 6.3 (kg/cm^2) compared with 6.3 for the control. That is, the fruit treated with CPPU were softer and sweeter at harvest. It was also observed that the fruit treated with CPPU reached their climacteric peak two days earlier that the control, that is, the CPPU treated fruit reached the maximum ethylene production point earlier than the control. Although the CPPU treated fruit reached the peak earlier both the CPPU and control fruits showed almost the same ethylene production. During storage the evolution of flesh firmness and soluble solids in both the control and CPPU treated fruits followed the same pattern with the CPPU fruits finishing (after 5 months) with slightly lower soluble solids and flesh firmness. Cruz-Castillo *et al.* (1999) also observed a reduction in flesh firmness in fruit treated with CPPU at final harvest. It would appear from the observations of Patterson *et al.* (1993) and Costa *et al.* (1995) and Cruz-Castillo *et al.* (1999) that CPPU advances *A. deliciosa* fruit maturity but does not significantly alter the storage capabilities of this fruit.

It can be concluded that CPPU does have an effect on fruit of both the major kiwifruit cultivars. However, the significance of the effect does appear to be affected by concentration, timing and method of application. What is not known is how CPPU causes an increase in fruit weight or how it interacts with other plant growth regulators, this will be discussed subsequently.

1.6. Effect of other plant growth regulators on A. chinensis and A. deliciosa fruit

Lorenzo *et al.* (2007) looked at the effects of one representative from each of the hormone groups associated with fruit growth of *A. deliciosa*, in Spain. Treatments of 2,4-D (2,4-dichlorophenoxyacetic acid) (auxin), CPPU, and GA_3, and all their possible combinations, were applied by spraying 43 days after full bloom at 25, 50, and 10 mgL^{-1} respectively. It was found that GA_3 alone did not affect fruit size or weight, and CPPU and 2,4-D increased both fruit size and weight. However, it was the combinations of hormones that gave the greatest increases of fruit size and weight. Application of CPPU plus GA_3 plus 2,4-D, and CPPU plus 2,4-D gave the greatest increase in fruit length and diameter, and the former combination gave the greatest increase in fruit weight. The study indicated that all three hormone groups may be limiting fruit growth in green kiwifruit. These results support those of Cruz-Castillo *et al.* (1991) and Cruz-Castillo *et al.* (1999). In the earlier study four treatments were applied to *A. deliciosa*, a control, CPPU (10 mgL^{-1}), CPPU (10 mgL^{-1}) plus 2,4-D (2.5 mgL^{-1}) plus GA_3 (5 mgL^{-1}), and CPPU (10 mgL^{-1}) plus 2,4-D (25 mgL^{-1}) plus GA_3 (50 mgL^{-1}). Fruit were dipped in the appropriate solution

for 5-10 seconds 21 days after full bloom. Although the concentrations and treatment applications were different between the two experiments the results were complementary, with the combination of hormones giving significantly heavier fruit than CPPU alone. It was also found that the higher concentrations also significantly increased fruit weight (Cruz-Castillo *et al.*, 1991). Famiani *et al.* (2007) achieved similar results under slightly different conditions; treatments were applied by dipping the fruit for approximately 5 seconds in the appropriate solution 15 days after full bloom. Again GA_3 was found to have no effect on fresh or dry weight of fruit, despite being applied at a higher concentration (50 mgL^{-1}). And again the greatest increase in fresh weight was obtained with the application of representatives from each hormone group or one from the cytokinin and auxin groups. Both studies used 2,4-D, however Famiani *et al.* (2007) used TDZ (thidiazuron) as their cytokinin representative at 10 mgL^{-1} and 2,4-D was applied at 20 mgL^{-1}. Hopping (1976b) found very similar results through testing a number of different synthetic and naturally occurring representatives from each of the main hormone groups. Treatment of fruits singly with auxin (2,4-D, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), NOA (β-Naphthoxyacetic acid), or IAA-asp), gibberellin (GA3, or GA4-7) or cytokinin (BA (6-Benzylamino-purine) or zeatin) did not increase fruit size. Combinations of auxin plus gibberellin and gibberellin plus cytokinin were also found to be ineffective in increasing fruit size. Instead it was the combinations of auxin plus cytokinin and auxin plus cytokinin plus gibberellin that were found to be effective in increasing fruit size. These results were corroborated by Lorenzo *et al.* (2007) and Famiani *et al.* (2007), though different representatives of the hormones were used.

Famiani *et al*. (2007) observed accelerated ripening, as determined by flesh firmness and soluble solid content, in fruit treated with TDZ, this observation was in contrast to results obtained by Patterson *et al*. (1993) when fruit were treated with CPPU (section 1.5). It was found that fruits treated with TDZ or 2,4-D had lower dry matter percentages than untreated ones.

Another synthetic auxin, 3,5,6-TPA (3, 5, 6-trichloro-2-pyridyloxyacetic acid), which is registered for use in citrus, has been tested on *A. deliciosa* in Italy (Bregoli *et al*., 2006). This chemical is available commercially as Maxim® which contains 10 percent w/w 3,5,6-TPA. The effect of 3,5,6-TPA was studied over 3 years. In Year One, it was applied at 7, 10, and 14 g/ha 70 days after full bloom; in Year Two at 1.5 g/ha 20 days after full bloom, and 7 and 10 g/ha 70 days after full bloom, and 10 g/ha 100 days after full bloom; and in Year Three at 1.5 and 7 g/ha at 20 and 70 days after full bloom, respectively. It was found that all concentrations produced an increase in fruit size though the greatest increase was achieved at 10 g/ha, with the fruit being 24 g heavier at final harvest than the control. It was also found that the date of application had a significant effect. Fruit sprayed at 20 days after full bloom did not differ in weight from the controls; applications at 70 and 100 days after full bloom, however, gave similar increases in fruit weight (Bregoli *et al*., 2006). It was observed from the same experiment that soluble solid content was lower and flesh firmness was higher in fruit treated with 3,5,6-TPA than controls regardless of treatment concentration or application date (Fabbrioni *et al*., 2007). That is, fruit maturity was delayed by treatment with 3,5,6-TPA. Fruit were stored for three months in a cold store with unaltered atmospheric

conditions. After one month in storage soluble solid content of 3,5,6-TPA treated fruit was still lower than the control but not significantly so, and the flesh firmness remained significantly higher. After three months in storage the soluble solid content of 3,5,6-TPA treated fruit was significantly lower than the control. There were no observed differences in flesh firmness, that is treated fruit had softened to the same point as control fruit.

Jindal *et al*. (2003) studied the effects of three plant growth regulators on fruit physiology and their effectiveness for chemical fruit thinning on *A. deliciosa* cultivar Allison in India. The plant growth regulators used were TDZ (25, 50, and 100 mgL^{-1}), ethrel (100, 200, and 400 mgL^{-1}) and carbaryl (500, 1000, and 1500 mgL^{-1}), these were applied at petal fall, which is approximately 15 days after full bloom, and again 10 days later. Fruit treated with TDZ (a synthetic cytokinin) were found to be larger than the control or either of the other treatments. There was a 74 percent increase in fruit weight from the 50 mgL^{-1} TDZ treatment when compared to the control. There was no significant difference between the different TDZ concentrations. TDZ is also thought to have induced a small amount of fruit thinning; an average of 8.3 percent across the different concentrations, fruit thinning on untreated vines was 1.48 percent. Ethrel was applied at 10 mgL^{-1} as a 5-10 second dip 56 days after full bloom in another experiment on *A. deliciosa* ‘Hayward’ in New Zealand and again no significant effect on fresh weight of fruit was observed (Cruz-Castillo *et al*., 1999).

The effect of Phytagro and Glucos P was studied on *A. deliciosa* in Italy over three consecutive years (Costa *et al.*, 2002). Phytagro is a plant extract with all three hormones represented IAA (70 mgL^{-1}), zeatin (1000 mgL^{-1}), and GA3 (700 mgL^{-1}), and Glucos P is a phosphorilate glucose which is credited with enhancing photosynthesis. Both of these bioregulators were applied at 15 days after full bloom and applications were repeated twice at two week intervals at a rate of 1mlL^{-1} and 3mlL^{-1} respectively. Return bloom was not effected in any of the years studied. The average fresh weight at final harvest over the three years of fruit treated with Phytagro was 7.4 g heavier than the control, whereas Glucos P did not significantly increase fruit weight.

These results perhaps indicate that gibberellins only become limiting when fruit growth is stimulated by other hormones. They also indicate that it is conceivably auxin and/or cytokinins that are initially limiting kiwifruit fruit growth, when other factors, such as water, are not limiting.

1.8. Interactions between plant growth regulators and other commercial practices:

1.8.1. Girdling:

Girdling is a common commercial practice whereby a thin ring of bark is removed around either the trunk or the canes of the kiwifruit vine; this restricts the flow of phloem sap and, therefore, the flow of photosynthates down to the roots. This allows the fruit to become more of a competitive sink against the vine, thus allowing the fruit to accumulate more photosynthates. Girdling

promotes fruit growth in both *A. deliciosa* (Lai, *et al.,* 1989; Woolley, *et al.*, 1991; Woolley & Cruz-Castillo, 2006) and *A. chinensis* (Woolley & Cruz-Castillo, 2006). It has been shown that *A. deliciosa* fruit on laterals that have been girdled are significantly ($p < 0.05$) larger than fruit on un-girdled laterals with the same leaf:fruit ratio of 2:1 when the lateral supported five fruit (Lai, *et al.*, 1989). An average increase of 14 g per fruit was achieved under these conditions (Lai, *et al.*, 1989). This indicates that the fruit are not the most competitive sink in the kiwifruit vine. By isolating the lateral from the rest of the vine, in terms of the import and export of photo-assimilates by way of the phloem, the fruit are able to rise in rank as it were and become the most competitive sink in the girdled fruit shoot system. They therefore accumulate more carbohydrates and increase in weight. Interactions have previously been studied between Benefit® and girdling, and between CPPU and girdling, on both *A. deliciosa* and *A. chinensis*.

There is a positive interaction between girdling and the application of Benefit® when applied to *A. chinensis* (Woolley & Cruz-Castillo, 2006), that is, there is a synergistic effect when these treatments are used together, and the average fruit weight when the treatments are used together is greater than the sum of treatments are applied singly. It was found that girdling of *A. chinensis* alone gave an average fruit weight of 77.2 g; this was an increase of 15.2 g from the control. Benefit® alone applied to *A. chinensis* produced an average fruit weight of 78.9 g; this was an increase of 16.9 g from the control. When the two treatments were applied together the average fruit weight was 101.8 g which was an increase of 39.8 g from the control (Woolley & Cruz-Castillo,

2006). It was, therefore, concluded that the two treatments caused a synergistic effect because the sum of the increase in fruit weight caused by the individual treatments is less than the increase caused when the two treatments are used together, that is, 39.8 g > 15.2 g + 16.9 g (Woolley & Cruz-Castillo, 2006). This indicates that although there are more photosynthates available to the fruit when the vine has been girdled the fruit do not accumulate them to their full potential. Therefore, Benefit® may act to increase their sink strength. These treatments did not result in the same synergistic interaction when applied to *A. deliciosa* (Woolley & Cruz-Castillo, 2006). The application of both treatments did not result in a greater increase in fruit weight than when the increase in fruit weight caused by the individual treatments is summed (Woolley & Cruz-Castillo, 2006). This result is not unexpected as Benefit® has been found to have a lesser effect on *A. deliciosa* than *A. chinensis.*

There was been found to be an additive interaction between girdling and CPPU on both *A. deliciosa* and *A. chinensis* (Woolley & Cruz-Castillo, 2006). It was found that girdling of *A. deliciosa* alone resulted in an average fruit weight of 106.8 g which was an increase of 22.2 g from the control. CPPU applied alone resulted in an average fruit weight of 132.5 g, an increase of 47.9 g. When the two treatments were applied together the average fruit weight was 146.5 g, an increase of 61.9 g from the control. However, although this increase is greater than for either of the treatments when applied alone it is not considered to be a synergistic interaction because the sum of the increase in fruit weight caused by the individual treatments is greater than the

increase caused by the application of both treatments, that is 61.9 g< 22.2 g + 47.9 g (Woolley & Cruz-Castillo, 2006). Similar results were obtained when the same experimental conditions were applied to *A. chinensis*, with girdling alone resulting in an increase in fruit weight of 15.2 g and the application of CPPU alone increasing fruit weight by 43.2 g. When the two treatments were applied together the average increase in fruit weight was 50.8 g, this again was not greater than the sum of the treatments alone (Woolley & Cruz-Castillo, 2006).

1.8.2. Crop load:

The effect of CPPU was studied at two different crop loads of *A. deliciosa*. Fruit were sprayed with 10 mgL^{-1} CPPU 21 days after full bloom; whole vines were used as one experimental unit. At a moderate crop load (35 fruit/m^2) fresh weight was increased by an average of 34.9 g per fruit, at a low crop load (25 fruit/m^2) the increase was 37 g per fruit; these increases were not found to be significantly different (Woolley *et al.*, 1991). These results support those found by girdling vines, that is, they indicated that it is the competitive ability of the fruit that was limiting fruit growth as opposed to the carbohydrate supply.

1.9. Overview of potential mechanisms of action:

Fruit growth and final size is dependent on both cell number and cell size, that is, the number of cells present at fruit set plus the number of subsequent cell divisions and the proportions to which the cells expand. It has been suggested that CPPU acts to increase fruit size by way of cell division rather than increasing the sink strength (Neri, *et al*., 1993). It was also observed in *A. deliciosa* ‘Monty’ fruit treated with CPPU that cell size did not increase and it was, therefore, concluded that the increase in fruit size and weight was due to an increase in cell number, that is cell division (Kurosaki & Mochizuki, 1990). This would seem to be a logical conclusion as CPPU is a cytokinin–like substance. However, it is unknown whether it acts directly as a cytokinin or indirectly to influence natural cytokinin production. Takahashi *et al.* (1978), found CPPU to exhibit strong cytokinin activity (as cited by Neri, *et al*., 1993). It has been found that by applying both CPPU and naturally occurring cytokinins a greater response, in terms of fruit size, is achieved (Woolley & Currie, 2006). It has, therefore, been suggested that rather than directly increasing fruit size by acting as a cytokinin CPPU may act to protect naturally occurring cytokinins from cytokinin oxidase (Woolley & Currie, 2006). Other studies, however, have had conflicting results.

Lewis *et al.* (1996) found that there was no difference in cell division between fruit treated with CPPU and control and therefore concluded that the differences in fruit size must be due to cell expansion. The same study also found a decrease in endogenous cytokinin levels. It was, therefore, suggested

that CPPU is an active cytokinin that may act directly to increase fruit size and the presence of CPPU causes a feedback loop which reduces the endogenous cytokinin production. Correspondingly Patterson *et al*. (1993), found a 30 and 22 percent increase in mean cross-sectional area of small parenchyma cells in the pericarp of *A. deliciosa* fruit dipped and sprayed with CPPU respectively when compared to untreated control fruit. It was suggested that the increase in fresh weight is due to cell expansion despite evidence that suggests cytokinins are associated with cell division. Increased fruit weight was found to be due to increased accumulation of both water and dry matter. CPPU dipped fruit had 2 percent more water than control fruit, however, the difference in fruit weight was 44 percent; this indicated a significant increase in dry matter accumulation. In contrast to this Woolley *et al*. (1991) found that both cell division and expansion were affected by CPPU application. *Actinidia deliciosa* fruit were dipped in 10 mgL^{-1} 24 days after full bloom; fruit were then harvested at intervals throughout the growing season. It was found that the period of rapid cell division in the OP of the control had ceased by 24 days after full bloom. However, in fruit treated with CPPU the period of cell division continued to between days 31 and 38. It was found that cell size in the OP of CPPU treated fruit was larger than the control by an average of 15 microns. This increase in cell division and expansion in the OP was proposed to be the cause of the increased proportion of OP in the CPPU treated fruit. It was found that the proportion of IP decreased and the core was unchanged by the application of CPPU, no explanation for this was given (Figure 1.7.).

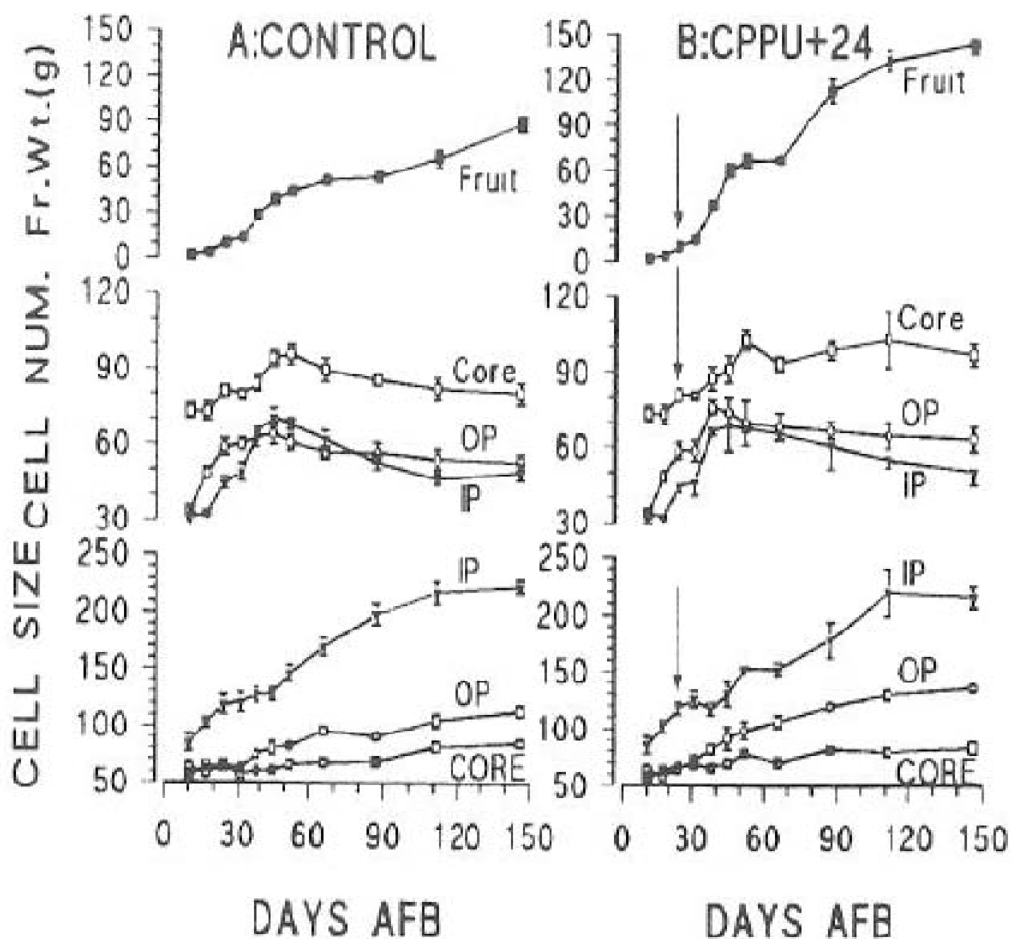


Figure 1.7. Effect of 10 mgL-1 CPPU applied 24 days after full bloom (AFB) on cell activity of *A. deliciosa* fruit. OP = outer pericarp; IP = inner pericarp; cell size in microns (Woolley *et al.*, 1991).

It has also been observed that fruit treated with CPPU were a darker green colour in the OP than control fruit. It was suggested that this was due to increased chlorophyll production. A reduction in tannin deposition was also observed in the sub-hypodermal layer of cells of CPPU treated fruit. It was noted that this could have contributed to the greener appearance of the fruit as tannins mask the underlying green chlorophyll in the skin (Patterson *et al.*, 1993). This phenomenon was also observed by Kurosaki & Mochizuki (1990), Lawes *et al.* (1991), Costa *et al.* (1995), and Cruz-Castillo *et al.* (1999). Costa

et al. (1995) also noted that fruit treated with CPPU did not lighten in colour as much as control fruit after five months in storage.

CPPU, along with *N*-phenyl-*N'*-(1,2,3-thiadiazol-5-yl)urea (another synthetic cytokinin), has been described as a strong positive regulator of cell division and shoot formation (Shudo, 1994). Another synthetic cytokinin-like substance, TDZ, is reported to stimulate endogenous adenine type cytokinin biosynthesis, or alter endogenous cytokinin metabolism and thus causes an increase in levels of endogenous cytokinins and increased cell division (Mok *et al.*, 1987). These are characteristics associated with naturally occurring cytokinins. TDZ was the cytokinin representative studied by Famiani *et al.* (2007) in relation to the effect hormones on *A. deliciosa* fruit. Mok *et al.* (1987) found that CPPU exhibited the same activities as zeatin. This does not, however, mean that CPPU acts as a cytokinin as Laloue & Fox (1989) found that CPPU strongly inhibited the activity of cytokinin oxidase which had been isolated from wheat (*Triticum aestivum* L.) germ . A potentially supporting hypothesis of cytokinin regulation of cell division involves a positive feedback loop where cytokinins or physiologically related cell division-promoting substances (such as CPPU) either inhibit their own degradation or induce their own biosynthesis (Meins & Binns, 1978; Meins, 1989 as cited by Meins Jr., 1994).

CPPU has been found to be effective only if applied to fruit (Woolley, personal communication) and completely ineffective if applied to leaves. This suggests that the compound has little or no mobility (Neri, *et al.*, 1993). In contrast to

CPPU, Benefit® is effective if applied to the leaves. It has in fact been shown that there is a greater increase in fruit growth if Benefit® is applied to the leaves as opposed to the fruit (Woolley & Cruz-Castillo, 2006). Application of Benefit® directly to the fruit resulted in an increase of 8.7 g per fruit, and application of Benefit® to only the leaves resulted in an increase of 16.9 g per fruit. This suggests that the active compound in Benefit® is readily transported (Woolley & Cruz-Castillo, 2006). Perhaps an inhibitor that is part of the natural plant extract that Benefit® is produced from is not as readily transported and therefore when Benefit® is applied to leaves the two compounds are separated. It has been found that a partially purified extract of Benefit® is more active than Benefit® itself, suggesting the presence of an inhibiting substance (Woolley, personal communication).

1.10. Conclusion:

Kiwifruit are of huge importance to New Zealand horticultural industry and it appears that this position is unlikely to change in the foreseeable future with planting areas increasing, consumer demand unwavering, and development of new cultivars. Fruit size is the major quality factor that growers and consumers are concerned about with dry matter content following close behind. Therefore an increase in the knowledge of factors that can improve these factors without adversely affecting others is necessary for New Zealand to continue to lead the way in quality and innovation.

Currently the only plant growth regulator used to increase fresh weight of fruit in New Zealand is the natural plant extract Benefit Kiwi® and this is only

widely commercially used on *A. chinensis*. There are some contradictory reports on its effectiveness on *A. deliciosa*; however, the predominant conclusion is that Benefit Kiwi® is much more effective in increasing fruit size of *A. chinensis*. Another plant growth regulator that is not used commercially on kiwifruit in New Zealand but shows promise in terms of increases in fresh weight is the synthetic cytokinin CPPU. CPPU appears to increase fresh weight of both *A. chinensis* and *A. deliciosa*. CPPU has also been shown to accelerate maturity. Both of these plant growth regulators have been shown to interact favorably with other current commercial practices such as pollination, girdling, and crop load.

It appears that, of the three hormones associated with fruit growth, fruit of both *A. chinensis* and *A. deliciosa* are limited by levels of cytokinins and/or auxins but that gibberellins only become limiting when fruit growth is stimulated by representatives from the other hormone groups. This conclusion, however, is dependent upon the assumption that the synthetic hormones studied, for example CPPU and 2,4, D, behave like naturally occurring hormones when applied to fruit. And while there is some evidence to support this there is also contradictory evidence as well. For example, CPPU has been shown to act as a strong regulator of cell division and shoot formation which are characteristics of naturally occurring cytokinins. However CPPU has also been shown to inhibit the activity of cytokinin oxidase, which is not a characteristic associated with naturally occurring cytokinins and while this does not rule out the possibility that CPPU also acts as a naturally occurring cytokinin it does not support the theory.

Therefore the objectives of the present research were:

- Identify what interactions occur between the plant growth regulators CPPU and Benefit® in both *A. chinensis* and *A. deliciosa* fruit
- Identify how endogenous hormones are affected by CPPU application
- Determine how fruit weight and percentage dry matter are affected by exogenous hormones
- Identify differences in endogenous hormones and physiological characteristics in *A. chinensis* and *A. deliciosa* when treated with the same plant growth regulators, in an attempt to understand the differences in their physiology.

Therefore experiments have been conducted on *A. chinensis* using CPPU and Benefit®, and on *A. deliciosa* using CPPU, Benefit®, NAA and 3,5,6 TPA.

