

Studies on the phenology and carbohydrate status of alternate bearing ‘Nadorcott’ mandarin trees

by
Izak Schalk van der Merwe

*Thesis presented in partial fulfilment of the requirements for the degree of
Master of Science in Agriculture (Horticultural Science) in the Faculty of AgriSciences at
Stellenbosch University*



Supervisor: Prof. K.I. Theron
Co-Supervisor: Dr. P.J.R. Cronjé
Faculty of AgriScience

December 2012

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to extent explicitly otherwise stated), that the reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2012

Copyright © 2012 Stellenbosch University

All rights reserved

Summary

Alternate bearing is a common phenomenon in most commercial perennial fruit trees. In citrus, the “on” year consists of a heavy crop load with mostly small fruit, often followed by an “off” year with few, large and coarse fruit. Carbohydrates play an important role in affecting alternate bearing, especially during fruit set, but also flowering and fruit maturation, and are essential in maintaining a regular bearing habit. Changes in starch and total sugar accumulation in the leaves of the alternate bearing prone ‘Nadorcott’ mandarin were followed over an entire season for both “on” and “off” trees to evaluate the possibility of using carbohydrate levels to predict bearing potential. Starch accumulation followed a distinct pattern with differences between “on” and “off” trees visible in April and May. Starch concentrations in April showed a moderate negative correlation with yield and a moderate positive correlation with return bloom. Rapid starch accumulation started prior to harvest with a peak at the beginning of flowering. Thereafter a sharp decrease in starch levels occurred until after full bloom followed by a steady decrease from physiological fruit drop towards fruit maturity. “On” trees bore 53% more fruit than “off” trees, but the return bloom of “off” trees was 140% more than “on” trees, thus illustrating the negative effect that a large crop has on the next season’s bloom. It was concluded that for ‘Nadorcott’ mandarin, leaf starch concentration in April can be used as an indication of bearing potential the following season.

Pruning is a well-established management tool to control alternate bearing. Summer pruned trees had more spring flush vegetative shoots, more nodes per shoot and also more growth per parent shoot overall, compared to unpruned, control trees. Control trees had higher light levels inside the tree compared to summer pruned trees. However, no differences in leaf starch or total sugar levels during April were measured between treatments. Production of new bearing sites should therefore be considered in this experiment. It was concluded that pruning during November followed by early regrowth management gave the best balance between light penetration and production of new bearing units. Pruning in November, rather than during winter, also allowed selective pruning of shoots with or without flowers, depending on whether it was an “on” or an “off” year.

When fruit thinning chemicals are applied at the optimum time and concentration, it is an effective way of moderating an alternate bearing cycle. Unfortunately no significant differences were obtained in this experiment even though the thinning treatments did show slightly higher starch levels in April 2012, indicating that the demand for energy was lower in these trees. This response was most likely due to the slightly lower yield and fruit number of the thinning treatments compared to the control. The dichlorprop treatment also showed a higher fruit growth rate, and future research should focus on timing of chemical thinning sprays in late mandarin cultivars.

Opsomming

Alternerende drag is 'n algemene verskynsel by die meeste meerjarige kommersiële vrugtebome. In die “aan” jaar by sitrus word 'n swaar oeslading gedra wat hoofsaaklik uit klein vrugte bestaan gevolg deur 'n “af” jaar met minder, groter en growwer vrugte. Koolhidrate speel 'n belangrike rol, veral gedurende vrugset, maar ook tydens blomtyd en vrugrypwording, en is noodsaaklik om 'n reëlmatige drasiklus te verseker. Veranderinge in stysel- en totale suiker akkumulاسie in die blare van ‘Nadorcott’ mandarynbome, is deur die loop van 'n volle seisoen gevolg op beide “aan” en “af” bome om die moontlikheid te ondersoek dat koolhidraatvlakke gebruik kan word om dragpotensiaal te bepaal. Verskille tussen “aan” en “af” bome was in April en Mei sigbaar. Styselvlakke in April het 'n matige negatiewe korrelasie met drag getoon en 'n matige positiewe korrelasie met die volgende seisoen se blom. Styselvlakke het voor oestyd begin toeneem en aan die begin van blomtyd 'n piek bereik waarna 'n skerp daling voorgekom het tot na volblom. Dit is gevolg deur 'n geleidelike afname vanaf fisiologiese vrugval totdat die vrugte ryp was. “Aan” bome het 53% meer vrugte gedra as “af” bome, maar die volgende seisoen se blom van “af” bome was 140% meer. Dit illustreer die negatiewe effek wat 'n groot oes op die volgende seisoen se blom het. Die gevolgtrekking is dat styselvlakke in blare gedurende April gebruik kan word as 'n aanduiding van die drag-potensiaal vir die komende seisoen vir ‘Nadorcott’ mandarynbome.

Snoei is 'n gevestigde manier om alternerende drag te beheer. Bome wat in die somer gesnoei is, het 'n groter aantal vegetatiewe lote in die lente, meer knoppe per loot en ook meer groei op ouer-lote gehad in vergelyking met die kontrole bome wat nie gesnoei is. Kontrole bome het hoër ligvlakke binne-in die boom gehad in vergelyking met die bome wat in die somer gesnoei is. Daar is egter in April geen verskille gemeet in die blare se stysel- en totale suikervlakke tussen behandelings nie. Produksie van nuwe dra-posisies moet dus vir hierdie eksperiment in ag geneem word. Die gevolgtrekking was dat, deur in November te snoei en vroeë bestuur van nuwe groei toe te pas, die beste boomvorm verkry is. Deur in November te snoei eerder as in die winter, kon daar ook selektief gesnoei word aan lote met of sonder blomme, afhangende of dit 'n “aan” of “af” jaar was.

Korrekte chemiese vruguitdunning is een van die mees effektiewe maniere om 'n alternerende drag-siklus te verminder. Ongelukkig is geen betekenisvolle verskille in hierdie eksperiment verkry nie, ten spyte van die feit dat die uitdunningsbehandelings wel ietwat hoër styselvlakke in April 2012 getoon het. Dit dui daarop dat die behoefte aan energie in hierdie bome laer was. Die reaksie was waarskynlik te wyte aan die effens laer oes en vruggetalle as gevolg van die uitdunningsbehandelings in vergelyking met die kontrole. Die dichlorprop-behandeling het ook 'n hoëvruggroei tempo gestimuleer. Navorsing in die toekoms behoort te fokus op die tydberekening waarvolgens die chemiese uitdunningsmiddels op laat mandarynkultivars toegedien word.

Acknowledgements

The following people and institutions helped me to complete my degree and are gratefully acknowledged:

My supervisor, Prof. Karen Theron, for her guidance, constructive criticism and expertise that was invaluable in compiling this thesis.

My co-supervisor, Dr. Paul Cronjé, for his expertise, support, guidance, constructive criticism and always trying to make the study as informative and enjoyable as possible.

Dr. Stephan Verreyne, for helping me start out with the project and giving me the necessary understanding of the outcomes to complete the study.

My parents, for their love, moral and financial support and for always believing in me. Thank you for teaching me that hard work and endurance always pays off.

Tim and Angie Payne, for believing in me, supporting me and for the selfless financial support.

Gustav Lötze and all the ladies in the lab; without your help completing my field work would not have been possible.

André Swartz, for his invaluable assistance in the field and for always going the extra mile, even when it was not expected of him.

Carin Pienaar and Dianah Daniels for administrative assistance.

The Citrus Academy and The Harry Crossley Foundation for providing me with bursaries, and also CRI for funding my project.

Doepie van Zyl, Bielie van Zyl and everyone from Rooihogte farm for providing me with trial sites and always being willing to help.

Abri, for his help with the carbohydrate analyses in the lab.

Elri, for her love and support.

My Heavenly Father, for giving me the courage to complete the study and for opening doors.

TABLE OF CONTENT

Declaration.....	i
Summary.....	ii
Opsomming.....	iii
Acknowledgements.....	iv
1. General introduction.	1
2. Literature review: Studies on the effect of time of harvest, pruning and fruit thinning on carbohydrates, flowering intensity, fruit set and yield of alternate bearing citrus	3
2.1 Introduction.....	3
2.2 Carbohydrates and flowering	4
2.2.1 Citrus flowering	4
2.2.2 Relationship between carbohydrates and flowering	5
2.2.3 Time of fruit removal.....	5
2.2.4 Effect of autumn girdling	6
2.3 The role of carbohydrates in fruit set.....	6
2.3.1 Leaf carbohydrate levels	7
2.3.2 Time of harvest	7
2.3.3 Sink and source relations	7
2.3.4 Sucrose and fruit set.....	9
2.4 The effect of fruit thinning, time of fruit removal and carbohydrates	9
2.4.1 Fruit thinning	9
2.4.2 Time of fruit removal.....	10
2.4.3 Carbohydrate-induced fruit abscission.....	13
2.5 Gibberellic acid (GA) and alternate bearing	13
2.5.1 Winter GA application.....	14
2.5.2 Role of GA during flowering	14
2.5.3 GA and parthenocarpy	14
2.6 Absciscic acid (ABA) and alternate bearing	15
2.6.1 ABA and bud sprouting	15
2.6.2 ABA and flowering.....	15
2.7 Pruning and alternate bearing	15

2.7.1 Pruning of citrus trees	16
2.7.2 Effect of pruning and re-growth on carbohydrate reserves.....	16
2.7.3 Pruning of alternate bearing trees	17
2.8 Conclusions.....	18
2.9 Literature cited.....	19
3. Paper 1. Changes in leaf carbohydrate levels over one season in alternate bearing ‘Nadorcott’ mandarin trees and the relationship between leaf carbohydrate status, return bloom, set percentage and subsequent yield ..	25
4. Paper 2. The effect of different pruning strategies on the phenology and carbohydrate status of alternate bearing ‘Nadorcott’ mandarin trees	48
5. Paper 3. Effect of chemical and hand thinning on the carbohydrate status and yield of ‘Nadorcott’ mandarin trees	63
6. General discussion and conclusions	71

This thesis represents a compilation of manuscripts where each chapter stands on its own. Repetition between chapters was therefore unavoidable.

1. General introduction.

The South African citrus industry consists of 60 355 ha with approximately two-thirds of all fruit produced, 95 million 15-kg equivalent cartons in 2011, being exported due to the high financial returns for export quality fruit (Citrus Growers' Association of Southern Africa, 2012). Mandarins are an important part of citrus production, especially in the colder production areas where most mandarins are planted. In recent years a few late mandarin cultivars, namely Nadorcott, Morri and Orri, have become available to producers in South Africa. These cultivars attain high prices in the export market compared to Navel and Valencia sweet orange cultivars due to high sugar levels and good acid to sugar ratios, and particularly the easy peeling characteristics of these cultivars. These cultivars have therefore become a very important component of the citrus industry in South Africa. These cultivars do, however, have some production problems, e.g. low yields in some cases and, more importantly, alternate bearing (Monselise and Goldschmidt, 1982). Alternate bearing impacts on consistent financial returns to producers, since crop load, fruit size and quality are often compromised.

This study focuses on 'Nadorcott' mandarin, and the main objective was to develop a technique to predict bearing potential of trees so that alternate bearing can be moderated in advance. It was also decided that carbohydrates should be used to determine bearing potential, especially leaf starch levels, since carbohydrates play an important role in alternate bearing (Goldschmidt, 1999) and is relatively easy to quantify. Another objective was to determine what cultural practises could be used to reduce alternate bearing once a citrus producer knows whether the fruit set should be reduced or increased. In addition the goal was to determine how the manipulations should be executed in the orchard.

A literature review was conducted to gain knowledge on how alternate bearing and carbohydrates are linked. The focus was on the factors that influence the starch or total sugar levels of citrus trees, since this information could help in planning the experiments. All relevant literature on the manipulations used in the study was also explored and discussed so that a better understanding of the underlying modes of action could be gained.

Predicting bearing potential would be a valuable tool to citrus producers to ensure a constant annual yield, but little research has been done on this aspect, and the only other study found of this nature was that of Okada (2004) on 'Aoshima' satsuma mandarin trees. This study attempted, therefore, to find a reliable indicator for bearing potential and also to ensure an easy method of prediction. The goal was, therefore, to conduct new research to see if predicting bearing potential was possible or practical. Thereafter different manipulations such as pruning, fruit thinning and time of harvest were studied to provide citrus producers with clear and concise information on how to manipulate alternate bearing orchards to ensure regular annual yields.

Previous research on how pruning can be used to reduce an alternate bearing cycle, did not focus on the effect that different pruning strategies have on carbohydrate levels (Moss, 1972; Procopiou 1972). Furthermore, ‘Nadorcott’ mandarin is known to have excessive vegetative regrowth when pruned. Research was therefore conducted to determine the best method of pruning for ‘Nadorcott’ mandarin, focusing on regrowth management, and also to determine the effect of different pruning strategies on carbohydrate levels. Although research has been conducted on thinning of different citrus types with Corasil E[®], e.g. dichlorprop, it is dangerous to extrapolate results to new cultivars such as ‘Nadorcott’ mandarin, without first conducting research on the effects of dichlorprop on ‘Nadorcott’ mandarin.

References

- Citrus Growers’ Association of Southern Africa. 2012. Key Industry Statistics for Citrus Growers 2012.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. Hort. Sci. 34:1020-1024.
- Monselise, S.P. and E.E. Goldschmidt. 1982. Alternate bearing in fruit trees. Hort. Rev. 4:128–173.
- Moss, G.I. 1972. Regrowth and flowering in sweet orange after pruning. Aust. J. Agric. Res. 24:101-109.
- Okada, M. 2004. Effectiveness of reserved nutrients for estimating productivity of Satsuma mandarin. J. Japan. Soc. Hort. Sci. 73(2):163-170.
- Procopiou, J. 1972. Severe pruning overcome alternate bearing of mandarin trees in Rhodes. Hort. Sci. 7:124.

2. Literature review: Studies on the effect of time of harvest, pruning and fruit thinning on carbohydrates, flowering intensity, fruit set and yield of alternate bearing citrus

2.1 Introduction

Alternate bearing of fruit trees is defined as a heavy crop load during the “on” year that is followed by a light crop load during the “off” year. Alternate bearing occurs in most fruit bearing trees, deciduous or evergreen, including citrus (Monselise and Goldschmidt, 1982). Goldschmidt (2005) described alternate bearing as a “phenomenon of homeostasis”, and pointed out that this behavior secured the longevity of fruit bearing trees in the wild, and that regular bearing is most probably a result of domestication of fruit bearing trees, whereby certain stresses such as drought and pests are removed. Alternate bearing can occur across an entire production region (usually initiated by adverse climatic conditions), between orchards, within an orchard or between branches on a single tree (Monselise and Goldschmidt, 1982).

Certain cultivars of mandarin species such as *Citrus unshiu* (Satsuma) and *C. reticulata*, including common mandarin hybrids within *C. reticulata* and crosses between *C. reticulata* and *C. paradisi* or *C. sinensis*, are prone to alternate bearing (Monselise et al., 1981). Some mandarin cultivars such as Wilking and Murcott mandarins (*C. reticulata* Blanco) are known as absolute alternate bearers due to the fact that there is a total lack of flowers in the “off” year (Goldschmidt and Golomb, 1982). These cultivars can alternate to such an extent that the trees collapse, or even die, during the “on” year (Monselise and Goldschmidt, 1982; Smith, 1976; Stewart et al., 1968). This collapse has been associated with a total depletion of reserve carbohydrates in the tree.

The main problem associated with alternate bearing in citrus is the irregular yield, i.e. a large number of small fruit the one season followed by few and large fruit the next season (Monselise et al., 1981). The net result is that each season relatively few fruit can be marketed for maximum financial returns. From an orchard management point of view it is currently not possible to predict whether a light or heavy crop will result in an “on” or “off” crop the next season. Furthermore, it has not been determined what will be the best indicator of alternate bearing, albeit starch reserves, nitrogen content, or any other factor. It seems likely that such a prediction strategy will be cultivar specific and will include the influence of cultural practices, for instance pruning, fruit thinning and time of harvest, to reduce the alternate bearing cycle sufficiently in the case where an “on” or “off” crop is predicted.

Carbohydrates (specifically starch) have an important role in the regulation and severity of alternate bearing in citrus trees (Jones et al., 1975; Schaffer et al., 1985). The large demand for energy during

spring when floral development, anthesis, fruit set and the spring vegetative flush occur, requires more energy than the current photosynthetic rates can supply (Goldschmidt, 1999; Goldschmidt and Koch, 1996). As a result, carbohydrate reserves are mobilized during this period and there is a decrease in carbohydrate levels from anthesis (Goldschmidt and Koch, 1996; Guardiola, 2000) until midwinter or just after harvest (García-Luis et al., 1995a; Mataa et al., 1996; Sanz et al., 1987). Although researchers have measured carbohydrate levels of “on” and “off” trees during certain critical stages of development, for instance flowering, no research has been conducted to determine the seasonal changes in starch reserves through the different phenological stages throughout an entire year and how this differs for “on” and “off” trees.

Alternate bearing of citrus cannot be fully explained by the effect of carbohydrate storage and mobilization on flowering and fruiting alone. Endogenous hormones such as gibberellins, abscisic acid (ABA) and auxins also play an important role (Talon et al., 1997). How all of these factors interact with each other to sustain an alternate bearing cycle is very complex and poorly understood. Nevertheless, the aim of this literature review is to gain insight into the carbohydrate balance of citrus trees and its effect on alternate bearing.

2.2 Carbohydrates and flowering

2.2.1 Citrus flowering

Flowering in citrus occurs on wood that is less than one year old (García-Luis et al., 1995b). In most cases, citrus trees flower once a year in the spring after a period of rest (either induced through low temperatures or controlled drought stress) (García-Luis et al., 1995b). Some types of citrus, such as certain lemon cultivars, flower two to three times a year (Monselise and Goldschmidt, 1982). For spring flowering, flower induction takes place in the winter and flower initiation takes place just before bud break in spring (García-Luis et al., 1988). The type of inflorescence can be a leafy or pure leafless inflorescence. Leafy inflorescences are more persistent and have a higher tendency to set fruit than leafless inflorescences. This is mainly due to the leaves that support the flowers by providing carbohydrates and hormones (Goldschmidt and Koch, 1996). In most cases, citrus trees exhibiting alternate bearing flower profusely during the “on” year (mostly in leafless inflorescences) and produce very few flowers (mostly in leafy inflorescences) in the following “off” year (Fig. 1.) (Moss, 1971; Verreyne and Lovatt, 2009).



Fig. 1. Typical flowering after the “off” year (left) for ‘Valencia’ citrus with many flowers and new vegetative shoots. During the “on” year fruit inhibit flowering and new vegetative growth the following spring (right).

2.2.2 Relationship between carbohydrates and flowering

It is evident that a threshold level of carbohydrates is necessary for sufficient flower bud differentiation (Goldschmidt, 1999; Goldschmidt and Koch, 1996). The fact that flowering only becomes a limiting factor in the “off” year, following an excessive crop during the previous “on” year which severely depletes carbohydrate levels, supports this notion (Guardiola, 2000). In regular bearing trees, leaf carbohydrate levels during flower induction and initiation do not seem to play a regulatory role (García-Luis et al., 1988). García-Luis et al. (1995a) found a consistent relationship between carbohydrate levels and flowering of ‘Owari’ satsuma mandarin (*C. unshiu* Marc.) trees, but concluded that the level of carbohydrates does not limit flower formation in *Citrus*. Evidence for the direct involvement of starch and other carbohydrates in flowering is inconclusive (García-Luis et al., 1988). Sanz et al. (1987) showed that the availability of mineral nutrients during flowering can also be a limiting factor since nitrogen, potassium and phosphate levels reached a minimum level at flower opening, coinciding with a peak in flower abscission. In conclusion, reserve carbohydrate levels are not the only factor influencing floral development and flowering intensity in *Citrus* and numerous studies have shown that endogenous hormones, particularly gibberellic acid, also play a very important role (Goldschmidt, 1999; Goldschmidt et al., 1985).

2.2.3 Time of fruit removal

Time of fruit removal, and therefore the duration that fruit remain on the tree, has a profound effect on flowering intensity through carbohydrate reserve accumulation or other controlling factors, e.g. endogenous hormones. In ‘Valencia’ orange [*C. sinensis* (L.) Osb.] trees, lower carbohydrate levels due to late harvest did not cause a lack of flowering, but rather an increase in the physiological fruit

drop in November (southern hemisphere) (Jones et al., 1970). In contrast, early removal of fruit from “on” ‘Owari’ satsuma mandarin trees increased the carbohydrate levels in the leaves and increased flowering during the following “off” year (García-Luis et al., 1995a). Yahata et al. (2004) found that early and very early harvest of ‘Yamakawa Wase’, ‘Okitsu Wase’ and ‘Aoshima Wase’ satsuma mandarin trees resulted in a rapid increase of starch in spring shoots followed by an increase in flower bud differentiation, even if the initial crop load was high. Goldschmidt and Golomb (1982) also found an increase in flower bud differentiation after fruit of ‘Wilking’ mandarin trees were removed in midsummer, which was correlated with an increase in starch reserve levels in the roots. Evidence suggests that early fruit removal increases flowering intensity of mandarin type cultivars, but not necessarily all sweet orange cultivars. However, in general it appears that starch levels increase due to early fruit removal for most *Citrus* types, mainly because the biggest sink for carbohydrates is removed allowing subsequent reserve buildup (Monselise and Goldschmidt, 1982).

