Fruit set and fruit size studies on ‘Forelle’ and ‘Abate Fetel’ pear (*Pyrus communis* L.)

by

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DECLARATION

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SUMMARY

Maintaining constant high yields in „Abate Fetel' and „Forelle' orchards in South Africa is challenging. Improving productivity in these orchards could be achieved by increasing fruit set and fruit size. Fruit size is an important marketing and quality parameter and has a significant effect on the economic value of fruit. Various protocols to improve fruit set are used by South African producers but these are not well researched. We therefore evaluated different combinations of plant growth regulators including gibberellic acid (GA_3), gibberellins 4+7 (GA_{4+7}), GA_{4+7} combined with 6-benzyladenine (6-BA), aminoethoxyvinylglycine (AVG) and prohexadione-calcium (P-Ca) in combination with trunk girdling during flowering on „Forelle' and „Abate Fetel' to determine the best fruit set strategy. All applied growth regulators improved fruit set relative to an untreated control over two consecutive seasons, but GA_3 and P-Ca reduced return bloom and AVG resulted in smaller fruit size relative to the other treatments.

The application of synthetic cytokinins are believed to enhance fruit size by stimulating and extending the cell division period in fruit when applied at the correct stage of fruit growth. In addition, combination of P-Ca with GA_{4+7} was used successfully on Japanese pear (*Pyrus pyrifolia* Nakai) and „Bing' sweet cherry to improve fruit size. This combination of GA_{4+7} and P-Ca was evaluated and combined with 6-BA treatments on European pear (*Pyrus communis* L.) cultivars, Forelle and Abate Fetel, to see if a similar effect on fruit size could be achieved under South African growing conditions. On both „Forelle' and „Abate Fetel' the combination of GA_{4+7} and P-Ca increased fruit size, but was more pronounced in „Abate Fetel'.

Growth regulators N-phenyl-N' -1,2,3-thiadiazol-5-ylurea (TDZ), N (2-chloro-4-pyridyl)-N' -phenylurea (CPPU), 6-BA and 2,4-dichlorophenoxyacetic acid (2,4-D) successfully increased fruit size in pear cultivars Coscia and Spadona in Israel. These

growth regulators were applied to „Forelle' and „Abate Fetel' to determine if a similar effect could be achieved. None of the synthetic cytokinins applied had a significant effect on increasing fruit size in these two cultivars over two consecutive seasons although 6-BA increased return bloom and 2,4-D application resulted in increased fruit set. The stage when the cell division period in „Forelle' and „Abate Fetel' ends was also determined as 34 and 45 days after full bloom respectively, which can be used in the future to better plan the timing of fruit size enhancement treatments.

Based on results from various fruit set and fruit size improvement trials, it can be recommended to use GA_{4+7} or AVG to increase fruit set on „Forelle' and „Abate Fetel', depending on the fruit set history of the orchard. Results from fruit size improvement trials were variable, and emphasises the fact that a balance between yield and fruit size must be determined for an orchard to achieve good fruit size and maximum return.

OPSOMMING

Die handhawing van konstante, hoë opbrengste in „Abate Fetel' en „Forelle' boorde in Suid-Afrika is „n uitdaging. Produktiwiteit in hierdie boorde kan verhoog word deur vrugset en vruggrootte te verbeter. Vruggrootte is „n belangrike bemarkings- en kwaliteitsparameter en het „n betekenisvolle effek op die ekonomiese waarde van vrugte. „n Verskeidenheid protokolle om vrugset te verbeter word deur Suid-Afrikaanse produsente gevolg, maar hierdie protokolle is nog nie goed nagevors nie. Verskillende kombinasies van plantgroeireguleerders insluitend gibberelliensuur (GA_3), gibberellien 4+7 (GA_{4+7}), GA_{4+7} in kombinasie met 6-bensieladenien (6-BA), aminoetoksievinielglisien (AVG) en prohexadioon-kalsium (P-Ca) in kombinasie met stamringelering is aan „Forelle' en „Abate Fetel' bome gedurende blomtyd toegedien om die beste vrugsetstrategie te bepaal. Alle plantgroeireguleerdes wat toegedien is het vrugset verbeter relatief tot „n onbehandelde kontrole oor twee opeenvolgende seisoene, maar GA_3 en P-Ca het die aantal blomme in die daaropvolgende seisoen verlaag en AVG het kleiner vruggrootte gelewer relatief tot alle ander behandelings.

Dit is wel bekend dat die toediening van sintetiese sitokiniene vruggrootte verbeter deur die stimulering en bevordering van seldeling in vrugte wanneer dit in die regte groeifase toegedien word. Die kombinasie van P-Ca en GA_{4+7} was suksesvol om vruggrootte te verbeter toe dit aan Japanese pere (*Pyrus pyrifolia* Nakai) en „Bing' kersies toegedien is. Hierdie kombinasie van GA_{4+7} en P-Ca is geëvalueer en gekombineer met 6-BA-behandelings op die Europese peer (*Pyrus communis* L.) kultivars, Forelle en Abate Fetel, om te bepaal of dieselfde effek op vruggrootte bereik kan word onder Suid-Afrikaanse groei kondisies. Op beide „Forelle' en „Abate Fetel' het die kombinasies van GA_{4+7} en P-Ca vruggrootte verbeter, maar dit was meer opmerklik in die geval van „Abate Fetel'.

Die groeireguleerders N-feniel-N' -1,2,3-thiadiazol-5-ylurea (TDZ), N (2-chloro-4-piridiel)-N' -fenielurea (CPPU), 6-BA en 2,4- dichloorfenoksieasynsuur (2,4-D) het vruggrootte verbeter in „Coscia' en „Spadona' pere in Israel**.** Hierdie plantgroeireguleerders is toegedien aan „Forelle' en „Abate Fetel' om vas te stel of dieselfde effek verkry kon word. Nie enige van die sintetiese sitokiniene wat toegedien is het „n betekenisvolle effek op die verbetering van vruggrootte in hierdie twee kultivars oor twee opeenvolgende seisoene getoon nie, alhoewel 6-BA die verbetering van blom in die daaropvolgende seisoen tot gevolg gehad en 2,4-D vrugset verbeter het. Die stadium waar seldeling in „Forelle' en „Abate Fetel' eindig is vasgestel as 34 en 45 dae na volblom, onderskeidelik, wat in die toekoms gebruik kan word om die beplanning en tydsberekening van vruggrootte behandelings te verbeter.

Na verskeie vrugset en vruggroote verbeterings proewe, kan aanbeveel word dat GA_{4+7} of AVG gebruik kan word om vrugset in „Forelle' en „Abate Fetel' te verbeter, afhangende van die vrugset geskiedenis van die boord. Resultate van vruggrootte verbeterings proewe het gevarieër en beklemtoon net weer die feit dat „n balans tussen opbrengs en vruggrootte bepaal moet word om optimale vruggrootte te handhaaf en so hoë winste te verseker.

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GENERAL INTRODUCTION

Currently maintaining constant high yields with optimal fruit size in „Forelle' and „Abate Fetel' orchards in South Africa is challenging. These cultivars are of the most profitable pear cultivars with a high percentage of plantings between 0 and 10 years old in South Africa (Hortgro tree census, 2011). Many orchards will still come into full production and therefore research to increase yields and fruit quality in these cultivars is of great importance.

Various protocols to improve fruit set are used by producers in the Elgin area, South Africa, which include plant growth regulators (PGR) such as gibberellic acid (GA_3), gibberellins 4+7 (GA_{4+7}), GA_{4+7} + 6-benzyladenine (6-BA), aminoethoxyvinylglycine (AVG) and prohexadione-calcium (P-Ca), without knowing whether these PGRs indeed improve set (Dr. J.J.B. Pretorius, personal communication). All these PGRs can potentially increase fruit set if applied at the correct phenological stage and rate (Lafer, 2008), but there are several other factors such as fruit size and return bloom which are also affected by PGRs. 6-BA is also widely applied in the industry to enhance fruit size on pears as various researchers have shown that synthetic cytokinins can extend the cell division period resulting in larger fruit, but research on the efficacy of 6-BA and other synthetic cytokinins on „Forelle' and „Abate Fetel' in South Africa is lacking.

In the literature review, various fruit set and fruit size strategies on different pear cultivars over the world are reviewed, with special emphasis on the use of PGRs, to determine potential options that can be evaluated under South African conditions. Other factors which determine high yields in pears were also reviewed, including the number and quality of flowers, the efficacy of pollination, the severity of natural or induced abscission of fruitlets and the degree and rate of cell division and expansion (Webster, 2002).

In South Africa, girdling is used in combination with GA application during bloom to improve fruit set in many pear orchards (Theron and Steyn, 2008). It is however not known whether girdling actually increases fruit set. Girdling was therefore combined with all PGR treatments to quantify its effect on fruit set. Fruit set trials were repeated on the same trees to determine treatment effects on the long term productivity of trees, as would be the case in practice. In fruit size trials, a combination of GA_{4+7} and P-Ca, as well as different synthetic cytokinins, were applied at different phenological stages of fruit growth to determine the effect on final fruit size. The idea of combining GA_{4+7} with P-Ca to improve fruit size stems from recent research on Japanese pear (Itai et al., 2009).

This thesis consists out of three chapters with the first: The evaluation of different GAs, in combination with 6-BA, AVG, P-Ca and girdling on fruit set and yield of „Forelle' and „Abate Fetel' pears, secondly: The efficacy of 6-BA, GA_{4+7} and P-Ca to increase fruit size in „Forelle' and „Abate Fetel' pear, and thirdly: Extending the cell division phase in „Forelle' and „Abate Fetel' pear with PGRs. All chapters have one common goal: to optimise yield and fruit quality for maximum return.

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LITERATURE REVIEW: Improving fruit set and size in pears (*Pyrus communis* L.)

1. Introduction

Optimal yield and good financial returns are dependent on high fruit set and optimal fruit size. Fruit size is an important quality parameter determining marketability of fruit and fruit prices (Webster, 2002). Currently maintaining constant high yields with optimal fruit size in „Abate Fetel' and „Forelle' orchards in South Africa is challenging. There are a number of factors that determines whether high yields are achieved in pears. These include the number and quality of flowers, the efficacy of cross-pollination, the severity of natural or induced abscission of fruitlets and the degree and rate of cell division and expansion, and therefore resultant fruit size of the persisting fruits (Webster, 2002). Many pear orchards display vigorous growth and consequently, low fruit set and biennial bearing (Lafer, 2008) which also have an effect on yield.

Different techniques are used to improve fruit set and size in pear orchards. Amongst others, plant growth regulators (PGRs), e.g. gibberellins (GAs) are applied to increase fruit set, but the outcome is not always positive because of smaller fruit size and a reduction in return bloom (Vanthournout et al., 2008; Deckers and Schoofs, 2002). An increase in fruit set can be observed when GAs are applied, but often this can also be partially lost again during June drop (Northern hemisphere) (Vercammen and Gomand, 2008).

The build-up in yield of „Abate Fetel' pear trees is slow, even though it is very precocious and develops flowers in abundance (Vilardell et al., 2008). Further plantings of this cultivar are limited because of the low initial fruit set and significant fruitlet drop by the end of May (Vilardell et al., 2008). „Abate Fetel' trees also show high vegetative vigour in spring

which creates significant competition with fruitlets which might be one of the reasons for low fruit set (Vilardell et al., 2008).

Fruit size is negatively correlated to crop load, and when average fruit weight is low it is most probably because of an increase in fruit set and yield (Lafer, 2008). It is therefore important to maintain a balance between fruit set and size to achieve optimal yield with good quality and the highest economic return the orchard can achieve year after year. To achieve the highest return from an orchard, a balance must therefore be determined between producing a high yield with many small to medium sized fruit or a lower yield with bigger sized fruit. By achieving this balance between fruit set and size one should be assured of correct tree vigour and tree health for constant yields year after year. In the following sections we explore the factors influencing fruit set and size in pears and how the use of PGRs and other cultural practices influence these.

2. Factors influencing fruit set in pears

Yields of pears are dependent upon the successful completion of a series of sequential processes: those associated with floral induction, flower differentiation, fruit set, fruitlet retention and growth (Webster, 2002). Floral buds must be initiated in sufficient numbers to facilitate the setting of enough fruit to produce a crop as large as possible, with fruit of adequate size and quality, to satisfy market requirements (Webster, 2002). A fact that is commonly overlooked is that conditions occurring prior to bloom or fruit set are just as important as those occurring after bloom or fruit set (Tukey, 1974).

Flower quality plays an important role in the percentage of fruit that will set (Webster, 2002). Poor quality flowers are either those that are incapable of setting fruit or those that can only set fruit if provided with the most favourable environmental conditions for pollination and

fertilization (Webster, 2002). One characteristic of poor flower quality is a short effective pollination period (EPP) (Williams, 1984). This is the difference between the longevity of the ovule and the time needed for pollen tube growth, which in other words are the time in days after anthesis during which the flowers remain capable of developing fertilized seed and setting fruit if pollinated with viable pollen (Williams, 1984). Flowers with EPPs of one day or less are considered of very "poor quality" (Webster, 2002). Fruit which do succeed in setting on "poor quality" flowers are more prone to subsequent fruitlet abscission and often develop into smaller than average sized or poorly shaped fruit (Webster, 2002). Vercammen and Gomand (2008) also found that high fruit set and a reasonable yield can still be obtained even if there are lower flower numbers. This leads to the conclusion that fewer flowers on a tree might still be of good quality and also result in a higher set percentage compared to trees with an abundance of flowers.

Climate during bloom plays an enormous role in determining flower quality and fruit set. Climatic conditions during flowering must be favourable for the activity of pollen vectors and for the subsequent germination and growth of the pollen tube once it is deposited on the stigma of the pear flower (Webster, 2002). Both pollen germination and the rate of pollen tube growth are highly dependent on the prevailing temperature (Petropoulou and Alston, 1998). Temperatures should be relative high (15°C to 25°C) with little or no wind and no rain to improve pollen vector activity, pollen germination and pollen tube growth (Webster, 2002). Choice of appropriate sites with favourable climatic conditions and the provision of adequate windbreaks can aid greatly in establishing optimum conditions for pollination and fertilization of pear flowers (Webster, 2002).

The nutritional status of the tree or of the reproductive buds themselves causes differences in flower quality (Webster, 2002). Williams (1984) proved this when he applied boron and urea sprays to trees in autumn, and improved the potential of the flowers formed in the

subsequent spring to set fruits. High levels of boron in floral organs such as the stigma and style may aid pollen germination and speed up pollen tube growth down the style and into the ovary, which might aid in fruit set when EPPs are short (Webster, 2002).

Important factors affecting the financial outcome of commercial fruit growing is the success of pollination and fertilization, which in turn are dependent on weather conditions, activity of pollinators, flowering overlap of the pollinizers and compatibility between cultivars (Kemp et al., 2008). There are three levels of compatibility between diploid cultivars. When two cultivars carry identical S-loci they will be incompatible with each other; if they share only one of their S-loci they will be semi-compatible; and if they differ in both S-loci they will be fully compatible, which leads to superior fruit set (Goldway et al., 2008). The majority of commercially grown European pear (*Pyrus communis* L.) cultivars are predominantly self-incompatible, requiring the transfer of pollen between different cultivars, where poor pollen transfer has been cited as a principal reason for poor fruit set (Webster, 2002). Because of self-incompatibility, pear orchards must contain at least two genetically compatible cultivars, which serve as pollinizers to each other (Goldway et al., 2008). However, in many cases the cultivars used are genetically semi-compatible, and semi-compatibility was shown to be correlated to low yield (Goldway et al., 2008). Semi-compatibility may also lead to reduced fruit quality and fruit set, because half of the pollen grains are rejected (Goldway et al., 1999). Stern et al. (2001) showed that increased honeybee visits between semi-compatible cultivars can increase the yield. It seems that, under sub-optimal conditions or in any case of potential pollen deficiency, full compatibility between adjacent cultivars is preferable (Goldway et al., 2008).

Rootstocks have an influence on flower quality because a dwarfing rootstock, possibly by altering assimilate partitioning in the tree, often improves the quality of flowers on pear trees and their ability to set fruitlets (Webster, 2002). The age of the tree also has an influence on flower

quality and on the number of flowers produced (Webster, 2002). Fewer flowers are formed and set more poorly during the first 4 to 6 years following planting, while flowers on young trees also exhibit a very short EPP (Webster, 2002). The reason for this is probably the greater competition and sink strength of strongly growing extension shoots on young trees and the reduced partitioning of assimilates and nutrients to the sites of floral initiation (De Pundert, 1980). Strong growing extension shoots in young trees are also associated with high levels of gibberellic acid (GA_3), which contributes to reducing floral initiation, which leads to fewer flowers formed on young trees than on more mature trees.

The position where reproductive buds develop on a pear tree has a significant effect on flower quality (Deckers and Daemen, 1998). Flowers formed in large clusters on the terminals of medium length extension shoots invariably have a higher set potential than flowers on spurs or axillaries (Deckers and Daemen, 1998). Pruning techniques can significantly influence the positions where reproductive buds are formed and hence on their intrinsic quality (Webster, 2002). It is essential to prune trees with the aim of producing as many strong terminal floral clusters for two years hence (Webster, 2002). Pruning severity and branch quality can also have an effect on fruit set as was shown by a 20% increase in fruit set of pears on short bearing units (28 cm) compared to long bearing units (56 cm) and a 70% increase in fruit set on thick bearing units (14 mm diameter) compared to thin bearing units (8 mm diameter) (Reynolds et al., 2005). Pruning „Packham's Triumph' at the intercalation between one- and two-year-old wood also increased fruit set, because of the negative effect shoots developing distal to fruitlets have on fruit set (Saunders, et al., 1991). Set is affected more negatively by new developing shoots distal to the young fruitlets, than shoot:fruit competition for limited metabolites (Reynolds et al., 2005). The better fruit set on short bearing units and thick bearing units may be due to increased

supply of xylem transported metabolites which increases sink strength of individual fruit (Reynolds et al., 2005).

Excessive vegetative growth in fruit trees can be controlled by trunk girdling, thus enhancing yields by increasing fruit set (Goren et al., 2004; Smit, et al., 2005). According to Goren et al. (2004) girdling entails a depression of hormone biosynthesis in roots which are associated with carbohydrate depletion and thus a reduction in root growth, which also leads to a decrease in shoot growth. When excessive vegetative growth are controlled by girdling, (Goren et al., 2004) less competition between fruitlets and shoots remains, thus increasing sink strength of fruitlets (Reynolds et al., 2005) which leads to better fruit set. Girdling entails the removal of a ring of bark around the full circumference of the tree trunk through the phloem, thus interrupting phloem transport (Goren et al., 2004). Raffo et al. (2011), using a 6 - 8 mm wide girdle, found no significant effect on yield or fruit size in „Bartlett' pear trees girdled 20 days after full bloom (d.a.f.b.). Although girdling can retard vegetative growth, the response to girding is still highly variable because of many other factors that could also play a role (Smit et al., 2005). Girdling performed between full bloom and three/four petal drop did not have an effect on fruit set on „Rosemarie', Forelle', „Packham's Triumph' and „Early Bon Chretien' for two consecutive seasons (Smit et al., 2005). Raffo et al. (2011) found that girdling could not replace practices such as pruning and thinning to ensure regular yield, although trials for more than two successive seasons might be more conclusive.

3. Fruit set and plant growth regulators (PGRs)

Improving fruit set in young pear trees by applying PGRs is a useful way to increase yield of young pear trees (Lafer, 2008). The characterization of fruit set and fruit drop has shown that the main factors influencing the final crop load manifest themselves during the first

three weeks following flowering (Silva and Herrero, 2008). This is probably why using PGRs during this crucial period might improve fruit set in some pear cultivars.

In fruit production one way to obtain high yields of high quality fruit is to reduce tree vigour, which is especially important in more vigorous cultivars (Asin et al., 2005). The best way to control the vegetative vigour of a fruit tree is to induce regularity in yield, which can be achieved by a treatment with GAs, but the results of these treatments are not consistent (Vanthournout et al., 2008). Applying PGRs such as GAs, aminoethoxyvinylglycine (AVG) and prohexadione-calcium (P-Ca) in intensive pear orchards is considered to be an important cultural practice to induce regularity of yield and to obtain good fruit quality (Lafer, 2008).

3.1 Prohexadione-calcium (P-Ca)

P-Ca is a plant bioregulator that is primarily used to inhibit excessive vegetative growth in fruit trees and reduces abortion of fruitlets, thereby increasing fruit set (Rademacher et al., 2006; Vilardell et al., 2008). P-Ca is an inhibitor of GA biosynthesis (Rademacher et al., 2006). Distinct dioxygenases involved in the GA biosynthesis pathway are blocked by P-Ca and as a result, less growth-active GAs which stimulates shoot growth, are formed (Rademacher et al., 2006).

P-Ca is very effective on apple trees, but much less effective on pear trees and can have a negative effect on return bloom (Deckers and Schoofs, 2004). Another difference between apples and pears is that a higher rate of P-Ca is needed on pear trees than for apple trees (Basak and Rademacher, 2000). The efficacy of P-Ca also varies according to several other factors including the cultivar, orchard, vigour and yield (Asin et al., 2005).

P-Ca caused a significant increase in fruit set on „Rosemarie', „Early Bon Chretien' (Meintjes et al., 2005; Smit et al., 2005) and „Forelle' pears (Smit et al., 2005). The application

of P-Ca at 250 mg.L^{-1} at full bloom significantly increased fruit set in two seasons on „Williams' pears (Lafer, 2008). Asin et al. (2005) found that the maximum seasonal rate of P-Ca to prevent a reduction in return bloom was 320 mg.L^{-1} and 480 mg.L^{-1} for „Conference' and „Blanquilla', respectively. Applications of 1000 mg.L^{-1} P-Ca at different times (full bloom and 10 d.a.f.b.) significantly increased yields in „Abate Fetel' pears for three consecutive years (Vilardell et al., 2008). Applications before flowering were not effective in increasing fruit set (Vilardell et al., 2008).

