

**Effect of exogenous bio-regulators on organically-managed Tahiti lime  
(*Citrus latifolia* Tanaka) fruit and essential oil productivity and quality**

By

Cecilia Coral Díaz Candelas

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Approved by:

\_\_\_\_\_  
José Pablo Morales Payán, Ph.D.  
President, Graduate Committee

\_\_\_\_\_  
Date

\_\_\_\_\_  
Sonia Martínez Garrastazú, M.S.  
Member, Graduate Committee

\_\_\_\_\_  
Date

\_\_\_\_\_  
Rodolfo J. Romañach, Ph.D.  
Member, Graduate Committee

\_\_\_\_\_  
Date

\_\_\_\_\_  
Duane A. Kolterman, Ph.D.  
Member, Graduate Committee

\_\_\_\_\_  
Date

\_\_\_\_\_  
Arq. Wilma Santiago  
Representative of Graduate Studies

\_\_\_\_\_  
Date

\_\_\_\_\_  
Skip Van Bloem, Ph.D.  
Chairperson of the Department

\_\_\_\_\_  
Date

## ABSTRACT

Tahiti or Persian lime productivity is limited because of its high abortion rate (>90%). Scientific literature is short of information regarding ethylene inhibitors, cytokinins and brassinolide in this crop and even less on organic management. Bioregulators accepted for organic agriculture were foliarly sprayed on Tahiti lime trees during flowering at the AEE-Lajas, to evaluate their effect on fruit and essential oil yield, fruit quality and post-harvest deterioration. During the October-December 2011 flowering period the following bioregulators were evaluated: aminoethoxyvinylglycine (AVG, 302 mg per tree, once four weeks before harvest), an *Ascophyllum nodosum* seaweed extract (AN, 0.6 mg eq. kinetin per tree, 4 times every three weeks), a polypeptide, amino acid and alkyl glycerol-triglycerides emulsion from hydrolyzed shark tissues (AHS, 3.4 mg a.i. per tree, 5 times every two weeks), a vitamin B, a triacontanol and brassinosteroids plant compost based formulation (TB, 0.04 mg a.i. per tree, 4 times every three weeks), gibberellic acid 3 (GA<sub>3</sub>, 12 mg per tree, two times), gibberellic acid 4/7 (GA<sub>4/7</sub>, 0.67 ng per tree, 5 times), and 2 applications of 24 mg GA<sub>3</sub> + 5 applications of 0.67 ng GA<sub>4/7</sub> per tree. The experiment was repeated during the April-June, 2012 flowering period adding two treatments and a slight augment in gibberellic acid doses: gibberellic acid 3 (GA<sub>3</sub>, 50 mg per tree, 2 times), gibberellic acid 4/7 (GA<sub>4/7</sub>, 3.36 ng per tree, 5 times), and 2 applications of 50 mg GA<sub>3</sub> + 5 applications of 3.36 ng GA<sub>4/7</sub> per tree. The added treatments were a brassinolide application (BR) sprayed at the beginning and five weeks after flowering and the other treatment consisted of the existent AVG application plus a second one four weeks after the first for a total of ten treatments including the check trees (Check). Concordant with Flores et al. (2010), the final flower retention was less than 10 percent. The main abscission period was during flower bud differentiation which was mainly affected by endogenous hormone levels. AN and GA<sub>4/7</sub> had higher fruit retention percentage than the check trees at the end of the project. AVG seemed to inhibit the decisive abscission effector ethylene for a period of time and along with GA<sub>4/7</sub> and AN enhanced fruit quality. GA<sub>4/7</sub> proved to be a weight, size, color and juice enhancer while GA<sub>3</sub> did not seem to have a positive effect on this citrus species' fruit yield nor quality apart of producing greener fruits. One single AVG application eight weeks before harvest had a consistent effect on juice content augmentation. AN and AHS applications produced a lasting and holistic fruit quality as long as water was available to the trees. TB did not seem to regularly affect fruit quality and storage. Terpenes in the lime essential oil were non-destructively confirmed in the fruit flavedo by near-infrared spectroscopy. Organically accepted pre-harvest application of exogenous plant growth regulators are useful for Tahiti lime fruit yield augmentation, post-harvest quality and storage enhancement.

## RESUMEN

La productividad del limón Tahití o lima Persa (*Citrus latifolia*) está limitada por su alta tasa de aborto de frutos (>90%). En la literatura científica hay poca información sobre efectos de inhibidores de etileno, citoquinina y brasinolida en este cultivo, y menos aun con manejo orgánico. Se asperjaron biorreguladores aceptables para producción orgánica al follaje de limón Tahití en floración en la EEA-Lajas, para evaluar su efecto en aceites esenciales en la cáscara, rendimiento y calidad de frutos y su deterioro post-cosecha. Durante la floración de octubre a diciembre de 2011 se evaluaron aminoetoxivinilglicina (AVG, 302 mg por árbol, aplicado una vez), un extracto del alga marina *Ascophyllum nodosum* (AN, 0.6 mg eq. kinetina por árbol, 4 veces), una emulsión de polipéptidos, aminoácidos y alquilglicerol-triglicéridos de tejidos hidrolizados de tiburón (AHS, 3.4 mg i.a. por árbol, 5 veces), una formulación de vitaminas, triacanol y brasinoesteroides (TB, 0.04 mg i.a. por árbol, 4 veces), ácido giberélico 3 (GA<sub>3</sub>, 12 mg por árbol, 2 veces), ácido giberélico 4/7 (GA<sub>4/7</sub>, 0.67 ng por árbol, 5 veces), y 2 aplicaciones de 24 mg GA<sub>3</sub> + 5 aplicaciones de 0.67 ng GA<sub>4/7</sub> por árbol. Se repitió el experimento durante la floración de abril a junio de 2012 añadiendo dos tratamientos y un leve aumento en las concentraciones de giberelinas: ácido giberélico 3 (GA<sub>3</sub>, 50 mg por árbol, 2 veces), ácido giberélico 4/7 (GA<sub>4/7</sub>, 3.36 ng por árbol, 5 veces), y 2 aplicaciones de 50 mg GA<sub>3</sub> + 5 aplicaciones de 3.36 ng GA<sub>4/7</sub> por árbol. Los tratamientos añadidos fueron uno de una aplicación de brasinolida (BR) a las cinco semanas de floración y el otro tratamiento consiste de la aplicación existente de AVG más una segunda aplicación cuatro semanas luego de la primera para un total de 10 tratamientos incluyendo el control (C). Concordando con Flores et al. (2010) la retención final de flores fue menos del 10 por ciento. El principal periodo de abscisión ocurrió durante la diferenciación de yemas florales la cual es afectada fundamentalmente por niveles endógenos hormonales. AN y GA<sub>4/7</sub> tuvieron el mayor porcentaje de retención final de frutos. AVG parece inhibir el efectuator decisivo de la abscisión, etileno, por un periodo de tiempo y junto con GA<sub>4/7</sub> y AN mejora la calidad del fruto. GA<sub>4/7</sub> probó mejorar el peso, tamaño, color y contenido de jugo mientras que el tan estudiado GA<sub>3</sub> no parece tener efecto positivo alguno en el rendimiento o calidad de esta especie a parte de producir frutos más verdes. Una sola aplicación de AVG ocho semanas antes de cosechar tuvo un efecto consistente en aumentar el contenido de jugo en ambos experimentos. AN y AHS mejoraron el fruto de manera duradera y holística siempre que no hubiera estrés hídrico en los arboles. TB no pareció afectar la calidad y almacenamiento del fruto de manera consistente. Usando espectroscopía de infrarrojo cercano (NIRS), un método no-destrutivo, se confirmó la presencia de terpenos en el flavedo. Estos resultados muestran que es posible aumentar la retención y la productividad de frutos, así como regular la post-cosecha en limón Tahití usando reguladores aceptables para producción orgánica.

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Es un mamey escribir una tesis  
viéndolo al lado de lo que has hecho  
por mi hermana y yo.  
No compara.

To my mother,

It's a mamey to write a thesis  
in view of what you have done  
for my sister and me.  
It doesn't compare.

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## INTRODUCTION

Puerto Rican, Dominican and Cuban lemons are usually what U.S. or Europeans would call key lime or Tahiti lime. Key lime is botanically known as *Citrus aurantifolia*, Tahiti lime as *Citrus latifolia*. In the Caribbean key lime is referred to as 'limón criollo' while Tahiti lime as 'limón Tahiti'. The fruit called lemon in Europe and the U.S. is scientifically known as *Citrus lemon*. *Citrus latifolia* is the superstar of my thesis and from this line on the terms 'lime' and 'lemon' might be used interchangeably for this same species unless otherwise stated.

Limes are mainly consumed fresh in the Caribbean and Latin America to produce all kinds of recipes from lemonade to complex home remedies. Some common recipes that call for fresh lime juice are foods like pico de gallo, fresh fried fish with lemon. Any kind of seafood seems to go well with lemon. Ceviche is an example of lemon juice used as a cooking agent by itself. Other recipes are drinks like 'mojito' or simply a piece of lime on a bottled beer. Also, lemon juice is constantly used as a remedy for common ailments like colds and even as a cleaning/antibacterial agent because of the terpene content and acidity. So it is pretty unambiguous that lemons are widely used on a daily basis on virtually all of Latin America and the immigrated Western countries as well. In fact they are used all over the world. Fresh lemons are constantly in demand.

The 2010 Census of the Agriculture Department of Puerto Rico reports a local production of 13% of the lemons consumed in the island so there is still ample room for growth in the local market. Added to this is the fact that, as time passes, there is less land to meet local demand so it is necessary to inquire into field practices that could increase production in an orchard. A first step to solve the problem is to seek local production practices that increase fruit retention.

A challenge for lemon fruit production and marketing is its lasting quality (Medina-Urrutía, 2000). The main use of a sold lemon is its juice which has to last long periods of storage as the fruit is transported from tropical and subtropical countries to the consumer. According to FAO the main lime importers are the U.S. and Europe taking from two to eight weeks from harvest to

table. This adds a challenge to farmers producing a high quality product and predicting its response to longer transport.

Pre-harvest bioregulator treatments have proven to have the most efficient effect in post-harvest quality (Agustí, 2000). A variety of growth regulating substances is available that might be used as pre-harvest enhancers of fruit quality in organic systems, but their effects need further evaluation. Most of the PGR pre-harvest experiments for fruit yield and quality improvement have been done with orange cultivars (*C. sinensis* Osbeck), and relatively little work has been done with lemons, which are grown in slightly warmer climates than oranges. Tahiti lime produces parthenocarpic fruit, which may drastically change the physiological aspects of fruit set and development and necessitates its own PGR research.

Puerto Rico can easily enter the lime essential oil industry as it is a highly mechanized process and has an economic potential on growing demand. A consumption of 1500 ton of lime essential oil was reported for 2007, valued at approximately \$99 million, and mainly used in the soft drink, candy, dairy products and fragrances industries. This is what has been officially reported, but it can be more. The price of standard or conventional (non-organic) essential lime oil was about \$ 66 per kilogram while the organically-produced essential oil was valued at \$ 138 per kg (Bovill, 2010). In addition, lime and lemon oil are priced higher than other citrus oils by lack of supply and growing demand (Di Giacomo, 2002). Apart from the fresh fruit and juice production a profitable niche where Puerto Rico would thrive is the essential oil industry.

## **OBJECTIVES**

1. To evaluate the effect of different growth regulators on retention, abscission and yield of Persian lime fruit.
2. To share our results using different bioregulators for citrus postharvest quality enhancement.
3. A brief discussion on the potential of NIRS for citrus oil quality assessment will be included. This method was utilized to evaluate whether bioregulators affect the content of terpenes in the flavedo.

### 3. Scientific Background

#### 3.1 Nomenclature

The origin of the genus *Citrus* is located in Southeast Asia, being *C. maxima* the most primitive species. It is thought that *C. maxima* (pomello), *C. medica* (citron), *C. reticulata* (mandarin) and *C. halimii* (a recently discovered taxon) are the parental species of the citrus we know today. *Citrus aurantiifolia* (Mexican lime) originated from a cross between citron and papeda (an Asian wild species without known commercial value). The Persian or Tahiti lime, *Citrus latifolia* Tan. is possibly a hybrid between *C. aurantiifolia* x *C. medica* (GRIN, 2011; Tropicos.org, 2011).

According to GRIN taxonomy for plants, *Citrus latifolia* (ex Yu Tanaka Tanaka) is classified as follows: Rutaceae family, Aurantioideae subfamily, Aurantieae tribe, subtribe Citrinae, genus Citrus, specific epithet latifolia Tan. A basionym of this species as in Tropicos.org is *Citrus* × *aurantiifolia* subsp. *latifolia* (ex Yu Tanaka Tanaka). Another synonym that may be found in literature is *Citrus aurantiifolia* var. *latifolia* Yu Tanaka (GRIN taxonomy for plants, 2011)

Common names used for this species are from the English: Bearss lime, Persian lime, Tahiti lime; French: limettier, limettier Perse, limettier Tahiti; German: Persische Limette, Tahitilimette and Spanish: lima, lima Persa or lima Tahití (GRIN taxonomy). Both Mexican lime (*C. aurantiifolia*) and the Persian are mostly grown in tropical and subtropical regions replacing the more temperate climate lemons [*Citrus limon* (L.) Burm. F].

Citrus fruits have been continually considered miraculous during different points in history from its origins in China until today because of its medicinal properties. Botanically, they are classified as hesperidia as inspired by a Greek legend where Heracles is required to bring the 'golden fruit' (a citrus) for the Garden of Hesperides' daughters: Arethusa, Aegles and Hyperetusa. Some examples that can be easily recalled of the *Citrus* healing record are Ayurvedic uses of lemon juice as an internal cleanser, scurvy treatment during colonial times, and currently it is seriously considered as a cancer preventive and some of its derivatives like limonene, as potential cancer treatments. The outer side of the pericarp is known as the flavedo

for the presence of flavonoids, whereas the inner side is called albedo for its whitish color and fluffy texture. The essential oil, where most of the terpenes as limonene is contained, is commercially extracted from the flavedo. Flavonoids and terpenes contained in the juice and essential oil are in part responsible for the so called extraordinary medicinal properties of the genus *Citrus* fruits.

### **3.2 Phases of Fruit Development, Growth and Maturity**

Fruit development relies on flower quality and quantity (Rebolledo, 2012) which are also coupled with hormonal signaling. Guardiola (2000) reported that increased flowering intensity heightens early abscission. The more flowers, more photoassimilates are used and less quality is produced. Also, the amount of leaves by flower is positively correlated to flower and fruit quality. There are five types of buds: individual flower with leaves, individual flower without leaves, multiple flowers with leaves, multiple flowers without leaves and vegetative buds. Iglesias et al. (2007) mention that ‘usually, late-opening flowers remain attached to the tree longer than early-opening flowers and flowering shoots with a high leaf-to-flower ratio have the highest fruit set. The presence of leaves increases GA levels and the chances of setting’. All of these associations point to nutrition as a limiting factor and hormonal signaling, which is also closely related to nutritional availability, as an essential part of the multifaceted fruit setting pathways.

Citrus fruit growth has a sigmoidal pattern (Rebolledo, 2012). There are three phases of fruit development all of which are affected by different physiological aspects which intertwine with each other. Phase I is the fruit set period and defined as a cell and flower bud differentiation stage. There are usually two main abscission waves and the first one typically occurs during the very first weeks of phase I where ovaries differentiate into fruit. During this stage is when the tree ‘decides’ which reproductive blossoms to keep and this choice is highly influenced by hormonal signals. The strongest abscission wave occurs during this flower differentiation phase. The fruit set promoting signals are generated during seed development as will be discussed in the fruit set physiology section.

Phase II is the cell enlargement/fruit growth stage where a significant amount of carbohydrates is needed due to a rapid growth. The transition time from phase I to phase II of fruit development is also known as the June drop period where the second main abscission wave occurs between six to twelve weeks after anthesis (flower opening). During this transition fruit set and abscission is determined by hormonal signals mainly on the base of carbohydrate accessibility which is influenced by ease of photoassimilation or transport (Guardiola, 2000). The fruit that was kept by hormonal regulation during phase I competes for the carbohydrate supply and is usually abscised during 'June drop' or might grow into a less quality fruit unless carbohydrate supply and transport is produced from leaves to fruit.

Fruit maturation occurs during phase III. Usually the fruit is out of risk of being abscised once it reaches this stage and should be harvested before it is too late to market. Fruit quality and set enhancement is focused on regulating phase I and II of fruit development and has many photoassimilates implicated on the process including carbohydrates and endogenous growth regulators.

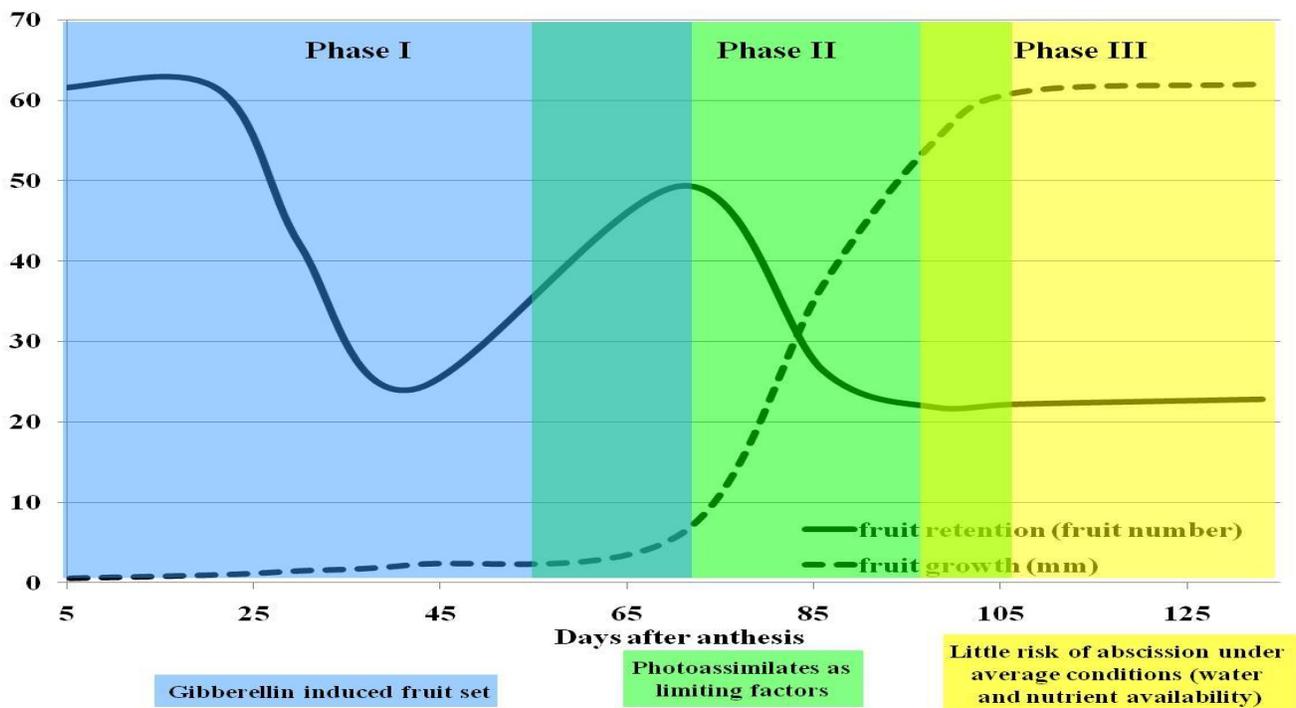


Figure 3.2.1 Fruit growth and fruit retention/abscission tendency through time as reported on the previous literature cited above.

### 3.3 Essential Oil Gland Development and Composition

Citrus essential oil is produced in oil glands developed in the fruit flavedo, leaves, petals and twigs. The citrus essential oil industry extracts the product mainly from the juiced fruit leftovers. In these tissues the flavedo contains the oil glands and consequently the majority of the essential oil. According Bosabalidis and Tsekos (1982) essential oil production in the cell is carried out by the plastids in *Citrus deliciosa*. As soon as a small cavity is formed, secretory cells enter a state of intense secretion. When the glandular cavity is completed, peripheral cell walls harden and plastids are transformed in leucoplasts. In *Citrus sinensis*, *Ruta graveolens* and *Dictamnus albus* plastids become chloroplasts with well-developed grana systems.

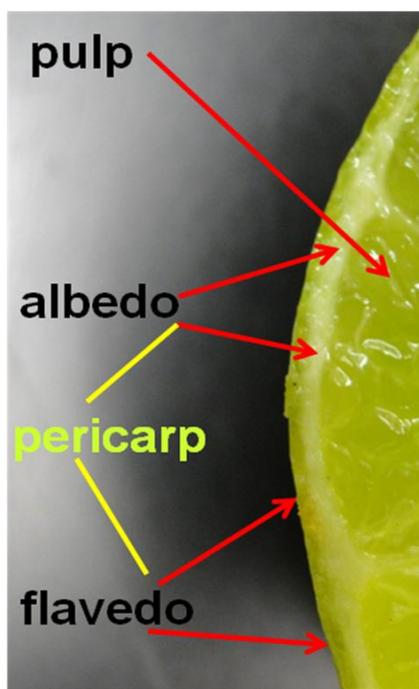


Figure 3.3.1 Image showing different tissues involving citrus fruit quality.

In a study conducted by Knight et al. (2001) the relationship between the maturity of the glands and fruit maturity was assessed in Washington Navel orange. After the fruit reached a 20 mm diameter, the number of glands did not increase. The glands reached maturity while the fruit was

still immature and green with a diameter between 32 and 52 mm. As the fruit ripened, glands continued to grow until the fruit reached 88 mm in diameter. At this point the sizes and shapes of the glands were varied. The depths were also varied and often reached to the albedo. Rafiei & Rajaei (2007) also study the development of oil producing organs in *C. aurantifolia* reporting that gland initiation happens until a certain point of cell differentiation during phase I of fruit development. Coinciding with Knight et al. (2001) observations, oil glands grow primarily while the fruit matures and grows.

Oil glands play an important role as a defense mechanism by the essential oil properties as a natural insect repellent, antibacterial and antifungal substance. Citral, a terpene contained in the essential oil, works as an antifungal agent (Klieber, Knight & Ben-Yehoshua, 2000). D-limonene, the predominant component of *Citrus* essential oils, has numerous properties as well as many other terpenes whose assessments are beyond the scope of this study. For the sake of simplifying the content of this work only the concentrations of the six most sought terpenes in *C. latifolia* essential oil are presented here as studied by reverse phase HPLC by Dugo et al. (1997).