2.2.4 Effect of autumn girdling

Autumn girdling increases flowering intensity in the following spring (Goldschmidt, 1999). There is evidence to support the hypothesis that carbohydrate levels are directly related to flower bud formation (García-Luis et al., 1995a) and it appears that autumn girdling increases the level of carbohydrates in the above ground parts of the tree. When small and large fruiting branches of ‘Murcott’ mandarin (*C. reticulata* Blanco) trees were girdled in autumn, a two to three times increase in starch levels were observed coinciding with a two to three times increase in flower number when compared to control trees (Goldschmidt et al., 1985).

2.3 The role of carbohydrates in fruit set

Citrus trees experience three stages of fruit abscission throughout the growing season, viz., bloom and post-bloom drop, November or physiological fruit drop, and summer drop. November drop is also referred to as the final fruit set period in the southern hemisphere, as most fruit that persist after the November drop will remain until they reach full maturity. Fruit set, rather than flower number, is the main factor influencing final yield (Ruiz et al., 2001; Sanz et al., 1987). Schaffer et al. (1985) found carbohydrate levels to be a limiting factor for fruit set for ‘Shamouti’ orange, but not for ‘Murcott’ mandarin trees. This illustrates that carbohydrates might be limiting during the initial fruit set period in some cultivars, but not in others. Hormones are most likely responsible for these differences since endogenous levels of hormones do differ between cultivars at certain critical times such as flowering and physiological fruit drop (Goldschmidt and Koch, 1996; Schaffer et al., 1985).

2.3.1 Leaf carbohydrate levels

It appears that carbohydrate limitation is the primary cause of fruitlet abscission during the November (physiological) fruit drop period (Goldschmidt, 1999; Ruan, 1993). Carbohydrate usage during this period seems to exceed the photosynthetic supply, since both developing fruit and leaves are sinks for carbohydrates at this stage, indicating that carbohydrate supply is a limiting factor at fruit set, albeit not the only limiting factor (García-Luis et al., 1988; Goldschmidt, 1999; Ruiz et al., 2001; Sanz et al., 1987). As soon as the leaves in a leafy inflorescence are fully expanded, usually before (Ruiz et al., 2001) or during the November fruit drop period, they become exporters of photosynthates. García-Luis et al. (1988) found a continuous decrease in carbohydrates in older leaves from flowering until the end of November (physiological) fruit drop. Furthermore, carbohydrate levels in inflorescence leaves started decreasing at the beginning of the November (physiological) fruit drop period. This coincided with these leaves converting from sinks to sources, supplying assimilates mainly to nearby fruits. Inflorescence leaves of ‘Washington’ navel oranges accumulate carbohydrates until the beginning of November (physiological) fruit drop and the carbohydrates levels drop drastically thereafter (Sanz et al., 1987). If the carbohydrates levels were not high enough before the sudden decrease, it might limit fruit growth and cause abscission (Sanz et al., 1987). Therefore, developing flowers, fruitlets and vegetative growth rely on older leaves to supply carbohydrates to meet their energy needs. As soon as inflorescence leaves are fully expanded, they start exporting carbohydrates to nearby developing fruit.

2.3.2 Time of harvest

Earlier harvest of ‘Valencia’ orange trees caused an increase in starch levels in the spring leaves and resulted in a higher set percentage the following year (Hilgeman et al., 1967b). Jones et al. (1970) reported that both early and late harvested ‘Valencia’ orange trees had sufficient flowers numbers, but the late harvested trees abscised more fruit during physiological fruit drop. They concluded that it was most likely due to a lack of reserve carbohydrates due to competition by mature fruit from the previous bloom, which was still on the tree.

2.3.3 Sink and source relations

Fruit set was increased by up to 70% by CO₂ enrichment in 3-year-old ‘Valencia’ orange trees (Downton et al., 1987), indicating that citrus is source-limited during the first fruitlet drop and November (physiological) fruit drop stage. Another important factor is the timing of the carbohydrate shortage during the November (physiological) fruit drop period, because that is when carbohydrates regulate fruiting in citrus. Guardiola (1997) identified the end of physiological fruit drop as the period during which carbohydrates regulate fruit set. This is supported by Mehouchi et al. (1995) who

2.3.4 Sucrose and fruit set

Sucrose is the main transport carbohydrate used by *Citrus* due to its non-reducing nature. Fruit set is essentially a case of supply and demand between the source (mainly from leaves close to the fruit) and the sink (developing fruitlets). By *in vivo* injection of sucrose into ‘Okitsu’ satsuma mandarin trees, Iglesias et al. (2003) decreased fruit abscission by 10–15% in both normal and partially defoliated trees. The difference in fruit set between treated and untreated trees became apparent at 60–65 days after anthesis (Iglesias et al., 2003), which is normally when physiological fruit drop is almost completed. Therefore, a smaller supply or higher demand will result in decreased fruit set (Iglesias et al., 2003). Since sucrose is metabolized from starch before transportation to the developing fruitlets (Mehouachi et al., 1995), these two carbohydrates cannot be viewed independently with regards to fruit set. Mehouachi et al. (1995) showed that sucrose levels at the fruit set period correlated negatively with fruit abscission. Hence, decreased sucrose levels lead to increased fruit abscission.

Researchers disagree on whether starch levels *per se* or sucrose mobilization from starch reserves are the limiting factor during fruit set. Studies that supports sucrose mobilization as the limiting factor was mostly done on regular bearing trees, and in this case sucrose mobilization is probably the limiting factor since fruit is a strong sink for carbohydrates. In alternate bearing trees, however, starch and sucrose levels probably become limiting factors resulting in some cases in very high levels of fruit abscission during physiological fruit drop in the “off” year. During the “on” year, starch is most likely not a limiting factor and sucrose mobilization is much less limiting than in a regular bearing tree (Schaffer et al., 1985).

2.4 The effect of fruit thinning, time of fruit removal and carbohydrates

The large crop produced in an “on” year is the single most important factor influencing the carbohydrate levels (especially starch) of the *Citrus* tree (García-Luis et al., 1995a; Hilgeman et al., 1967b; Monselise and Goldschmidt, 1982; Verreyne, 2005). The large number of fruit during an “on” year requires a continuous supply of energy in the form of carbohydrates that must be sourced from current photosynthates, and when this is not sufficient, from reserves (Monselise and Goldschmidt, 1982). Therefore, the number of fruit on the tree and the length of the fruit development period (early, normal or late) are arguably the most important factors influencing starch levels and degree of alternate bearing of *Citrus*.

2.4.1 Fruit thinning

By hand thinning ‘Valencia’ sweet orange trees at different times and intensities throughout the season, Jones et al. (1974) showed that early and more intense fruit thinning of “on” trees increased leaf starch levels just before bloom when compared to progressively later and less intense thinning.

The increased starch levels led to a higher degree of set and subsequently a higher yield the following season. When naphthaleneacetic acid (NAA) was applied as a thinning agent during the physiological fruit drop period to “on” ‘Wilking’ mandarin trees, it decreased that current yield and increased the yield during the following “off” season (Lewis et al., 1964). It did not, however, increase the carbohydrate status of the leaves. The difference in the above mentioned results may be due to cultivar differences (sweet orange vs. mandarin types) or different thinning techniques (NAA vs. hand thinning). If the difference was due to the use of NAA, it indicates that this hormone could cause some physiological change in the tree, which is not the case in hand thinning.

Galliani et al. (1975) found that thinning of ‘Wilking’ mandarin trees by applying NAA during the “on” year sufficiently reduced the alternate bearing cycle. They concluded that thinning agents should be applied annually to successfully control alternate bearing. With high input costs and very competitive international markets, a small crop every second season is a high financial risk for producers and thinning of “on” crops is therefore critical for cultivars that are prone to alternate bearing. Choice of thinning agent, rate and timing of application need to be determined per cultivar to ensure that the degree of thinning is not too light or too severe.

2.4.2 Time of fruit removal

2.4.2.1 Maturing fruit and starch

Verreynne (2005) showed that removing all fruit from “on” trees in December in California (NH) increased the shoot starch concentration measured in January by 5.5 times. Root starch concentration also increased, but it did not differ significantly from “off” trees in January. Similarly, the effect of maturing fruit on carbohydrate levels is clearly illustrated by autumn girdling which usually increases bloom the following spring. However, the effect is nullified when there are still fruit present on the tree, because the carbohydrates are utilized by the maturing fruit (Goldschmidt et al., 1985).

For ‘Wilking’ mandarin trees, leaves sampled in both spring and autumn from “on” trees, had lower levels of all carbohydrates than leaves from “off” trees (sampled on the same dates) with little or no fruit (Lewis et al., 1964). The biggest differences between “on” and “off” trees were seen in starch concentrations with the “off” trees having almost twice as high starch concentration in their leaves compared to “on” trees. Furthermore, Monselise and Goldschmidt (1981) defruited “on” ‘Wilking’ mandarin trees in July (NH) and found that by February (spring) the next year defruited “on” trees had a 30% higher starch concentration in the leaves than “on” trees that were hand thinned. Li et al. (2003) found that defruiting “on” ‘Murcott’ mandarin trees in October (NH) changed the expression of *STPH-L*, *STPH-H*, *Agps*, *RI*, *AATP*, *PGM-P*, *PGM-C*, *CitSuS1*, *HK*, *SUT1* and α -AMY genes in the roots to reflect that of “off” trees. Shortly after the fruit were removed, carbohydrate depletion

stopped and accumulation started. Thus, in most cases, maturing fruit do not allow starch accumulation to occur throughout the entire growing season.

2.4.2.2 Timing of crop inhibition and its effect on tree morphology

Maturing fruit on heavy cropping trees can exert their inhibitory effect on the next season's yield as early in the season as June (NH) (Verreynne and Lovatt, 2009). Furthermore, García-Luis et al. (1995b) found that fruit increased the depth of paradormancy of buds. Martínez-Fuentes et al. (2010) defruited branches at different times on 'Valencia' trees and found that return bloom was reduced by 80% in the period from fruit reaching 90% of their final size until bud sprouting in the following spring. The reduction in flowering was mainly due to a reduction in bud sprouting, and not bud formation, as was also observed for "on" 'Pixie' mandarin trees (Verreynne and Lovatt, 2009). This reduces the number of nodes that can bear inflorescences and spring shoots (which usually bear the most flowers) in the "off" year (Verreynne and Lovatt, 2009).

2.4.2.3 Time of harvest and the alternate bearing cycle

'Valencia' orange fruit are unique among citrus cultivars and need a very long maturation time, in some cases longer than 12 months to reach maturity (Jones et al., 1974). Mature fruit from the previous season are therefore still on the tree when the flowers and fruitlets of the new season are formed. This makes 'Valencia' orange selections prone to alternate bearing and in this instance the number of fruit and the time of on-tree 'storage' (sometimes up to 7 months later than the normal harvest date) affects the degree of alternate bearing (Jones et al., 1974). Therefore, most research on time of harvest was done on 'Valencia' orange trees and relevant research on this subject for others cultivars are lacking. Extrapolating from 'Valencia' orange to others *Citrus* types that are harvested before flowering occurs for the new crop is therefore risky.

Jones et al. (1974) found that late harvesting during the "on" season resulted in fewer buds developing and the vegetative shoots produced were shorter than following a normal harvest season. This reduction in bearing wood for the following season, together with a large yield and late harvest further intensifies the alternate bearing cycle. Hilgeman et al. (1967a) showed that harvesting 'Valencia' oranges in February (NH) increased the yield of the next season with an average of 28% when compared to harvesting in May (NH). They found that harvesting trees in May (late) during the "on" season and in February (early) during the "off" season augmented the alternate bearing cycle, but early harvest of "on" trees and late harvest of "off" trees decreased the intensity of the alternate bearing cycle.

2.4.2.4 Time of harvest and carbohydrates

Leaves of 'Valencia' orange trees, sampled during January (NH), from early harvested trees contained higher total carbohydrate concentrations than trees harvested during mid-season or later (Jones et al. 1964). These authors reported that the relationship between relative yield in a season and time of harvest of the previous season's crop, were curvilinear for 'Valencia' orange, and that early harvest was proportionately more effective in increasing the subsequent yield than mid-season harvest. Similarly, late harvest of 'Valencia' orange decreased fruit set the following season and this can also initiate an alternate bearing cycle (Hilgeman et al. 1967b). The decreased set was reflected in the carbohydrate concentration in the leaves, since trees harvested before the set period had significantly higher carbohydrate levels than trees harvested at a later stage. The carbohydrate levels in the leaves of 'Valencia' orange trees just before flowering was correlated negatively with the fruit load (now reaching maturity) of the previous season, but it was also positively correlated with the yield of the current season (Jones et al., 1964; Jones et al., 1974). Identifying relationships such as these mentioned above can be important in predicting and preventing alternate bearing and research needs to be done to see if such relationships exist for other cultivars as well.

'Owari' satsuma mandarin trees harvested one month before the normal harvest date had higher starch and non-reducing sugar levels in the leaves and bark in comparison to trees harvested at the normal harvesting date (García-Luis et al., 1995a). The difference was most prominent in leaves sampled during October (NH). The early fruit removal caused a 3.4-fold increase in the number of flowers and a 1.3-fold increase in shoot formation. The number of reproductive and mixed shoots were increased and the number of vegetative shoots were decreased.

Although many researchers have studied the effect of early and late harvest on return bloom, subsequent yield and also alternate bearing, the harvest intervals are usually very long (a month or longer) making it impractical for producers due to the inferior quality of fruit harvested too early. The effect that a short harvest interval (especially during an "on" year) has on reserve carbohydrate levels and return yield has not been thoroughly researched. It still needs to be determined whether a difference in harvest time as short as 2 weeks or less will make a difference in starch levels and subsequently return bloom, fruit set and yield the next season. If it does improve these parameters, it can help producers reduce alternate bearing with minimum loss of potential profit. It also needs to be determined whether partial fruit removal shortly before normal harvest times will influence starch levels and return yield and if this practice can be used to reduce alternate bearing. Another factor that needs to be addressed is the effect that postponing harvest by as little as 2 weeks or less has on starch levels, return bloom, fruit set and yield the next season. If it influences these parameters negatively, as illustrated by the literature, it can initiate or worsen alternate bearing in an orchard and needs to be avoided.

2.4.3 Carbohydrate-induced fruit abscission

The mechanism by which ABA causes abscission has been described for citrus (Fig. 3.). In ‘Clausellina’ and ‘Okitsu’ satsuma mandarin trees, fruitlet abscission was induced by carbohydrate shortage (caused by defoliation). The process starts with an increase in ABA levels in the fruit followed by an increase in ACC, which in turn promotes ethylene production, which causes the fruit to abscise. This is what would happen during the final period of physiological fruit drop (Talon et al., 1997) when carbohydrate accumulation is the most important factor for fruit persistence. In terms of alternate bearing, if ABA levels are higher during spring in trees following an “on” year, it could promote flower abscission and contribute to the “on”/“off” cycle. This does not, however, explain why certain cultivars do not flower at all during the “off” year, indicating that ABA does not play a direct role in flowering, but is the most important contributing factor during abscission.

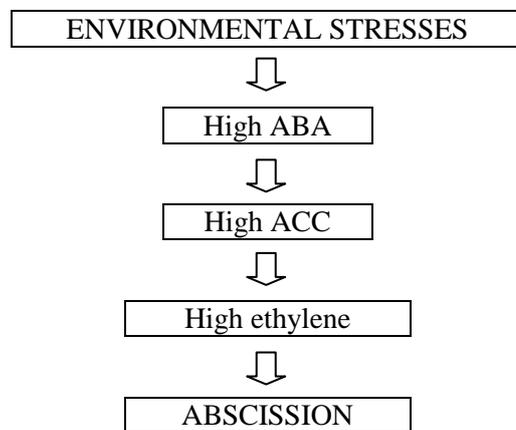


Fig. 3. The chain of events leading to abscission in reproductive and vegetative tissues of citrus (Talon et al., 1997).

2.5 Gibberellic acid (GA) and alternate bearing

Flowering and fruit set cannot be discussed without giving considerable attention to endogenous hormones. Currently, gibberellins are identified as the most important of these. GA are important in many plant physiological processes such as stem elongation, pollen development, seed germination, floral development and fruit set (Davies and Sponsel, 2010). GA production within the citrus fruit itself is important for fruit retention (Turnbull 1989); however, seeds do not appear to be an important source of GA (Ben Cheikh et al., 1997; Plummer et al., 1989; Turnbull, 1989). In citrus, GA₃ [ProGibb® (Philagro SA (Pty) Ltd)] is the most important form of this hormone since it gives the best physiological response when applied exogenously and is almost exclusively used in the industry.

2.5.1 Winter GA application

GA₃ applied during the winter has an inhibitory effect on flowering the subsequent spring (García-Luis et al., 1988; Goldschmidt et al., 1985; Monselise and Goldschmidt, 1982). This can be used to reduce alternate bearing when GA is applied during winter when an “on” year is expected. In alternate bearing ‘Wilking’ mandarin trees, higher level of GAs were found in the tracheal sap of “on” trees at the end of the season in comparison with “off” trees (Saidha et al., 1983) indicating that flower formation is most likely inhibited by endogenous GA levels from developing fruit during the growing season (García-Luis et al., 1988). Carbohydrates and GA regulate flowering independently of each other (Goldschmidt et al., 1985).

GA₃ application to buds in mid-December (NH) reduced the number of sprouted nodes and increased the number of vegetative shoots of ‘Owari’ satsuma mandarin trees in comparison with the control, an effect that could not be mimicked by ABA and kinetin (Garcia-Luis et al., 1986). Plummer et al. (1989) obtained the same results with ‘Valencia’ orange trees. Shoots bearing only flowers were most sensitive to exogenous GA₃ application and were significantly reduced, while mixed type shoots (having both flowers and leaves) were unaffected by GA₃ (Garcia-Luis et al., 1986). Shy flowering trees appear to be more sensitive to GA₃ application than profuse flowering trees (Garcia-Luis et al., 1988).

2.5.2 Role of GA during flowering

Mehouachi et al. (2000) found that GA levels did not differ between treatments from anthesis until 84 days later when fruitlet abscission was induced in ‘Clausellina’ satsuma mandarin by removing either old or young leaves. GA application during flowering increases the initial growth rate of ovaries and decreases the extent of abscission shortly after anthesis; however, GA application 4 to 6 weeks later has little or no effect on physiological fruit drop (Guardiola, 2000). These results indicate that GA does not play a significant role during the physiological fruit drop period. GAs do, however, play an important role during the first abscission wave just after anthesis, since they are activators of cell division and enlargement processes (Talon et al., 1997) and they are therefore important during the initial phase of cell division. ProGibb[®] (Philagro SA (Pty) Ltd) has long since been used in the South African citrus industry as a formulation of GA₃ to increase fruit set when it is applied at 100% petal fall.

2.5.3 GA and parthenocarpy

Interestingly, Talon et al. (1990) showed that sterile satsuma mandarins have a spike in GA levels at anthesis that is absent in self-incompatible clementine mandarins. They also found that satsuma mandarin trees set 23% of the initial flowers formed compared to the 3% in clementine trees.

Therefore, it would seem that clementine trees do not reach a certain GA threshold at anthesis that is conducive to sufficient fruit set (Talon et al., 1990). To illustrate this, exogenous application of GA₃ at, or shortly after, anthesis increases set in clementine trees (Guardiola, 2000). This indicates that GA is important for fruit development during the initial cell division period, possibly by increased mobilization of metabolites to fruitlets containing high GA levels (Kojima, 1996). This shows that GA could be responsible for the difference in set between cultivars with strong and poor parthenocarpic ability.

2.6 Absciscic acid (ABA) and alternate bearing

Goldschmidt (1984) found that ABA concentrations during the flower induction period were significantly higher in leaves, stems and buds from “on” ‘Wilking’ mandarin trees in comparison to “off” trees. High ABA levels in the leaves appeared to be associated with the high fruit load, even though the fruit were not a source of ABA. Therefore, the increased ABA in “on” trees may be synthesized in the leaves in response to the unique stress experienced by the tree due to the high crop load (Goldschmidt, 1984).

2.6.1 ABA and bud sprouting

One of the major problems with alternate bearing is increased dormancy of buds after an “on” year that causes delayed bud sprouting and reduced growth (Garcia-Luis et al., 1995b). Garcia-Luis et al. (1986) showed that ABA application delayed bud sprouting of ‘Owari’ satsuma mandarin. Exogenous GA₃ has the opposite effect; therefore, the delayed bud sprouting caused by the high fruit load could be due to accumulation of ABA in the buds (Garcia-Luis et al., 1986).

2.6.2 ABA and flowering

Okuda et al. (1996) and Okada (2000) found increased levels of ABA in leaves of defruited ‘Aoshima’ satsuma mandarin trees, a heavy alternate bearer, during the flower induction period when compared to control trees. The control trees produced almost no flowers the next spring and Okada (2000) suggested that a minimum ABA threshold level is necessary during this period for adequate flowering the next spring. Since “on” trees usually have higher ABA levels during the flower induction period (Goldschmidt, 1984), ABA cannot be the deciding factor in flowering when considering the evidence to the contrary.

2.7 Pruning and alternate bearing

Pruning is important to achieve sustainable, marketable yields each year (Bilge et al., 2010), and there are many different strategies with equally different responses depending on cultivar, fruiting habit,

growing conditions, tree age and tree vigour (Tucker et al., 1994). Under normal growing conditions, pruning is only used to remove weak, complex and unproductive bearing branches (Krajewski and Pittaway, 2000) and to confine trees to their allotted space in the orchard (Wheaton, 1992).