The mode of action of P-Ca is two-fold when applied post bloom. Firstly, it reduces the competition between fruitlets and strong vegetative shoot growth (Vilardell et al., 2008) and secondly, it also leads to reduced ethylene formation due to the structural similarities with ascorbic acid, the co-substrate of aminocyclopropanecarboxylic acid (ACC) oxidase which is involved in ethylene biosynthesis (Rademacher et al., 2006). P-Ca has a strong effect as a growth retardant in „Abate Fetel' pears (Vilardell et al., 2008), and inhibits the effect of the synthesis of ethylene, which is possibly responsible for the massive drop of „Comice' fruitlets in June (Northern hemisphere) (Lombard and Richardson, 1982).

The June drop process starts 2 to 4 weeks after full bloom (w.a.f.b.) when the fruit that will drop are already determined (Vercammen and Gomand, 2008). The number of fruit that will drop depends on the amount of stress the trees suffer during these 2 weeks (Vercammen and Gomand, 2008). When stress occurs, ethylene is produced and it is thought by many that this is the way the signal for fruit drop is transmitted within the tree (Bangerth, 1978; Vercammen and Gomand, 2008). It is very important that the timing of the P-Ca application after full bloom is correct and that the application is applied before stress occurs (Vercammen and Gomand, 2008). The residual properties of P-Ca are not sufficient to span the crucial 14 days of stress with only one application 2 w.a.f.b., for this reason many growers apply a second application of P-Ca, but

when 240 $mg.L^{-1}$ P-Ca was applied twice, return bloom in „Conference' pear was reduced significantly (Vercammen and Gomand, 2008). The result of two P-Ca applications was counterbalanced the next season, because although there were fewer reproductive buds, fruit set was better and June drop was also reduced (Vercammen and Gomand, 2008). Lowered ethylene levels, together with the elevated availability of assimilates no longer needed for shoot growth, explain how P-Ca can increase fruit set (Rademacher et al., 2006). According to Vercammen and Gomand (2008), the treatment of pear trees with GAs can be combined with P-Ca, however this combination is not advisable in the case of trees with a sufficient number of reproductive buds, because this can lead to excessive fruit set and fruit that are too small at harvest.

3.2 Gibberellin (GA)

GAs are synthesized in seeds, young leaves and roots (Goodwin et al., 1978) and function at cellular level by elongating and expanding cells as well as stem elongation at the whole plant level (Brock and Kaufman, 1991). Fruit set improvement on young pear trees with GA treatments can be considered as an important tool to improve early productivity (Deckers and Schoofs, 2002). Different gibberellins like GA_3 (gibberellic acid), GA_{4+7} and mixtures of GA_3 + GA_{4+7} can be applied to improve fruit set (Lafer, 2008). GA_3 is the most widely used PGR in the chemical induction of parthenocarpic fruit set, however different responses were seen in different pear cultivars (Gyuro et al., 1978). The success of GA applications is also related to the tree physiology and climatic conditions during the time of application and up to 5 days later (Chitu et al., 2008).

Applications of GA_{4+7} together with very low concentrations of GA_3 improved yields, resulted in good fruit shape without deleterious effects on return bloom of „Conference' (Webster, 2002). Deckers and Schoofs (2002) found that 5 $mg.L^{-1}$ GA_3 had a stronger effect on

fruit set than 5 mg.L^{-1} GA_{4+7} in „Conference' pears, but for this fertile cultivar it is not always necessary or desirable to induce the highest fruit set. In most pear cultivars the fruit set effect following a GA_3 treatment was stronger than the effect with GA_{4+7} applied at the same rate and at the same time (Deckers and Schoofs, 2002). Return bloom is reduced more after the application of GA_3 in the previous season in most pear cultivars than with GA_{4+7} (Deckers and Schoofs, 2002). Early applications of GA_3 to „Conference' reduced seed number and increased fruit length, thereby reducing fruit quality (Vercammen and Gomand, 2008). The application of especially GA_3, which is effective in many pear cultivars, show little efficacy on „Abate Fetel' (Vilardell et al., 2008).

3.3 Aminoethoxyvinylglycine (AVG)

AVG is an ethylene biosynthesis inhibitor that increases fruit set if applied to pear trees at or soon after full bloom (Dussi et al., 2002). AVG is a potent inhibitor of ACC synthase thereby actively preventing the formation of ACC, the natural precursor of ethylene (McGlasson, 1985).

AVG applied towards the end of bloom on „Williams' pear trees significantly improved the yield compared to an untreated control (Lafer, 2008). Fruit set of „Packham's Triumph' trees sprayed with AVG 2 w.a.f.b. at two rates, 200 mg.L^{-1} and 400 mg.L^{-1}, and an untreated control were evaluated after "June drop" (Dussi et al., 2002). Fruit set was increased most by the highest rate, although the lower rate also increased fruit set relative to the control (Dussi et al., 2002). Smaller fruit diameters were found in treated fruit and the lateral and terminal shoot growth was reduced significantly by both treatments, but was more marked following the 400 mg.L^{-1} application (Dussi et al., 2002). Dussi et al. (2011) also found that when AVG was applied at full bloom, fruit set was not statistically increased above that of the control in „Abate Fetel' and Packhams' Triumph' pears, but that the best application time for these two cultivars was 2

w.a.f.b. Fruit set in „Abate Fetel' and „Packham's Triumph' pear also increased with higher AVG rates with the optimum of 300 mg.L^{-1} on „Abate Fetel' and 200 mg.L^{-1} on „Packham's Triumph' (Dussi et al., 2011). A rate of 150 mg.L^{-1} AVG showed the best balance between fruit set and fruit size in „Abate Fetel' pear (Dussi et al., 2011). Lombard and Richardson (1982) found a significant increase in fruit set following an application of AVG to seven-year-old „Comice' trees in the USA. However, applications at full bloom or up to 4 w.a.f.b., did cause some leaf injury and fruit russeting and when used at full bloom, also reduced final fruit size (Lombard and Richardson, 1982).

3.4 Combinations of gibberellin (GA), aminoethoxyvinylglycine (AVG), cytokinin and prohexadione-calcium (P-Ca)

GAs can be applied in combination with cytokinins in products such as Promalin™ (Valent BioSciences Corporation, USA) which contains GA_{4+7} and 6-benzyladenine (6-BA). The active components of Promalin™ (GA_{4+7} + 6-BA) operate as growth promoters at cellular level and improve the development of fruitlets immediately after flowering (Vilardell et al., 2008). Increased fruit set in trees treated with the combination of GA_{4+7} and 6-BA was found in different pear cultivars worldwide (Vilardell et al., 2008). Applications of GA_{4+7} + 6-BA at different phenological stages around bloom improved yield in „Abate Fetel' pears, but the efficacy varied depending on the location and on the year (Vilardell et al., 2008). The results indicated that even though the product has an effect on improving fruit set and yield, its efficacy is conditioned by weather conditions of a particular year (Vilardell et al., 2008).

The combination of GA_{4+7} + 6-BA and P-Ca applications improved yield in „Abate Fetel' pears significantly compared to GA_{4+7} + 6-BA applied alone (Vilardell et al., 2008). On „Abate Fetel' the most effective strategy to improve fruit set was the combination of a GA_{4+7} + 6-BA

application during bloom with a P-Ca spray 15 days after petal drop (Vilardell et al., 2008). With this strategy, a 40% increase in yield and a 55% increase in the number of fruit per tree were recorded (Vilardell et al., 2008). The interest in the combined application of both products is that GA_{4+7} + 6-BA should increase fruit set whereas P-Ca applied after bloom should reduce fruit drop as a consequence of reduced fruit-shoot competition during the months May and early June (Northern hemisphere) (Vilardell et al., 2008). A combination of Promalin™ (1 $ml.L^{-1}$) applied at full bloom followed by Retain™ (2 $g.L^{-1}$) 15 days after full bloom increased the total number of fruit on the tree and thus the yield on „Packham's Triumph' significantly (Rufato et al., 2011).

3.5 Auxins

The application of the synthetic auxins naphthlacetic acid (NAA) and naphthylacetamide (NAD) on „Abate Fetel' at flowering did not increase fruit set or yield significantly, as was also found in earlier studies with these auxins (Vilardell et al., 2008). The lowest fruit set was found on „Williams' trees treated with auxins NAD, NAA and naphthoxyacetic acid (NAO) compared to an untreated control, P-Ca, AVG and GA_3, but fruit size was improved, thus auxins had a thinning effect (Lafer, 2008). NAD reduced fruit set significantly and did not affect return bloom compared to an untreated control in „Early Bon Cretien' pear (Theron et al., 2011).

4. Fruit size

Assuming that larger fruit size of good quality is desirable to consumers and therefore important marketing parameters, treatments that may increase average fruit diameter and quality may have significant economic value (Flaishman et al., 2001). There are two commercial practices commonly applied to enlarge fruit (Stern, 2008); one is the indirect method of thinning

flowers or fruitlets to reduce competition between fruit for assimilates, resulting in larger fruit (Stern et al., 2003); the second method directly enhances fruit size by stimulating and extending cell division, e.g. application of synthetic cytokinins (Shargal et al., 2006). Chemical and manual thinning reduces total yield, but because of increased fruit size the remaining fruit should obtain higher prices. If fruit size can be enhanced by thinning, the economic value of the crop would most probably be greater when compared to a crop with smaller fruit and greater volume, but the optimal balance between crop load (total yield) and fruit size needs to be determined for every orchard every season.

Fruit size of pears is also dependant on the sink strength of the fruit (Reynolds et al., 2005). Sink strength is the product of two components: sink activity, which is a measure of the potential flux of assimilate accumulation, and sink size, which is a measure of a potential volume for biomass gain (Patrick, 1988). Increasing the leaf to fruit ratio by thinning and thus increasing the size of the source relative to the sink is offered as an explanation for an improved fruit size (Lakso, 1994). Excessive vegetative growth within the first 50 d.a.f.b. is also a very strong sink which coincides with the cell division stage resulting in smaller fruit (Costa et al., 2002; Dussi, 2011). Excessive vegetative growth can be controlled by pruning, the use of dwarfing rootstocks, girdling and the use of PGRs, resulting in better fruit size (Meintjes et al., 2005; Smit et al., 2005).

4.1 The role of cell division and cell enlargement in fruit size

There are two distinct stages in fruit growth, Stage I, the first 42 to 56 days of development which is the main cell division period and stage II, the cell enlargement period which is the remainder of the time until harvest (Bain, 1961). Final pear fruit size depends on the combined contributions of the number of cells present at fruit set, the number of subsequent

cell divisions, and on cell expansion (Shargal et al., 2006). In apple, cell division that accounts for the greatest expansion in fruit continues for 3 to 4 w.a.f.b. (Tukey, 1974). The ultimate size of the fruit is also determined by the number of cells at anthesis (Tukey, 1974). Therefore both the floral differentiation phase prior to anthesis as well as the development of the fruit after anthesis will determine eventual fruit size (Theron, 2011). It was determined by Zhang et al. (2006) that cell division is more important than cell enlargement in determining the final fruit size in *Pyrus pyrifolia* Nakai.

In most pear fruit size studies the cortex is interpreted as equivalent to the flesh, with the pith separated from it by a ring of 10 main vascular bundles, surrounding the five carpels (Bain, 1961). The core is defined as the five carpels and the pith (Bain, 1961). Bain (1961) found that cell division in the outer cortex, cortex and pith is very active approximately 7 to 14 d.a.f.b., with the main cell division period lasting until approximately 28 d.a.f.b.

4.2 The role of plant growth regulators (PGRs) in fruit size

The use of PGRs offers an effective means of modifying fruit growth and development (Tukey, 1974). Therefore it must be determined if applications made during the cell division phase can improve fruit size by enhancing the cell division stage.

Cytokinins are primarily synthesized in root tips and transported through the xylem to various plant organs, with concentrations highest in young organs like seeds, fruits and leaves (Salisbury and Ross, 1992). Early cell division is influenced by endogenous plant growth hormones, especially cytokinins, while exogenous applied cytokinins induce non-dividing fruit cells to enter the cell cycle (Looney, 1993). Progression through the cell cycle is controlled by the activities of cyclin-dependant kinases (CDKs) at two transition points, G1-S and G2-M and the stimulatory effect of cytokinins on cell division may occur at both points (Shargal et al.,

2006). Cytokinins are required to initiate cell proliferation in non-dividing tissues, and their continued presence is also needed to maintain mitotic activity (Werner et al., 2001).

An increase in fruit size in pear following application of the synthetic cytokinins CPPU [N (2-chloro-4-pyridyl)-N' -phenylurea] or TDZ [N-phenyl-N' -1,2,3-thiadiazol-5-ylurea] suggested that endogenous cytokinin levels are a major factor controlling fruit growth (Shargal et al., 2006). Exogenous applied cytokinin, 6-BA at 100 $mg.L^{-1}$, 2 w.a.f.b. resulted in increased fruit size in the pear cultivars Spadona and Coscia (Stern and Flaishman, 2003). In „Spadona', 6-BA increased fruit size without causing a dramatic thinning effect (Flaishman et al., 2001), which suggests that the increase in fruit size can be attributed mainly to a direct effect on increasing the rate in cell division or the length of the cell division period in the fruit cortex (Stern and Flaishman, 2003). In „Coscia' however, the increase in large fruit was accompanied by a heavy thinning effect, therefore the increase was achieved in two ways; directly through cell division and indirectly through thinning indicating that each cultivar may respond differently to the same treatment (Stern and Flaishman, 2003). 6-BA at 100 $mg.L^{-1}$ stimulated fruit growth in „Coscia' and „Spadona' with no negative influence on fruit shape, seed number and return bloom (Stern and Flaishman, 2003). Fruit size enhancement was also achieved in „Williams' pear with 6-BA, and it was established that the most consistent effective time of 6-BA application was when fruitlet diameter was between 10 and 15 mm, which is approximately 10 to 15 days after petal fall (Gimenez et al., 2010). The reason for this positive response can be because the application was made during the rapid cell division phase (Wismer et al., 1995).

The increase in fruit size observed following CPPU- or TDZ application could be due to an increase in cell number due to the induction and/or extension of the period of mitotic activity, or to an increase in cell size, or to a combination of both (Shargal et al., 2006). With fluorescence-activated cell sorter (FACS) analysis it was determined that prolonged mitotic

activity in CPPU-treated fruit occurred (Shargal et al., 2006). The G2 population persisted in fruitlets of diameter >27mm for at least 2 weeks following treatment, which suggested prolonged mitotic activity (Shargal et al., 2006). In a non-treated fruit the G2 population was evident in fruitlets until 33 d.a.f.b., whereas in CPPU-treated fruitlets the G2 population persisted until 45 d.a.f.b. (Flaishman et al., 2001).

Shargal et al., (2006) concluded that the endogenous levels of cytokinins may restrict expression of the full developmental potential of pear fruit and that exogenous applied cytokinins indeed are effective in increasing fruit size by increasing the number of cell divisions. The timing of the applications appeared to be crucial, but Flaishman et al., (2001) showed an increase in „Spadona' pear fruit size when CPPU was applied twice, at 14 and 21 d.a.f.b. These results indicated that endogenous cytokinin is still a limiting factor in the fruitlet development one week after the first application, and an additional application of 5 $\mu l.L^{-1}$ could further induce an increase in „Spadona' pear fruit size (Flaishman et al., 2001).

Exogenously applied cytokinins specifically enhance cell division in pulp parenchyma cells, leading to a significant increase in fruit size (Shargal et al., 2006). Applications of both synthetic cytokinins CPPU and TDZ at 10 mm fruit diameter had no effect on the size or number of stone cells during fruit development; however the parenchyma that forms the fruit flesh, between the epidermis and the seed layers had significantly smaller cells and produced higher numbers of cells compared to untreated fruit (Shargal et al., 2006). The increase in parenchyma cell numbers correlated with a prolonged phase of cell division, as demonstrated by the detection of G2 nuclei using FACS analysis (Shargal et al., 2006). Shargal et al., (2006) found that a single application of CPPU or TDZ can induce an increase in the diameter of pear fruit by extending the phase of cell division in pulp parenchymal cells. Histological measurements across the pulp radius showed significantly higher numbers of parenchymal cells in cytokinin-

treated fruits (230 to 250 cells) compared to untreated fruit (170 cells) (Flaishman et al., 2001), which suggests that an increase in cell number made a major contribution to the larger fruit size (Shargal et al., 2006).

GAs are used commercially to promote pear fruit growth, however it was found that exogenous GA application were not effective in promoting pear growth (Itai et al., 2009). The reason for GAs being ineffective in promoting pear growth is based on the expression analysis of GA biosynthesis genes, due to higher catabolising active GAs in fruit (Itai et al., 2009). In the GA biosynthesis pathway inactive GA_9 is converted to GA_4 (active) which in turn is metabolised to GA_3 (active) (Rademacher et al., 2006). P-Ca inhibits both these processes (Rademacher et al., 2006). When exogenous GA_{4+7} was applied in combination with P-Ca, the breakdown of the applied GA_{4+7} to inactive forms was inhibited, resulting in the persistence of the applied GA_{4+7} which then contributed to cell enlargement (Itai et al., 2009).

Commercial application of GA_3 at full bloom for enhancement of fruit set of „Spadona' and „Coscia' pear is prone to induce fruit malformation, due to asymmetrical enlargement of the cells at the blossom-end of the fruit (Stern, 2008). Addition of the synthetic auxins 2,4-dichlorophenoxyacetic acid (2,4-D) and NAA in a product trading as Bolero™ (manufactured by Lainco, Barcelona, Spain) eliminated this problem and also noticeably increased fruit size in both cultivars (Stern, 2008). Bolero™ applied during full bloom at 1200 $\mu l.l^{-1}$ (containing 3 $mg.L^{-1}$ GA^3, 6 $mg.L^{-1}$ 2,4-D and 6 $mg.L^{-1}$ NAA) increased fruit size (Stern, 2008). A split application of two 600 $\mu l.L^{-1}$ Bolero™ applications at 30% and 100% bloom did not have any additional effect. A combination of the cytokinin 6-BA and gibberellins GA_{4+7} each at 25 $mg.L^{-1}$ applied 14 d.a.f.b., greatly increased fruit size without causing any malformation (Stern, 2008).

4.3 The role of water availability and temperature in fruit size

An important factor influencing apple growth is the availability of water to the fruit during the cell enlargement phase (Tukey, 1974). Azevedo et al., (2008) found that the total yield per „Rocha' pear tree increased with water supply (from full bloom until harvest) to a certain limit, but varied for each of the six years of observation. A regular increase in yield was observed with water supply reaching a maximum close to 10 litres per fruit per season (Azevedo et al., 2008). All water applied in excess of 10 litres per fruit had no effect on yield (Azevedo et al., 2008). During a period of moisture stress, fruit growth can be drastically reduced or ceased, especially when it occurs during the cell enlargement phase (Tukey, 1974). The amount of fruit growth lost during such periods appears to be non-recoverable by later irrigation (Tukey, 1974). Temperature also plays a very important role in determining the growth rate of apples (Tukey, 1974). In apple, fruit growth is negatively affected by low spring temperatures due to a reduction in the rate of cell division, while during the cell enlargement phase the fruit are remarkably insensitive to temperature (Corelli-Grappadelli and Lakso, 2004).

4.4 The role of pruning severity, branch quality and girdling in fruit size

By reducing the number of fruiting shoots in peach trees, but keeping fruit numbers per tree constant, fruit size was increased (Marini, 2003), which implies that the size of the source was not affected but sink strength of individual fruit was increased by the treatments that in turn improved fruit size (Reynolds et al., 2005). The increase in fruit set and fruit size on short bearing units and thick bearing units could be due to an increase in the source or an increase in the sink strength of individual fruits, or both (Reynolds et al., 2005). The sink strength of fruit on thick bearing units exceeds the sink strength of fruit on thin bearing units by far; the sink

strength is possibly related to a better supply of xylem transported metabolites to fruit on thick and short bearing units (Reynolds et al., 2005).

5. Conclusion

From this review, it can be concluded that there are numerous factors that determine fruit set and fruit size. Many of these factors can be manipulated to improve yields and thus return. Maximum return from an orchard can be achieved by a high fruit set percentage with good fruit quality, which includes good fruit size. There is a fine line between factors playing a role in fruit set and combining these factors in such a way to achieve constant good yields. Fruit size is dependent on the fruit set and by achieving a full-bearing orchard with a balance between yield and vegetative growth, one would most likely also achieve good fruit size.

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PAPER 1: The evaluation of different gibberellins, in combination with 6-benzyladenine, aminoethoxyvinylglycine , prohexadione-calcium and girdling on fruit set and yield of 'Forelle' and 'Abate Fetel' pears

Keywords: gibberellic acid (GA_3), gibberellins 4+7 (GA_{4+7}), 6-benzyladenine (6-BA), aminoethoxyvinylglycine (AVG), prohexadione-calcium (P-Ca), fruit set, plant growth regulator

Abstract

Maintaining constant high yields in 'Abate Fetel' and 'Forelle' orchards in South Africa is challenging. One way of improving productivity in these orchards is to increase fruit set. Various protocols to improve fruit set are used by South African producers without knowing whether these indeed improve set. Different combinations of plant growth regulators including gibberellic acid (GA_3), gibberellins 4+7 (GA_{4+7}), (GA_{4+7} + 6-benzyladenine (6-BA)), aminoethoxyvinylglycine (AVG) and prohexadione-calcium (P-Ca) in combination with trunk girdling were applied during flowering to determine the best fruit set strategy. All applied growth regulators improved fruit set relative to an untreated control over two consecutive seasons, but GA_3 and P-Ca reduced return bloom and AVG resulted in smaller fruit size relative to the other treatments.