Chapter Two

2. The effect of a natural plant extract and synthetic plant growth regulators on the physiological characteristics of Actinidia chinensis and Actinidia deliciosa fruit.

2.1. Introduction

The physiological characteristics of both *Actinidia* sp., such as size, shape, dry matter content, are important quality factors for growers, Zespri, and consumers. These factors determine whether the fruit will be rejected outright, what the consumer is willing to pay and what payment the grower will receive. Many plant growth regulators both natural and synthetic have been studied and some have been shown to have a positive effect on one or more of these physiological characteristics on either *A. chinensis*, *A. deliciosa* or both.

Previous studies have shown that application of the natural plant extract Benefit® to *A. chinensis* can significantly increase fruit weight (Woolley & Cruz-Castillo, 2006). However, there have been contrasting results reported when Benefit® is applied to *A. deliciosa*. Woolley & Cruz-Castillo (2006) found that there was no significant increase in fresh fruit weight of *A. deliciosa* whereas Costa *et al*. (2002) found there was a significant increase, although the conditions of these two experiments did differ. Benefit® is currently being used commercially on *A. chinensis* fruit in New Zealand to increase fruit size and has been recently recommended for use on *A. deliciosa*. The synthetic

cytokinin CPPU is not used commercially in New Zealand despite the fact that it has been shown in multiple experiments to significantly increase the fresh weight of both *A. chinensis* and *A. deliciosa* fruit (Lawes *et al.*, 1991; Patterson *et al.*, 1993; Cruz-Castillo *et al.*, 1999; Woolley & Cruz-Castillo, 2006; Woolly & Currie, 2006). Although experimental conditions such as application time and concentration were shown to influence responses. The synthetic auxin 3,5,6 TPA has been shown to increase the fresh weight of *A. deliciosa* fruit in Italy (Bregoli *et al.*, 2006). Other synthetic auxins studied, in terms of the response of either *A. chinensis* or *A. deliciosa* fruit, include: 2,4-D (Hopping, 1976; Cruz-Castillo *et al.*, 1991; Cruz-Castillo *et al.*, 1999; Famiani *et al.*, 2007; Lorenzo *et al.*, 2007), 2,4,5 T, NOA, and IAA-asp (Hopping, 1976). It would appear from previous studies (Hopping, 1976b; Cruz-Castillo *et al.*, 1991; Cruz-Castillo *et al.*, 1999; Famiani *et al.*, 2007; Lorenzo *et al.*, 2007) that gibberellins only become limiting when fruit growth is stimulated by other hormones. It is also feasible to conclude from previous studies that it is auxin and/or cytokinins that are initially limiting kiwifruit fruit growth, where other factors, such as water, are not limiting.

Therefore the purpose of the experiments described in this chapter were to apply the natural plant extract Benefit®, the synthetic cytokinin-like compound CPPU, and the synthetic auxins 3,5,6 TPA and NAA in a variety of different experiments to both *A. chinensis*, and *A. deliciosa* and observe the effect these treatments had on the weight, dry matter content, and proportions of different tissues within the fruit.

2.2. Materials and Methods

2.2.1. Introduction

Experiments one and two were conducted on *A. chinensis* cv. 'Hort16A' in Hawkes Bay, New Zealand. These vines were grown on a pergola trellis and apart from the applied treatments they were grown in a normal commercial manner, with the exception that Benefit® was not applied except where stated. Experiments three, four, five, and six were conducted on *A. deliciosa* cv. 'Hayward' in Palmerston North New Zealand, at the Massey University plant growth unit. These vines were grown on T-Bar trellis and with the exception of the treatments applied they were grown in the normal commercial manner. All of the experiments were conducted over the 2007/2008 growing season. All experiments were randomised block design with each vine being a complete block. In all experiments corrugated plastic sheets were used to protect canes from spray drift and potential contamination. The first spray date for the experiments on *A. chinensis* was 20 days after full bloom, and the first spray date for the experiments on *A. deliciosa* was 26 days after full bloom. Full bloom was determined by the grower. Each spray was made up with reverse osmosis water, contained one drop per litre of the wetting agent Tween 20, and was applied at a rate of one litre per six canes. When fruit were cut into the IP and OP the IP included the seeds and core and the OP included the skin.

2.2.2. Experiment one, the growth response of *A. chinensis* to CPPU over time.

Four vines were used with one replicate (cane) per vine and two treatments: 1) control, and 2) CPPU (SKW Trotsberg, Germany). CPPU was applied three times to the same fruit at 0, 10, and 20 days. These spray dates were chosen based on the commercial application of Benefit®, the reason for which will be explained in the following experiment. CPPU was applied at 5 mgL^{-1}, and both fruit and leaves on the appropriate cane were sprayed.

2.2.2.1. Data collection

Twenty fruit were harvested per treated cane into polythene bags at 0, 10, 23, 34, 43, 52, 73, 133, and 156 days. All fruit were weighed within one day after collection (except for the final harvest). Fruit collected from days 0, 10, 23, 34, 43, and 52 days were left whole or roughly chopped to reduce the size for processing. Fruit collected from days 73, 133, and 156 were cut into IP and OP, which were then weighed again separately. The fruit were then freeze-dried to obtain the dry weight and for preparation for further analysis.

2.2.3. Experiment two, the interaction between Benefit® and CPPU on *A. chinensis*.

Four vines were used with two replicates per vine. The treatments were: 1) control, 2) CPPU, 3) Benefit® (Valagro, Italy), and 4) CPPU plus Benefit®. All treatments were applied three times, at 0, 10, and 20. CPPU was applied at 5 mgL^{-1}, and Benefit® was applied at the commercial application rate of 2.5 mlL^{-1}. Commercially Benefit® is applied three times during the season at 10-day intervals. Both fruit and leaves on the appropriate cane were sprayed

2.2.3.1. Data collection

All fruit were harvested from the first wire out on the commercial harvest date, 156 days after full bloom. Fruit were weighed as soon as possible after harvest and 10 fruit were separated into the IP and OP, these were also weighed. These ten fruit were then freeze-dried to obtain the dry weight and for preparation for further analysis.

2.2.4. Experiment three, the growth response of *A. deliciosa* fruit to CPPU over time

Four vines were used with one replicate per vine and two treatments: 1) control, and 2) CPPU. CPPU was applied three times to the same fruit at 0, 11, and 20 days, at 1 mgL^{-1}, to both fruit and leaves.

2.2.4.1. Data collection

Ten fruit were harvested per treatment at 0, 11, 20, 30, 54, 66, and 146 days. All fruit were weighed within one day after collection (except for the final harvest). Fruit collected from days 0, 11, 20, 30, and 54 days were left whole or roughly chopped to reduce the size for processing. Fruit collected from days 66 and 146 were cut into IP and OP, which were then weighed again separately. The fruit were then freeze-dried to obtain the dry weight and for preparation for further analysis.

2.2.5. Experiment four, the interaction between Benefit® and CPPU on *A. deliciosa* fruit

Four vines were used with two replicates per vine. The treatments were: 1) control, 2) CPPU, 3) Benefit®, and 4) CPPU plus Benefit®. All treatments were applied three times, at 0, 11, and 20 days. CPPU was applied at 1 mgL^{-1}, and Benefit® was applied at the commercial application rate of 2.5 mlL^{-1}.

Commercially Benefit® is applied three times during the season at 10-day intervals. Both fruit and leaves on the appropriate cane were sprayed.

2.2.5.1. Data collection

All fruit were harvested from the first wire out on the commercial harvest date; this was 146 days after full bloom. Fruit were weighed as soon as possible after harvest and 10 fruit were separated into the IP and OP, these were also weighed. Those 10 fruit were then freeze-dried to obtain the dry weight and for preparation for further analysis.

2.2.6. Experiment five, the effect of concentration and time of application of 3,5,6-TPA on growth of *A. deliciosa* fruit

Six vines were used with one replicate per vine and seven treatments: 1) control, 2) 0.1 mgL^{-1} 3,5,6-TPA (Chem Service, USA) early, 3) 1 mgL^{-1} 3,5,6-TPA early, 4) 10 mgL^{-1} 3,5,6-TPA early, 5) 0.1 mgL^{-1} 3,5,6-TPA late, 6) 1 mgL^{-1} 3,5,6-TPA late, and 7) 10 mgL^{-1} 3,5,6-TPA late. The early and late spray dates were 26 and 46 days after full bloom respectively. Both fruit and leaves on the appropriate cane were sprayed.

2.2.6.1. Data collection

The collection of fruit was as for experiment four.

2.2.7. Experiment six, the effect of NAA at different application dates and the interaction between CPPU and NAA at multiple application dates on the growth of *A. deliciosa* fruit

Six vines were used with one replicate per vine and nine treatments: 1) control, 2) NAA early, 3) NAA late, 4) CPPU early, 5) CPPU late, 6) NAA plus CPPU early, 7) NAA plus CPPU late, 8) NAA early plus CPPU late, and 9) CPPU early plus NAA late. The early and late application dates were as per experiment 5. NAA was applied at 10 mgL^{-1}, and CPPU was applied at 1 mgL^{-1}. Both fruit and leaves on the appropriate cane were sprayed.

2.2.7.1. Data collection

The collection of fruit was as for experiment four.

2.2.8. Data analysis

All data from fruit less than 60 g was discarded as those fruit were likely to be poorly pollinated and previous studies have shown that regardless of the treatment poorly pollinated fruit will not reach the size of a well pollinated fruit. Also fruit greater than two standard deviations away from the average fresh weight were disregarded and no data from these fruit were used for further analysis. SAS 9.1 was used for statistical analysis, using the general linear

model procedure. Duncan's multiple range test was used for the analysis of significant differences for experiments 1-4 and 6. Fisher's protected least significant difference (LSD) method was used for experiment 5 as Duncan's is not suitable for factorial experiments (MacKay, 2007). An Anova-two factor with replication analysis in Excel 2007 was used to analyse the interaction between CPPU and Benefit® in experiments two and four.

2.3. Results

2.3.1. Experiment one, the growth response of *A. chinensis* to CPPU over time.

A. chinensis fruit treated with CPPU were significantly heavier ($p<0.05$) at final harvest than control fruit. This increase in fresh weight became evident after day 20 and continued throughout the growing season, however the fruit from final harvest were lighter in weight than those harvested 23 days earlier (Figure 2.1.). At final harvest fruit treated with CPPU were on average 46.98 g heavier than the untreated control fruit.

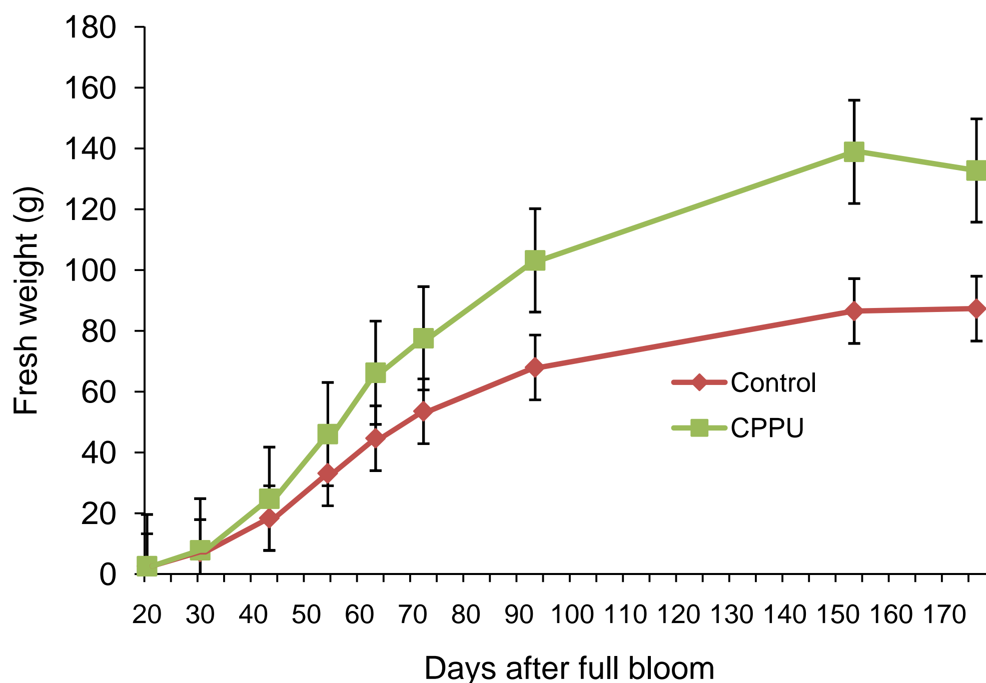


Figure 2.1. Fresh weight growth curve of *A. chinensis* fruit treated with 5 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) three times at ten day intervals, first treatment date was 20 days after full bloom, and untreated control. n varied from 70 to 107.

The proportion of the IP and OP was found to be different between the fruit treated with CPPU and the untreated control at the final harvest. Fruit treated with CPPU had a significantly higher proportion of OP, correspondingly the proportion of IP of the fruit treated with CPPU was significantly smaller than the untreated control fruit (Table 2.1.).

Table 2.1. Percentage of inner and outer pericarps of *A. chinensis* fruit treated with 5 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea), and untreated control.

	Control	CPPU
Inner pericarp (%)	21.95 a*	19.04 b
	(38)†	(40)
Outer pericarp (%)	77.31 a	80.49 b

* rows sharing the same letter are not significantly different (P<0.05).
† bracketed number = n

The percentage dry matter changed dramatically throughout the growing season; decreasing rapidly from the first collection date to the second ten days later (Figure 2.2.). There did not appear to be a correlation between percentage dry matter and the treatment and although the fruit treated with CPPU did have a higher percentage dry matter at the final harvest this difference was not found to be significant.

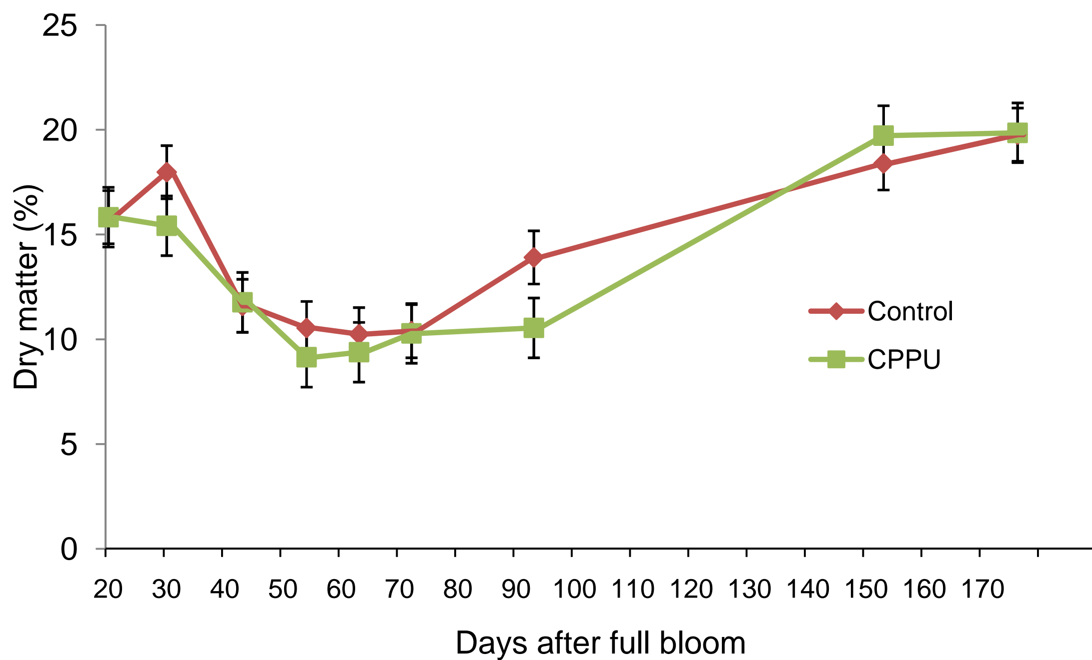


Figure 2.2.Percentage dry matter of *A. chinensis* fruit treated with 5 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea), and untreated control. n varied from 40 to 80.

2.3.2. Experiment two, the interaction between Benefit® and CPPU on *A. chinensis*.

Treatment with either CPPU and/or Benefit® significantly increased the fresh weight ($P<0.05$) of *A. chinensis* fruit (Table 2.2.). Fruit treated with Benefit® alone were 26.38 g heavier than the control, and fruit treated with CPPU

alone were 47.78 g heavier than the untreated control. *Actinidia chinensis* fruit treated with both CPPU and Benefit® were 52.74 g heavier than the control, an increase in fruit weight significantly higher than the CPPU and Benefit® treatments alone. Fruit treated with Benefit® and Benefit® plus CPPU had a significantly lower percentage of dry matter when compared with those treated with CPPU and the untreated control. The proportions of IP and OP were found to be significantly affected by the treatments. Fruit treated with Benefit® had on average significantly smaller IP and correspondingly significantly larger OP, when compared with the untreated control, as did fruit treated with CPPU and CPPU plus Benefit®, those fruit also had significantly smaller IP and larger OP when compared with fruit treated with Benefit® alone (Table 2.3.).

Table 2.2. Fresh weight (g) at final harvest of *A. chinensis* fruit when treated with 5 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) and/or 2.5 mlL^{-1} Benefit®.

	Control	Benefit®	CPPU	CPPU + Benefit®
Fresh weight (g)	89.65 a* (264)†	116.03 b (266)	137.42 c (272)	142.39 d (245)
Percentage dry matter (%)	17.49 a (70)	15.99 b (80)	17.11 a (80)	15.58 b (70)

* rows sharing the same letter are not significantly different (P<0.05).
† bracketed number = n

Table 2.3. Inner and outer pericarp percentages of *A. chinensis* fruit when treated with 5 mgL-1 CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) and/or 2.5 mgL^{-1} Benefit®.

	Control	Benefit®	CPPU	CPPU + Benefit®
Inner pericarp (%)	22.68 a* (81)†	20.69 b (80)	19.84 c (80)	19.45 c (80)
Outer pericarp (%)	76.66 a	78.68 b	79.71 c	80.07 c

* rows sharing the same letter are not significantly different (P<0.05).
† bracketed number = n

2.3.3. Experiment three, the growth response of *A. deliciosa* fruit to CPPU over time

A. deliciosa fruit treated with CPPU were significantly heavier ($p<0.05$) at final harvest than the untreated control fruit (figure 2.3.). The increase in fresh weight became evident after day 10 and continued throughout the growing season. At final harvest fruit treated with CPPU were on average 31.34 g heavier than the untreated control. There was no significant difference in the percentage dry matter between the fruit treated with CPPU and the untreated control (Appendix 6.3.2.). Nor was there any significant difference in the proportions of IP and OP (Appendix 6.3.3. and 6.3.4.).

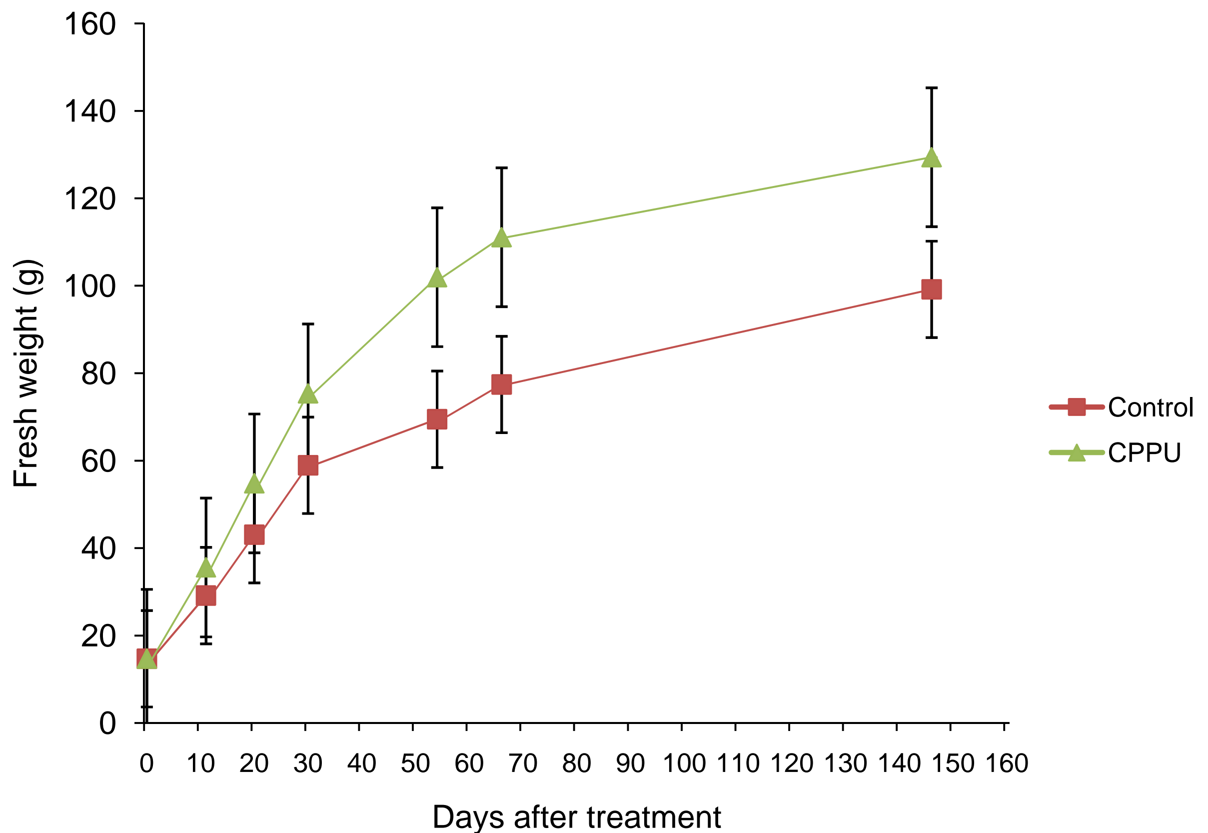


Figure 2.3. Fresh weight growth curve of *A. deliciosa* fruit treated with 1 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea), and untreated control. n varied from 33 to 66.

2.3.4. Experiment four, the interaction between Benefit® and CPPU on *A. deliciosa* fruit

Treatment of *A. deliciosa* fruit with Benefit® had no significant impact on the fresh weight. Treatment with CPPU and CPPU plus Benefit® did, however, significantly increase fresh weight (p<0.05), with increases in fresh weight being 31.31 and 28.38 g respectively when compared to the untreated control fruit (Table 2.4.). There were no significant differences in the percentage dry matter between any of the treatments (Table 2.4.). Neither CPPU nor Benefit® altered the proportion of IP or OP when compared with the control, however treatment with CPPU and CPPU plus Benefit® did significantly increase the percentage of OP when compared with fruit treated with Benefit® alone (Figure 2.5.).

Table 2.4. Fresh weight (g) of *A. deliciosa* fruit when treated with 1 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) and/or 2.5 mlL^{-1} Benefit®.

	Control	Benefit®	CPPU	CPPU + Benefit®
Fresh weight (g)	106.45 a* (76)†	110.26 a (96)	137.76 b (94)	134.83 b (105)
Dry matter (%)	15.65 a (38)	15.73 a (34)	14.71 a (31)	15.35 a (38)

*rows sharing the same letter are not significantly different (P<0.05).
† bracketed number = n

Table 2.5. Percentage of inner and outer pericarps of *A. deliciosa* fruit treated with 1 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea), and/or 2.5 mlL^{-1} Benefit®, and untreated control.

	Control	Benefit®	CPPU	CPPU + Benefit®
Inner pericarp (%)	32.49 a* b (40)†	32.86 a (34)	31.15 b c (36)	30.61 c (42)
Outer pericarp (%)	67.25 a b	66.68 b	68.78 a	68.67 a

*rows sharing the same letter are not significantly different (P<0.05).
† bracketed number = n

2.3.5. Experiment five, the effect of concentration and time of application of 3,5,6-TPA on growth of *A. deliciosa* fruit

Treatment of *A. deliciosa* fruit with 3,5,6 TPA did not significantly increase fresh weight of fruit at any concentration or application date when compared with the untreated control fruit (Figure 2.4.). It did, however, induce a significant decrease in fruit weight when it was applied at 0.1 mgL^{-1} at the late application date. However there does appear to have been an effect on the percentage dry matter with all treated fruit having a significantly higher percentage dry matter than the untreated control fruit (Figure 2.5.).