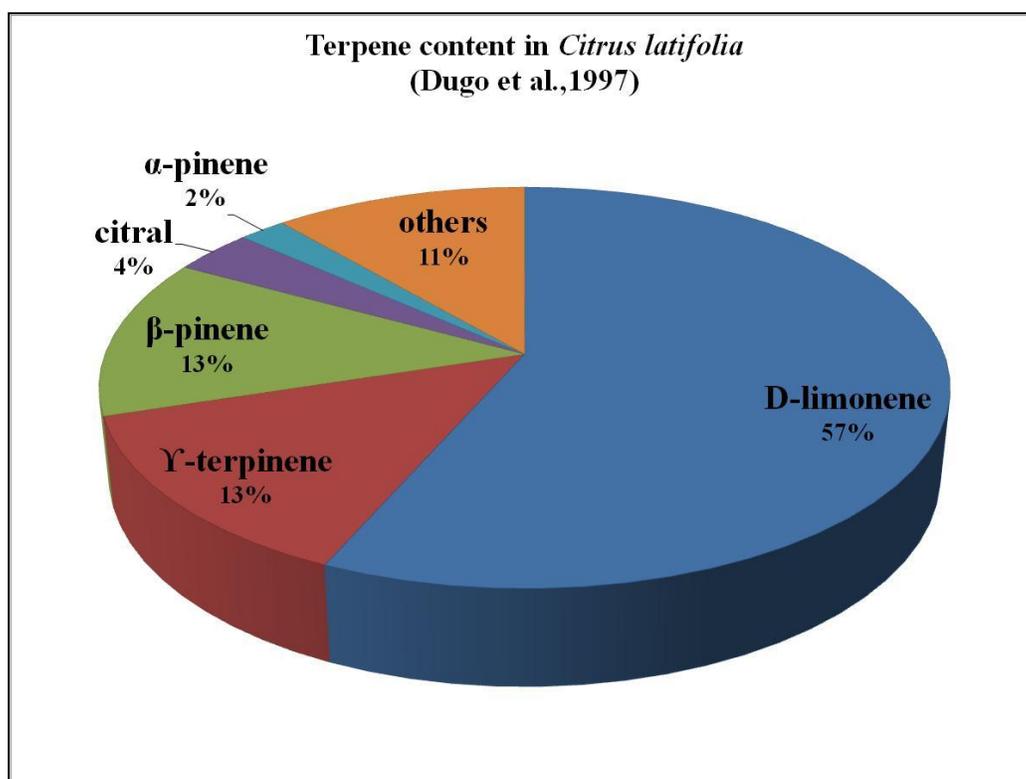


Figure 3.3.2 Pie chart of the most solicited terpenes in *C. latifolia* essential oil quantitatively assessed via reverse phase HPLC by Dugo et al. (1997).

Works studying the relationship of fruit ripening and development of oil gland includes *C. sinensis* by Knight et. al. (2001), *C. medica* by Liang et. al. (2006) and *C. aurantifolia* by Rafiei & Rajaei (2007). Also, one work on the effect that growth regulators exert on terpene content of essential oil in the flavedo, Ortuño-Tomás et al. (1993), where etephon is added to *C. paradisi* in order to augment nootkatone content and, as a result, it is positively increased. Many studies like these are yet to be completed and might also concern the effect of growth regulators in oil gland development in addition to certain terpene concentrations. The regulation of *C. latifolia* oil gland development or terpene content has not been reported so it can be a pioneer work to consider in both fields.

### **3.4 Citrus Fruit Physiology**

The role of endogenous and exogenous regulators as well as their precursors and modes of action will be discussed in the following sections.

#### ***3.4.1 Endogenous Growth Regulators: what are they, what they do and how they do it?***

The main endogenous growth regulators concerning fruit set and studied so far are: ethylene, gibberellin, auxin, abscisic acid, and cytokinins. It is of wide acceptance that gibberellic acid and cytokinins regulate and promote fruit set as they are naturally synthesized during pollination and seed development. It has been widely proved as well that ethylene has an important or ‘the’ most important role in regulating and promoting fruit abscission. Exogenous auxins seem to have an ambiguous aspect to their effect on fruit set and abscission. At first auxins appear to lower fruit abscission and stimulate fruit growth but these induce ethylene synthesis. Auxins have proven effective as thinning agents to produce larger fruits but the effects on fruit number are not yet clear and usually induce a very low yield at the end of the harvest period. Agustí (2000) reports that exogenous auxins ‘have a thinning effect when applied during phase I of fruit growth, and a growth enhancing effect when applied as phase III starts’. Auxins do not enhance absolute fruit set (Iglesias et al. 2007) and are considered as part of the same list of abscising agents, as Cecilia C. Díaz Candelas, tesis MS, 2013

ethylene and ABA, when applied before the cell enlargement phase which is not always definite as fruit growth phases on tree commonly overlap with new flower production.

Flower bud and flower abscission occur at the pedicel (abscission zone A, abbreviated AZ A) while fruitlet abscission occurs at the calyx (abscission zone C, abr. AZ C) (Guardiola, 2000). Iglesias et al. (2006) made AVG and ACC treatments to citrus trees to study the effect of carbohydrate and ethylene levels in the fruitlet drop through AZ A. As a result AVG significantly decreased abscission, ethylene and ACC. Branch girdling lowered abscission and increased hexose and sucrose availability. In Iglesias et al. (2006) own words ‘...induced ethylene, combined or not with ACC treatments was unable to re-activate the AZ A after June drop, during the natural period of AZ A inactivation (90-100 days after anthesis)’ concluding that ‘carbohydrate content and ethylene release are two main components of the abscission process through the AZ A while ethylene might act as the last effector for AZ C.’

Studies with 1-methylcyclopropene (1-mcp), another ethylene inhibitor, showed that it prevents degreening and ethylene production in *Citrus paradisi*. In a different study it also had the effect of inhibiting abscission, blocking ethylene induced degreening and increasing decay and chilling injury. 1-mcp did not affect soluble solids nor tritrable acid content in Shamouti oranges. It was suggested that endogenous ethylene is an important regulator of the defense system of the plant (Blankenship & Dole, 2003).

Carbohydrate supply might be a factor affecting fruit yield and quality according to Goldschmidt (2000). In a study by Duarte & Guardiola (1996) GA<sub>3</sub> sprays to 'Fortune' hybrid mandarin at flowering ‘retarded abscission of reproductive organs, but failed to increase the final fruit set’. On the contrary girdling did not affect the main wave of fruit drop, but reduced late abscission, which resulted in an increase in set and final yield. Hormonal balance in fruit trees has been affected by girdling as it eliminates sugar competition by the root system (Guardiola, 2000). Iglesias et al. (2003) conclude that carbon shortage induces ethylene synthesis seeing that ‘nutritional factors are limiting factors but hormonal compounds are effectors of the regulation of the abscission process’. Also ‘modulation of sugar effects by hormones and vice-versa, has also been reported for several plant hormones including gibberellins and ethylene.’

**Table 3.4.1 Endogenous bioregulators implicated in citrus fruit development, set and growth as reported in the cited literature above.** Most regulators are derived from amino acids. According to Lehninger, Nelson & Cox (1993) amino acids themselves are derived from glycolysis, the citric acid cycle or the pentose phosphate pathway intermediates. Fruit set and fruit growth is regulated by a complex combination of carbohydrate supply and hormonal balance.

<b>PGR</b>	<b>Effect on fruit development</b>	<b>Physiological role</b>	<b>Precursor</b>	<b>Reference</b>
Ethylene	Abscission inducer, growth inhibitor	Polygalacturonase and b-1,4-endogalacturonase gene (hydrolases) activator, it's synthesis mediates abscission	Methionine Immediate precursor is ACC Exogenous inhibitors: AVG and/or 1-MCP	Blankenship & Dole (2003); Bonghi & Tonutti (2000); Gómez-Cadenas et al. (2000); Iglesias et al. (2003); Iglesias et al. (2006); Ladaniya (2008); Sinclair (1984)
Gibberellins	Fruit set promoter, vegetative growth inducer, fruit quality enhancer, exogenously enhances partenocarpic fruit set, inhibits flowering	Promotes cell growth and vegetative growth, retards ethylene effects, Inhibits abscission during phase I, increased by pollination on seeded cultivars, may decrease facultative partenocarpic fruit set	Pyruvate Produced during seed development	Duarte & Guardiola (1996); Gómez-Cadenas et al. (2000); Guardiola (2000); Iglesias et al. (2003); Iglesias et al. (2007); Ladaniya (2008); Sinclair (1984); Talon, Mehouchi & Primo-Millo (2000)
Auxins	Fruit set promoter, growth inducer, fruit quality enhancer, thinning agent	Promotes cell differentiation, stimulates ethylene production, promotes photosynthate movement to the roots	Tryptophan	Agusti (2000); Iglesias et al. (2007); Ladaniya (2008); Sinclair (1984);
Cytokinins	Fruit set promoter, growth inducer, partenocarpic fruit quality enhancer	Promotes cell differentiation, facilitates photoassimilate transport, reduces ethylene effects, increases sink strength	Adenine Produced during seed development	Iglesias et al. (2007); Ladaniya (2008); Sinclair (1984)
Abscisic acid (ABA)	Growth inhibitor, abscission inducer, Stress signal	Induces growth discontinuation and ethylene synthesis	Pyruvate Produced during stress	Iglesias et al. (2007); Ladaniya (2008); Sinclair (1984)
1-Aminocyclopropane-1-carboxylic acid (ACC)	Growth inhibitor	Immediate ethylene precursor	Methionine→SAM→ACC→ethylene	Iglesias et al. (2007); Ladaniya (2008);
Brassinolide	Fruit set promoter, growth inducer, fruit quality enhancer, thinning agent	Considered to be involved with auxin and ethylene metabolism with a similar effect as auxins	Acetyl-CoA	Chul Chang et al. (2004); Iwahori et al. (1990); Kim et al. (2007); Kuraishi et al. (1991); Nakamura (2006)
Carbohydrates*, nutrients*	Limiting factor, scarcity limits fruit set and growth while availability eases it	Provides energy and/or biochemical precursors Sugar shortage induces ethylene synthesis	Sunlight and soil fertility *Not regulators per se but highly implicated in fruit set and growth regulation	Duarte & Guardiola (1996); Goldschmidt (2000); Gómez-Cadenas et al. (2000); Guardiola (2000); Iglesias et al. (2003); Iglesias et al. (2006);

### 3.4.2 Parthenocarpic fruit set

A fruit is conferred parthenocarpic characteristics when it develops without seeds or in other terms the ovary develops into a fruit while the ovules stay undeveloped, never existed or are aborted. There are three main reasons for parthenocarpy to occur: a. stenospermocarpy (embryo

abortion), b. chromosomic imbalance as with triploid cultivars, c. auto-incompatibility as with some *C. sinensis* varieties that may or may not produce fertilized seeds usually induced by endogenous and/or exogenous regulators. Tahiti lime is a triploid hybrid that will always produce parthenocarpic fruit, which means it cannot develop seeds.

Seed development in the fruit unleashes a series of hormonal events that may not be present in parthenocarpic fruits. In fact it is known that commercially acclaimed seeded mandarins produce much less when exogenously induced parthenocarpic fruit is produced. This might also depend in the type of parthenocarpy. Talon, Mehouchi & Primo-Millo (2000) report that at anthesis GA levels increase in the ovaries of seeded and low abscising parthenocarpic cultivars. In these last cultivars exogenous GA has no significant effect on fruit set. Moreover in high abscising, self-incompatible parthenocarpic cultivars exogenous GAs considerably increases fruit set by suppressing post-anthesis fruit drop. Iglesias et al. (2007) reported that 'self-incompatible parthenocarpic genotypes contain lower GA levels than 'naturally' parthenocarpic varieties'. Contrasting with seeded cultivars where pollination induces GA levels augment and reduces fruit abscission. In parthenocarpic species the GA levels rise is developmentally regulated. At first fruit set and seedless fruit development is induced in "facultative" and "truly" parthenocarpic cultivars but fruit growth is fully accomplished until ripening in facultative varieties only (Iglesias et al. 2007).

Cytokinins stimulate cell division and also serves as transport facilitator of a variety of growth metabolite biosynthesis. Iglesias et al. (2007) also report that 'cytokinin levels increase at anthesis while the ovaries are developing'. Applying exogenous cytokinins enhances parthenocarpic fruit development and stimulates sink strength in developing fruits of certain cultivars. Sinclair (1984) reported that BA retards ethylene treatments. Kinetin has a role in photosynthate transport making it a versatile biostimulant for different metabolic pathways that the plant might need.

### ***3.4.3 Exogenous Growth Regulators: Bioregulator use in Citriculture***

With the information discussed until now a fruit set enhancing program may be developed by using exogenous gibberellic acid and cytokinins and/or inhibiting ethylene synthesis along with an adequate fertilization program. Nutritional supply during flowering may promote vegetative growth delaying the flower bud development process so any fertilization should be done way before anthesis, in order not to inhibit flowering, or preferably right after the harvest season. For this reason once anthesis has started bioregulators are the norm in a fruit set regulation program. *Citrus latifolia* might flower and fruit in an ongoing way throughout the year making the fertilization program complicated.

Concerning fruit quality, it is more cost-effective to control pre-harvest factors (Agustí, 2000). Moreover, pre-harvest applications of plant growth regulators (PGRs) and other chemicals significantly affect quality and storage life of citrus fruit. Several studies have been published about using gibberellins (GA) and auxins separately or combined as pre-harvest treatments to enhance fruit quality. Postharvest effects vary widely depending on timing of application and active ingredient concentration. These include thinning to enhance fruit size and weight and total soluble solids (TSS), longer retention of green and increased juice quantity (Ladaniya, 2008). Publications by Guardiola et al. (1993) and Guardiola (2000) demonstrate that for a cell differentiation effect, early applications during flowering or fruit development are more efficacious.

Gómez-Cadenas et al (2000) found that ‘AVG decreased ACC up to 65% and abscission up to 40%. Exogenous gibberellins had no effect on abscission. Fruitlet abscission induced by carbon shortage in citrus is regulated by ABA and ACC originating in the fruits, while gibberellins are apparently implicated in the maintenance of growth. GAs are not directly implicated in the control of fruit abscission induced by carbon shortage.’ There is a lack of literature regarding the effect of pre-harvest application of aminoethoxyvinylglycine (AVG) on lime fruit storage life. AVG mode of action according to Even-Chen et al. (1981): Methionine > SAM > ACC > ethylene were AVG affects ACS and SAM eventually turns into spermidine. Spermidine applied

exogenously increased proline and growth in drought stressed *C. aurantifolia* seedlings (Amri & Shahsavar, 2010).

A common practice in organic agriculture in Latin America is the application of algae extracts. Numerous works have been done in relation to the seaweed *Ascophyllum nodosum* extract applications. Fornes et al. (1995) published a study showing an increase in TSS and decrease of acidity. Also better colored peel was reported by Koo (1988) when applying seaweed to 'Valencia' orange trees. Seaweed extract to Tahiti lime application by Flores-Torres et al. (2010) significantly reduced fruit abscission in Tahiti lime trees. Being a plant derived extract it contains several biostimulant components like auxin, cytokinin and others which have not been isolated.

Another composting practice applied in the Caribbean since the time of the Taínos is fertilizing fields with fish waste. Here a wider variety in biostimulant components is expected because of the fact that fish has a higher rank in the food chain. The effects of fish waste on citrus fruit yield and quality has yet to be reported.

The effects of other plant biorregulators on citrus seem to have been eclipsed by the reliable outcomes of GA, auxin and ethylene inhibitor applications (Guardiola, 2000). Notably, most of the PGR pre-harvest experiments for fruit quality improvement have been done with orange cultivars (*C. sinensis* Osbeck), and relatively little work has been done with limes. Tahiti lime produces parthenocarpic fruits; a fact that may drastically change the physiological aspects of fruit set and necessitates its own PGR research. Adding to this is the limited literature concerning bioregulator applications in organically managed agriculture and post-harvest systems.

A regulator whose effects on abscission are still under studied is brassinolide. The function of this hormone has not been clearly elucidated but there is a relationship with auxin (Nakamura, 2006; Kim et al., 2007) and ethylene (Chul Chang et al., 2004). According to Kim et al. and Nakamura the relation with auxin is negative and Chul Chang considers it positive with ethylene although the metabolic pathway is somewhat different. Brassinolide has been reported to delay fruit abscission in citrus fruits having a analogous result as auxin (Iwahori et al. 1990). Kuraishi

et al. (1991) report its abscission reducing effect in *C. aurantifolia* and Brix/acid ratio (BAR) increasing effect in Navel orange. As the case with auxins, its effects on fruit abscission is not yet clearly understood and needs further assessment.

Triacontanol is another biorregulator that needs additional study on its effect on abscission and fruit quality. Preharvest application reduced orange acidity and increased total soluble solids (TSS) but did not affect acidity in grapefruit (Wilson et al. 1988). Proline foliar sprays decreased ascorbic acid content and TSS in lime juice (Ladaniya, 2008). No effects have been reported concerning their use as fruit set biostimulants. A variety of growth regulating substances are available that might be used as pre-harvest enhancers of fruit quality, but their effects need further evaluation.

During this experiment auxin was not considered for assessment since there is vast evidence showing its thinning effects contrasting with the abscission inhibition objective of this study. One example is Agustí's (2000) report of auxin as a thinning agent when applied during physiological fruit drop and growth enhancing effect when applied during the cell enlargement stage. Exogenous auxin accepted for organic production exists in the market though.

### **3.5 Near Infrared Spectroscopy techniques used in essential oil evaluation**

Near infrared spectroscopy has proven useful on intact fruit quality evaluations. Several studies have been published concerning the use of this technique to assess fruit maturity of apple, pear, tomato, apricot and fruits with thin peel. Many of these evaluate the fruit soluble solids as a maturity index. Furthermore a publication by Jamshidi et al. (2011) reported the effect of citrus fruit peel on the NIR reflectance of intact citrus internal quality. Citrus fruit peel is not as thin and they wanted to assess if the peel components affected the internal fruit reflectance. For fruit evaluations the spectra interpretation analysis is based on main peak absorbance. Spectra of intact fruit, peeled fruit a fruit peel was taken and evaluated for main peak detection. They concluded that the main absorbance peaks for peel did not interfere with absorbance wavelength used in soluble solid assessment (Jamshidi et al., 2011).

In another work by Steuer, Schulz & Läger (2001) different *Citrus* oils were assessed by NIR spectroscopy and report that vibrations in the area of 1634-1766 nm and 2250-2350 nm were the predominant overtones in the loading factor 1 of C-H-stretching bands. These might be converted to obtain 6117-5662 and 4444-4255  $\text{cm}^{-1}$  respectively. Limonene, sabinene, nootkatone and other demanded terpenes were successfully quantified on different *Citrus* fruits. Moreover Wilson (2002) published a technique for citral quantification in lemongrass and lemon oils. Lemongrass oil contains up to 70% citral while lemon oil around 1.5%. He observed that the most appropriate mathematical pre-treatment for these oils was standard normal variate with second derivative. Wavelength peaks statistically selected for citral were 2212 nm in lemongrass oil and 2212 and 2258 nm in lemon oil, which is the same as 4520 and 4428  $\text{cm}^{-1}$  respectively. In this work citral concentration in lemongrass oil could be quantified while not in lemon. Near Infrared Spectroscopy (NIRS) can be used as a non-destructive, real time analysis method to evaluate the terpene content in the essential oils in citrus fruits. No work has been published on measuring terpene contents on intact *Citrus* fruit with NIR.

A statistical discipline used for analysis of vast amounts of chemical data as produced by NIRS is known as chemometrics and is combined with multivariate analysis in order to be able to classify the wide categories of parameters caught up by complicated chemical mixtures and extract the highest amount of information possible from the ample data. Within multivariate analysis, Principal Component Analysis (PCA) is an orthogonal transformation to reduce any linear correlation between the data points other than a true relationship amongst them. PCA is related to multiple linear regressions and is useful for exploratory analysis. Data should be normalized prior to PCA. Two normalization techniques employed in this work are Standard Normal Variate (SNV) and 2<sup>nd</sup> derivative. Data is pretreated with SNV to remove the scatter that may develop from the equipment. This pretreatment consists of subtracting every data point from the mean and dividing it by its standard deviation. The NIRS analysis presented in this work consists of these three pretreatments, in order of employment: SNV, 2<sup>nd</sup> der. and PCA.

## 4. EXOGENOUS BIOREGULATOR EFFECT ON ORGANIC TAHITI FRUIT PRODUCTIVITY AND QUALITY

### 4.1 Introduction

Limes are grown mainly for the fruit juice and valuable essential oils synthesized in the fruit flavedo but a problem in Tahiti lime production is its high fruit abscission rate, over 90% (Flores-Torres *et al.*, 2010). As with many agricultural products, markets for organic citrus juice and essential oils are on the rise and making similar or higher profits as conventional non-organic. Organic orchards are supplied with compost, animal based or plant based fertilizers which might have a bioregulating effect on the crops. Treatments with traditional synthetic and/or organically approved bioregulators may lower citrus fruit abscission rates while increasing juice and essential oil content (Ladaniya, 2007).

Numerous studies have been conducted to understand fruit set/abscission physiology with gibberellin and ethylene acting usually as the main and opposing characters. Gibberellins have been related to promoting fruit set and post-harvest quality, reducing fruit abscission and the effects of ethylene whereas ethylene has been related to growth and fruit set inhibition, promoting abscission in general. On the other hand there is ample evidence that ethylene is the main effector of abscission as an activator of hydrolysis enzyme genes (Bonghi and Tonutti, 2000) while for gibberellin there is little evidence of the mode of action other than it is produced endogenously during seed development and that when applied exogenously it might promote fruit set or parthenocarpic fruit set (Talon *et al.*, 2000). In the case of gibberellin promoted parthenocarpic fruit set, as is the case of some mandarin cultivars, a high abscission rate usually follows as a secondary effect while in self-incompatible cultivars GA levels are relatively low and exogenous GA increases fruit set (Duarte & Guardiola, 1996; Guardiola, 2000; Talon, *et al.*, 2000). In some cases gibberellins retard ethylene effects but exogenous GA's are usually applied during early fruit development (first to sixth week) in order to be able to have a post-harvest effect in which case its effect on June drop (eighth to twelfth week) might be null.

Other important plant growth regulators on the fruit-set promoting side are cytokinins working as a photosynthate transportation facilitator as well as ethylene-effect retardant (Sinclair, 1984). Gibberellins and cytokinins are synthesized during seed development at the fruit setting stage. On the ethylene's fruit abscission promotion side, auxins would be of oscillating nature as they perform as fruit set supporters at first but a small amount of fruit makes it to the end of the harvesting season (Iglesias et al., 2007). The fruit ends up being usually larger with increased shelf-life. Auxins promote growth as well as ethylene synthesis (Goren et al., 2000; Sinclair, 1984), with ambiguous results, where good quality fruit is produced at the expense of a small amount of fruit. Exogenous auxins do not decrease fruit abscission by themselves but are used in combination with gibberellins to promote fruit thinning and facilitating mechanized harvesting. Their use for fruit set is complicated and requires a specific precision on doses and timing of application for each *Citrus* species as well as combining with other growth promoting bioregulators. A biosynthesized regulator still under study is brassinolide. It is known to be related to the auxin and ethylene pathways and have similar effects as auxins (Iwahori et al., 1990; Kuraishi et al., 1991). With the available information a focus to promote fruit setting and decrease abscission could be trying to regulate the gibberellic acids and related pathways and/or down-regulate ethylene and related pathways.

Now, parthenocarpic fruit setting, as it naturally happens in the triploid Tahiti lime, is a different saga. Seeds are not developed in parthenocarpic fruits making the gibberellic acid and cytokinin synthesis (and fruit setting with it) yet another mystery to be understood. As with any plant regulating project it is important to understand the metabolic pathways as much as possible specially when working in organic systems where a variety of biostimulating components are present in the composts, plant and animal based extracts applied. Here is where this scientific experiment fits in the thread as an advancement project.

## **4.2 Objective**

The objective of this study is to quantify with this work the effect of selected bioregulators on the yield and quality of organically produced Tahiti lime.

### 4.3 Materials and methods

The field research was conducted during October 2011-February 2012, and repeated during April 2012-November 2012 using a 6-year old organically-managed Persian lime orchard at the Agricultural Experimental Station in Lajas. For the 2011 experiment the orchard had flowered out of season right after the main harvest season. Small fruits (<20 mm) and flowers were sprayed together starting October 21, 2011. The 2012 experiment was conducted during the heaviest flowering season and any small fruit that could be found was manually thinned during the first day, April 2nd, 2012, in order to start the applications with only flowers.