2.7.1 Pruning of citrus trees

The two basic pruning cuts that are widely used in citriculture are heading back, where the terminal portion of a shoot is removed, and thinning out, where an entire branch is removed from the tree to increase light into the inner canopy (Tucker et al., 1994). Mechanical hedging and topping is a type of heading back cut that is done on a large scale (Tucker et al., 1994) in order to save time and reduce labour costs. Krajewski and Pittaway (2000) classified strong bearing branch units as follows: an upright orientation in close proximity to a strong limb, short and thick, not complex, bearing leafy inflorescences, large fruit size, and many active leaves that are dark green in color and originating from the top of the old limb. They also classified weak bearing branch units as: downward orientation, far from a strong limb, long and thin, complex, bearing leafless inflorescences, small fruit size, few and inactive leaves that are yellowish in color and originating from the bottom of the old limb.

2.7.2 Effect of pruning and re-growth on carbohydrate reserves

Literature on the effect of re-growth caused by pruning on carbohydrate reserves is lacking. Okuda et al. (2003) observed that 'Haraguchi Wase' satsuma mandarin shoots pruned during early spring sprouted seven times more shoots compared to the unpruned shoots. In addition, pruning decreased the carbohydrate concentration in the bark of 2- and 3-year-old branches. Although the effect of different pruning techniques on tree morphology has been documented, many aspects are largely unexplored. Detailed studies on the effect of varying intensities of re-growth on tree carbohydrates and morphology are still lacking. It is necessary to determine how important re-growth management is for producers to ensure sustainable, regular yields each year. This is especially important for vigorous cultivars such as the late maturing mandarins 'Nadorcott', 'Morri' and 'Orri' that are prone to have excessive re-growth if not controlled as well as a tendency to exhibit alternate bearing. It is also important to explore the effect that different re-growth management strategies, i.e. early, regular management or late management, have on starch concentration of trees and how this affects fruiting. The relationship between pruning, whole tree carbohydrate reserves and flowering and fruit set is also unexplored. This relationship can potentially be important to better control alternate bearing with effective pruning techniques and to better understand the effect that pruning has on alternate bearing trees.

2.7.3 Pruning of alternate bearing trees

Two strategies can be followed when pruning alternate bearing trees. Firstly, trees can be pruned heavily during the winter of an “off” year, before an expected “on” year, thereby reducing the number of fruit bearing sites during the “on” year (Krajewski, 1996; Tucker et al. 1994). The second strategy is to prune trees during the spring of the “on” year, thereby removing part of the crop for that year. Another possible strategy that is not well documented is summer pruning instead of normal winter pruning. This practice must be explored further at different severities to see whether or not it is an effective means to control alternate bearing.

2.7.3.1 Pruning of ‘Valencia’ orange trees to decrease alternate bearing

Moss (1972) found that moderate pruning of ‘Valencia’ sweet orange trees during spring of the “on” year was not effective in controlling alternate bearing because the re-growth on pruned trees did not flower for the following two seasons and therefore part of two crops were effectively removed. By contrast, Bevington and Bacon (1978) found that light hedging of ‘Valencia’ orange trees during the fruit set period of an expected “on” year decreased yield in that season, but resulted in a significantly increased yield during the following “off” year when compared to un-pruned trees. The cumulative yield over four seasons did not differ significantly from un-pruned trees. The cumulative yield of severely pruned trees, however, was significantly lower than in un-pruned trees, presumably because the excessive re-growth that followed could not produce enough flowers even during the second season after pruning (Bevington and Bacon, 1978). Shoot length of re-growth after pruning is positively correlated with the circumference of the pruned branch (Bacon 1981; Moss, 1972) and re-growth of severely pruned branches most likely grows longer and harden off later before it can form flowers when compared to thinner branches. ‘Valencia’ sweet orange trees that were pruned during autumn, thereby delaying re-growth until the following spring (Bevington, 1980), developed excessively long vegetative shoots the following spring (Bacon, 1981). Shoot length was negatively correlated with the number of fruit per stem for that crop the following year (Bacon, 1981). This shows that timing of pruning is very important and trees should be pruned as soon after harvest as possible (Krajewski and Pittaway, 2000). For ‘Valencia’ sweet orange trees, pruning should be done during spring or early summer (Bevington, 1980).

2.7.3.2 Pruning of other *Citrus* types to decrease alternate bearing

Khurshid and Krajewski (2010) found that mechanical hedging of ‘Washington’ navel orange trees during full bloom of an “on” year increased the percentage leafy inflorescences and vegetative shoots and decreased the percentage leafless inflorescences during the following spring. It also increased fruit size in the “on” year. For severely alternating trees, pruning can be done only during the spring

of the “on” year, thereby reducing the crop and also inducing more vegetative growth that can bear fruit during the following “off” year (Wheaton, 1992). Severe pruning of ‘Mediterranean’ mandarin (*C. deliciosa* Tenore) trees during an “off” year resulted in consistent yields over the following five years, after the initial deleterious effect of severe pruning on yield was overcome, and fruit quality during the “on” years was also improved with better fruit size and a higher °Brix:acid ratio (Procopiou, 1972). Bilge et al. (2010) found increased fruit size for ‘Star Ruby’ grapefruit (*C. paradisi* Macf.) trees that were mechanically topped and hedged when compared to control trees. Hield and Hilgeman (1969) reported that light pruning of alternate bearing ‘Wilking’ mandarins had no effect on yield or the alternate bearing cycle when compared to un-pruned trees. The pruned trees did yield fewer, but larger fruit. Galliani et al. (1975) reported that summer pruning of alternate bearing ‘Wilking’ mandarin trees during the “on” year reduced the yield to the same extent as 350 mg.kg⁻¹ NAA applied at 1 cm fruit diameter and increased the average fruit weight and °Brix:acid ratio.

2.8 Conclusions

Currently, the alternate bearing phenomenon of citrus is not well understood, especially in relation to the role of carbohydrate, GA₃ and ABA levels at the different phenological stages. Furthermore, flowering and fruiting are very complex processes that are affected by many factors at any given time (Fig. 2.). Another problem is the large variation between different *Citrus* spp. and even different cultivars of the same species that lead to contradictory results in response to various treatments. It is necessary to determine how important carbohydrates for instance are for flowering, fruit set and subsequent yield on a cultivar by cultivar basis.

In terms of the effect of carbohydrates on flowering, it seems that a minimum threshold level is necessary for sufficient flowering, but any effect of carbohydrates beyond this point becomes unclear. Carbohydrates are a deciding factor in fruitlet abscission during the late stages of physiological fruit drop. The ability of a fruitlet to persist might be increased by application of GA₃ shortly after anthesis by increasing the ability of the fruitlet to utilize carbohydrates, particularly in weak parthenocarpic cultivars. Maturing fruit is arguably the most important factor influencing return bloom and subsequently the alternate bearing cycle of any given tree. Earlier harvest will usually ensure sufficient carbohydrate reserve build-up and the result is sufficient flowering intensity and fruit set in the following season. Late harvest usually has the opposite effect, because mature fruit continue to utilize carbohydrates while they are still on the tree and flowering intensity and fruit set are reduced significantly the following spring. This cannot be related to the utilization of carbohydrate reserves by the fruit in all cases, since different results have been found for different cultivars. In most cases, fruit thinning during the “on” year is effective in reducing alternate bearing.

GA₃ influences flowering in different ways; it inhibits flower induction during the winter rest period, thereby decreasing flowering intensity the following spring, and increases fruit set when applied during full bloom. Exogenous application of GA₃ during spring increases set in parthenocarpic cultivars lacking an inherent GA₃ peak during full bloom. Higher fruit loads, such as those during an “on” year, usually increase GA₃ levels in the tree. Therefore it can be concluded that GA₃ plays an integral part in regulating alternate bearing in citrus. ABA is an important part of abscission, whether of shoots, leaves, flowers or fruit. There is no clear relationship between ABA and flower formation, but it appears that the GA:ABA ratio is important in determining whether abscission will occur or not. Fruit abscission induced by carbohydrate shortage is also mediated by ABA.

Pruning has been used to control alternate bearing with varying results. In theory, severe pruning before an expected “on” crop will reduce the yield of that crop and result in better flowering and fruit set during the subsequent “off” season. This practice was successful in some cases, but not in others. Severe pruning will also result in lower cumulative yield over the following few years. Light pruning does not seem to reduce the alternate bearing cycle of citrus trees, but it is important for regular bearing trees to remove unproductive and dead wood. Although little research has been done on the effect of different pruning severities on carbohydrate reserves of citrus, it appears that the vigorous vegetative growth that results due to pruning may use large quantities of carbohydrate from the parent shoot.

2.9 Literature cited

- Bacon, P.E. 1981. The effect of hedging time on re-growth and flowering of mature Valencia orange trees. *Aust. J. Agric. Res.* 32:61-68.
- Ben-Cheikh, W., J. Perez-Botella, F.R. Tadeo, M. Talon, and E. Primo-Millo. 1997. Pollination increases gibberellin levels in developing ovaries of seeded varieties of citrus. *Plant Physiol.* 114:557-564.
- Bevington, K.B. 1980. Response of Valencia orange trees in Australia to hedging and topping. *Proc. Fla. State Hort. Soc.* 93:65-66.
- Bevington, K.B., and P.E. Bacon. 1978. Effect of hedging on the productivity of Valencia orange trees. *Aust. J. Exp. Agric. Anim. Husb.* 18:591-596.
- Bilge, Y., Y. Turgut, I. Meral, K. Müge, Ö. Faruk, T. Onder, and K.A. Yildiz Aka. 2010. The effects of mechanical pruning on fruit yield and quality in ‘Star Ruby’ grapefruit. *Intl. J. Food. Agric. Environ.* 8:834-838.

- Davies, P.J. and V. Sponsel. 2010. Gibberellins: Regulators of plant height and seed germination. p.581-619 In: L. Taiz and E. Zeiger (eds.). Plant Physiology Fifth Edition. Sinauer Associations, Inc., Sunderland, MA.
- Downton, W.J.S., W.J.R. Grant and B.R. Loveys. 1987. Carbon dioxide enrichment increases yield of Valencia orange. Aust. J. Plant. Physiol. 14:493-501.
- Erner, Y. 1989. Citrus fruit set: Carbohydrate, hormone, and leaf mineral relationships. p.233-242. In: C.J. Wright (ed.). Manipulation of fruiting. The Anchor Press Ltd., Tiptree, Essex.
- Galliani, S., S.P. Monselise, and R. Goren. 1975. Improving fruit size and breaking alternate bearing in 'Wilking' mandarins by ethephon and other agents. HortScience 10:68-69.
- García-Luis, A., V. Almela, C. Monerri, M. Agusti, and J.L. Guardiola. 1986. Inhibition of flowering in vivo by existing fruits and applied growth regulators in *Citrus unshiu*. Physiol. Plant. 66:515-520.
- García-Luis, A., F. Fornes, and J.L. Guardiola. 1995a. Leaf carbohydrates and flower formation in Citrus. J. Amer. Soc. Hort. Sci. 120:222-227.
- García-Luis, A., F. Fornes, A. Sanz, and J.L. Guardiola. 1988. The regulation of flowering and fruit set in Citrus: Relationship with carbohydrate levels. Israel J. Bot. 37:189-201.
- García-Luis, A., M. Kanduser, and J.L. Guardiola. 1995b. The influence of fruiting on the bud sprouting and flower induction responses to chilling in Citrus. J. Hort. Sci. 70:817-825.
- Goldschmidt, E.E. 1984. Endogenous abscisic acid and 2-trans-abscisic acid in alternate bearing 'Wilking' mandarin trees. Plant Growth Regulat. 2:9-13.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. HortScience 34:1020-1024.
- Goldschmidt, E.E. 2005. Regulatory aspects of alternate bearing in fruit tree. Italus Hortus 12:11-17
- Goldschmidt, E.E., N. Aschkenazi, Y. Herzano, A.A. Schaffer, and S.P. Monselise. 1985. A role for carbohydrate levels in the control of flowering in citrus. Scientia Hort. 26:159-166.
- Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate bearing citrus trees and the significance of reserves for flowering and fruiting. J. Amer. Soc. Hort. Sci. 107:206-208.

- Golschmidt, E.E. and K.E. Koch. 1996. Citrus, p.797-823. In: E. Zamski and A. A. Schaffer (eds.). Photoassimilate distribution in plants and crops. Marcel Dekker, New York.
- Guardiola, J.L. 2000. Regulation of flowering and fruit development: Endogenous factors and exogenous manipulation. Proc. Intl. Soc. Citricult. 9:342-346.
- Guardiola, J.L. 1997. Competition for carbohydrates and fruit set, p.43-61. In: S.H. Futch and W.J. Kender (eds.). Citrus flowering and fruiting short course. Citrus Res. Ed. Ctr., Lake Alfred, Fla.
- Hield, H.Z. and R.H. Hilgeman. 1969. Alternate bearing and chemical fruit thinning of certain citrus varieties. Proc. Intl. Citrus Symp. 3:1145–1153.
- Hilgeman, R.H., J.A Dunlap, and F.O. Sharp. 1967a. Effect of time of harvest of Valencia oranges in Arizona on fruit grade and size and yield the following year. Proc. Amer. Soc. Hort. Sci. 90:103–109.
- Hilgeman, R.H., J.A. Dunlap, and G.C. Sharples. 1967b. Effect of time of harvest of Valencia oranges on leaf carbohydrate content and subsequent set of fruit. Proc. Amer. Soc. Hort. Sci. 90:110–116.
- Iglesias, D.J., F.R. Tadeo, E. Primo-Millo, and M. Talon. 2003. Fruit set dependence on carbohydrate availability in citrus trees. Tree Physiol. 23:199-204.
- Jones, W.W., T.W. Embleton, E.L. Barnhart, and C.B. Cree. 1974. Effect of time and amount of fruit thinning on leaf carbohydrates and fruit set in Valencia oranges. Hilgardia 42:441–449.
- Jones, W.W., T.W. Embleton, and C.W. Coggins, Jr. 1975. Starch content of roots of ‘Kinnow’ mandarin trees bearing fruit in alternate years. HortScience 10:514.
- Jones, W.W., T.W. Embleton, M.L. Steinacker, and C.B. Cree. 1964. The effect of time of fruit harvest on fruiting and carbohydrate supply in the Valencia orange. Proc. Amer. Soc. Hort. Sci. 84:152–157.
- Jones, W.W., T.W. Embleton, M.L. Steinacker, and C.B. Cree. 1970. Carbohydrates and fruiting of ‘Valencia’ orange trees. J. Amer. Soc. Hort. Sci. 95:380–381.
- Khurshid, T., and A. Krajewski. 2010. Bearing branch units developed on branches hedged during flowering produce large ‘Washington’ navel (*Citrus sinensis* L. Osbeck) oranges. Intl. J. Fruit. Sci. 10:201-227.

- Kojima, K. 1996. Changes of abscisic acid, indole-3-acetic acid and gibberellin-like substances in the flowers and developing fruitlets of citrus cultivar 'Hyuganatsu'. *Scientia Hort.* 5:263-272
- Krajewski A.J. 1996. Pruning of Citrus in Southern Africa: A Hacker's Guide. *Citrus Journal* 6(4):19-23
- Krajewski, A., and T. Pittaway. 2000. Manipulation of citrus flowering and fruiting by pruning. *Proc. Intl. Soc. Citricult. Congr.* 9:357-360.
- Lewis, L.N., C.W. Coggins, Jr., and H.Z. Hield. 1964. The effect of biennial bearing and NAA on the carbohydrate and nitrogen composition of Wilking mandarin leaves. *Proc. Amer. Soc. Hort. Sci.* 84:147-151.
- Li, C.Y., D. Weiss, and E.E. Goldschmidt. 2003. Effects of carbohydrate starvation on gene expression in citrus root. *Planta* 217:11-20.
- Martínez-Fuentes, A., C. Mesejo, C. Reig, and M. Agusti. 2010. Timing of the inhibitory effect of fruit on return bloom of 'Valencia' sweet orange (*Citrus sinensis* (L.) Osbeck). *J. Sci. Food. Agric.* 90:1936-1943.
- Mataa, M., S. Tominaga, and I. Kozaki. 1996. Seasonal changes of carbohydrate constituents in Ponkan (*Citrus reticulata* Blanco). *J. Japan. Soc. Hort. Sci.* 65:513-523.
- Mehouachi, J., D. Serna, S. Zaragosa, M. Agusti, M. Talon, and E. Primo-Millo. 1995. Defoliation increase fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107:189-197.
- Mehouachi, J., D.J. Iglesias, F.R. Tadeo, M. Agusti, E. Primo-Millo, and M. Talon. 2000. The role of leaves in citrus fruitlet abscission: Effect on endogenous gibberellin levels and carbohydrate content. *J. Hort. Sci. Biotech.* 75:79-85.
- Monselise, S.P. and E.E. Goldschmidt. 1982. Alternate bearing in fruit trees. *Hort. Rev.* 4:128-173.
- Monselise, S.P., E.E. Goldschmidt, and A. Golomb. 1981. Alternate bearing in citrus and ways of control. *Proc. Intl. Soc. Citricult.* 1:239-242.
- Moss, G.I. 1971. Effect of fruit on flowering in relation to biennial bearing in sweet orange (*Citrus sinensis*). *J. Hort. Sci.* 46:177-184.
- Moss, G.I. 1972. Regrowth and flowering in sweet orange after pruning. *Aust. J. Agric. Res.* 24:101-109.

- Okuda, H. 2000. A Comparison of IAA and ABA levels in leaves and roots of two citrus cultivars with different degrees of alternate bearing. *J. Hort. Sci. Biotech.* 75:355-359.
- Okuda, H., T. Kihara, and I. Iwagaki. 1996. Effects of fruit removal on photosynthesis, stomatal conductance and ABA level in the leaves of vegetative shoots in relation to flowering of Satsuma mandarin. *J. Japan. Soc. Hort. Sci.* 65:15-20.
- Okuda, H., Y. Yonemoto, and T. Takahara. 2003. Effects of the timing of cut back pruning for the whole tree canopy on sprouting and growth and on the carbohydrate and nitrogen content in the branches of Satsuma mandarin trees in a systemized alternate-bearing orchard. *Hort. Res. (Japan)* 2:279-281.
- Plummer, J.A., M.G. Mullins, J.H. Vine, and R.P. Pharis. 1989. The role of endogenous hormones in shoot emergence and abscission in alternate bearing Valencia orange trees. *Acta Hort.* 239:341-344.
- Powell, A.A., and A.H. Krezdorn. 1977. Influence of fruit-setting treatment on translocation of ¹⁴C-metabolites in citrus during flowering and fruiting. *J. Amer. Soc. Hort. Sci.* 102:709-714.
- Procopiou, J. 1972. Severe pruning overcome alternate bearing of mandarin trees in Rhodes. *HortScience* 7:124.
- Ruan, Y. 1993. Fruit set, young fruit and leaf growth of *Citrus unshiu* in relation to assimilate supply. *Scientia Hort.* 53:99-107.
- Ruiz, R., A. García-Luis, C. Monerri, and J.L. Gaurdiola. 2001. Carbohydrate availability in relation to fruitlet abscission in *Citrus*. *Ann. Bot.* 87:805-812.
- Saidha, T., E.E. Goldschmidt, and S.P. Monselise. 1983. Endogenous growth regulators in tracheal sap of Citrus. *HortScience* 18:231-232.
- Sanz, A., C. Monerri, J. González-Ferrer, and J.L. Guardiola. 1987. Changes in carbohydrates and mineral elements in *Citrus* leaves during flowering and fruit set. *Physiol. Plant.* 69:93-98.
- Schaffer, A.A., E.E. Goldschmidt, R. Goren, and D. Galili. 1985. Fruit set and carbohydrate status in alternate and nonalternate bearing *Citrus* cultivars. *J. Amer. Soc. Hort. Sci.* 110:574-578.
- Smith, P.F. 1976. Collapse of 'Murcott' tangerine trees. *J. Amer. Soc. Hort. Sci.* 101:23-25.
- Stewart, I., T.A. Wheaton and R.L. Reese. 1968. 'Murcott' collapse due to nutritional deficiencies. *Proc. Fla. State Hort. Soc.* 81:15-18.

- Talon, M., F.R. Tadeo, W. Ben-Cheikh, A. Gomez-Cadenas, J. Mehouchi, J. Pérez-Botella, and E. Primo-Millo. 1997. Hormonal regulation of fruit set and abscission in citrus: Classical concepts and new evidence. *Acta Hort.* 463:209-217.
- Talon, M., L. Zacarias, and E. Primo-Millo. 1990. Hormonal changes associated with fruit set and development in mandarins differing in their parthenocarpic ability. *Physiol. Plant.* 79:400-406.
- Tucker, D.P.H., T.A. Wheaton, and R.P. Muraro. 1994. Citrus tree pruning principles and practices. Horticultural Science Department. Florida Cooperative Extension Service. Institute of Food and Agricultural Science. University of Florida. Fact Sheet HS-144. Reviewed: June 1994.
- <<<http://cms.cnr.edu.bt/cms/files/docs/File/Jeanette/PDF/citrus%20pruning.pdf>>>
- Turnbull, C.G.N. 1989. Gibberellins and control of fruit retention and seedlessness in Valencia orange. *Acta Hort.* 239:335–340.
- Verreynne, J.S., 2005. The mechanism and underlying physiology perpetuating alternate bearing in ‘Pixie’ mandarin (*Citrus reticulata* Blanco). Phd Thesis. University of California, USA.
- Verreynne, J.S., and C.J. Lovatt. 2009. The effect of crop load on budbreak influences return bloom in alternate bearing ‘Pixie’ mandarin. *J. Amer. Soc. Hort. Sci* 134:299-307.
- Wheaton, T.A. 1992. Alternate bearing of citrus. *Proc. Intl. Sem. Citricult.* 1:224–228.
- Yahata, D., K. Matsumoto, and K. Ushijima. 2004. Relationship between flower-bud differentiation and carbohydrate content in spring shoots of very-early, early and late maturing cultivars of Satsuma mandarin. *J. Japan. Soc. Hort. Sci.* 73:405-410.