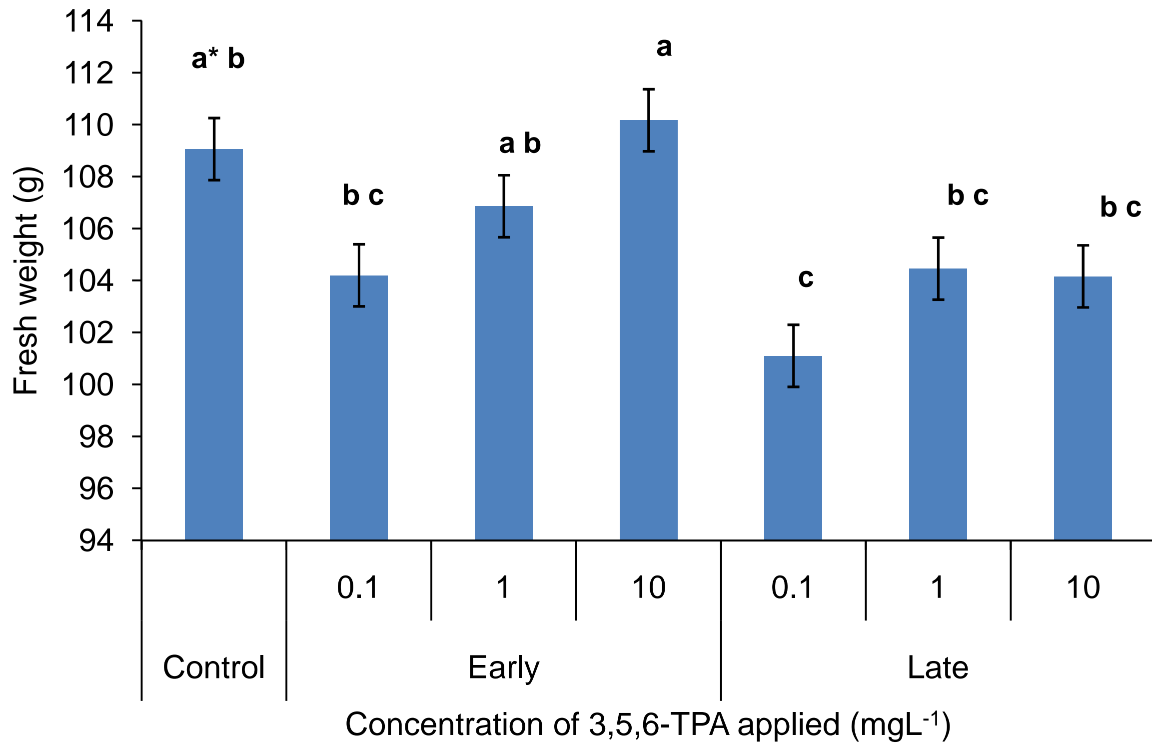


Figure 2.4.Fresh weight (g) of *A. deliciosa* fruit treated with 3,5,6 TPA (3, 5, 6-trichloro-2-pyridyloxyacetic acid) at two application dates early (26 days after full bloom), and late (46 days after full bloom). *Bars sharing the same letter are not significantly different ($p<0.05$). (n = 81, 77, 101, 115, 96, 74, and 127 respectively)

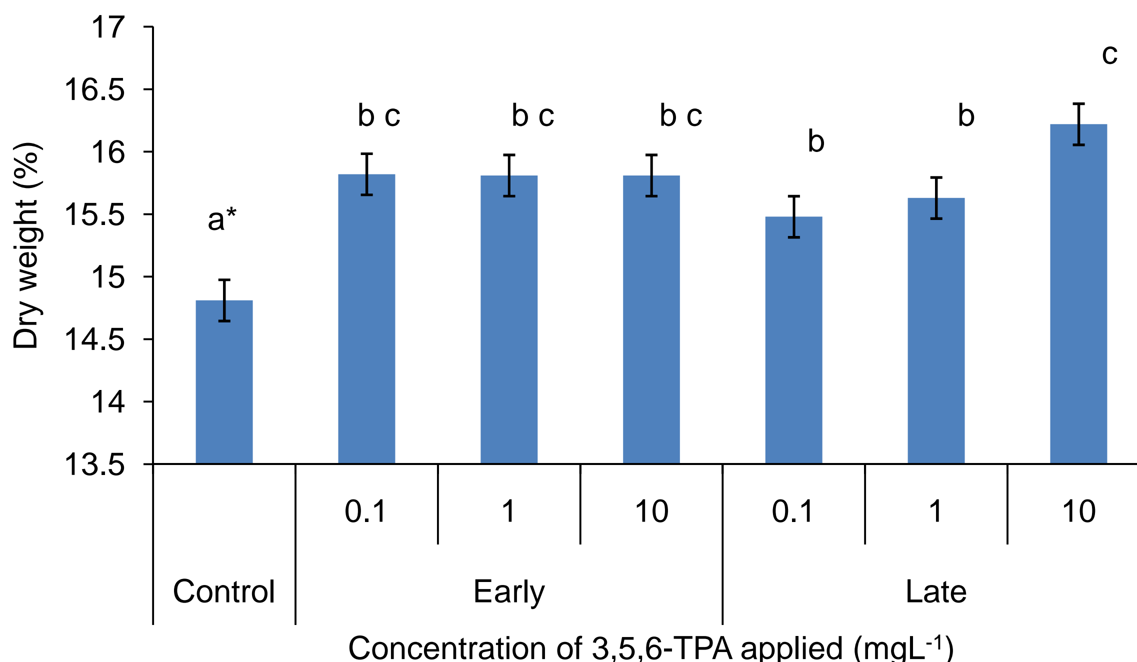


Figure 2.5. Percentage dry matter of *A. deliciosa* fruit treated with 3,5,6 TPA (3, 5, 6-trichloro-2-pyridyloxyacetic acid) at two application dates early (26 days after full bloom), and late (46 days after full bloom). *Bars sharing the same letter are not significantly different ($p<0.05$). (n = 40, 37, 53, 37, 53, 40, and 68 respectively)

2.3.6. Experiment six, the effect of NAA at different application dates and the interaction between CPPU and NAA at multiple application dates on the growth of *A. deliciosa* fruit

Treatment of *A. deliciosa* fruit with NAA resulted in both a significant decrease and increase in fresh weight depending on the application date (Table 2.6.). Fruit treated early in the season were 7.03 g lighter than untreated control fruit and fruit treated late were 13.32 g heavier. CPPU at 1 mgL^{-1} was found to have no significant effect when applied alone early, though when applied later in the season an increase of 13.54 g, when compared to the untreated control fruit was observed. However when fruit were treated with both CPPU early and NAA late they were 33.88 g heavier than the untreated control, this was

found to be a positive synergistic effect as the increase observed when fruit were treated with CPPU early (4.56 g) plus the increase observed when fruit were treated with NAA late (13.32 g) is less than 33.88 g. All other treatments, CPPU late plus NAA early, CPPU plus NAA early and late, also significantly increased the fresh weight of fruit when compared to the untreated control, however none of the other combinations resulted in a positive synergistic response. All treatments reduced percentage dry matter of fruit, though it was not significantly reduced in fruit treated with CPPU late plus NAA early and CPPU plus NAA early. There were no correlations between treatments and the proportions of the pericarps (Appendix 6.6.3. and 6.6.4.).

Table 2.6. Fresh weight and percentage matter of *A. deliciosa* fruit treated with 10 mgL^{-1} NAA (napthalene acetic acid) and or 1 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea) on one or both of two treatment dates early (26 days after full bloom) and late (46 days after full bloom).

	Control	NAA		CPPU		CPPU early NAA late	CPPU late NAA early	CPPU + NAA	
		Early	Late	Early	Late			Early	Late
Fresh weight (g)	105.37 a (43)†	98.34 b (86)	118.69 c (87)	109.93 a d (56)	118.91 c (66)	139.25 e (48)	116.80 c d (56)	115.268 c d (88)	118.00 c (64)
Dry matter (%)	18.79 a (18)	16.20 b c (25)	16.59 b c (27)	14.92 c (13)	16.09 b c (25)	16.22 b c (29)	17.73 a b (29)	17.51 a b (20)	16.35 b c (40)

*rows sharing the same letter are not significantly different (P<0.05).
† bracketed number = n

2.4. Discussion

2.4.1. Experiments one and three:

Both *A. chinensis* and *A. deliciosa* fruit treated with CPPU were significantly heavier than the untreated control fruit. The fruit were on average 46.98 and 31.34 g heavier than the controls, respectively. These results support those obtained by Woolley & Currie (2006); Woolley & Cruz-Castillo (2006); and for *A. deliciosa* alone, Patterson *et al.* (1993); Iwahori *et al.* (1998); and Lawes *et al.* (1991), despite the fact that the treatment conditions did differ between some experiments. *Actinidia deliciosa* fruit were treated with 1 mgL^{-1} CPPU and thus the results replicate those acquired by Woolley & Currie (2006), where an increase of 37.9 g was observed. However, while an increase in fresh weight is of interest to growers and Zespri, based on what consumers will pay, the percentage of dry matter is also a major concern.

Actinidia chinensis fruit treated with CPPU had a lower percentage dry matter than the untreated control and while the decrease was not statistically significant it may be of significance to growers as a portion of what they are paid is determined by the percentage of dry matter. The decrease in percentage dry matter was also observed in *A. deliciosa* fruit. However the decrease was greater (0.21 as opposed to 0.09 percent dry matter). The overall percentage dry matter differs between *A. chinensis* and *A. deliciosa* fruit, 19.86 and 14.67 percent for untreated controls respectively.

The ratio between OP and IP was significantly altered in *A. chinensis* fruit treated with CPPU but not in *A. deliciosa* fruit. The percentage of the OP was found to be significantly larger in *A. chinensis* fruit treated with CPPU, this agrees with results obtained by Woolley *et al.* (1991). However those results were observed in *A. deliciosa* fruit not *chinensis* but the fruit were dipped in 10 mgL^{-1} CPPU, this increase in concentration may be the reason for the different observations. It has been found previously that CPPU has little or no mobility in the plants (Neri *et al.*, 1993; D.J. Woolley, personal communication). This may be, in part, why the proportion of the OP was increased in fruit treated with CPPU as opposed to the fruit increasing in size equally in all fruit tissue. Another explanation is that cell division in the OP continues for approximately seven to fourteen days longer in fruit treated with CPPU than untreated control fruit (Woolley *et al.*, 1991). It was also found in the same experiment that cell size in the OP was increased. These potential mechanisms for an increase in the proportion of the OP in fruit treated with CPPU are not mutually exclusive. These experiments looking at the affect of CPPU on cell size versus cell enlargement have all, at this time, been conducted on *A. deliciosa* fruit, it would be interesting and informative to repeat them on *A. chinensis* fruit.

2.4.2. Experiments two and four:

When treated with CPPU alone the fresh weight of *A. chinensis* was 137.42 g, a statistically significant 48.77 g increase from the control. When fruit from the same experiment were treated with Benefit® the average fruit weight was

116.03 g. This was significantly larger than the control (26.38 g) but significantly smaller than the fruit treated with CPPU (22.39 g difference). Fruit from the same experiment treated with CPPU plus Benefit® had an average fresh weight of 142.39 g, which was also significantly heavier than the control (52.74 g increase). Thus the increase due to CPPU was 48.77 g and the increase due to Benefit® was 26.38 g, the gain when both were applied was only 52.74 g. There does appear to be a statistically significant interaction between Benefit® and CPPU when both are applied to *A. chinensis*. When the same experiment was conducted on *A. deliciosa* CPPU was found to significantly increase the fresh weight (31.31 g increase when compared to the untreated control) but application of Benefit® had little effect. These results support those found by Woolley & Currie (2006) and Woolley & Cruz-Castillo (2006). When CPPU plus Benefit® was applied to *A. deliciosa* the increase in fresh weight was not significantly different from that achieved by CPPU alone.

Treatment with Benefit® and Benefit® plus CPPU was found to significantly decrease the percentage dry matter in *A. chinensis* fruit. whereas, the decrease with CPPU alone was not significant suggesting that the deleterious effect on percentage dry matter is mainly due to Benefit®. None of the treatments in this experiment significantly altered the percentage dry matter of *A. deliciosa* fruit.

It was observed in this experiment that treatment with CPPU significantly altered the ratio of the pericarps, of *A. chinensis* fruit, as did Benefit®. The mobility of the active component of Benefit® is unknown, but if it is mobile this

would suggest that the effects is mainly due to a longer cell division phase in the outer pericarp. The ratio of the pericarps of *A. deliciosa* were not altered by any treatment when compared to the untreated control fruit. However, the percentage of OP in fruit treated with Benefit® was significantly smaller when compared to fruit treated with CPPU and CPPU plus Benefit®. It is likely then that the effect was due to CPPU in the fruit treated with both CPPU and Benefit®.

These results indicate that application of Benefit® to *A. deliciosa* fruit at 2.5 mlL^{-1} at 26, 36, and 46 days after full bloom is ineffective in altering fruit physiology. Although another possibility is that *A. deliciosa* is less sensitive to Benefit® than *A. chinensis* and could potentially respond to a higher concentration. These results suggest that either that CPPU and Benefit® act in the same way to increase fruit weight, or that the interaction is hidden behind perhaps a limitation of photo-assimilates, other hormones, or minerals. Alternatively the response could have become saturated by CPPU, that is, the maximum growth of the fruit may have been reached.

2.4.3. Experiment five:

Treatment of *A. deliciosa* with 3,5,6 TPA did not significantly increase fruit weight at any concentration or application date. Application of 3,5,6 TPA at 0.1 mgL^{-1} on the late application date, in fact, significantly decreased the fresh weight by 7.96 g. This is in contrast to results obtained by Bregoli *et al.* (2006) who found that application of 3,5,6 TPA increase fruit weight, although

experimental conditions did differ somewhat. 3,5,6-TPA was applied at either 7, 10 or 14 g/ha at either 20 plus 70, 70, or 100 days after full bloom, and it was found that the application of 10 g/ha at all application dates had the greatest effect on fresh fruit weight, application date appeared to have little influence on the effect of the treatment. There did, however, appear to be a relationship between fruit weight and concentration at the early application date with fruit treated with 10 mgL^{-1} being significantly heavier than those treated with 0.1 mgL^{-1}. Auxin is predominantly associated with cell expansion in fruit, though it is the ratio of hormones that are usually found to affect plant development and often subtle changes in the ratio of different hormones can significantly alter cell processes. It could be then that the early application of the synthetic auxin 3,5,6-TPA, at the concentrations tested, was not sufficient to increase fresh weight of fruit by way of cell expansion. But that it was sufficient to alter the ratio of auxins and cytokinins required for cell division. Therefore, the late application of 0.1 mgL^{-1}, which significantly decreased the fresh weight, could have done so because the concentration was not sufficient to increase cell expansion and, due to the alteration of the ratio of auxin to cytokinins, could have actually decreased cell expansion. Therefore further study with higher concentrations of 3,5,6-TPA could, potentially, produce enhanced growth.

In contrast with the other plant growth regulators studied 3,5,6 TPA did appear to significantly increase percentage dry matter at every concentration, regardless of application date. The greatest increase was observed when 3,5,6 TPA was applied at 10 mgL^{-1} on the late application date.

2.4.4. Experiment six:

Date of application appears to affect the response of *A. deliciosa* to both NAA and CPPU. An early application of NAA significantly reduced fresh weight of fruit while a late application significantly increased it, this could perhaps be explained in the same way as the result observed in experiment five was. An early application of CPPU did not alter fresh weight of fruit, this does differ from previous results however CPPU is usually applied at higher concentrations such as 5 or 10 mgL^{-1}. Whereas a late application of CPPU induced almost the exact response obtained from the late application of NAA. This reaction differs to that observed in experiment three where application of 1 mgL^{-1} CPPU induced a significant increase in fruit weight, however, in experiment three CPPU was applied three times whereas in this experiment it was applied either early or late. When both NAA plus CPPU were applied together early the increase in fruit weight was significant when compared to the untreated control and the fruit treated early with NAA alone, though not when compared to fruit treated early with CPPU alone. Furthermore when fruit were treated with both CPPU plus NAA late the response was almost exactly the same as that obtained from a late application of NAA and CPPU separately. A positive synergistic response was observed when CPPU was applied early and NAA was applied late, that is, the increase due to the early application of CPPU alone (4.56 g) plus the increase due to the late application of NAA alone (13.32 g) is less than the increase due to the early application of CPPU plus the late application of NAA (33.88 g). This indicates

that the two compounds do, in fact, increase fruit weight by different mechanisms and is significant when related to activity of naturally occurring auxin and cytokinins and the stages of fruit growth at the application dates.

Auxin is predominantly associated with cell expansion in fruit and cytokinins are predominantly associated with cell division, although there is interaction with auxin and gibberellins and most processes are dependent on a ratio of hormones. Therefore it is significant that application of a synthetic cytokinin like compound during the period of cell division plus the application of a synthetic auxin during the period of cell expansion increased the fresh weight of the fruit so substantially. These results support the mechanisms of action proposed for CPPU, for example that CPPU acts to protect endogenous cytokinins, or acts directly as a cytokinin or indirectly to promote a positive feedback loop for naturally occurring cytokinins, though they do not strengthen any one over the others. Previous studies which have looked at the effect of CPPU on cell division and or expansion have had conflicting results. It has been suggested that CPPU increases fresh weight of fruit by way of increasing cell expansion (Patterson *et al*., 1993; Lewis *et al*., 1996), however it has also been suggested to increase both cell division and expansion (Woolley *et al*., 1993).

2.4.5. Conclusion:

CPPU was shown to increase the fresh weight of fruit of both *A. chinensis* and *A. deliciosa*, thus supporting the results found in previous studies. Percentage

dry matter was not significantly altered in either cultivar, though the slight decrease observed in fruit treated with CPPU could have an effect on the payment growers receive. CPPU also increased the percentage of the OP, again supportive of previous research.

While Benefit® did significantly increase the fresh weight of *A. chinensis* fruit, which supports previous studies, it does not appear to have any effect on the fruit of *A. deliciosa*. The physiological basis for this is, as yet, unknown. Benefit® was found to significantly decrease percentage dry matter of *A. chinensis* fruit. There was found to be a positive interaction between CPPU and Benefit® when both were applied to *A. chinensis*, but not *A. deliciosa*, which could be indicative of a related mechanism of action though this would not explain why CPPU is effective in increasing the fresh weight of both cultivars while Benefit® increases the fresh weight of *A. chinensis* fruit and has no physiological effect on *A. deliciosa* fruit. Or the interaction could potentially be hidden behind a limitation of photo-assimilates or the maximum fruit growth, though this would still leave the aforementioned question, of why Benefit® had no physiological effect on *A. deliciosa*, unanswered, although it could simply be that *A. deliciosa* requires a higher concentration of Benefit® before a reaction will be observed.

Under the conditions of Experiment five 3,5,6 TPA had no positive effect on fresh weight of *A. deliciosa*. However percentage dry matter was increased.

Application date appears to influence the effectiveness of both NAA and CPPU. A positive synergistic response was obtained by applying CPPU early and NAA late, this result was particularly interesting when related to naturally occurring auxin and cytokinins and the stages of fruit growth at the dates of application.

Chapter Three

3. The effect of a natural plant extract and synthetic plant growth regulators on endogenous hormones of *Actinidia chinensis* and *Actinidia deliciosa* fruit.

3.1 Introduction:

It is currently unknown how plant growth regulators such as CPPU and natural plant extracts such as Benefit® induce an increase in fruit size of *Actinidia* fruit, or why there is a difference in response between the two main *Actinidia sp*. to the natural plant extract Benefit®. The hypothesis of this experiment is that one or both of these compounds increases fruit size by altering the endogenous hormone content of the fruit. It had been shown previously that CPPU acts, in some circumstances, as a naturally occurring cytokinin (Takahashi *et al*., 1978 as cited by Neri, *et al*., 1993; Shudo, 1994; Mok *et al*., 1987 .

However, when naturally occurring cytokinins (zeatin (Z), zeatin riboside (ZR), dihydrozeatin (DZ), isopentenyl adenine (2iP), or isopentenyl adenosine (IPA)) were applied to *A. deliciosa* no increase in fruit weight was observed (Woolley & Currie, 2006). Application of any one of the naturally occurring cytokinins (Z, DZ, 2iP, or their ribosides) and CPPU appeared to have an additive effect. This suggests that either the CPPU is protecting the cytokinin

in some way or that they act in different ways but that the presence of CPPU also enables the effect of the cytokinin to be observed.

The potentially protective role of CPPU has been studied previously in terms of the compound cytokinin oxidase, with conflicting results. Laloue & Fox (1989) found that CPPU inhibited the activity of cytokinin oxidase isolated from wheat germ. This was also observed by Woolley and Currie (2006) when it was found that *A. deliciosa* fruit tissue had high cytokinin oxidase activity, the activity of which could be completely abated by the addition of CPPU. It was, therefore, reasoned that if CPPU did play a protective role for cytokinins in fruit tissue by suppressing the activity of cytokinin oxidase then the concentration of one or more naturally occurring cytokinins would be increased in tissue treated with CPPU. However results showed only limited support for this hypothesis; endogenous levels of Z and ZR were found to be reduced in both the IP and OP of fruit treated with CPPU when compared to untreated control fruit, although there was a slight increase in endogenous levels of 2iP and IPA. There are, however, a large number of other identified cytokinins which could have had altered levels due to treatment with CPPU, though the aforementioned ones are the most common. Woolley and Currie (2006) mention the potential presence of a novel cytokinin, the identification of which requires further investigation. This novel cytokinin could potentially be one of the recently discovered aromatic cytokinins, the topolins, which appear to be more common than previously thought (Holub *et al.*, 1998).

Lewis *et al.* (1996) found that in *A. deliciosa* fruit treated with CPPU endogenous cytokinin levels were reduced, and therefore suggested that perhaps CPPU acts directly to increase fruit size and also causes a feedback loop which reduces cytokinin production. It was found that zeatin levels were highest in untreated control fruit 10 days after anthesis and due to low levels in unpollinated fruit it was hypothesised that “an increase in zeatin is the critical change in cytokinin metabolism required for the initiation of cell division and fruit growth” (Lewis *et al.*, 1996, p.187). However in fruit treated with CPPU there was found to be no detectable zeatin. Two independent studies have found that the increase in *A. deliciosa* fruit size induced by application of CPPU is caused by cell expansion as opposed to cell division (Lewis *et al.*, 1996; Patterson *et al.*, 1993), another study however found that the increase was due to both cell division and expansion (Woolley *et al.*, 1991) and two others suggested that the increase was due to cell division (Kurosaki & Mochizuki, 1990; Neri *et al.*, 1993). Naturally occurring cytokinins are usually associated with cell division in fruit, however naturally occurring auxin are associated with both cell division and expansion (Atwell, *et al.*, 1999). It was, therefore, hypothesised that CPPU may act by altering the concentration of endogenous auxin and/or perhaps by altering the ratio of auxin and cytokinins.

Therefore, the purpose of this experiment was to look at the endogenous content of auxin and cytokinins of *A. chinensis* and *A. deliciosa* fruit both treated with CPPU and an untreated control. However, due to time constraints

and the limited previous research in this field the experiments were required to be reduced and will be continued at a later date.

3.2 Materials and Methods

3.2.1 Introduction

A new experimental method was developed in an attempt to quantify IAA directly using a fluorescence detector and to also determine cytokinin levels in same extract. The fruit for this experiment came from experiment one as described in Chapter Two.

3.2.2 Method development

3.2.2.1 Extraction

Freeze dried fruit were ground using a mortar and pestle and two extraction solutions were tested. These were methanol/water (4/1 v/v) and methanol/water/formic acid (15/4/1). These extraction solutions were obtained from Woolley (personal communication) and Dobrev and Kamínek (2002), respectively. Both extraction solutions were tested at 5 mlg^{-1} fresh weight, as suggested by Dobrev and Kamínek (2002). The radioactive isotope ^{14}C-IAA (Sigma, America) was added to both samples to track the progression and

breakdown of auxin (IAA), samples were then left in a -20°C freezer overnight. The following morning both extracts were centrifuged, the supernatants were removed and the pellets re-suspended and after a further two hours in the freezer were centrifuged again. The supernatants were pooled and a sample taken from each to test the radioactivity of each extraction to ensure that the IAA (both the ^{14}C and endogenous) was in the supernatant not the pellet. It was found that essentially 100 percent of the ^{14}C-IAA was in the supernatant. It was decided that the extraction method would follow that outlined in Dobrev and Kamínek (2002), because this had been shown to reduce cytokinin nucleotide breakdown. Thus an extraction solution of methanol/water/formic acid (15/4/1) at -20°C was used, samples extracted overnight at -20°C and the pellet re-extracted for two hours following centrifugation the following day. The only change made to the extraction method developed by Dobrev and Kamínek (2002) was the volume of extraction solution. This was altered to 12 mlg^{-1}, as freeze dried tissue was being used instead of fresh. The mortar and pestle were replaced by a coffee grinder.