INTRODUCTION

Maintaining constant high yields in „Abate Fetel' and „Forelle' orchards in South Africa is challenging. The reason for this may be due to vigorous growth, and consequently low fruit set which can also lead to biennial bearing (Lafer, 2008). Exogenous gibberellins (GAs) are

often applied to increase fruit set, but the outcome is not always positive because of smaller fruit size and a reduction in return bloom. An increase in initial fruit set can be observed when GAs are applied, but often this is partially lost again during June drop (Northern hemisphere) (Vercammen and Gomand, 2008).

The build-up of yield in „Abate Fetel' orchards is slow, even though it is very precocious and develops flowers in abundance (Vilardell et al., 2008). Further plantings of this cultivar are limited because of the low initial fruit set and significant fruitlet drop by the end of May (Northern hemisphere) (Vilardell et al., 2008). One of the reasons for low fruit set in „Abate Fetel' trees can be the vigorous growth that occurs during spring, which creates competition with fruitlets (Vilardell et al., 2008).

Improvement of fruit set in young pear trees by applying plant growth regulators (PGRs) is a useful way to increase yield, because young trees often lose most of their fruit due to excessive June drop (Northern Hemisphere) (Lafer, 2008). Final fruit set in „Rocha' pear is determined during the first three weeks following flowering (Silva and Herrero, 2008). This is probably why using growth regulators in this crucial period may improve fruit set and alleviate fruitlet drop in some pear cultivars.

In more vigorous cultivars, reducing tree vigour is one way to obtain high yields and high quality fruit (Asin et al., 2005). The best way to control the vegetative vigour of a fruit tree is to induce regularity in yield, which can be achieved by a treatment with GAs, but the results of these treatments are not consistent (Vanthournout et al., 2008). Excessive vegetative growth in fruit trees can also be controlled by trunk girdling, thus enhancing yields by increasing fruit set (Goren et al., 2004; Smit, et al., 2005). Girdling entails the removal of a ring of bark around the full circumference of the tree trunk through the phloem thus interrupting phloem transport (Goren et al., 2004).

Prohexadione-calcium (P-Ca) is a plant growth regulator that is primarily used to inhibit excessive vegetative growth in fruit trees and thus reduces abortion of fruitlets, thereby increasing fruit set (Rademacher et al., 2006). Fruit set on pear trees can also be improved by GA applications to improve young tree productivity (Deckers and Schoofs, 2002). Different GAs like GA_3, GA_{4+7} and mixtures of GA_3 and GA_{4+7} can be applied to improve fruit set (Lafer, 2008). It was suggested by García-Martínez and García-Papí (1979) that in seedless Clementine mandarins, an increase in fruit set after application of GA_3 is due to increased availability of nutrients from leaves, thereby increasing the sink strength of fruitlets. Increased fruit set was observed in different pear cultivars worldwide when treated with a combination of GA_{4+7} and 6-benzyladenine (6-BA) (Vilardell et al., 2008). Applications of (GA_{4+7} + 6-BA) at different phenological stages around bloom also improved yield in „Abate Fetel' pears, but the efficacy varied depending on the location and the year (Vilardell et al., 2008). When (GA_{4+7} + 6-BA) are applied in combination around full bloom, they function like growth promoters at cellular level and improve the development of fruitlets immediately after flowering (Vilardell et al., 2008). Another growth regulator that could play a role in fruit set is aminoethoxyvinylglycine (AVG), an ethylene biosynthesis inhibitor that increased fruit set when applied to „William' and „Packham's Triumph' pear two weeks after full bloom (w.a.f.b.) (Dussi et al., 2002). AVG is an inhibitor of amino-cyclopropane-1-carboxylic acid (ACC) synthase, thereby actively preventing the formation of ACC, the natural precursor of ethylene which could result in a reduction in "November drop" (McGlasson, 1985). Therefore, applying PGRs such as GAs, AVG and P-Ca in intensive pear growing systems is considered to be an important cultural practice to regulate yield and to obtain good fruit quality (Lafer, 2008).

In this paper we report on the evaluation of combinations of different plant growth regulators and girdling to maximize fruit set in „Forelle' and „Abate Fetel' orchards in South Africa.

MATERIALS AND METHODS

Site background, experimental design and treatments

Trials were conducted on the same „Abate Fetel' and „Forelle' pear trees during the 2010/2011 and 2011/2012 seasons. The trial sites were on separate farms in the Elgin Valley, Western Cape, (Mediterranean type climate) South Africa. „Abate Fetel' trees on BP1 rootstock were planted in 1996 at a spacing of 4 x 1.2 m on the farm Oak Valley. „Forelle' trees on BP1 rootstock were planted in 2001 at a spacing of 4 x 1.5 m on the farm Restanwold. Trees uniform in height, stem circumference and blossom density were chosen during spring 2010. Phenological stages and treatment application dates for „Forelle' and „Abate Fetel' for the two consecutive seasons are presented in Table 1.

The trials were arranged as a randomized complete split block design with 10 replications. Different chemical applications served as the main factor and girdling as the sub factor. The girdling treatment comprised approximately 3 mm (the width of the blade) of the phloem removed right around the trunk, approximately 50 cm above the ground around full bloom with a Felco 600™ saw (Table 1). During 2011/2012 the chain of a chainsaw was used to girdle „Abate Fetel' trees to create a more rigorous girdle of about 6 mm wide. Each main plot consisted of four trees treated with the same PGR application, with two trees girdled (G) and two trees not girdled (C). Different combinations of PGRs at different phenological stages were used as summarised in Table 2. All treatments were applied using a Stihl™ motorized knapsack sprayer at 1000 $L.ha^{-1}$. Dash™ ec (BASF (Pty) Ltd.) at 60 $ml.100\ L^{-1}$ was added to the GA_{4+7}

(Regulex™; Valent BioSciences Corporation, USA) and P-Ca (Regalis™; BASF (Pty.) Ltd.) combination applications and 5 ml.100 L^{-1} Biodew™, a non-ionic surfactant was added to GA_3 (ProGibb™; Valent BioSciences Corporation, USA) applications.

Trial adjustments in 2011/2012 and additional trial

During 2011/2012 slight adjustments were made to the trial treatments. As it was difficult to determine 75% full bloom accurately, all applications applied during 2010/2011 at 75% full bloom were applied at full bloom.

A second trial on „Forelle' and „Abate Fetel' was added on the farm Oak Valley. The additional „Abate Fetel' trial was conducted in the same orchard as the initial trial and the additional „Forelle' trial was conducted on trees on BP1 rootstock planted in 1996 at a spacing of 4 x 1 m. Trees uniform in height, stem circumference and blossom density were used.

The trial was laid out as a randomized complete block design with 10 replications. Each plot consisted of a single tree with unsprayed buffer trees between each treated plot. Different concentrations of PGRs and different phenological stages were used as summarized in Table 4. All treatments were applied using a Stihl™ motorized knapsack sprayer at 1000 $L.ha^{-1}$. Data collection and analysis was as described in section 3.2.3.

Data collection and analysis

In all trials, two representative branches were tagged in the lower half of the tree canopy during full bloom. Flower clusters on tagged branched were counted. Fruit set per flower cluster was recorded after the natural fruit abscission period just prior to hand thinning (Table 1 and 3). Total number of fruit thinned per plot was recorded during commercial hand thinning. The standard farm practice was used which was to thin all fruit clusters to two fruitlets per cluster.

Trunk circumference was recorded approximately 5 cm above the graft union during July each year. Yield per treatment was recorded at commercial harvest (Table 1 and 3) by weighing all fruit harvested per two-tree plot. At harvest a sample of 30 fruit per plot was collected randomly from trees at chest height. The fruit sample was analysed for fruit diameter, weight, length, number of developed seeds per fruit and number of malformed fruit. As there were little malformed fruit, fruit were classed in a malformed (1) or normal (0) category, with any kind of malformation classified as malformed (1). During July following each season, the average one-year-old shoot length per plot was determined by measuring 20 one-year-old shoots on tagged branches per plot, starting from the distal end of the branch, measuring 10 shoots per side of each treated plot. Return bloom was recorded on the tagged branches during full bloom the following spring. The total number of flower clusters relative to the total number of buds sprouting was recorded to determine the return bloom percentage.

The general linear models (GLM) procedure of the Statistical Analysis System (SAS Enterprise Guide 4) was used to analyse the data. Pairwise t-tests were used to compare treatment means when the ANOVA showed significant differences ($P < 0.05$) between treatments.

RESULTS

'Forelle': Interactions and girdling

There were no significant interactions between the PGR treatments and girdling at full bloom in fruit set, fruit weight, fruit diameter, fruit length, yield efficiency, final one-year-old shoot length and return bloom following the 2010/2011 (Table 5) and the 2011/2012 seasons (Table 6). There were also no significant interactions between PGR treatments and girdling in

the percentage of malformed fruit and the number of fully developed seeds during both seasons (Table 7).

Girdling during full bloom, significantly reduced fruit weight, diameter and fruit length relative to ungirdled trees (C) during the 2010/2011 season (Table 5). Girdling at full bloom did not affect fruit set, one-year-old shoot length or return bloom during both seasons (Table 5 and 6). There were also no significant differences in yield efficiency between girdled (G) and ungirdled (C) trees during the 2010/2011 season although yield efficiency was significantly increased on girdled trees relative to ungirdled control trees during the 2011/2012 season (Table 6). A trend to increase fruit set per flower cluster was also observed following the 2011/2012 season although it was not significant (P = 0.0720) (Table 6). There was also no significant differences between girdled and control (ungirdled) trees during both seasons in the percentage of malformed fruit or the number of fully developed seeds per fruit (Table 7).

'Forelle': PGR effect on fruit set, fruit quality, yield efficiency and seed development

Fruit set, as determined by the number of fruitlets thinned by hand, was significantly increased by all PGR treatments relative to the control during the 2010/2011 season (Table 5). The highest number of fruitlets (309.2) was thinned from trees treated with a GA_{4+7} and P-Ca tank-mix at 75% bloom although this was not significantly higher than the treatment combining GA_{4+7} with (GA_{4+7} + 6-BA) (282.9) (Table 5). During the 2011/2012 season the highest number of fruitlets (437.2) were thinned from trees treated with two AVG applications, but it did not differ significantly from one AVG application (367.6) or the GA_{4+7} application (420.6) (Table 6). The untreated control had the lowest number of fruitlets (222.6) to thin during 2011/2012 and did not differ significantly from the combination treatment of GA_3, (GA_{4+7} + 6-BA) and GA_3 (277.06). Fruit set per flower cluster was significantly increased by all PGR treatments relative

to the control during the 2010/2011 (Table 5) and the 2011/2012 seasons (Table 6). During both seasons the highest fruit set per flower cluster was evident on trees treated with the combination of GA_3, (GA_{4+7} + 6-BA) and GA_3 (2.01 and 3.1 fruit/flower cluster, respectively), the GA_{4+7} and P-Ca combination (1.99 and 3.2 fruit/flower cluster, respectively) and the treatment containing GA_{4+7} and (GA_{4+7} + 6-BA) (1.99 and 2.6 fruit/flower cluster, respectively) (Table 5 and 6).

During the 2010/2011 season average fruit weight of fruit harvested from the untreated control treatment yielded fruit with significantly higher fruit weight (133.1 g) relative to all other treatments, with the GA_{4+7} and P-Ca combination treatment yielding fruit with the lowest fruit weight (102.6 g) (Table 5). Fruit diameter resulted in the same trend as fruit weight during 2010/2011 with the untreated control yielding fruit with significantly greater diameter relative to all other treatments (Table 5). During 2011/2012 there were slight differences between PGR treatments in fruit weight, with significantly smaller fruit following treatments containing AVG while there were no significant differences in fruit diameter between PGR treatments (Table 6). There were significant differences between treatments in fruit length in both seasons with no similar trend between treatments in the two seasons. During 2010/2011 fruit length was the greatest in the untreated control (77.9 mm) and did not differ significantly from the GA_3, (GA_{4+7} + 6-BA) and GA_3 combination treatment (76.7 mm) or the GA_{4+7} treatment (77.6 mm) (Table 5). During 2011/2012 fruit length was the greatest on trees treated with the (GA_{4+7} + 6-BA) and GA_3 combination treatment (80.6 mm) and did not differ significantly from the GA_{4+7} and P-Ca treatment (80.0 mm) (Table 6).

Covariate analysis was undertaken to determine the effect of fruitlets thinned (set) and yield efficiency on fruit weight, diameter and fruit length. During the 2010/2011 season yield efficiency and the number of fruitlets thinned as covariates only reduced the treatment significance level slightly for fruit weight, diameter and fruit length and treatment effects

remained highly significant (Table 5). During the 2011/2012 season, including yield efficiency as covariate removed the significant differences between PGR treatments for fruit weight but not fruit length (Table 6). Using the number of fruitlets thinned as covariate for fruit weight and fruit length during the 2011/2012 season was significant, after including the covariate there were no significant differences between PGR treatments in fruit weight and fruit length (Table 6). Yield efficiency during the 2011/2012 season showed an exact opposite trend than during the 2010/2011 season. The untreated control and the two treatments that included AVG resulted in the highest yield in 2011/2012, while yielding the lowest relative to all other treatments during 2010/2011 (Table 5 and 6).

Overall the average number of fully developed seeds per treatment was low in both seasons, ranging from 0.5 to 3.4 (Table 7). There were significant differences between PGR treatments in the average number of fully developed seeds per fruit during both seasons. The untreated control had significantly more developed seeds relative to all PGR treatments in 2010/2011 but did not differ from the two AVG treatments during the 2011/2012 season (Table 7). All four treatments containing GAs contained significantly fewer developed seeds relative to the untreated control and two AVG treatments in both seasons (Table 7). The Pearson correlation coefficient between fruit diameter and the total number of developed seeds over two seasons was only $r^2 = 0.072$ with a p value of 0.0024 which indicates that the model is significant (Fig. 1a). There were no significant differences between PGR treatments in the percentage of malformed fruit during both seasons (Table 7).

'Forelle': Shoot length and return bloom

There were no significant differences between treatments in average one-year-old shoot length on two tagged branches during the 2010/2011 season or 2011/2012 season (Table 5 and

6). There were significant differences between treatments in return bloom on two tagged branches after the 2010/2011 season (Table 5). Control trees had the highest return bloom percentage differing significantly from all other treatments except the treatment containing two AVG applications. Return bloom percentage between treatments containing one and two AVG applications did not differ significantly and one AVG application did not differ significantly from the GA_{4+7} treatment. The treatment containing two GA_3 applications and a (GA_{4+7} + 6-BA) application and the GA_{4+7} and P-Ca combination treatment significantly differed from all other treatments with the lowest return bloom percentage during the 2010/2011 season (Table 5). There were no significant differences between PGR treatments in return bloom percentage following the 2011/2012 season (Table 6). The return bloom percentage of the 2010/2011 season was used as covariate to see if it had an effect on fruit set during 2011/2012 (Table 6). The covariate was not significant for the number of fruitlets thinned by hand but was highly significant for the number of fruitlets per flower cluster (Table 6). After including the return bloom percentage there was no significant differences between treatments in the number of fruitlets per flower cluster (Table 6).

'Forelle': Second fruit set trial (2011/2012)

Fruit set as quantified by the number of fruitlets thinned by hand was the highest in trees treated with two low rate GA_{4+7} applications (30% bloom and full bloom) (24.7) and AVG (24.2) at full bloom, although it was not significant (Table 8). There were also no significant differences between PGR treatments in fruit set per flower cluster on two tagged branches. Trees treated with a combination of GA_3 at 30% bloom and AVG at full bloom yielded the smallest fruit relative to all other treatments as determined by fruit weight and diameter although it was only significant for fruit weight. Using yield efficiency and the number of fruitlets thinned as

covariate for fruit weight was not significant. Yield efficiency was the highest on trees treated with a high rate of GA_{4+7} (0.09) at 30% bloom and full bloom and differed significantly from GA_3 (0.05) at full bloom, GA_3 (0.06) at 30% bloom and full bloom and the high rate of (GA_{4+7} + 6-BA) (0.06) at 30% bloom and full bloom. There were no significant differences between PGR treatments in the average number of developed seeds that fruit contained or the return bloom percentage (Table 8).

'Abate Fetel': Interactions and girdling

There were no significant interactions between PGR treatments and girdling at full bloom in fruit set, fruit size, fruit length, yield efficiency, return bloom, one-year-old shoot length, the percentage of malformed fruit and the number of fully developed seeds per fruit during both seasons (Table 9, 10 and 11). Girdling did not have any significant effect on fruit set, fruit diameter, fruit weight, fruit length, yield efficiency, one-year-old shoot length, the percentage of malformed fruit and the number of fully developed seeds per fruit during both seasons (Table 9, 10 and 11). Girdling during full bloom had no significant effect on return bloom following the 2010/2011 season, although it increased return bloom after the 2011/2012 season (Table 10).

'Abate Fetel': PGR effect on fruit set, fruit quality, yield efficiency and seed development

In „Abate Fetel' all PGR treatments increased the number of fruitlets thinned by hand significantly relative to the controls in both seasons (Table 9 and 10). During the 2010/2011 season, the highest number of fruitlets was thinned on trees treated with two AVG applications (474.3) which differed significantly from all other treatments (Table 9). During the 2011/2012 season there were again significant differences between PGR treatments in the number of fruitlets thinned, with the treatment consisting of GA_{4+7} and P-Ca and the treatment consisting of

two AVG applications resulting in the highest number of thinned fruitlets (397.9 and 352.3, respectively) (Table 10). Smaller differences between treatments occurred in terms of fruit set per flower cluster on two tagged branches in comparison with the number of fruitlets thinned between treatments during the 2010/2011 season (Table 9). The number of fruitlets thinned by hand however gives a better indication of overall fruit set on the tree as the two tagged branches represented only the lower canopy of the tree. During both seasons the treatment containing GA_{4+7} and P-Ca increased fruit set per flower cluster most relative to the control (Table 9 and 10).

The untreated control trees and trees treated with GA_{4+7} on its own yielded fruit with significantly higher fruit weight relative to all other treatments except the treatment combination of GA_{4+7} and (GA_{4+7} + 6-BA) during the 2010/2011 season which was however not the case during 2011/2012 (Table 9 and 10). The untreated control trees, the GA_{4+7} treatment and the GA_{4+7} and (GA_{4+7} + 6-BA) combination treatment yielded fruit with significantly greater diameter relative to the GA_{4+7} and P-Ca combination treatment and the two treatments containing AVG (Table 9). Fruit length was the greatest in the GA_{4+7} treatment but not significantly different from the untreated control and the combination treatment with GA_{4+7} and (GA_{4+7} + 6-BA) during 2010/2011 (Table 9). During 2011/2012 the two treatments containing 6-BA yielded the largest fruit in terms of fruit weight, diameter and length (Table 10).

Covariate analysis was undertaken to determine if the number of fruitlets thinned and yield efficiency had an effect on fruit weight, diameter and fruit length. After including the number of fruitlets thinned as covariate for fruit weight, diameter and fruit length, there were no significant differences between treatments (Table 9). Yield efficiency included as covariate to determine the effect on fruit weight, diameter and fruit length during 2010/2011 reduced the treatment significance for these three parameters slightly, although treatment effects still

remained highly significant (Table 9). Including the number of fruitlets thinned as covariate for fruit weight, diameter and fruit length during 2011/2012 reduced the treatment significance slightly for all three fruit size parameters, with treatment effects still remaining highly significant (Table 10). Yield efficiency as covariate for fruit weight, diameter and fruit length during the 2011/2012 season was not significant in explaining the differences in fruit size and did not affect treatment significance much (Table 10).

There were no significant differences between treatments in yield efficiency during both seasons (Table 9 and 10). Very few „Abate Fetel' pears contained developed seeds although there were still significant differences between treatments in terms of the total number of fully developed seeds per fruit during both seasons ranging from 0.2 to 0.9 seeds per fruit but these differences are of no horticultural significance (Table 11). The Pearson correlation coefficient between fruit diameter and the total number of developed seeds over two seasons was only r^2 = 0.0033 with a p value of 0.5017 which indicates that the model is not significant (Fig. 1b). There were no significant differences between PGR treatments in the percentage malformed fruit during either season (Table 11).

'Abate Fetel': Shoot length and return bloom

There were no significant differences between treatments in terms of the average one-year-old shoot length during both seasons (Table 9 and 10). There were significant differences between treatments in return bloom during both seasons. The untreated control, the treatment containing one AVG application and the treatment containing two AVG applications did not differ significantly from each other and resulted in the highest return bloom percentage during both seasons (Table 9 and 10). These three treatments differed significantly from all other treatments in return bloom percentage. The GA_3 and (GA_{4+7} + 6-BA) combination, the GA_{4+7}

and P-Ca combination and the treatment containing GA_{4+7} and (GA_{4+7} + 6-BA) did not differ significantly and resulted in the lowest return bloom percentage during 2010/2011 (Table 9). During 2011/2012 all treatments containing GAs resulted in a significantly lower return bloom percentage than the control and AVG treatments, with the GA_{4+7} and P-Ca combination treatment resulting in significantly lower return bloom percentage than all other treatments (Table 10). The 2010/2011 return bloom percentage was used as a covariate to determine if it had an effect on 2011/2012 fruit set (Table 10). The covariate was not significant in explaining the number of fruitlets thinned by hand, but was significant for the number of fruitlets per flower cluster. Although the covariate was significant for the number of fruitlets per flower cluster, treatment differences remained highly significant after inclusion of the covariate (Table 10).