All the bioregulators (except AVG) were first applied early in the fruit development phase (fruits <20 mm in diameter). The bioregulators evaluated were (1) aminoethoxyvinylglycine (AVG, 302 mg per tree applied once, four weeks before harvest); (2) a commercial extract of *Ascophyllum nodosum* (AN, 0.6 mg kinetin equivalent per tree, applied every three weeks), (3) a commercial blend of B vitamins, triacontanol, and brassinolide (TB, 0.04 mg a.i. per tree, applied every three weeks), (4) gibberellic acid 3 (GA<sub>3</sub>, 12 mg a.i. per tree applied twice, at the start of fruiting and 2 weeks later), (5) gibberellic acid 4/7 (GA<sub>4/7</sub>, five weekly 0.672 mg applications per tree starting at fruiting), (6) GA<sub>3</sub> and GA<sub>4/7</sub>, applied separately to the same tree at the rates and times described above, and (7) a blend of amino acids from hydrolyzed shark tissues (AHS, five bi-weekly applications of 3.4 mg a.i. each). In this first experiment there were 7 treatments plus the check trees. For EII GA concentrations were augmented and two more treatments added. The new treatments was a brassinolide (BR) application five weeks after anthesis and a second AVG treatment (AVG II) where 302 mg AVG per tree were added four weeks before the first harvest and another 302 mg right after the first harvest. Gibberellic acid 3 was augmented to 50 mg per tree and GA<sub>4/7</sub> to 3.36 ng per tree. The GA<sub>3</sub>+GA<sub>4/7</sub> was augmented accordingly. Adding the BR and a second AVG treatment gave a total of nine treatments plus the check trees. All but AHS were foliarly sprayed early in the morning. The AHS treatment was applied to the roots 8 to 12 inches from the trunk. The GA and AVG treatments were accompanied by a non-anionic surfactant.

For 2011 biorregulator applications started on October 21, 2011 ending on December 19, 2011. Field and yield evaluations were done from November 28, 2011 to February 6, 2012. For 2012 Cecilia C. Díaz Candelas, tesis MS, 2013

bioregulator applications ran from April 2<sup>nd</sup>, 2012 to July 9, 2012, field evaluations from April 2<sup>nd</sup>, 2012 to October 8, 2012 and yield evaluations from July 9, 2012 to October 29, 2012.

Leaf chlorophyll concentration was determined every 14 days for 2011 and monthly for 2012 to three leaves per tree using a SPAD meter. All the flowers produced per tree were counted every two weeks. Fruit retention rate was determined by counting the number of fruit every two weeks and dividing the number of fruit by the number of the last count. Fruit size was measured using a caliper. Fruit was harvested at the mature stage, with a size >45mm but looking for >60mm. FAO size code is: (1) 58-67mm, (2) 53-62 mm, (3) 48-57, (4) 45-52mm and (5) 42-49mm. For the sake of uniformity large fruits in this project would fall in the 1 and 2 categories of the FAO code while the medium fruit would be code 4 and 5. No small fruits were collected. Yield as total number and weight of harvested fruit per tree was determined for the 2012 experiment.

Within 24 hours after fruit harvesting, whole fruits were non-destructively analyzed using near-infrared spectroscopy (NIRS) to assess fruit essential oil content and quality in the fruit peel. After NIRS analysis, we determined percentage of fruit juice per weight, as well as juice acidity and soluble sugar content.

## **4.4 Results and Discussion**

### ***4.4.1 Chlorophyll concentrations***

GA<sub>3</sub>, AVG y GA<sub>4/7</sub> had significantly higher chlorophyll content in 2011 (Fig. 4.4.1) (Appendix D.1). For 2012, GA<sub>3</sub>+GA<sub>4/7</sub> kept a significantly higher chlorophyll concentration followed by GA<sub>3</sub> which kept a steady trend through time (Fig. 4.4.2) (Appendix C.1).

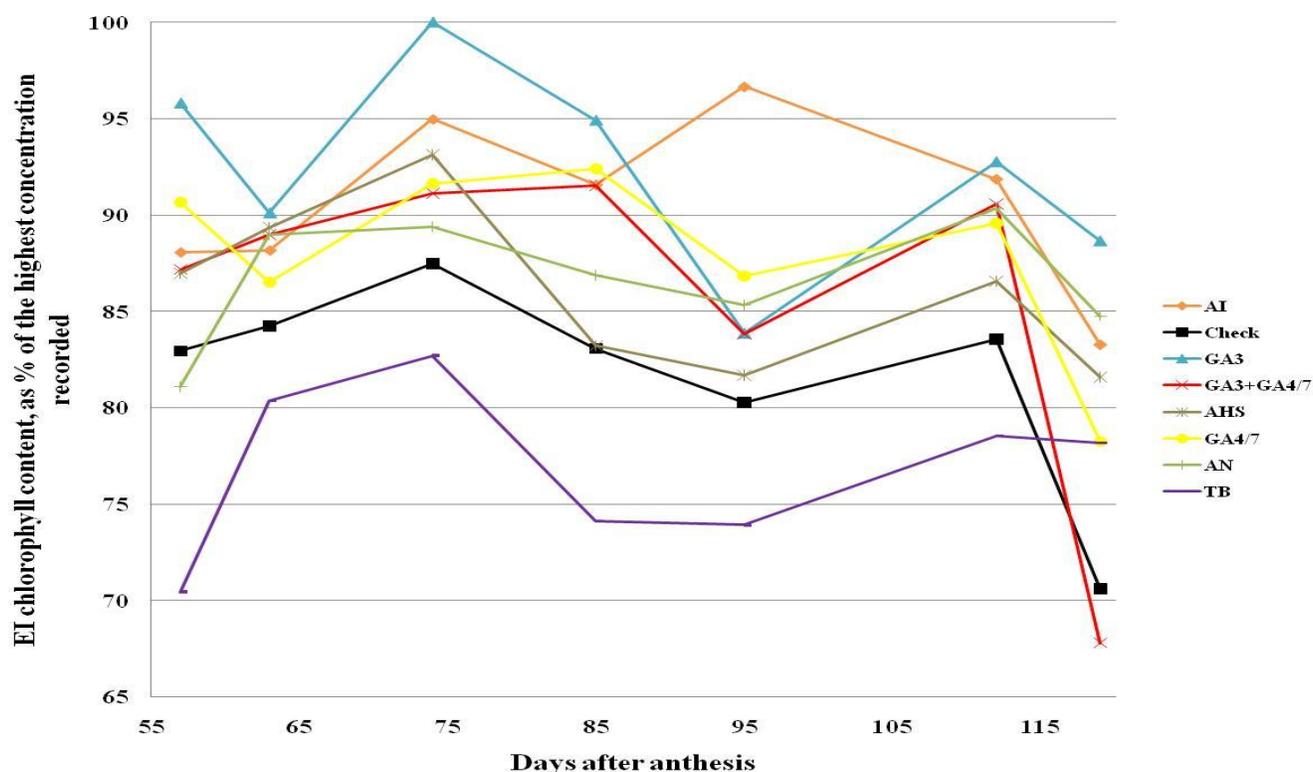


Figure 4.4.1. 2011 Tree chlorophyll content, as % of the highest chlorophyll recorded.

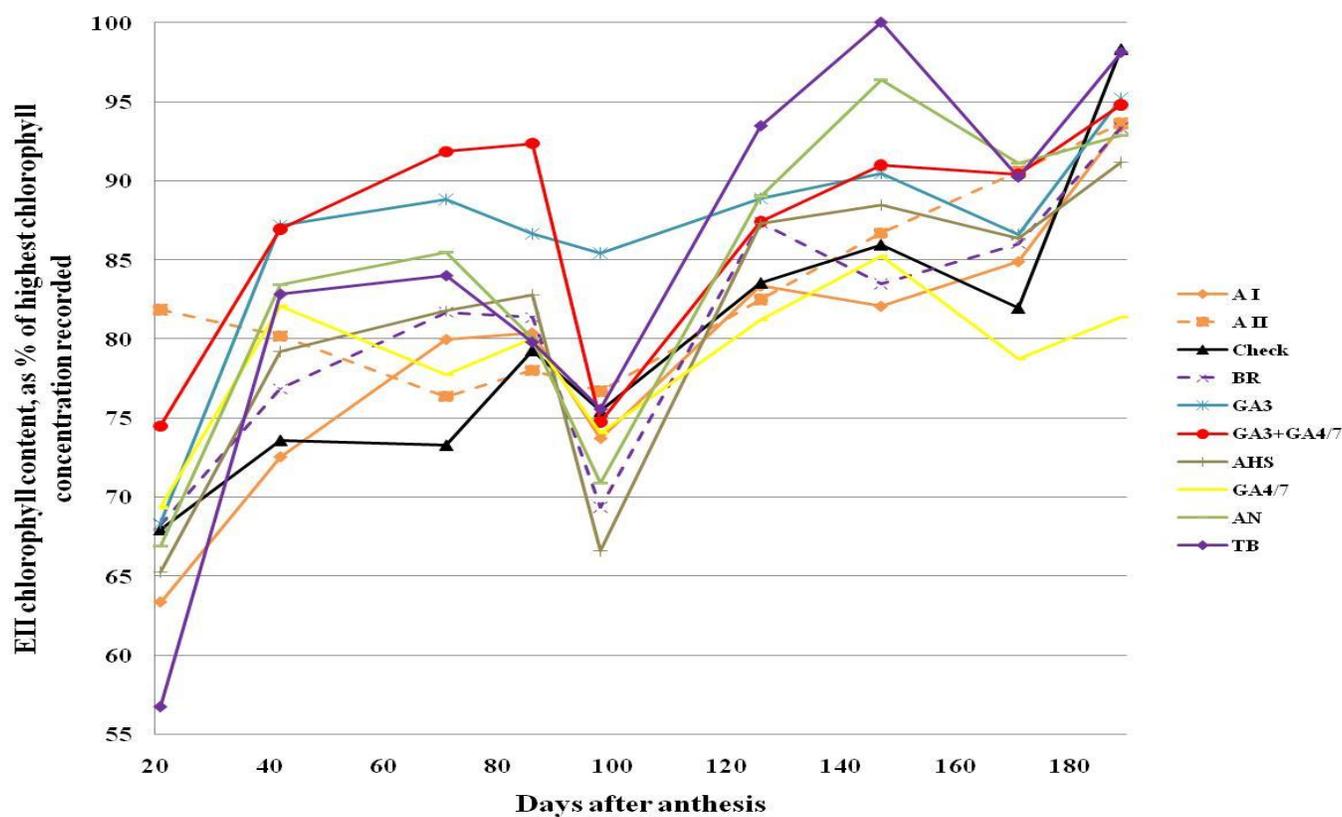
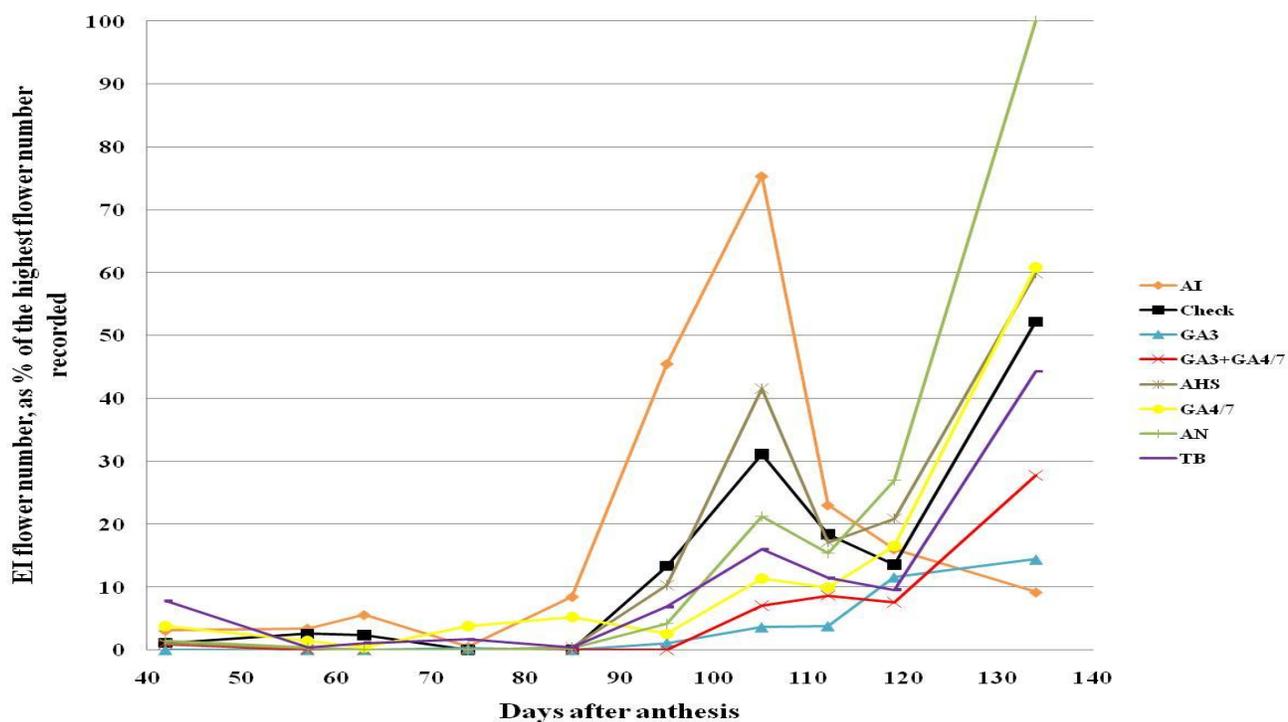


Figure 4.4.2 2012 Tree chlorophyll content, as % of the highest chlorophyll recorded.

#### 4.4.2 Flower Production

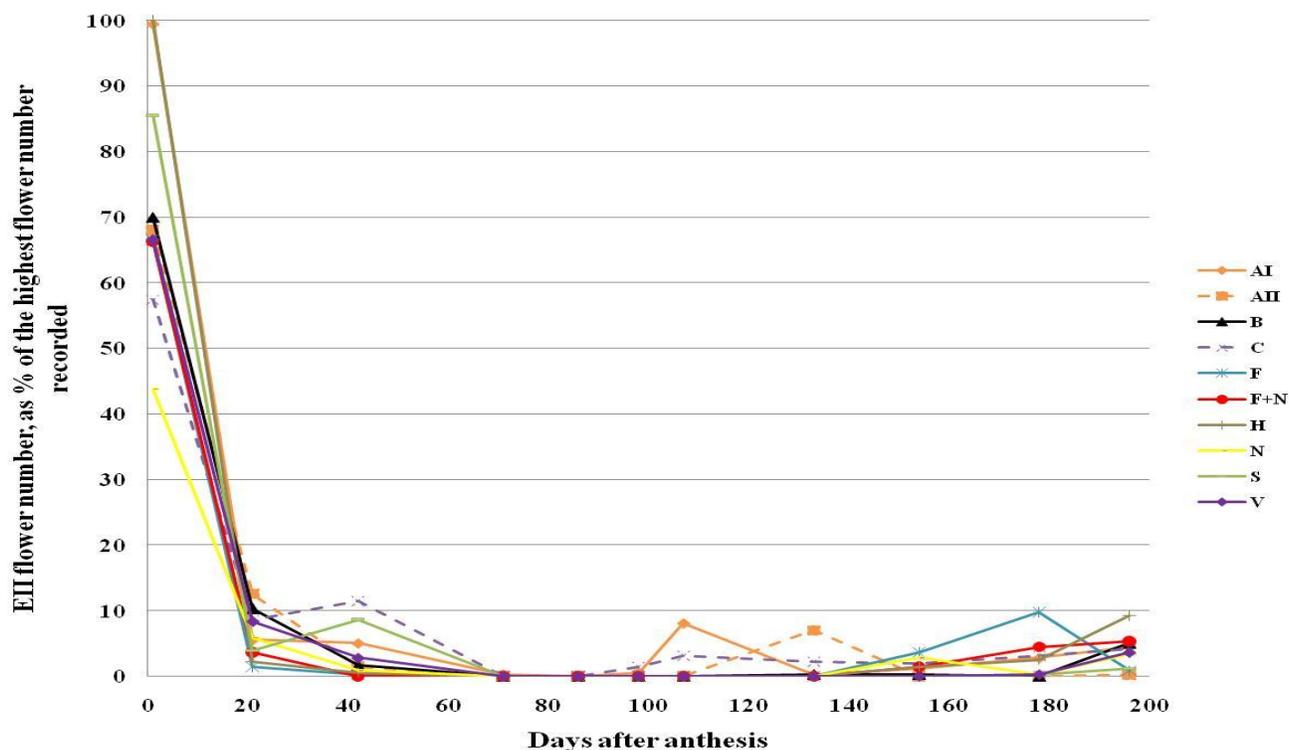
Through the 2011 test the number of flowers significantly increased in trees treated with AVG, AN and AHS as compared to check trees (Fig. 4.4.3) (Appendix C.1). Moreover, 37 days after the AVG treatment profuse leafless multifloral inflorescences were observed and for AN a considerable increase was observed 71 days after the last treatment.  $GA_3$  and  $GA_3+GA_{4/7}$  did inhibit flowering as was expected to happen. Interestingly,  $GA_{4/7}$  did not inhibit flowering as significantly as  $GA_3$ , in fact it had the most flowers compared to the other treatments on day 32 after the last application (Appendix C.1).

During the 2012 study flower content drastically dropped for all treatments during the first three weeks after flower opening (Fig. 4.4.4). AI and AII had a flower increase 36 and 35 days after the last application respectively. This was about the same time it took for the AVG drastic flower increase during EI Appendix C.1).



**Figure 4.4.3 2011 Effect of bioregulator application on flower increase and retention over time after first application.** All but AVG were applied just after petal drop and early fruit development from days 1 to 80 according to their respective recommendations. AVG was applied on day 63, four weeks before first harvest. Quantity presented as % of the highest yielding treatment.

The low flower production throughout 2012 might have been the effect of a long drought that extended from February to June 2012. Rebolledo (2012) reports an acute effect of drought in Tahiti lime abscission. Furthermore the trees included in this experiment were liberally pruned by the main author of this work during February 2011 which might have caused a substantial loss of photosynthesizing material. Drought and pruning were an unhelpful combination for flower production in this experiment.

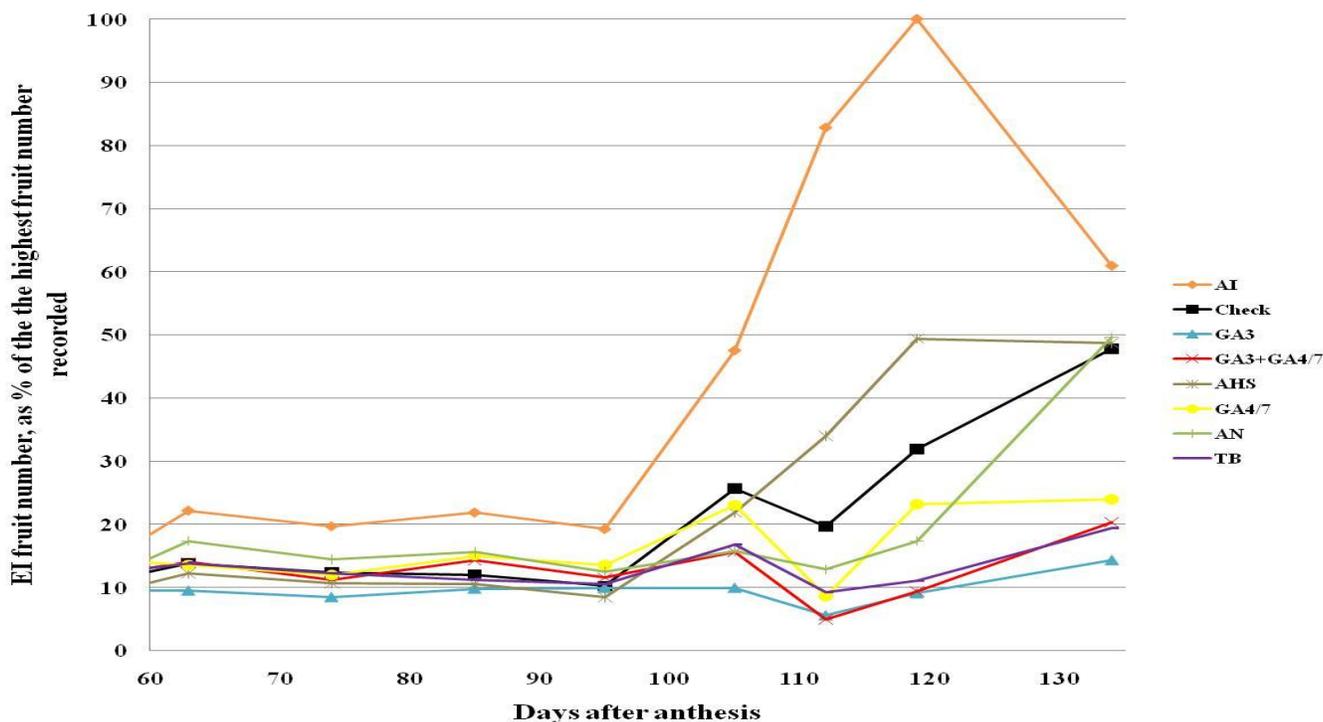


**Figure 4.4.4. 2012 Effect of bioregulator application on flower increase and retention over time after first application.** All but AVG were applied just after petal drop and early fruit development from days 1 to 71 according to their respective recommendations. AVG was applied on day 71 for AI and AII, four weeks before first harvest and applied again on day 98 four weeks before second harvest. Quantity presented as % of the highest flower number.

#### 4.4.3 Fruit Production

In the course of 2011 AVG and AHS treatments had significantly the highest fruit retention, yield and quality during a period of time (49-71 days after initiation of treatments), but fruit abscission rate augmented drastically ten weeks after the last treatment (Fig. 4.4.5) (Appendix C.1). GA<sub>3</sub> treated trees produced the smallest fruit number and size.

While for 2012 AN, AHS and AI had the highest fruit retention until the famous June drop after which all treatments ended with equal fruit retention. These treatments seem to be able to support fruit set during phase I even under drought conditions (Fig. 4.4.6).

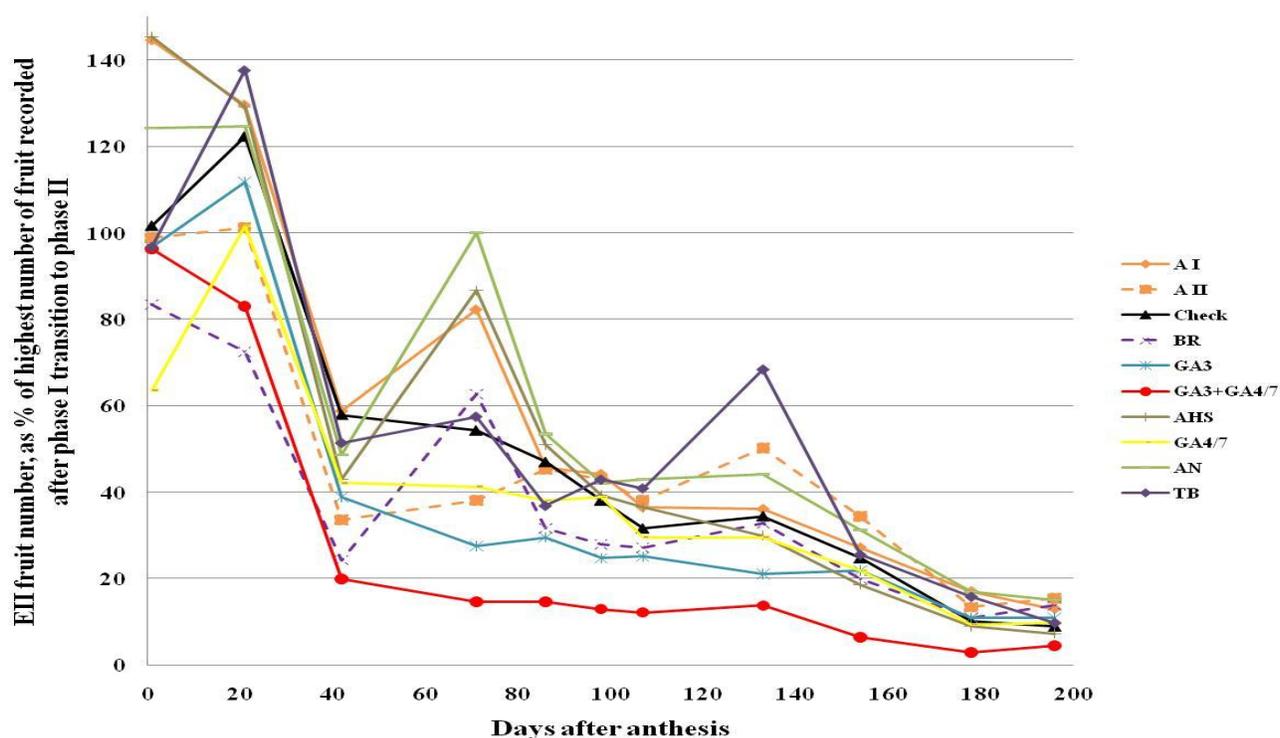


**Figure 4.4.5 2011 Effect of bioregulator application on fruit retention over time.** All but AVG were applied just after petal drop and early fruit development from days 1 to 80 according to their respective recommendations. AVG was applied on day 63, four weeks before first harvest. Quantity presented as % of the highest yielding treatment.