3.2.2.2 Purification

Two methods of initial purification were tested. The first was using Waters 360 mg C18 Sep-pak® cartridges for solid phase extraction (Waters, Ireland), as suggested by Dobrev and Kamínek (2002). The Sep-pak® was first preconditioned with 5 ml 100 percent methanol, then the appropriate extraction solution and then loaded with 100 ml of the corresponding sample (the pooled supernatant) and finally rinsed with 5 ml of 100 percent methanol

to ensure all compounds of interest had been extracted (Figure 3.1.). Solutions were pushed through at ~5 ml per minute (Dobrev and Kamínek, 2002). The Sep-pak® cartridges were used three times each and were rinsed with 10 ml of ether between usage. Each of the extraction solutions were tested.

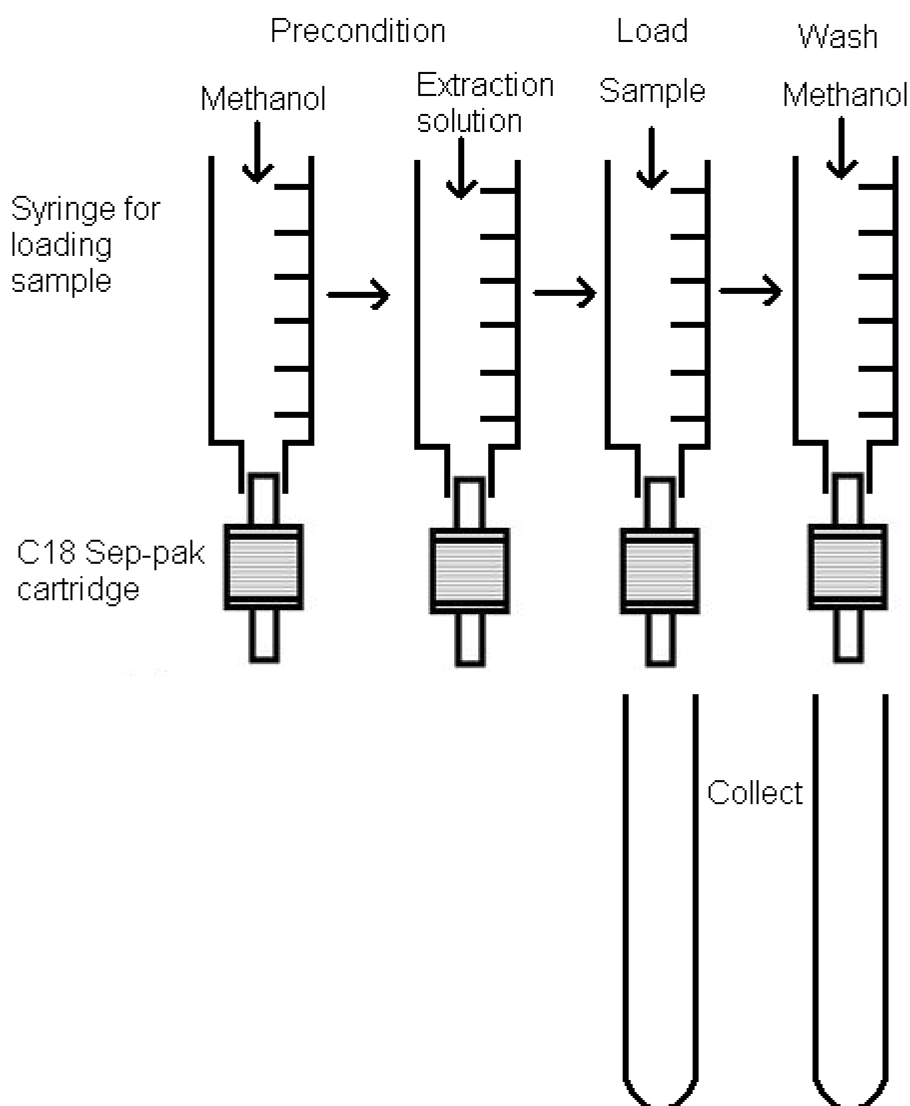


Figure 3.1. Initial purification method used for the purification of auxin and cytokinins from freeze dried *Actinidia chinensis* and *A. deliciosa* fruit. Solutions were pushed through at ~5 ml per minute (Dobrev and Kamínek, 2002)

The second method tested for initial purification was dichloromethane partitioning, as outlined in Powell (1964). A 100 ml sample from each of the two extraction solutions being tested was used. The samples were dried down on a Watson Victor Ltd Büchi rotavapor-R rotary evaporator and then re-suspended in 1 M ammonia. The ammonia solution was then mixed with dichloromethane in a flask. After the two solutions (ammonia and dichloromethane) separated, partitioning the kiwifruit sample between them, the dichloromethane was drained out. A further 100 ml of dichloromethane was added and the mixing and partitioning of the sample was repeated three times, with all of the dichloromethane pooled at the end and a sample counted on the scintillation counter to ensure that no IAA had partitioned into the dichloromethane. The aqueous phase (ammonia) was kept as this contained the IAA.

It was decided that the Sep-pak® method would be used for the initial purification of the kiwifruit samples. This was due to the large number of samples being processed, and the more time consuming nature of the dichloromethane partitioning, it was also observed that the solution collected from the Sep-pak® method was cleaner than that obtained from the partitioning. Another advantage with using the Sep-pak® was that they contained the same packing as the HPLC (high pressure liquid chromatography) column to be used for HPLC separation of the samples. This meant that due to the chemistry of the Sep-pak®, a somewhat indelicate version of the HPLC column, any compound that would irreversibly bind to the

HPLC column should, theoretically, bind to the comparably inexpensive Sep-pak® cartridge.

As the method outlined by Dobrev and Kamínek (2002) was being used it was decided to continue with their method for the next phase of the sample purification. The predominant reason for choosing this method, however, was that it had been shown that auxin and cytokinins could be separated and purified within the same process, using the Oasis® (Waters, Ireland) MCX cartridges.

The next step in the purification process was to dry down each of the samples following separation by the Sep-pak® cartridge. Samples were then re-suspended in 1 M formic acid to prepare them for further purification by a 150 mg Oasis® MCX cartridge (Dobrev and Kamínek, 2002). This was later modified as the dried samples were difficult to re-suspend and often would not entirely re-suspend, and 60 mg Oasis® MCX cartridges were used. Therefore, each sample was reduced to 10 ml (in Genetech miVac quattro concentrator) thus ensuring that all of the methanol was removed from the solution. The pH was found to be within the range specified by Dobrev and Kamínek (2002) so the sample was loaded onto a preconditioned Oasis® MCX column. The column was preconditioned with 5 ml 100 percent methanol followed by 5 ml 1M formic acid. After the sample was loaded it was washed with 5 ml formic acid, followed by 5 ml 100 % methanol. The second wash removed auxin and abscisic acid (ABA) from the column and was, therefore, collected. Following the second wash there were three elutes: the first, 5 ml 0.35 M ammonia,

removed cytokinin nucleotides; the second, 5 ml 0.35 M ammonia in 60 % methanol, removed cytokinin bases, ribosides, and glucosides; and the third, 5 ml 0.7 M ammonia in 100 % methanol, removed remaining compounds from the column (Figure 3.2.).

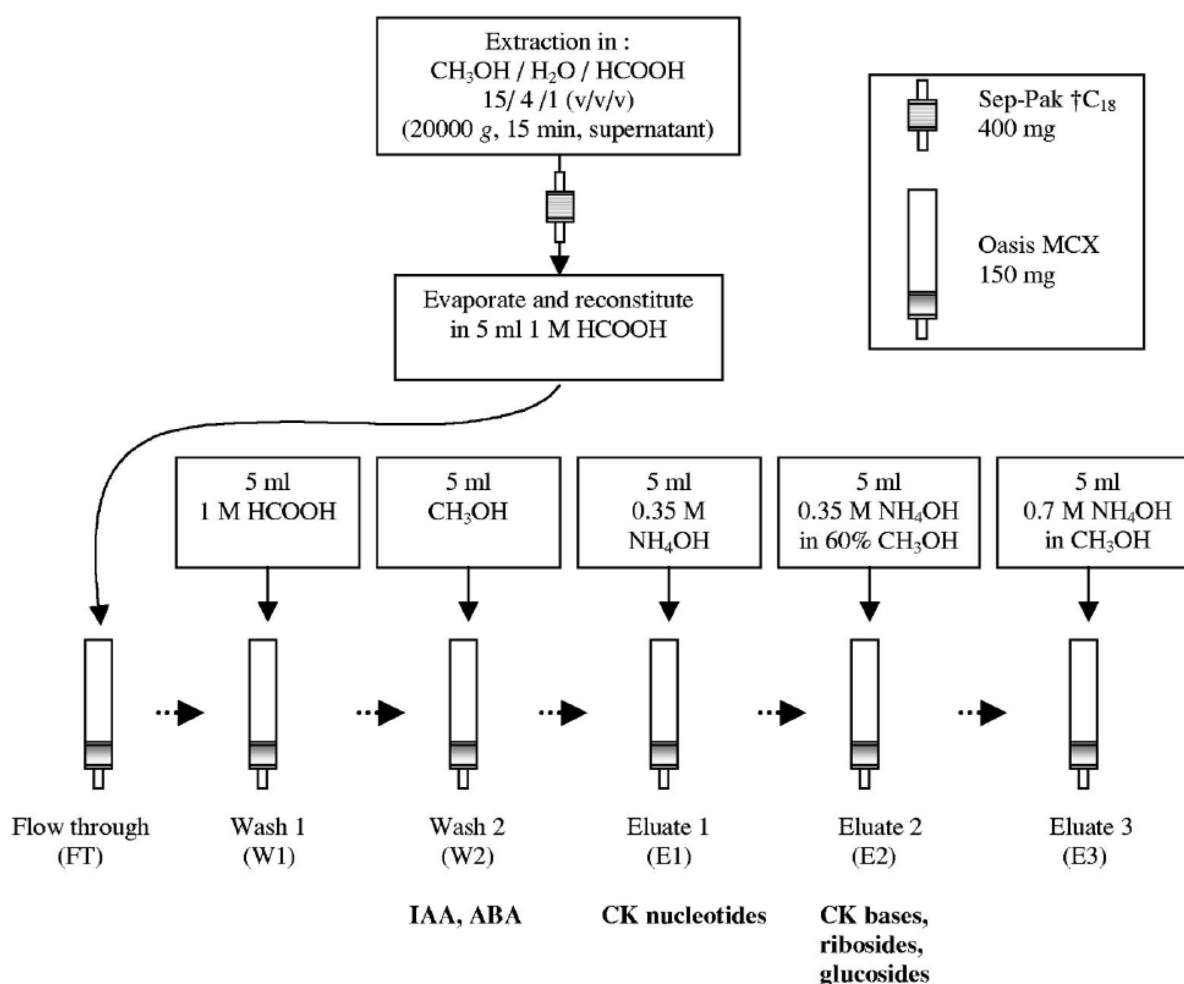


Figure 3.2. Extraction and purification protocol for cytokinins (CK), auxin (IAA) and abscisic acid ABA. Plant material is homogenised in liquid nitrogen and dropped in cold (-20°C) extraction mixture of methanol/water/formic acid (15/4/1, v/v/v) at 5 ml g^{-1} fresh weight containing labelled internal standards. After overnight extraction at -20°C, solids are separated by centrifugation (20000 g, 15min) and re-extracted for 30 min in an additional 5 ml g^{-1} extraction mixture. Pooled supernatants are passed through a Sep-pak® Plus C18 cartridge to remove lipids and part of plant pigments and evaporated to near dryness or until methanol is removed. The residue is dissolved in 5 ml 1 M formic acid and applied to Oasis® MCX column pre-conditioned with 5 ml of methanol followed by 5 ml 1 m formic acid. The column is washed and eluted with indicated order of solutions. After passing of each solvent the columns are purged briefly with air. Solvents are evaporated at 40°C under vacuum. (Dobrev and Kamínek, 2002, p. 26).

This was sufficient to prepare the tissue used by Dobrev and Kamínek (2002) for HPLC. However when the sample containing auxin was obtained from the Oasis® MCX column, dried down, re-suspended in the HPLC start solution and injected it was still too contaminated to separate the auxin peak using either a fluorescence or UV detector (Figure 3.3.). The trace obtained contained too many peaks and the putative IAA peak did not reach baseline nor was it Gaussian in shape. This indicated that it was composed of more than one compound, and consequently the putative IAA peak could not be quantified from this trace.

It was, therefore, decided to try a further method of purification for the fraction containing auxin. The Waters 60 mg Oasis® MAX column was chosen for testing as it was designed to separate acids and auxin is a weak acid. The basic method outlined by Waters (Anonymous, 2005) was tested. Wash Two (the fraction containing auxin from the Oasis® MCX method) was dried down and re-suspended in 5 ml 5 percent ammonia. This was then loaded on a preconditioned Oasis® MAX column. The column was preconditioned with 5 ml 100 percent methanol followed by 5 ml 5 % ammonia. Following loading of the sample the column was first washed with 5 ml 5 % ammonia, then 5 ml of 100 % methanol and finally one elution of 5 ml of 2 % formic acid in 100 % methanol. Eluate one contained auxin and was collected (Figure 3.4.).

Initially samples were passed through the Sep-pak® and Oasis® cartridges by hand, later a 24 port Phenomenix speed vac, using a water vacuum, was

used, the flow rate was kept at ~5 ml per minute. Each step of the method was tested to ensure that the endogenous hormones were not being lost in some way or broken down. Radioactive isoptopes were used as internal standards to monitor the progress and break down of the hormones of interest throughout the extraction and purification methods (Figure 3.5.). IAA in particular is relatively unstable and breaks down quickly if exposed to light and/or heat. The antioxidant butylated hydroxytoluene was tested with an IAA standard but appeared to accelerate breakdown, the reason for this is unknown. The samples were then sufficiently purified to be injected into the HPLC for identification and quantification of auxin (Figure 3.6.).

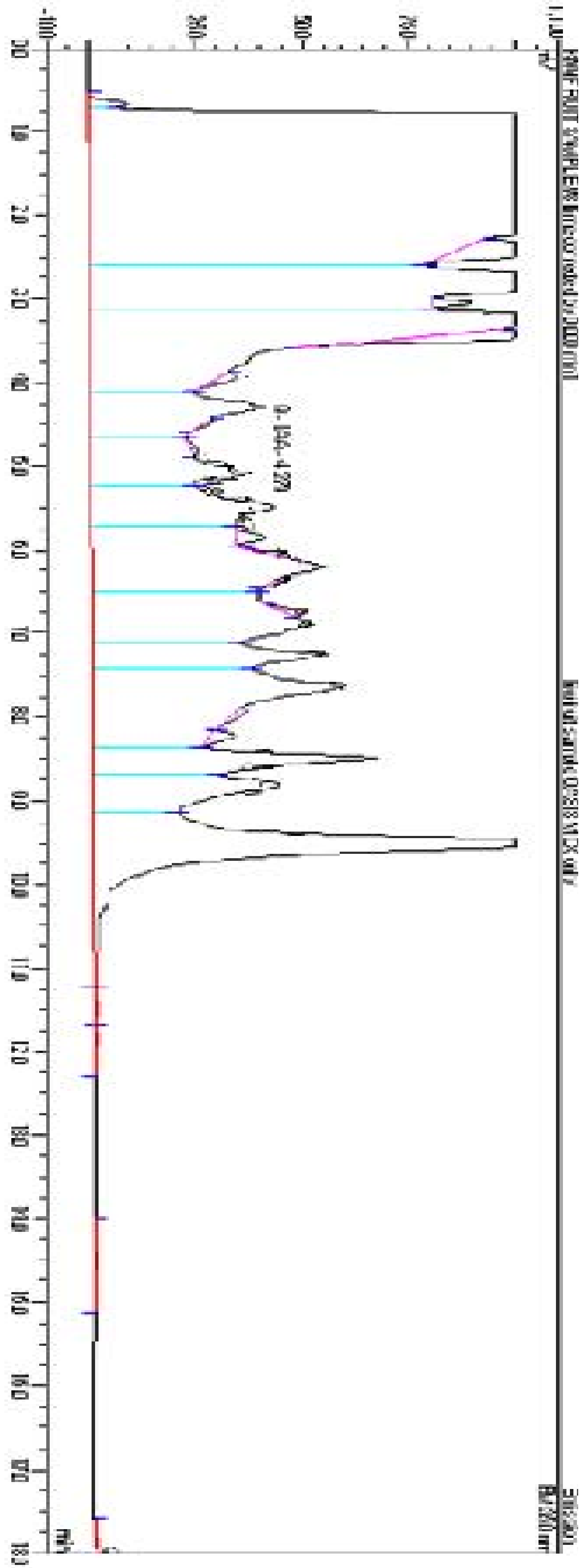


Figure 3.3. High pressure liquid chromatography fluorescence detector trace of a kiwifruit sample that was purified with an Oasis® MCX column only. Note that the putative IAA (indole-3-acetic acid) peak does not reach baseline and is not separated from other peaks, also it is not Gaussian in shape thus suggesting it is composed of more than one compound.

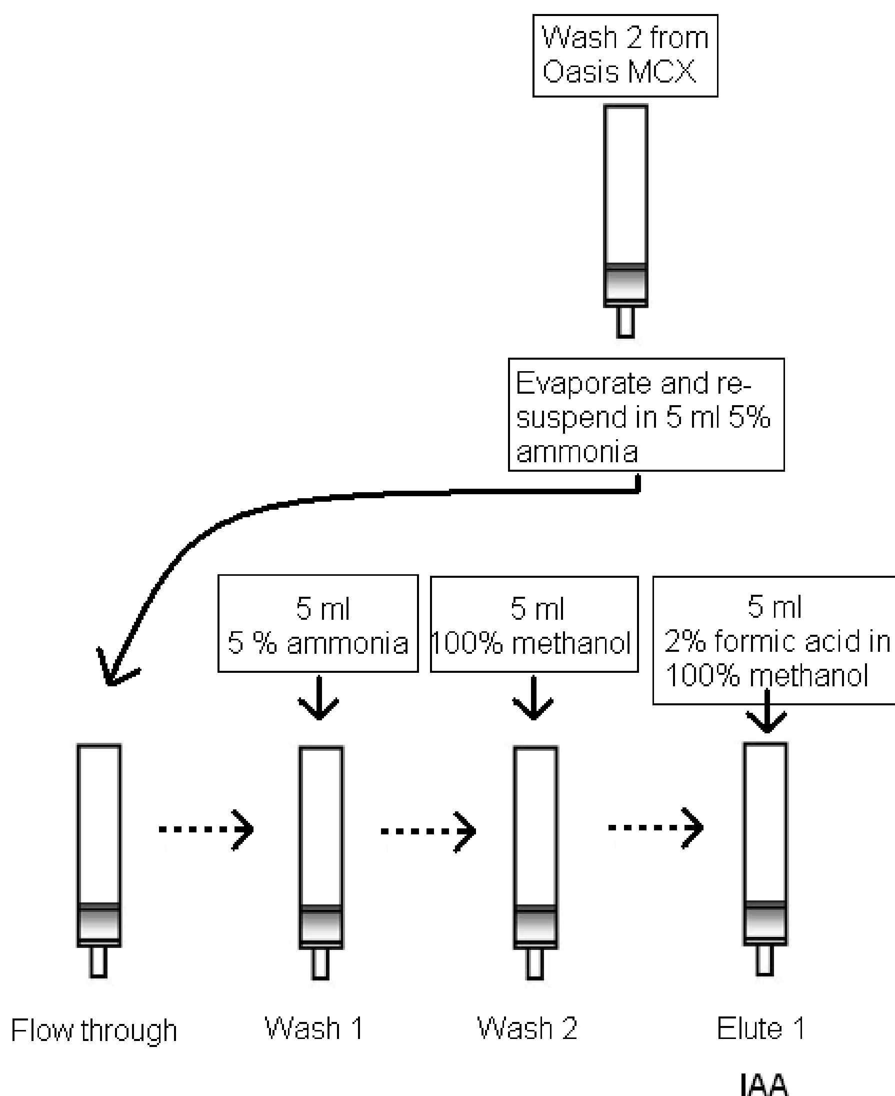


Figure 3.4. Purification protocol for auxin in kiwifruit fruit tissue. Wash 2 from Oasis® MCX is dried down and re-suspended in 5 ml 5 percent ammonia and applied to a Oasis® MAX column pre conditioned with 5 ml 100 percent methanol followed by 5 ml 5 percent ammonia. The column is washed and eluted with the indicated order of solutions. After passing each solvent the columns are purged briefly with air.

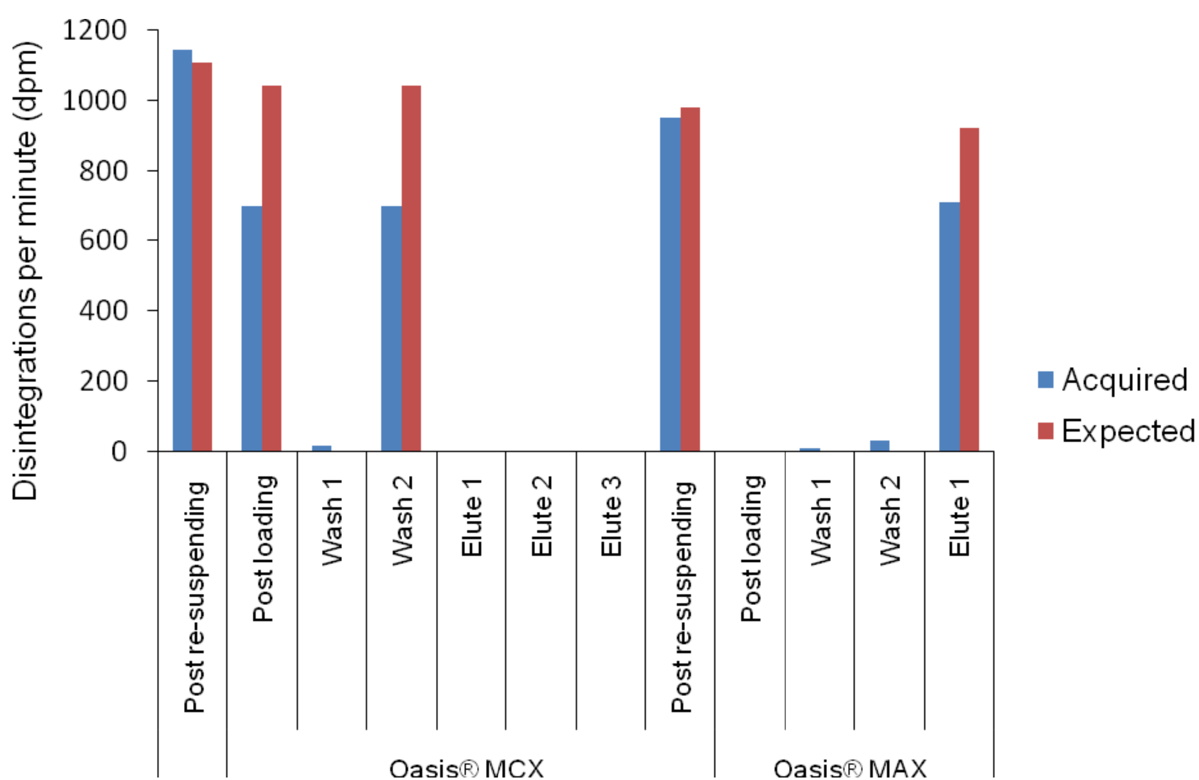


Figure 3.5. The expected and acquired scintillation counts (disintegrations per minute, dpm) obtained from counting the ^{3}H indole-3-acetic acid in 100 µl samples taken while testing an extraction and purification method for endogenous indole-3-acetic acid in *Actinidia chinensis* and *A. deliciosa* fruit.