'Abate Fetel': Second fruit set trial (2011/2012)

The highest number of fruitlets was thinned from trees treated with AVG at full bloom although it was not significant (Table 12). The GA_3 and AVG combination treatment and the treatment containing only AVG resulted in significantly higher fruit set per flower cluster relative to all other treatments. Fruit weight from trees treated with a combination of GA_3 + AVG and AVG only, was significantly lower than all other treatments and the same trend was evident in fruit diameter although differences between treatments were smaller. Yield efficiency did not have an effect on fruit weight as determined by covariate analysis. Yield efficiency as covariate for fruit diameter was significant, but differences between treatments still remained highly significant after including the covariate. The number of fruitlets thinned was highly significant as covariates for both fruit weight and diameter although treatment differences still remained highly significant after including this covariate (Table 12). There were no significant

differences between treatments in fruit length, yield efficiency, the number of developed seeds per fruit or the return bloom percentage (Table 12).

DISCUSSION

The efficacy of different PGRs to improve fruit set of „Forelle' and „Abate Fetel' proved to be complex.

The inability of girdling to improve set, fruit size, yield or reduce shoot growth during the 2010/2011 season on „Forelle' could be because girdling was performed one day before full bloom (Table 1) while higher set and yield was achieved in 2011/2012 when girdling was performed a week before full bloom even though shoot growth was unaffected. The earlier girdling could have resulted in a greater correlative advantage to the inflorescences resulting in better fruit set. Saunders et al. (1991) found that pruning „Packham's Triumph' at the intercalation between one- and two-year-old wood before flowering also increased fruit set significantly due to the correlative advantage to inflorescences compared to pruning during or after anthesis. In the case of „Abate Fetel' girdling was performed at full bloom, but had no significant effect in either season even though a more aggressive girdle was made during the 2011/2012 season. With girdling, the aim is to reduce tree vigour, but it seems that the type of girdle used was not aggressive enough to achieve this in these two cultivars as one-year-old shoot length was not affected. Alternative measures to reduce tree vigour to increase fruit set, e.g. more dwarfing rootstocks should rather be used (Webster, 2002). Raffo et al. (2011) found that girdling 20 d.a.f.b. did not cause any significant improvement in yield and fruit size in „Bartlett' pear and it cannot replace practices such as pruning and thinning. Although girdling can retard vegetative growth, the response to girding is still highly variable because of many other factors that could also play a role (Smit et al., 2005; Theron and Steyn, 2008). The current

practice of girdling during full bloom used by growers therefore appears to be ineffective in increasing set and yield in „Forelle' and „Abate Fetel' pear orchards.

In all fruit set trials on „Forelle and „Abate Fetel', GA_{4+7} did increase fruit set during both seasons relative to the untreated control, although the fruit set increase was not as pronounced as achieved with combinations of GA_{4+7} with 6-BA and P-Ca. The mode of action for GAs is that it functions at cellular level by elongating and expanding cells (Brock and Kaufman, 1991), thus increasing the sink strength of fruitlets which leads to increased fruit set. GA_{4+7} did not have as negative an effect on return bloom as GA_3 on both cultivars after the first season, which was however not the case after the second season, with varying return bloom percentages which was due to treatments repeated on the same trees.

In „Forelle', the combination of GA_{4+7} with P-Ca improved fruit set during the first season but did not show the same effect during the following season. This was mainly because the trial was repeated on the same trees and P-Ca caused a reduction in return bloom after the 2010/2011 season, leading to some plots with less potential for fruit set. This was confirmed by the covariate analysis, where there were no significant differences between treatments in fruit set on two tagged branches during the second season after including the first season's return bloom percentage as covariate. The reason for repeating the trial on the same trees was to determine the possibility of carry over effects of such treatments in practice, and to establish if it would contribute towards creating a balance in fruit set and yield over two consecutive seasons. Vanthournout et al. (2008) also found that P-Ca applied at the end of bloom on „Conference' pear, resulted in a reduction in return bloom. Although the P-Ca treatment reduced return bloom in „Forelle' and „Abate Fetel', fruit set per flower cluster was still the highest among treatments during the second season. The combined effect of GA_{4+7} and P-Ca performed satisfactory, with GA_{4+7} that increases the 'sink' activity of fruitlets through cell expansion (Brock and Kaufman,

1991) and P-Ca, that decreased the ethylene effect which resulted in a reduced „November' drop (Rademacher et al., 2006; Vilardell et al., 2008). P-Ca reduced the competition between fruitlets and strong vegetative shoot growth, as it is a shoot growth retardant when applied post bloom (Vilardell et al., 2008). P-Ca applied during flowering at 50 $mg.L^{-1}$ also increased fruit set on „Rosemarie', whereas 75 $mg.L^{-1}$ P-Ca increased fruit set on „Forelle' (Smit et al., 2005). Fruit set treatments can be weighed up against one another to determine if a reduction in return bloom can be justified by a saving of hand thinning costs during the following season, but this can only be considered for an orchard that flowers profusely every year.

Combinations of GA_{4+7} with 6-BA or GA_3 in the GA_3 + (GA_{4+7} + 6-BA) + GA_3 and GA_{4+7} + (GA_{4+7} + 6-BA) combination treatments also increased fruit set in both „Forelle' and „Abate Fetel', although this was more pronounced during the first season of the trials. Both these combination treatments caused a significant reduction in return bloom in „Forelle' and „Abate Fetel'. This was therefore probably the reason for the lower fruit set during the second season as was confirmed by covariate analysis in „Forelle', where the 2010/2011 return bloom percentage removed treatment differences in the number of fruitlets per flower cluster during the 2011/2012 season. There was a difference in return bloom between these two combination treatments, where the treatment containing GA_3 resulted in a lower return bloom percentage than the treatment that contained GA_{4+7}. Deckers and Schoofs, (2002) also found that return bloom on „Conference' was reduced more after the application of GA_3 than GA_{4+7} in the previous season.

In „Abate Fetel', AVG applications resulted in the highest fruit set during both seasons in all fruit set trials. The effect of AVG on fruit set was not as pronounced on „Forelle' as on „Abate Fetel', although fruit set was also increased. AVG acts as an ethylene biosynthesis inhibitor that increases fruit set if applied to pear trees at or after full bloom, by reducing fruitlet abscission (Dussi et al., 2002). Dussi et al. (2011) found that fruit set in „Abate Fetel' pear

increased with higher AVG rates with the optimum of 300 $mg.L^{-1}$ for the highest fruit set, although a rate of 150 $mg.L^{-1}$ AVG resulted in the best balance between fruit set and fruit size.

Fruit size was affected significantly by the number of fruitlets on the tree after flowering, as was seen in covariate analyses on both „Forelle' (2011/2012) and „Abate Fetel' (2010/2011) where fruit set had a greater effect on fruit size than yield of the current season. Therefore, too big an increase in fruit set could lead to small fruit. In „Forelle', treatments containing 6-BA and GA_{4+7} improved fruit set and size when compared to AVG treatments. As AVG treatments only improved fruit set, the 6-BA component must have had an effect in stimulating cell division in fruitlets. In „Abate Fetel', AVG treatments also resulted in the smallest fruit size during both seasons. In the second fruit set trial on „Forelle', the combination of GA_3 and AVG resulted in a smaller fruit size relative to all other treatments, which was also evident in „Abate Fetel'. During the second season on „Abate Fetel', treatments containing 6-BA resulted in larger fruit, which was also found by Stern and Flaishman (2003) on „Spadona' and „Coscia' pears. There also appeared to be no correlation between seed number and fruit diameter in both cultivars, but seed numbers were very low, making a strong correlation difficult.

In the second „Forelle' trial there were very few significant differences between treatments in fruit set, probably because trees used for the trial had relatively few flowers and variation between trees occurred. There was also no control included in this trial, which could have attributed to the fact that there were very few significant differences between treatments. Average temperatures during full bloom of „Forelle', when most treatments were applied, were between 7 and 17 ºC for the specific day of application, where after it rose to a maximum temperature of 23 ºC within 7 days after the application date. During September 2011, temperatures were moderate and no heat waves occurred, which could have had an effect on fruit set treatments.

In conclusion, GA_{4+7} appears to be a promising treatment to increase fruit set, while maintaining good fruit size and optimal yields on both „Forelle' and „Abate Fetel' pear. AVG can also be considered to increase fruit set in both cultivars in orchards with a poor fruit set history, because AVG might decrease fruit size indirectly if fruit set is abundant. Other combinations of PGRs can also be considered depending on the fruit set history of an orchard, but care should be exercised when using P-Ca or GA_3 to increase fruit set, as it can also reduce return bloom significantly. GA_{4+7} also reduced return bloom on „Abate Fetel' more severely during the second season. Further research is needed to determine the correct rate for GA_{4+7} for optimal fruit set and return bloom.

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Table 1: Dates of treatment applications, girdling, hand thinning and harvest for „Forelle' and „Abate Fetel' for the initial fruit set trial.

Phenological stage	2010/2011 season	2011/2012 season
	„Forelle'	
30% full bloom	7 September 2010	12 September 2011
75% full bloom	14 September 2010	-*
Girdling	15 September 2010	15 September 2011
Full bloom	16 September 2010	22 September 2011
Petal drop	24 September 2010	28 September 2011
Hand thinning	15 October 2010	12 October 2011
Harvest	1 March 2011	7 March 2012
	„Abate Fetel'	
30% full bloom	11 September 2010	14 September 2011
75% full bloom	16 September 2010	-*
Girdling	16 September 2010	23 September 2011
Full bloom	17 September 2010	21 September 2011
Petal drop	27 September 2010	28 September 2011
Hand thinning	19 October 2010	20 October 2011
Harvest	1 February 2011	6 February 2012

* All 75% full bloom applications were changed to full bloom during the 2011/2012 season

Table 2: Treatments applied to increase fruit set on „Forelle' and „Abate Fetel' pears during the 2010/2011 and the 2011/2012 season.

	Treatment by active ingredient	Treatment by trade name
1	Untreated Control	Untreated Control
2	10 mg.L^{-1} GA*$_3$ (30% flowering) + (22.5 mg.L^{-1} 6-BA* + 22.5 mg.L^{-1} GA$_{4+7}$*)(Full bloom) + 10 mg.L^{-1} GA$_3$ (Petal drop)	ProGibb™ (30% flowering) + Promalin™ (Full bloom) + ProGibb™ (Petal drop)
3	30 mg.kg^{-1} GA$_{4+7}$ (Full bloom)	Regulex™ (Full bloom)
4	30 mg.kg^{-1} GA$_{4+7}$ + 70 mg.kg^{-1} P-Ca* (75% flowering)** (Applied in a tank-mix)	Regulex™ + Regalis™ (75% flowering)
5	75 mg.kg^{-1} AVG* (Full Bloom)	Retain™ (Full Bloom)
6	30 mg.kg^{-1} GA$_{4+7}$ (75% flowering)** + (22.5 mg.L^{-1} 6-BA + 22.5 mg.L^{-1} GA$_{4+7}$) 1 week later	Regulex™ (75% flowering) + Promalin™ 1 week later
7	75 mg.kg^{-1} AVG (Full Bloom) + 75 mg.kg^{-1} AVG (1 week later)	Retain™ (Full Bloom) + Retain™ (1 week later)

*GA$_3$, Gibberellic acid; 6-BA, 6 benzyl adenine; GA$_{4+7}$, Gibberellic acid; P-Ca, Prohexadione-calcium; AVG, aminoethoxyvinylglycine

**during the 2011/2012 season 75% flowering sprays were sprayed at full bloom

Table 3: Dates of treatment applications, hand thinning and harvest for „Forelle' and „Abate Fetel' for the 2nd fruit set trial.

Phenological stage	2011/2012 season
„Forelle'	
30% full bloom	7 September 2011
Full bloom	14 September 2011
Petal drop	23 September 2011
Hand thinning	18 October 2011
Harvest	5 March 2012
„Abate Fetel'	
30% full bloom	14 September 2011
Full bloom	21 September 2011
Petal drop	28 September 2011
Hand thinning	20 October 2011
Harvest	6 February 2012

Table 4: Treatments applied to increase fruit set in a second fruit set trial during the 2011/2012 season on „Forelle' and „Abate Fetel' pears.

	Treatment by active ingredient	Treatment by trade name
1.	10 $mg.L^{-1}$ GA_3* (full bloom)	ProGibb™
2.	10 $mg.L^{-1}$ GA_3 (30% flowering) + 10 $mg.L^{-1}$ GA_3 (full bloom)	ProGibb™
3.	10 $mg.L^{-1}$ GA_3 (30% flowering) + 125 $mg.kg^{-1}$ AVG* (full bloom)	ProGibb™ + Retain™
4.	125 $mg.kg^{-1}$ AVG (full bloom)	Retain™
5.	10 $mg.kg^{-1}$ GA_{4+7}* (30% flowering) + 10 $mg.kg^{-1}$ GA_{4+7} (full bloom)	Regulex™ + Regulex™
6.	20 $mg.kg^{-1}$ GA_{4+7} (30% flowering) + 20 $mg.kg^{-1}$ GA_{4+7} (full bloom)	Regulex™ + Regulex™
7.	(11 $mg.L^{-1}$ 6-BA* + 11 $mg.L^{-1}$ GA_{4+7}) (30% flowering) + (11 $mg.L^{-1}$ 6-BA + 11 $mg.L^{-1}$ GA_{4+7}) (full bloom)	Promalin™ + Promalin™
8.	(22.5 $mg.L^{-1}$ 6-BA + 22.5 $mg.L^{-1}$ GA_{4+7}) (30% flowering) + (22.5 $mg.L^{-1}$ 6-BA + 22.5 $mg.L^{-1}$ GA_{4+7}) (full bloom)	Promalin™ + Promalin™

*GA_3, Gibberellic acid; AVG, aminoethoxyvinylglycine; GA_{4+7}, Gibberellic acid;

6-BA, 6 benzyl adenine

Table 5: Effect of different combinations of growth regulators on the number of fruits thinned by hand, fruit set on two tagged branches, fruit diameter, fruit weight, fruit length, yield, one-year-old shoot length and return bloom on „Forelle' pear during the 2010/2011 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average number of fruitlets thinned by hand	Average fruit set per cluster on two tagged branches	Average fruit diameter (mm)	Average fruit weight (g)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average 1-year-old shoot length (cm)	% Return bloom on two tagged branches
Untreated Control	138.61 e	0.85 c	60.20 a	133.05 a	77.90 a	0.08 d	16.87 ns	27.76 a
GA_3 + (GA_{4+7} + 6-BA)* + GA_3	259.50 bc	2.01 a	56.69 c	113.44 c	76.66 ab	0.11 b	18.30	6.11 e
GA_{4+7}	227.83 cd	1.64 b	58.86 b	124.60 b	77.55 ab	0.10 bc	14.13	18.82 c
GA_{4+7} + P-Ca	309.17 a	1.99 a	54.78 d	102.63 d	73.64 c	0.13 a	16.77	4.87 e
AVG	215.28 d	1.48 b	57.98 bc	117.14 bc	73.68 c	0.10 c	24.41	21.39 bc
GA_{4+7} + (GA_{4+7} + 6-BA)*	282.89 ab	1.99 a	56.92 c	113.28 c	75.90 b	0.12 ab	16.89	12.09 d
AVG + AVG	208.67 d	1.55 b	58.49 b	122.82 d	75.90 b	0.10 c	16.88	25.5 ab
Girdling								
Control	225.60 ns	1.637 ns	58.06 a	120.97 a	76.73 a	0.10 ns	14.044 ns	16.75 ns
Girdled	243.52	1.651	57.35 b	115.31 b	75.05 b	0.11	16.447	16.57
Significance level								
Treatment	*<0.0001*	*<0.0001*	*<0.0001*	*<0.0001*	*0.0071*	*<0.0001*	*0.0750*	*<0.0001*
Girdling	*0.1313*	*0.840*	*0.0499*	*0.0136*	*0.001*	*0.1581*	*0.1085*	*0.8873*
Treatment x Girdling	*0.3705*	*0.0847*	*0.3150*	*0.1024*	*0.3016*	*0.1250*	*0.3539*	*0.2211*
Covariate yield efficiency			*<0.0001*	*<0.0001*	*<0.0001*			
Treatment			*0.0019*	*0.0198*	*0.0240*			
Covariate fruitlets thinned			*<0.0001*	*<0.0001*	*<0.0001*			
Treatment			*0.0034*	*0.0353*	*0.0194*			

*Promalin™

Table 6: Effect of different combinations of growth regulators on the number of fruits thinned by hand, fruit set on two tagged branches, fruit diameter, fruit weight, fruit length, yield, one-year-old shoot length and return bloom on „Forelle' pear during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average number of fruitlets thinned by hand	Average fruit set per cluster on two tagged branches	Average fruit diameter (mm)	Average fruit weight (g)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average 1-year old shoot length (cm)	% Return bloom on two tagged branches
Untreated Control	222.61 d	1.31 e	59.26 ns	122.20 ab	76.36 de	0.28 ab	28.69 ns	18.54 ns
GA_3 + (GA_{4+7} + 6-BA)* + GA_3	277.06 cd	3.07 ab	59.46	129.52 a	80.61 a	0.20 d	29.35	26.04
GA_{4+7}	420.56 ab	2.26 cd	58.95	124.62 a	78.29 bc	0.26 bc	27.32	23.19
GA_{4+7} + P-Ca	325.83 c	3.24 a	58.39	124.48 a	79.97 ab	0.22 cd	31.99	22.30
AVG	367.61 abc	1.97 d	57.67	114.60 bc	75.40 ef	0.31 a	30.07	27.61
GA_{4+7} + (GA_{4+7} + 6-BA)*	328.11 bc	2.60 bc	58.39	122.86 a	78.11 cd	0.23 cd	32.52	23.38
AVG + AVG	437.22 a	1.99 cd	57.69	113.64 c	74.59 f	0.30 ab	26.63	27.65
Girdling								
Control	334.55 ns	2.19 ns	58.53 ns	122.06 ns	77.95 ns	0.24 b	29.14 ns	23.52 ns
Girdled	345.70	2.52	58.56	121.03	77.26	0.28 a	28.87	24.68
Significance level								
Treatment	*0.0092*	*0.0001*	*0.2703*	*0.0507*	*0.0001*	*0.0014*	*0.3261*	*0.4188*
Girdling	*0.8030*	*0.0720*	*0.6301*	*0.9504*	*0.2153*	*0.0048*	*0.6687*	*0.5678*
Treatment x Girdling	*0.5040*	*0.3368*	*0.7891*	*0.6402*	*0.0611*	*0.6554*	*0.6763*	*0.6326*
Cov. yield efficiency				*<0.0001*	*0.8208*			
Treatment				*0.6826*	*0.0540*			
Cov. fruitlets thinned				*0.0058*	*0.0345*			
Treatment				*0.1132*	*0.9082*			
Cov. 2010/2011 return bloom	*0.3616*	*<0.0001*						
Treatment	*0.0074*	*0.8042*						

*Promalin™

Table 7: Effect of different combinations of growth regulators on the percentage of malformed fruit and the number of fully developed seeds per fruit over two consecutive seasons in „Forelle' pear.