3. Paper 1. Changes in leaf carbohydrate levels over one season in alternate bearing ‘Nadorcott’ mandarin trees and the relationship between leaf carbohydrate status, return bloom, set percentage and subsequent yield

Abstract

Alternate bearing is a common phenomenon in most commercial perennial fruit trees. In Citrus, the “on” year consist of a heavy crop load with mostly small fruit followed by an “off” year with few and large fruit. Carbohydrates play an important role, especially during fruit set, but also in flowering and fruit maturation and are essential in maintaining a regular bearing habit. Late mandarin types such as ‘Nadorcott’ mandarin are prone to alternate bearing. The aim of this study was to follow the change in starch and total sugar accumulation in the leaves across an entire season for both “on” and “off” trees and to see if leaf carbohydrate levels can be used to predict bearing potential. Ten “on” and ten “off” trees were chosen in a randomized complete block design. Vegetative leaves from the previous spring flush were sampled on a monthly basis from April 2010 until March 2011 for starch and sugar analysis. Phenology was followed by determining the yield and counting the number of flowers in the return bloom. Fruit growth was also measured from after physiological fruit drop until harvest the following year. Starch accumulation followed a distinct pattern with differences between “on” and “off” trees visible in April and May. Starch levels in April showed a moderate negative correlation with yield and a moderate positive correlation with return bloom. Rapid starch accumulation started prior to harvest with a peak at the beginning of flowering. Thereafter a sharp decrease in starch levels occurred until after full bloom and a steady decrease was observed from physiological fruit drop onwards as fruit matured. Total sugar levels were more constant, but the increase in yield overall in 2011 compared to 2010 was reflected in the leaf total sugar levels as the demand for carbohydrates was increased by the higher fruit load. “On” trees yielded 53% more than “off” trees, but the return bloom for “off” trees was 140% more than “on” trees, thus illustrating the negative effect that a large crop has on the next season’s bloom. We conclude that leaf starch levels in April can be used as an indication for bearing potential the following season for ‘Nadorcott’ mandarin trees.

Keywords: *late mandarin; phenology; starch; total sugar; predicting bearing potential*

3.1 Introduction

Alternate bearing of fruit trees refers to when a heavy crop load during the “on” year is followed by a light crop load during the “off” year. Alternate bearing occurs in most fruit trees, deciduous or evergreen, including citrus (Monselise and Goldschmidt, 1982). Goldschmidt (2005) described alternate bearing as a “phenomenon of homeostasis”, and pointed out that this behavior secured the longevity of fruit trees in the wild, and that regular bearing is most probably a result of domestication of fruit trees, whereby certain stresses such as drought and pests are removed. Alternate bearing can occur across an entire production region (usually initiated by adverse climatic conditions), between orchards, within an orchard or between branches on a single tree (Monselise and Goldschmidt, 1982).

Certain cultivars of mandarin species such as *Citrus unshiu* (Satsuma) and *C. reticulata*, including common mandarin hybrids within *C. reticulata* and crosses between *C. reticulata* and *C. paradisi* or *C. sinensis*, are prone to alternate bearing (Monselise et al., 1981). Some mandarin cultivars such as Wilking and Murcott mandarins (*C. reticulata* Blanco) are known as absolute alternate bearers due to the fact that there is a lack of flowers in the “off” year (Goldschmidt and Golomb, 1982). These cultivars can alternate to such an extent that the trees collapse, or even die, during the “on” year (Stewart et al., 1968). This collapse is associated with a total depletion of carbohydrates in the tree.

The main problem associated with alternate bearing in citrus is the irregular yield, i.e., a large number of small fruit during the one season followed by a few and large fruit the next season (Monselise et al., 1981). The net result is that relatively few fruit can be marketed for maximum financial returns each season. From an orchard management point of view, it is not currently possible to predict whether a light or heavy crop will result in an “on” or “off” crop the next season. Furthermore, it has not been determined what will be the best indicator of alternate bearing, whether it is starch reserves, nitrogen content, or any other factor. It seems likely that such a prediction strategy will be cultivar specific and will include the influence of cultural practices, for instance pruning, fruit thinning and time of harvest, to reduce the alternate bearing cycle sufficiently in the case where an “on” or “off” crop is predicted.

Carbohydrates (specifically starch) play an important role in the regulation and severity of alternate bearing in citrus trees (Jones et al., 1975; Schaffer et al., 1985). It is important to remember that endogenous hormones, such as gibberellins, also play an important role, but do not form part of this study. The large demand for energy during spring when floral development, anthesis, fruit set and the spring vegetative flush develop at the same time, is more than current photosynthesis can supply (Goldschmidt, 1999; Goldschmidt and Koch, 1996). Carbohydrate reserves are mobilized during this period and there is a decrease in carbohydrate levels from anthesis (Guardiola, 2000; Goldschmidt and Koch, 1996) until mid-winter or just after harvest (García-Luis et al., 1995; Mataa et al., 1996; Sanz et

al., 1987). Although researchers have measured carbohydrate levels of “on” and “off” trees during certain critical stages of development, for instance during flowering, no research has been conducted to determine the seasonal changes in starch reserves during all the different phenological stages throughout an entire growing season and how this differs for “on” and “off” trees.

The aim of this study was to determine whether leaf starch or total sugar levels during a certain critical period of phenological development can be identified for possible use to predict bearing potential for the following season. This could enable mandarin producers to manage alternate bearing orchards early on, thereby minimizing financial losses. Another objective was to determine how “on” and “off” trees differ in phenological development. The hypothesis that was tested is that leaf carbohydrate levels can be used as an indicator of bearing potential for ‘Nadorcott’ mandarin.

3.2 Materials and Methods

3.2.1 Plant material

The study was conducted in the 2010/2011 season on ‘Nadorcott’ mandarin (*C. reticulata* Blanco) trees on Carizzo citrange rootstock in a commercial orchard with a history of alternate bearing in the Porterville area (33°04’54.70’’S; 18°51’24.64’’E), South Africa. The orchard was planted in a north-south row direction in 2000 with a between row spacing of 5 m and a between tree spacing of 2 m.

3.2.2 Treatments and data collection

The two treatments used were: “on” (heavy crop load) and “off” (light crop load) trees that were visually selected in 2010 for their current crop load (Fig. 1.). Therefore, no treatment was carried out *per se* and the two groups of 10 trees were used to follow the phenology and leaf carbohydrate accumulation throughout the growing season. Trees were chosen for their uniform size and large differences in yield between trees in the same orchard.

From April 2010 until March 2011 ten leaf samples from vegetative shoots from the previous spring flush were randomly taken from all trees in the experiment during the first two weeks of every month to later analyze the starch and total sugars levels. The leaves were picked during the morning, brought to the lab in a cooler bag with ice bags upon which they were washed with distilled water and wiped dry using a paper towel before the midribs were removed. Thereafter the leaf samples were dried in an oven at 65°C for 3 days before being ground to a fine powder and stored at -40°C until analysis.

The phenology of the two sets of trees was recorded by randomly selecting and tagging five vegetative and five reproductive shoots on each tree prior to harvest in 2010. The number of fruit on the reproductive shoots and the number of nodes per shoot were recorded for all the shoots in addition

to counting the flowers as soon as the first flowers reached the balloon stage. An inflorescence was defined as the structure that sprouted from a single auxiliary bud. The inflorescences were divided into leafy (consisting of both flowers and leaves) and leafless (consisting of only flowers, Fig. 2.). The number of inflorescences, number of flowers per inflorescence and the number of vegetative shoots were recorded for each shoot. This was repeated 1 week later to record any newly developed flowers.

In Dec. 2010, after physiological fruit drop, the number of persistent fruit on each shoot was recorded. The set percentage was then calculated by dividing the number of fruit by the number of flowers for each tree. At this time, 10 fruit per tree were tagged to follow fruit growth until harvest. Fruit diameter was measured at monthly intervals from December 2010 until June 2011 using an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan). However, many tagged fruit had abscised by January 2011 due to the use of a thinning agent (3,5,6-Trichloro-2-pyridinyloxyacetic acid) and new fruit were tagged in February 2011 to replace abscised fruit.

Trees were harvested on 12 August 2010 and the fruit harvested from each tree were weighed on an electronic balance (W22 Series, UWE Co, Hsin Tien, Taiwan) to determine yield (kg/tree). Fruit size distribution was determined by measuring 130 fruit (diameter) per tree in the field using an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan). Twelve fruit were randomly sampled from each tree for external and internal quality analysis as described below.

The following fruit quality parameters were determined. Rind colour was assessed using a No. 36 CRI colour chart for mandarins [Citrus Research International (CRI), 2004]. Fruit diameter was measured using an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan). The fruit were cut in half on the equatorial line and the flesh juiced using a citrus juicer (Sunkist®, Chicago, USA). The juice was strained through a muslin cloth to remove any solid particles and the juice percentage was determined by dividing the weight of the juice by the total weight of the fruit. Total soluble solids (TSS) of the juice was determined by using an electronic refractometer (PR-32 Palette, Atago Co, Tokyo, Japan) and titratable acidity (TA) was determined by titrating 20 ml of juice against 0.1 N sodium hydroxide. Phenolphthalein was used as indicator and titration was complete when the liquid turned pink in colour. Acid was expressed as citric acid content. The TSS:TA ratio was determined by dividing TSS values by TA values.

3.2.3 Starch and total sugars analysis

The anthrone method, adapted for citrus tissue, was used to determine the starch and total soluble sugar (in 80% ethanol) concentration of the dried leaf samples (Dische, 1962; Hamid et al., 1985; Hettasch, 1999; Reed et al., 2004). From here onward the total soluble sugars in 80% ethanol will be referred to as "total sugars", similar to Tibschraeny (1995). Total sugars are primarily composed of sucrose, glucose and fructose (Ruiz et al., 2001).

Step 1. Total sugars extraction: Five ml of 80% ethanol was added to 0.1 g of the dry leaf powder, briefly vortexed, and placed in a heating block at 80°C for 1 hour. The solution was centrifuged for 12 min. at 4 000 rpm at 20°C before transferring the supernatant to a new tube. Five ml of 80% ethanol was again added to the leaf powder, vortexed, and placed in a heating block for 30 min. at 80°C. The solution was centrifuged and the supernatant was combined with the supernatant from the first extraction. Five millilitres of deionised water was added to the solution and it was stored at -20°C until the starch extraction was done.

Step 2. Starch extraction: The tubes containing the pellet that was left after the total sugars extraction were placed on their sides and the alcohol was allowed to evaporate. Two ml of 5 mM acetate buffer (pH 4.8) was then added to the pellet, vortexed, and placed in a heating block to gelatinize the solution at 100°C for 1 hour. Amyloglucosidase enzyme (AGS) was prepared by adding 5 mM acetate buffer in a ratio of 1 mg AGS to 10 ml buffer solution. Two ml of this solution (equal to 14 units of AGS) was then added to the pellet, vortexed, and incubated in a heating block at 60°C for 18 hours. Incubation was terminated by placing the solution in a boiling water bath for 5 min. The solution was then centrifuged and the supernatant transferred to another tube. Five ml of deionised water was added to dilute the sample.

Step 3. Preparation for spectrophotometric readings: Twenty µl of the soluble sugar solution extracted in step 1 was added to 480 µl of deionised water. For the starch solution (extracted in step 2), 30 µl of solution was added to 470 µl of deionised water.

A standard curve was prepared by diluting glucose, made up to 200 µg/ml, with deionised water to concentrations of 0, 6.667, 13.333 and 20 µg/ml, respectively. All four solutions were made up to 500 µl.

The anthrone solution was prepared by adding 2 g of anthrone to 1000 ml of H₂SO₄. The tubes containing the starch and sugar solutions, as well as the standard solutions, were placed in wet ice to absorb heat produced by adding anthrone to the solutions. One ml of the anthrone solution was then added to the sugar, starch and standard solutions and kept in the wet ice for 5 min.. The tubes were vortexed, placed in a boiling water bath for 5 min. and then immediately placed back into the ice water.

Step 4. Spectrophotometric readings: The sugar, starch and standard solutions were placed in plastic cuvettes and the absorbance was determined on a spectrophotometer (Cary 50 Bio, Varian, Varian Australia, Australia) at 620 nm against a blank prepared for the standard (0 µg/ml glucose).

Total carbohydrates were determined by adding the starch and total sugars value for each sample.

3.2.4 Experimental design and statistical analysis

The trial consisted of 10 single tree replications for each treatment in a completely randomized design. Analysis of variance (ANOVA) was carried out using Enterprise Guide (version 4.1, SAS Institute Inc., Cary, NC, USA). Least significant difference (LSD, $p=0.05$) was used to separate means where data were normally distributed. The Wilcoxon rank sum test was used in cases where data were not normally distributed. Pearson's product-moment correlation coefficient was used to analyse correlations and it was carried out using Enterprise Guide (version 4.1, SAS Institute Inc., Cary, NC, USA). Regressions were carried out using Microsoft Excel 2007.

3.3 Results

3.3.1 Carbohydrate accumulation over one season

Starch. The average leaf starch concentration for both “on” and “off” trees is presented in Fig. 3 for the period April 2010 until March 2011. Differences occurred during fruit maturation (April 2010 until June 2010) between “on” and “off” trees. On the first and second sampling dates (7 April 2010 and 18 May 2010) this difference was significant with “on” trees having an average of $15.3 \text{ mg}\cdot\text{g}^{-1}$ dry weight (DW) of starch in the leaves in April compared to the $28.7 \text{ mg}\cdot\text{g}^{-1}$ DW in “off” trees'. Rapid starch accumulation in the leaves took place from the beginning of July until the first week in September in both “on” and “off” trees. Leaf starch levels between “on” and “off” trees differed significantly on 6 August 2010 with $52.4 \text{ mg}\cdot\text{g}^{-1}$ DW starch in “on” tree leaves compared to $39.5 \text{ mg}\cdot\text{g}^{-1}$ DW in “off” tree leaves. After the peak in leaf starch levels (7 September 2010), a rapid decrease was seen until middle October. From this period onwards until March 2011, there was a steady decrease in starch levels in leaves of both “on” and “off” trees coinciding with the demand for carbohydrates from new developing fruit.

Total sugars. There was a small, insignificant difference in total sugar levels between leaves from “on” and “off” trees in April 2010 (Fig. 4) with “off” trees having marginally more total sugars in the leaves ($71.7 \text{ mg}\cdot\text{g}^{-1}$ DW) compared to “on” trees ($67 \text{ mg}\cdot\text{g}^{-1}$ DW). During the first week of June, just prior to the onset of starch accumulation (Fig. 3), there was a bigger (yet insignificant) difference in leaf total sugar levels between “on” ($58.3 \text{ mg}\cdot\text{g}^{-1}$ DW) and “off” ($68.7 \text{ mg}\cdot\text{g}^{-1}$ DW) trees. A notable difference in total sugars levels also occurred by the end of the first week of September, coinciding with the onset of flowering. Overall, total sugar levels in March 2011 were lower than April 2010.

Total carbohydrates. Total carbohydrates (a combination of starch and total sugars) concentration in leaves followed a similar pattern to that of starch with the only important significant difference between “on” ($87.0 \text{ mg}\cdot\text{g}^{-1}$ DW) and “off” ($104.3 \text{ mg}\cdot\text{g}^{-1}$ DW) trees occurring in April 2010 (Fig. 5).

3.3.2 Phenology (2010)

Flower number and fruit set. During the return bloom there was a significant difference between “on” and “off” trees in the number of all inflorescence types (Table 1). “Off” trees had 80% more leafy inflorescences (LY infl) compared to “on” trees and also 155% more leafy (LY) flowers. “Off” trees had 134% more leafless inflorescences (LL infl) and 135% more leafless (LL) flowers. Overall, “off” trees had 140% more flowers than “on” trees. One parameter that did not differ significantly was the number of vegetative shoots that sprouted. There was no significant difference in fruit set (number of fruit set per shoot) between “on” and “off” trees (Table 1).

Fruit growth. Fruit growth was measured during the season following the “on” and “off” season (i.e. “return” fruit growth). There was no significant difference in growth rate between “on” and “off” trees (Table 1).

Yield and fruit quality. There was a significant difference in yield in 2010 with “on” trees yielding 53% more than “off” trees (Table 2). However, in 2011 no significant difference was found between “on” and “off” trees, but yield was on average 108% higher than in 2010. On average, trees bearing an “off” crop in the 2010 season yielded 8% more in 2011 compared to “on” trees (Table 2). There were no significant differences in any of the internal and external fruit quality parameters during 2010 or 2011 (Table 2).

3.3.3 Correlations

Starch. In previous research on alternate bearing of mandarin, linear correlations with r-values higher than 0.5 were interpreted as physiologically significant (Verreynne, 2005). Therefore, only r-values larger than 0.5 will be discussed in this study. Data from “on” and “off” trees were combined when calculating the correlations. When leaf starch levels for April 2010 were correlated with the physiological parameters listed in Table 3, r-values higher than 0.5 were observed for leafless flowers per 100 nodes (LL/100 nodes), total flowers per 100 nodes (total flowers/100 nodes) and yield. Yield showed the highest correlation of -0.66 (p-value, 0.0014). The correlation between yield and leaf starch levels continued during May '10 and June '10 with r-values of -0.60 (p-value, 0.0055) and -0.59 (p-value, 0.0058) respectively. These negative correlations indicate that as one parameter (leaf starch levels) increases, the other parameter (yield) decreases. The relationship is, therefore, equally important in comparison with a positive correlation. There was also a physiologically significant correlation with an r-value of 0.57 (p-value, 0.0089) between leaf starch levels in April and total flowers/100 nodes for the return bloom. Furthermore, May '10 showed a positive correlation of 0.58 (p-value, 0.0078) with LL/100 nodes and leaf starch levels in March '11 showed a negative correlation of -0.53 (p-value, 0.017) with LY/100 nodes.

Significant correlations between set percentage and starch levels were observed during October 2010, December 2010 and January 2010. The r-values were as follows: October 2010 0.55 (p-value, 0.0127), December 2010 0.6 (p-value, 0.0052) and January 2011 0.57 (p-value, 0.0093). Curiously, the r-value for November 2010 during fruit set was only -0.02 indicating no correlation.

Total sugars and total carbohydrates. Total soluble sugar levels in February 2011 had a r-value of -0.52 (p-value, 0.020) when correlated with LY/100 nodes (Table 4). For total leaf carbohydrate levels in February 2011 there were a r-value of -0.53 (p-value, 0.016) when correlated with LY/100 nodes. Total leaf carbohydrates in August 2010 showed a correlation with a r-value of 0.50 (p-value, 0.026) with LL/100 nodes (Table 5).

3.4 Discussion

The changes in leaf starch levels provide insight into the energy demand of the tree during different phenological stages. This should lead to a better understanding of when the tree produces sufficient carbohydrates and also when reserve mobilization might become necessary. The difference in leaf carbohydrate levels of “on” and “off” trees for one entire season has not been well documented with authors typically focusing on only a few months (Hilgeman et al., 1967; Mataa et al., 1996) or specific dates or phenological stages such as flowering (Jones et al., 1970; Lewis et al., 1964; Sanz et al., 1987). Tibschraeny (1995) did investigate changes in starch and total sugars in ‘Satsuma’ mandarin over 12 months, but only bark and wood tissue were sampled and the link to tree phenology was not discussed.

It is interesting to note that starch levels in the leaves started to increase approximately 5 weeks prior to the actual harvest date. Although it was only significant in April 2010, from then onwards “on” trees had lower starch levels compared to “off” trees and even though “on” trees had a slightly lower minimum level of leaf starch and also lower levels in early August, “on” and “off” trees reached a similar maximum point at the onset of bud break in September. This is an interesting phenomenon since research has shown that starch levels in ‘Owari’ satsuma mandarin starts to accumulate after harvest (García-Luis et al., 1995). However, Tibschraeny (1995) also found an increase in bark starch levels in ‘Satsuma’ mandarin roughly one month before harvest. Since ‘Nadorcott’ mandarin trees are harvested in August (SH) and maximum fruit TSS is reached a few weeks prior to harvest (data not shown), it can be argued that the demand for carbohydrates by the fruit is decreasing at this point since fruit at this stage only need carbohydrates for respiration. This could allow starch accumulation in the leaves if the sink strength of the fruit is reduced (García-Luis et al., 1988). Studies on other late mandarin cultivars such as Orri, Morri and also clementine mandarins, which is harvested in May, will show whether or not this phenomenon occurs for all mandarin trees or only certain cultivars such as Nadorcott mandarin and Owari satsuma mandarin.

After the peak in leaf starch levels early in September (Fig. 3.), coinciding with the flowering period, the trend in leaf starch levels was reversed between “on” and “off” trees with “on” trees having slightly higher values until mid-October. This reversion is most likely due to “off” trees having a larger return bloom compared to “on” trees. This illustrates the amount of energy required by the tree during this period of not only flower development and nectar production, but also rapid growth of the spring vegetative flush (Guardiola, 2000). The rate of starch mobilization from leaves steadily declined from full bloom onwards as fruit developed and no significant differences between “on” and “off” trees occurred. This is probably because no significant difference in yield was found between previously “on” and “off” trees in 2011.