#### 4.4.4 Fruit Yield

In the end of the 2012 study trees treated with AN yielded the highest fruit number followed by AHS and TB.  $GA_{4/7}$  and AI seemed to have a timid effect on yield since they had higher yields than the Check trees for the first two harvests but not as distinctive overall (Fig. 4.4.7). AN trees also yielded the heaviest harvest weight followed by  $GA_{4/7}$ , AHS and TB (Fig. 4.4.8). For individual fruit weight  $GA_{4/7}$  and  $GA_3 + GA_{4/7}$  treatments yielded a significantly higher individual fruit weight and size (Appendix C.1).

The fruit and weight yield curves suggest there are at least two different processes happening. AN, AHS, TB, GA<sub>4/7</sub> and AI curves seem to have a similar tendency among them with the Check curve being in the middle of these and the rest of the treatments having a whole different tendency with a somewhat sigmoidal pattern (Figs. 4.4.7 & 4.4.8).



**Figure 4.4.6. 2012 Effect of bioregulator application on fruit retention over time.** All but AVG were applied just after petal drop and early fruit development from days 1 to 71 according to their respective recommendations. AVG was applied on day 71 for AI and AII, four weeks before first harvest and applied again on day 98 four weeks before second harvest. Quantity presented as % of the highest yielding treatment after the first abscission wave.

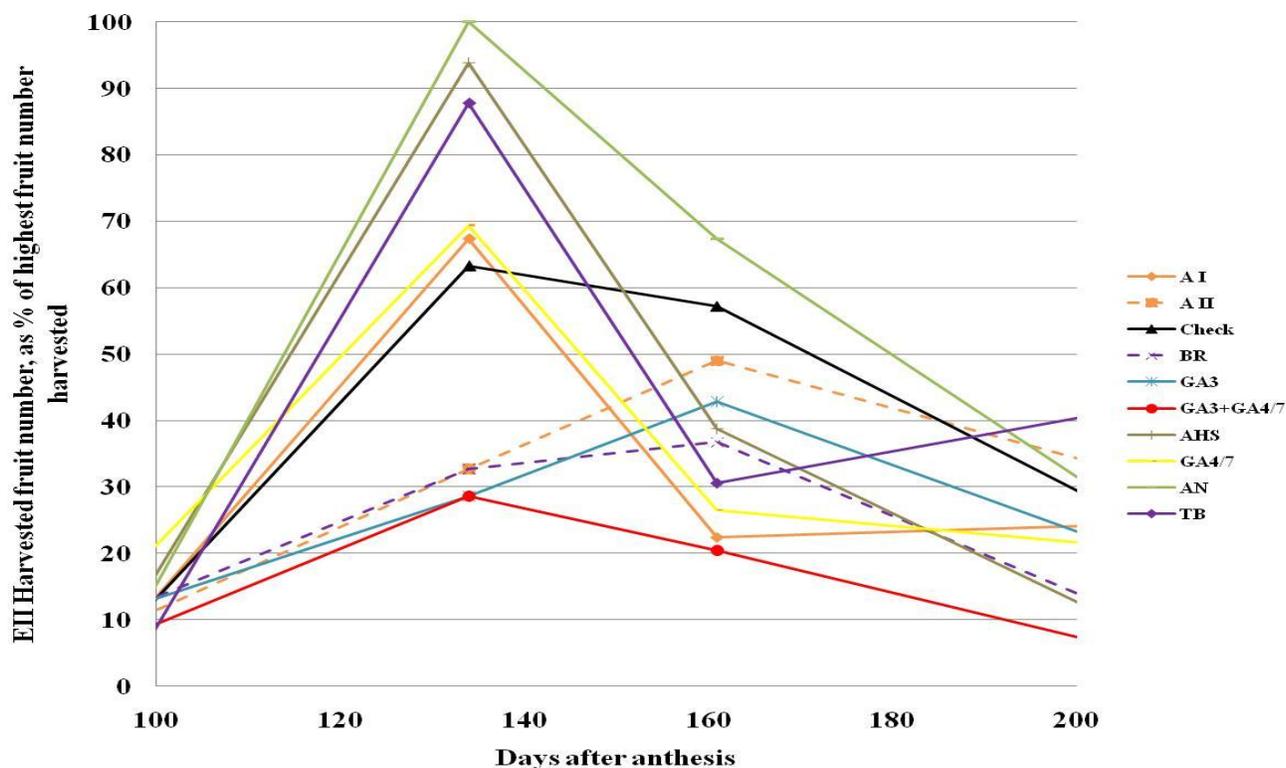


Figure 4.4.7. 2012 Effect of bioregulator application on fruit number yield over time. All but AVG were applied just after petal drop and early fruit development from days 1 to 71 according to their respective recommendations. AVG was applied on day 71 for AI and AII, four weeks before first harvest and applied again on day 98 four weeks before second harvest. Quantity presented as % of the highest yielding treatment.

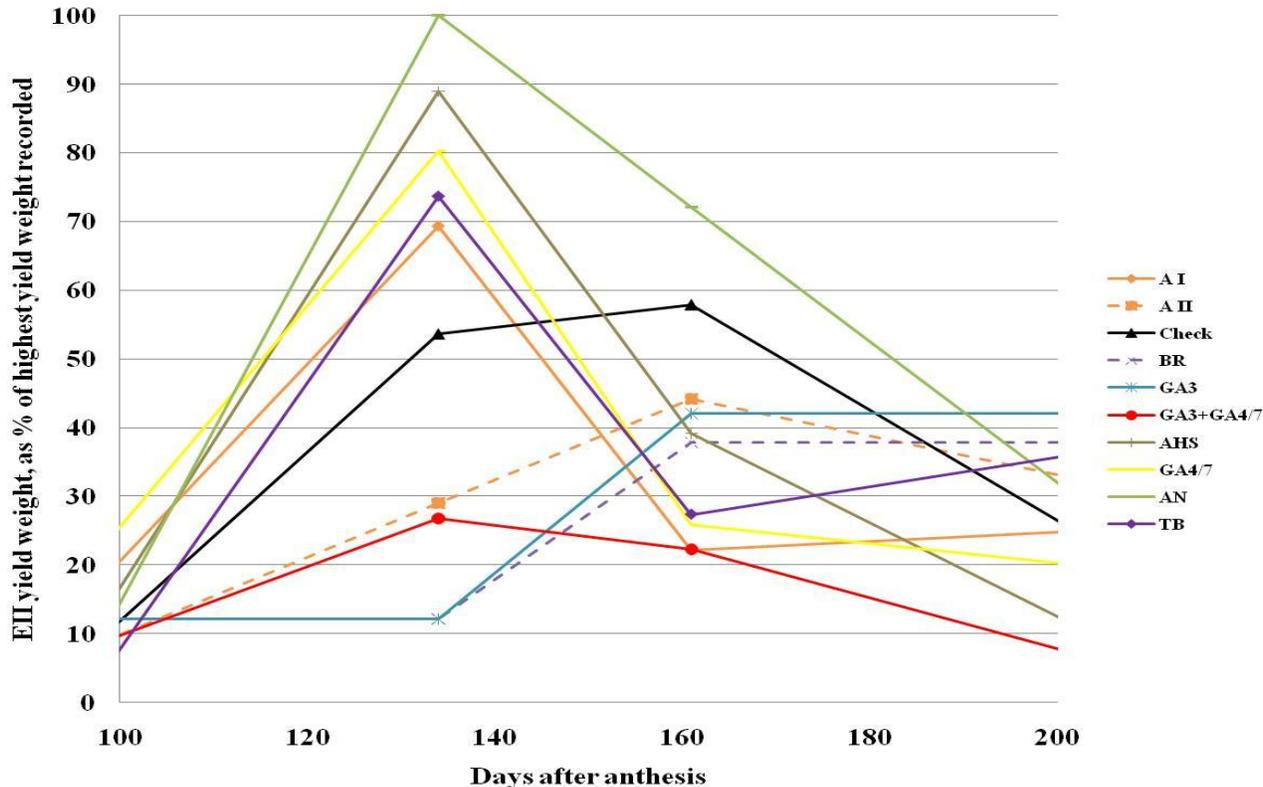


Figure 4.4.8 2012 Effect of bioregulator application on fruit weight yield over time. All but AVG were applied just after petal drop and early fruit development from days 1 to 71 according to their respective recommendations. AVG was applied on day 71 for AI and AII, four weeks before first harvest and applied again on day 98 four weeks before second harvest. Quantity presented as % of the highest yielding treatment.

Overall bioregulators extracted from seaweed or marine juices performed better even during stress conditions as well as the ethylene inhibitor AVG. AN, AVG and AHS gave constant results as higher fruit retention and higher yielding treatments in both years. AN contains kinetin which probably assisted in sink strength and transport of photoassimilates and AHS contains a variety of amino acids (Appendix B) from which proteins and many plant growth factors derive. TB treatments showed a promising effect under drought and low carbohydrate conditions but not as marked as AN, AI and AHS. This might hint into the possibility that AHS amino acids and AN components have the potential of being straightforwardly metabolized into the factors the plant needs to grow without the need of much more photosynthetically produced energy.

TB yield might be related to chlorophyll content since for 2011 chlorophyll concentration was significantly the lowest with fruit retention among the lower treatments through the experiment while for 2012 it was among the highest values for chlorophyll concentration as well as fruit production and final yield. TB and AN significantly augmented chlorophyll content at the end of 2012, TB having the highest concentration (Appendix C.1). Also for day 100 of the 2012 assessment AN and AHS had the lowest chlorophyll content as well as higher abscission rate.

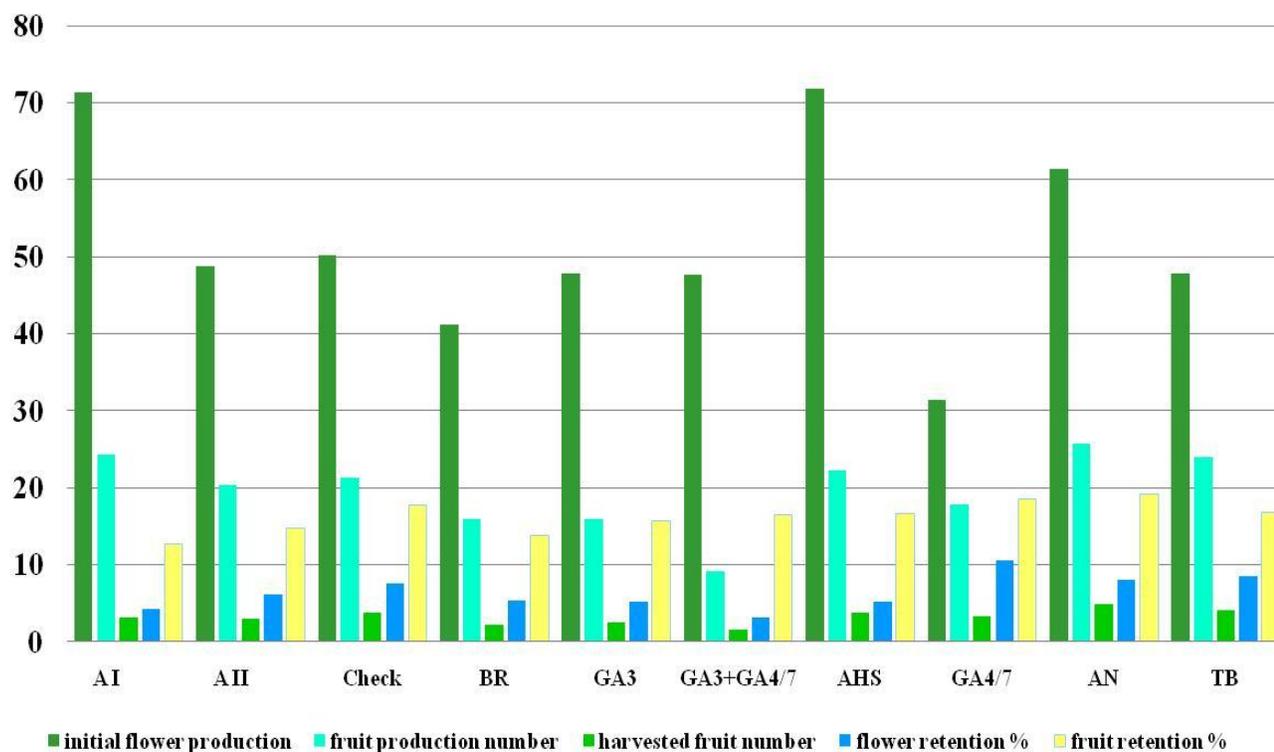
AVG performed better during lower temperatures and high precipitation periods. AVG did not seem to affect chlorophyll content during 2012 which may confirm that the abscission hindrance mode of action during stress is mainly as ACC inhibitor. It is likely that SAM turns into spermidine through another metabolic pathway as reported by Even-Chen et al (1981). Another work by Amri & Shahsavari (2010) shows that exogenous spermidine acts as a growth supporter of *C. aurantifolia* seedlings. This effect might be induced in the tree by AVG application and sustaining, not only ethylene inhibited fruit set but also, a polyamine-mediated fruit quality enhancement of this treatment.

A second AVG application four weeks after the first application had a negative effect on fruit retention, yield and quality. This product may be applied every two to three weeks under tropical conditions as the AVG is photodegraded easily. This plant growth regulator was not applied until four weeks before harvest. In order to assess its effect on fruit retention during phase I, it should

be applied strategically during this stage. If ethylene is the final effector of abscission, AVG should be able to inhibit the first and second abscission waves during these stages.

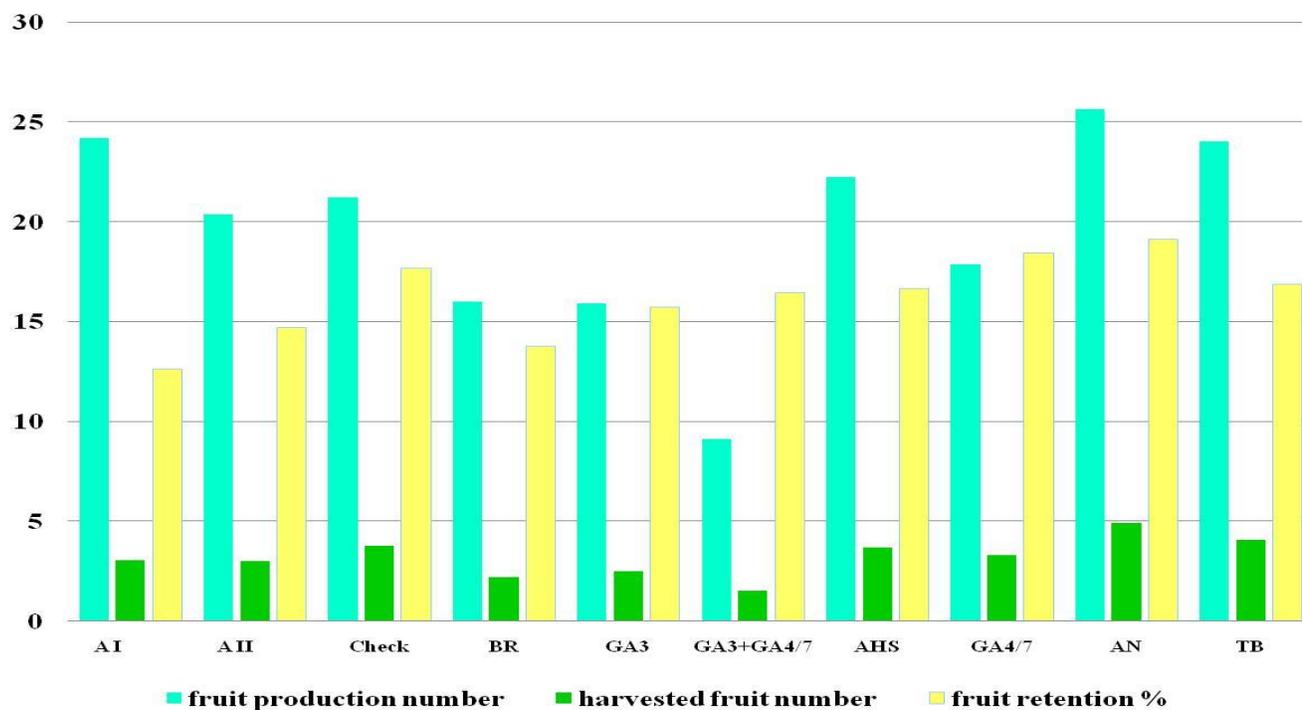
The GAs affected neither fruit retention nor abscission, but did have a positive and significant effect on individual fruit weight and post-harvest quality.  $GA_{4/7}$  might have lowered the first abscission wave by inhibiting flowering but did not affect yield compared to other treatments. Guardiola (2000) mentions that hormone levels in ovaries are associated to parthenocarpic fruit set. Also  $GA_3$  application at flowering is supposed to reduce the magnitude of the peak abscission period right after anthesis during phase I as it enhances fruit set due to initial ovary growth augment. With the huge amount of literature supporting the fact that gibberellins enhance fruit set it has to be concluded that gibberellic acid concentrations should be changed. Either the fruit somehow produces its own gibberellins and the dose should be lessened, or most likely the fruit has a low gibberellin production, as of its lack of seeds, and the dose should be increased. The evidence in this project inclines towards increasing the dose in light of the significant GA induced fruit quality enhancement. In another work by Iglesias et al. (2007) it is mentioned that gibberellin treatment augments fruit set in low gibberellin producing parthenocarpic cultivars, but fruit growth is fully completed to ripening in facultative parthenocarpic cultivars only. In the case of Tahiti lime, a triploid hybrid which only produces parthenocarpic fruit, GA applications might have to be combined with other PGRs to generate a final fruit retention increase. Fruit quality enhancement might have been the result of chlorophyll content increase by these treatments. Fruit quality and post-harvest storage enhancement by pre-harvest plant growth regulation will be discussed in the next chapter.

### Accumulated Ovary Retention



**Figure 4.4.9 Accumulated ovary retention.** AHS, AI and AN had a higher flower production than check trees as well as fruit production with TB. Flower retention percentage was measured dividing total harvested fruit by total flower production and is an indicator of physiological fruit drop. GA4/7 had the higher accumulated ovary retention which means that it hindered physiological fruit drop more than the other treatments. Fruit retention was measured dividing total harvested fruit by total fruit production and is an indicator of 'June drop'. AN had higher fruit retention during 'June drop'.

### Accumulated Fruit Retention



**Figure 4.4.10 Accumulated fruit retention.** Fruit retention was measured dividing total harvested fruit by total fruit production and is an indicator of 'June drop'. AN had higher fruit retention during 'June drop'.

## 4.5 Conclusions

Concordant with Flores et al. (2010) accumulated flower retention was less than 10 percent (Fig. 4.4.8). The main abscission period was during flower bud differentiation which is mainly affected by endogenous hormone levels. AN and GA<sub>4/7</sub> had eventually higher fruit retention percentage than the check trees. This means that, though AI, TB and AHS produced significantly more fruits, only AN and GA<sub>4/7</sub> could retain it through the abscission waves. No treatment was able to significantly reduce physiological fruit drop though, a fact that draws us to conclude that the considerably higher AN, TB and AHS yields might be due to a myriad of metabolic pathway enhancement including sink strength increase and nutrient availability through June drop. Flower retention was higher than 10% for GA<sub>4/7</sub> though, meaning that this treatment might have an effect on physiological fruit drop if applied on higher doses. On the other hand AVG does seem to inhibit the decisive abscission effector ethylene for a period of time and, along with GA<sub>4/7</sub> and AN, enhances fruit quality.

## 5. SELECTED BIOREGULATOR EFFECTS ON ORGANIC TAHITI LIME POST-HARVEST DETERIORATION

### 5.1. Introduction

Lime trees prefer tropical to subtropical climates to grow and produce optimally, but its fresh fruit is widely consumed even in non-tropical climates. FAO statistics show that the main lemon and lime importers are the United States of America and the European Union. This means that after harvest, the fruits have to endure a considerable amount of time in shipping to end at the consumer's table with the optimal quality possible. The duration of Tahiti lime storage and transport can be up to six to eight weeks when stored at 10°C with a relative humidity from 90-95%. A main challenge to improve lime exportation is postharvest technology (Medina-Urritia & Robles-González, 2000).

The storage maximization harvest index, according to Arpaia & Kader (2002), suggests/warrants that the fruit contain a minimum of 30% juice and still maintain a green color. The yellow fruit should be rapidly commercialized. FAO commercialization standard is a minimum of 42% juice and a 42 millimeter size (Ladaniya, 2008). The FAO size code is: (1) 58-67mm, (2) 53-62 mm, (3) 48-57, (4) 45-52mm and (5) 42-49mm.

A variety of growth regulating substances is available that might be used as pre-harvest enhancers of fruit quality, but their effects need further evaluation. However, little has been reported on the effects of bioregulators approved for organic production on the yield and essential oil of citrus in general and lime in particular. Several studies have been published about using gibberellins (GA) and auxin separately or combined as pre-harvest treatments to enhance fruit quality. The effects of other plant bioregulators on citrus seem to have been eclipsed by the reliable outcomes of GA and auxin applications (Guardiola, 2000). Moreover, most of the PGR pre-harvest experiments for fruit yield and quality improvement have been done with orange cultivars (*C. sinensis* Osbeck), and relatively little work has been done with limes, which are

grown in slightly warmer climates than oranges. Tahiti lime produces parthenocarpic fruits, which may drastically change the physiological aspects of fruit set and development and necessitates its own PGR research.

## 5.2 Objective

This section of the project has the intention of assessing the effect of pre-harvest bioregulator applications to fruit quality and storage enhancement.

## 5.3 Materials and Methods

Bioregulators accepted for organic agriculture were foliarly sprayed to Tahiti lime trees during flowering at the AEE-Lajas, to evaluate their effect on fruit and essential oil yield, fruit quality and post harvest deterioration. During the October-December 2011 flowering the evaluated bioregulators were aminoethoxyvinylglycine (AVG, 302 mg per tree, once four weeks before harvest), an *Ascophyllum nodosum* seaweed extract (AN, 0.6 mg eq. kinetin per tree, 4 times every three weeks), a polypeptide, amino acid and alkyl glycerol-triglycerides emulsion from hydrolyzed shark tissues (AHS, 3.4 mg a.i. per tree, 5 times every two weeks), a vitamin B, triacontanol y brasinosteroids plant compost based formulation (TB, 0.04 mg a.i. per tree, 4 times every three weeks), gibberellic acid 3 (GA<sub>3</sub>, 12 mg per tree, two times), gibberellic acid 4/7 (GA<sub>4/7</sub>, 0.67 ng per tree, 5 times), and 2 applications of 24 mg GA<sub>3</sub> + 5 applications de 0.67 ng GA<sub>4/7</sub> per tree.