Treatment	Average % malformed fruit		Average number of fully developed seeds	
	2010/2011	2011/2012	2010/2011	2011/2012
Untreated Control	0 ns	9.72 ns	2.93 a	3.39 a
GA_3 + (GA_{4+7} + 6-BA)* + GA_3	0	6.39	0.57 ef	2.06 b
GA_{4+7}	0.22	8.06	1.22 d	1.85 b
GA_{4+7} + P-Ca	0	3.89	0.51 f	0.94 c
AVG	0.67	8.33	2.17 c	3.00 a
GA_{4+7} + (GA_{4+7} + 6-BA)*	0.44	4.17	0.90 de	1.69 b
AVG + AVG	0.22	6.67	2.54 b	2.97 a
Girdling				
Control	0.19 ns	6.83 ns	1.56 ns	2.37 ns
Girdled	0.25	6.67	1.54	2.16
Significance level				
Treatment	*0.4624*	*0.0933*	*<0.0001*	*<0.0001*
Girdling	*0.7500*	*0.8789*	*0.7679*	*0.0956*
Treatment x Girdling	*0.9117*	*0.5223*	*0.4039*	*0.9490*

*Promalin™

Table 8: Effect of different combinations of growth regulators on the number of fruits thinned by hand, fruit set on two tagged branches, fruit diameter, fruit weight, yield and return bloom on the second „Forelle' pear trial during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average number of fruitlets thinned by hand	Average fruit set per cluster on two tagged branches	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fully developed seeds	% Return bloom on two tagged branches
GA_3	9.00 ns	1.88 ns	125.10 a	58.33 ns	77.68 ns	0.054 d	1.03 ns	11.02 ns
GA_3 + GA_3	17.67	2.25	131.04 a	59.19	78.49	0.064 bcd	0.66	5.57
GA_3 + AVG	22.17	1.78	108.35 b	55.84	75.45	0.083 abc	0.98	11.19
AVG	24.17	2.22	120.90 ab	58.30	76.89	0.077 abcd	1.34	9.73
GA_{4+7} + GA_{4+7} (low rate)	24.67	1.98	130.61 a	58.58	78.79	0.086 ab	0.66	8.83
GA_{4+7} + GA_{4+7} (high rate)	10.67	1.44	125.89 a	58.71	77.06	0.092 a	0.68	14.08
(GA_{4+7} + 6-BA)*+ (GA_{4+7} + 6-BA) (low rate)	21.83	1.67	123.54 a	58.79	78.38	0.083 abc	0.90	9.24
(GA_{4+7} + 6-BA)* + (GA_{4+7} + 6-BA) (high rate)	15.17	1.38	129.79 a	58.83	81.79	0.059 cd	0.99	6.69
Significance level	*0.0607*	*0.3199*	*0.0511*	*0.0741*	*0.1532*	*0.0507*	*0.6321*	*0.6777*
Covariate yield efficiency			*0.5096*					
Treatment			*0.0416*					
Covariate fruitlets thinned			*0.9493*					
Treatment			*0.0557*					

*Promalin™

Table 9: Effect of different combinations of growth regulators on the number of fruits thinned by hand, fruit set on two tagged branches, fruit diameter, fruit weight, yield, one-year-old shoot length and return bloom on „Abate Fetel' pear during the 2010/2011 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average number of fruitlets thinned by hand	Average fruit set per cluster on two tagged branches	Average fruit diameter (mm)	Average fruit weight (g)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average 1-year old shoot length (cm)	% Return bloom on two tagged branches
Untreated Control	204.85 e	2.26 c	60.58 a	182.74 a	114.71 ab	0.10 ns	21.50 ns	23.39 a
GA_3 + (GA_{4+7} + 6-BA)* + GA_3	334.05 bc	2.85 ab	59.30 bc	172.19 bc	114.07 abc	0.12	18.18	14.24 c
GA_{4+7}	266.15 d	2.92 ab	60.35 ab	182.30 a	116.10 a	0.10	21.35	18.17 b
GA_{4+7} + P-Ca	374.25 b	3.12 a	58.84 c	168.86 c	112.57 bcd	0.12	17.47	13.61 c
AVG	356.25 b	3.00 ab	58.99 c	165.67 c	110.33 d	0.10	20.82	24.71 a
GA_{4+7} + (GA_{4+7} + 6-BA)*	282.30 cd	2.59 bc	60.21 ab	179.54 ab	114.23 ab	0.11	19.67	15.15 c
AVG + AVG	474.25 a	2.97 ab	58.26 c	164.02 c	111.50 cd	0.13	20.72	21.83 a
Girdling								
Control	334.26 ns	2.823 ns	59.48 ns	173.69 ns	112.95 ns	0.12 ns	20.829 ns	18.86 ns
Girdled	320.63	2.806	59.53	173.54	113.77	0.11	19.086	18.86
Significance level								
Treatment	*<0.0001*	*0.0245*	*0.0020*	*0.0024*	*0.0020*		*0.7831*	*<0.0001*
Girdling	*0.3624*	*0.8653*	*0.8658*	*0.9502*	*0.8658*		*0.2094*	*0.7370*
Treatment x Girdling	*0.9288*	*0.6824*	*0.8571*	*0.9895*	*0.8571*		*0.4869*	*0.1906*
Covariate yield efficiency			*<0.0001*	*<0.0001*	*<0.0001*			
Treatment			*0.0099*	*0.0048*	*0.0177*			
Covariate fruitlets thinned			*<0.0001*	*<0.0001*	*<0.0001*			
Treatment			*0.4582*	*0.4979*	*0.2139*			

*Promalin™

Table 10: Effect of different combinations of growth regulators on the number of fruits thinned by hand, fruit set on two tagged branches, fruit diameter, fruit weight, yield, one-year-old shoot length and return bloom on „Abate Fetel' pear during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average number of fruitlets thinned by hand	Average fruit set per cluster on two tagged branches	Average fruit diameter (mm)	Average fruit weight (g)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average 1-year old shoot length (cm)	% Return bloom on two tagged branches
Untreated Control	201.50 e	1.17 c	57.73 bc	161.94 b	114.49 c	0.13 ns	43.30 ns	22.43 a
GA_3 + (GA_{4+7} + 6-BA)* + GA_3	297.40 cd	2.01 b	59.30 a	170.98 a	119.03 a	0.14	43.40	15.33 b
GA_{4+7}	280.95 d	1.67 b	57.44 cd	160.53 bc	117.40 ab	0.15	47.29	8.43 c
GA_{4+7} + P-Ca	397.85 a	2.98 a	57.13 cd	153.89 cd	115.43 bc	0.15	41.59	3.37 d
AVG	332.50 bc	1.75 b	56.68 d	147.93 d	110.18 d	0.14	40.92	19.43 a
GA_{4+7} + (GA_{4+7} + 6-BA)*	278.45 d	1.89 b	58.60 ab	167.86 ab	119.34 a	0.14	41.21	10.85 c
AVG + AVG	352.30 ab	1.69 b	56.76 cd	150.28 d	111.40 d	0.14	42.98	23.01 a
Girdling								
Control	300.80 ns	1.77 ns	57.47 ns	158.05 ns	115.30 ns	0.14 ns	43.11 ns	13.08 b
Girdled	310.90	1.98	57.85	160.07	115.35	0.14	42.80	16.30 a
Significance level								
Treatment	*<0.0001*	*<0.0001*	*0.0008*	*<0.0001*	*<0.0001*	*0.6742*	*0.2408*	*<0.0001*
Girdling	*0.4468*	*0.1197*	*0.1562*	*0.3386*	*0.9345*	*0.5488*	*0.7155*	*0.0036*
Treatment x Girdling	*0.6121*	*0.5093*	*0.6160*	*0.4828*	*0.5022*	*0.8865*	*0.0511*	*0.1444*
Cov. yield efficiency			*0.1236*	*0.4567*	*0.4963*			
Treatment			*0.0094*	*0.0005*	*<0.0001*			
Cov. fruitlets thinned			*0.0352*	*0.0052*	*0.0030*			
Treatment			*0.0165*	*0.0011*	*<0.0001*			
Cov. 2010/2011 return bloom	*0.2747*	*<0.0001*						
Treatment	*<0.0001*	*0.0044*						

*Promalin™

Table 11: Effect of different combinations of growth regulators on the percentage of malformed fruit and the number of fully developed seeds per fruit over two consecutive seasons in „Abate Fetel' pear.

Treatments	Average % malformed fruit		Average number of fully developed seeds	
	2010/2011	2011/2012	2010/2011	2011/2012
Untreated Control	0.75 ns	5.50 ns	0.49 ab	0.73 ab
GA_3 + (GA_{4+7} + 6-BA)* + GA_3	1.50	6.50	0.23 c	0.48 bc
GA_{4+7}	1.50	5.25	0.33 bc	0.46 bc
GA_{4+7} + P-Ca	0.75	5.25	0.24 c	0.27 c
AVG	0	6.00	0.52 a	0.88 a
GA_{4+7} + (GA_{4+7} + 6-BA)*	0.75	6.75	0.34 bc	0.42 c
AVG + AVG	1.25	5.75	0.51 a	0.77 a
Girdling				
Control	0.79 ns	6.21 ns	0.38 ns	0.53 ns
Girdled	1.07	5.50	0.38	0.63
Significance level				
Treatment	*0.2463*	*0.9898*	*0.0006*	*0.0006*
Girdling	*0.3994*	*0.4169*	*0.9606*	*0.2056*
Treatment x Girdling	*0.4798*	*0.7250*	*0.9064*	*0.9850*

Table 12: Effect of different combinations of growth regulators on the number of fruits thinned by hand, fruit set on two tagged branches, fruit diameter, fruit weight, yield and return bloom on the second „Abate Fetel' pear trial during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average number of fruitlets thinned by hand	Average fruit set per cluster on two tagged branches	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fully developed seeds	% Return bloom on two tagged branches
GA_3	208.11 ns	1.62 b	156.69 a	57.44 a	120.41 ns	0.26 ns	0.42 ns	14.82 ns
GA_3 + GA_3	215.33	1.64 b	157.95 a	57.22 ab	114.42	0.28	0.40	18.39
GA_3 + AVG	250.00	2.43 a	142.87 b	55.89 c	110.65	0.24	0.62	10.51
AVG	301.89	2.29 a	142.68 b	56.14 bc	110.75	0.28	0.83	15.33
GA_{4+7} + GA_{4+7} (low rate)	212.22	1.46 b	155.80 a	57.48 a	114.12	0.24	0.51	13.20
GA_{4+7} + GA_{4+7} (high rate)	244.33	1.58 b	158.68 a	57.38 ab	116.50	0.30	0.49	12.57
(GA_{4+7} + 6-BA)*+ (GA_{4+7} + 6-BA) (low rate)	196.44	1.52 b	158.38 a	57.67 a	115.47	0.26	0.51	18.99
(GA_{4+7} + 6-BA)* + (GA_{4+7} + 6-BA) (high rate)	212.56	1.72 b	161.60 a	58.22 a	115.23	0.24	0.51	12.12
Significance level	*0.0852*	*0.0027*	*0.0007*	*0.0077*	*0.1439*	*0.0675*	*0.1647*	*0.1907*
Covariate yield efficiency			*0.2046*	*0.0028*				
Treatment			*0.0008*	*0.0088*				
Covariate fruitlets thinned			*<0.0001*	*0.0004*				
Treatment			*0.0044*	*0.0309*				

*Promalin™

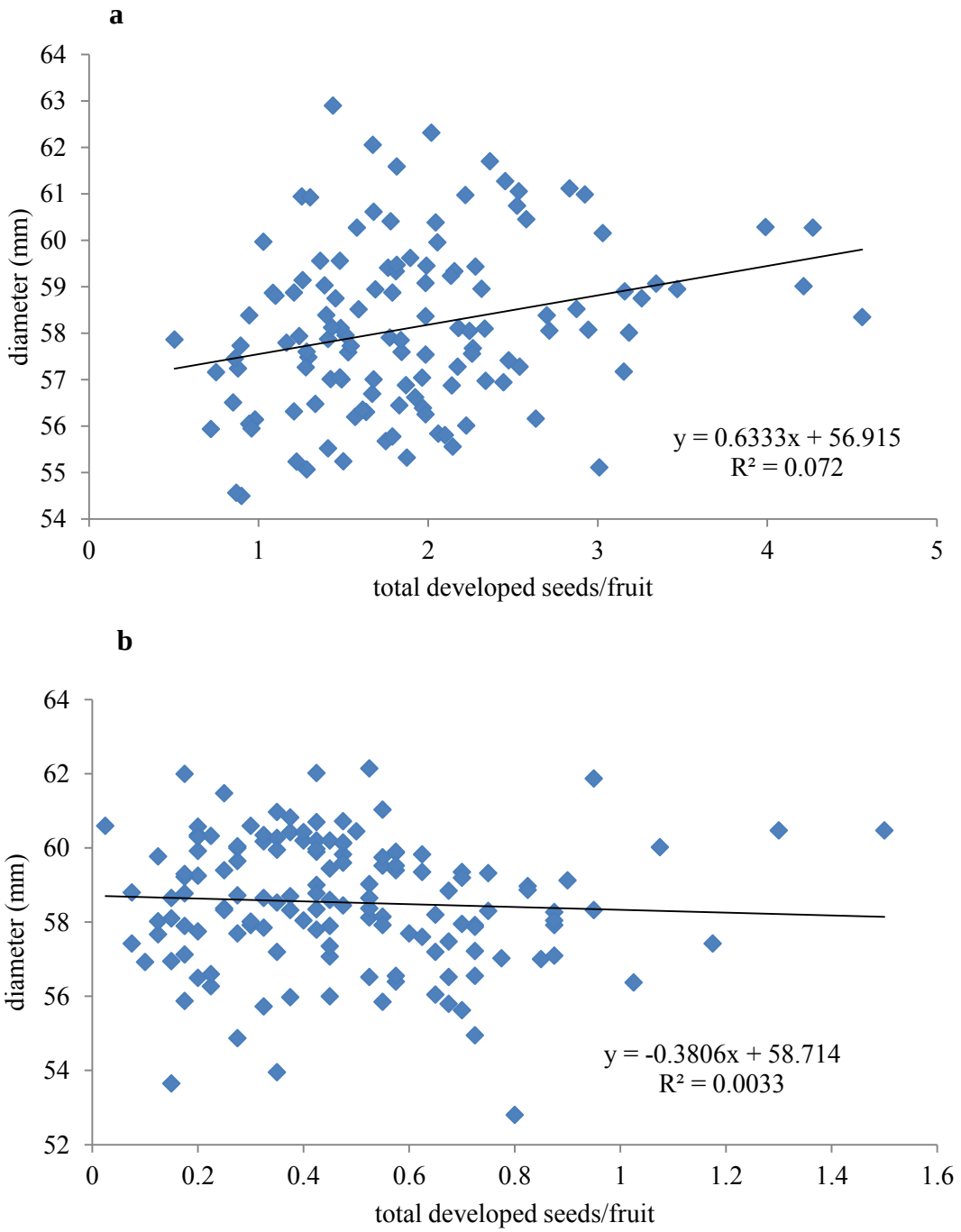

Figure 1: The correlation between the amount of fully developed seeds and fruit diameter (mm) for (a) „Forelle' (p = 0.0024) and (b)„Abate Fetel' (p = 0.5017) as recorded during the 2010/2011 and 2011/2012 seasons.

PAPER 2: The efficacy of 6-benzyladenine, gibberllins$_{4+7}$ and prohexadione-calcium to increase fruit size in 'Forelle' and 'Abate Fetel' pear

Keywords: gibberellins$_{4+7}$ (GA$_{4+7}$), 6-benzyladenine (6-BA), prohexadione-calcium (P-Ca), fruit size

Abstract

Fruit size is an important marketing and quality parameter and has a significant effect on the economic value of fruit. The application of synthetic cytokinins are believed to enhance fruit size by stimulating and extending the cell division period in fruit when applied at the correct stage of fruit growth. In addition, the combination of prohexadione-calcium (P-Ca) with gibberellins$_{4+7}$ (GA$_{4+7}$) was used successfully on Japanese pear (*Pyrus pyrifolia* Nakai) and 'Bing' sweet cherry to improve fruit size. This combination of GA$_{4+7}$ and P-Ca was evaluated and combined with 6-benzyladenine (6-BA) treatments on European pear (*Pyrus communis* L.) cultivars, Forelle and Abate Fetel to see if a similar effect on fruit size could be achieved under South African growing conditions. On both 'Forelle' and 'Abate Fetel' the combination of GA$_{4+7}$ and P-Ca increased fruit size but this was more pronounced in 'Abate Fetel'. 6-BA did not improve fruit size in these two cultivars over two consecutive seasons.

INTRODUCTION

Assuming that larger fruit size and good fruit quality are desirable attributes and important marketing parameters, treatments that increase average fruit diameter and quality may have significant economic value (Flaishman et al., 2001). There are two commercial

practices commonly applied to enlarge fruit (Stern, 2008); one is the indirect method of thinning flowers or fruitlets to reduce competition between fruit for assimilates, resulting in larger fruit (Stern et al., 2003); the second method directly enhances fruit size by stimulating and extending cell division, e.g. through the application of synthetic cytokinins (Shargal et al., 2006).

There are two distinct stages in pear fruit growth, Stage I, the first 42 to 56 days of development which is the cell division period and is followed by stage II, the cell enlargement period which continues until harvest (Bain, 1961). Early cell division during the first stage of fruit growth is influenced by endogenous plant growth hormones, especially cytokinins, while exogenously applied cytokinins induce non-dividing fruit cells to enter the cell cycle (Looney, 1993). During stage II endogenous gibberellins (GAs) function at cellular level by elongating and expanding cells (Brock and Kaufman, 1991), thus increasing fruit size. The use of exogenous growth regulators thus offers an effective means of modifying fruit growth and development (Tukey, 1974).

Exogenous cytokinin, 6-benzyladenine (6-BA), applied two weeks after full bloom resulted in an increase in fruit size in the pear cultivars Spadona and Coscia (Stern and Flaishman, 2003). In „Spadona', 6-BA increased fruit size without causing a dramatic thinning effect (Flaishman et al., 2001), which suggests that the increase in fruit size can be attributed mainly to a direct effect on an increased rate of cell division in the fruit cortex (Stern and Flaishman, 2003). In „Coscia' however, the increase in large fruit was accompanied by a heavy thinning effect, therefore the increase was possibly achieved in two ways; directly through cell division and indirectly through thinning indicating that cultivars respond differently to the same treatment (Stern and Flaishman, 2003). Currently many growers in the Elgin area, South African, are applying 6-BA on pears around 30 days after

full bloom (d.a.f.b.) believing it increases fruit size (Dr. J.J.B. Pretorius, personal communication).

A combination of gibberellins$_{4+7}$ (GA_{4+7}) and prohexadione-calcium (P-Ca) have been used successfully to promote pear fruit growth in *Pyrus pyrifolia* Nakai (Itai et al., 2009). In the GA biosynthesis pathway, inactive GA_9 is converted to GA_4 (active), which in turn is metabolised to GA_3 (active) (Rademacher et al., 2006). P-Ca inhibits both these processes (Rademacher et al., 2006). If exogenous GA_4 is applied in combination with P-Ca, the breakdown of the applied GA_4 to inactive forms will be inhibited, resulting in the persistence of the applied GA_4 which should contribute to cell enlargement (Itai et al., 2009).

In this paper we report on the efficacy of the combination of GA_{4+7} and P-Ca alone or together with 6-BA to improve fruit size in European pear (*Pyrus communis* L.) cultivars, Forelle and Abate Fetel under South African growing conditions.

MATERIALS AND METHODS

Site background, experimental design and treatments

Trials on „Abate Fetel' and „Forelle' pear were conducted in the Elgin Valley, Western Cape (Mediterranean type climate), South Africa during the 2010/2011 and 2011/2012 seasons on the farm Oak Valley. „Abate Fetel' trees on BP1 rootstock, planted in 1996 at a spacing of 4 x 1.2 m and „Forelle' trees on BP1 rootstock, planted in 1998 at a spacing of 4 x 1 m were used. Trees uniform in height, stem circumference and blossom density were chosen. Full bloom and harvest dates for the two consecutive seasons are summarized in Table 1.

The trials were laid out as randomized complete block designs with four treatments and ten replicates for both „Forelle' and „Abate Fetel'. Each plot consisted of two trees receiving the same treatment with one buffer tree between treatments. All trees were trunk

girdled with a Felco 600™ saw during full bloom and flower initiation (November) as a standard farm practice. The following plant growth regulators (PGRs) were applied in different combinations at different phenological stages as summarised in Table 2; MaxCel™ (Valent BioSciences Corporation, USA) containing 6-BA, Regulex™ (Valent BioSciences Corporation, USA) containing GA_{4+7} and Regalis™ (BASF (Pty.) Ltd.) containing P-Ca. All treatments were applied using a Stihl™ motorized knapsack sprayer at 1000 $L.ha^{-1}$. A non-ionic surfactant, Dash™ ec (BASF (Pty.) Ltd.) at 60 ml.100 L^{-1} water was added to Regulex™ and Regalis™ applications. The trials were repeated during the second season (2011/2012) on the same trees as well as on a new set of trees in the same orchards, but on single tree plots, with one buffer tree between treatments.

Data collected

The total number of fruit thinned per plot was recorded during commercial hand thinning as an indication of fruit set. The standard farm practice was used which was to thin all fruit clusters to two fruitlets per cluster. Trunk circumference was recorded approximately 5 cm above the graft union during July. Yield per treatment was recorded at harvest by weighing all fruit harvested per plot and expressed as kg per trunk cross sectional area. At harvest a sample of 30 fruit per plot was collected randomly at chest height from the trees. The fruit sample was analysed for fruit diameter, weight, length, number of developed seeds per fruit, firmness with an 8 mm penetrometer (Southtrade™) on two pared sides of the fruit (during the first season) and recording of malformed fruit. Fruit were classed as malformed (1) or normal (0), with any kind of malformation classified as malformed (1). Two representative branches were tagged in the lower half of the tree during winter. During July following each season, the average one-year-old shoot length per plot was determined by measuring 20 one-year-old shoots on the tagged branches per plot, starting from the tip of the

branch, measuring 10 shoots per side of each treated plot. Return bloom was recorded on the same tagged branches during full bloom following both seasons. The total number of flower clusters relative to the total number of buds sprouting was recorded to determine the return bloom percentage.

The general linear models (GLM) procedure of the Statistical Analysis System (SAS Enterprise Guide 4) was used to analyse the data. Pairwise t-tests were used to compare treatment means when the ANOVA showed significant differences ($P < 0.05$) between treatments.

RESULTS AND DISCUSSION

'Forelle'

There were no significant differences between treatments in fruit weight, diameter or fruit length during the 2010/2011 season (Table 3). There were also no significant differences between treatments in fruit weight and fruit length during the 2011/2012 season, although there was a significant increase in fruit diameter with the 6-BA, GA_{4+7} and P-Ca combination treatment and the GA_{4+7} and P-Ca combination treatment yielding fruit with significantly larger diameter relative to the untreated control (60.8 mm, 60.3 mm versus 58 mm, respectively) (Table 4). In this trial, repeated during the second season (2011/2012) on a new set of trees, there were no significant differences between treatments in average fruit weight, diameter, fruit length or yield efficiency (Table 5). The effect on fruit diameter that occurred during 2011/2012 in the initial fruit size trial could be due to a direct PGR effect or differences in crop load. There were no significant differences between treatments in yield efficiency during the 2010/2011 season (Table 3), but differences occurred during the 2011/2012 season (Table 4). The untreated control treatment yielded significantly more fruit (0.07 $kg.cm^2$) than the two treatments containing GA_{4+7} and P-Ca (0.05 $kg.cm^2$ and 0.06

kg.cm^2, respectively) (Table 4). These differences could be due to the untreated control treatment having a significantly higher return bloom percentage relative to these two treatments following the 2010/2011 season (Table 3). Yield efficiency was used as a covariate in the 2011/2012 season to determine if differences in yield could account for the differences between treatments in average fruit diameter, but although the covariate was not significant it made the treatment differences more significant, indicating that differences in fruit size was partially due to yield differences (Table 4). The number of fruitlets thinned by hand after physiological fruit drop was also used as covariate on fruit diameter. The covariate was significant and removed treatment differences indicating that treatment effects could have been attributed to a difference in initial fruit set. These differences were removed by hand thinning and are therefore no longer reflected in the yield efficiency. The number of fruitlets thinned by hand however did not differ significantly between treatments in either season, but as the covariate indicated, was still correlated to the fruit diameter (Table 3 and 4).

There were no significant differences between treatments in fruit firmness during the first season of the trial (Table 3), and therefore this parameter was not measured the following season. During both seasons, there were no significant differences between treatments in the average number of developed seeds formed per fruit (Table 3 and 4). In the trial repeated during the second season (2011/2012) on a new set of trees, there were no significant differences between treatments in the number of fruitlets thinned or the seed content (Table 5).