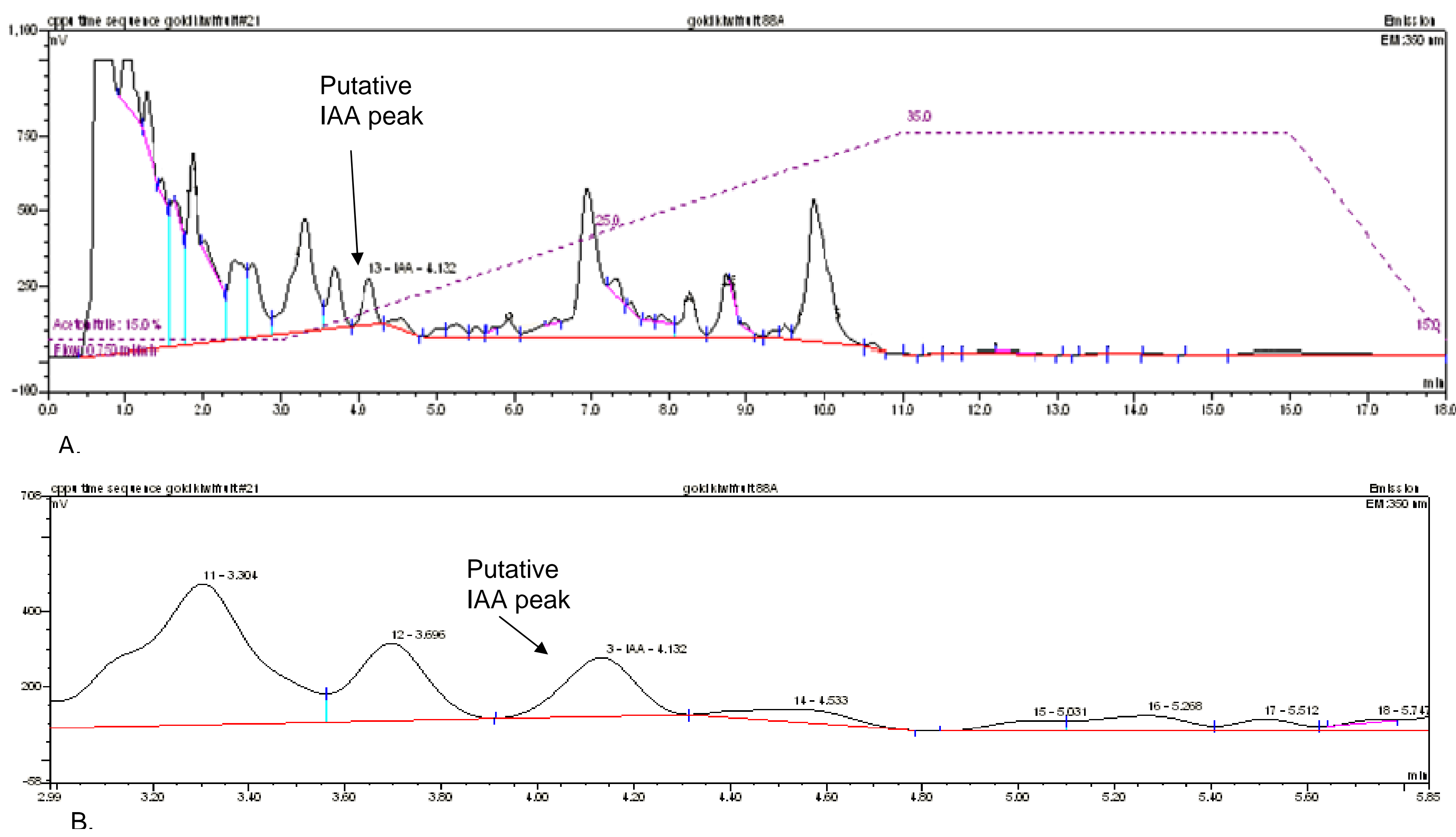


Figure 3.6. A. High pressure liquid chromatography fluorescence detector trace of a kiwifruit sample that has undergone the entire purification process, that is Sep-pak®, Oasis® MCX and Oasis® MAX. B. The same trace as A. with the section containing the putative IAA (indole-3-acetic acid) peak expanded. Note the baseline separation and Gaussian shape of the peak.

3.1.2.3 Internal standards

Initially the internal standard used for testing the methods of extraction and purification was ^{14}C-IAA. As this was the exact structure of the hormone auxin it was used to monitor where the endogenous IAA was during the extraction and purification process, and by counting the radioactivity of samples (with a Perkin elmer liquid scintillation counter Tri-Carb 2900TR using Quanta Smart Tri-Carb LSC software on a three minute count time) any losses were able to be accounted for and minimised. Using ^{14}C-IAA however posed a problem with quantification of endogenous auxin via the HPLC. This was because the two compounds would be seen as one peak due to their identical chemical structure. This would mean subtracting the ^{14}C-IAA peak from the overall peak obtained and could lead to inaccuracies. It was therefore decided that ^{14}C-IAA-Me, a methylated form of ^{14}C-IAA, would be used as the internal standard. This compound would separate from the endogenous IAA on the HPLC and thus losses could be calculated without compromising the quantification of the endogenous IAA. However, methylated IAA is no longer an acid and did not partition with the endogenous IAA on the Oasis® MAX column (Figure 3.7.).

It was therefore decided that high specific activity ^{3}H-IAA (GE Healthcare, United Kingdom) would be used as the internal standard (Figure 3.8.), as this fulfilled the requirements of an internal standard. That is, it would follow the same path through the extraction and purification method as the endogenous IAA. The advantage of using ^{3}H-IAA over ^{14}C-IAA was that ^{3}H-IAA was

required in such a small concentration that it would not be detected by the fluorescence detector in the HPLC, and thus the peak generated would be wholly endogenous IAA, while still providing sufficient radioactivity as to be detected by the scintillation counter, thus allowing a correction to be made for losses during processing.

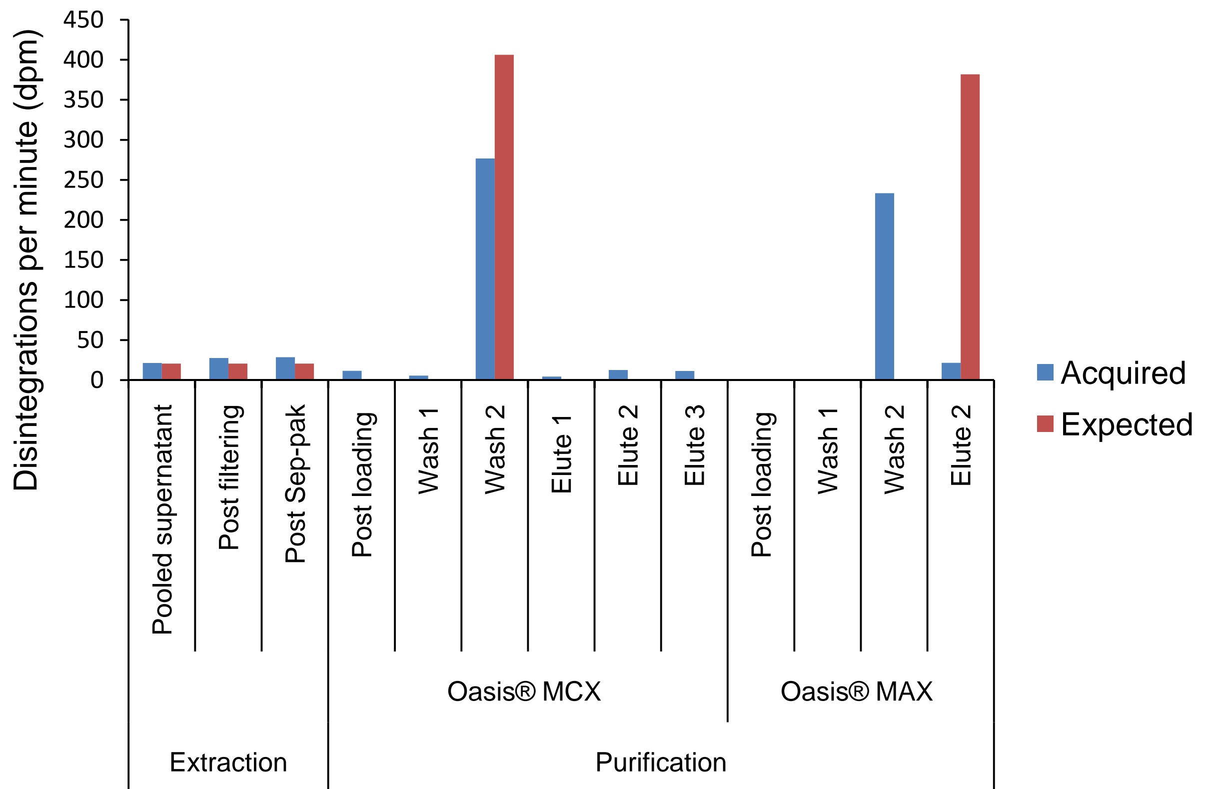


Figure 3.7. The expected and acquired scintillation counts (dpm) obtained from counting the ^{14}C indole-3-acetic acid methyl ester (^{14}C-IAA-Me) in 100 µl samples taken while testing an extraction and purification method for endogenous indole-3-acetic acid in *Actinidia chinensis* and *A. deliciosa* fruit.

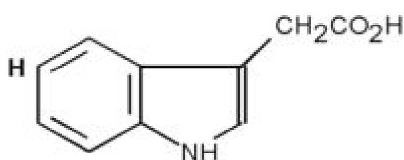


Figure 3.8. Structure of ^{3}H Indole-3-acetic acid (GE Healthcare, United Kingdom)

A second internal standard was also added to monitor the progress and losses of cytokinins. Initially ^{14}C-zeatin (Sigma, America) was chosen to test the method. However, the same problems as were encountered with ^{14}C-IAA were likely to occur again. Therefore ^{3}H-ZR (zeatin riboside) and ^{3}H-IPA (isopentenyl adenosine) were tested. Samples of freeze dried *A. chinensis* fruit were spiked with 20,000 dpm (disintegrations per minute) of either ^{3}H-ZR or ^{3}H-IPA and the extraction and purification method executed. It was found, however, upon counting the dpm of the aliquots taken at each step of the method that as much as 50 percent of the radioactivity of the ^{3}H-ZR and a significant amount of the ^{3}H-IPA had been lost after drying down with the mivac (Figure 3.9.). It was hypothesised that due to the age of the ^{3}H-ZR and ^{3}H-IPA compounds that they had broken down and a proportion of the ^{3}H was no longer associated with the ZR and IPA and was instead associated with the water and or methanol that the ZR and IPA were suspended in. The compounds were 13 years old and had been re purified two years before they were used. This theory was tested by again putting a sample of ZR and IPA through the mivac to dry down and upon re-suspension and the subsequent counting of the aliquots taken it was found that again significant amounts of each isotope had been lost. This supported the previous hypothesis and therefore ^{14}C-Z was used for the cytokinin internal standard. This was deemed a suitable internal standard as the cytokinins were not going to be quantified on the HPLC.

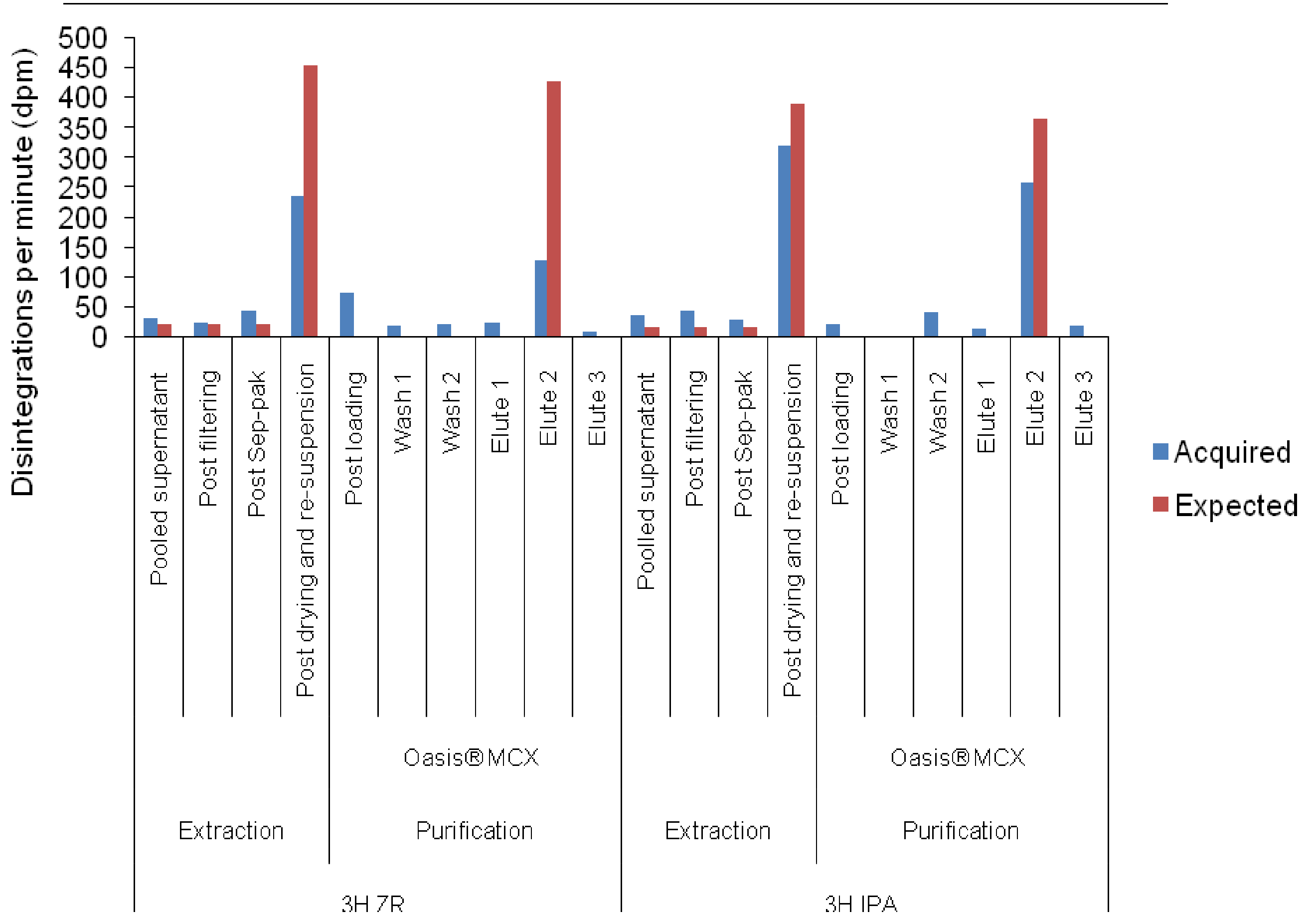


Figure 3.9. The expected and acquired scintillation counts (disintegrations per minute) obtained from counting the ^{3}H-zeatin riboside (ZR) and ^{3}H-isopentenyl adenosine in 100 µl samples taken while testing an extraction and purification method for endogenous cytokinins in *Actinidia chinensis* and *A. deliciosa* fruit.

3.1.2.4 High pressure liquid chromatography (HPLC)

The HPLC used was composed of a Dionex Ultimate 3000 pump, column compartment, and variable wavelength detector, Dionex RF 2000 fluorescence detector, Rheodyne 7725i injection port with a 20 µl loop, and an Agilent SB-C18 1.8 µm 3.0 x 50 mm column. The software used was Dionex Chromeleon® version 6.80. The mobile phase used in this HPLC system was a gradient of 40 mM acetic acid and 100 percent acetonitrile. The 40 mM acetic acid was made by combining 4.6 ml acetic acid and 1994.6 ml

nanopure water. Both mobile phases were filtered through 0.2 µm nylon filters. The gradient was developed to separate out the IAA peak from the other compounds which had not been purified from the kiwifruit solid phase extraction. A gradient had been previously developed on another column for the separation and quantification of IAA in apple sap (Table 3.1). This gradient was however not found to be suitable for the kiwifruit extract and had to be modified through a process of trial and error and shortened for the new column (Table 3.2). The fluorescence detector was set at excitation 280 nm and emission 350 nm which have been shown to be the correct excitation and emission wavelengths for IAA (Horgan, 1995). The sensitivity of the detector was set at sensitivity medium and gain 2 (magnification x128). The ultra violet (UV) lamp of the variable wavelength detector was set at 280 nm.

Table 3.1. The HPLC (high pressure liquid chromatography) gradient developed for the separation and quantification of indole-3-acetic acid in apple xylem sap at Massey University.

Flow rate (ml/minute)	Time (minutes from injection)	40mM acetic acid (%)	Acetonitrile (%)
1	0	80	20
1	1	80	20
1	6	64	36
1	19	64	36
1	24	2	98
1	32	2	98
1	35	80	20

Table 3.2. The HPLC (high pressure liquid chromatography) gradient developed for the separation and quantification of indole-3-acetic acid in *A. chinensis* and *A. deliciosa* fruit.

Flow rate (ml/minute)	Time (minutes from injection)	40mM acetic acid (%)	Acetonitrile (%)
0.75	0	85	15
0.75	3	85	15
0.75	7	75	25
0.75	11	65	35
0.75	16	65	35
0.75	18	85	15

3.1.2. Final method:

It was concluded that the most efficient and accurate method of extracting and purifying both the IAA and cytokinins from the *A. chinensis* and *A. deliciosa* fruit was by following the method outlined by Dobrev and Kamínek (2002) with some modifications (detailed in 3.2.2.2). Following this the fraction containing IAA was further purified with an Oasis® MAX column, fractions containing cytokinins were frozen for processing at a later date and will not be discussed further in this thesis. The samples containing IAA were then injected into an HPLC for quantification. Finally the IAA peak was collected from the HPLC and the ^{3}H-IAA used for the internal standard was counted by a scintillation counter so the losses could be calculated per sample and accurate quantification could be obtained.

3.3 Results:

A standard curve was produced by running known concentrations of IAA (Korn Light Laboratories Ltd, England) on the HPLC and under the same conditions as the samples were run (Figure 3.10.). From this the concentration of IAA in the samples could be calculated, using the formula y=21.806x + 2.9467.

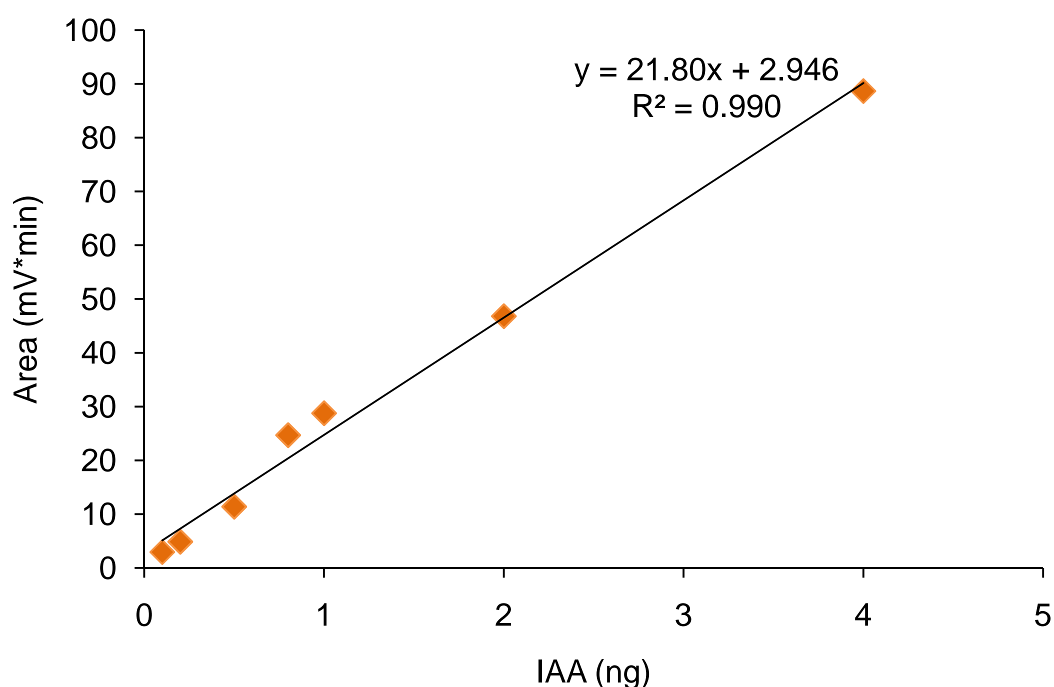


Figure 3.10. Standard curve of IAA (indole-3-acetic acid) (Korn Light Laboratories Ltd, England).

The IP and OP data was pooled so data shown represents whole fruit including seeds and skin. Changes in IAA concentration over time differed between control and CPPU treated *A. chinensis* fruit (Figure 3.11). High concentrations of IAA correlated well with the two phases of rapid fruit growth, particularly for the CPPU treated fruit (Figure 3.12) and there appears to be a relationship between the IAA content and fresh weight of fruit (Figure 3.12).

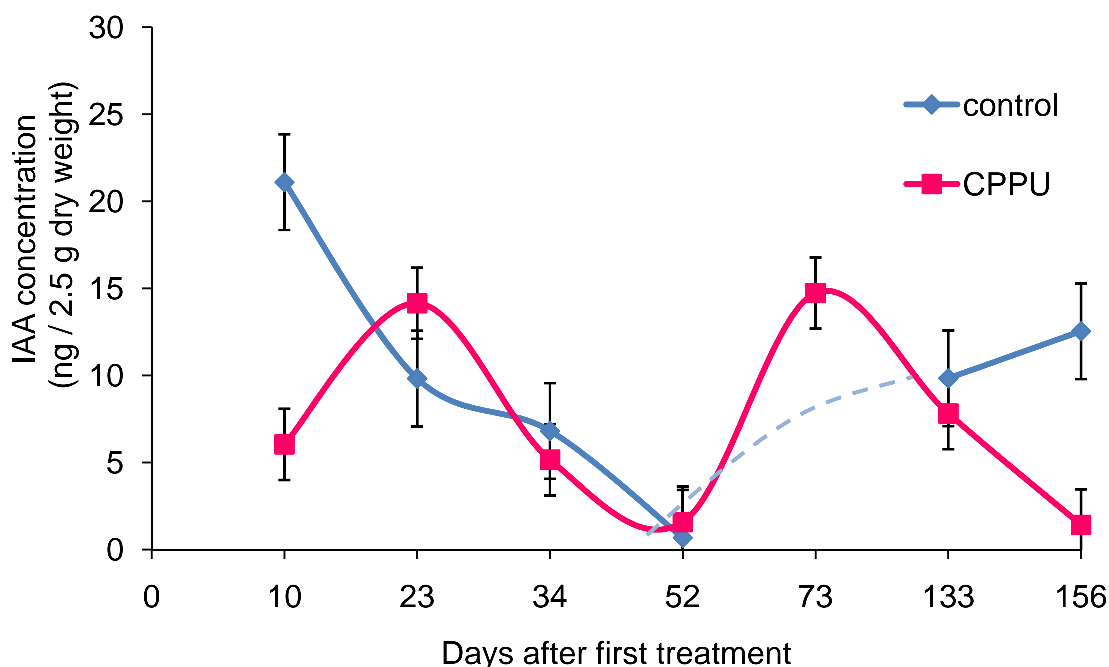


Figure 3.11. IAA (indole-3-acetic acid) concentration in 2.5 g freeze dried *A. chinensis* fruit treated with 5 mgL^{-1} CPPU N-(2-chloro-4-pyridyl)-N'-phenylurea and untreated control. Dashed line represents missing data point. Error bars = standard error.

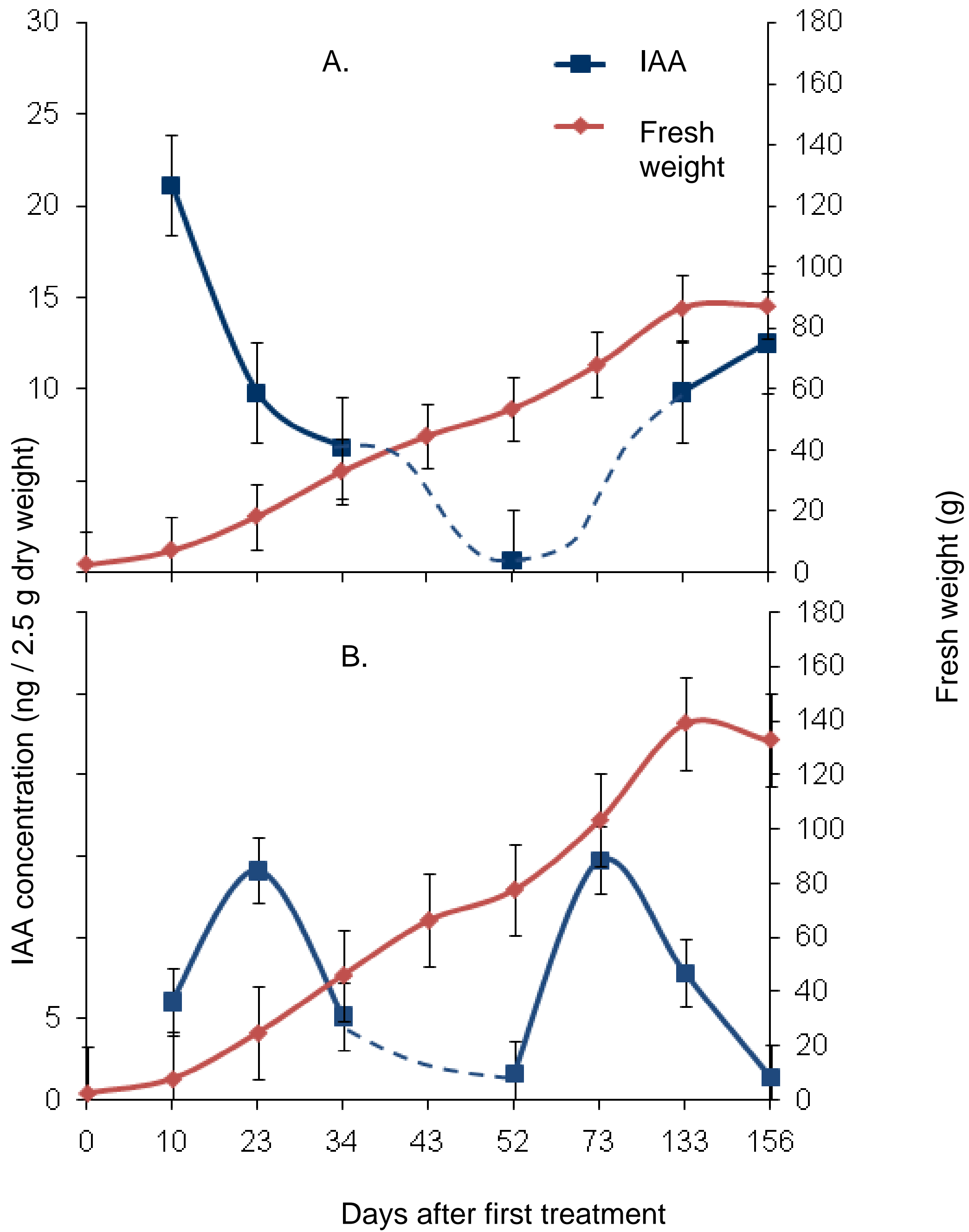


Figure 3.12. IAA (indole-3-acetic acid) concentration (ng / 2.5 g dry weight) and fresh weight of A. chinensis fruit. A. untreated control, B. treated with 5 mgL^{-1} CPPU (N-(2-chloro-4-pyridyl)-N'-phenylurea). Dashed line represents missing data point(s). Error bars = standard error.