It is interesting to note that total sugar levels remained much more constant throughout the season (Fig. 4) and no definite pattern of accumulation or depletion was visible. This concurs with previous research that showed differences in soluble sugar levels between “on” and “off” trees to be much smaller compared to starch (Goldschmidt and Golomb, 1982). Sucrose, a component of total sugars, is important in citrus physiology since it is the main transport carbohydrate in the phloem due to its non-reducing nature. Glucose and fructose, the constituents of sucrose, are very important in respiration and are substrates for glycolysis which produce the energy and other substrates needed by plant. Furthermore, it would seem that in citrus starch is used to keep total sugar levels constant throughout the year to ensure that the constant demand for energy by plant organs are met.

The reduction in return bloom for “on” trees ($\pm 50\%$ compared to “off” trees) without a difference in set percentage confirmed the results of Moss (1971), who reported that there were roughly twice as many ‘Late Valencia’ sweet orange flowers after an “off” year in comparison to an “on” year without a significant differences in set percentage, albeit over different seasons. This shows that a critical factor with regards to alternate bearing is a lack (or abundance) of flowers (Gaurdiola, 2000), which is not an important factor in regular bearing trees. It has been established that fruit inhibit bud break of floral shoots the following season (García-Luis et al., 1995; Verreynne and Lovatt, 2009) and the reduction in flowering can easily be explained by this phenomenon. It was observed in ‘Nadorcott’ mandarin that shoots bearing fruit do not flower the next spring, therefore drastically reducing bearing wood for the next season during an “on” year. Another reason is that the summer and autumn vegetative flushes are often absent during “on” years, thereby further reducing bearing units for the next season (Verreynne and Lovatt, 2009).

April was identified as the month during which leaf starch levels displayed the highest correlations with current yield (negative) and return bloom (positive). Moss (1971) correlated current yield with return bloom for ‘Late Valencia’ and found that it was not a physiologically significant relationship ($r=0.451$). It could be construed that starch levels in April, rather than current yield, might give the best indication of how the tree will react to the current yield the following season. Similar to the data

presented in this paper, Okada (2004) found a strong negative correlation between current yield and non-structural carbohydrates in the leaves of ‘Aoshima’ satsuma mandarin trees sampled in Nov. (NH). In addition, a high positive correlation with return bloom was reported and they concluded that reserve nutrients can be effective predictors of bearing potential in satsuma mandarin trees. In support of this, Agusti et al. (1992) has shown that girdling just prior to or during Oct. (NH) will increase flowering in the following season and that this treatment will induce starch accumulation in leaves and twigs (García-Luis et al., 1995; Goldschmidt et al., 1985). It seems that higher leaf starch levels in April result in good return bloom and we propose that starch levels in April can be used as an indicator of bearing potential for the following season. At this stage a single threshold level cannot be established for ‘Nadorcott’ mandarin and producers should use historical yield data to identify an alternate bearing cycle if present and then subsequently use leaf starch levels in April to determine the threshold for each orchard. This could give producers the ability to prevent or reduce the impact of an “on” year by removing small and scarred fruit in April instead of at harvest three to five months later. Furthermore, certain horticultural practices, such as girdling in an “off” year, can be used early in the following season to alter fruit load and pruning methods can be adapted.

A physiologically significant correlation was found between leaf starch levels in October, December and January and fruit set percentage. This period coincides with the onset and end of physiological fruit drop. Ruiz et al. (2001) also reported starch mobilization, rather than total sugar supply was the limiting factor during this period. Mehouchi et al. (1995) implicated total sugar supply to be the most important factor influencing physiological fruit drop, but it was not confirmed in this experiment, since there was only a small reduction of leaf total sugar levels and no physiological significant correlations were found for fruit set.

The fact that 2010 was an “off” year for the orchard as a whole compared to the “on” year in 2011 illustrates the phenomenon that alternate bearing can occur across an orchard or between trees in an orchard (Monselise and Goldschmidt, 1982). Furthermore it shows that ‘Nadorcott’ mandarin have a tendency to alternate bearing if crop load is not well managed. This tendency is also reflected in the leaf total sugars levels since a steady decrease was visible from April 2010 (“off” year) until March 2011 (“on” year). As reported by Lewis et al. (1964), it seems that although leaf total sugar levels remain much more constant than starch levels it will decrease with an increase in demand by developing fruit.

Alternate bearing of citrus cannot be fully explained by the effect of carbohydrate storage and mobilization on flowering and fruiting alone. It is important to remember that endogenous hormones such as gibberellins, abscisic acid (ABA) and auxins also play an important role (Talon et al., 1997).

In conclusion, ‘Nadorcott’ mandarin trees displayed a distinct pattern of leaf starch accumulation and mobilization during one season. “On” trees had lower leaf starch levels from April until May

compared to “off” trees due to the larger demand for carbohydrates by the larger crop. Starch accumulation began prior to harvest, presumably when fruit TSS levels had reached its maximum for the season, and peaked at the onset of flowering. A sharp decrease in leaf starch levels followed until after full bloom followed by a steady decrease during physiological fruit drop and fruit maturation. Total sugars levels remained more constant throughout the season with “on” trees having lower levels just prior to the onset of starch accumulation and at the onset of flowering. “Off” trees had significantly more flowers during the return bloom compared to “on” trees even though the number of vegetative shoots did not differ. Leaf starch levels in October, December and January correlated well with fruit set percentage illustrating that the tree was source-limited during this period and that starch mobilization is an important factor influencing fruit set. In April, leaf starch levels had a negative linear correlation with yield indicating that a large yield at this stage will induce low leaf starch levels and *vice versa*. Leaf starch levels in April were positively correlated to flower number the next spring. This illustrates the negative effect that crop load has on return bloom. No significant correlation between flowering and yield was observed for leaf total sugars and total carbohydrate levels during any one month. The time and costs involved with taking leaf samples more than once in a season reduces the practical use of these parameters to predict bearing potential. Leaf starch levels in April could therefore potentially be used as an indicator of bearing potential for the following season, allowing producers an ‘early warning system’ for an upcoming “on” or “off” year, thereby allowing producers to adjust cultural practises on time.

3.5 References

- Agusti, M., V. Almela, and J. Pons. 1992. Effect of girdling on alternate bearing in citrus. *J. Hort. Sci.* 67(2):203-210.
- Dische, Z. 1962. Colour reactions of carbohydrates. In: RL Whistler, ML Wolfrom (eds.). *Methods in carbohydrate chemistry*, Academic Press, New York, 475-514.
- Garcia-Luis, A., F. Fornes, and J.L. Guardiola. 1995. Leaf carbohydrates and flower formation in Citrus. *J. Amer. Soc. Hort. Sci.* 120:222–227.
- Garcia-Luis, A., F. Fornes, A. Sanz, and J.L. Guardiola. 1988. The regulation of flowering and fruit set in *citrus*: Relationship with carbohydrate levels. *Isr. J. Bot.* 37:189-201.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. *HortScience* 34:1020-1024.
- Goldschmidt, E.E. 2005. Regulatory aspects of alternate bearing in fruit tree. *Italus Hortus* 12:11-17.
- Goldschmidt, E.E., N. Aschkenazi, Y. Herzano, A.A. Schaffer, and S.P. Monselise. 1985. A role for carbohydrate levels in the control of flowering in citrus. *Scientia Hort.* 26:159–166.
- Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate bearing citrus trees and the significance of reserves for flowering and fruiting. *J. Amer. Soc. Hort. Sci.* 107:206–208.
- Golschmidt, E.E. and K.E. Koch. 1996. Citrus, p.797-823. In: E. Zamski and A. A. Schaffer (eds.). *Photoassimilate distribution in plants and crops*. Marcel Dekker, New York.
- Guardiola, J.L. 2000. Regulation of flowering and fruit development: Endogenous factors and exogenous manipulation. *Proc. Intl. Soc. Citricult.* 9:342-346.
- Hamid, G.A., S.D. Van Gundy and C.J. Lovatt. 1985. Citrus nematode alters carbohydrate partitioning in the ‘Washington’ navel orange. *J. Amer. Soc. Hort. Sci.* 110(5):642-646.
- Hettasch, H. 1999. Studies of the vegetative development of *Protea* cv. ‘Sylvia’ and ‘Cardinal’. MSc Thesis. Dept. Horticultural Science, University of Stellenbosch, South Africa.
- Hilgeman, R.H., J.A. Dunlap, and G.C. Sharples, 1967. Effect of time of harvest of Valencia oranges on leaf carbohydrate content and subsequent set of fruit. *Proc. Amer. Soc. Hort. Sci.* 90:110–116.

- Jones, W.W., T.W. Embleton, M.L. Steinacker and C.B. Cree. 1970. Carbohydrate and flowering of 'Valencia' orange trees. *J. Amer. Soc. Hort. Sci.* 95(3): 380-381.
- Jones, W.W., T.W. Embleton, and C.W. Coggins, Jr. 1975. Starch content of roots of 'Kinnow' mandarin trees bearing fruit in alternate years. *Hort. Sci.* 10:514.
- Lewis, L.N., C.W. Coggins Jr. and H.Z. Hield. 1964. The effect of biennial bearing and NAA on the carbohydrate and nitrogen composition of Wilking mandarin leaves. *J. Amer. Soc. Hort. Sci.* (84): 147-151.
- Mataa, M., S. Tominaga, and I. Kozaki. 1996. Seasonal changes of carbohydrate constituents in Ponkan (*Citrus reticulata* Blanco). *J. Japan. Soc. Hort. Sci.* 65:513-523.
- Mehouachi, J., D. Serna, S. Zaragosa, M. Agusti, M. Talon, and E. Primo-Millo. 1995. Defoliation increase fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci.* 107:189-197.
- Monselise, S.P. and E.E. Goldschmidt. 1982. Alternate bearing in fruit trees. *Hort. Rev.* 4:128-173.
- Monselise, S.P., E.E. Goldschmidt, and A. Golomb. 1981. Alternate bearing in citrus and ways of control. *Proc. Intl. Soc. Citricult.* 1:239-242.
- Moss, G.I. 1971. Effect of fruit on flowering in relation to biennial bearing in sweet orange (*Citrus sinensis*). *J. Hort. Sci.* 46:177-184.
- Okada, M. 2004. Effectiveness of reserved nutrients for estimating productivity of Satsuma mandarin. *J. Japan. Soc. Hort. Sci.* 73(2):163-170.
- Reed, A.B., C.J. O'Connor, L.D. Melton and B.G. Smith. 2004. Determination of sugar composition in grapevine rootstock cuttings used for propagation. *Am. J. Enol. Vitic.* 55(2):181-186.
- Ruiz, R., A. García-Luis, C. Monerri, and J.L. Guardiola. 2001. Carbohydrate availability in relation to fruitlet abscission in *Citrus*. *Ann. Bot.* 87:805-812.
- Sanz, A., C. Monerri, J. González-Ferrer, and J.L. Guardiola. 1987. Changes in carbohydrates and mineral elements in *Citrus* leaves during flowering and fruit set. *Physiol. Plant.* 69:93-98.
- Schaffer, A.A., E.E. Goldschmidt, R. Goren, and D. Galili. 1985. Fruit set and carbohydrate status in alternate and nonalternate bearing *Citrus* cultivars. *J. Amer. Soc. Hort. Sci.* 110:574-578.
- Stewart, I., T.A. Wheaton and R.L. Reese. 1968. 'Murcott' collapse due to nutritional deficiencies. *Proc. Fla. State Hort. Soc.* 81:15-18.

- Talon, M., F.R. Tadeo, W. Ben-Cheikh, A. Gomez-Cadenas, J. Mehouchi, J. Pérez-Botella, and E. Primo-Millo. 1997. Hormonal regulation of fruit set and abscission in citrus: Classical concepts and new evidence. *Acta Hort.* 463:209-217.
- Tibschraeny, C. 1995. Studies on light improvement, reserve fluctuations and crop estimation in various *Citrus spp.* M.Sc. Thesis, Dept. of Horticultural Science, Stellenbosch University, South Africa.
- Verreynne, J.S., 2005. The mechanism and underlying physiology perpetuating alternate bearing in 'Pixie' mandarin (*Citrus reticulata* Blanco). Phd Thesis. University of California, USA.
- Verreynne, J.S., and C.J. Lovatt. 2009. The effect of crop load on budbreak influences return bloom in alternate bearing 'Pixie' mandarin. *J. Amer. Soc. Hort. Sci* 134:299-307.

Table 1. Effect of alternate bearing on inflorescence type, flower number, number of vegetative shoots, set percentage and fruit growth for “on” and “off” ‘Nadorcott’ mandarin trees in the Porterville area, South Africa (2010/2011). Set percentage was calculated by dividing the number of fruit by the number of flowers for each tagged shoot.

Treatment	LY infl/ 100 nodes	LY/100 nodes	LL infl/100 nodes	LL/100 nodes	Total flowers/ 100 nodes	Veg/100 nodes	Fruit set %	Fruit growth (mm/day)
“On” tree	5.7b ^z	5.8a	13.1a	21.7a	27.5a	25.9 ^{ns}	26.0 ^{ns}	0.28 ^{ns}
“Off” tree	10.2a	14.8b	30.7b	51.0b	65.8b	29.3	16.3	0.27
<i>p-value</i>	<i>0.0440</i>	<i>0.0312</i>	<i>0.0020</i>	<i>0.0006</i>	<i>0.0004</i>	<i>0.5807</i>	<i>0.1702</i>	<i>0.0648</i>

^zMeans with different letters differ significantly at the 5% level (Wilcoxon rank sum test)

LY infl (Leafy inflorescence)

LL infl (Leafless inflorescence)

LY (Leafy flowers)

LL (Leafless flowers)

Veg (Vegetative shoots)

Table 2. Effect of an “on” and “off” bearing habit on internal quality, final fruit size and yield of ‘Nadorcott’ mandarin trees in the Porterville area, South Africa (2010/2011).

Year	Treatment	TSS (°Brix)	TA (% citric acid)	TSS:TA	Juice %	Fruit diameter (mm)	Yield (kg/tree)
2010	“On” tree	13.8 ^{ns}	1.2 ^{ns}	11.2 ^{ns}	47.2 ^{ns}	56.7 ^{ns}	34.7a ^z
	“Off” tree	13.4	1.3	10.5	48.0	58.6	22.7b
	<i>p-value</i>	<i>0.2491</i>	<i>0.2806</i>	<i>0.0748</i>	<i>0.4031</i>	<i>0.0640</i>	<i>0.0093</i>
2011	“On” tree	9.1 ^{ns}	0.84 ^{ns}	10.8 ^{ns}	49.1 ^{ns}	68.2 ^{ns}	57.3 ^{ns}
	“Off” tree	9.1	0.81	11.2	49.9	69.7	62.0
	<i>p-value</i>	<i>0.9264</i>	<i>0.2271</i>	<i>0.3980</i>	<i>0.4765</i>	<i>0.0592</i>	<i>0.3146</i>

^zMeans with different letters differ significantly at the 5% level (Fischer’s LSD)

Table 3. Pearson correlation coefficients (r) between different physiological parameters and leaf starch levels from April 2010 until March 2011 on ‘Nadorcott’ mandarin trees in the Porterville area, South Africa. P-values appear in brackets underneath r-values. Fruit were harvested on 12 August 2010, flowers were counted during September 2010 and fruit set was determined during December 2010.

Physiological parameter	Apr. '10	May '10	Jun. '10	Jul. '10	Aug. '10	Sep. '10	Oct. '10	Nov. '10	Dec. '10	Jan. '11	Feb. '11	Mar. '11
LY/100 nodes	0.29 [0.29]	-0.06 [0.79]	0.18 [0.45]	-0.17 [0.47]	0.21 [0.37]	0.10 [0.67]	-0.17 [0.48]	0.47 [0.038]	-0.05 [0.83]	-0.31 [0.18]	-0.21 [0.36]	-0.53 [0.017]
LL/100 nodes	0.60 [0.0055]	0.58 [0.0078]	0.35 [0.13]	-0.02 [0.93]	0.43 [0.056]	-0.09 [0.69]	-0.25 [0.28]	0.30 [0.20]	-0.26 [0.28]	-0.36 [0.12]	-0.20 [0.40]	-0.30 [0.20]
Total flowers/100 nodes	0.57 [0.0089]	0.43 [0.057]	0.34 [0.14]	-0.08 [0.75]	0.42 [0.069]	-0.04 [0.87]	-0.26 [0.27]	0.40 [0.084]	-0.22 [0.35]	-0.39 [0.086]	-0.23 [0.33]	-0.42 [0.068]
Fruit set ^z	-0.12 [0.63]	-0.16 [0.51]	-0.12 [0.62]	-0.18 [0.45]	-0.09 [0.69]	0.13 [0.59]	0.55 [0.013]	-0.02 [0.95]	0.60 [0.0052]	0.57 [0.0093]	0.43 [0.058]	0.30 [0.20]
Yield ^y	-0.66 [0.0014]	-0.60 [0.0055]	-0.59 [0.0058]	-0.12 [0.61]	-0.36 [0.12]	-0.26 [0.26]	0.07 [0.77]	-0.35 [0.13]	-0.23 [0.34]	0.25 [0.29]	0.04 [0.85]	0.18 [0.46]

^zFruit set was expressed as percentage

^yYield for 2010 expressed as kilogram/tree

LY (Leafy flowers)

LL (Leafless flowers)

Table 4. Pearson correlation coefficients (r) between different physiological parameters and leaf total soluble sugar levels from Apr. 2010 until Mar. 2011 on ‘Nadorcott’ mandarin trees in the Porterville area, South Africa. P-values appear in brackets underneath r-values. Fruit were harvested on 12 Aug. 2010, flowers were counted during Sept. 2010 and fruit set was determined during Dec. 2010.

Physiological parameter	Apr. '10	May '10	Jun. '10	Jul. '10	Aug. '10	Sep. '10	Oct. '10	Nov. '10	Dec. '10	Jan. '11	Feb. '11	Mar. '11
LY/100 nodes	-0.31 [0.18]	-0.45 [0.045]	-0.03 [0.90]	-0.41 [0.072]	-0.01 [0.98]	0.28 [0.23]	-0.10 [0.67]	-0.16 [0.50]	-0.18 [0.44]	-0.02 [0.94]	-0.52 [0.020]	-0.20 [0.39]
LL/100 nodes	-0.14 [0.54]	0.17 [0.48]	0.41 [0.074]	0.03 [0.90]	0.38 [0.099]	0.40 [0.085]	0.26 [0.26]	0.25 [0.30]	-0.03 [0.89]	0.00 [0.99]	-0.06 [0.79]	0.30 [0.20]
Total flowers/100 nodes	-0.22 [0.35]	-0.02 [0.92]	0.31 [0.18]	-0.12 [0.62]	0.30 [0.20]	0.41 [0.074]	0.17 [0.47]	0.14 [0.56]	-0.09 [0.71]	-0.01 [0.97]	-0.23 [0.34]	0.17 [0.48]
Fruit set ^z	-0.14 [0.54]	-0.13 [0.58]	-0.21 [0.38]	-0.24 [0.30]	-0.18 [0.44]	-0.15 [0.53]	-0.37 [0.11]	-0.29 [0.22]	0.07 [0.77]	-0.04 [0.85]	-0.05 [0.85]	-0.19 [0.43]
Yield ^y	0.01 [0.97]	-0.27 [0.24]	-0.19 [0.42]	0.06 [0.81]	0.23 [0.34]	0.17 [0.47]	-0.22 [0.35]	-0.35 [0.13]	0.33 [0.16]	-0.15 [0.53]	-0.07 [0.77]	-0.37 [0.11]

^zFruit set was expressed as percentage

^yYield for 2010 expressed as kilogram/tree

LY (Leafy flowers)

LL (Leafless flowers)

Table 5. Pearson correlation coefficients (r) between different physiological parameters and leaf total carbohydrate levels from Apr. 2010 until Mar. 2011 on ‘Nadorcott’ mandarin trees in the Porterville area, South Africa. P-values appear in brackets underneath r-values. Fruit were harvested on 12 Aug. 2010, flowers were counted during Sept. 2010 and fruit set was determined during Dec. 2010.

Physiological parameter	Apr. '10	May '10	Jun. '10	Jul. '10	Aug. '10	Sep. '10	Oct. '10	Nov. '10	Dec. '10	Jan. '11	Feb. '11	Mar. '11
LY/100 nodes	0.00 [0.98]	-0.28 [0.23]	0.04 [0.87]	-0.40 [0.084]	0.15 [0.54]	0.23 [0.34]	-0.19 [0.41]	0.19 [0.42]	-0.15 [0.53]	-0.23 [0.32]	-0.53 [0.016]	-0.32 [0.17]
LL/100 nodes	0.31 [0.18]	0.40 [0.078]	0.44 [0.054]	0.02 [0.93]	0.50 [0.026]	0.11 [0.65]	-0.05 [0.84]	0.35 [0.13]	-0.20 [0.40]	-0.26 [0.27]	-0.14 [0.55]	0.22 [0.36]
Totalflowers/100 nodes	0.25 [0.30]	0.22 [0.35]	0.36 [0.12]	-0.12 [0.62]	0.44 [0.051]	0.16 [0.49]	-0.11 [0.66]	0.34 [0.14]	-0.21 [0.38]	-0.29 [0.22]	-0.30 [0.20]	0.06 [0.80]
Fruit set ^z	-0.17 [0.47]	-0.16 [0.51]	-0.2 [0.39]	-0.25 [0.28]	-0.16 [0.50]	0.04 [0.86]	0.22 [0.35]	-0.2 [0.40]	0.47 [0.038]	0.38 [0.10]	0.16 [0.49]	-0.11 [0.65]
Yield ^y	-0.44 [0.050]	-0.47 [0.035]	-0.36 [0.12]	0.03 [0.91]	-0.14 [0.56]	-0.15 [0.52]	-0.07 [0.76]	-0.45 [0.045]	0.04 [0.87]	0.08 [0.72]	-0.04 [0.87]	-0.31 [0.18]

^zFruit set was expressed as percentage

^yYield for 2010 expressed as kilogram/tree

LY (Leafy flowers)

LL (Leafless flowers)



Fig. 1. Examples of “on” (left) and “off” (right) ‘Nadorcott’ mandarin trees typically used as treatment trees in this study.