There were no significant differences between treatments in one-year-old shoot length for either of the 2 seasons (Table 3 and 4). This is in contrast to results from Meintjies et al., (2005), who found a reduction in shoot growth in „Forelle' with one application of P-Ca (125 mg.L^{-1}) at 5-10 cm shoot length. Smit et al. (2005) also found a slight reduction in shoot

growth with 125 mg.L^{-1} P-Ca applied at 5-10 cm shoot length, but only during the second season of the trial.

After treatments were applied during the 2010/2011 season, there were significant differences in return bloom, with the two treatments containing GA_{4+7} and P-Ca resulting in a significantly lower return bloom percentage (5.9% and 6.9%, respectively) than the untreated control (11%) (Table 3). As mentioned, this could have impacted on yield efficiency in the 2011/12 season. Vanthournout et al. (2008) also found that P-Ca (100 mg.L^{-1}) reduced return bloom on „Conference' pear when applied at the end of bloom, followed by four weekly applications of 25 mg.L^{-1}. P-Ca, applied at 70 mg.kg^{-1} at 75% bloom, resulted in a significant reduction in return bloom relative to the control during the first season of the trial reported on in Paper 1. Smit et al. (2005) also found that P-Ca (250 mg.L^{-1}) applied at 5-10 cm shoot length reduced return bloom in „Forelle'. There were however no significant differences in return bloom following the 2011/2012 season, in the trial repeated on the same trees or on the trial conducted on the set of new trees (Table 4 and 5). The variation in return bloom over seasons can partly be because P-Ca forced the tree into an alternate bearing pattern as can be seen in the lower yield efficiency during the second season, leading to a higher return bloom percentage after the second season of P-Ca treatment.

The combination of GA_{4+7} and P-Ca in the initial fruit size trial resulted in a slight improvement in fruit size (diameter) in „Forelle' during the 2011/2012 season. It may be worthwhile to evaluate this combination further, increasing application rates and changing to earlier applications. The fact that the treatment containing only 6-BA did not increase fruit size in either of the seasons, is in contrast to Stern and Flaishman (2003) who found an increase in fruit size in „Spadona' and „Coscia' following a 6-BA application at 100 mg.L^{-1}, 14 d.a.f.b.. This proves that pear cultivars do not all respond significantly to 6-BA applications to improve fruit size.

'Abate Fetel'

During the 2010/2011 season, there were no significant differences in average fruit weight (P = 0.0650) between treatments, although trees treated with GA_{4+7} in combination with P-Ca yielded the largest fruit relative to the untreated control with 191.3 g and 179.6 g average fruit weight, respectively (Table 6). During the 2011/2012 season there were also no significant differences between treatments in fruit weight (P = 0.0576), although the treatment containing 6-BA, GA_{4+7} and P-Ca achieved the highest fruit weight relative to the untreated control with 180.4 g and 166.1 g average fruit weight, respectively (Table 7). The GA_{4+7} and P-Ca combination treatment significantly improved average fruit diameter relative to the untreated control and the 6-BA treatment during 2010/2011 (61.4 mm versus 59.6 mm and 59.6 mm, respectively). There were no significant differences between treatments in average fruit length (Table 6). During the 2011/2012 season the two treatments containing GA_{4+7} and P-Ca yielded fruit with significantly larger diameters than the untreated control and the 6-BA treatment, while there were no significant differences between treatments in fruit length (Table 7). No significant differences between treatments were found in yield efficiency or fruit set, as determined by the number of fruitlets thinned by hand, during both seasons (Table 6 and 7). Yield efficiency and the number of fruitlets thinned were used as covariates to determine it's effect on fruit weight and diameter. Although none of the covariates were significant, both reduced the significance level of the treatments for fruit weight and diameter during both seasons, indicating that fruit size differences occurred mainly due to a direct effect of the applied PGRs (Table 6 and 7). In the trial repeated during the second season (2011/2012) on the new set of trees, there were no significant differences between treatments for average fruit weight, diameter, fruit length, yield efficiency or the number of fruitlets thinned during hand thinning (Table 8). Although not

significant, the trend indicated that GA_{4+7} and P-Ca increased fruit size. As one-tree plots was used in this trial compared to the two-tree plots in the initial trial, more variation probably occurred between treated plots.

There were no significant differences between treatments for fruit firmness during the 2010/2011 season. There were also no significant differences for the number of developed seeds in fruit over two consecutive seasons in the initial fruit size trial or in the trial repeated on a new set of trees during 2011/2012. Malformation of fruit was noted over both seasons, but only 0.63% of fruit displayed slight malformation but no significant differences were found between treatments (data not shown) and malformation is therefore not a problem.

There were no significant differences between treatments in average one-year-old shoot length after each season for 2 consecutive seasons (Table 6 and 7). P-Ca thus did not reduce shoot growth, as was also the case in the „Forelle' trial. There were also no significant differences in return bloom percentage after the 2010/2011 season, although a trend showed that treatments containing GA_{4+7} and P-Ca responded with a lower return bloom percentage than the untreated control and 6-BA treatment (Table 6). According to Deckers and Schoofs (2004), P-Ca is very effective as a growth retardant on apple trees, but much less effective on pear trees and potentially has a negative effect on return bloom. After the 2011/2012 season, there were significant differences between treatments in return bloom with the 6-BA treatment, resulting in a significantly higher return bloom percentage relative to all other treatments (Table 6). In the trial repeated on the new set of trees during the 2011/2012 season, there were no significant differences between treatments for return bloom. However, there was a trend indicating improved return bloom after a 6-BA application (Table 7). Theron at al. (2011), found that 6-BA at 50 $mg.L^{-1}$ significantly increased return bloom in „Early Bon Chrétien' pear.

As with „Forelle', 6-BA applied 30 and 50 d.a.f.b. did not improve fruit size in „Abate Fetel', although it increased return bloom after the second season of application. Therefore the application of 6-BA as currently practised by growers should only be recommended for improving return bloom, and be discouraged for application to improve fruit size. GA_{4+7} and P-Ca applied 65 and 80 d.a.f.b. had a significant effect on improving fruit diameter during both seasons, in „Abate Fetel', similarly to „Forelle'.

CONCLUSION

PGRs offered an effective way of modifying fruit growth and development, albeit slightly, as was also concluded by Tukey (1974). On both cultivars 6-BA on its own did not improve fruit size and this practice by growers should be discontinued. On both „Forelle' and „Abate Fetel' the combination of GA_{4+7} and P-Ca increased fruit size, although it was more pronounced in „Abate Fetel'. However it had a negative effect on return bloom which could not be alleviated by the addition of 6-BA. Zhang and Whiting (2011) also reported that a combination of GA_{4+7} (30 $mg.L^{-1}$) with P-Ca (150 $mg.L^{-1}$) applied to „Bing' cherry 30 days after anthesis improved fruit size. According to Itai et al. (2009), the mechanism of action of the simultaneous application of GA_{4+7} and P-Ca is explained by the inhibition of the breakdown of GA_4 into inactive forms, resulting in the persistence of GA_4 in the fruit, which then contribute towards cell enlargement. More research is needed on the phonological stage of application, application rate, number of applications per seasons, and GA_{4+7} and P-Ca persistence in the fruit, to determine whether these PGRs can be used commercially for these two pear cultivars.

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Table 1: Dates of full bloom, petal drop, hand thinning and harvest for „Forelle' and „Abate Fetel' during two consecutive seasons.

Phenological stage	2010/2011 season	2011/2012 season
	„Forelle'	
Full bloom	9 September 2010	14 September 2011
Petal drop	16 September 2010	23 September 2011
Hand thinning	5 October 2010	18 October 2011
Harvest	2 March 2011	5 March 2012
	„Abate Fetel'	
Full bloom	17 September 2010	21 September 2011
Petal drop	27 September 2010	28 September 2011
Hand thinning	19 October 2010	20 October 2011
Harvest	1 February 2011	6 February 2012

Table 2: Treatments applied to increase fruit size on „Forelle' and „Abate Fetel' during the 2010/2011 and the 2011/2012 season.

Treatments
1. Untreated control
2. 95 $mg.L^{-1}$ 6-BA at 30 and 50 d.a.f.b. + 30 $mg.kg^{-1}$ GA_{4+7} and 70 $mg.kg^{-1}$ P-Ca 65 at 80 d.a.f.b.
3. 95 $mg.L^{-1}$ 6-BA at 30 and 50 d.a.f.b
4. 30 $mg.kg^{-1}$ GA_{4+7} and 70 $mg.kg^{-1}$ P-Ca at 65 and 80 d.a.f.b.

Table 3: Effect of different combinations of plant growth regulators on fruit weight, fruit diameter, fruit length, yield efficiency, average number of fruitlets thinned by hand, fruit firmness, seed percentage, shoot length and return bloom on „Forelle' during the 2010/2011 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fruitlets thinned by hand	Average fruit firmness (kg)	Average number of fully developed seeds	Average 1-year-old shoot length (cm)	% Return bloom on two tagged branches
Untreated control	108.64 ns	56.82 ns	71.82 ns	0.09 ns	127.70 ns	6.07 ns	0.59 ns	18.18 ns	11.04 a
6-BA 30 and 50 d.a.f.b.* + GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	103.18	55.69	70.63	0.09	177.80	6.25	0.48	18.24	5.92 b
6-BA 30 and 50 d.a.f.b.	109.82	56.75	71.84	0.09	173.50	6.30	0.57	20.81	7.67 ab
GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	113.53	57.43	73.72	0.10	134.50	6.03	0.43	16.87	6.89 b
Significance level	*0.0935*	*0.1161*	*0.2950*	*0.8248*	*0.0821*	*0.3936*	*0.7238*	*0.8196*	*0.0386*

*days after full bloom

Table 4: Effect of different combinations of plant growth regulators on fruit weight, fruit diameter, fruit length, yield efficiency, average number of fruitlets thinned by hand, seed percentage, shoot length and return bloom on „Forelle' during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fruitlets thinned by hand	Average number of fully developed seeds	Average 1-year-old shoot length (cm)	% Return bloom on two tagged branches
Untreated control	120.99 ns	58.00 c	76.19 ns	0.07 a	69.10 ns	0.49 ns	31.48 ns	13.79 ns
6-BA 30 and 50 d.a.f.b.* + GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	135.91	60.78 a	77.52	0.05 c	58.40	0.95	32.67	16.73
6-BA 30 and 50 d.a.f.b.	123.65	58.48 bc	76.54	0.07 ab	75.40	0.40	30.97	14.80
GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	131.47	60.29 ab	76.62	0.06 bc	53.00	0.79	30.02	11.37
Significance level	*0.1355*	*0.0290*	*0.8208*	*0.0021*	*0.2496*	*0.0643*	*0.9091*	*0.7311*
Covariate (yield efficiency)		*0.0745*						
Treatment		*<0.0001*						
Covariate (fruitlets thinned)		*0.0016*						
Treatment		*0.0708*						

*days after full bloom

Table 5: Effect of different combinations of plant growth regulators on fruit weight, fruit diameter, fruit length, yield efficiency, average number of fruitlets thinned by hand, seed percentage and return bloom on „Forelle' on a new set of trees during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fruitlets thinned by hand	Average number of fully developed seeds	% Return bloom on two tagged branches
Untreated control	113.03 ns	56.96 ns	74.95 ns	0.07 ns	36.75 ns	1.07 ns	8.43 ns
6-BA 30 and 50 d.a.f.b.* + GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	120.54	58.16	77.08	0.07	35.50	0.99	7.42
6-BA 30 and 50 d.a.f.b.	114.65	57.16	75.58	0.07	36.50	0.69	10.02
GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	117.55	57.71	75.20	0.10	31.50	0.66	9.88
Significance level	*0.4761*	*0.5327*	*0.3314*	*0.0868*	*0.9527*	*0.7372*	*0.9267*

Table 6: Effect of different combinations of plant growth regulators on fruit weight, fruit diameter, fruit length, yield efficiency, average number of fruitlets thinned by hand, fruit firmness, seed percentage, shoot length and return bloom on „Abate Fetel' during the 2010/2011 season. Treatment means followed by the same letter are not significantly different at 5 % (LSD).

Treatments	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fruitlets thinned by hand	Average fruit firmness (kg)	Average number of fully developed seeds	Average 1-year-old shoot length (cm)	% Return bloom on two tagged branches
Untreated control	179.60 ns	59.63 b	116.03 ns	0.07 ns	127.50 ns	6.55 ns	0.26 ns	19.72 ns	17.95 ns
6-BA 30 and 50 d.a.f.b.* + GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	183.59	60.17 ab	117.40	0.07	141.63	6.71	0.24	19.55	16.82
6-BA 30 and 50 d.a.f.b.	175.65	59.55 b	114.35	0.06	126.00	6.92	0.15	20.33	19.08
GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	191.32	61.42 a	116.37	0.05	105.50	6.41	0.10	29.42	12.84
Significance level	*0.0650*	*0.0246*	*0.4811*	*0.2321*	*0.4952*	*0.1244*	*0.3745*	*0.0563*	*0.1326*
Covariate (yield efficiency)	*0.8148*	*0.7083*							
Treatment	*0.1394*	*0.0608*							
Covariate (thinned fruit)	*0.4018*	*0.7200*							
Treatment	*0.0964*	*0.0456*							

Table 7: Effect of different combinations of plant growth regulators on fruit weight, fruit diameter, fruit length, yield efficiency, average number of fruitlets thinned by hand, seed percentage, shoot length and return bloom on „Abate Fetel' during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fruitlets thinned by hand	Average number of fully developed seeds	Average 1-year-old shoot length (cm)	% Return bloom on two tagged branches
Untreated control	166.14 ns	58.64 b	117.27 ns	0.14 ns	227.50 ns	0.45 ns	42.37 ns	13.57 b
6-BA 30 and 50 d.a.f.b.* + GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	180.42	60.70 a	118.78	0.15	252.75	0.83	43.74	10.79 b
6-BA 30 and 50 d.a.f.b.	170.40	58.50 b	118.11	0.13	267.25	0.44	47.20	20.96 a
GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	177.18	60.26 a	116.88	0.13	246.38	0.63	44.90	11.63 b
Significance level	*0.0576*	*0.0021*	*0.7967*	*0.2512*	*0.9147*	*0.2097*	*0.4334*	*0.0109*
Covariate (yield efficiency)	*0.7807*	*0.5248*						
Treatment	*0.0586*	*0.0039*						
Covariate (thinned fruit)	*0.5274*	*0.7639*						
Treatment	*0.0672*	*0.0027*						

Table 8: Effect of different combinations of plant growth regulators on fruit weight, fruit diameter, fruit length, yield efficiency, average number of fruitlets thinned by hand, seed percentage and return bloom on „Abate Fetel' on a new set of trees during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5 % level (LSD).

Treatments	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm^2 cross sectional area)	Average number of fruitlets thinned by hand	Average number of fully developed seeds	% Return bloom on two tagged branches
Untreated control	189.98 ns	61.02 ns	122.40 ns	0.18 ns	120.75 ns	0.88 ns	4.54 ns
6-BA 30 and 50 d.a.f.b.* + GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	185.48	61.16	120.11	0.16	129.88	0.96	8.76
6-BA 30 and 50 d.a.f.b.	189.02	60.63	123.20	0.17	120.25	0.52	11.97
GA_{4+7} and P-Ca 65 and 80 d.a.f.b.	196.51	61.99	121.90	0.16	98.63	0.84	8.83
Significance level	*0.3402*	*0.3188*	*0.6279*	*0.5960*	*0.4335*	*0.2182*	*0.2413*

PAPER 3: Extending the cell division phase in 'Forelle' and 'Abate Fetel' pear using plant growth regulators

Keywords: [N (2-chloro-4-pyridyl)-N' -phenylurea] CPPU, [N-phenyl-N' -1,2,3-thiadiazol-5-ylurea] TDZ, 6-benzyladenine (6-BA), [2,4-dichlorophenoxyacetic acid] (2,4-D), cell division

Abstract

Fruit size is an important marketing and quality parameter and has a significant effect on the economic value of fruit. The application of synthetic cytokinins and other plant growth regulators (PGRs) are believed to enhance fruit size by stimulating and extending the cell division period in fruit or improving cell size when applied at the correct stage of fruit growth. The PGRs N-phenyl-N' -1,2,3-thiadiazol-5-ylurea (TDZ), N (2-chloro-4-pyridyl)-N' -phenylurea (CPPU), 6-benzyladenine (6-BA) and 2,4-dichlorophenoxyacetic acid (2,4-D) successfully increased fruit size in the pear cultivars Coscia and Spadona in Israel. These PGRs were applied to 'Forelle' and 'Abate Fetel' to determine if a similar effect could be achieved. None of the growth regulators applied had a significant effect on fruit size. The stage when the cell division period in 'Forelle' and 'Abate Fetel' ends was also determined as being 34 and 45 days after full bloom, respectively which can be used in the future to better schedule the time of fruit size enhancement treatments.

INTRODUCTION

„Forelle' and „Abate Fetel' are two of the most profitable pear cultivars planted in South Africa, however it remains a challenge to achieve regular high yields with good fruit size. Final pear fruit size depends on the combined contributions of the number of cells present at fruit set, the number of subsequent cell divisions, and on cell expansion (Shargal et al., 2006).

It was determined by Zhang et al. (2006) that cell division is more important than cell enlargement in determining the final fruit size in Japanese pear (*Pyrus pyrifolia* Nakai). Bain (1961) found that cell division in the outer cortex, inner cortex and pith of „Williams Bon Chretien' pear became very active approximately 7 to 14 days after full bloom (d.a.f.b.), with the main cell division period lasting until approximately 28 d.a.f.b. Plant growth regulators (PGRs) offer a means of modifying fruit growth and development during this crucial period (Tukey, 1974).

Early cell divisions are influenced by endogenous plant growth hormones, especially cytokinins, while exogenous applied cytokinins induce non-dividing fruit cells to enter the cell cycle (Looney, 1993). Cytokinins are required to initiate cell proliferation in non-dividing tissues, and their continued presence is also needed to maintain mitotic activity (Werner et al., 2001). An increase in fruit size in pear following application of the synthetic cytokinins CPPU [N (2-chloro-4-pyridyl)-N' -phenylurea] or TDZ [N-phenyl-N' -1,2,3-thiadiazol-5-ylurea] also suggested that endogenous cytokinin levels are a major factor controlling fruit growth (Shargal et al., 2006). Exogenously applied cytokinin 6-benzyladenine (6-BA) also resulted in increased fruit size in „Spadona' and „Coscia' pear (Stern and Flaishman, 2003). Shargal et al. (2006) concluded that the endogenous levels of cytokinins may restrict expression of the full

developmental potential of pear fruit and that exogenous applied cytokinins indeed are effective in increasing fruit size by increasing the number of cell divisions.

Exogenously applied cytokinins specifically enhance cell division in pulp parenchyma cells, leading to a significant increase in fruit size (Shargal et al., 2006). Single applications of synthetic cytokinins CPPU or TDZ at 10 mm diameter fruit size (14 d.a.f.b.) had no effect on the size or number of stone cells during fruit development; however the parenchyma that forms the fruit flesh, between the epidermis and the seed layers had significantly smaller but more cells compared to untreated fruit (Shargal et al., 2006). This resulted in an increase in the diameter of the fruit by extending the phase of cell division (Shargal et al., 2006). Over five consecutive years, the application of 0.12% (v/v) Bolero™ [8.6 mg l–1 gibberellic acid (GA3) plus 6 mg l–1 2,4-dichlorophenoxyacetic acid (2,4-D) plus 6 mg l–1 naphthaleneacetic acid (NAA)] at full-bloom also resulted in a significant increase in fruit size in „Coscia' and „Spadona' pear in Israel (Stern et al., 2007).

The objective of this trial was to evaluate the efficacy of the growth regulators CPPU, TDZ, 6-BA and 2,4-D on „Abate Fetel' and „Forelle' pear to stimulate cell division during the first phase of fruit growth and observe the effect on final fruit size at harvest. The duration of the cell division period of these two cultivars was also determined with an aim to link it to the most effective stage of PGR applications in order to stimulate cell division.

MATERIALS AND METHODS

Site background, experimental design and treatments

The trials on „Abate Fetel' and „Forelle' pear were conducted on the farm Oak Valley in the Elgin Valley, Western Cape, (Mediterranean type climate) South Africa during the

2010/2011 and 2011/2012 seasons. „Abate Fetel' trees on BP1 rootstock planted in 1996 at a spacing of 4 x 1.2 m and „Forelle' trees on BP1 rootstock planted in 1998 at a spacing of 4 x 1 m were used. Trees uniform in height, stem circumference and blossom density were selected. Full bloom and harvest dates for the two consecutive seasons are summarized in Table 1.

The trials were designed in randomized complete blocks with five treatments and ten replicates for both „Forelle' and „Abate Fetel'. Each plot consisted of two trees receiving the same treatment with one buffer tree between treatments. During 2010/2011, different synthetic cytokinins were applied 14 d.a.f.b. when fruitlets were approximately 10 mm in diameter. In 2011/2012 the trial was repeated on the same trees but slight changes in treatments were made as summarised in Table 2. The 95 $mg.L^{-1}$ 6-BA treatment was substituted with 12 $mg.L^{-1}$ 2,4-D applied at full bloom and the 6-BA rate for the fifth treatment was doubled to 50 $mg.L^{-1}$ per application. All treatments were applied using a Stihl™ motorized knapsack sprayer at 1000 $L.ha^{-1}$.

Data collected

Fruit set, yield and fruit quality

The total number of fruit thinned per treated plot was recorded during commercial hand thinning as an indication of fruit set. The standard farm practice was used, which is to thin all fruit clusters to two fruitlets per cluster. Trunk circumference was recorded approximately 5 cm above the graft union. Yield per treatment was recorded at harvest by weighing all fruit harvested per two-tree plot and expressed as kg per cm cross sectional area. At harvest a sample of 30 fruit per plot was collected randomly from trees at chest height. The fruit sample was analysed for fruit diameter, weight, length, number of developed seeds per fruit, fruit firmness

with an 8 mm penetrometer (Southtrade™) on two pared sides and recording of malformed fruit as described in Paper 2. Two representative branches were tagged in the lower half of the tree during full bloom. Return bloom was recorded on these tagged branches during full bloom in the following season by counting the total number of flower clusters relative to the total number of buds sprouting and expressed as a percentage.