3.4 Discussion:

Much more work is required in the thorough analysis and quantification of endogenous IAA in kiwifruit fruit and the effect of CPPU on the endogenous IAA content and therefore the conclusions drawn here from the data available now are tentative and relationships and correlations may become clearer with more data. Not all of the samples have been analysed at the time of writing, and it is yet to be confirmed that the putative IAA peak contains solely IAA. Mass spectrometry can be employed to determine the purity of the putative IAA peak and obtain the relationship between specific ions and the fluorescence peak. Also not all of the putative IAA peaks from the samples were resolved to base line in the fluorescence trace and therefore more work is required to adjust the gradient on the HPLC.

There does appear, at this stage, to be a correlation between the IAA content in fruit and application of CPPU (Figure 3.11 and 3.12). The initial concentration in the control fruit is high (day 10), this decreases rapidly to day 20, levels off and then decreases rapidly to day 52, after which IAA concentration increased until maturity. When these changes are related to the growth curve and stages of growth defined by Hopping (1976a) it would appear that the increases in endogenous IAA content correlates firstly with periods of cell division and then with cell enlargement. The IAA concentration follows a very similar pattern to that of peach fruits (Millar *et al.*, 1987). Peach fruit also follow a double sigmoid growth curve, and this curve has been broken down into stages very similar to that of kiwifruit. It was found in peach

fruit that the IAA concentration was also relatively high during the first stage, which is also associated with cell division in peach fruits (Miller *et al.*, 1987). No explanation for this was given even though IAA is usually associated with cell expansion and division. The lowest concentrations of IAA were also reached during the second stage of growth in peach fruit (Miller *et al.*, 1987). Furthermore concentration of IAA was found to increase during the third stage of growth. A very similar pattern was also observed in blackcurrant berries which also follow a double sigmoidal growth curve (Wright, 1956 as cited by Wareing & Phillips, 1970).

The initial concentration of IAA in the fruit treated with CPPU (at day 10) was lower than the control but did increase to a peak at day 23, though overall was lower than the IAA content in the control fruit. The delayed increase in IAA in fruit treated with CPPU could possibly be related to cell activity during the first stage of fruit growth when cell division is very active. Cell division is associated with a relatively high ratio of cytokinin to auxin (Das *et al.*, 1956) and prolonging this ratio may also prolong stage one of fruit growth, as found in CPPU treated *A. deliciosa* fruit (Woolley *et al.*, 1991). The IAA content continued to decline in CPPU treated fruit until day 52, where the trough in IAA concentration appears to be correlated with slowing of growth. Following that there was a rapid increase to a second peak in IAA concentration at day 73 and then a decline until the final harvest. The IAA concentration appears to have decreased more rapidly in fruit treated with CPPU whereas the concentration in the control fruit was increasing. The concentration in fruit treated with CPPU was lower than in the untreated control fruit. This could

account for the advanced maturity previously observed in fruit treated with CPPU (Patterson *et al.*, 1993; Costa *et al.*, 1995; Cruz-Castillo *et al.*, 1999). Auxin has previously been shown to delay ripening in other fruits such as strawberry. It was found, by Manning 1994 & 1995, that in strawberry the expression of almost all known ripening genes were suppressed by auxin (Civello *et al.*, 1999). It was suggested that declining levels of auxin activates the expression of ripening genes which therefore initiate the process of fruit ripening (Manning 1994 & 1995 as cited in Civello *et al.*, 1999).

The results obtained in the current experiment appear to follow a similar initial pattern to that determined by Ohara *et al.* (1997). However the majority of the paper was published in Japanese and all conclusions and comparisons drawn here are based off the information provided in the abstract and figure legends which were published in English. Ohara *et al.* (1997) extracted and purified an IAA-like substance from the seeds alone or flesh alone of fresh *A. deliciosa* fruit which had been treated once with an unknown concentration of CPPU approximately 20 days after full bloom and untreated control fruit. The results have been compared between the IAA concentrations found in the seeds of both the treated and untreated fruit, as determined by Ohara *et al.* (1997), and the results presented here because although the inner and outer pericaps were separated in this experiment the data was pooled in figure 3.11. The IAA-like substance was quantified from both the “seeds and pericarp” of the treated and untreated fruit. The initial IAA pattern of concentration was very similar to that determined in the present experiments (Figure 3.11) and the first measurement taken in the current experiments has been compared to the

Ohara *et al*. (1997) results (Figure 3.13). Unlike the results shown in figure 3.11 the IAA concentration in both control fruit and those treated with CPPU were very similar at 35 days after full bloom, following this time however the pattern is the same as the results shown in figure 3.11 in that the IAA concentration declined in seeds of control fruit whereas, the concentration in the seeds of the fruit treated with CPPU peaked at day 23 and approximately day 50 (Figures 3.12 and 3.13 respectively). From this point onwards though the results of the two experiments differed. IAA concentration in the control fruit followed a similar pattern but in the CPPU treated fruit there was no second peak and the final concentration is slightly higher than that of control. However the significance of this difference cannot be determined from the data available.

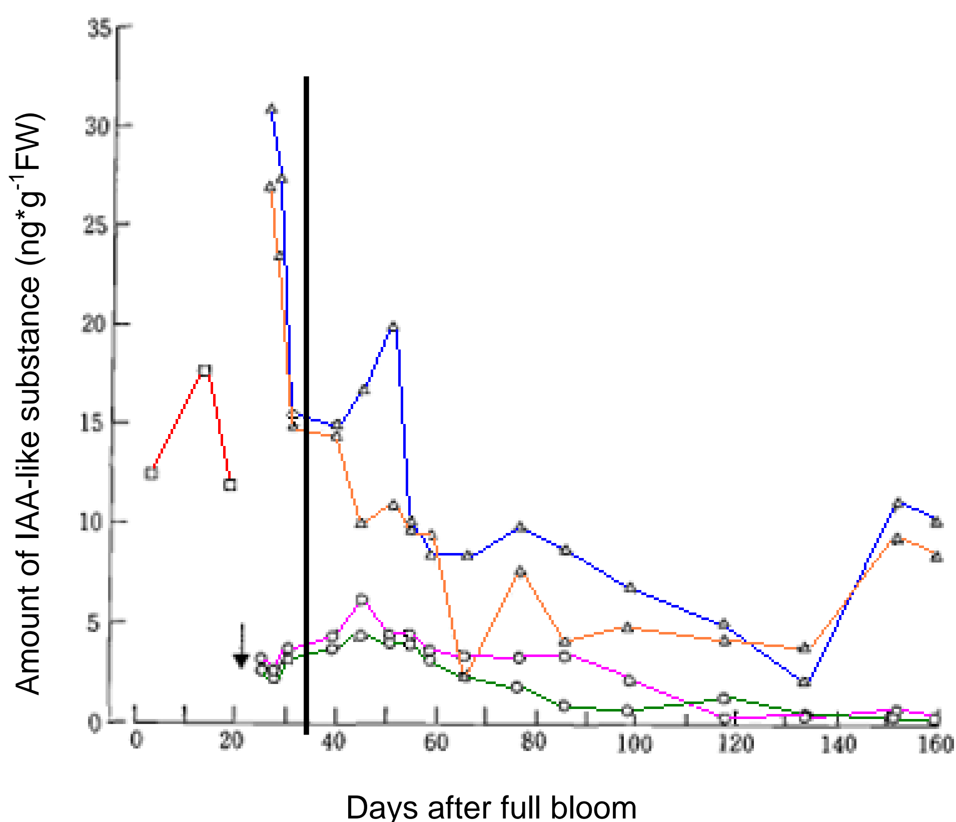


Figure 3.13. Seasonal changes in the amount of IAA-like substances in the seeds and pericarp of 'Hayward' kiwifruit. □-□ = whole untreated fruit. △-△ = seed of CPPU-treated fruit. △-△ = seed of untreated fruit. ○-○ = pericarp of CPPU-treated fruit. ○-○ = pericarp of untreated fruit. An arrow indicated the day of CPPU treatment. (Ohara *et al*., 1997). The line at approximately day 35 indicates the position of the first IAA measurement in figure 3.11.

Application of CPPU may increase fruit size by indirectly increasing sink strength by way of increasing either or both cell division and cell expansion and therefore increasing the flow of photo-assimilates to fruit. Alternatively, though not mutually exclusively, sink strength could be increased directly by influencing the phloem transportation of photo-assimilates by way of phloem unloading or transport. Thus if competition for photo-assimilate supply is limiting this limiting factor would be lessened and cell division and/or enlargement could increase. It has been suggested that endogenous IAA concentration is associated with sink strength in potatoes (Marschner *et al*., 1984). Sink strength is a product of sink size and sink activity and the results

of an experiment with potatoes suggests that IAA concentration may influence sink activity. It was found that when fast growing tubers were removed the tubers which were left (formerly slow growing tubers) increased in growth rate. It was determined that the fast growing tubers had a higher IAA content than the slow growing ones but when the fast growing tubers were removed (after a lag phase) both the IAA content and the growth rate increased. It was also observed that for a given growth rate the IAA content was higher in tubers beginning a high growth rate after the tubers which initially had a high growth rate were removed, compared to the un-pruned control tubers. Marschner *et al*. (1984) suggested that this indicated that the increase in IAA content occurred before the increase in the growth rate of the tuber. The proposed mechanisms of action for IAA in regards to this increase in tuber growth rate were through an effect on cell elongation or by increased phloem transport to sites with a higher IAA concentration. It was observed by Patrick (1979) that when endogenous auxin levels were reduced by treatment with 1-(2'-carboxyphenyl)-3-phenylpropane-1,3-dione (ACP1.55) (which had been shown previously to inhibit auxin transport) ^{14}C-phtotosynthate transfer was reduced by 50 percent. This and other experiments have led to the suggestion that the mobilising ability of a sink may extend beyond its physical confines through the influence of exported auxins (Patrick, 1979). If IAA concentration is associated with phloem transport of photo-assimilates then this could be one possible mechanism behind the increase in fresh weight associated with CPPU application as IAA peaked in the CPPU treated fruit early in the third stage of growth and not in the control fruit.

CPPU could simply be increasing fresh weight by stimulating cell division but can also stimulate cell enlargement (Woolley et al., 1991; Patterson et al., 1993; and Lewis et al., 1996) possibly via effects on IAA concentration. It would be problematic to establish which (if either) of the two mechanisms described here is responsible for the increase in fresh weight of fruit induced by the application of CPPU. Repeating the experiments conducted to count cell number and measure cell size on both *A. chinensis* and *A. delicisoa* fruit on fruit treated with CPPU, and perhaps other compounds shown to increase fruit size, could shed further light on the mechanisms behind the increases. Also it would be interesting to observe the effect of restricting the flow of IAA to and from fruit with an inhibitor such as ACP1.55 (Patrick, 1979); ^{14}C IAA could be used to determine the direction of flow.

An unresolved problem encountered in the purification of kiwifruit fruit samples was that a high percentage of the ^{3}H-IAA tracer was lost during the purification process. Therefore investigation on where the ^{3}H-IAA is being lost is required, it may be in part of the purification process or it may be that is it simply being broken down by light, heat or chemical oxidase. Therefore greater care needed to be taken to protect the samples. Anti-oxidants are often used in extraction solutions but appeared to increase losses in our sample. More investigation is therefore required into suitable anti-oxidants.

If, after further study, it becomes apparent that there is no effect of CPPU on IAA levels then it may be that another hormone or group of hormones is being affected and further study of other hormones such as cytokinins is required. It

has been shown previously that the endogenous content of cytokinins is altered in *A. deliciosa* fruit treated with CPPU (Woolley & Currie 2006). It was found that Z and ZR levels in fruit treated with CPPU was lower than in untreated control fruit, as determined by radio-immunoassays. There was also found to be a slight transient increase in 2iP and IPA content. In another experiment overall cytokinin levels were found to be decreased in fruit treated with CPPU when compared to the cytokinin content of untreated control fruit (Lewis *et al.,* 1996), but this was probably due to the early CPPU application inhibiting seed development. It was also found in that experiment that both Z and ZR were undetectable in fruit treated with CPPU and concentration of other cytokinins tested (iP, IPA, zeatin-0-glucoside, zeatin-9-glucoside, zeatin riboside-0-glucoside, dihydro zeatin riboside-0-glucoside and, isopentenyladenine-9-glucoside), were decreased in fruit treated with CPPU. However fruit were only harvest once, ten days after flowering, whereas Woolly & Currie (2006) harvested throughout the growing season which gives a more complete representation than a single harvest. Given that *A. deliciosa* and *A. chinensis* respond differently to Benefit® there is no reason to assume that the endogenous hormone response to CPPU would be the same.

Chapter Four

4. General discussion

It has been shown that fresh weight of both *A. chinensis* and *A. deliciosa* can be increased by the application of a natural plant extract and synthetic plant growth regulators. However, the effect of the natural plant extract (Benefit®) does appear to differ between *A. chinensis* and *A. deliciosa*, although the response to CPPU is similar.

4.1. Response of fruit growth to Benefit® and CPPU and interactive effects

Applications of both Benefit® and CPPU were shown to significantly increase the fresh weight of *A. chinensis*. These results support those found in previous studies (Woolley & Cruz-Castillo, 2006). Moreover there appears to be a statistically significant positive interaction between Benefit® and CPPU, observed when Benefit® plus CPPU was applied. Application of CPPU also significantly increased the fresh weight of *A. deliciosa* fruit; again this result supports previous studies (Iwahori *et* al., 1988; Lawes *et al*., 1991; Patterson *et al*., 1993; Lewis *et al*., 1996; Cruz-Castillo *et al*., 1999; Woolley & Cruz-Castillo, 2006; Woolley & Currie, 2006). However, application of Benefit® to *A. deliciosa* did not significantly alter the fresh weight of fruit, despite being applied following the recommended protocol. This result supported that found by Woolley & Cruz-Castillo (2006) but was in contrast to the results obtained

by Costa *et al.* (2002). Costa *et al.* (2002), found in experiments repeated over three years, that application of Benefit® did significantly increase the fresh weight of *A. deliciosa* fruit. There appears to be no simple reason for the difference based on a comparison of the methodology other than a possible environmental influence due to temperature differences. The main difference, apart from the locations, New Zealand and Italy, was rather small in that Benefit® was applied at 2.5 mlL^{-1} when it had no effect and 3 mlL^{-1} when it did also the initial date of application was ten days earlier when Benefit® was found to have an effect. These subtle differences appear to indicate that is it not that *A. deliciosa* is insensitive to Benefit® but that it may be highly sensitive to concentration and/or application date. Application of Benefit® plus CPPU resulted in a significant increase in fresh weight of fruit when compared to the untreated control fruit (Table 2.4), however when compared to the fruit treated solely with CPPU there was no significant difference. These results indicate that the natural plant extract Benefit® is not effective in increasing the fresh weight of *A. deliciosa* fruit under the conditions of application followed in these experiments. Therefore, both time and concentration of application need to be tested before conclusions can be drawn as to the effect of Benefit® on *A. deliciosa* grown in New Zealand.

At this stage though the question remains to be answered as to why the two major kiwifruit cultivars ‘Hort16A’ and ‘Hayward’ are not affected in the same way when treated with Benefit®. The reaction of both *A. chinensis* and *A. deliciosa* to CPPU indicates that they share a limiting factor for fruit growth, however, their differing reactions to Benefit® suggest that either the limiting

factors are not the same or that *A. deliciosa* has a limiting factor that is not met by Benefit®, but that Benefit® does supply for *A. chinensis*. Previous studies with multiple representatives from all of the hormone groups suggest that gibberellins are not an initial limiting factor for either cultivar (Hopping, 1976b; Cruz-Castillo *et al.*, 1991; Cruz-Castillo *et al.*, 1999; Famiani *et al.*, 2007; and Lorenzo *et al.*, 2007). This leaves cytokinins and auxin. However, Benefit® has been shown to have very little cytokinin activity (D.J. Woolley, personal communication). Benefit® has also been shown to induce a greater or equal increase in fruit growth if applied to the leaves as opposed to the fruit (Woolley & Cruz-Castillo, 2006). This indicates that the active component(s) is readily transported. It has been found that by fractioning Benefit® via chromatography and applying the fractions separately to *A. chinensis* vines, one fraction will increase the fruit size more than when Benefit® is applied whole (D.J. Woolley, personal communication). It is possible that perhaps there is an inhibitor that is part of the natural plant extract which is not readily transported and is therefore separated from the active component of Benefit®. It is conceivable that perhaps *A. deliciosa* vines are lacking in something required to separate the inhibitor from the active component. This suggests that the hypothetical inhibitor and the active component are able to be separated *in vitro*. It may be reasonable to suggest that through further purification the natural plant extract Benefit® may be able to induce the same response when applied to *A. deliciosa* as it does now when applied to *A. chinensis*. Alternatively it could be as simple as *A. deliciosa* requiring a higher concentration of Benefit® to elicit the same response as is achieved in *A.*

chinensis. This would suggest that both *A. chinensis* and *A. delicisoa* have the same limiting factor which is met by Benefit®, but different sensitivities.

4.1.1. Proportions of inner and outer pericarps

Application of CPPU altered the proportion of some of the fruit tissues of *A. chinensis* fruit. That is, the percentage of the OP was increased and, correspondingly, the percentage of the IP was decreased. One could suggest several hypotheses as to why the tissues did not increase in size proportionately and none are mutually exclusive. The first is that CPPU has been shown to have little or no mobility within the plant (Neri *et al.*, 1993; D.J. Woolley, personal communication). Thus it could be that CPPU is only active in the OP because it is not readily transported within the fruit. It has also been shown previously that cell division continues for between seven and fourteen days longer in the OP of fruit treated with CPPU (Woolley *et al.*, 1991). The same experiment also showed that cell size in the OP of CPPU treated fruit was increased compared to the untreated control fruit. However, in the experiments conducted here the physiology of *A. chinensis* and *A. deliciosa* fruit were found to differ, in that the proportions of the pericarps of *A. deliciosa* fruit were not significantly altered by CPPU treatment. This is in contrast to the results reported by Woolley *et al.* (1991). However it should be noted that CPPU was applied to *A. deliciosa* at a lower rate than to *A. chinensis* (1 and 5 mgL^{-1} respectively (Section 2.2.5. and 2.2.3.)) and this could have influenced these results; also the application of 1 mgL^{-1} is lower than what appears to be

the standard concentration used in other studies, including that by Woolley *et al*. (1991).

4.1.2. Percentage dry matter

The percentage dry matter content was not significantly altered in either *A. chinensis* fruit or *A. delicisoa* fruit by the application CPPU. Nonetheless, there did appear to be a tendency for a decrease in percentage dry matter that may be of significance to growers, Zespri, and consumers, given that a high proportion of the payment that growers receive is determined by the average percentage dry matter of their crop and consumers have shown a preference for the flavour of fruit with a high percentage dry matter (Burdon *et al*., 2004). Application of Benefit®, again, had different results depending on the cultivar. Percentage dry matter was significantly decreased in *A. chinensis* fruit treated with both Benefit® and Benefit® plus CPPU from 17.49 percent to 15.99 and 15.58 respectively (Table 2.2). Neither of these treatments had any significant effect on the percentage dry matter of *A. deliciosa* fruit and nor did the effect of CPPU alone (Table 2.4) suggesting that the decrease in percentage dry matter observed in *A. chinensis* was mainly due to Benefit®.

4.1.3. Endogenous IAA content

There are several theories behind the mechanisms by which exogenous compounds such as CPPU induce an increase in fresh weight of fruit of the

two main *Actinidia sp.* (these are discussed in detail in sections 1.9 and 3.1). However none of these theories are conclusive. The rationale behind the quantification of endogenous hormones over time was that CPPU may act by altering the overall concentration of one or more hormones or perhaps by altering the ratio between hormones. CPPU had been shown previously to act as a naturally occurring cytokinin (Takahashi *et al.*, 1978 as cited by Neri, *et al.*, 1993; Shudo, 1994; Mok *et al.*, 1987 . It has also been suggested that it may act in a protective role for naturally occurring cytokinins (Woolley & Currie, 2006). The increase in fruit size must be due to an increase in cell division and/or cell expansion. However when the cells of fruit treated with CPPU have been studied the results have been conflicting with some researcher reporting an increase in cell expansion (Lewis et al., 1996; and Patterson et al., 1993), others reporting an increase in cell division (Kurosaki & Mochizuki, 1990; and Neri et al., 1993), and others reporting that the increase is due to both (Woolley et al., 1991). Naturally occurring cytokinins are usually associated with cell division in fruit and auxins with both cell division and expansion (Atwell, *et al.*, 1999). Thus it was hypothesised that CPPU may be altering the concentration of endogenous auxin and/or the ratio of auxin and cytokinins, and so endogenous auxin was quantified first, and partially purified fractions for the same extracts have been stored for future cytokinin analysis.

Endogenous IAA content was quantified in *A. chinensis* fruit treated with CPPU and untreated control fruit. The methodology required a considerable amount of development. The main reasons for the amount of method

development required was that quantification of both IAA and cytokinins was initially being attempted, and appropriate internal standards needed to be identified. The final method involved passing ground extracted samples through a Sep-pak® cartridge, an Oasis® MCX column, then the fraction containing IAA was passed through an Oasis® MAX and finally samples were put through an HPLC for separation, identification, and quantification. The fractions containing cytokinins were stored in a freezer for quantification at a later date due to time constraints.

It was found that there does appear to be an alteration in endogenous IAA pattern and concentration in *A. chinensis* fruit treated with CPPU, though the data at this time is limited. The delayed and lower peak of IAA could allow the extended period of cell division in fruit treated with CPPU observed by Woolley *et al.* (1991) because of the high cytokinin to auxin ratio. Quantification of cytokinin concentrations may help strengthen this theory. It has been shown that while cytokinins are predominantly associated with cell division auxin is also required and it is the high ratio of cytokinin to auxin that determines cell division (Das *et al.*, 1956). The decrease in IAA at final harvest in fruit treated with CPPU could explain, in part, why fruit treated with CPPU appear to mature earlier than control fruit, as observed by Patterson *et al.* (1993); Costa *et al.* (1995); and Cruz-Castillo *et al.* (1999). IAA is associated with delaying maturity and a decline in the concentration is related to the expression of ripening genes (Manning 1994 & 1995 as cited in Civello *et al.*, 1999). The endogenous IAA concentration of the control fruit follows the same pattern observed by Millar *et al.* (1987) in peach fruit and Wright (1956)

(as cited by Wareing & Phillips, 1970) in blackcurrent berries, both of which also follow similar double sigmoidal growth patterns as kiwifruit.