The experiment was repeated during the April-June, 2012 flowering adding two treatments and a slight augment in gibberellic acid doses: gibberellic acid 3 (GA<sub>3</sub>, 50 mg per tree, 2 times), gibberellic acid 4/7 (GA<sub>4/7</sub>, 3.36 ng per tree, 5 times), and 2 applications of 50 mg GA<sub>3</sub> + 5 applications of 3.36 ng GA<sub>4/7</sub> per tree. The added treatments were a brasinolide application (BR) sprayed at the beginning of flowering and five weeks after and the other treatment consisted of the existent AVG application plus a second one four weeks after the first for a total or ten treatments including the check trees (Check).

For the 2011 experiment fruits were collected from the trees four times at three-week intervals while for 2012 it was at four week intervals. Of each 2011 harvest, a sample of 12 fruits per treatment were used for post-harvest evaluation and 18 fruits for 2012, six for immediate juice quality assessment, six for juice assessment after four weeks of storage and the last six fruits were stored at 10 °C and 85% RH, with their peel color change assessed weekly with a MiniScan XE Hunter Lab colorimeter. Fruit weight loss was also weekly determined during storage. For the sake of uniformity large fruits in this project would fall in the 1 and 2 categories of the FAO code while the medium fruit would be code number 4 and 5. No small fruits were collected.

Four and eight weeks after storage, juice content was determined relating juice weight and fruit weight, and soluble solids content was measured with a r<sup>2</sup> mini Reichert refractometer for 2011 fruit and a Leica refractometer for 2012 fruit. Results were submitted to ANOVA and LSD Fisher tests ( $\alpha=0.05$ ) using InfoStat.

## **5.4 Results and Discussion**

### ***5.4.1 Fruit size and weight***

#### *2011*

GA<sub>3</sub>-treated trees produced smaller fruits than check trees (Fig. 5.3.1). The size decrease in GA<sub>3</sub> treated fruits may be due to competition between increased sink (fruit) numbers. Parthenocarpic fruit set is related to ovary hormone levels (Guardiola, 2000). GA<sub>3</sub> application at flowering increases the ovary initial growth and reduces the physiological abscission during the cell enlargement phase of the fruit development (Rabe, 2000). The retained fruit compete for carbohydrates during the cell enlargement phase, which may be the cause of smaller fruit for the GA<sub>3</sub> treatment. Trees treated with other bioregulators produced fruit of the same size than check trees.

At harvest GA<sub>3</sub> treated trees produced lighter fruit than check trees. Trees treated with other bioregulators produced significantly higher fruit yield than check trees (Appendix D.2). After eight weeks of storage, AN treated fruit had the least weight loss, followed by fruit AVG, TB and AHS.

2012

The 2012 experiment resulted in GA<sub>4/7</sub> having the significantly heaviest individual fruit weight (Fig. 5.3.2) followed by GA<sub>3</sub>+GA<sub>4/7</sub> and AHS for harvest one (HI). For harvest two (HII) GA<sub>4/7</sub> was still the significantly heaviest fruit until the end of the eight week storage while for HIII AN was the significantly heaviest fruit and at the end of the storage GA<sub>4/7</sub> was the lightest fruit (Appendix C.3). In the same manner HII fruit resulted in a significantly larger size for GA<sub>4/7</sub> followed by AHS and TB and for the end of the storage GA<sub>4/7</sub> was still the only significantly larger sized fruit. AN fruit resulted the significantly largest in size for HIII (Appendix C.3). For HIII, GA<sub>4/7</sub> seemed to lose its effect on fruit quality while GA<sub>3</sub>+GA<sub>4/7</sub> lost it for HII (Appendix C.3). For EII, AII and GA<sub>3</sub> fruit were constantly among the smallest and lightest.

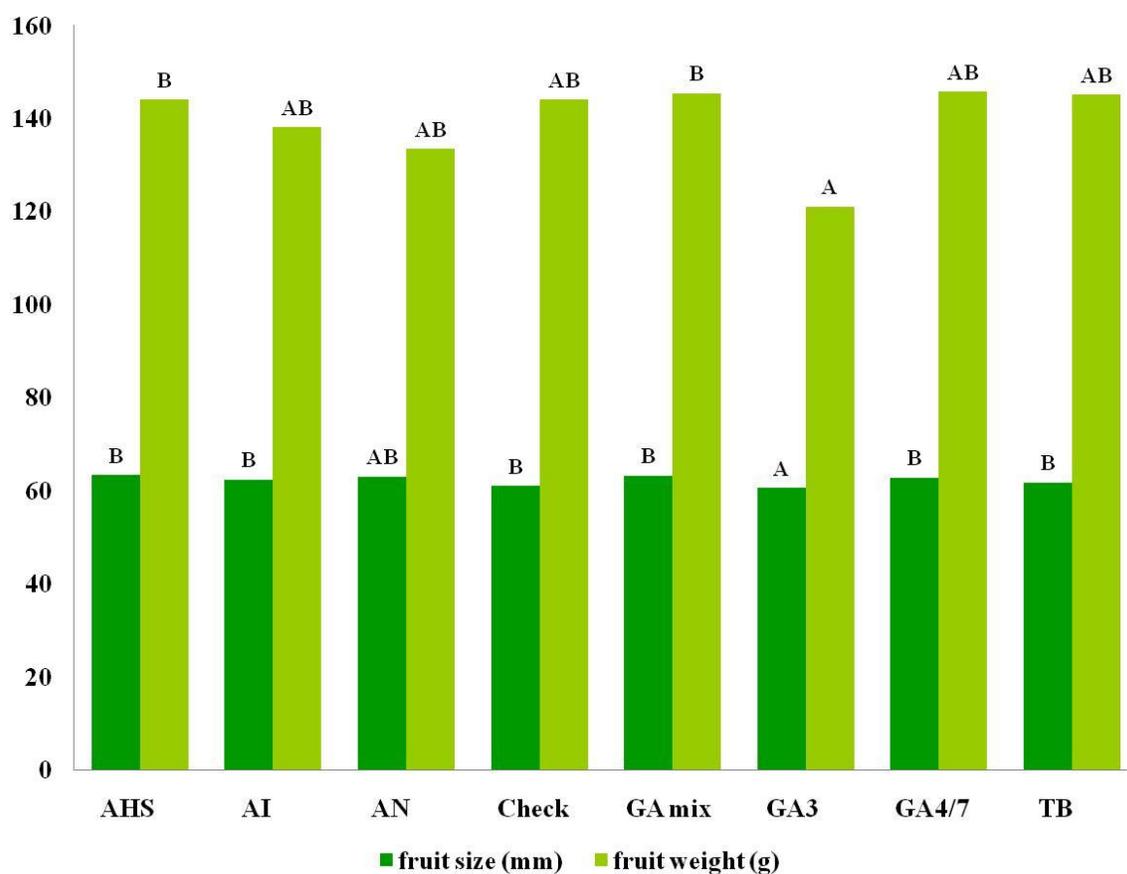


Figure 5.3.1 2011 Individual fruit weight and size

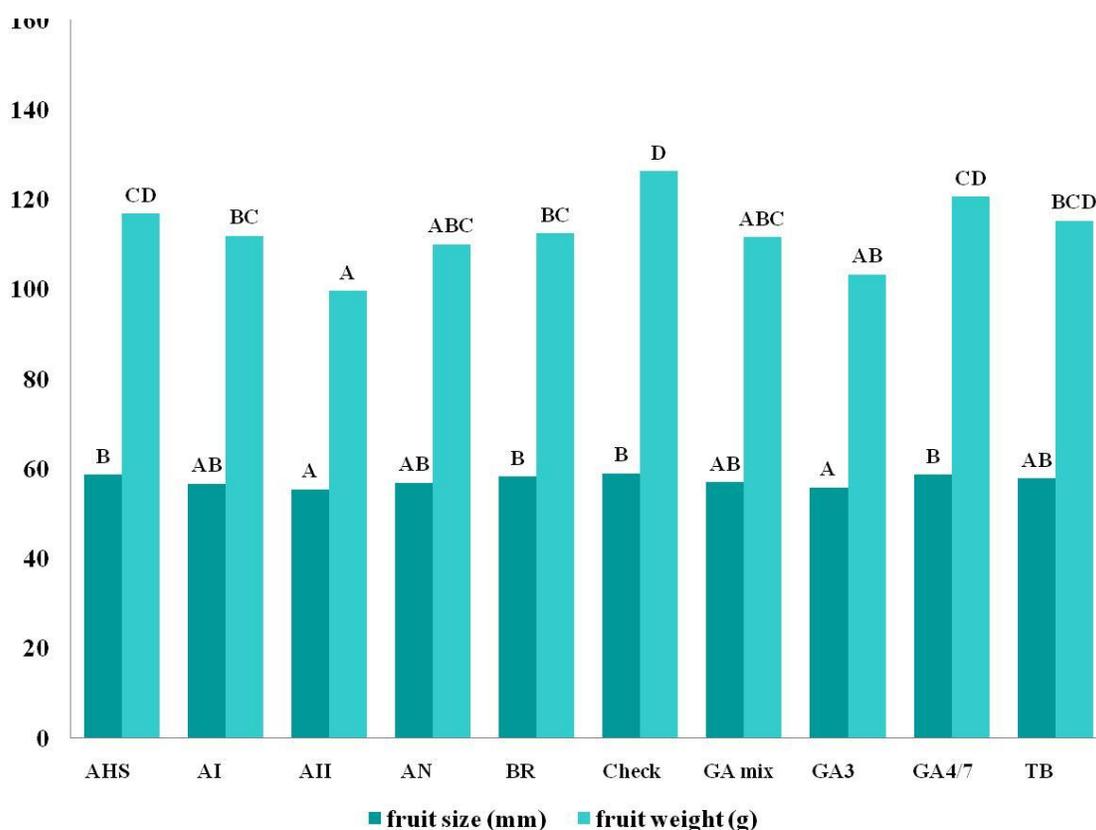


Figure 5.3.2 2012 Individual fruit weight and size

#### 5.4.2 Juice quality attributes

2011

AVG treated fruit had the highest juice content percentage at harvest, followed by GA<sub>4/7</sub> and the GA<sub>3</sub>+GA<sub>4/7</sub> (Fig. 5.3.3). Fruit from the other treatments had juice contents similar to that of the check treatment. After storage, fruit from AVG-treated trees continued to have the highest juice percentage, followed by those of trees treated with AN and GA<sub>4/7</sub>.

Fruit from check, AN-, and AHS-treated trees had a significantly higher acidity (averaging 1.12) as compared to fruit from other treatments (average of 2.02) (Appendix C.3). Moreover, the juice of fruit from check trees and AN- and AHS- treated trees had a significantly lower TSS content as well, although not enough as to affect their brix/acid ratio (BAR).

2012

For HI GA<sub>3</sub>+GA<sub>4/7</sub> fruit had a significantly highest juice percentage followed by GA<sub>4/7</sub>. For HII AN started with the highest juice percentage but ended among the lowest while the AVG treatments had a significantly higher juice content along GA<sub>3</sub>+GA<sub>4/7</sub> towards the end of storage (Fig. 5.3.4). For HIII BR, AII, AN and GA<sub>3</sub>+GA<sub>4/7</sub> fruit had the significantly highest juice percentage (Appendix C.3). Overall GA<sub>4/7</sub>, AII and AHS juice had considerably higher BAR values (Appendix C.3).

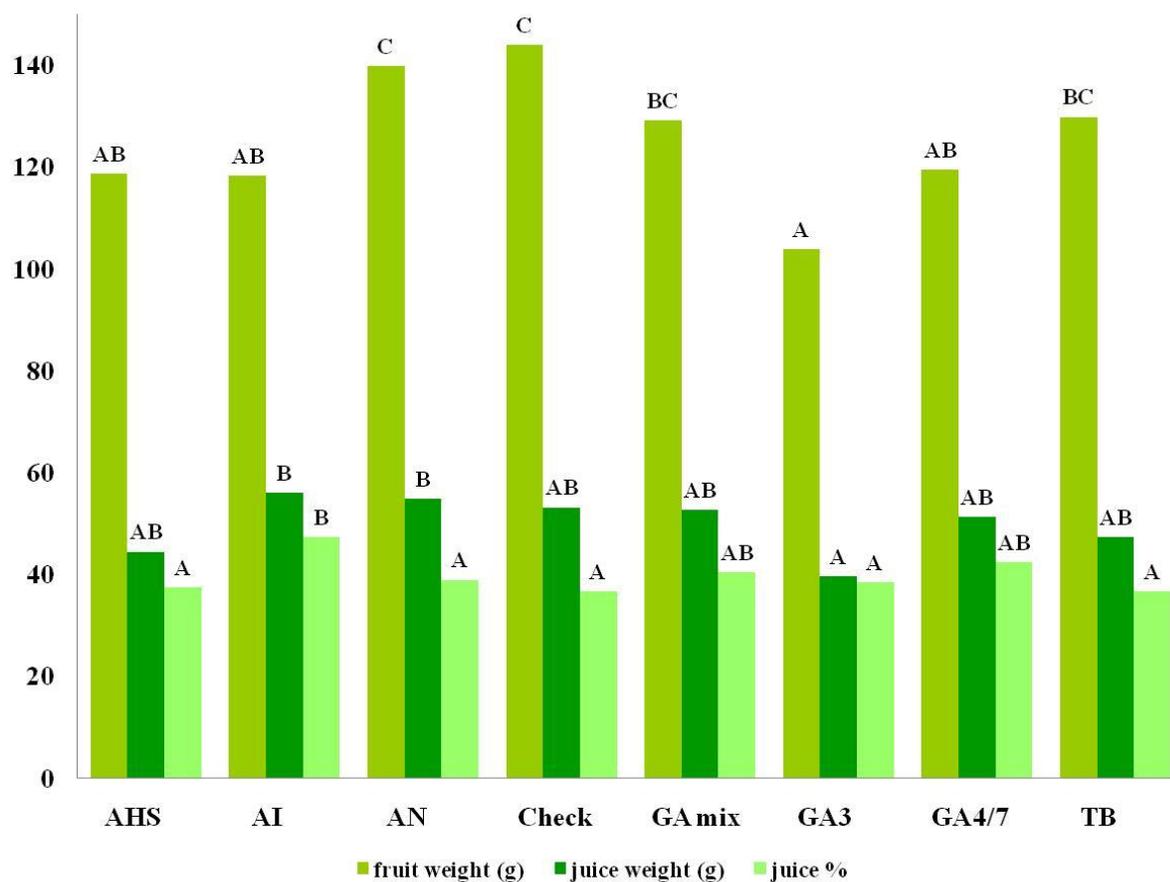
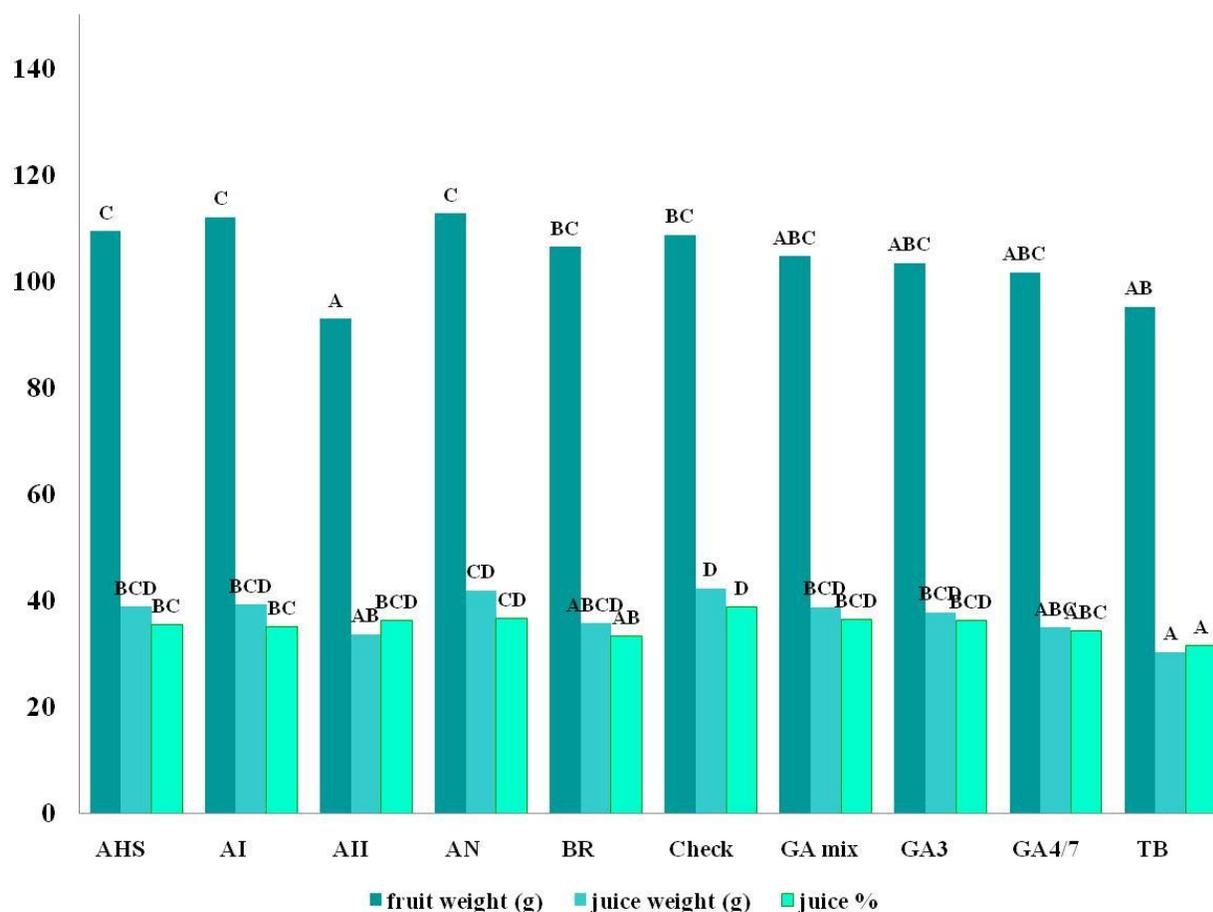


Figure 5.3.3 2011 Individual fruit juice content. Juice % is juice weight as percentage of fruit weight.



**Figure 5.3.4 2012 Individual fruit juice content.** Juice % is juice weight as percentage of fruit weight.

### 5.4.3 Color and appearance

#### 2011

At harvest and the beginning of the storage period, all the fruit peels were the same green color. After four weeks in storage, fruit from GA-treated trees remained the same color as at the beginning of the storage period. In contrast, the peel of fruit from all the other treatments changed color each passing week, becoming more yellow over time.

#### 2012

GA<sub>3</sub> fruit resulted to be greener at the beginning of storage but equalized from week two. GA<sub>4/7</sub> fruit also displayed an extraordinary chilling injury resistance over the other treatments. Fruit

was accidentally submitted to 5° C during a week. All but the GA<sub>4/7</sub> treated fruit had an aggressive chilling injury which made them unacceptable for fresh fruit marketing.

### Control

### GA<sub>4/7</sub> treated



**Figure 5.3.5 2012 Photograph of chilling injured Check treatments vs. GA<sub>4/7</sub> treated fruit after eight weeks of storage.**

## 5.4 Conclusions

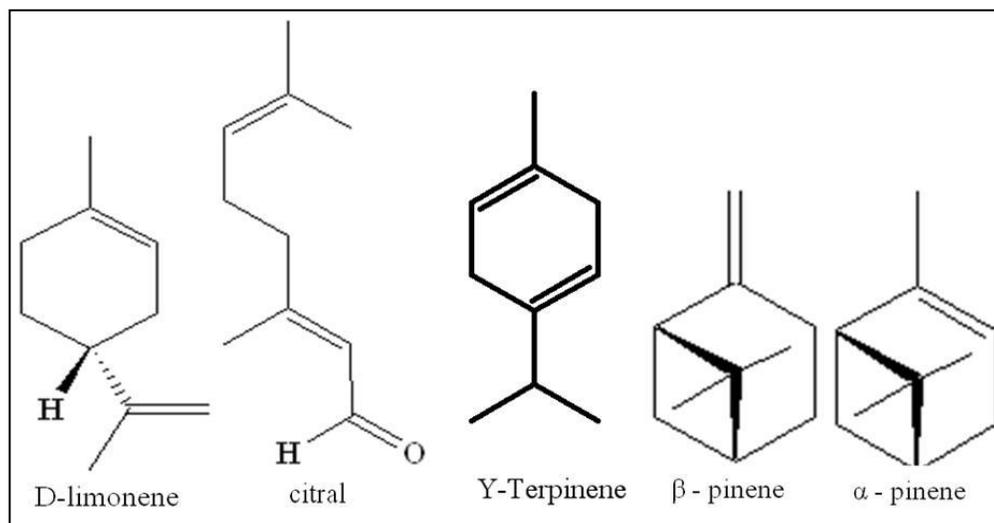
Fruit quality is the outcome of countless physiological pathways occurring from reproductive bud differentiation to fruit storage, being the earlier the most decisive stage over the latter. GA<sub>4/7</sub> proved to be a weight, size, color and juice enhancer while the assessed GA<sub>3</sub> does not seem to have a positive effect on this citrus species' fruit yield nor quality apart of producing greener fruits which might actually be a sign of immaturity, given the small size remarks. One single AVG application eight weeks before harvest had a consistent effect on juice content augmentation. AN and AHS applications effected a lasting and holistic fruit quality as long as water was available to the trees. TB did not seem to regularly affect fruit quality and storage. Pre-harvest application of exogenous plant growth regulators is practical for Tahiti lime post-harvest quality and storage enhancement.

## 6. NEAR INFRARED SPECTROSCOPY AS A NON-DESTRUCTIVE CITRUS ESSENTIAL OIL ASSESSMENT TECHNIQUE

### 6.1. Introduction

Tahiti, Persian or Bearss lime (*Citrus latifolia* Tanaka) is a seedless variety of lime, possibly a hybrid of key lime and citron (*C. aurantiifolia* x *C. medica*). It is commercially found in the USA as a green lime and is slightly larger than key lime. It also has a thicker peel and longer shelf-life than key lime.

Essential oil is a valuable by-product of the citrus industry related with fruit quality. Lime and lemon oil trade is growing due to their increasing use by a wide range of industries; namely, pharmaceutical as the new nutraceutical branch develops (Basu, Thomas & Acharya, 2007), and the traditional use in the perfumery industry and the widening food and beverage industry (Di Giacomo, 2002). The largest essential oil consumer is the soft-drink industry (Brud, 2010). However studies on the pre-harvest factors influencing its quality have been close to absent. A reason of this lack of study might be the complicated and time-consuming analyses used that requires expensive sample processing. Near Infrared spectroscopy (NIRS) can be used as a non-destructive, real time analysis method to evaluate the terpene content in the essential oils in citrus fruits.



**Figure 6.1.1 Terpene chemical structures.** Note the numerous olefinic C-H stretchings.

The important commercial value of the extracted essential oils has led to several spectroscopic studies. Commercial citrus species have been studied by NIR spectroscopy, and by mid-IR spectroscopy using attenuated total reflectance and by NIR-FT Raman Spectroscopy. These previous studies have reported the analysis of extracted citrus oils, however, this study further extends the application of the NIR spectroscopy to intact Tahiti limes.