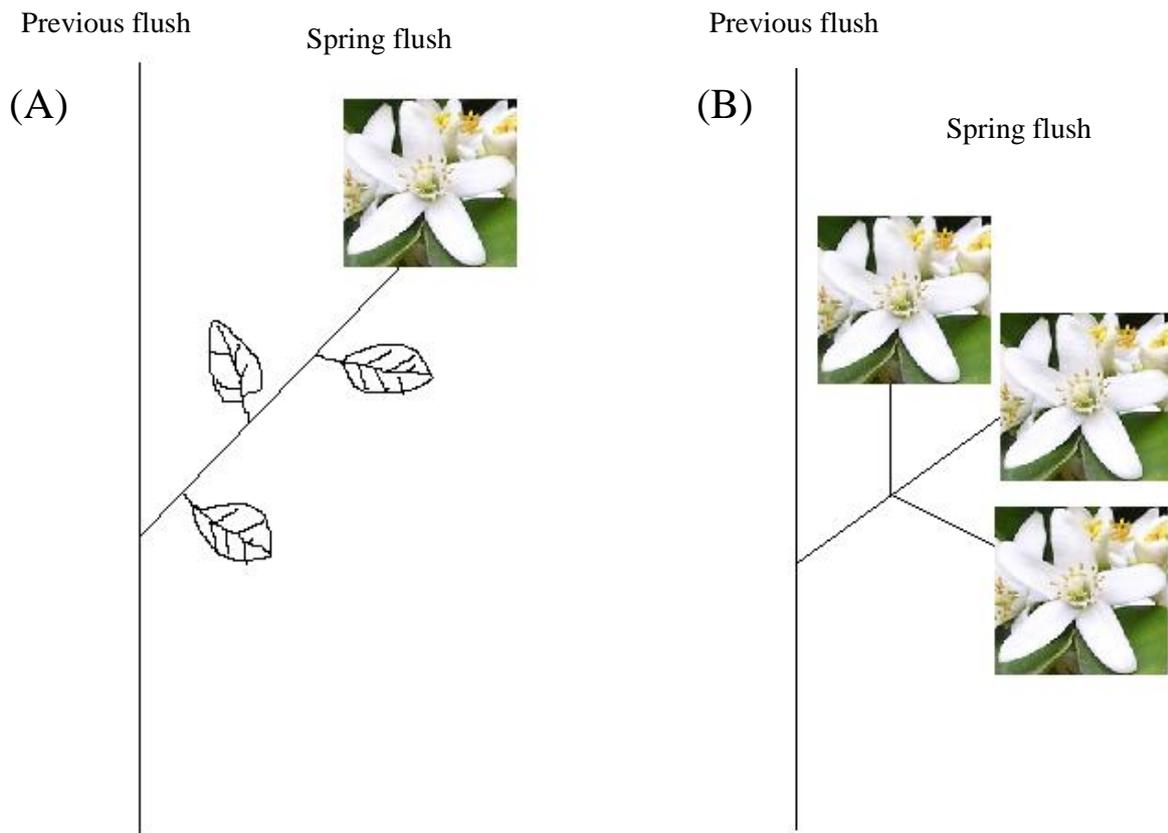


Fig. 2. Schematic illustration of a leafy inflorescence (LY infl; A) with three newly formed leaves and one leafy flower (LY) and (B) a leafless inflorescence (LL infl) with three leafless flowers (LL) and no newly formed leaves. Leafy flowers, therefore, develop on leafy inflorescences and leafless flowers develop on a leafless inflorescence (there may be more than one flower per inflorescence).

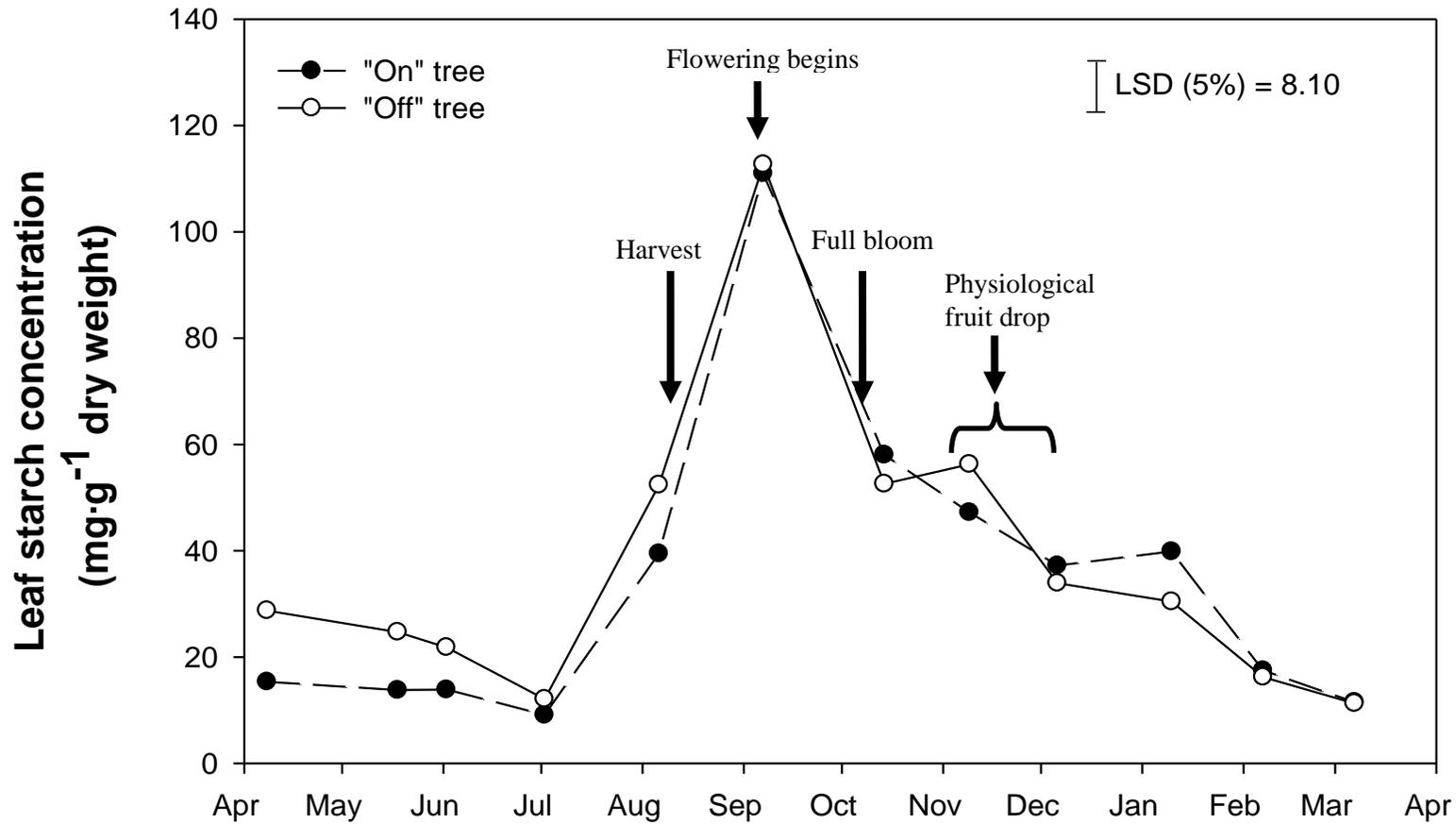


Fig. 3. Changes in total leaf starch levels ($\text{mg}\cdot\text{g}^{-1}$ dry weight) of alternating 'Nadorcott' mandarin trees for the 2010/2011 season. The bar represent least significant difference (LSD) between treatments as well as sampling dates of the same treatment ($n=10$). Important phenological development stages are shown on the graph.

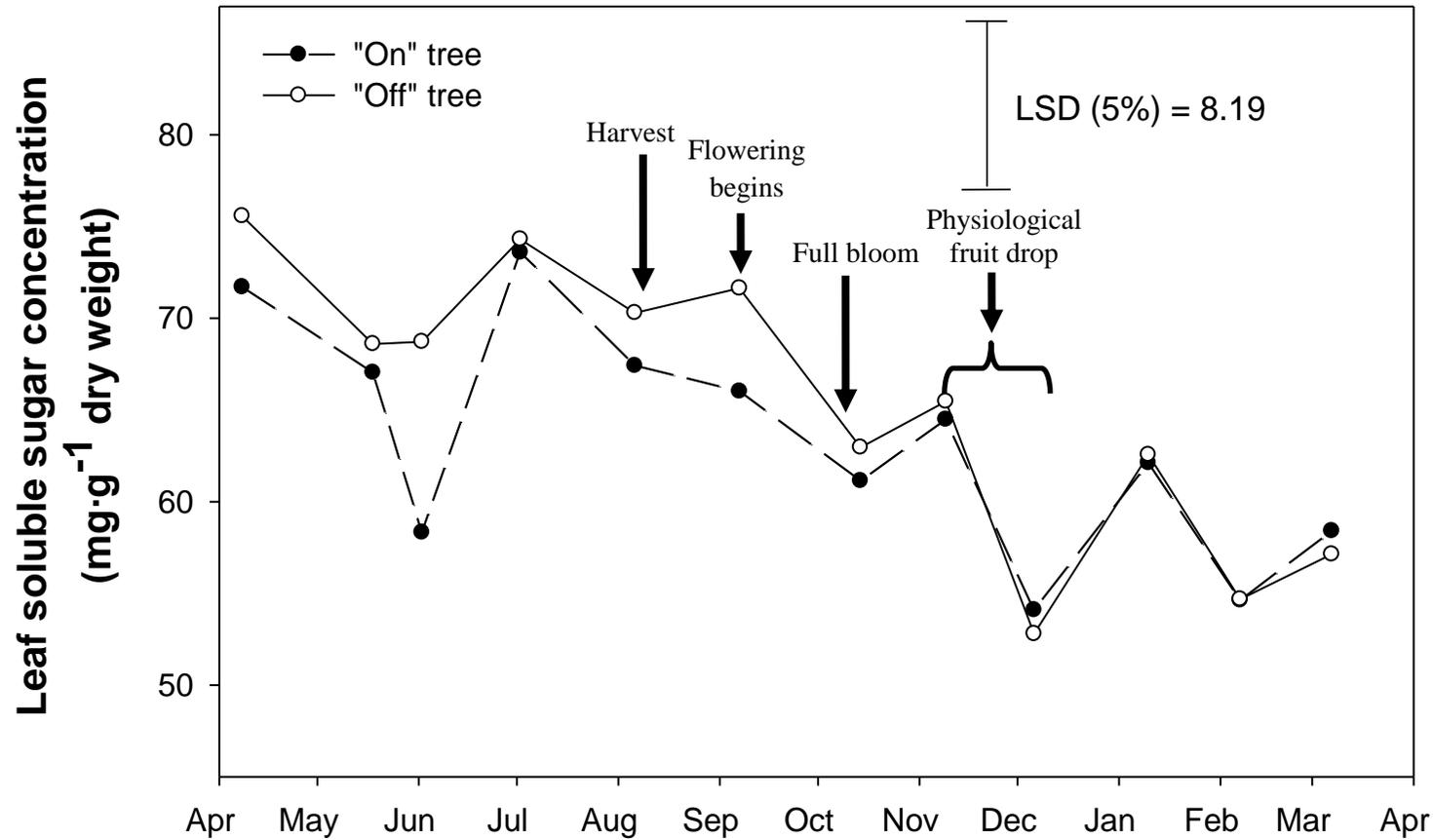


Fig. 4. Changes in leaf total soluble sugars levels (mg·g⁻¹ dry weight) of alternating 'Nadorcott' mandarin trees for the 2010/2011 season. The bar represent least significant difference (LSD) between treatments as well as sampling dates of the same treatment (n=10). Important phenological development stages are shown on the graph.

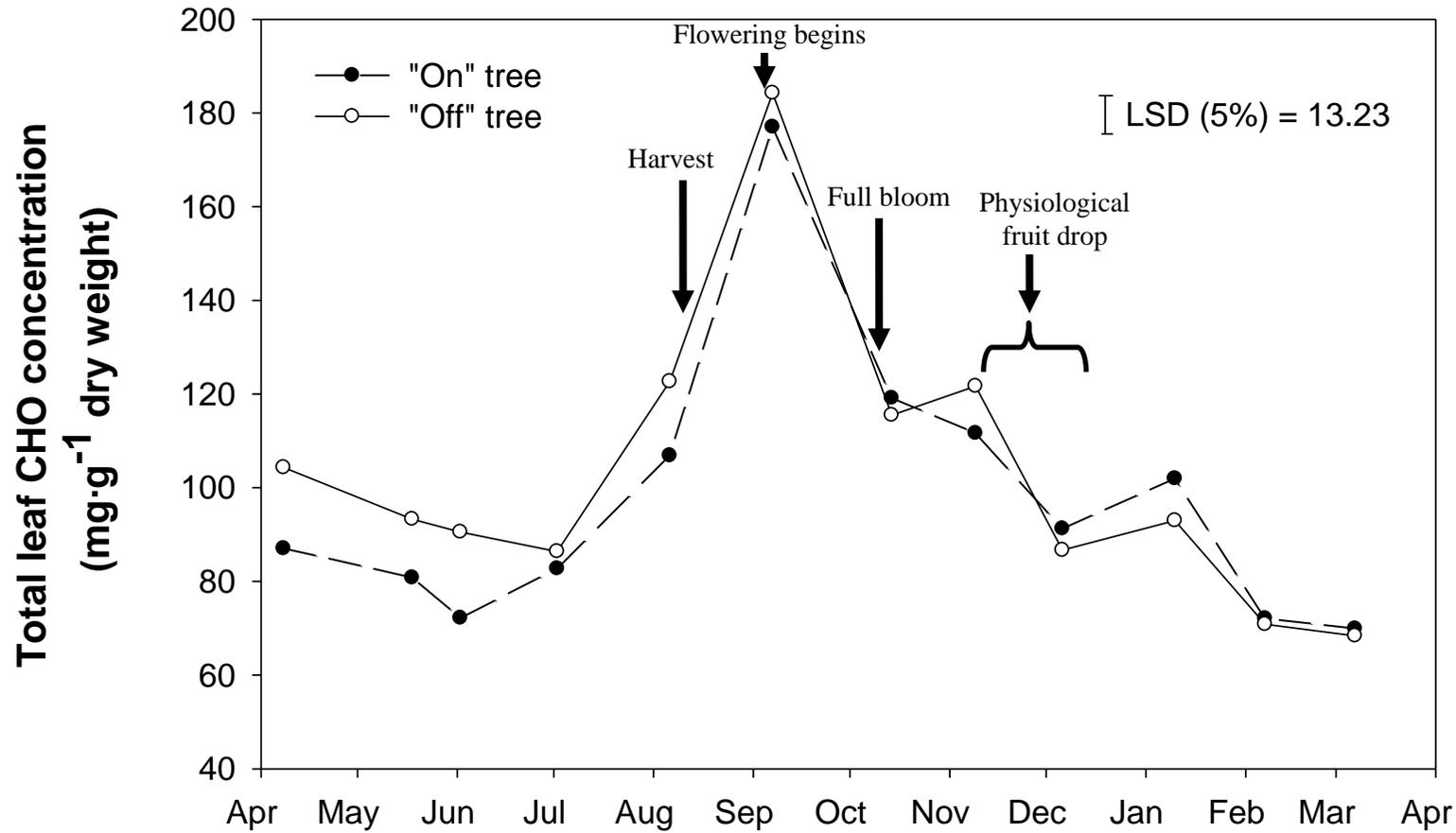


Fig. 5. Changes in total leaf carbohydrates (Tot CHO) levels (mg·g⁻¹ dry weight) for alternating 'Nadorcott' mandarin trees for the 2010/2011 season. The bars represent least significant difference (LSD) between treatments as well as sampling dates of the same treatment (n=10). Important phenological development stages are shown on the graph.

4. Paper 2. The effect of different pruning strategies on the phenology and carbohydrate status of alternate bearing ‘Nadorcott’ mandarin trees

Abstract

Alternate bearing is a common phenomenon in citrus trees. The “on” year consists of a heavy crop load with mostly small fruit followed by an “off” year with few and large fruit. Mandarin cultivars such as Nadorcott are prone to alternate bearing and one method that has been researched in the past to control alternate bearing is pruning. In general, heavy pruning before an expected “on” year should reduce the yield and light pruning before an “off” year can ensure the highest yield for that year. Five treatments were used in a randomized complete block design with 10 single tree replications. All treatments except the summer pruning treatment were pruned on 3 November 2011 by removing old regrowth and three to five vigorous and complex shoots from the centre of the tree. The summer pruning treatment was exactly the same as the control except that trees were pruned on 26 January 2012 instead of 3 November 2011. The control and summer pruned trees were left as is for the rest of the experiment. For the early regrowth treatment, regrowth was thinned in late January 2012 so that shoots were spaced 15 cm apart in all directions. Vigorous shoots were pinched and new shoots that formed after the initial thinning were removed throughout the rest of the season. For the late regrowth treatment all regrowth was simply removed from the tree on 26 March. For the uniconazol-P treatment, trees were treated twice, once when the summer flush had reached 5 cm in length and the same for the autumn flush. The number of spring flush vegetative shoots were determined on 26 September 2011 and the vegetative growth (in mm) was determined on 22 March 2012. PAR measurements were taken for the control and summer pruning treatments in late January 2012. Leaf total sugar and starch levels were determined during September 2011 and April 2012. Summer pruned trees had a higher number of spring flush vegetative shoots, more nodes per shoot and also more growth per parent shoot compared to control trees. Control trees had higher light levels inside the tree compared to summer pruned trees. No differences in leaf starch or total sugar levels during April were found between treatments. Favourable tree architecture should therefore be considered in this experiment. We conclude that pruning during November with early regrowth management will give the best balance between light penetration and production of good bearing units. Pruning in November, rather than during winter, also allows selective pruning of shoots with or without flowers, depending on whether it is an “on” or an “off” year, in an alternate bearing orchard.

Keywords: *late mandarin; summer pruning; starch; light measurements, regrowth management*

4.1 Introduction

Goldschmidt (2005) described alternate bearing, where a heavy crop load during the “on” year is followed by a light crop load during the “off” year, as a “phenomenon of homeostasis”, and pointed out that this behavior secured the longevity of fruit bearing trees in the wild. He also pointed out that regular bearing is most probably a result of domestication of fruit bearing trees, whereby certain stresses such as drought and pests are removed. Alternate bearing occurs in most fruit bearing trees, deciduous or evergreen, including citrus (Monselise and Goldschmidt, 1982), and has a negative impact on the economic sustainability of an orchard. Alternate bearing can occur across an entire production area (usually initiated by adverse climatic conditions), between orchards, within an orchard or between branches on a single tree (Monselise and Goldschmidt, 1982).

The main problem associated with alternate bearing is the irregular yield, i.e., a large number of small fruit the one season followed by a few large fruit with coarse rinds the next season (Monselise et al., 1981). The result is that little or no fruit can be marketed for maximum financial returns each season. Certain cultivars of mandarin species such as *Citrus unshiu* (Satsuma) and *C. reticulata*, including common mandarin hybrids within *C. reticulata* and crosses between *C. reticulata* and *C. paradisi* or *C. sinensis*, are prone to alternate bearing (Monselise, et al. 1981). Some mandarin cultivars such as Wilking and Murcott mandarins (*C. reticulata* Blanco) are known as absolute alternate bearers as there is a lack of flowers in the “off” year (Goldschmidt and Golomb, 1982). These cultivars that develop an absolute alternate bearing habit can alternate to such an extent that the trees collapse, or even die, during the “on” year (Monselise and Goldschmidt, 1982; Smith, 1976; Stewart et al., 1968). This collapse has been associated with a total depletion of carbohydrates in the tree (Jones et al., 1975).

Carbohydrates (specifically starch) play an important role in the regulation and severity of alternate bearing in citrus trees (Jones et al., 1975; Schaffer et al., 1985). The large demand for energy during spring when floral development, anthesis, fruit set and the spring vegetative flush occur, require more energy than the current photosynthetic rates can supply (Goldschmidt, 1999; Goldschmidt and Koch, 1996). Carbohydrate reserves are mobilized during this period and there is a decrease in carbohydrate levels from anthesis (Guardiola, 2000; Goldschmidt and Koch, 1996) until midwinter or just after harvest (García-Luis et al., 1995; Mataa et al., 1996; Sanz et al., 1987). Horticultural management practices, such as irrigation, nutrition and manipulation of the vegetative and reproductive growth, are vital to reduce the impact of alternate bearing.

Pruning, a well-established horticultural tool for reducing vegetative growth, allows sustainable, marketable yields each year in ‘Star Ruby’ grapefruit (Bilge et al., 2010). There are, however, many different pruning strategies in citriculture with equally variable responses depending on cultivar,

fruiting habit, growing conditions, tree age and tree vigour (Tucker et al., 1994). Under normal growing conditions, pruning is only used to remove weak, complex and unproductive bearing branches (Krajewski and Pittaway, 2000) and to confine trees to their allotted space in the orchard (Wheaton, 1992). Literature on the effect that re-growth caused by pruning has on carbohydrate reserves are lacking. Okuda et al. (2003) observed that ‘Haraguchi Wase’ satsuma mandarin shoots pruned during early spring sprouted seven times more shoots compared to the unpruned shoots. In addition, pruning decreased the carbohydrate concentration in the bark of two- or three-year-old branches.