The results were similar in some ways to those obtained by Ohara *et al.* (1997). Though the conclusions drawn from this paper are restricted due to language barriers and the limited amount of the paper published in English. As such the date of CPPU application is known but not the rate, which has been shown to influence the outcome previously. However the overall pattern of IAA concentration can be determined and it was similar to the results obtained from the present experiments. The pattern of IAA concentration in the untreated control fruit was similar between experiments, though the initial measurement was taken later in this experiment than that by Ohara *et al.* (1997). Both showed the concentration to be high in the early stages of fruit growth and declining through into the end of the third stage of growth where it increased into the final harvest, though the Ohara *et al.* (1997) results showed a slight decline at final harvest whereas the current experiment showed a continued increase. The IAA concentration determined in the fruit treated with CPPU also followed a similar pattern in the two experiments. However the concentration at day 35 (which corresponds with day 10, the first day of IAA quantification, in the current experiment) was similar between the control and CPPU treated fruit in the Ohara *et al.* (1997) results while in the current experiment the IAA concentration in control fruit was determined to be significantly higher than in the CPPU treated fruit. Following this though the IAA concentration appeared to follow a very similar peak in both experiments and then declined in the second stage of growth. The subsequent results,

however, show differing patterns of IAA concentration in the CPPU treated fruits. In the current experiment it was shown that there was a second IAA peak higher than the first in the early period of stage three and then the IAA concentration declined until the final harvest. In the Ohara *et al*. (1997) results the IAA concentration in CPPU treated fruit follows a similar pattern to that of the control although it finished slightly higher at the final harvest. These observations and comparisons have been surmised from limited information however.

From these results it appears evident that CPPU is altering the concentration of endogenous IAA. What is still unclear at this time is whether CPPU is inducing an increase in fruit weight directly by increasing the sink activity of fruit. That is, the fruit size and weight could be increased because the photo-assimilates being imported into the fruit are being imported at a higher rate and therefore cell division and or cell expansion increased as those processes are less limited by photo-assimilates. Conversely application of CPPU could induce an increase in fruit weight indirectly by increasing the sink size by way of increase cell division and/or cell expansion and therefore more photo-assimilates are transported to the fruit.

What can be concluded, at this time, is that CPPU is having an effect on the endogenous IAA content of *A. chinensis* fruit. More quantification of endogenous IAA is required to strengthen these results and quantification of cytokinins may make the overall picture somewhat clearer.

4.2. Response to 3,5,6 TPA

It was found that, contrary to results previously observed by Bregoli *et al.* (2006), 3,5,6-TPA did not increase the fresh weight of *A. deliciosa* fruit. This synthetic auxin compound was applied at three different concentrations and on two different application dates. It did not induce an increase in fruit weight in any of the conditions applied. In fact application of 3,5,6-TPA at 0.1 mgL^{-1} on the late application date significantly decreased the fresh weight of the fruit, though there did appear to be a correlation between fresh weight and concentration at the early date (Figure 2.4) with the fresh weight increasing with concentration. One possibility as to why the fresh weight was not increased is that the early application date was during the period of cell division and auxins are predominantly associated with cell expansion. An early application of NAA was also found to have a deleterious effect on fresh weight in experiment six. The concentration applied may not have been sufficient to increase cell expansion but could have, potentially, altered the subtle balance between cytokinins and auxins required to induce cell division, hence the deleterious effect of both 3,5,6-TPA and NAA. The late application of 3,5,6-TPA also failed to increase fresh weight even though it was during the period of predominant cell expansion, and higher concentrations could feasibly increase fresh weight. However, 3,5,6-TPA was the only plant regulator applied in this set of experiments that induced a significant increase in percentage dry matter. The greatest increase was achieved by the application of 10 mgL^{-1} for the late treatment date.

4.3. Response to NAA and interaction with CPPU

Early application of another synthetic auxin, NAA, to *A. deliciosa* fruit also failed to induce an increase in fresh weight of fruit. In contrast to the 3,5,6-TPA results though a late application resulted in a significant increase in the fresh weight of fruit. Also in contrast to the results of the 3,5,6-TPA experiment application of NAA did not increase percentage dry matter at either date. Application of CPPU on the late application date to *A. deliciosa* in the same experiment also resulted in a greater increase in fruit weight than when it was applied on the early application date, although it must be noted that the concentration used was only 1 mgL^{-1} compared to the more common 5 or 10 mgL^{-1}. An additive response was achieved by applying both CPPU and NAA at either the early or late application date. However, perhaps the most interesting result was observed when CPPU was applied early and NAA was applied late. This combination of treatments resulted in a synergistic interaction whereby the increase in weight induced by applying CPPU early plus the increase induced by applying NAA late on separate fruit was less than that induced by the application of CPPU early plus NAA late on the same fruit. This interaction cannot be tested statistically as there was no internal replication due to a limitation of canes and the large nature of this experiment, but this could be tested in future experiments. The significance of this result may be related to the timing of application and the activity of natural cytokinins and auxin. The early application of CPPU, the synthetic cytokinin-like compound, was during the period of cell division and cytokinins are predominantly associated with cell division in fruit (Atwell, *et al.*, 1999).

Furthermore the late application of NAA, the synthetic auxin, was during the period of cell expansion and auxin is predominantly associated with cell expansion (Atwell, *et al.*, 1999). These results do not support or disprove any one of the theories behind the potential mechanisms of action put forward for CPPU over any others. That is, the protective role of CPPU for endogenous cytokinins perhaps by interfering with the activity of cytokinin oxidase, or the direct role of CPPU acting like a naturally occurring cytokinin, or the indirect role whereby CPPU acts by forming a positive feedback loop where the concentration of naturally occurring cytokinins is increased. These results do not correspond with those obtained by Patterson *et al.* (1993) and Lewis *et al.* (1996) which suggest that CPPU causes an increase in cell expansion, nor do they support the hypothesis put forward in this thesis that CPPU application causes an increase in fresh weight simply by altering the concentration of endogenous IAA. However the results can be tentatively interpreted in terms of optimal auxin/cytokinin ratios at specific stages of fruit growth.

4.4. Summary

Both the natural plant extract Benefit® and the synthetic plant growth regulator CPPU increased the fresh weight of fruit when applied to *A. chinensis* (Figure 2.1), as found in previous studies (Woolley & Cruz-Castillo, 2006). There was a statistically significant ($p < 0.05$) interaction between Benefit® and CPPU when they were applied together, whereby the fruit treated with both were significantly heavier than when either was applied alone (Table 2.4). However the increase was not found to be greater than when the increase caused by

each individually was added together. While this result could suggest that both Benefit® and CPPU induce an increase in fruit weight by the same means this is not necessarily the case. There have been conflicting results as to whether application of CPPU increases fresh weight by way of cell division (Kurosaki & Mochizuki, 1990; and Neri et al., 1993) or cell expansion (Lewis et al., 1996; and Patterson et al., 1993) or both (Woolley et al., 1991). Although, given that the fresh weight has increased without a proportional increase in percentage dry matter, it may be reasonable to conclude that the increase is at least partially due to cell expansion. However, Benefit® has been shown to have little or no cytokinin activity. It would, therefore, seem unlikely that Benefit® and CPPU induce an increase in fruit weight by means of the same mechanism. Benefit® was also shown to decrease the percentage dry matter of fruit significantly when applied to *A .chinensis*, while application of CPPU did not (Table 2.2).

Not all treatments applied to *A. deliciosa* increased the fresh weight of fruit. Application of CPPU increased fresh weight in both of the experiments it was tested in (Figure 2.3 and Table 2.6), thus supporting previous results (Iwahori *et* al., 1988; Lawes *et al*., 1991; Patterson *et al*., 1993; Lewis *et al*., 1996; Cruz-Castillo *et al*., 1999; Woolley & Cruz-Castillo, 2006; Woolley & Currie, 2006). However application of Benefit® did not affect fresh weight, proportions of pericarps, or percentage dry matter, in any way (Table 2.4 and 2.5). This results supported of one previous study (Woolley & Cruz-Castillo, 2006) but not another (Costa *et al*., 2002). It could be suggested that *A. deliciosa* is less sensitive to Benefit® than *A. chinensis* and thus requires a higher

concentration. The differing outcome of Benefit® application between *A. chinensis* and *A. deliciosa* fruit suggests a difference in their physiology, either in terms of their limiting factors or their ability to metabolise Benefit®. Application of 3,5,6-TPA also failed to induce an increase in *A. deliciosa* fruit weight (Figure 2.4) which was in contrast to previous studies (Bregoli *et al.*, 2006). Percentage dry matter was, however, significantly altered and concentration and application date affected this (Figure 2.5). The effect of NAA application was dependent on date with an early application having a negative effect on fresh fruit weight and a late application date significantly increasing the fresh weight (Table 2.6). Reaction to CPPU was also found to be affected by application date. The most intriguing result came from the application of CPPU early plus NAA late as this resulted in a synergistic interaction. However, none of the NAA/CPPU combinations resulted in an increase in percentage dry matter (Table 2.6).

A large amount of method development was required for the extraction and purification of both endogenous auxin and cytokinins from *A. chinensis* fruit. The method, at this stage, is by no means absolute and requires improvement due to high losses of the internal standard and the putative IAA peak did not reach baseline in all of the traces from the fluorescence detector. Also it has not been determined that the putative IAA peak does in fact contain solely IAA. Nonetheless there does appear to be a correlation between treatment of *A. chinensis* fruit with CPPU and concentration of endogenous IAA (Figure 3.11 and 3.12). However, results at this point in time are limited and inconclusive. Though the endogenous IAA in the control fruit does appear to

follow a similar pattern over the development of fruit as has been previously determined for *A. deliciosa* fruit, peach, and blackcurrant berries (Ohara *et al.*, 1997; Millar *et al.*, 1987; and Wright, 1956 as cited by Wareing & Phillips, 1970 respectively) and this supports the validity of the results.

4.5. Recommendations for further research

Further work is required in the study of the differing physiology of *A. chinensis* and *A. deliciosa* fruit in relation to how the fruit respond when treated with different substances. To this end, more trials where the same plant growth regulators and the natural plant extract Benefit® are applied to both cultivars should assist in a better understanding of these differences. Also repeating the experiment with the CPPU plus NAA interaction with an experimental design that allowed internal replication so that the interaction could be tested statistically would be very useful. Trials with different concentrations and application times of Benefit® on *A. deliciosa* may help with its commercial application. With this in mind it may also be useful to look further at the commercial preparation that is Benefit®, particularly in terms of a further refinement given previous results observed when fractions of Benefit® were applied to *A. chinensis* (D. J. Woolley, personal communication). It would also be interesting and informative to repeat the experiments done previously on *A. deliciosa* with regards to the effect of CPPU on cell size and cell division as the results to date have been contradictory and inconclusive. It would also be advantageous to conduct the same experiments on *A. chinensis* fruit and on fruit from both cultivars treated with Benefit® and both CPPU and Benefit®

as this may help uncover how an increase in fruit size is achieved. The aforementioned experiments should assist in the understanding of the mechanisms of action of CPPU and Benefit®, and may also yield results which explain why CPPU appears to have little effect on percentage dry matter in both *A. chinensis* and *A. deliciosa* fruit while Benefit® appears to significantly decrease percentage dry matter in *A. chinensis* fruit.

The methodology described in Chapter Three, while it has come a long way and did yield useful results, requires further refinement to ensure that less of the internal standard is lost. Also the gradient on the HPLC needs adjusting to ensure that all of the putative IAA peaks are separated from the other compounds and they reach baseline so they can be accurately quantified. It also needs to be confirmed by mass spectrometry that the putative IAA peak does in fact contain solely IAA. Following these improvements, continuation of the quantification of IAA in fruit treated with CPPU and untreated control fruit should produce valuable results and these results should further the understanding of the mechanism(s) behind the observed increases in fresh weight. Identification and quantification of endogenous cytokinins will also greatly improve understanding of the mechanism(s) as it is likely to be an interaction between hormones as opposed to a single one or even a single group of hormones that is inducing the changes in fruit growth. Also the identification of the unknown cytokinin observed by Woolley and Currie (2006), and an investigation of aromatic cytokinins, could help in this. Repeating these experiments on *A. deliciosa* fruit and fruit of both *A. chinensis* and *A. deliciosa* treated with Benefit® could also uncover

mechanism(s) behind the different reactions of both *A. chinensis* and *A. deliciosa* when treated with the same compounds and the mechanism(s) behind the increase in fresh weight of *A. chinensis* induced by Benefit®.

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6. Appendices

The following is an example of the code used in the statistical analysis program SAS:

```
proc glm data=thisrun;
title 'experiment 4 fresh weight';
class treatment block rep;
model fwt = treatment block rep /ss3;
means treatment /lsd duncan;
run;
```

This was used for the analysis of all of the parameters tested in experiments one through six, that is fresh weight, dry weight, and the percentage of IP and OP. The GLM (General Linear Model) is a mathematical formula that relates one factor to another and is used as a basis for both the t-test and analysis of variance (ANOVA) (Trochim, 2006). These experiments were tested with both the t-test and the two way ANOVA without replication. The fresh weight results from experiments two and four were also tested with the two way ANOVA with replication test. This was used to test the interaction between treatments, it could not be used on the other experiments as they had no internal replication.

6.1. Statistical analysis of final harvest data from experiment one, the growth response of A. chinensis to CPPU over time

6.1.1. Fresh weight

Class Level Information

Class	Levels	Values
treatment	2	1 = Control 2 = CPPU
block	4	1 2 3 4

Number of Observations Read 196
Number of Observations Used 196

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	191
Error Mean Square	462.3125
Critical Value of t	1.97246
Least Significant Difference	6.0844
Harmonic Mean of Cell Sizes	97.17347

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	134.009	107	2
B	87.026	89	1

Duncan's Multiple Range Test for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	191
Error Mean Square	462.3125
Harmonic Mean of Cell Sizes	97.17347

Number of Means	2
Critical Range	6.084

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	134.009	107	2
B	87.026	89	1

6.1.2. Percentage dry matter

Class Level Information

Class	Levels	Values
treatment	2	1 = Control 2 = CPPU
block	4	1 2 3 4

Number of Observations Read	78
Number of Observations Used	78

The GLM Procedure

Dependent Variable: dwt dwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	23.4992905	5.8748226	0.64	0.6387
Error	73	674.6646775	9.2419819		
Corrected Total	77	698.1639680			

R-Square	Coeff Var	Root MSE	dwt Mean
0.033659	15.34222	3.040063	19.81501

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	0.10792534	0.10792534	0.01	0.9142
block	3	23.35294448	7.78431483	0.84	0.4751

t Tests (LSD) for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	73
Error Mean Square	9.241982
Critical Value of t	1.99300
Least Significant Difference	1.3725
Harmonic Mean of Cell Sizes	38.97436

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	19.8595	38	1
A	19.7728	40	2

Duncan's Multiple Range Test for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	73
Error Mean Square	9.241982
Harmonic Mean of Cell Sizes	38.97436

Number of Means	2
Critical Range	1.373

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	19.8595	38	1
A	19.7728	40	2

6.1.3. Percentage inner pericarp

Class Level Information

Class	Levels	Values
treatment	2	1 = Control 2 = CPPU
block	4	1 2 3 4

Number of Observations Read	78
Number of Observations Used	78

The GLM Procedure

Dependent Variable: inner pericarp inner pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	291.6290531	72.9072633	23.04	<.0001
Error	73	231.0232649	3.1647023		
Corrected Total	77	522.6523179			

R-Square	Coeff Var	Root MSE	inner pericarp Mean
0.557979	8.696677	1.778961	20.45564

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	175.7574846	175.7574846	55.54	<.0001
block	3	126.9706094	42.3235365	13.37	<.0001

t Tests (LSD) for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	73
Error Mean Square	3.164702
Critical Value of t	1.99300
Least Significant Difference	0.8032
Harmonic Mean of Cell Sizes	38.97436

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	21.9463	38	1
B	19.0395	40	2

Duncan's Multiple Range Test for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	73
Error Mean Square	3.164702
Harmonic Mean of Cell Sizes	38.97436

Number of Means	2
Critical Range	.8032

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	21.9463	38	1
B	19.0395	40	2

6.1.4. Percentage of outer pericarp

Class Level Information

Class	Levels	Values
treatment	2	1 = control, 2 = CPPU
block	4	1 2 3 4

Number of Observations Read	78
Number of Observations Used	78

The GLM Procedure

Dependent Variable: outer pericarp   outer pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	340.5549577	85.1387394	27.02	<.0001
Error	73	230.0053164	3.1507578		
Corrected Total	77	570.5602740			

R-Square	Coeff Var	Root MSE	outer pericarp Mean
0.596878	2.248621	1.775037	78.93892

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	210.1107638	210.1107638	66.69	<.0001
block	3	143.6856815	47.8952272	15.20	<.0001

t Tests (LSD) for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	73
Error Mean Square	3.150758
Critical Value of t	1.99300
Least Significant Difference	0.8014
Harmonic Mean of Cell Sizes	38.97436

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	80.4874	40	2
B	77.3089	38	1

Duncan's Multiple Range Test for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	73
Error Mean Square	3.150758
Harmonic Mean of Cell Sizes	38.97436

Number of Means	2
Critical Range	.8014

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	80.4874	40	2
B	77.3089	38	1

6.2. *Statistical analysis of experiment two, the interaction between Benefit® and CPPU on A. chinensis*

6.2.1. Fresh weight

Class Level Information

Class	Levels	Values
Treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
Block	4	1 2 3 4
Rep	2	1 2

Number of Observations Read 1047
Number of Observations Used 1047

The GLM Procedure

Dependent Variable: fwt fwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	527096.555	75299.508	142.78	<.0001
Error	1039	547961.185	527.393		
Corrected Total	1046	1075057.740			

R-Square	Coeff Var	Root MSE	fwt Mean
0.490296	18.98200	22.96504	120.9832

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	482226.6664	160742.2221	304.79	<.0001
Block	3	28281.4546	9427.1515	17.88	<.0001
Rep	1	38716.3045	38716.3045	73.41	<.0001

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	1039
Error Mean Square	527.3929
Critical Value of t	1.96225
Least Significant Difference	3.9421
Harmonic Mean of Cell Sizes	261.346

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	142.393	245	4
B	137.517	272	3

C	116.034	266	2
D	89.067	264	1

Duncan's Multiple Range Test for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	1039
Error Mean Square	527.3929
Harmonic Mean of Cell Sizes	261.346

NOTE: Cell sizes are not equal.

Number of Means	2	3	4
Critical Range	3.942	4.150	4.290

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	142.393	245	4
B	137.517	272	3
C	116.034	266	2
D	89.067	264	1

An Anova-two factor with replication analysis in Excel 2007 was used to analyse the interaction between CPPU and Benefit®.

Block		Control	CPPU	Benefit®	CPPU + Benefit®
	1	98.01	137.013	101.448	154.168
		89.615	109.255	115.746	127.013
	2	84.54	132.07	122.11	163.325
		83.11	111.11	107.36	169.73
	3	83.16	157.97	108.45	113.37
		78.88	128.71	116.13	115.93
	4	104.413	160.28	121.16	158.25
		95.44	136.9	115.04	153.81

Anova: Two-Factor With Replication

SUMMARY	Control	CPPU	Benefit®	CPPU + Benefit®	Total
1					
Count	2	2	2	2	8
Sum	187.625	246.268	217.194	281.181	932.268
Average	93.8125	123.134	108.597	140.5905	116.5335
Variance	35.23801	385.2533	102.2164	368.697	470.64
2					
Count	2	2	2	2	8
Sum	167.65	243.18	229.47	333.055	973.355
Average	83.825	121.59	114.735	166.5275	121.6694
Variance	1.02245	219.6608	108.7812	20.51201	1047.865
3					

Count	2	2	2	2	8
Sum	162.04	286.68	224.58	229.3	902.6
Average	81.02	143.34	112.29	114.65	112.825
Variance	9.1592	428.0738	29.4912	3.2768	623.2402
4					
Count	2	2	2	2	8
Sum	199.853	297.18	236.2	312.06	1045.293
Average	99.9265	148.59	118.1	156.03	130.6616
Variance	40.25736	273.3122	18.7272	9.8568	639.5715
Total					
Count	8	8	8	8	
Sum	717.168	1073.308	907.444	1155.596	
Average	89.646	134.1635	113.4305	144.4495	
Variance	78.33681	350.0642	50.79355	493.0341	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	1433.926	3	477.9752	3.724115	0.033251	3.238872
Columns	14097.54	3	4699.181	36.61338	2.15E-07	3.238872
Interaction	3318.14	9	368.6822	2.872565	0.031589	2.537667
Within	2053.536	16	128.346			
Total	20903.14	31				

6.2.2. Percentage dry matter

Class Level Information

Class	Levels	Values
Treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
Block	4	1 2 3 4
Rep	2	1 2

Number of Observations Read 320
Number of Observations Used 300

The GLM Procedure

Dependent Variable: dwt dwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	195.569114	27.938445	5.12	<.0001
Error	292	1593.363146	5.456723		
Corrected Total	299	1788.932260			

R-Square	Coeff Var	Root MSE	dwt Mean
0.109322	14.12138	2.335963	16.54203

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	168.8011043	56.2670348	10.31	<.0001

Block	3	17.3609259	5.7869753	1.06	0.3662
Rep	1	1.6710135	1.6710135	0.31	0.5804

t Tests (LSD) for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	292
Error Mean Square	5.456723
Critical Value of t	1.96812
Least Significant Difference	0.7524
Harmonic Mean of Cell Sizes	74.66667

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	17.4863	70	1
A	17.1064	80	3
B	15.9890	80	2
B	15.5849	70	4

Duncan's Multiple Range Test for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	292
Error Mean Square	5.456723
Harmonic Mean of Cell Sizes	74.66667

NOTE: Cell sizes are not equal.

Number of Means	2	3	4
Critical Range	.7524	.7921	.8186

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	17.4863	70	1
A	17.1064	80	3
B	15.9890	80	2
B	15.5849	70	4

6.2.3. Percentage inner pericarp

Class Level Information

Class	Levels	Values
Treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK

block	4	1 2 3 4
rep	2	1 2

Number of Observations Read	321
Number of Observations Used	321

The GLM Procedure

Dependent Variable: inner pericarp inner pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	785.477105	112.211015	23.42	<.0001
Error	313	1499.388325	4.790378		
Corrected Total	320	2284.865431			

R-Square	Coeff Var	Root MSE	inner pericarp Mean
0.343774	10.58708	2.188693	20.67324

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	501.5965571	167.1988524	34.90	<.0001
block	3	267.7523617	89.2507872	18.63	<.0001
rep	1	16.5236879	16.5236879	3.45	0.0642

t Tests (LSD) for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	313
Error Mean Square	4.790378
Critical Value of t	1.96757
Least Significant Difference	0.6799
Harmonic Mean of Cell Sizes	80.24768

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	22.6814	81	1
B	20.6934	80	2
C	19.8405	80	3
C	19.4526	80	4

Duncan's Multiple Range Test for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	313
Error Mean Square	4.790378
Harmonic Mean of Cell Sizes	80.24768

Number of Means	2	3	4
Critical Range	.6799	.7157	.7397

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	22.6814	81	1
B	20.6934	80	2
C	19.8405	80	3
C	19.4526	80	4

6.2.4. Percentage of outer pericarp

Class Level Information

Class	Levels	Values
Treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
block	4	1 2 3 4
rep	2	1 2

Number of Observations Read 321
Number of Observations Used 320

The GLM Procedure

Dependent Variable: outer pericarp outer pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	816.968052	116.709722	26.96	<.0001
Error	312	1350.664367	4.329052		
Corrected Total	319	2167.632419			

R-Square	Coeff Var	Root MSE	outer pericarp Mean
0.376894	2.641356	2.080638	78.77156

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	563.8134506	187.9378169	43.41	<.0001
block	3	236.5459143	78.8486381	18.21	<.0001
rep	1	14.2279278	14.2279278	3.29	0.0708

t Tests (LSD) for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	312
Error Mean Square	4.329052
Critical Value of t	1.96760
Least Significant Difference	0.6473
Harmonic Mean of Cell Sizes	79.99375

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treatment
A	80.0684	80	4
A	79.7141	79	3
B	78.6831	80	2
C	76.6589	81	1

Duncan's Multiple Range Test for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	312
Error Mean Square	4.329052
Harmonic Mean of Cell Sizes	79.99375

NOTE: Cell sizes are not equal.