Fruit growth measurements

The diameter of ten fruit per treatment per block was measured every week from 19 d.a.f.b. until harvest. Five fruit were selected at chest height on each side of the plot. The first five fruit from the tip of a tagged branch were used for the weekly measurements. These measurements were used to set up growth curves during the first season of the trial (2010/2011).

Histological examinations

Histological examinations were made to determine when the cell division period ends in „Forelle' and „Abate Fetel' fruit. Two fruitlets (one from each side of the treated plot) from five replications of the untreated control (10 fruit), were collected weekly from 19 d.a.f.b. until 63 d.a.f.b. in „Forelle' and until 73 d.a.f.b. in „Abate Fetel', respectively. Fruit collection took place from uniform clusters where one fruit was removed from a cluster of two fruitlets. Collected fruit were stored in an ethanol (50%), formaldehyde (10%), acetic acid (5%) and distilled water (35%) (FAA) solution at room temperature until further examination. Fruitlet diameter at the widest part of the fruit was measured. Cell number and cell length were measured according to a slightly modified version of the method developed by Zhang et al. (2005). A cross sectional cut was made through the widest part of the pear and the core (the central part which includes seeds

and vascular bundles) diameter was measured. The mesocarp width was determined by subtracting the core diameter from the fruit diameter. A transverse slice of mesocarp along the widest part of the fruitlet was removed and dehydrated in an increasing ethanol series and stored in acetone before exposing it to a critical drying process. The sample was then coated with a thin layer of gold in order to make the sample surface electrically conducting. Imaging from the centre of the mesocarp was accomplished using a Leo® 1430VP Scanning Electron Microscope. Beam conditions during surface analysis were 7 KV and approximately 1.5 nA, with a working distance of 13 mm and a spot size of 150. Cell length of seven adjacent cells from ten observation zones per transverse slice was measured to determine cell size, where after the average cell length was calculated by dividing the total length measured per transverse slice by seventy. Cell number in the mesocarp was then calculated by dividing the mesocarp width by the average cell length which was used as an indicator of the total number of cells in the fruit.

Data analysis

The general linear models (GLM) procedure of the Statistical Analysis System (SAS Enterprise Guide 4) was used to analyse the data. Pairwise t-tests were used to compare treatment means when the ANOVA showed significant differences between treatments ($Pr > 0.05$).

RESULTS

'Forelle'

No growth regulator treatment increased fruit weight or diameter significantly relative to the untreated control during the 2010/2011 season, although a significant difference in fruit

weight occurred between the CPPU, TDZ and 4 x 24 mg.L^{-1} 6-BA treatments relative to the 1 x 95 mg.L^{-1} 6-BA treatment, which resulted in the lowest fruit size and diameter (Table 3). During 2010/2011, CPPU treated trees yielded fruit with significantly greater length relative to all other treatments and the unsprayed control (Table 3). There were no significant differences between treatments in the number of fruitlets thinned by hand, after physiological fruit drop, or yield efficiency during the 2010/2011 season (Table 3). There were also no significant differences between treatments in the number of fully developed seeds in fruit during 2010/2011 (Table 3). There were no significant differences between treatments in fruit firmness during 2010/2011 (Table 3). There was however significant differences in the return bloom percentage following the 2010/2011 season, with the 95 mg.L^{-1} 6-BA treatment resulting in the highest return bloom percentage which differed significantly from all other treatments (Table 3). CPPU and TDZ treated trees resulted in a significantly lower return bloom percentage relative to the control (Table 3). The number of fruitlets thinned and yield efficiency did not have any significant effect as covariate on fruit weight or length during 2010/2011 (Table 3).

During the 2011/2012 season there were significant differences between treatments in fruit weight (Table 4). Following the CPPU and TDZ treatments, fruit weight was significantly higher than in 6-BA and 2,4-D treatments, while the 6-BA treatment resulted in significant lower fruit weight relative to the control (Table 4). Fruit diameter during 2011/2012 was significantly smaller in the 6-BA treatment relative to the untreated control, CPPU and TDZ treatments (Table 4). There were significant differences in fruit length during the 2011/2012 season with the CPPU treatment resulting in the greatest fruit length, not differing significantly from TDZ, but differing significantly from the untreated control, 2,4-D and 6-BA treatment (Table 4). There were significant differences between treatments in yield efficiency during the 2011/2012 season

with the CPPU and TDZ treatments resulting in significantly lower yields relative to all other treatments and the control (Table 4). There were significant differences between treatments in the number of fruitlets thinned during the 2011/2012 season, with the 2,4-D treatment resulting in significantly higher fruit set relative to all other treatments (Table 4). During 2011/2012 there were significant differences in the number of developed seeds per fruit with the 2,4-D treatment resulting in the highest seed content, but not differing significantly from the untreated control (Table 4). There were no significant differences between treatments in fruit firmness during 2011/2012 (Table 4). There were significant differences between treatments in return bloom following the 2011/2012 season, with the 6-BA treatment and control resulting in the highest return bloom (Table 4). The TDZ treatment resulted in the lowest return bloom percentage and differed significantly from the control and the 6-BA treatment (Table 4). The 2,4-D treatment also resulted in a significant lower return bloom percentage than the 6-BA treatment (Table 4). Covariate analysis were undertaken where the number of thinned fruitlets and yield efficiency were included to see if it had an effect on fruit weigh, diameter and length during 2011/2012 (Table 4). The number of fruitlets thinned as covariate for fruit weight was significant and removed significant differences between treatments (Table 4). Yield efficiency as covariate for fruit weight was not significant in explaining treatment differences (Table 4). The number of fruitlets thinned and yield efficiency as covariates was not significant in explaining differences between treatments in diameter during 2011/2012 (Table 4). Both the number of fruitlets thinned and yield efficiency as covariates were significant for fruit length during 2011/2012, and treatment significance levels were less significant after inclusion of the covariates (Table 4).

'Abate Fetel'

There were no significant differences between treatments during the 2010/2011 season in fruit weight, diameter or fruit length (Table 5). There were also no significant differences between treatments in the number of fruitlets thinned, yield efficiency, the number of developed seeds per fruit or fruit firmness (Table 5). There was significant differences between treatments in return bloom following the 2010/2011 season (Table 5). The 4 x 24 $mg.L^{-1}$ 6-BA treatment resulted in the highest return bloom percentage but it did not differ significantly from the untreated control or the single BA treatment. CPPU resulted in the lowest return bloom percentage and differed significantly from the control and the two 6-BA treatments (Table 5). The TDZ treatment also resulted in a low return bloom percentage and differed significantly from the 4 x 24 $mg.L^{-1}$ 6-BA treatment (Table 5).

During 2011/2012, there were significant differences in fruit weight with CPPU and TDZ resulting in the highest average fruit weight relative to the 2,4-D and 6-BA treatment, but it did not differ significantly from the untreated control (Table 6). CPPU and TDZ treated trees also yielded fruit with significantly greater diameter relative to all other treatments and the control (Table 6). There were no significant differences between treatments in fruit length and yield efficiency during 2011/2012 (Table 6). The number of fruitlets thinned differed between treatments, with the 2,4-D treatment resulting in a significant higher fruit set relative to all other treatments and the untreated control (Table 6). TDZ resulted in the highest number of seeds per fruit, which differed significantly from the untreated control and the CPPU treatment in seed content, but not from the 2,4-D and 6-BA treatments (Table 6). There were no significant differences in average fruit firmness during 2011/2012 (Table 6). Significant differences occurred in return bloom following the 2011/2012 season with the 6-BA and untreated control

treatment resulting in a significantly higher return bloom percentage relative to the CPPU, TDZ and 2,4-D treatments (Table 6). Both the number of fruitlets thinned and yield efficiency were significant as covariates for fruit weight, but the treatment effect became non-significant after using the thinned fruit covariate and less significant following the yield efficiency as covariate (Table 6). After doing covariate analysis on fruit diameter, both covariates were significant but the differences between treatments stayed significant, although slightly less so (Table 6).

No significant malformation was observed during both seasons in both cultivars with less than 1.2% of all sampled fruit that were malformed (data not shown).

Fruit and cell measurements

With weekly fruit measurements on „Forelle' and „Abate Fetel' during 2010/2011 there were no differences in growth rate between treatments as all lines on the graph had a similar gradient (Fig. 1). In „Forelle' the curve started to flatten at approximately 150 d.a.f.b. after an initial linear increase in fruit size, which indicates that fruit growth rate decreased with approaching maturity but overall fruit growth increased at approximately 0.33 $mm.day^{-1}$ (Fig. 1a). In „Abate Fetel' fruit diameter increased linearly from 18 d.a.f.b. until harvest at approximately 0.44 $mm.day^{-1}$ (Fig. 1b).

The increase in cortex cell number of „Forelle' and „Abate Fetel' control fruit during 2010/2011 was explained by fitting a logarithmic curve on data points, as it is suspected that points before 18 d.a.f.b. (before fruit collection started) would have a steeper gradient that decreased as fruit developed (Fig. 2). The Pearson correlation coefficient between cell number and days after full bloom during the 2010/2011 season was $r^2 = 0.8062$ and $r^2 = 0.9185$ on „Forelle' and „Abate Fetel', respectively (Fig. 2). The critical point when the slope of the fitted

curve was below 0.5 cells.day^{-1} was regarded as the point where the cell division rate started to decrease or ended (Zhang et al., 2005). This critical point was 34 and 45 d.a.f.b. for „Forelle' and „Abate Fetel', respectively (Fig.2). After the critical points were reached for both „Forelle' and „Abate Fetel', cell division still continued at a fairly rapid rate as curves did not flatten after the critical points. The increase in cell length in both „Forelle' and „Abate Fetel' was linear with Pearson correlation coefficients of $r^2 = 0.8351$ and $r^2 = 0.9711$, respectively (Fig. 2). In SEM images of „Forelle' mesocarp, there were still a few dividing cells visible at 63 d.a.f.b, although they were much fewer than at 19 d.a.f.b. (Fig. 3). In „Abate Fetel' there were also only a few dividing cells visible at 73 d.a.f.b. with many more dividing cells visible at 19 d.a.f.b (Fig. 4).

DISCUSSION

Final fruit size is influenced by many factors starting from flower initiation during the previous season until harvest, which includes the number of cells present at full bloom (Shargal et al., 2006), and the growth rate of the fruit itself until harvest (Theron, 2011). Treatments with synthetic cytokinins CPPU and TDZ yielded significantly larger fruit than the 2,4-D and 6-BA treatment during the second season of the trials on „Forelle' and „Abate Fetel', but not significantly larger than the untreated control. In contrast, Shargal et al. (2006) found an increase in fruit size in „Coscia' and „Spadona' pear, with 20 µl.L^{-1} CPPU and 15 µl.L^{-1} TDZ applied at 10 mm fruit size (14.d.a.f.b.). In both „Forelle' and „Abate Fetel', we found no increase in fruit size with 6-BA application at 10 mm fruit size during both seasons. This is in contrast with Stern and Flaishman (2003), who found an increase in fruit size on „Coscia' and „Spadona' with 6-BA at 100 mg.L^{-1}, applied at the same phenological stage. Fruit size enhancement was also achieved on „Williams' pear with 6-BA when applied at 10-15 mm fruit

diameter (Gimenez et al., 2010). More research is therefore needed on these synthetic cytokinins to determine if a higher rate or more applications could have a greater effect in stimulating cell division which would lead to bigger fruit. The positive effect of 6-BA on return bloom was again confirmed by these trials, also reported in Paper 2 on „Abate Fetel'. Theron et al. (2011) also found that 6-BA at 50 $mg.L^{-1}$ significantly increased return bloom on „Early Bon Chrétien' pear. In contrast to 6-BA, synthetic cytokinins CPPU and TDZ had an overall negative effect on return bloom on both „Forelle' and „Abate Fetel'.

The application of 2,4-D at full bloom during the second season of the trial did not increase fruit size in „Forelle' and „Abate Fetel'. This was in contrast with the increase observed by Stern et al. (2007) on „Coscia' and „Spadona' with Bolero™ [8.6 mg l–1 gibberellic acid (GA_3) plus 6 mg l–1 2,4-dichlorophenoxyacetic acid (2,4-D) plus 6 mg l–1 naphthaleneacetic acid (NAA)]. Bolero™ could have been more effective due to the combination of GA_3 and NAA with 2,4-D in Bolero™. In citrus, 2,4-D improves fruit size successfully in various cultivars. 2,4-D (40 $mg.L^{-1}$) applied at 13 mm fruit diameter on „Nova' mandarin increased fruit size (Greenberg et al., 2006), while Guiardiola and Lazaro (1988) determined that 2,4-D applied 26 d.a.f.b. on 'Satsuma' mandarin at 7.5 $mg.L^{-1}$ had a significant effect on improving fruit size without a thinning effect. Although 2,4-D did not have an effect on increasing fruit size in „Forelle' and „Abate Fetel' it did however have a significant effect on increasing fruit set in both cultivars. An increase in fruit set by a synthetic auxin such as 2,4-D can be due to an increase in chlorophyll content and a carbon dioxide assimilation increase in sources leaves (Serciloto et al., 2003) supplying more carbohydrates to fruitlets thus leading to increased fruit set. However, synthetic auxins such as naphthylacetamide (NAD) have also been applied successfully as post bloom thinning agent on various European pear cultivars (Dussi et al., 2008). The lowest fruit

set was found on „Williams' trees treated with the auxins naphthylacetamide (NAAm), NAA and naphthoxyacetic acid (NAO) compared to an untreated control, thus auxins had a thinning effect (Lafer, 2008). Auxins therefore have different effects on pears at different rates and stage of application, and can act as a thinning agent when competition between fruitlets and leaves are increased.

In „Williams Bon Chretien' pear cell division occurs during the first 42 to 56 days after anthesis (Bain, 1961). We determined that the cell division rate started to decrease 34 and 45 d.a.f.b. in „Forelle' and „Abate Fetel', respectively, but still continued slowly thereafter. Zhang et al. (2006) found that cell division is more important than cell enlargement in determining the final fruit size in *Pyrus pyrifolia* Nakai, therefore any treatment that might extend the cell division period must probably be applied before the cell division rate starts to decline to have an effect on final cell number and therefore fruit size. Tukey (1974) also found that, in apples, the cell division period continues until 3 to 4 weeks after full bloom and accounts for the greatest expansion in fruit size.

Currently 6-BA can be recommended to increase return bloom, but after various trials with 6-BA, CPPU, TDZ and 2,4-D, none of these PGRs can be recommended to increase fruit size on „Forelle' and „Abate Fetel'. More research is needed to establish if different rates or application stages of these PGRs might be effective. For future research of the cell division and enlargement period of „Forelle' and „Abate Fetel', cell measurements must be taken from full bloom until harvest to determine the rate of cell division from anthesis and to gain a better understanding of cell division and enlargement in these two cultivars.

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Table 1: Full bloom, petal drop, hand thinning and harvest dates for „Forelle' and „Abate Fetel' during two consecutive seasons.

2010/2011 season	2011/2012 season	Growth stage
	„Forelle'	
9 September 2010	14 September 2011	Full bloom
16 September 2010	23 September 2011	Petal drop
5 October 2010	18 October 2011	Hand thinning
2 March 2011	5 March 2012	Harvest
	„Abate Fetel'	
17 September 2010	21 September 2011	Full bloom
27 September 2010	28 September 2011	Petal drop
19 October 2010	20 October 2011	Hand thinning
1 February 2011	6 February 2012	Harvest

Table 2: Treatments applied 14 d.a.f.b. to increase fruit size on „Forelle' and „Abate Fetel' during the 2010/2011 and the 2011/2012 season.

Treatments
1. Untreated control
2. 20 $mg.L^{-1}$ CPPU
3. 15 $mg.kg^{-1}$ TDZ
4. 95 $mg.L^{-1}$ 6-BA*
5. 24 $mg.L^{-1}$ 6-BA applied at 14 d.a.f.b. and repeated weekly thereafter for 3 weeks**

*During the second season (2011/2012) the 95 $mg.L^{-1}$ 6-BA treatment was substituted with 12 $mg.L^{-1}$ 2,4-D applied at full bloom.

** During the second season the 6-BA dose for the fifth treatment was doubled to 50 $mg.L^{-1}$ per application.

Table 3: Effect of different growth regulators applied at 10 mm fruit size (14 d.a.f.b.) on fruit weight, fruit diameter, fruit length, yield, the number of fruitlets thinned by hand, the average developed seed percentage and fruit firmness on „Forelle' pear during the 2010/2011 season. Treatment means followed by the same letter are not significantly different at 5% level (LSD).

	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm trunk circumference)	Average number of fruitlets thinned by hand	Average number of developed seeds per fruit	Average fruit firmness	% Return bloom on two tagged branches
Treatment:								
Untreated Control	109.42 ab	56.68 ns	72.82 b	0.10 ns	250.90 ns	0.38 ns	6.00 ns	9.34 b
20 mg.L^{-1} CPPU	118.02 a	57.68	76.43 a	0.09	276.00	0.38	6.14	3.18 c
15 mg.kg^{-1} TDZ	112.47 a	57.22	74.02 b	0.08	238.50	0.79	6.07	3.40 c
95 mg.L^{-1} 6-BA	102.77 b	55.43	72.34 b	0.10	252.50	0.53	6.19	16.01 a
24 mg.L^{-1} x 4 6-BA	111.81 a	57.17	73.85 b	0.09	225.10	0.43	6.18	10.76 b
Significance level	*0.0205*	*0.0619*	*0.0079*	*0.1736*	*0.6706*	*0.0547*	*0.8154*	*<0.0001*
Covariate (thinned fruit)	*0.7559*		*0.5907*					
Treatment	*0.0241*		*0.0068*					
Covariate (yield)	*0.2466*		*0.1340*					
Treatment	*0.0311*		*0.0116*					

Table 4: Effect of different growth regulators applied at 10 mm fruit size (14 d.a.f.b.) on fruit weight, fruit diameter, fruit length, yield, the number of fruitlets thinned by hand, the average developed seed percentage and fruit firmness on „Forelle' pear during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5% level (LSD).

	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm trunk circumference)	Average number of fruitlets thinned by hand	Average number of developed seeds per fruit	Average fruit firmness	% Return bloom on two tagged branches
Treatment:								
Untreated Control	136.83 ab	60.53 a	79.50 bc	0.08 a	62.90 bc	0.56 ab	7.55 ns	12.95 ab
20 mg.L^{-1} CPPU	141.68 a	60.93 a	83.14 a	0.05 b	56.40 bc	0.57 ab	4.00	9.24 abc
15 mg.kg^{-1} TDZ	141.76 a	61.12 a	81.83 ab	0.05 b	49.70 c	0.59 a	9.55	4.28 c
12 mg.L^{-1} 2,4-D	127.41 bc	59.90 ab	76.03 d	0.08 a	101.70 a	0.54 b	4.05	7.14 bc
50 mg.L^{-1} x 4 6-BA	121.86 c	57.93 b	78.03 dc	0.07 a	71.90 b	0.58 a	4.83	15.36 a
Significance level	*0.0174*	*0.0443*	*0.0012*	*<0.0001*	*0.0002*	*0.0406*	*0.0650*	*0.0132*
Covariate (thinned fruit)	*0.0171*	*0.0753*	*0.0019*					
Treatment	*0.0676*	*0.0774*	*0.0301*					
Covariate (yield)	*0.2540*	*0.5806*	*0.0074*					
Treatment	*0.0360*	*0.0556*	*0.0244*					

Table 5: Effect of different growth regulators applied at 10 mm fruit size (14 d.a.f.b.) on fruit weight, fruit diameter, fruit length, yield, the number of fruitlets thinned by hand, the average developed seed percentage and fruit firmness on „Abate Fetel' pear during the 2010/2011 season. Treatment means followed by the same letter are not significantly different at 5% level (LSD).

	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm trunk circumference)	Average number of fruitlets thinned by hand	Average number of developed seeds per fruit	Average fruit firmness	% Return bloom on two tagged branches
Treatment:								
Untreated Control	173.96 ns	59.82 ns	112.86 ns	0.08 ns	202.50 ns	0.42 ns	6.51 ns	15.22 ab
20 mg.L^{-1} CPPU	175.10	59.66	113.73	0.07	188.90	0.37	6.71	7.92 c
15 mg.kg^{-1} TDZ	176.21	60.11	114.16	0.06	205.50	0.64	6.85	11.03 bc
95 mg.L^{-1} 6-BA	171.32	59.60	111.31	0.07	227.30	0.54	6.64	13.01 ab
24 mg.L^{-1} x 4 6-BA	177.16	60.24	113.11	0.07	226.80	0.54	6.48	17.05 a
Significance level	*0.8523*	*0.8382*	*0.4946*	*0.6047*	*0.7301*	*0.0654*	*0.2496*	*0.0024*

Table 6: Effect of different growth regulators applied at 10 mm fruit size (14 d.a.f.b.) on fruit weight, fruit diameter, fruit length, yield, the number of fruitlets thinned by hand, the average developed seed percentage and fruit firmness on „Abate Fetel' pear during the 2011/2012 season. Treatment means followed by the same letter are not significantly different at 5% level (LSD).