The aim of this study was to determine how different pruning strategies affect leaf carbohydrate status during certain critical periods of phenological development. It is hypothesized that late regrowth management in late March will have a detrimental effect on leaf carbohydrate status compared to early regrowth management (from January onwards) and that the application of a growth retardant will reduce vegetative growth and increase leaf carbohydrate levels.

4.2 Materials and Methods

4.2.1 Plant material

The study was conducted in the 2011/2012 season on ‘Nadorcott’ mandarin (*C. reticulata* Blanco) trees on Carizzo citrange rootstock in a commercial orchard in the Porterville area (33°04’54.70’’S; 18°51’24.64’’E), South Africa. The orchard was planted in a north-south row direction in 2000 with a between row spacing of 5 m and a between tree spacing of 2 m.

4.2.2 Experimental layout and treatments

A randomized complete block design of 10 single tree replications per treatment was used. Treatments are summarised in Table 1. With the control treatment some of the regrowth was removed shortly after harvest on 9 September 2011, but it was decided that more shoots needed to be removed in order to get the desired effect and this was done on 3 November 2011 for all treatments except the summer pruning treatment. Previously uncontrolled regrowth and three to five vigorous and complex shoots from the centre of each tree were removed to increase light penetration into the tree and to remove unproductive bearing wood. The same protocol was followed for the summer pruning treatment as for the control treatment, except that it was done during summer on 26 January 2012.

In the early regrowth treatment, regrowth was managed on 26 January 2012. Vigorous shoots were pinched and the remaining shoots were spaced evenly, roughly 15 cm apart in all directions. All unnecessary shoots were removed. On 29 February, any newly formed shoots were removed and vigorous shoots were pinched. Also, regrowth that resulted from pinching in November 2011 was thinned to two shoots per main shoot. The late regrowth treatment consisted of removing all summer

flush shoots on 26 March 2012 and no follow up shoot removal or thinning was done to illustrate the effect of poor regrowth management.

A growth retardant, uniconazol-P (Sunny[®], Sumitomo Chemical Australia), was applied as a soil drench with buffer trees left between treated and untreated trees. Uniconazol-P was applied when the shoots of the summer (January 2012) and autumn (March 2012) vegetative flushes had reached 5 cm in length, at a rate of 3 L/ha, which equates to 3 ml per tree for this specific orchard. It was applied under the drip irrigators (to ensure the presence of roots) closest to the base of the tree. The uniconazol-P was mixed with 2 L of water and applied to the soil using a watering can. Another 2 L of clean water was then also applied to ensure that it was washed into the root zone.

4.2.3 Data collection

The time table for data collection is summarised in Table 2. Five fruit bearing and five vegetative “parent” shoots were randomly selected prior to harvest in 2011 on each of the control and summer pruned trees, to follow phenology throughout the 2012 season. In addition to the floral development (see Paper 1), the number of nodes and fruit per shoot were counted. On 26 September 2011 flowers and vegetative shoots that developed on the tagged parent shoots were counted. Due to the absolute “off” year experienced by the orchard in 2011/2012, very few flowers were counted and none persisted, therefore only vegetative data are shown. In addition, on 22 March 2012 (after vegetative growth had seized), the number of nodes and length of the vegetative shoots formed on the 10 parent shoots were determined to gauge any differences between the two treatments. Five fruit per tree were tagged after physiological fruit drop (November 2011) and fruit diameter was measured at monthly intervals from December 2011 until July 2012 using an electronic calliper (CD-6”C, Mitutoyo Corp, Tokyo, Japan).

Photosynthetically active radiation (PAR) measurements were taken for each replication of the control and summer pruning treatments on 26 January 2012, since there was no difference between the control and other three treatments at this stage, using a light meter (LI-250 light meter with a Li-1905A quantum meter, LI-COR[®], Lincoln, NE, USA). Readings were taken between 1100h and 1300h on the eastern side of the trees at ground level and 1 m above the soil from the trunk towards the outside in increments of 0, 30, 60, 90 cm. Full sunlight was measured for percentage sunlight calculations after readings for each level of each tree were completed.

Leaf samples for starch and total sugars analyses were taken on 26 September 2011, coinciding with flowering, for the control and summer pruning treatment. On 6 April 2012, leaf samples were again taken from all treatments (see Paper 1 for the full handling and analyses protocols).

On 19 July 12 fruit were sampled from each tree in the trial for external quality and fruit maturity measurements. Standard procedures as described in Paper 1 were followed.

4.2.4 Statistical analysis

Analysis of variance (ANOVA) was carried out using Enterprise Guide (version 4.1, SAS Institute Inc., Cary, NC, USA). Least significant difference (LSD, $p \leq 0.05$) was used to separate means.

4.3 Results and Discussion

4.3.1 Vegetative growth

The summer pruning treatment had a significantly higher number of vegetative shoots per 100 nodes compared to the control treatment (pruned November 2011) (Table 3). Furthermore, by March 2012 the summer pruned trees had 51% more growth per parent shoot and 49% more nodes per parent shoot compared to the control. There was no significant difference between treatments for average length of growth per node. More vegetative growth recorded for the summer pruned trees can be ascribed to these trees being pruned more than two months later than the control trees. Large amounts of shoots and, more importantly, leaves were removed from the control trees earlier, thereby shifting regrowth during the summer flush from the outside of the tree to the centre, where cuts were made. Furthermore, the control trees' photosynthetic ability could have been reduced due to the number of leaves removed. Therefore, summer pruned trees could probably produce more energy for longer during the season compared to control trees. It can be argued that control trees shifted vegetative growth during the summer flush from the outside of the canopy to the inside where regrowth resulted in response to pruning (Krajewski and Pittaway, 2000) done in November.

4.3.2 Light measurements

The control treatment had significantly higher average light levels 1 m above the ground ($169.4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$) compared to the unpruned summer pruning treatment ($14.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), but there was no significant difference at ground level (Table 4). Although these levels seem low even for the heavily pruned control trees (7.2 - 0.4 % of full sun), it has to be kept in mind that overshadowing is at its maximum with the sun overhead (Greene and Gerber, 1967), since leaves have mostly a horizontal aspect. Therefore, during the morning or evening when the sun is at a more horizontal position, light levels for the control treatment will most likely be higher inside the canopy while light levels will only change slightly in the summer pruning treatment.

Diffuse sunlight, which plays a role in photosynthesis of citrus trees (Greene and Gerber, 1967), will be higher in the control trees since these trees contained open "holes" or "windows" in the leaf canopy. This allows light to penetrate the lower part of the canopy directly as well as via diffuse light,

thereby increasing photosynthesis. Higher sunlight in the canopy could improve the quality of previously shaded fruit by increasing parameters such as soluble solid content (Sites and Reitz, 1949). Transport of important mineral nutrients will also be increased due to the increase in transpiration from leaves close to these fruit (Cosgrove and Holbrook, 2010).

The control treatment had significantly higher light levels at all increments, except 30 cm from the base of the tree (Table 4). Numerically there is a large difference (Control: $200.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$; summer pruned: $4.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{sec}^{-1}$), but due to the large variation in readings, the difference was not statistically significant. This illustrates the favourable and consistent light distribution throughout the control trees compared to the summer pruned trees. Furthermore, these data indicate that a larger portion of the control trees will experience more than 25% full sunlight, which is the minimum light level for maximum photosynthesis in citrus (Kriedemann, 1968).

4.3.3 Carbohydrate analysis

Leaf starch and total sugar levels did not differ significantly between treatments during bloom (26 September 2011) (Table 5) and in April 2012 (Table 6). Although the control and late regrowth management treatments were expected to have lower starch levels due to the higher demand for energy by the excessive regrowth, it can be argued that any disadvantage was revoked by early April since leaves start to export photosynthates when they are 80% expanded (Ruiz et al., 2001). A possible detrimental effect on leaf starch levels could have been observed if an excessive autumn flush occurred, but this did not happen in this trial. Although it was not significant, the early regrowth treatment did have slightly lower leaf starch levels compared to the control. This is most likely due to the removal of the photosynthetic factory when excess shoots were removed and vigorous shoots were pinched and also since most reserve carbohydrates in citrus are stored in leaves and twigs (Monselise et al., 1981). Overall, however, these results would suggest that none of the treatments will improve or decrease return bloom by altering whole tree carbohydrate levels (see Paper 1).

‘Nadorcott’ mandarin trees seem to be very sensitive to pruning and produce excessive regrowth that must be managed to form reproductive bearing wood, otherwise it can intensify the alternate bearing cycle (Hield and Hilgeman, 1969). Therefore, early regrowth management is the best strategy to manipulate the trees to become reproductive, since flowers can be induced as soon as growth has stopped (Moss, 1973). As seen in Fig. 1., by the end of February the early regrowth shoots had already hardened-off due to pinching while the control shoots were not hardened-off yet and will most likely only flower a year after the early regrowth shoots (Moss, 1971). It is important to remember that subsequent flushes should also be thinned and pinched at the proper shoot height of roughly 30 cm.

Producers should start by removing complex and unproductive shoots as described by Krajewski (1996) and Krajewski and Pittaway (2000) in the winter or spring to open “windows” into the canopy. More shoots can be removed when an “on” year is expected. Trees should then be monitored for when the next vegetative flush starts and as soon as the flush reaches the desired height (roughly 30 cm in this experiment), shoots should be pinched and thinned to 15 cm apart in all directions. Orchards should be monitored every 3 weeks hereafter and newly formed shoots should be removed. When the next flush occurs on the previous flush, shoots should be thinned to two new shoots per previous flush shoot and pinched. It is important that trees should rather be lightly pruned from an early stage of tree canopy development before problems such as overcrowding occurs to avoid the need for large cuts later on (Krajewski, 1996), although it is important to remember that no pruning of branches should be done before trees are 3 years old (Tucker et al., 1994). Light pruning is apparently advantageous to establish the balance between vegetative and reproductive growth (Krajewski and Pittaway, 2000). Furthermore, when pruning is done in spring as in this experiment, regrowth will only result from the summer and autumn flush and not from the spring flush as well (as would be the case if pruning was done in the winter). This practice could reduce the amount of work needed to control the regrowth.

No significant differences were found between any of the fruit quality parameters measured at harvest (Table 7). This is most likely due to the extent of the “off” year that was experienced in this orchard. Some trees had no crop and other trees as little as four fruit. Differences in fruit quality due to the treatments could therefore not be determined.

Uniconazol-P did not alter any of the parameters measured in this experiment and there was no visual difference in regrowth amount or length compared to the control (Table 3). However, in orchards where uniconazol-P had been used for an extended period, trees become more complex after approximately three years of continued use compared to untreated orchards (Personal observation). It can be argued, therefore, that the duration of this experiment was not long enough for uniconazol-P to result in a change in canopy architecture and complexity that could affect leaf carbohydrate levels.

In conclusion, pruning trees during the flowering period can be advantageous in alternate bearing orchards since shoots can selectively be removed to either remove flowers during the “on” year, or be vegetative during the “off” year. This practice also reduces the work necessary to control regrowth for that season. Summer pruning will significantly reduce light penetration into the canopy before trees are pruned and this might result in fewer fruit of high quality compared to conventionally pruned trees. The different pruning manipulations used in this experiment did not alter leaf carbohydrate levels and return bloom will therefore not be increased through improved starch levels. It is suggested that early regrowth management is the best pruning strategy for ‘Nadorcott’ mandarin trees and allows formation of the right type of bearing wood for the next season and is also favourable in forming the desired tree shape.

4.4 References

- Bilge, Y., Y. Turgut, I. Meral, K. Müge, Ö. Faruk, T. Onder, and K.A. Yildiz Aka. 2010. The effects of mechanical pruning on fruit yield and quality in 'Star Ruby' grapefruit. *Intl. J. Food. Agric. Environ.* 8:834-838.
- Cosgrove, D.J. and N.M. Holbrook. 2010. Water balance of plants, p.85-105. In: L. Taiz and E. Zeiger (eds.). *Plant Physiology*, Sinauer Associates, Inc., Sunderland, MA, U.S.A.
- Garcia-Luis, A., F. Fornes, and J.L. Guardiola. 1995. Leaf carbohydrates and flower formation in Citrus. *J. Amer. Soc. Hort. Sci.* 120:222–227.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. *HortScience* 34:1020-1024.
- Goldschmidt, E.E. 2005. Regulatory aspects of alternate bearing in fruit tree. *Italus Hortus* 12:11-17.
- Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate bearing citrus trees and the significance of reserves for flowering and fruiting. *J. Amer. Soc. Hort. Sci.* 107:206–208.
- Goldschmidt, E.E. and K.E. Koch. 1996. Citrus, p.797-823. In: E. Zamski and A. A. Schaffer (eds.). *Photoassimilate distribution in plants and crops*. Marcel Dekker, NY.
- Greene, B.A. and J.F. Gerber. 1967. Radiant energy distribution in citrus trees. *Proc. Am. Soc. Hort. Sci.* 90:77-85.
- Guardiola, J.L. 2000. Regulation of flowering and fruit development: Endogenous factors and exogenous manipulation. *Proc. Intl. Soc. Citricult.* 9:342-346.
- Hield, H.Z. and R.H. Hilgeman. 1969. Alternate bearing and chemical fruit thinning of certain citrus varieties. *Proc. Intl. Citrus Symp.* 3:1145–1153.
- Jones, W.W., T.W. Embleton, and C.W. Coggins, Jr. 1975. Starch content of roots of 'Kinnow' mandarin trees bearing fruit in alternate years. *HortScience* 10:514.
- Krajewski A.J. 1996. Pruning of citrus in Southern Africa: A hacker's guide. *Citrus J.* 6(4):19– 23.
- Krajewski, A., and T. Pittaway. 2000. Manipulation of citrus flowering and fruiting by pruning. *Proc. Intl. Soc. Citricult. Congr.* 9:357-360.

- Kriedemann, P.E. 1968. Some photosynthetic characteristics of citrus leaves. *Aust. J. biol. Sci.* 21:895-905.
- Mataa, M., S. Tominaga, and I. Kozaki. 1996. Seasonal changes of carbohydrate constituents in Ponkan (*Citrus reticulata* Blanco). *J. Japan. Soc. Hort. Sci.* 65:513-523.
- Monselise, S.P. and E.E. Goldschmidt. 1982. Alternate bearing in fruit trees. *Hort. Rev.* 4:128–173.
- Monselise, S.P., E.E. Goldschmidt, and A. Golomb. 1981. Alternate bearing in citrus and ways of control. *Proc. Intl. Soc. Citricult.* 1:239–242.
- Moss, G.I. 1971. Effect of fruit on flowering in relation to biennial bearing in sweet orange (*Citrus sinensis*). *J. Hort. Sci.* 46:177–184.
- Okuda, H., Y. Yonemoto, and T. Takahara. 2003. Effects of the timing of cut back pruning for the whole tree canopy on sprouting and growth and on the carbohydrate and nitrogen content in the branches of Satsuma mandarin trees in a systemized alternate-bearing orchard. *Hort. Res. (Japan)* 2:279-281.
- Ruiz, R., A. García-Luis, C. Monerri, and J.L. Gaurdiola. 2001. Carbohydrate availability in relation to fruitlet abscission in *Citrus*. *Ann. Bot.* 87:805-812.
- Sanz, A., C. Monerri, J. González-Ferrer, and J.L. Guardiola. 1987. Changes in carbohydrates and mineral elements in *Citrus* leaves during flowering and fruit set. *Physiol. Plant.* 69:93-98.
- Schaffer, A.A., E.E. Goldschmidt, R. Goren, and D. Galili. 1985. Fruit set and carbohydrate status in alternate and nonalternate bearing *Citrus* cultivars. *J. Amer. Soc. Hort. Sci.* 110:574-578.
- Sites, J.W. and H.J. Reitz. 1949. The variation in individual oranges from different locations of the tree as a guide to sampling methods and spot-picking for quality I. Soluble solids in the juice. *Proc. Am. Soc. Hort. Sci.* 54:1-10.
- Smith, P.F. 1976. Collapse of ‘Murcott’ tangerine trees. *J. Amer. Soc. Hort. Sci.* 101:23–25.
- Stewart, I., T.A. Wheaton and R.L. Reese. 1968. ‘Murcott’ collapse due to nutritional deficiencies. *Proc. Fla. State Hort. Soc.* 81:15–18.

Tucker, D.P.H., T.A. Wheaton, and R.P. Muraro. 1994. Citrus tree pruning principles and practices. Horticultural Science Department. Florida Cooperative Extension Service. Institute of Food and Agricultural Science. University of Florida. Fact Sheet HS-144. Reviewed: June 1994.

<<<http://cms.cnr.edu.bt/cms/files/docs/File/Jeanette/PDF/citrus%20pruning.pdf>>>

Wheaton, T.A. 1992. Alternate bearing of citrus. Proc. Intl. Sem. Citricult. 1:224–228.

Table 1. Summary of manipulations for five different pruning strategies on “off” ‘Nadorcott’ mandarin trees in the Porterville area, South Africa 2011/2012.

	2011		2012		
Treatment	November, December	January	February	March	July
Control	Remove old regrowth and 3 – 5 complex shoots from centre of the tree. 3 November 2011				Harvest 26 July 2012
Summer Pruning		Remove old regrowth and 3 – 5 complex shoots from centre of the tree. 26 January 2012			Harvest 26 July 2012
Early regrowth	Remove old regrowth and 3 – 5 complex shoots from centre of the tree. 3 November 2011	Thin regrowth and space 15 cm apart in all directions and pinch all vigorous shoots. 26 January 2012	Remove any additional new regrowth. Pinch vigorous shoots from first thinning. 29 February 2012		Harvest 26 July 2012
Late regrowth	Remove old regrowth and 3 – 5 complex shoots from centre of the tree 3 November 2011			Remove all regrowth. 26 March 2012	Harvest 26 July 2012
Uniconazol-P	Remove old regrowth and 3 – 5 complex shoots from centre of the tree. 3 November 2011	Apply uniconazol-P soil drench at a rate of 3 ml/tree. 9 January 2012		Apply soil drench. 22 March 2012	Harvest 26 July 2012

Table 2. Time table of data collection for five different pruning strategies on “off” ‘Nadorcott’ mandarin trees in the Porterville area, South Africa 2011/2012.

	2011		2012		
Treatment	September	January	March	April	July
Control	Count flowers and vegetative shoots. Take leaf samples. 26 September 2011	Take light measurements. 26 January 2012	Measure vegetative growth and count nodes. 22 March 2012	Take leaf samples. 6 April 2012	Take fruit samples for fruit maturity indexing. 19 July 2012
Summer Pruning	Count flowers and vegetative shoots. Take leaf samples. 26 September 2011	Take light measurements. 26 January 2012	Measure vegetative growth and count nodes. 22 March 2012	Take leaf samples. 6 April 2012	Take fruit samples for fruit maturity indexing. 19 July 2012
Early regrowth				Take leaf samples. 6 April 2012	Take fruit samples for fruit maturity indexing. 19 July 2012
Late regrowth				Take leaf samples. 6 April 2012	Take fruit samples for fruit maturity indexing. 19 July 2012
Uniconazol-P				Take leaf samples. 6 April 2012	Take fruit samples for fruit maturity indexing. 19 July 2012

Table 3. Differences in the vegetative growth of spring (3 November 2011) and summer pruned (26 January 2012) ‘Nadorcott’ mandarin trees in the Porterville area, South Africa. Measurements were made on 22 March 2012.

Treatment	Vegetative shoots/ 100 nodes	Growth/parent shoot (cm)	Nodes/shoot	Growth/node (cm)
Control	40.9 ^{bz}	206.6 ^b	16.3 ^b	10.1 ^{ns}
Summer pruning	50.1 ^a	311.6 ^a	24.3 ^a	11.5
<i>p-value</i>	0.0246	0.0482	0.0154	0.0891

^zMeans with different letters differ significantly at the 5% level (Fischer’s LSD)

Table 4. Differences in PAR for control (pruned in spring) and summer pruned ‘Nadorcott’ mandarin trees in the Porterville area, South Africa (2011). Readings were taken on the eastern side on ground level and 1 m above ground level from the base of the tree outwards at 30 cm increments on 26 January 2012. Trees from the summer pruning treatment were pruned after the measurements were taken.

Treatment	-----% full sun-----		-----PAR (μmol·m ⁻² ·sec ⁻¹)-----					
	Ground level	1 m level	Ground level avg	1 m avg	1 m base	1 m 30 cm	1 m 60 cm	1 m 90 cm
Control	2.0 ^{ns}	7.2 ^{az}	45.9 ^{ns}	169.4 ^a	26.1 ^a	200.3 ^{ns}	171.9 ^a	270.2 ^{ns}
Summer pruning	0.4	0.6 ^b	10.3	14.0 ^b	2.7 ^b	4.0	6.7 ^b	42.8
<i>p-value</i>	0.1000	0.0058	0.1034	0.0059	<0.0001	0.147	0.0372	0.2776

^zMeans with different letters differ significantly at the 5% level (Fischer’s LSD)

Table 5. Leaf starch and total sugar levels taken on 26 September 2011 from ‘Nadorcott’ mandarin trees in the Porterville area, South Africa.

Treatment	Total sugars (mg·g ⁻¹ dry weight)	Starch (mg·g ⁻¹ dry weight)
Control	63.9 ^{ns}	90.7 ^{ns}
Summer pruning	61.2	94.1
<i>p-value</i>	0.5116	0.7624

Table 6. Leaf total sugar and starch levels of ‘Nadorcott’ mandarin trees pruned using different strategies in the Porterville area, South Africa. Leaf samples were taken on 6 April 2012.