Number of Means	2	3	4
Critical Range	.6473	.6815	.7043

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	80.0684	80	4
A	79.7141	79	3
B	78.6831	80	2
C	76.6589	81	1

6.3. Statistical analysis of final harvest data from experiment three, the growth response of A. deliciosa fruit to CPPU over time

6.3.1. Fresh weight final harvest

Class Level Information

Class	Levels	Values
treatment	2	1 = control, 2 = CPPU
block	4	1 2 3 4

Number of Observations Read	122
Number of Observations Used	122

The GLM Procedure

Dependent Variable: fwt fwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	65453.2731	16363.3183	32.08	<.0001
Error	117	59683.8602	510.1185		
Corrected Total	121	125137.1333			

R-Square	Coeff Var	Root MSE	fwt Mean
0.523052	19.75824	22.58580	114.3108

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	27960.66996	27960.66996	54.81	<.0001
block	3	35688.92438	11896.30813	23.32	<.0001

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	117
Error Mean Square	510.1185
Critical Value of t	1.98045
Least Significant Difference	8.1267
Harmonic Mean of Cell Sizes	60.59016

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	128.698	66	2
B	97.354	56	1

Duncan's Multiple Range Test for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	117
Error Mean Square	510.1185
Harmonic Mean of Cell Sizes	60.59016

Number of Means	2
Critical Range	8.127

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	128.698	66	2
B	97.354	56	1

6.3.2. Percentage dry matter

Class Level Information

Class	Levels	Values
treatment	2	1 = control, 2 = CPPU
block	4	1 2 3 4

Number of Observations Read 40
Number of Observations Used 40

The GLM Procedure

Dependent Variable: dwt dwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	18.31971605	4.57992901	3.89	0.0102
Error	35	41.17586145	1.17645318		
Corrected Total	39	59.49557750			

R-Square	Coeff Var	Root MSE	dwt Mean
0.307917	7.448585	1.084644	14.56175

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	0.44682605	0.44682605	0.38	0.5417
block	3	17.85770747	5.95256916	5.06	0.0051

t Tests (LSD) for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	35
Error Mean Square	1.176453
Critical Value of t	2.03011
Least Significant Difference	0.6972
Harmonic Mean of Cell Sizes	19.95

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	14.6747	19	1
A	14.4595	21	2

Duncan's Multiple Range Test for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	35
Error Mean Square	1.176453
Harmonic Mean of Cell Sizes	19.95

Number of Means	2
Critical Range	.6972

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	14.6747	19	1
A	14.4595	21	2

6.3.3. Percentage inner pericarp

Class Level Information

Class	Levels	Values
treatment	2	1 = control, 2 = CPPU
block	4	1 2 3 4

Number of Observations Read	40
Number of Observations Used	40

The GLM Procedure
Dependent Variable: inner pericarp inner pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	253.9711500	63.4927875	6.83	0.0004
Error	35	325.4348275	9.2981379		
Corrected Total	39	579.4059775			

R-Square	Coeff Var	Root MSE	inner pericarp Mean
0.438330	9.230258	3.049285	33.03575

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	4.9632025	4.9632025	0.53	0.4699
block	3	249.0079475	83.0026492	8.93	0.0002

t Tests (LSD) for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	35
Error Mean Square	9.298138
Critical Value of t	2.03011
Least Significant Difference	1.9576

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	33.3880	20	1
A	32.6835	20	2

Duncan's Multiple Range Test for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 35
Error Mean Square 9.298138
Number of Means 2
Critical Range 1.958

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	33.3880	20	1
A	32.6835	20	2

6.3.4. Percentage outer pericarp

Class Level Information

Class	Levels	Values
treatment	2	1 = control, 2 = CPPU
block	4	1 2 3 4

Number of Observations Read 40
Number of Observations Used 40

The GLM Procedure

Dependent Variable: outer pericarp outer pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	326.7409100	81.6852275	8.90	<.0001
Error	35	321.0758275	9.1735951		
Corrected Total	39	647.8167375			

R-Square	Coeff Var	Root MSE	outer pericarp Mean
0.504372	4.589343	3.028794	65.99625

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	1	11.9574225	11.9574225	1.30	0.2613
block	3	314.7834875	104.9278292	11.44	<.000

t Tests (LSD) for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha 0.05
Error Degrees of Freedom 35
Error Mean Square 9.173595
Critical Value of t 2.03011
Least Significant Difference 1.9444

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	66.5430	20	2
A	65.4495	20	1

Duncan's Multiple Range Test for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	35
Error Mean Square	9.173595
Number of Means	2
Critical Range	1.944

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	66.5430	20	2
A	65.4495	20	1

6.4. Statistical analysis of experiment four, the interaction between Benefit® and CPPU on A. deliciosa fruit

6.4.1. Fresh weight

Class Level Information

Class	Levels	Values
treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
block	4	1 2 3 4
rep	2	1 2

Number of Observations Read	372
Number of Observations Used	371

The GLM Procedure

Dependent Variable: fwt fwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	128655.2194	18379.3171	49.19	<.0001
Error	363	135621.1787	373.6121		
Corrected Total	370	264276.3981			

R-Square	Coeff Var	Root MSE	fwt Mean
0.486821	15.66234	19.32905	123.4110

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	3	72818.03146	24272.67715	64.97	<.0001
block	3	57110.62141	19036.87380	50.95	<.0001
rep	1	44.44715	44.44715	0.12	0.7304

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	363
Error Mean Square	373.6121
Critical Value of t	1.96652
Least Significant Difference	5.621
Harmonic Mean of Cell Sizes	91.45644

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	137.761	94	3
A			
A	134.830	105	4
B	110.295	96	2
B			
B	106.454	76	1

Duncan's Multiple Range Test for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	363
Error Mean Square	373.6121
Harmonic Mean of Cell Sizes	91.45644

Number of Means	2	3	4
Critical Range	5.621	5.918	6.116

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	137.761	94	3
A			
A	134.830	105	4
B	110.295	96	2
B			
B	106.454	76	1

An Anova-two factor with replication analysis in Excel 2007 was used to analyse the interaction between CPPU and Benefit®.

Block	Control	Benefit®	CPPU	CPPU + Benefit®
1	94.52	101.24	129.97	117.4
	107.1	91.41	123.95	103.53
2	112.08	103.55	142.12	176.65
	100.18		146.04	144.69
3	111.25	137.16	147.99	146.81
	151.51	144.16	137.22	183.74

4	99.27	106.81	164.26	135.96
	105.13	108.9	131.32	140.42

Anova: Two-Factor With Replication

SUMMARY	Control	Benefit®	CPPU	CPPU + Benefit®	Total
1					
Count	2	2	2	2	8
Sum	201.62	192.65	253.92	220.93	869.12
Average	100.81	96.325	126.96	110.465	108.64
Variance	79.1282	48.31445	18.1202	96.18845	192.2277
2					
Count	2	2	2	2	8
Sum	212.26	207.1	288.16	321.34	1028.86
Average	106.13	103.55	144.08	160.67	128.6075
Variance	70.805	0	7.6832	510.7208	770.0351
3					
Count	2	2	2	2	8
Sum	262.76	281.32	285.21	330.55	1159.84
Average	131.38	140.66	142.605	165.275	144.98
Variance	810.4338	24.5	57.99645	681.9124	402.449
4					
Count	2	2	2	2	8
Sum	204.4	215.71	295.58	276.38	992.07
Average	102.2	107.855	147.79	138.19	124.0088
Variance	17.1698	2.18405	542.5218	9.9458	511.1805
Total					
Count	8	8	8	8	
Sum	881.04	896.78	1122.87	1149.2	
Average	110.13	112.0975	140.3588	143.65	
Variance	316.0223	340.8989	161.944	725.0745	

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	5368.991	3	1789.664	9.616599	0.000724	3.238872
Columns	7692.659	3	2564.22	13.77861	0.000106	3.238872
Interaction	2460.962	9	273.4402	1.469307	0.240421	2.537667
Within	2977.624	16	186.1015			
Total	18500.24	31				

6.4.2. Percentage dry matter

The GLM Procedure

Class Level Information

Class	Levels	Values
treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
block	4	1 2 3 4
rep	2	1 2

Number of Observations Read	176
Number of Observations Used	141

The GLM Procedure

Dependent Variable: dwt dwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	83.8018659	11.9716951	2.48	0.0199
Error	133	641.0887142	4.8202159		
Corrected Total	140	724.8905801			

R-Square	Coeff Var	Root MSE	dwt Mean
0.115606	14.27279	2.195499	15.38241

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	3	20.86053108	6.95351036	1.44	0.2333
block	3	45.05403065	15.01801022	3.12	0.0284
rep	1	18.84192623	18.84192623	3.91	0.0501

t Tests (LSD) for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	133
Error Mean Square	4.820216
Critical Value of t	1.97796
Least Significant Difference	1.0382
Harmonic Mean of Cell Sizes	34.99519

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	15.7291	34	2
A	15.6497	38	1
A	15.3497	38	4
A	14.7145	31	3

Duncan's Multiple Range Test for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	133
Error Mean Square	4.820216
Harmonic Mean of Cell Sizes	34.99519

Number of Means	2	3	4
Critical Range	1.038	1.093	1.129

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treatment
A	15.7291	34	2
A	15.6497	38	1
A	15.3497	38	4
A	14.7145	31	3

6.4.3. Percentage inner pericarp

Class Level Information

Class	Levels	Values
treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
block	4	1 2 3 4
rep	2	1 2

Number of Observations Read 162
Number of Observations Used 152

The GLM Procedure
Dependent Variable: inner pericarp inner pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	379.606119	54.229446	4.71	<.0001
Error	144	1657.358727	11.509436		
Corrected Total	151	2036.964847			

R-Square	Coeff Var	Root MSE	inner pericarp Mean
0.186359	10.68962	3.392556	31.73691

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	3	151.0414153	50.3471384	4.37	0.0056
block	3	211.9986536	70.6662179	6.14	0.0006
rep	1	45.0408948	45.0408948	3.91	0.0498

t Tests (LSD) for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	144
Error Mean Square	11.50944
Critical Value of t	1.97658
Least Significant Difference	1.5437
Harmonic Mean of Cell Sizes	37.73618

Means with the same letter are not significantly different.

t Grouping	Mean	N	treatment
A	32.8621	34	2
B A	32.4902	40	1

Duncan Grouping		Mean	N	treatment
B	C	31.1514	36	3
	C	30.6105	42	4

Duncan's Multiple Range Test for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	144
Error Mean Square	11.50944
Harmonic Mean of Cell Sizes	37.73618

Number of Means	2	3	4
Critical Range	1.544	1.625	1.679

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	treatment
	A	32.8621	34	2
B	A	32.4902	40	1
B	C	31.1514	36	3
	C	30.6105	42	4

6.4.4. Percentage of outer pericarp

Class Level Information

Class	Levels	Values
treatment	4	1 = control, 2 = BK, 3 = CPPU, 4 = CPPU + BK
block	4	1 2 3 4
rep	2	1 2

Number of Observations Read	164
Number of Observations Used	156

The GLM Procedure

Dependent Variable: outer pericarp outer pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	495.962259	70.851751	4.21	0.0003
Error	148	2489.290262	16.819529		
Corrected Total	155	2985.252521			

R-Square	Coeff Var	Root MSE	outer pericarp Mean
0.166137	6.038859	4.101162	67.91287

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	3	119.4420347	39.8140116	2.37	0.0732
block	3	302.0428224	100.6809408	5.99	0.0007
rep	1	70.9921536	70.9921536	4.22	0.0417

t Tests (LSD) for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	148
Error Mean Square	16.81953
Critical Value of t	1.97612
Least Significant Difference	1.8437
Harmonic Mean of Cell Sizes	38.6463

Means with the same letter are not significantly different.

t Grouping		Mean	N	treatment
	A	68.7756	41	3
	A	68.6652	42	4
B	A	67.2548	40	1
B		66.6811	33	2

Duncan's Multiple Range Test for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	148
Error Mean Square	16.81953
Harmonic Mean of Cell Sizes	38.6463

Number of Means	2	3	4
Critical Range	1.844	1.941	2.005

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	treatment
	A	68.7756	41	3
	A	68.6652	42	4
B	A	67.2548	40	1
B		66.6811	33	2

6.5. *Statistical analysis of experiment five, the effect of concentration and time of application of 3,5,6-TPA on growth of A. deliciosa fruit*

6.5.1. Fresh weight

Class Level Information

Class	Levels	Values
Treatment	7	1 = control,
		2 = 3,5,6-TPA 0.1mgL^{-1} early,
		3 = 3,5,6-TPA 0.1mgL^{-1} late,
		4 = 3,5,6-TPA 1mgL^{-}early,

5 = 3,5,6-TPA 1mgL^{-1} late,
6 = 3,5,6-TPA 10mgL^{-1} early,
7= 3,5,6-TPA 10mgL^{-1} late

Block	6	1 2 3 4 5 6
Rep	2	1 2

Number of Observations Read	671
Number of Observations Used	671

The GLM Procedure

Dependent Variable: fwt fwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	19012.7499	1584.3958	4.85	<.0001
Error	658	215139.1353	326.9592		
Corrected Total	670	234151.8852			

R-Square	Coeff Var	Root MSE	fwt Mean
0.081198	17.14551	18.08201	105.4621

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	6	5929.12865	988.18811	3.02	0.0064
Block	5	10868.79503	2173.75901	6.65	<.0001
Rep	1	1458.25483	1458.25483	4.46	0.0351

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	658
Error Mean Square	326.9592
Critical Value of t	1.96358
Least Significant Difference	5.2228
Harmonic Mean of Cell Sizes	92.42934

Means with the same letter are not significantly different.

t Grouping		Mean	N	Treatment
	A	110.168	74	6
B	A	109.058	81	1
B	A	106.855	115	4
B	C	104.458	96	5
B	C	104.199	77	2
B	C	104.157	127	7
	C	101.102	101	3

Duncan's Multiple Range Test for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	658
Error Mean Square	326.9592
Harmonic Mean of Cell Sizes	92.42934

NOTE: Cell sizes are not equal.

Number of Means	2	3	4	5	6	7
Critical Range	5.223	5.499	5.683	5.820	5.926	6.013

Means with the same letter are not significantly different.

Duncan Grouping			Mean	N	Treatment
	A		110.168	74	6
B	A		109.058	81	1
B	A	C	106.855	115	4
B		C	104.458	96	5
B		C	104.199	77	2
B		C	104.157	127	7
		C	101.102	101	3

6.5.2. Percentage dry matter

Class Level Information

Class	Levels	Values
Treatment	7	1 = control, 2 = 3,5,6-TPA 0.1mgL^{-1} early, 3 = 3,5,6-TPA 0.1mgL^{-1} late, 4 = 3,5,6-TPA 1mgL^{-}early, 5 = 3,5,6-TPA 1mgL^{-1} late, 6 = 3,5,6-TPA 10mgL^{-1} early, 7= 3,5,6-TPA 10mgL^{-1} late
Block	6	1 2 3 4 5 6
Rep	2	1 2

Number of Observations Read 337
Number of Observations Used 328

The GLM Procedure

Dependent Variable: dwt dwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	12	251.3161231	20.9430103	13.23	<.0001
Error	315	498.6828693	1.5831202		
Corrected Total	327	749.9989924			

R-Square	Coeff Var	Root MSE	dwt Mean
0.335089	8.015501	1.258221	15.69735

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	6	26.17114614	4.36185769	2.76	0.0127
Block	5	57.95027924	11.59005585	7.32	<.0001
Rep	1	21.87115358	21.87115358	13.82	0.0002

t Tests (LSD) for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	315
Error Mean Square	1.58312
Critical Value of t	1.96752
Least Significant Difference	0.5235
Harmonic Mean of Cell Sizes	44.72964

Means with the same letter are not significantly different.

t Grouping		Mean	N	Treatment
	A	16.2168	68	7
B	A	15.8178	37	2
B	A	15.8105	37	4
B	A	15.8100	53	3
B		15.6298	40	6
B		15.4751	53	5
	C	14.8110	40	1

Duncan's Multiple Range Test for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	315
Error Mean Square	1.58312
Harmonic Mean of Cell Sizes	44.72964

Number of Means	2	3	4	5	6	7
Critical Range	.5235	.5511	.5695	.5831	.5938	.6024

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	16.2168	68	7
B	A	15.8178	37	2
B	A	15.8105	37	4
B	A	15.8100	53	3
B		15.6298	40	6
B		15.4751	53	5
	C	14.8110	40	1

6.6. Statistical analysis of experiment six, the effect of NAA at different application dates and the interaction between CPPU and NAA at multiple application dates on the growth of A. deliciosa fruit

6.6.1. Fresh weight

Class Level Information

Class	Levels	Values
Treatment	9	1 = control, 2 = NAA early, 3 = NAA late, 4 = CPPU early, 5 = CPPU late, 6 = CPPU early NAA late, 7 = CPPU late NAA early, 8 = CPPU + NAA early, 9 = CPPU + NAA late
Block	6	1 2 3 4 5 6

Number of Observations Read	595
Number of Observations Used	594

The GLM Procedure

Dependent Variable: fwt fwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	95632.1785	7356.3214	21.25	<.0001
Error	580	200812.6966	346.2288		
Corrected Total	593	296444.8751			

R-Square	Coeff Var	Root MSE	fwt Mean
0.322597	16.19704	18.60722	114.8804

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	8	46396.99300	5799.62413	16.75	<.0001
Block	5	35162.04435	7032.40887	20.31	<.0001

t Tests (LSD) for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	580
Error Mean Square	346.2288
Critical Value of t	1.96406
Least Significant Difference	6.5617
Harmonic Mean of Cell Sizes	62.04084

Means with the same letter are not significantly different.

t Grouping		Mean	N	Treatment
	A	139.249	48	6
	B	118.913	66	5
	B	118.688	87	3
	B	118.004	64	9
	B	116.801	56	7
C	B	115.268	88	8
C	D	109.927	56	4
	D	105.369	43	1
	E	98.341	86	2

Duncan's Multiple Range Test for fwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	580
Error Mean Square	346.2288
Harmonic Mean of Cell Sizes	62.04084

Number of Means	2	3	4	5	6	7	8	9
Critical Range	6.562	6.908	7.140	7.311	7.445	7.554	7.646	7.724

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	139.249	48	6
	B	118.913	66	5
	B	118.688	87	3
	B	118.004	64	9
C	B	116.801	56	7
C	B	115.268	88	8
C	D	109.927	56	4
	D	105.369	43	1
	E	98.341	86	2

6.6.2. Percentage dry matter

Class Level Information

Class	Levels	Values
Treatment	9	1 = control, 2 = NAA early, 3 = NAA late, 4 = CPPU early, 5 = CPPU late, 6 = CPPU early NAA late, 7 = CPPU late NAA early, 8 = CPPU + NAA early, 9 = CPPU + NAA late
Block	6	1 2 3 4 5 6

Number of Observations Read	244
Number of Observations Used	226

The GLM Procedure

Dependent Variable: dwt dwt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	730.503189	56.192553	5.88	<.0001
Error	212	2027.454434	9.563464		
Corrected Total	225	2757.957623			

R-Square	Coeff Var	Root MSE	dwt Mean
0.264871	18.50802	3.092485	16.70889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	8	137.7200937	17.2150117	1.80	0.0785
Block	5	539.8211267	107.9642253	11.29	<.0001

t Tests (LSD) for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	212
Error Mean Square	9.563464
Critical Value of t	1.97122
Least Significant Difference	1.8026
Harmonic Mean of Cell Sizes	22.87276

Means with the same letter are not significantly different.

t Grouping		Mean	N	Treatment
	A	18.7894	18	1
B	A	17.7255	29	7
B	A	17.5130	20	8
B	C	16.5863	27	3
B	C	16.3523	40	9
B	C	16.2193	29	6
B	C	16.2044	25	2
B	C	16.0940	25	5
	C	14.9200	13	4

Duncan's Multiple Range Test for dwt

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	212
Error Mean Square	9.563464
Harmonic Mean of Cell Sizes	22.87276

Number of Means	2	3	4	5	6	7	8	9
Critical Range	1.803	1.897	1.961	2.007	2.044	2.073	2.098	2.119

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	18.7894	18	1
B	A	17.7255	29	7
B	A	17.5130	20	8
B	C	16.5863	27	3

		Mean	N	Treatment
B	C	16.3523	40	9
B	C	16.2193	29	6
B	C	16.2044	25	2
B	C	16.0940	25	5
	C	14.9200	13	4

6.6.3. Percentage of inner pericarp

Class Level Information

Class	Levels	Values
Treatment	9	1 = control, 2 = NAA early, 3 = NAA late, 4 = CPPU early, 5 = CPPU late, 6 = CPPU early NAA late, 7 = CPPU late NAA early, 8 = CPPU + NAA early, 9 = CPPU + NAA late
Block	6	1 2 3 4 5 6

Number of Observations Read 250
Number of Observations Used 247

The GLM Procedure

Dependent Variable: inner pericarp inner pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	1241.570303	95.505408	6.26	<.0001
Error	233	3552.553843	15.247012		
Corrected Total	246	4794.124146			

R-Square	Coeff Var	Root MSE	inner pericarp Mean
0.258978	12.69614	3.904742	30.75534

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	8	236.3130204	29.5391275	1.94	0.0554
Block	5	957.1279081	191.4255816	12.55	<.0001

t Tests (LSD) for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	233
Error Mean Square	15.24701
Critical Value of t	1.97020
Least Significant Difference	2.0863
Harmonic Mean of Cell Sizes	27.19363

Means with the same letter are not significantly different.

t Grouping			Mean	N	Treatment
	A		32.406	31	8
B	A		31.944	23	4
B	A	C	31.212	25	3

Duncan Grouping	Mean	N	Treatment
B A C	31.184	30	5
B D A C	31.029	27	2
B D A C	30.514	27	6
B D C	30.248	29	9
D C	29.340	25	1
D	28.970	30	7

Duncan's Multiple Range Test for inner pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	233
Error Mean Square	15.24701
Harmonic Mean of Cell Sizes	27.19363

Number of Means	2	3	4	5	6	7	8	9
Critical Range	2.086	2.196	2.270	2.324	2.366	2.400	2.429	2.453

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	Treatment
A	32.406	31	8
A	31.944	23	4
B A	31.212	25	3
B A	31.184	30	5
B A	31.029	27	2
B A	30.514	27	6
B A	30.248	29	9
B	29.340	25	1
B	28.970	30	7

6.6.4. Percentage of outer pericarp

Class Level Information

Class	Levels	Values
Treatment	9	1 = control, 2 = NAA early, 3 = NAA late, 4 = CPPU early, 5 = CPPU late, 6 = CPPU early NAA late, 7 = CPPU late NAA early, 8 = CPPU + NAA early, 9 = CPPU + NAA late
Block	6	1 2 3 4 5 6

Number of Observations Read	251
Number of Observations Used	248

The GLM Procedure

Dependent Variable: outer pericarp outer pericarp

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	1328.008093	102.154469	5.54	<.0001
Error	234	4315.159613	18.440853		

Corrected Total	247	5643.167706

R-Square	Coeff Var	Root MSE	outer pericarp Mean
0.235330	6.243177	4.294281	68.78359

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	8	248.682927	31.085366	1.69	0.1026
Block	5	1038.829030	207.765806	11.27	<.0001

t Tests (LSD) for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	234
Error Mean Square	18.44085
Critical Value of t	1.97015
Least Significant Difference	2.2898
Harmonic Mean of Cell Sizes	27.30275

Means with the same letter are not significantly different.

t Grouping			Mean	N	Treatment
	A		70.213	27	2
	A		70.209	30	7
B	A		69.461	25	1
B	A		69.333	28	6
B	A	C	68.793	29	9
B	A	C	68.422	30	5
B	A	C	68.051	25	3
B		C	67.470	23	4
		C	67.023	31	8

Duncan's Multiple Range Test for outer pericarp

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	234
Error Mean Square	18.44085
Harmonic Mean of Cell Sizes	27.30275

Number of Means	2	3	4	5	6	7	8	9
Critical Range	2.290	2.410	2.491	2.550	2.597	2.634	2.666	2.692

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	Treatment
	A	70.213	27	2
	A	70.209	30	7
B	A	69.461	25	1
B	A	69.333	28	6
B	A	68.793	29	9
B	A	68.422	30	5
B	A	68.051	25	3
B		67.470	23	4
B		67.023	31	8

6.7. *Radioactivity calculations*

2.5 g ground kiwifruit + 30 ml extraction solution + 100 µl ^{3}H-IAA (55,500 dpm)

Expected DPM

55,500 dpm / 30100 µl	(+ 30 ml extraction solution
55,500 dpm / 60100 µl	(+ 5 ml methanol
55,500 dpm / 65100 µl	(dried down to 10 ml
55,500 dpm / 10,000 µl	(pushed through Oasis® column, washed off in 5 ml
55,500 dpm / 5000 µl	(dried down and re-suspended in 5 ml
55,500 dpm / 5000 µl	(pushed through Oasis® column, eluted in 5 ml
55,500 dpm / 5000 µl	(dried down and re-suspended in 1 ml
55,500 dpm / 1000 µl	(20 µl injection into HPLC
1110 dpm / 20 µl	(collected over 2 mins at 0.75 ml / min flow rate
1110 dpm / 1500 µl	