Near Infrared Spectroscopy (NIRS) can be used as a non-destructive, real time analysis method to evaluate the terpene content in the essential oils in citrus fruits. The C-H stretch bands have confirmed the presence of terpenes in Tahiti lime flavedos analyzed directly without solvent extractions.

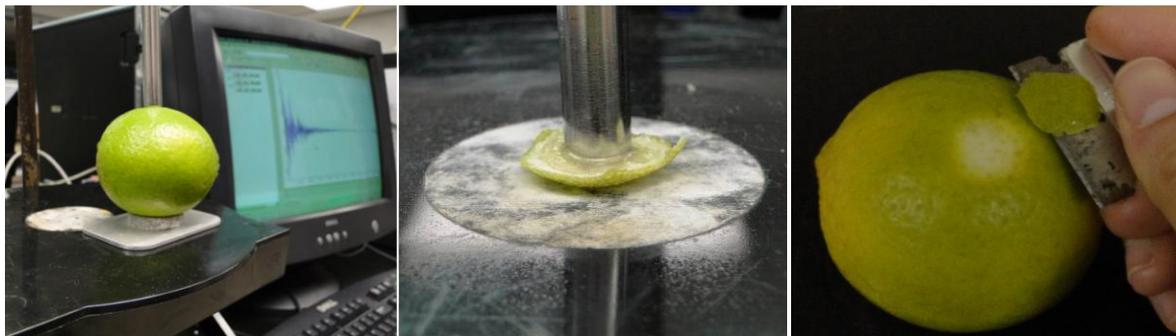
## **6.2 Objectives**

To evaluate Near Infrared Spectroscopy as a non-destructive real-time analysis tool for Citrus essential oil assessment by: 1. discriminating oil-containing tissue from other tissues by NIR spectroscopy, 2. detecting the main absorbance peaks reflected by terpenes and 3. quantifying the terpene concentration in the intact lemon.

## **6.3 Materials and methods**

### ***6.3.1 Tissue discrimination***

Using a Bruker Multi-Purpose Analyzer, six lemons were scanned six times each. The absorbed spectra was in the range of  $15000-4000\text{ cm}^{-1}$  with a  $8\text{ cm}^{-1}$  resolution and 128 sample scan time. The fiber optic probe was always placed in direct contact with the flavedo. The flavedo is the green part of the peel that comes into contact with the fiber optic probe when the intact limes are analyzed. This is followed by the albedo (white section of the peel) and then pulp. The flavedo was removed and isolated, allowing the acquisition of spectra from the flavedo, albedo and pulp. The raw data was pre-treated with SNV and 2<sup>nd</sup> derivative normalizations.



**Figure 6.3.1** Non-destructive NIR analysis on the intact Tahiti lemon, albedo and pulp.

### ***6.3.2 AVG effect on intact lime 6360-5442 $\text{cm}^{-1}$ spectra***

Exogenous AVG effect on Tahiti lime fruit quality was evaluated on April-June 2011. Five year old trees were sprayed with 200 mg/L AVG 69 days before harvest. NIR spectra were obtained from six points along the equatorial line of six fruits.

### ***6.3.3 Fruit size effect on 6360-5442 $\text{cm}^{-1}$ spectra***

Different sized fruit were collected during August 2011 and classified according to weight: large (135-162g), medium (75-100g), small (43-60g), premature (9-17g). Six fruits per size were submitted to NIR spectroscopy on six points on the ecuatorial line.



**Figure 6.3.2** Different sized fruits: large (135-162g), medium (75-100g), small (43-60g), premature (9-17g).

**6.3.4**

### 6.3.4 Terpene main absorbance peak determination

Terpene standard spectra were also obtained and compared with intact flavedo and albedo spectra in order to study the correlation between the terpenes and citrus tissues. Standards >95% pure of D-limonene,  $\alpha$ -pinene,  $\beta$ -pinene, citral,  $\alpha$ -terpinene and linalyl acetate were purchased for NIRS spectra determination. A total of 0.2 ml of the substance was placed into the spectrum cup with a vacuum syringe and spectra were taken three times with the parameters above. Main absorbance peaks were determined for each terpene standard by making a 6360-5442 and 4900-4200  $\text{cm}^{-1}$  exclusion sets and detecting the most intense peaks downwards as would the 2<sup>nd</sup> derivative indicate. Principal Component Analysis (PCA) terpene 1<sup>st</sup> loadings were correlated with intact lemon PCA 1<sup>st</sup> loadings to determine any relationship amongst them. PCA is a chemometric analysis tool where the data is visualized from orthogonal standpoints to reduce any correlation other than a factual association between the analyzed mixture and the substance to detect and quantify on it.



Figure 6.3.3 Terpene standard spectra acquisition for calibration.

### 6.3.5 Understanding diffuse reflectance behavior through pericarp tissue

Theoretically NIR beam penetrates up to 5 mm deep into matter but only the first two millimeters of the surface are detected for diffuse reflectance spectrometry. Stereoscopic grade photography was taken to freshly peeled pericarp to determine depth of tissues and consequently of penetration. In this technique professional photograph cameras are equipped with stereoscopic

magnification lenses which may take pictures of up to 40X of magnification. The pictures are then observed with a calibrated digital picture processor.

Three lime sizes were collected at the AEE – Lajas classified as: large (~140g), medium (~80g), small (~45g). The flavedo and albedo thickness and oil gland depth was determined using stereoscopic magnification photography with calibrated Adobe Photoshop scale to transversal pericarp cuts. In the same manner 12 × 18mm dorsal pictures were taken to flavedo peelings to measure oil gland count and size.



Figure 6.3.4 Stereoscopic magnification photography with calibrated Adobe Photoshop scale.

For analysis the spectrum data was transformed with SNV and 2<sup>nd</sup> derivative and plotted in a PCA with Pirouette software.

## 6.4 Results and Discussion

### 6.4.1 Tissue discrimination

NIR spectra of intact limes, albedo and pulp produced the spectrum shown in Figure 6.4.1. After normalizing the data with an SNV and 2<sup>nd</sup> derivative there is a clear peak differentiation for each of the tissues (Fig. 6.4.2). The most striking feature of this spectrum is the strong band(s) at 6117 cm<sup>-1</sup> indicative of olefinic C-H stretch in the NIR region. The olefinic bands are practically the

same strength or stronger than the C-H aliphatic hydrocarbon bands at  $5920\text{ cm}^{-1}$  and  $5840\text{ cm}^{-1}$ . These peaks agree with previous work by Steuer, Schulz & Läger (2001) where NIR spectra of cold pressed extracted citrus oils have predominant peaks in the  $6119\text{--}5662\text{ cm}^{-1}$  range. These results indicate that the limes have a high olefinic content, as the aliphatic bands are usually stronger because of a higher dipole moment. Figure 6.4.3 shows a clear different tendency for each tissue. This simple experiment clearly confirms that the essential oils reside mainly in the flavedo.

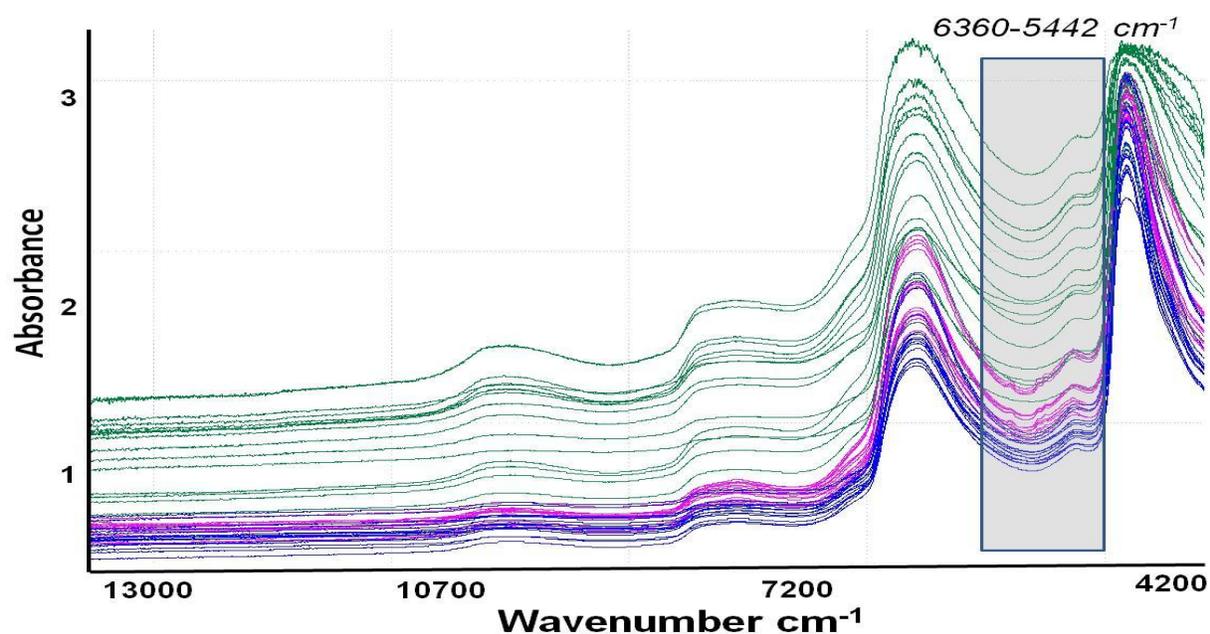


Figure 6.4.1 Raw Spectra of flavedo, albedo and pulp.

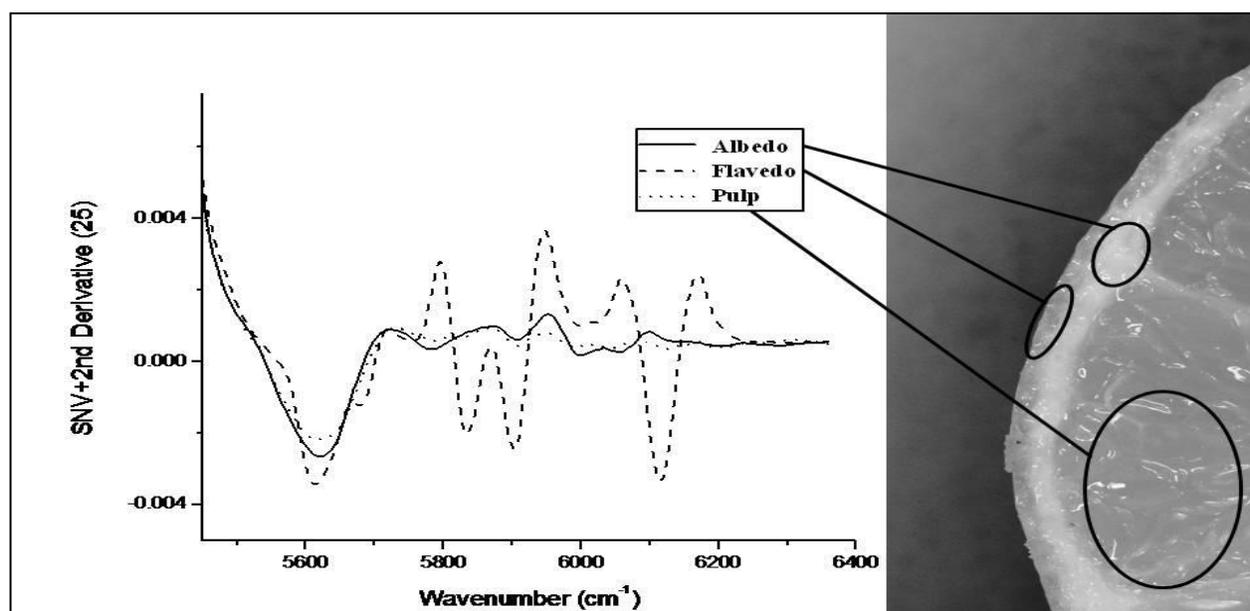


Figure 6.4.2 Standard Normal Variate and Second Derivative of flavedo, albedo and pulp. In a second derivative the most intense peaks are actually lower and viceversa. The substance with the most intense peaks downwards is strongly suggesting a dominance in composition. The flavedo is showing an extraordinary dominance near  $6100\text{ cm}^{-1}$  where bond insaturation (as in terpenes) is expected to reflect.

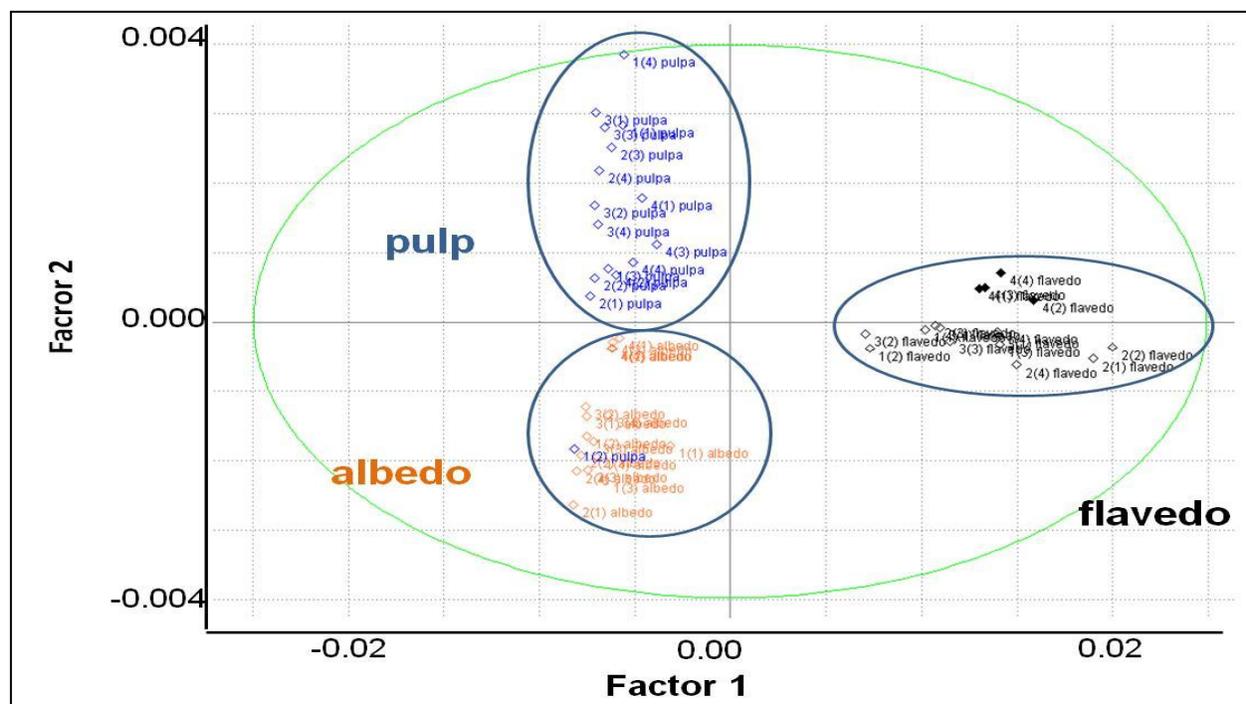


Figure 6.4.3 Principal Component Analysis of pre-treated flavado, albedo and pulp spectra showing clear different tendencies on the factor 1 with factor 2 level.

#### 6.4.2 AVG effect on intact lime 6360-5442 $\text{cm}^{-1}$ spectra

AVG application augmented fruit size and affected the NIR response. A substantial discrimination may be observed in the spectra and the PCA show a considerable difference in tendency for both. This may be indicative of an increase in terpene content since olefinic peaks are represented near  $6100 \text{ cm}^{-1}$  (Figs. 6.4.4 & 6.4.5).

#### 6.4.3 Fruit size effect on 6360-5442 $\text{cm}^{-1}$ spectra

The PCA score plot of different sized fruits show a differentiation among large, small and premature fruits. No difference among large and medium fruits is detected. The AVG difference was probably not due to different size as they were large and medium fruits as well (Fig. 6.4.6).

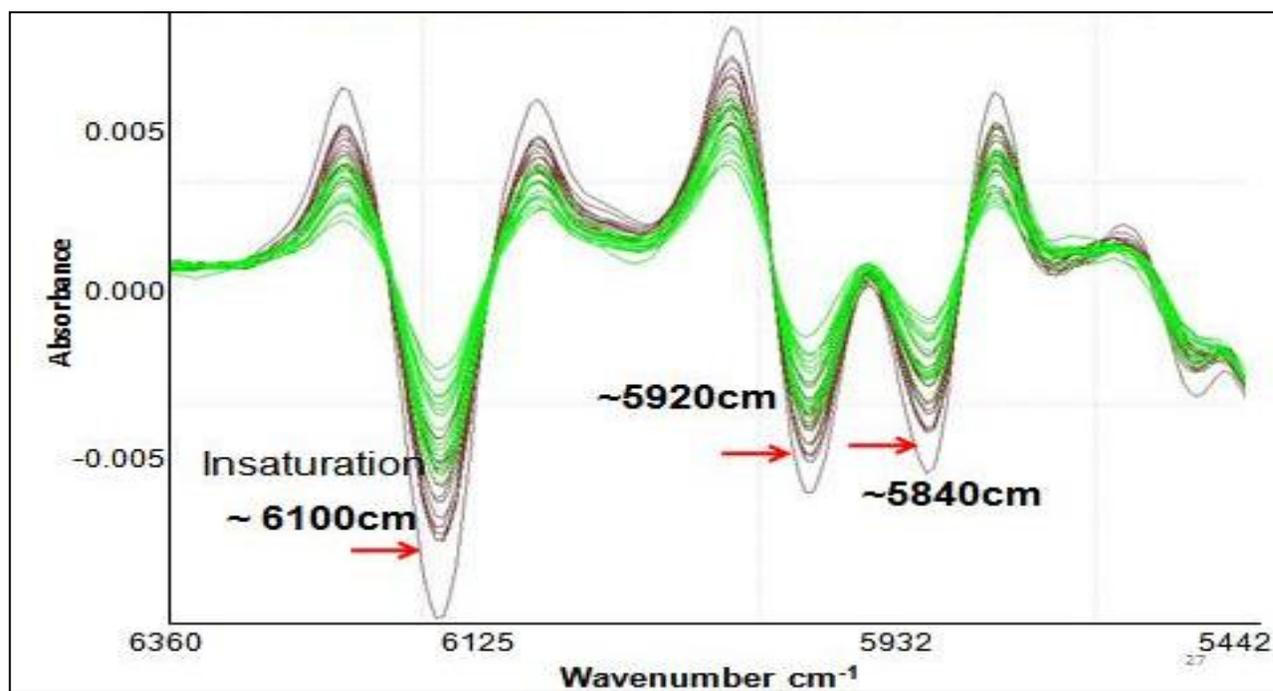


Figure 6.4.4 Second derivative of SNV treated flavedo spectra. AVG treated fruit (brown colored) had a considerable effect on olefinic stretch peaks compared to check trees (green colored) but another trait they had was that they were significantly larger.

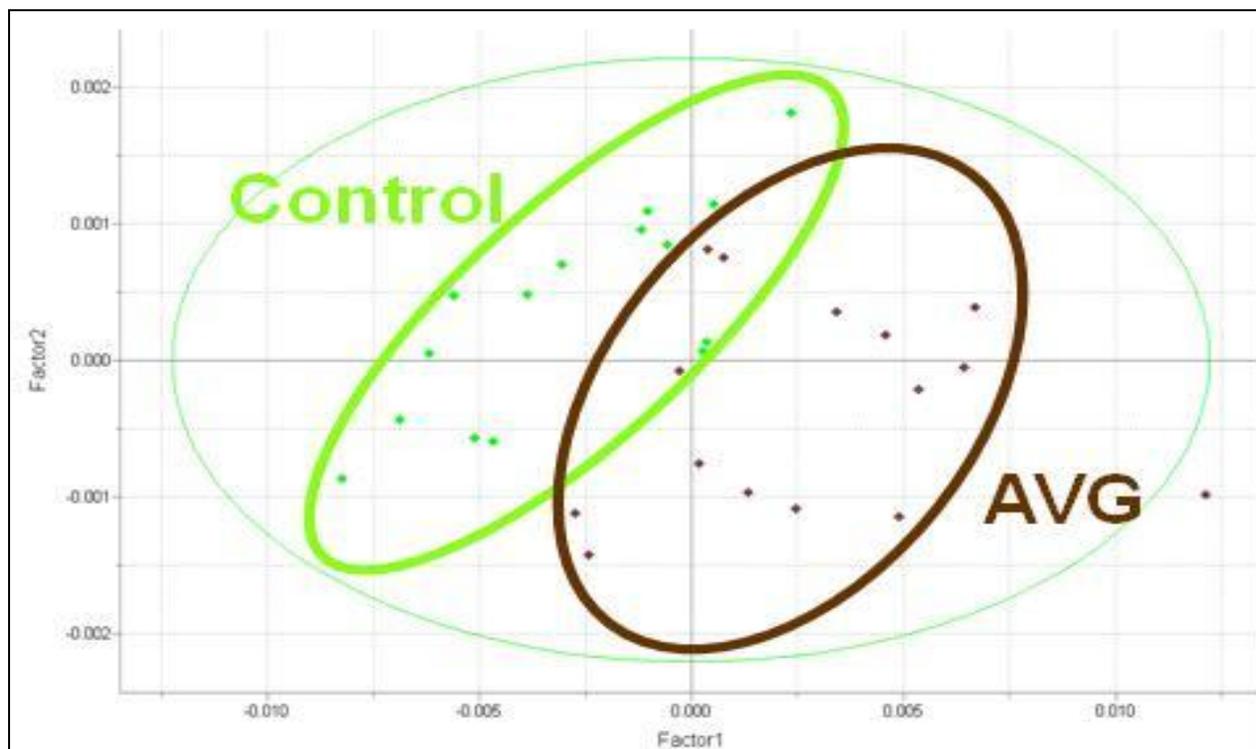


Figure 6.4.5 Principal Component Analysis of AVG treated fruit and Check showing a different tendency on the factor 1 with factor 2 level.

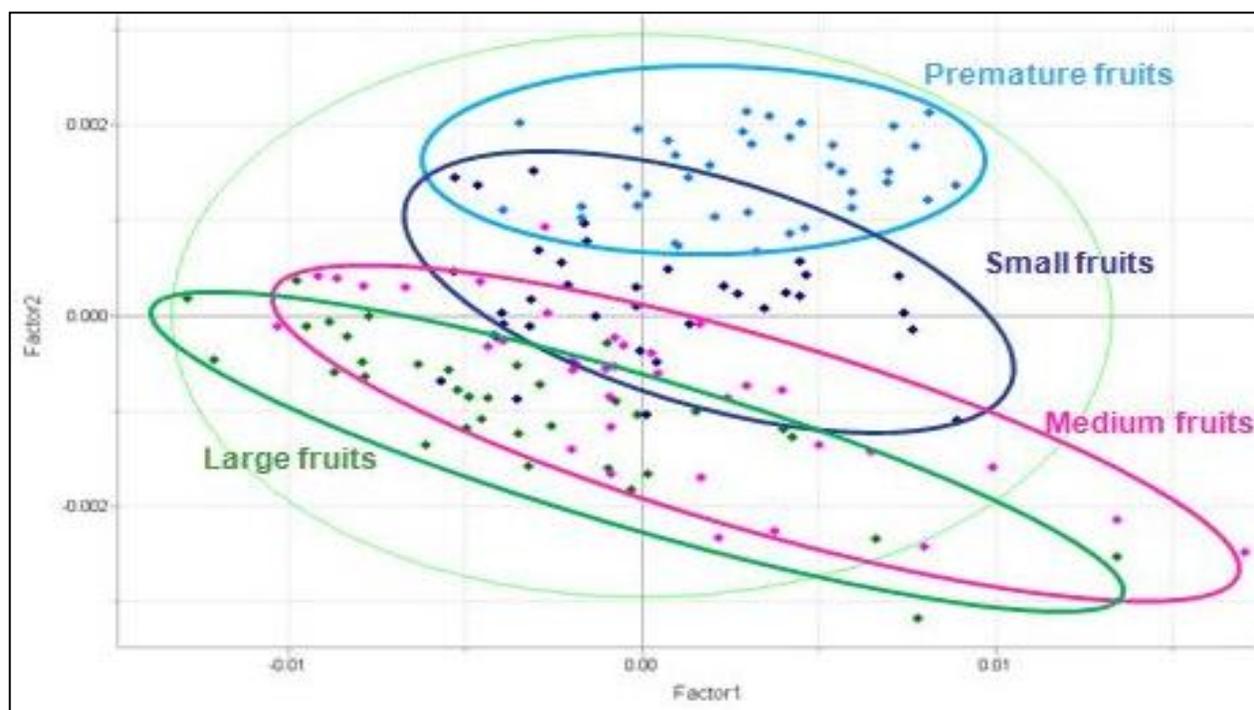


Figure 6.4.6 Principal Component Analysis of different sized fruit show different tendencies.