Treatment	Total sugars (mg·g ⁻¹ dry weight)	Starch (mg·g ⁻¹ dry weight)
Control	98.7 ^{ns}	93.8 ^{ns}
Summer pruning	88.3	95.6
Early regrowth ^z	127.1	79.3
Late regrowth ^y	96.7	94.0
Uniconazol-P	93.6	75.3
<i>p-value</i>	0.3575	0.0588

^zEarly regrowth management (± 2 months after pruning)

^yLate regrowth management (± 4 months after pruning)

Table 7. Fruit quality at harvest of ‘Nadorcott’ mandarin trees pruned using different strategies in the Porterville area, South Africa. Fruit were sampled on 19 July 2012.

Treatment	TSS (°Brix)	TA (%)	Colour (No. 36 CRI colour chart)	Juice content (%)	Fruit size (mm)
Control	11.7 ^{ns}	1.7 ^{ns}	1.0 ^{ns}	47.3 ^{ns}	61.8 ^{ns}
Summer pruning	11.4	1.7	1.1	47.8	59.6
Early regrowth ^z	11.6	1.7	1.2	45.6	60.7
Late regrowth ^y	11.1	1.7	1.1	47.9	62.1
Uniconazol-P	12.0	1.8	1.4	44.5	58.2
<i>p-value</i>	0.5643	0.5209	0.1016	0.5911	0.1170

^zEarly regrowth management (± 2 months after pruning)

^yLate regrowth management (± 4 months after pruning)



Fig. 1. Differences in regrowth of early regrowth management (A, view from bottom of the tree with pinched regrowth shoots shown by red arrows) and untreated control (B, view from top of tree with regrowth inside red circle) trees.

5. Paper 3. Effect of chemical and hand thinning on the carbohydrate status and yield of ‘Nadorcott’ mandarin trees

Abstract

Alternate bearing is a common phenomenon in most commercial fruit trees. In citrus, the “on” year consist of a heavy crop load with mostly small fruit followed by an “off” year with few and large fruit. When application is optimized in terms of timing and rate of application, fruit thinning is one of the most effective ways of reducing an alternate bearing cycle. Chemical thinning is an easy and effective method and hand thinning can be used in orchards where there is large variation in yield between trees. Three treatments were used in this experiment in a randomized complete block design. All trees were sprayed with 10 mg.L^{-1} GA₃ at 80% petal fall to ensure a large set and control trees were left as is for the rest of the experiment. Dichlorprop was applied when most fruit had reached 8 mm in diameter and hand thinning was done in January by removing all fruit with a diameter smaller than 15 mm. Fruit growth was measured throughout the season and leaf starch and total sugar levels were determined for April 2012. Yield, fruit size and fruit number were determined at harvest. Unfortunately no significant results were obtained from the experiment although the thinning treatments did show slightly higher starch levels in April 2012, indicating that the demand for energy was lower on these trees. This is most likely due to the slightly lower yield and fruit number for the thinning treatments compared to the control. The dichlorprop treatment also showed a slightly higher fruit growth rate. It is suggested that more research be done in future on thinning methods for late mandarin cultivars, especially on the timing of chemical thinning sprays, as this is a valuable tool in managing fruit load throughout the citrus industry.

Keywords: *late mandarin; dichlorprop; starch; fruit growth*

5.1 Introduction

Alternate bearing of fruit trees is defined as a heavy crop load during the “on” year that is followed by a light crop load during the “off” year. Alternate bearing occurs in most fruit bearing trees, deciduous or evergreen, including citrus (Monselise and Goldschmidt, 1982). Goldschmidt (2005) described alternate bearing as a “phenomenon of homeostasis”, and pointed out that this behavior secured the longevity of fruit bearing trees in the wild, and that domestication of fruit bearing trees, whereby certain stresses such as drought and pests are removed, most likely resulted in the trees exhibiting a regular bearing habit. Alternate bearing can occur across an entire production region (usually initiated by adverse climatic conditions), between orchards, within an orchard or between branches on a single tree (Monselise and Goldschmidt, 1982).

Carbohydrates (specifically starch) play an important role in the physiology and severity of alternate bearing in citrus trees (Jones et al., 1975; Schaffer et al., 1985). The large demand for energy during spring when floral development, anthesis, the spring vegetative flush and fruit set occur simultaneously, require more energy than current photosynthesis can supply (Goldschmidt, 1999; Goldschmidt and Koch, 1996). This energy deficit results in carbohydrate reserves being mobilized during this period and there is a decrease in carbohydrate levels from anthesis (Goldschmidt and Koch, 1996; Guardiola, 2000) until midwinter or just after harvest (García-Luis et al., 1995; Mataa et al., 1996; Sanz et al., 1987).

Certain cultivars of mandarin species such as *Citrus unshiu* (Satsuma) and *C. reticulata*, including common mandarin hybrids within *C. reticulata* and crosses between *C. reticulata* and *C. paradisi* or *C. sinensis*, are prone to alternate bearing (Monselise et al., 1981). Some mandarin cultivars such as Wilking and Murcott mandarins (*C. reticulata* Blanco) are known as absolute alternate bearers due to the fact that there is a total lack of flowers in the “off” year (Goldschmidt and Golomb, 1982). These cultivars can alternate to such an extent that the trees collapse, or even die, during the “on” year (Monselise and Goldschmidt, 1982; Smith, 1976; Stewart et al., 1968). This collapse is associated with a total depletion of reserve carbohydrates in the tree.

By hand thinning ‘Valencia’ sweet orange trees at different times and intensities throughout the season, Jones et al. (1974) showed that early and more intense fruit thinning of “on” trees increased leaf starch levels just before bloom when compared to progressively later and less intense thinning. The increased starch levels led to higher set and subsequently higher yields the following season. When naphthaleneacetic acid (NAA) was applied as a thinning agent during the physiological fruit drop period to “on” ‘Wilking’ mandarin trees, it decreased that current yield and increased the yield during the following “off” season (Lewis et al., 1964). It did not, however, increase the carbohydrate status of the leaves. The difference in the above mentioned results may be due to cultivar differences

(sweet orange vs. mandarin types) or different thinning techniques (NAA vs. hand thinning). If the difference was due to the use of NAA, it indicates that this hormone causes some physiological change in the tree that is not the case in hand thinning.

The aim of this study was to determine the influence of hand and chemical thinning on leaf starch concentration of 'Nadorcott' mandarin trees. The effect of hand and chemical thinning on yield, fruit number and fruit weight were also determined, as this could give an indication of the ability of the tree to produce a large enough crop the following season. It is hypothesized that the chemical thinning (earlier than hand thinning) will give a better thinning effect than hand thinning and should be used during an "on" year.

5.2 Materials and Methods

5.2.1 Plant material

The study was conducted in the 2011/2012 season on 'Nadorcott' mandarin (*C. reticulata* Blanco) trees on Carizzo citrange rootstock in a commercial orchard in the De Doorns area (33°30'42.36"S; 19°31'01.42"E), South Africa. The orchard was planted in a north-south row direction in 2004 with a between row spacing of 5 m and a between tree spacing of 2 m.

5.2.2 Experimental layout and treatments

A randomized complete block design with 10 single tree replicates per treatment was used. To induce a heavy set ("on" year), GA₃ [Progibb[®], Philagro SA (Pty) Ltd] was applied to all trees at 80% petal fall at a rate of 10 mg.L⁻¹. The chemical thinning agent dichlorprop [as 2-butoxyethyl ester, 50 g.L⁻¹, Corasil.E[®], Nufarm Agriculture (Pty) Ltd] was applied at a rate of 150 ml.100 L⁻¹ of water when the majority of the fruit had a diameter of 8 mm (2 December 2011). A non-ionic wetting agent (Break-Thru[®], Goldschmidt Chemical Corporation) with active ingredient polymethylsiloxanecopolymer (100 mg.L⁻¹) was added to all spray solutions at a rate of 5 ml.100 L⁻¹ water. An untreated control was included. All applications were done at dusk to ensure slow drying conditions. Hand thinning was done on 12 January 2012 according to the following protocol. Fruit diameters of 100 fruit were measured. These values were used in a growth curve for 'Nadorcott' mandarin and it was determined that only fruit larger than 15 mm would reach minimum export size (in this case 55 mm). Subsequently all fruit smaller than 15 mm (\pm 30 fruit per tree) were removed using metal rings as size guides.

5.2.3 Data collection

Fruit growth throughout the season was measured from December 2011 onwards, and leaves for carbohydrate analyses were sampled in April 2012 (see Paper 1 for protocol). Fruit were harvested at

commercial maturity on 27 August 2012 and yield as well as the number of fruit per tree were determined. Average fruit weight was determined by dividing the yield (in grams) by the number of fruit for each tree. Fruit diameter of roughly 130 fruit per tree was measured using an electronic calliper (CD-6"C, Mitutoyo Corp, Tokyo, Japan) in the field at harvest to determine average fruit size. Samples were taken from each tree for maturity indexing in the laboratory as described in Paper 1. Rind colour was not determined since all fruit had developed full colour and a score of 1 on the No. 36 CRI colour chart for mandarins [Citrus Research International (CRI), 2004].

5.2.4 Statistical analysis

Analysis of variance (ANOVA) was carried out using Enterprise Guide (version 4.1, SAS Institute Inc., Cary, NC, USA). Least significant difference (LSD, $p=0.05$, unless indicated otherwise) was used to separate means where data was normally distributed.

5.3 Results and Discussion

The treatments in this experiment unfortunately did not yield any significant differences, indicating that the contrast created between the control and two thinning treatments were not large enough. In addition, it was observed that fruit abscised unexpectedly from the control trees during January, just after physiological fruit drop, and this could be due to a period of drought stress, since only a single drip line irrigation is used in the orchard. It is possible that applying a higher concentration of GA_3 , viz. 20 mg.L^{-1} and not 10 mg.L^{-1} , a higher fruit set could have been achieved, thereby creating a better contrast.

The differences in yield between the control and thinning treatments were not significant (Table 1). Leaf starch and total sugar levels measured in April 2012 did not differ significantly between treatments (Table 2). Hand and chemical thinning did, however, result in slightly higher starch levels indicating that the sink strength of fruit in these treatments were slightly lower on these trees. The reduced sink strength is most likely due to the slightly lower fruit number (Table 1.) Another possibility could be a reduction in photosynthesis in response to the lower fruit load (Syvertson et al., 2003) following thinning treatments. The reduced photosynthesis could therefore nullify the expected positive effect in leaf starch levels in response to the thinning treatments. However, since yield, fruit number and fruit weight did not differ significantly between treatments (even though yield and fruit number were lower for the thinning treatments), this is an unlikely scenario.

Fruit growth throughout the season did not differ at the 5% level, but differences were found at the 10% level (Table 2). Fruit from the chemical thinning treatment had a higher growth rate of 0.18 mm/day compared to 0.17 mm/day for the control. The hand thinning treatment did not differ

significantly from the other two treatments. The chemical thinning treatment also had marginally bigger fruit at harvest, although this was not significant at either the 5% or 10% levels.

No differences were found for any of the quality parameters at harvest except for juice percentage where the hand thinning treatment had significantly less juice compared to the other treatments (Table 2). This small difference of less than 3% is, however, not of economic importance. Fruit number and yield was consistently lower for both thinning treatments, once again indicating that some measure of thinning was achieved, but not enough to measure any differences.

Lewis et al. (1964) applied naphthaleneacetic acid (NAA) in the “on” year as a thinning agent during the physiological fruit drop period and recorded favourable reduction of yield in the “on” year and also an increase in yield during the following “off” year. This indicates that the dichlorprop used in this experiment could have been applied at least 2 weeks earlier (middle November) to coincide with the physiological fruit drop period. The timing of dichlorprop thinning sprays should therefore be investigated further as it could be a helpful tool for producers when used correctly.

In conclusion, thinning is a very important tool to control alternate bearing since it directly reduces the sink size of fruit in the “on” year. It is important, therefore, that protocols for hand thinning and the use of thinning agents are developed for cultivars such as ‘Nadorcott’ mandarin that are prone to alternate bearing. In this experiment the amount of thinning was not sufficient and more research is needed to “fine-tune” the application of the thinning methods.

5.4 References

- Garcia-Luis, A., F. Fornes, and J.L. Guardiola. 1995. Leaf carbohydrates and flower formation in Citrus. *J. Amer. Soc. Hort. Sci.* 120:222–227.
- Goldschmidt, E.E. 1999. Carbohydrate supply as a critical factor for citrus fruit development and productivity. *HortScience* 34:1020-1024.
- Goldschmidt, E.E. 2005. Regulatory aspects of alternate bearing in fruit tree. *Italus Hortus* 12:11-17.
- Goldschmidt, E.E. and A. Golomb. 1982. The carbohydrate balance of alternate bearing citrus trees and the significance of reserves for flowering and fruiting. *J. Amer. Soc. Hort. Sci.* 107:206–208.
- Goldschmidt, E.E. and K.E. Koch. 1996. Citrus, p.797-823. In: E. Zamski and A. A. Schaffer (eds.). *Photoassimilate distribution in plants and crops*. Marcel Dekker, NY.
- Guardiola, J.L. 2000. Regulation of flowering and fruit development: Endogenous factors and exogenous manipulation. *Proc. Intl. Soc. Citricult.* 9:342-346.
- Jones, W.W., T.W. Embleton, E.L. Barnhart, and C.B. Cree. 1974. Effect of time and amount of fruit thinning on leaf carbohydrates and fruit set in Valencia oranges. *Hilgardia* 42:441–449.
- Jones, W.W., T.W. Embleton, and C.W. Coggins, Jr. 1975. Starch content of roots of ‘Kinnow’ mandarin trees bearing fruit in alternate years. *HortScience* 10:514.
- Lewis, L.N., C.W. Coggins, Jr., and H.Z. Hield. 1964. The effect of biennial bearing and NAA on the carbohydrate and nitrogen composition of Wilking mandarin leaves. *Proc. Amer. Soc. Hort. Sci.* 84:147–151.
- Mataa, M., S. Tominaga, and I. Kozaki. 1996. Seasonal changes of carbohydrate constituents in Ponkan (*Citrus reticulata* Blanco). *J. Japan. Soc. Hort. Sci.* 65:513-523.
- Monselise, S.P. and E.E. Goldschmidt. 1982. Alternate bearing in fruit trees. *Hort. Rev.* 4:128–173.
- Monselise, S.P., E.E. Goldschmidt, and A. Golomb. 1981. Alternate bearing in citrus and ways of control. *Proc. Intl. Soc. Citricult.* 1:239–242.
- Sanz, A., C. Monerri, J. González-Ferrer, and J.L. Guardiola. 1987. Changes in carbohydrates and mineral elements in *Citrus* leaves during flowering and fruit set. *Physiol. Plant.* 69:93-98.

- Schaffer, A.A., E.E. Goldschmidt, R Goren, and D. Galili. 1985. Fruit set and carbohydrate status in alternate and nonalternate bearing *Citrus* cultivars. *J. Amer. Soc. Hort. Sci* 110:574-578.
- Smith, P.F. 1976. Collapse of 'Murcott' tangerine trees. *J. Amer. Soc. Hort. Sci.* 101:23-25.
- Stewart, I., T.A. Wheaton and R.L. Reese. 1968. 'Murcott' collapse due to nutritional deficiencies. *Proc. Fla. State Hort. Soc.* 81:15-18.
- Syvertson, J.P., C. Goñi, and A. Otero. 2003. Fruit load and canopy shading affect leaf characteristics and net gas exchange of 'Spring' navel orange trees. *Tree Physiol.* 23:899-906.

Table 1. Fruit growth, fruit quality and yield of ‘Nadorcott’ mandarin trees treated with different fruit thinning techniques in the De Doorns area, South Africa (2011/2012).

Treatment	Fruit growth (mm/day)	Fruit size (mm)	Fruit number	Yield (kg/tree)	Fruit weight (g)	Juice %	TSS (°Brix)	Titratable acidity (%)
Control	0.168b	51.4 ^{ns}	689.7 ^{ns}	48.1 ^{ns}	70.3 ^{ns}	50.3a	13.7 ^{ns}	1.5 ^{ns}
Chemical thinning	0.178a	52.6	623.0	43.7	70.5	49.3a	14.1	1.6
Hand thinning	0.169ab	51.9	601.5	41.6	71.2	47.6b	13.8	1.7
<i>p-value</i>	0.1532 ^c	0.2628	0.6064	0.5169	0.9719	0.0019	0.3182	0.1231

^zDetermined with p=0.10

Table 2. Leaf starch and total sugar levels as determined in April 2012 of ‘Nadorcott’ mandarin trees treated with different fruit thinning techniques in the De Doorns area, South Africa.

Treatment	Total sugar (mg·g ⁻¹ dry weight)	Starch (mg·g ⁻¹ dry weight)
Control	118.2 ^{ns}	46.5 ^{ns}
Chemical thinning	116.6	50.0
Hand thinning	120.8	54.4
<i>p-value</i>	0.6454	0.5579

6. General discussion and conclusions

Starch accumulation follows a distinct pattern throughout the season in 'Nadorcott' mandarin trees. Rapid starch accumulation started prior to harvest with a peak at the beginning of flowering. Thereafter a sharp decrease in starch levels occurred until after full bloom and a steady decrease was observed from physiological fruit drop onwards as fruit matured. The accumulation of starch starting prior to harvest is suggested to coincide with fruit reaching maximum TSS levels. It is possible that the sink strength of the fruit is reduced after maximum sugar accumulation in the pulp, allowing the tree to accumulate starch in the leaves. Initially it was hypothesized that "on" trees would reach a lower maximum starch level compared to "off" trees, but both "on" and "off" trees reached similar maximum starch levels at flowering. Differences in leaf starch levels for "on" and "off" trees did occur during April and May with no significant differences during any of the other months. The peak at flowering was followed by a sharp decrease as large amounts of starch needed to be mobilized to support the growth of floral parts and vegetative shoots. A steady decline was observed from then onwards as fruit matured. Whereas most previous studies only focused on certain growth stages or critical periods during a season, this data shows the continuous accumulation and mobilization of starch and total sugars across an entire season, thereby giving the "bigger picture" of the carbohydrate economy of the tree during all phenological stages.

Correlations between leaf starch and total sugar levels for each month, and yield, return bloom and set showed that April has a moderate negative correlation ($r = -0.66$) with yield and a moderate positive correlation with return bloom ($r = 0.57$). This illustrates that April starch levels will be low when there is a large crop on the tree, due to the high demand for carbohydrates of the developing fruit, and this will result in a reduction in bloom the following year. The opposite will happen when there is a small crop. Correlations show that starch levels in April could give an indication of bearing potential for 'Nadorcott' mandarin and whether the current crop load is too heavy, adequate or too light. Identifying an "on" or "off" year at this stage is difficult since fruit are still small in size and have a dark green colour, especially in a regular bearing orchard where an "on" or "off" year was initiated. Therefore, if producers take leaf samples during the first two weeks of April and combine this with historical yield data, an alternate bearing orchard can easily be identified. Producers will already know by April that the next year will either be an "on" or an "off" year, depending on the leaf starch levels, and this will give them adequate time to manipulate trees into producing more or fewer fruit the next year, depending on the situation. Leaf starch levels in October and December (coinciding with the beginning and end of physiological fruit drop) showed moderate correlations with set percentage, confirming results from previous research that showed that starch has a direct relationship with fruit set. Future research should be done on other citrus cultivars to determine if leaf starch concentration gives a good indication of bearing potential for these cultivars as well. It would be

especially interesting to determine if other late maturing mandarins follow the same starch accumulation pattern and to compare it to early mandarin types such as ‘Nules’ clementine mandarin, which is harvested in May.

No significant differences in leaf starch levels were recorded in April for any of the pruning treatments. Little research has been done on the effect that different pruning techniques have on the carbohydrate levels of trees and the current research suggests that it has little or no effect and the focus of pruning research should remain on producing trees with many and good bearing units. Pruning of ‘Nadorcott’ mandarin trees can result in excessive regrowth, which has a negative effect on producing good bearing units. The early regrowth treatment where vigorous shoots were pinched and shoots were thinned to 15 cm apart in all directions showed great potential for producing good bearing units while maintaining a favourable tree size and this strategy is recommended to producers. Furthermore, it was observed that pruning in November instead of just after harvest (September) can be helpful when pruning alternate bearing orchards. With the flowers already formed, shoots with no or little flower can be pruned during the “off” year. During the “on” year shoots with flowers can be removed and more shoots can be removed compared to a normal year, thereby effectively thinning the crop for that year. Future research should focus on long-term management of regrowth and how it should be pruned after two years from starting the treatment, as this information is currently lacking.

Unfortunately the thinning trial in this study did not yield any significant results. It did, however, give an insight into the potential that thinning (especially chemical thinning) has for managing alternate bearing of ‘Nadorcott’ mandarin. Chemical thinning increased the fruit growth rate slightly as well as reduced the number of fruit and yield compared to the control treatment (albeit not significant differences). This illustrates that research should be done on optimizing the timing and rate of dichlorprop application for ‘Nadorcott’ mandarin trees, as this will give producers a good tool for reducing the yield during “on” years.

Alternate bearing is a complex phenomenon with many different factors controlling the extent thereof. This study showed that trying to turn an alternate bearing orchard into a regular bearing orchard is difficult. A way to reduce alternate bearing in citrus is by better understanding all the factors involved (carbohydrates, hormones, fruit load, climate) and how they interact. Research should therefore be continued in order to better understand this complex phenomenon.