	Average fruit weight (g)	Average fruit diameter (mm)	Average fruit length (mm)	Yield efficiency (kg/cm trunk circumference)	Average number of fruitlets thinned by hand	Average number of developed seeds per fruit	Average fruit firmness	% Return bloom on two tagged branches
Treatment:								
Untreated Control	163.49 ab	58.12 b	116.85 ns	0.12 ns	244.40 b	0.43 c	6.47 ns	12.98 a
20 mg.L^{-1} CPPU	174.66 a	59.82 a	119.45	0.10	212.40 b	0.64 bc	6.39	6.60 b
15 mg.kg^{-1} TDZ	174.33 a	60.41 a	120.0	0.08	203.60 b	1.00 a	6.58	7.52 b
12 mg.L^{-1} 2,4-D	157.13 b	57.32 b	115.48	0.11	346.10 a	0.78 ab	6.47	4.87 b
50 mg.L^{-1} x 4 6-BA	159.86 b	58.10 b	116.09	0.12	232.20 b	0.72 abc	6.39	14.29 a
Significance level	*0.0076*	*0.0003*	*0.1169*	*0.1340*	*0.0009*	*0.0259*	*0.5226*	*0.0002*
Covariate (thinned fruit)	*<0.0001*	*<0.0001*						
Treatment	*0.0625*	*0.0033*						
Covariate (yield)	*0.0020*	*0.0094*						
Treatment	*0.0109*	*0.0004*						

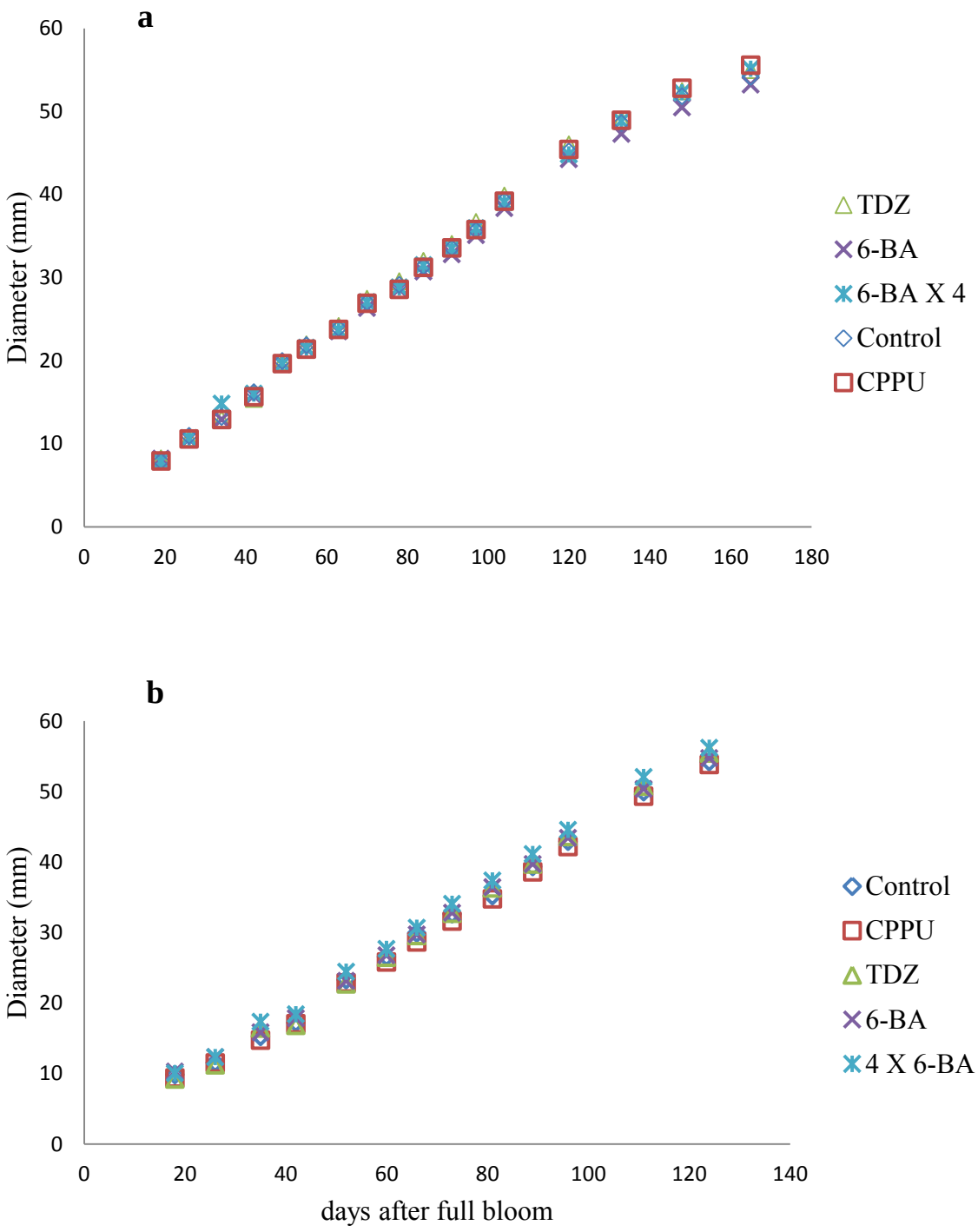

Figure 1: A growth curve of „Forelle' (a) and „Abate Fetel' (b) set up by measuring 10 fruit per repetition weekly from 18 d.a.f.b. until a week before harvest. Fruit were marked to measure the same fruit weekly.

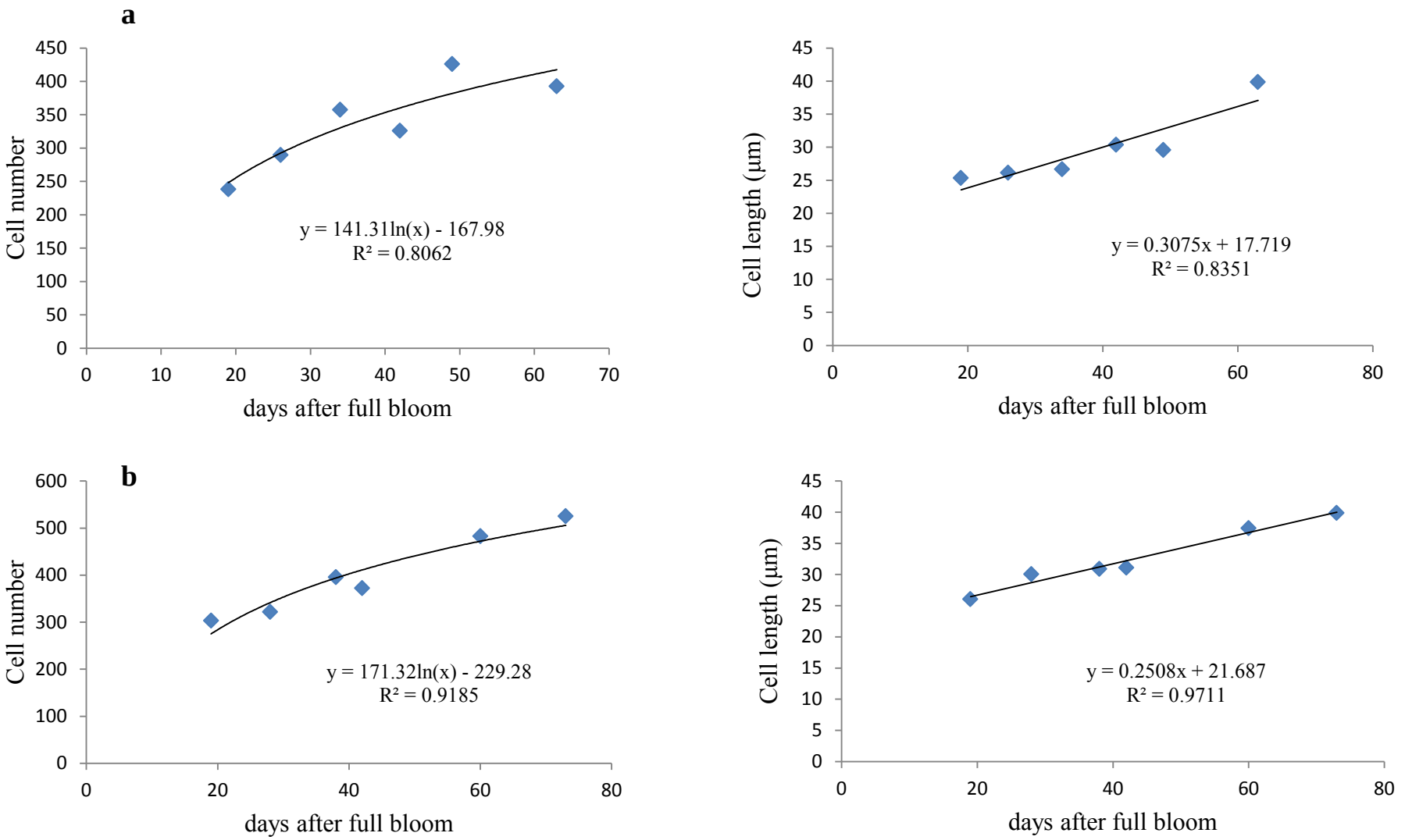

Figure 2: Changes in cell number and cell length in „Forelle' (a) and „Abate Fetel' (b) during 2010/2011 in the mesocarp along the equatorial part of the fruit from 18 to 70 d.a.f.b. Increasing cell number were fitted with logarithmic curves which were used to determine when the cell division period seizes. Linear graphs were used to explain the increase in cell length

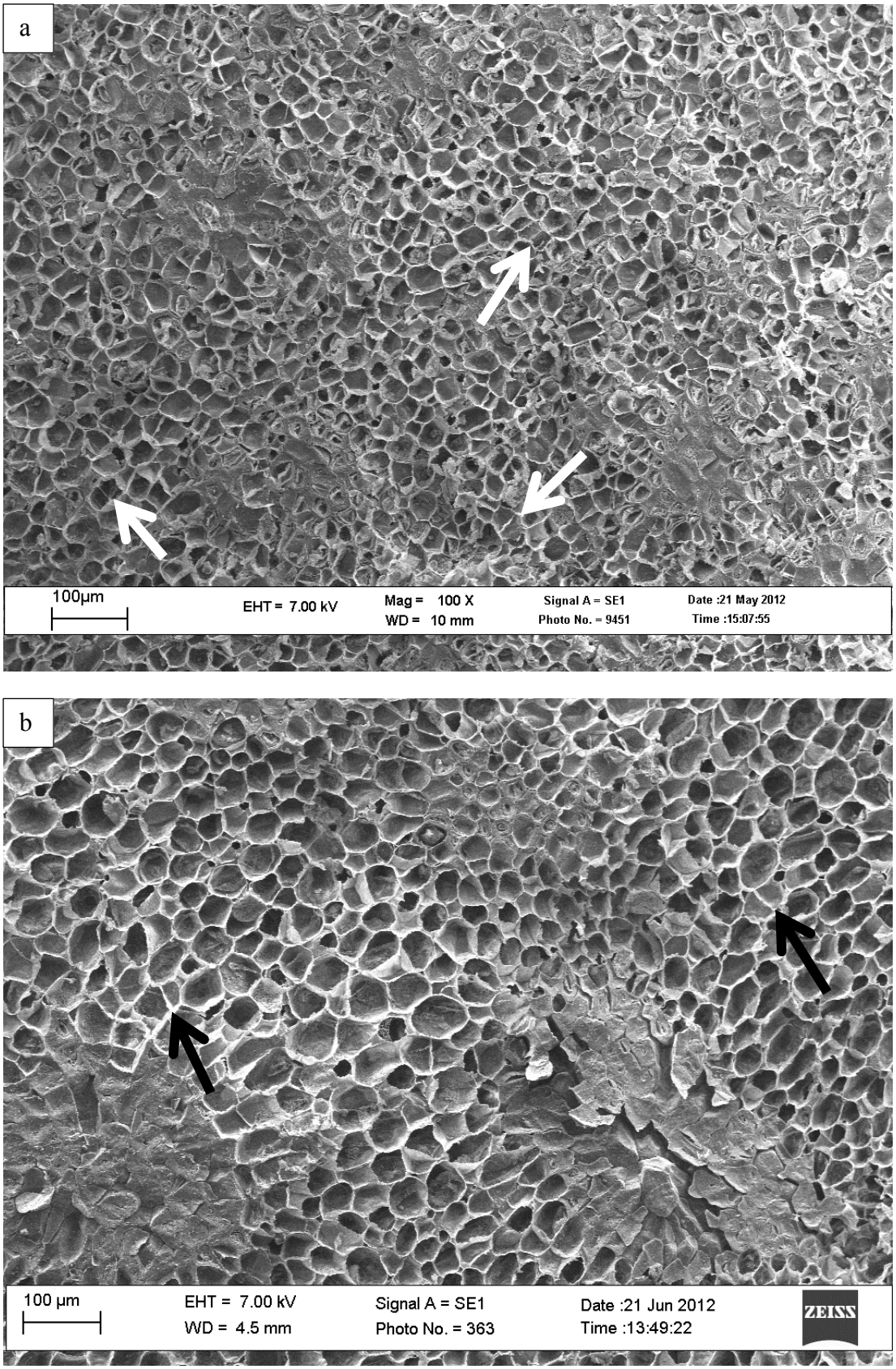

Figure 3: Photos taken through a scanning electron microscope (SEM) of the middle of the mesocarp of „Forelle' pear at 19 (a) and at 63 (b) d.a.f.b. Arrows indicate cells that are in the cell division phase.

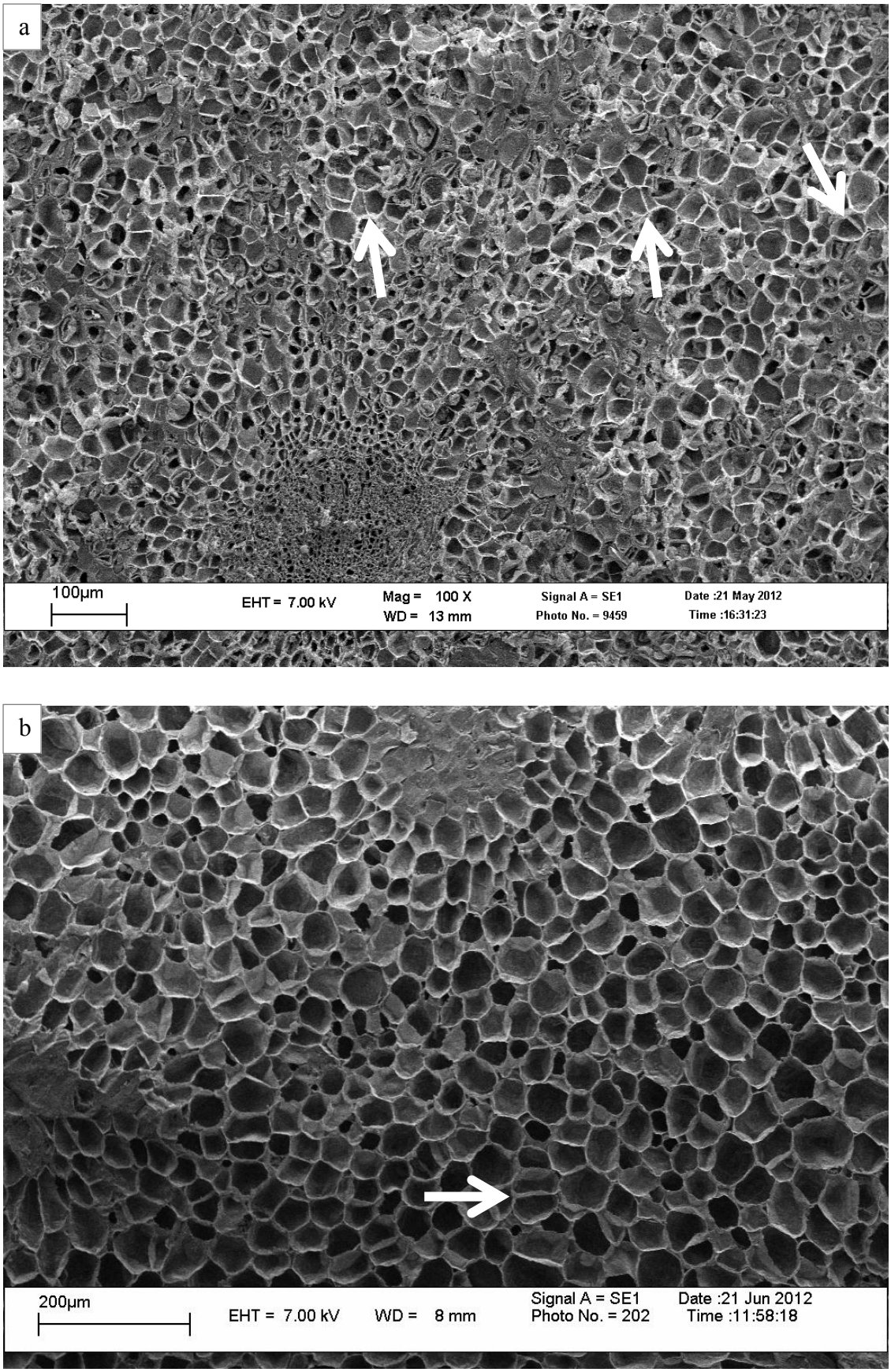

Figure 4: Photos taken through a scanning electron microscope (SEM) of the middle of the mesocarp of „Abate Fetel' pear at 19 (a) and at 73 (b) d.a.f.b. Arrows indicate cells that are in the cell division phase.

GENERAL CONCLUSIONS

From the different trials conducted with different plant growth regulators (PGRs) including gibberellins (GAs), cytokinins and auxins on „Forelle' and „Abate Fetel', the response proved to be quite complex. The main aim with the trials was to promote regular bearing orchards with high yields and good fruit size, which was partially achieved, but should be developed further.

From the fruit set trials it was confirmed that GAs increase fruit set but have a negative effect on return bloom, with GA_3 more so than GA_{4+7}. GA_{4+7} in combination with 6-BA applied as Promalin™ (Valent BioSciences Corporation, USA) Ltd.) during flowering had a slight positive effect on fruit size in addition to the fruit set improvement, compared to other treatments that only improved fruit set. Aminoethoxyvinylglycine (AVG) also showed promise in improving fruit set, especially in „Abate Fetel', without a negative effect on return bloom, but resulted in a slight decrease in fruit size which was due to application of this PGR. Prohexadione-calcium (P-Ca) increased fruit set significantly in both „Forelle' and „Abate Fetel' during both seasons, but it also reduced return bloom significantly, which is not a wanted effect. Even though there were fewer flowers during the second season on P-Ca treated trees, fruit set per flower cluster was still the highest on both cultivars. Although the fruit set percentage was high on a reduced number of flowers during the second season, yield on „Forelle' was significantly lower relative to the untreated control treatment, which was however not the case on „Abate Fetel'. An ideal situation is to have enough flower clusters on the tree with constant fruit set of one to two fruitlets per cluster, rather than four or five fruit setting in a small number of flower clusters and the rest of the flower clusters aborting. Smaller fruit clusters can reduce hand thinning cost and have a significant effect on fruit size. Girdling to increase fruit set must be reconsidered as a cultural practice as it is time consuming and expensive. If performed about a week before full bloom and with a more aggressive girdle cut, it could possibly increase fruit set, but this needs further investigating.

From Paper 2 and 3 it could be concluded that 6-BA did not have any effect in increasing fruit size, even though it was applied at different rates and phenological stages. It did however increase

return bloom in „Forelle' and „Abate Fetel', which could be ideal to regulate an alternate bearing orchard. The combination of P-Ca and GA_{4+7} to improve fruit size has potential for future applications and also showed success in recent research in Japan (Itai et al., 2009). Although this treatment did not result in a significant increase in fruit size, it increased fruit size over two consecutive seasons in „Forelle' and „Abate Fetel'. With the synthetic cytokinins, N-phenyl-N' -1,2,3-thiadiazol-5-ylurea (TDZ) and N (2-chloro-4-pyridyl)-N' -phenylurea (CPPU) applied 14 days after full bloom (d.a.f.b.) to increase fruit size, no significant results were obtained in „Forelle' and „Abate Fetel'. This is in contrast to results on „Coscia' and „Spadona' pear in Israel, where a significant increase in fruit size occurred with there PGRs (Shargal et al., 2006). TDZ and CPPU reduced return bloom in both „Forelle' and „Abate Fetel'. The synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) was applied during the second season of the trial to improve fruit size, because it was recently found that 2,4-D used commercially in a product trading as Bolero™ in Israel improved pear fruit size. 2,4-D did not have the same effect on fruit size as in Israel, which could partly be due to the formulation of Bolero™ which in addition also contains small amounts of GA_3 and naphthaleneacetic acid. 2,4-D did however have a significant effect in increasing fruit set in both cultivars, probably due to an increased supply of assimilates from source leaves.

The main cell division period in apples and pears continues until more or less 40 d.a.f.b. where after cell enlargement continues (Bain, 1961). To be able to extend the cell division period with PGRs, it must be applied before cell division ends. From cell measurements in the mesocarp it was determined when the cell division rate decrease in these two cultivars. In „Forelle' it was determined as 34 d.a.f.b. and „Abate Fetel', at 45 d.a.f.b. This should aid in optimising timing manipulations for fruit size improvement.

The following recommendations in terms of trial design can be made following this research project. It would have been better if all trials had been repeated on the same trees and on a new set of trees to evaluate carry over effects better. Repeated applications of

treatments on the same trees occur in practice. This influences results and makes interpretation difficult, as trees are not starting at the same level at the beginning of the second season. In addition fruit sampling to determine how long the cell division period lasts should already commence at full bloom, to determine when the cell division curves flatten out.

In conclusion, after various fruit set and fruit size improvement trials, we recommend GA_{4+7} or AVG to increase fruit set on „Forelle' and „Abate Fetel', but further trials are required to verify these results in other pear production areas of South Africa. It seems that a balance between yield and fruit size for an orchard must be reached to achieve maximum economical return. Research in future to improve fruit size could entail the evaluation of the combination of P-Ca and GA_{4+7} on European pear cultivars at different phenological stages and rates to determine if there is significant value in this.

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