#### 6.4.4 Terpene main absorbance peak determination

Pure substance characteristic peaks are a tool for chemometric analysis of these in a mixture. Table 6.4.1 shows the predominant peaks in the olefinic bond range. Predominant peaks in intact lemons concur with Steuer, Schulz & Läger (2001) pressed citrus oil values in the 6119-5662

Table 6.4.1 Terpene main absorbance peaks at 6360-5442  $\text{cm}^{-1}$  and 4900-4200  $\text{cm}^{-1}$  exclusion sets. Numbers in bold are the most intense for that given terpene.

Terpinene standard	Predominant peak in 6360-5442 $\text{cm}^{-1}$ range	Predominant peak in 4900-4200 $\text{cm}^{-1}$ range
$\beta$ -pinene	<b>6102</b>	4341
Limonene	<b>6117</b>	4304
Citral	-	<b>4512</b>
$\alpha$ -terpinene	<b>5897</b>	<b>4408</b>
$\alpha$ -pinene	5619	<b>4424</b>

and  $4444\text{-}4255\text{ cm}^{-1}$ . Another fact that confirms intact lemon NIR terpene detection is the high correlation linking intact lemon and terpene standard loadings.

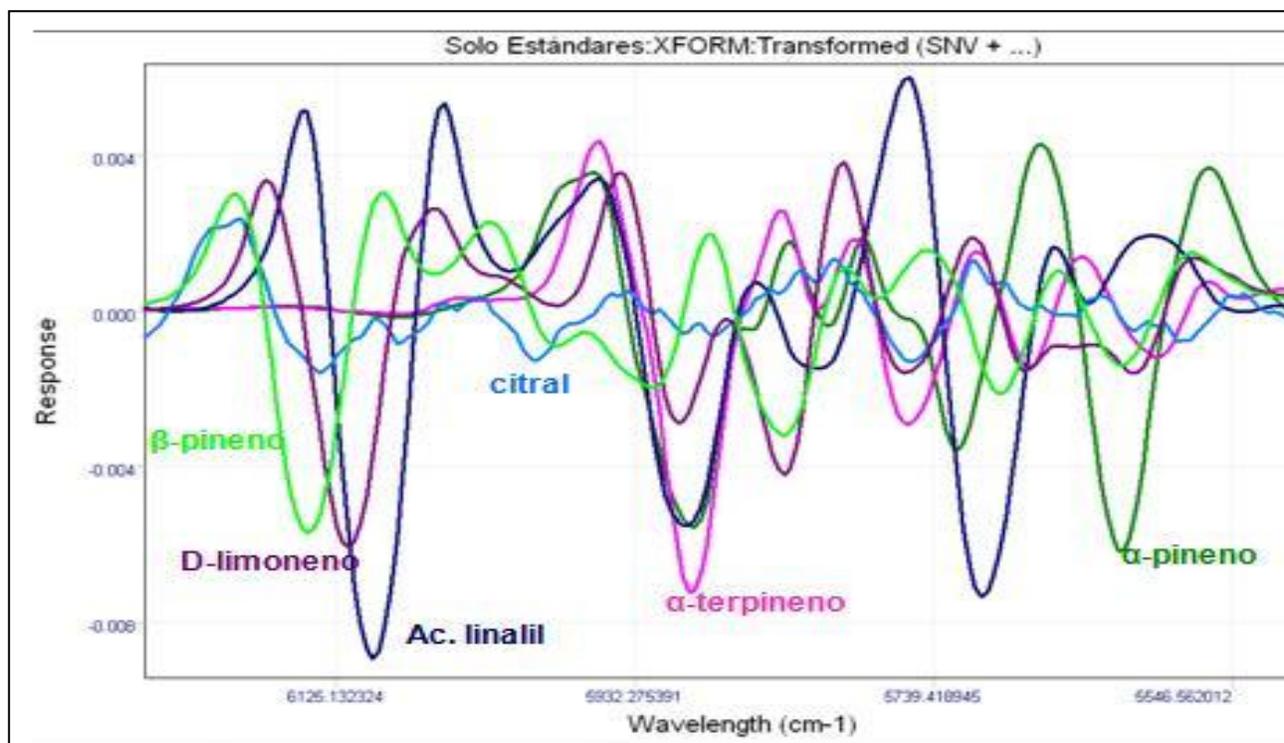


Figure 6.4.7 SNV and 2nd derivative treated terpene standard spectra.

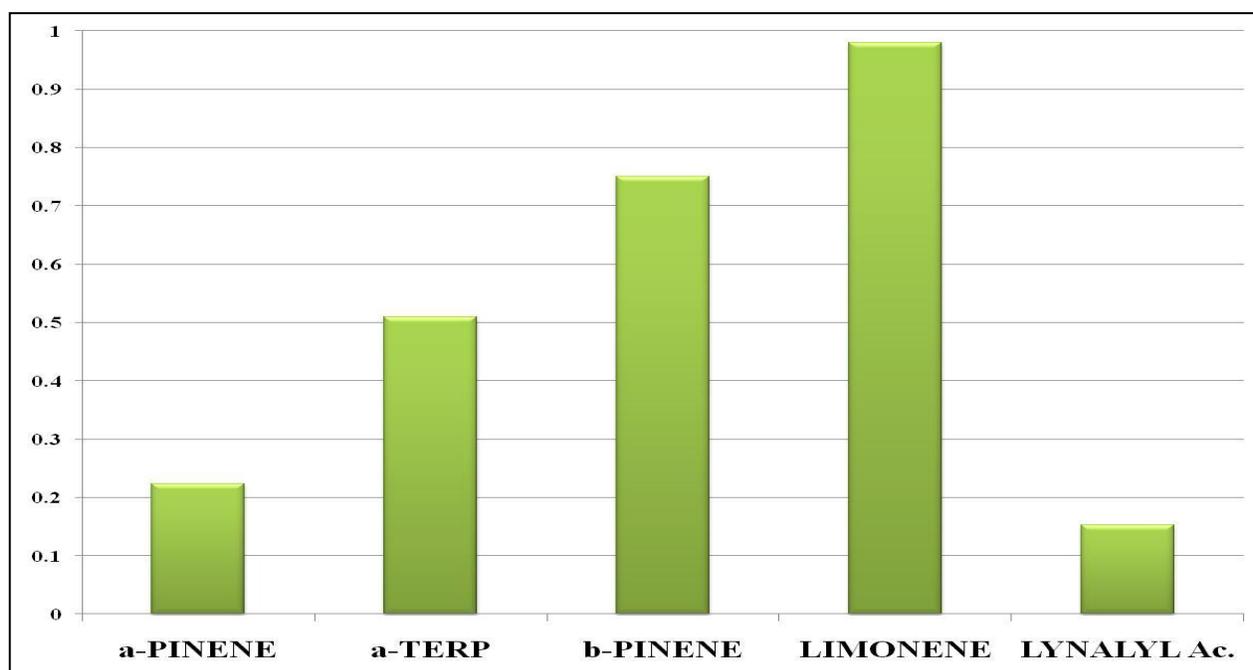


Figure 6.4.8 Terpene standard and intact flavedo correlation of loadings factor 1 values.

### 6.4.5 Understanding diffuse reflectance behavior throughout pericarp tissue

It is clear that tissues of different maturity have different gland spatial arrangement. Figure 6.4.9 shows oil glands are in fact in the flavedo, not crossing to the albedo and there is no significant difference in gland depth. Also gland quantity per mm<sup>2</sup> is obliquely correlated to gland size (Fig. 6.4.10). These last figures may be another important fact to understand diffuse reflectance inside a lemon as to how the light is absorbed, dispersed or simply lost and should be further assessed.

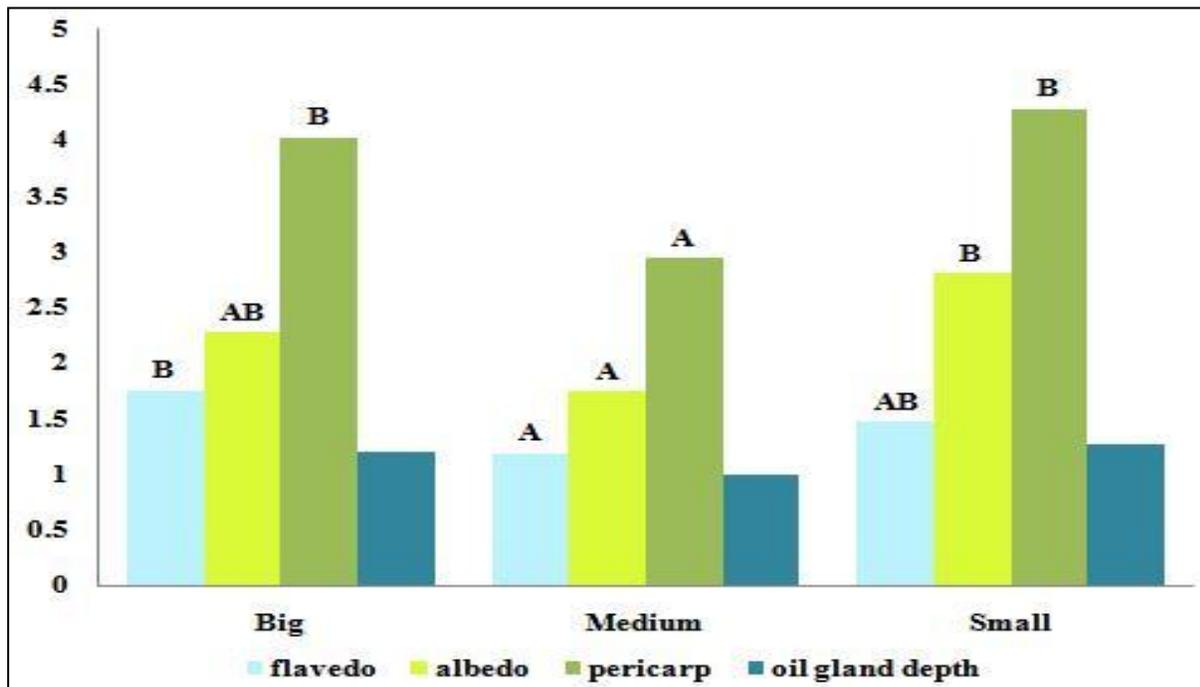


Figure 6.4.9 Bar chart depicting thickness of flavedo, albedo and pericarp on large, medium and small fruits as well as oil gland depth assessed by stereoscopic grade photography of pericarp transversal sections. Different letters indicate value significant difference.

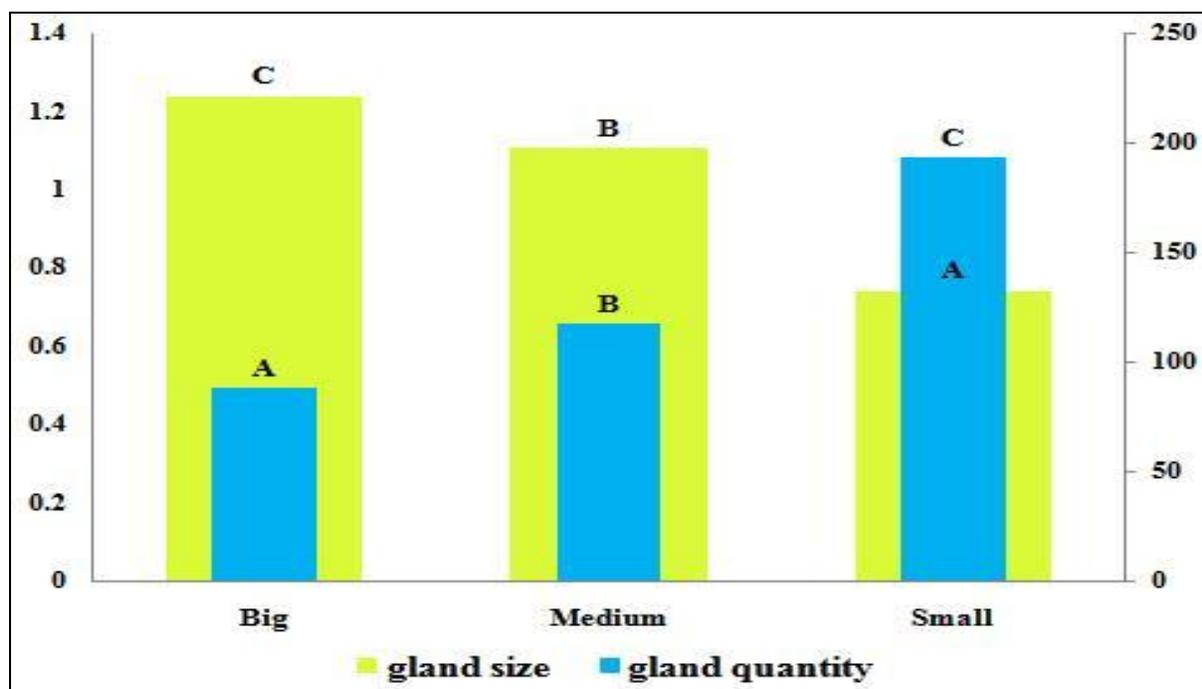


Figure 6.4.10 Bar chart depicting gland size and quantity on large, medium and small fruits assessed by stereoscopic grade photography of pericarp dorsal sections. Different letters indicate value significant difference.

## 6.5 Conclusions

These initial results indicate that NIR spectroscopy is a promising technique for monitoring the growth process of Tahitian limes. NIR diffuse reflectance monitors the flavedo where the essential oils reside and can straightforwardly detect specific terpenes contained on its essential oil without time consuming or expensive sample processing techniques. Furthermore multivariate analysis can be used to quantify certain terpenes by way of a terpene standard calibration model.

## 7. CONCLUSIONS AND RECOMENDATIONS

A fruit retention model could be derived preferably from the EII curve because this experiment was conducted during the main flowering and harvest season and it was assessed from flowering to the fourth harvest while the EI experiment assessment started in an ambiguous stage where there were flowers mixed with fruit. With EII as a model it can be predicted that the first abscission wave starts within the second and fourth week after anthesis and may extend until the seventh week after anthesis for Tahiti lime. This is also the typical cell differentiation period when the ovaries are turned into fruit. The second main abscission wave takes place between the ninth and 17<sup>th</sup> week where fruit is growing. In this experiment a third abscission wave was recorded from week 19 without recuperation.

Harvests were ongoing every month since week 14 and the harvested fruit number was added as part of the fruit retention record. This first collection during week 14 of EII was not considered characteristic because of a small amount of fruit number although it is part of the results here presented and analyzed. The second and third harvests collected during the 19<sup>th</sup> and 23<sup>rd</sup> week respectively were the most characteristic of the experiment showing significant yield and quality differentiations between treatments. For this experiment phase II ended between week 19 and 23 and a considerable and characteristic yield was obtained from day 134 to 161. A fourth harvest was collected on week 30 but it had already lost the bioregulators' effects and was considered non-characteristic as the first one.

For EI fruit could be collected much faster after flowering. A characteristic harvest was picked 110 to 130 days after the start of the experiment though. This might be due to higher water availability for cell enlargement to occur. Final fruit set was higher for EI than for EII and it is most probably due to water availability too. Water accessibility supplies for a faster cell growth and might shorten the June drop period. Data for this fact is not shown because of a lack of record of fruit number for EI, but the difference was too large not to mention.

Fruit retention yield may be augmented using AN, AVG and GA<sub>4/7</sub> though the latter should be further assessed, augmented and/or combined with another fruit-set supporting bioregulator.

These reduced June drop but no evaluated biorregulator proved to significantly reduce physiological fruit drop which is the most forceful abscission wave. In order to reduce physiological drop further evaluations should be made including endogenous GA, ACC and cytokinin level assessment during various points of the tree phenology as well as GA identification specially for an exceptional case as is Tahiti lime. These bioregulator level evaluations may be combined with exogenous applications to estimate their effect on endogenous levels, fruit abscission and production. So far, no literature has been published on this key information.

As overall recommendations *Ascophyllum nodosum* extract is advisable for fresh fruit local marketing specially if there is drought stress. As long as there is water availability AVG might augment fruit and juice yield as well as storage life. Previous works show how cytokinins combined with gibberellins excel in final fruit yield (Guardiola, 2000). For international fruit marketing GA<sub>4/7</sub> showed a promising fruit quality enhancer so it can be combined with AN to enhance yield and quality but research on their combination doses should be expanded for this citrus species. For essential oil production AVG, AHS and gibberellins may increase oil yield by expanding fruit size.

NIR spectroscopy is a promising technique for monitoring the growth process of Tahitian limes. NIR diffuse reflectance monitors the flavedo where the essential oils reside and can straightforwardly detect specific terpenes contained on its essential oil without time consuming nor expensive sample processing techniques. Furthermore multivariate analysis can be used to quantify certain terpenes by way of a terpene standard calibration model. Additional work may be pointed towards understanding diffuse reflectance behavior through *Citrus* tissues among other plants.

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## APPENDIX A. LIST OF ABBREVIATIONS

ABA	abscisic acid
ACC	1-Aminocyclopropane-1-carboxylic acid
ACS	ACC synthase
AHS	Hydrolyzed shark tissues
AN	<i>Ascophyllum nodosum</i> extracts
AVG	Aminoethoxyvinylglycine
AZ A	Abscission zone A, peduncle
AZ C	Abscission zone C, calyx
BAR	Brix/acid ratio
BR	brassinolide formulation
EI	Experiment I
EII	Experiment II
FAO	Food and Agriculture Organization
GA3	Gibberellic acid 3
GA4/7	Gibberellic acid 4/7
HI	Harvest I
HII	Harvest II
HIII	Harvest III
NIR	Near Infra-Red
SAM	<i>S</i> -Adenosyl methionine
TB	Tricontanol with Brassinolide formulation
TSS	Total Soluble Solids

## APPENDIX B. AHS AND TB COMPONENTS

Concentraciones de amino acidos libres y totales en muestras de bioestimulantes analizado por HPLC con detection por fluorescencia

Amino acido	amino acidos libres <sup>1,2,3,5</sup>		amino acido totales <sup>1,2,4,5</sup>	
	TIBURON	ALGA	TIBURON	ALGA
	pmol/ $\mu$ L	pmol/ $\mu$ L	pmol/ $\mu$ L	pmol/ $\mu$ L
ASP	274	ND	337	ND
SER	18	ND	101	ND
GLY	446	ND	611	ND
ARG	259	ND	142	ND
THR	594	ND	594	ND
ALA	154	ND	407	ND
PRO	872	ND	326	ND
CYS	586	ND	1704	ND
VAL	160	ND	125	ND
MET	73	ND	114	ND
LYS	42	ND	706	ND
ILE	219	ND	720	ND
LEU	770	ND	333	ND
PHE	208	ND	264	ND

1) muestras no replicadas; 2) diferencia entre total y libres puede ser a lo heterogeneo de las muestras; 3) extraccion de aa libres fue en 0.1N HCL, sonificado 15 min; 4) extraccion aa totales fue en digestion 6N HCL por 24 h, luego evaporacion alicuota y reconstituir con 0.1N HCL. Todos los analisis fueron por derivatizacion a los correspondientes hidrosilatos

**Ingredients in TB:**

- **Brassinosteroids (including homobrassinolide, dolicholide, homodolicholide, and brassinone).....0.0022%**  
**(0.022mg/ml)**
- **1-triacontanol.....0.013%**  
**(0.13 mg/ml)**
- **Vitamin B1 (thiamin).....0.00035%**  
**(0.35 mg/100g)**
- **Vitamin B2 (riboflavin).....0.00002%**  
**(0.03 mg/100g)**
- **Vitamin B6 (pyridoxine).....0.0015%**  
**(0.15 mg/100g)**

## APPENDIX C. STATISTICAL SUMMARY TABLES

For EII:

B = Check,  
 C=BR,  
 H=AHS,  
 S=AN,  
 F=GA3,  
 N=GA4/7,  
 F+N=GAmix,  
 V=TB

### C.1 ANOVA of Field Evaluations

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
clorofila	280	0.14	0.12	13.38

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	2138.15	7	305.45	6.30	<0.0001
Tratamientos	2138.15	7	305.45	6.30	<0.0001
Error	13192.33	272	48.50		
Total	15330.48	279			

#### Test:LSD Fisher Alfa=0.05 DMS=3.27750

Error: 48.5012 gl: 272

Tratamientos	Medias	n	E.E.			
TB	46.50	35	1.18	A		
Check	49.43	35	1.18	A	B	
GA3+GA4/7	51.93	35	1.18		B	C
AHS	52.20	35	1.18		B	C
AN	52.42	35	1.18		B	C
GA4/7	53.21	35	1.18			C D
AVG	54.83	35	1.18			C D
GA3	55.82	35	1.18			D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
clorofila	450	0.04	0.02	15.94

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	1185.66	9	131.74	2.26	0.0179
tratamiento	1185.66	9	131.74	2.26	0.0179
Error	25692.80	440	58.39		
Total	26878.46	449			

**Test:LSD Fisher Alfa=0.05 DMS=3.16616**

Error: 58.3927 gl: 440

tratamiento	Medias	n	E.E.				
N	45.82	45	1.14	A			
A I	46.07	45	1.14	A	B		
B	46.44	45	1.14	A	B		
C	46.97	45	1.14	A	B		
H	47.05	45	1.14	A	B	C	
A II	48.18	45	1.14	A	B	C	D
S	48.81	45	1.14	A	B	C	D
V	49.11	45	1.14		B	C	D
F	50.19	45	1.14			C	D
F+N	50.61	45	1.14				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
flores	400	0.04	0.02	216.42

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	13083.68	7	1869.10	2.34	0.0240
trat flores	13083.68	7	1869.10	2.34	0.0240
Error	313511.50	392	799.77		
Total	326595.18	399			

**Test:LSD Fisher Alfa=0.05 DMS=11.12000**

Error: 799.7742 gl: 392

trat flores	Medias	n	E.E.				
F	3.90	50	4.00	A			
F+N	5.76	50	4.00	A	B		
V	10.26	50	4.00	A	B	C	
N	12.86	50	4.00	A	B	C	
C	14.94	50	4.00	A	B	C	
H	16.82	50	4.00		B	C	
S	18.86	50	4.00			C	
A	21.14	50	4.00				C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia flores	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
95	flores	40	0.47	0.36	156.97

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	9582.18	7	1368.88	4.11	0.0025
trat flores	9582.18	7	1368.88	4.11	0.0025
Error	10655.20	32	332.98		
Total	20237.38	39			

**Test:LSD Fisher Alfa=0.05 DMS=23.50783**

Error: 332.9750 gl: 32

trat flores	Medias	n	E.E.	
F+N	0.00	5	8.16	A
F	1.20	5	8.16	A
N	2.80	5	8.16	A
S	4.60	5	8.16	A
V	7.60	5	8.16	A
H	11.40	5	8.16	A
C	14.80	5	8.16	A
A	50.60	5	8.16	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia flores	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
134	flores	40	0.33	0.18	96.16

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	37462.80	7	5351.83	2.21	0.0600
trat flores	37462.80	7	5351.83	2.21	0.0600
Error	77573.60	32	2424.18		
Total	115036.40	39			

**Test:LSD Fisher Alfa=0.05 DMS=63.42913**

Error: 2424.1750 gl: 32

trat flores	Medias	n	E.E.	
A	10.20	5	22.02	A
F	16.00	5	22.02	A
F+N	30.80	5	22.02	A
V	49.20	5	22.02	A B
C	58.00	5	22.02	A B
H	66.60	5	22.02	A B
N	67.60	5	22.02	A B
S	111.20	5	22.02	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

dia flores	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
105	flores	40	0.36	0.22	131.43

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	24649.60	7	3521.37	2.55	0.0334
trat flores	24649.60	7	3521.37	2.55	0.0334
Error	44270.80	32	1383.46		
Total	68920.40	39			

**Test:LSD Fisher Alfa=0.05 DMS=47.91710**

Error: 1383.4625 gl: 32

trat flores	Medias	n	E.E.
F	4.00	5	16.63 A
F+N	7.80	5	16.63 A
N	12.60	5	16.63 A
V	13.80	5	16.63 A
S	23.60	5	16.63 A
C	34.60	5	16.63 A
H	46.20	5	16.63 A B
A	83.80	5	16.63 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia flores	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
134	flores	40	0.33	0.18	96.16

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	37462.80	7	5351.83	2.21	0.0600
trat flores	37462.80	7	5351.83	2.21	0.0600
Error	77573.60	32	2424.18		
Total	115036.40	39			

**Test:LSD Fisher Alfa=0.05 DMS=63.42913**

Error: 2424.1750 gl: 32

trat flores	Medias	n	E.E.
A	10.20	5	22.02 A
F	16.00	5	22.02 A
F+N	30.80	5	22.02 A
V	49.20	5	22.02 A B
C	58.00	5	22.02 A B
H	66.60	5	22.02 A B
N	67.60	5	22.02 A B
S	111.20	5	22.02 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia flores	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
95	flores	40	0.47	0.36	156.97

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	9582.18	7	1368.88	4.11	0.0025
trat flores	9582.18	7	1368.88	4.11	0.0025
Error	10655.20	32	332.98		
Total	20237.38	39			

**Test:LSD Fisher Alfa=0.05 DMS=23.50783**

Error: 332.9750 gl: 32

trat flores	Medias	n	E.E.	
F+N	0.00	5	8.16	A
F	1.20	5	8.16	A
N	2.80	5	8.16	A
S	4.60	5	8.16	A
V	7.60	5	8.16	A
H	11.40	5	8.16	A
C	14.80	5	8.16	A
A	50.60	5	8.16	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	65557.19	7	9365.31	8.14	<0.0001
trat frutos	65557.19	7	9365.31	8.14	<0.0001
Error	404989.47	352	1150.54		
Total	470546.66	359			

**Test:LSD Fisher Alfa=0.05 DMS=14.06381**

Error: 1150.5383 gl: 352

trat frutos	Medias	n	E.E.	
F	13.09	45	5.06	A
F+N	17.27	45	5.06	A B
V	17.78	45	5.06	A B
N	22.42	45	5.06	A B
S	25.49	45	5.06	A B
C	28.16	45	5.06	B
H	31.24	45	5.06	B
A	59.27	45	5.06	C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
cantidad de frutos	100	0.10	0.01	76.31

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	2268.93	9	252.10	1.12	0.3570
tratamiento	2268.93	9	252.10	1.12	0.3570
Error	20256.15	90	225.07		
Total	22525.08	99			

**Test:LSD Fisher Alfa=0.05 DMS=13.32904**

Error: 225.0684 gl: 90

tratamiento	Medias	n	E.E.
F+N	9.12	10	4.74 A
F	15.92	10	4.74 A B
C	15.98	10	4.74 A B
N	17.88	10	4.74 A B
A II	20.38	10	4.74 A B
B	21.20	10	4.74 A B
H	22.24	10	4.74 A B
V	24.04	10	4.74 B
A I	24.20	10	4.74 B
S	25.64	10	4.74 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
frutos cosechados	200	0.03	0.00	161.86

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	172.35	9	19.15	0.72	0.6938
tratamiento	172.35	9	19.15	0.72	0.6938
Error	5081.05	190	26.74		
Total	5253.40	199			

**Test:LSD Fisher Alfa=0.05 DMS=3.22569**

Error: 26.7424 gl: 190

tratamiento	Medias	n	E.E.
F+N	1.50	20	1.16 A
C	2.20	20	1.16 A B
F	2.50	20	1.16 A B
A II	3.00	20	1.16 A B
A I	3.05	20	1.16 A B
N	3.30	20	1.16 A B
H	3.70	20	1.16 A B
B	3.75	20	1.16 A B
V	4.05	20	1.16 A B
S	4.90	20	1.16 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso	200	0.03	0.00	161.07

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	1930098.42	9	214455.38	0.72	0.6936
trat	1930098.42	9	214455.38	0.72	0.6936
Error	56884785.68	190	299393.61		
Total	58814884.10	199			

**Test:LSD Fisher Alfa=0.05 DMS=341.30642**

Error: 299393.6088 gl: 190

trat	Medias	n	E.E.		
F+N	164.58	20	122.35	A	
C	236.45	20	122.35	A	B
F	267.95	20	122.35	A	B
A II	298.35	20	122.35	A	B
A I	358.10	20	122.35	A	B
B	370.73	20	122.35	A	B
V	379.83	20	122.35	A	B
H	388.73	20	122.35	A	B
N	391.98	20	122.35	A	B
S	540.38	20	122.35		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**C.2 Análisis de la varianza de Evaluaciones Post-cosecha Experimento I****Peso individual****Análisis de la varianza**

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
10	peso	45	0.36	0.24	14.13

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6901.74	7	985.96	3.02	0.0129
trat peso	6901.74	7	985.96	3.02	0.0129
Error	12075.62	37	326.37		
Total	18977.35	44			

**Test:LSD Fisher Alfa=0.05 DMS=22.06928**

Error: 326.3680 gl: 37

trat peso	Medias	n	E.E.		
FLO	99.50	6	7.38	A	
STX	121.12	6	7.38		B
HSK	129.32	6	7.38		B
AVG	132.17	6	7.38		B
CONTROL	133.55	6	7.38		B
NVB	134.15	6	7.38		B
VTZ	138.45	6	7.38		B
F+N	141.70	3	10.43		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
17	peso	45	0.37	0.25	14.35

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	7045.08	7	1006.44	3.13	0.0107
trat peso	7045.08	7	1006.44	3.13	0.0107
Error	11896.86	37	321.54		
Total	18941.95	44			

**Test:LSD Fisher Alfa=0.05 DMS=21.90533**

Error: 321.5368 gl: 37

trat peso	Medias	n	E.E.
FLO	96.47	6	7.32 A
STX	118.57	6	7.32 B
HSK	125.93	6	7.32 B
NVB	129.63	6	7.32 B
AVG	129.77	6	7.32 B
CONTROL	130.58	6	7.32 B
VTZ	135.92	6	7.32 B
F+N	140.70	3	10.35 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
2	peso	45	0.36	0.24	13.87

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6930.72	7	990.10	2.97	0.0142
trat peso	6930.72	7	990.10	2.97	0.0142
Error	12333.66	37	333.34		
Total	19264.38	44			

**Test:LSD Fisher Alfa=0.05 DMS=22.30383**

Error: 333.3422 gl: 37

trat peso	Medias	n	E.E.
FLO	103.42	6	7.45 A
STX	124.52	6	7.45 A B
HSK	133.28	6	7.45 B
AVG	135.48	6	7.45 B
CONTROL	136.55	6	7.45 B
NVB	138.83	6	7.45 B
VTZ	141.87	6	7.45 B
F+N	146.47	3	10.54 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
28	peso	45	0.37	0.25	14.87

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	6892.77	7	984.68	3.07	0.0119
trat peso	6892.77	7	984.68	3.07	0.0119
Error	11867.09	37	320.73		
Total	18759.86	44			

#### Test:LSD Fisher Alfa=0.05 DMS=21.87789

Error: 320.7320 gl: 37

trat peso	Medias	n	E.E.	
FLO	91.72	6	7.31	A
STX	114.28	6	7.31	B
HSK	122.02	6	7.31	B
CONTROL	125.50	6	7.31	B
AVG	125.90	6	7.31	B
NVB	126.60	6	7.31	B
VTZ	131.10	6	7.31	B
F+N	132.80	3	10.34	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
37	peso	45	0.37	0.25	15.19

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	6835.06	7	976.44	3.06	0.0121
trat peso	6835.06	7	976.44	3.06	0.0121
Error	11810.42	37	319.20		
Total	18645.48	44			

#### Test:LSD Fisher Alfa=0.05 DMS=21.82559

Error: 319.2004 gl: 37

trat peso	Medias	n	E.E.	
FLO	88.82	6	7.29	A
STX	112.05	6	7.29	B
HSK	119.10	6	7.29	B
CONTROL	121.88	6	7.29	B
AVG	123.45	6	7.29	B
NVB	123.78	6	7.29	B
VTZ	128.07	6	7.29	B
F+N	130.03	3	10.32	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
44	peso	45	0.37	0.25	15.31

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6646.25	7	949.46	3.05	0.0123
trat peso	6646.25	7	949.46	3.05	0.0123
Error	11515.94	37	311.24		
Total	18162.19	44			

**Test:LSD Fisher Alfa=0.05 DMS=21.55178**

Error: 311.2416 gl: 37

trat peso	Medias	n	E.E.	
FLO	86.78	6	7.20	A
STX	109.97	6	7.20	B
HSK	116.53	6	7.20	B
CONTROL	119.58	6	7.20	B
AVG	121.07	6	7.20	B
NVB	121.12	6	7.20	B
VTZ	125.78	6	7.20	B
F+N	127.07	3	10.19	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
53	peso	45	0.37	0.25	15.38

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6560.72	7	937.25	3.10	0.0112
trat peso	6560.72	7	937.25	3.10	0.0112
Error	11173.91	37	302.00		
Total	17734.63	44			

**Test:LSD Fisher Alfa=0.05 DMS=21.22932**

Error: 301.9975 gl: 37

trat peso	Medias	n	E.E.	
FLO	84.67	6	7.09	A
STX	108.03	6	7.09	B
HSK	113.72	6	7.09	B
CONTROL	117.37	6	7.09	B
NVB	118.55	6	7.09	B
AVG	119.12	6	7.09	B
VTZ	123.88	6	7.09	B
F+N	123.93	3	10.03	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dias	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
63	peso	45	0.37	0.26	15.49

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	6430.11	7	918.59	3.16	0.0101
trat peso	6430.11	7	918.59	3.16	0.0101
Error	10743.07	37	290.35		
Total	17173.18	44			

#### Test:LSD Fisher Alfa=0.05 DMS=20.81601

Error: 290.3531 gl: 37

trat peso	Medias	n	E.E.	
FLO	82.15	6	6.96	A
STX	104.80	6	6.96	B
HSK	110.32	6	6.96	B
CONTROL	114.53	6	6.96	B
NVB	115.35	6	6.96	B
AVG	116.33	6	6.96	B
F+N	120.27	3	9.84	B
VTZ	121.27	6	6.96	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

#### dia 1 peso

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 1 peso	48	0.21	0.08	10.87

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	2357.37	7	336.77	1.56	0.1758
trat peso	2357.37	7	336.77	1.56	0.1758
Error	8638.01	40	215.95		
Total	10995.38	47			

#### Test:LSD Fisher Alfa=0.05 DMS=17.14741

Error: 215.9503 gl: 40

trat peso	Medias	n	E.E.	
GA3	118.28	6	6.00	A
Check	133.42	6	6.00	A B
GA mix	133.67	6	6.00	A B
GA4/7	137.18	6	6.00	B
AHS	137.92	6	6.00	B
AVG	138.05	6	6.00	B
TB	139.42	6	6.00	B
AN	143.20	6	6.00	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 11 peso**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 11 peso	48	0.25	0.11	11.08

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	2759.23	7	394.18	1.86	0.1026
trat peso	2759.23	7	394.18	1.86	0.1026
Error	8484.20	40	212.11		
Total	11243.43	47			

**Test:LSD Fisher Alfa=0.05 DMS=16.99407**

Error: 212.1050 gl: 40

trat peso	Medias	n	E.E.
GA3	113.02	6	5.95 A
GA mix	128.58	6	5.95 A B
Check	131.42	6	5.95 B
GA4/7	132.73	6	5.95 B
AHS	134.67	6	5.95 B
AVG	136.33	6	5.95 B
TB	136.42	6	5.95 B
AN	138.62	6	5.95 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 18 peso**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 18 peso	48	0.26	0.13	11.34

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	3009.64	7	429.95	1.99	0.0804
trat peso	3009.64	7	429.95	1.99	0.0804
Error	8635.78	40	215.89		
Total	11645.42	47			

**Test:LSD Fisher Alfa=0.05 DMS=17.14520**

Error: 215.8944 gl: 40

trat peso	Medias	n	E.E.
GA3	110.77	6	6.00 A
GA mix	125.67	6	6.00 A B
Check	130.10	6	6.00 B
GA4/7	130.30	6	6.00 B
AHS	132.27	6	6.00 B
TB	135.03	6	6.00 B
AVG	135.25	6	6.00 B
AN	137.48	6	6.00 B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 25 peso**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 25 peso	48	0.27	0.14	11.35

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	3102.79	7	443.26	2.11	0.0642
trat peso	3102.79	7	443.26	2.11	0.0642
Error	8388.73	40	209.72		
Total	11491.51	47			

**Test:LSD Fisher Alfa=0.05 DMS=16.89818**

Error: 209.7182 gl: 40

trat peso	Medias	n	E.E.		
GA3	108.75	6	5.91	A	
GA mix	123.08	6	5.91	A	B
Check	127.80	6	5.91		B
GA4/7	128.18	6	5.91		B
AHS	129.77	6	5.91		B
TB	133.28	6	5.91		B
AVG	133.60	6	5.91		B
AN	135.85	6	5.91		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 35 peso**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 35 peso	48	0.29	0.17	11.48

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	3469.98	7	495.71	2.38	0.0391
trat peso	3469.98	7	495.71	2.38	0.0391
Error	8324.82	40	208.12		
Total	11794.80	47			

**Test:LSD Fisher Alfa=0.05 DMS=16.83369**

Error: 208.1205 gl: 40

trat peso	Medias	n	E.E.		
GA3	106.45	6	5.89	A	
GA mix	119.72	6	5.89	A	B
GA4/7	125.30	6	5.89		B
Check	126.78	6	5.89		B
AHS	127.77	6	5.89		B
TB	131.77	6	5.89		B
AVG	132.58	6	5.89		B
AN	134.77	6	5.89		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 53 peso**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 53 peso	48	0.29	0.16	11.76

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	3264.98	7	466.43	2.32	0.0436
trat peso	3264.98	7	466.43	2.32	0.0436
Error	8031.69	40	200.79		
Total	11296.67	47			

**Test:LSD Fisher Alfa=0.05 DMS=16.53466**

Error: 200.7923 gl: 40

trat peso	Medias	n	E.E.		
GA3	101.67	6	5.78	A	
GA mix	115.17	6	5.78	A	B
Check	119.83	6	5.78		B
GA4/7	121.43	6	5.78		B
AHS	124.42	6	5.78		B
TB	124.85	6	5.78		B
AVG	126.72	6	5.78		B
AN	129.82	6	5.78		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 67 peso**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 67 peso	48	0.31	0.19	12.09

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	3525.59	7	503.66	2.53	0.0298
trat peso	3525.59	7	503.66	2.53	0.0298
Error	7968.18	40	199.20		
Total	11493.77	47			

**Test:LSD Fisher Alfa=0.05 DMS=16.46916**

Error: 199.2045 gl: 40

trat peso	Medias	n	E.E.		
GA3	97.32	6	5.76	A	
GA mix	110.75	6	5.76	A	B
Check	116.75	6	5.76		B
GA4/7	118.57	6	5.76		B
TB	120.03	6	5.76		B
AHS	120.98	6	5.76		B
AVG	122.77	6	5.76		B
AN	127.03	6	5.76		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
tamano	45	0.35	0.22	5.78

**Cuadro de Análisis de la Varianza (SC tipo I)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	241.91	7	34.56	2.81	0.0188
trat tam	241.91	7	34.56	2.81	0.0188
Error	454.67	37	12.29		
Total	696.58	44			

**Test:LSD Fisher Alfa=0.05 DMS=4.28233**

Error: 12.2883 gl: 37

trat tam	Medias	n	E.E.	
GA mix	sd	0	sd	A
FLO	55.17	6	1.43	B
STX	59.83	6	1.43	C
HSK	60.67	6	1.43	C
CONTROL	61.50	6	1.43	C
NVB	61.67	6	1.43	C
AVG	61.83	6	1.43	C
VTZ	62.33	6	1.43	C
F+N	63.33	3	2.02	C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza****dia 25 a**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 25 a	48	0.29	0.17	14.38

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	14.27	7	2.04	2.34	0.0424
trat color a	14.27	7	2.04	2.34	0.0424
Error	34.87	40	0.87		
Total	49.14	47			

**Test:LSD Fisher Alfa=0.05 DMS=1.08946**

Error: 0.8717 gl: 40

trat color a	Medias	n	E.E.	
F	-7.55	6	0.38	A
N	-6.90	6	0.38	A
A	-6.76	6	0.38	A
H	-6.43	6	0.38	B
V	-6.30	6	0.38	B
F+N	-6.28	6	0.38	B
C	-6.15	6	0.38	B
S	-5.59	6	0.38	C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 35 a**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 35 a	48	0.37	0.26	31.55

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	43.62	7	6.23	3.30	0.0073
trat color a	43.62	7	6.23	3.30	0.0073
Error	75.50	40	1.89		
Total	119.12	47			

**Test:LSD Fisher Alfa=0.05 DMS=1.60313**

Error: 1.8875 gl: 40

trat color a	Medias	n	E.E.	
F	-6.70	6	0.56	A
A	-4.74	6	0.56	B
F+N	-4.40	6	0.56	B
C	-3.97	6	0.56	B
N	-3.95	6	0.56	B
V	-3.79	6	0.56	B
H	-3.67	6	0.56	B
S	-3.63	6	0.56	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 53 a**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 53 a	48	0.47	0.38	140.05

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	99.63	7	14.23	5.09	0.0003
trat color a	99.63	7	14.23	5.09	0.0003
Error	111.95	40	2.80		
Total	211.58	47			

**Test:LSD Fisher Alfa=0.05 DMS=1.95214**

Error: 2.7988 gl: 40

trat color a	Medias	n	E.E.	
F	-4.93	6	0.68	A
F+N	-1.11	6	0.68	B
N	-0.98	6	0.68	B
V	-0.78	6	0.68	B
H	-0.70	6	0.68	B
C	-0.43	6	0.68	B
A	-0.31	6	0.68	B
S	-0.30	6	0.68	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 67 a**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 67 a	48	0.64	0.57	753.82

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	104.27	7	14.90	10.06	<0.0001
trat color a	104.27	7	14.90	10.06	<0.0001
Error	59.25	40	1.48		
Total	163.52	47			

**Test:LSD Fisher Alfa=0.05 DMS=1.42021**

Error: 1.4814 gl: 40

trat color a	Medias	n	E.E.		
F	-3.52	6	0.50	A	
N	-0.11	6	0.50		B
H	0.40	6	0.50		B C
C	0.54	6	0.50		B C
S	0.70	6	0.50		B C
A	0.75	6	0.50		B C
V	0.76	6	0.50		B C
F+N	1.77	6	0.50		C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza****jugo dia 2**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
jugo dia 2	48	0.28	0.15	14.98

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	545.69	7	77.96	2.20	0.0551
trat jugo	545.69	7	77.96	2.20	0.0551
Error	1419.74	40	35.49		
Total	1965.44	47			

**Test:LSD Fisher Alfa=0.05 DMS=6.95179**

Error: 35.4935 gl: 40

trat jugo	Medias	n	E.E.		
V	36.61	6	2.43	A	
C	36.70	6	2.43	A	
H	37.43	6	2.43	A	
F	38.43	6	2.43	A	
S	38.93	6	2.43	A	
F+N	40.37	6	2.43	A	B
N	42.34	6	2.43	A	B
A	47.30	6	2.43		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**jugo dia 28**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
jugo dia 28	48	0.47	0.37	13.70

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	501.62	7	71.66	5.03	0.0004
trat jugo	501.62	7	71.66	5.03	0.0004
Error	570.19	40	14.25		
Total	1071.81	47			

**Test:LSD Fisher Alfa=0.05 DMS=4.40556**

Error: 14.2547 gl: 40

trat jugo	Medias	n	E.E.			
F	21.26	6	1.54	A		
C	24.78	6	1.54	A	B	
H	26.48	6	1.54		B	C
V	26.80	6	1.54		B	C
F+N	28.99	6	1.54		B	C D
N	29.87	6	1.54			C D
S	30.32	6	1.54			C D
A	31.96	6	1.54			D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**ph dia 28**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
ph dia 28	48	0.86	0.83	11.80

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	9.39	7	1.34	34.03	<0.0001
trat jugo	9.39	7	1.34	34.03	<0.0001
Error	1.58	40	0.04		
Total	10.97	47			

**Test:LSD Fisher Alfa=0.05 DMS=0.23175**

Error: 0.0394 gl: 40

trat jugo	Medias	n	E.E.		
C	1.10	6	0.08	A	
H	1.12	6	0.08	A	
S	1.13	6	0.08	A	
F	1.87	6	0.08		B
N	2.03	6	0.08		B
A	2.04	6	0.08		B
F+N	2.08	6	0.08		B
V	2.09	6	0.08		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**brix dia 28**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
brix dia 28	48	0.30	0.18	13.28

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	23.58	7	3.37	2.49	0.0318
trat jugo	23.58	7	3.37	2.49	0.0318
Error	54.02	40	1.35		
Total	77.60	47			

**Test:LSD Fisher Alfa=0.05 DMS=1.35603**

Error: 1.3505 gl: 40

trat jugo	Medias	n	E.E.		
C	7.23	6	0.47	A	
S	8.15	6	0.47	A	B
H	8.53	6	0.47	A	B
V	8.97	6	0.47		B
F	9.15	6	0.47		B
F+N	9.22	6	0.47		B
N	9.28	6	0.47		B
A	9.47	6	0.47		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**C.3 2012 post-harvest evaluations****Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso	57	0.49	0.40	11.82

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	7512.89	9	834.77	5.08	0.0001
trat peso	7512.89	9	834.77	5.08	0.0001
Error	7730.61	47	164.48		
Total	15243.50	56			

**Test:LSD Fisher Alfa=0.05 DMS=15.44918**

Error: 164.4811 gl: 47

trat peso	Medias	n	E.E.		
AII	92.40	5	5.74	A	
S	99.20	5	5.74	A	B
B	99.70	5	5.74	A	B
V	102.25	6	5.24	A	B
AI	105.50	5	5.74	A	B
F	106.58	6	5.24	A	B
C	107.08	6	5.24	A	B
H	109.67	6	5.24		B
F+N	115.13	4	6.41		B
N	131.83	9	4.28		C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia peso	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
1	peso	60	0.54	0.46	10.81

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	9225.40	9	1025.04	6.49	<0.0001
trat peso	9225.40	9	1025.04	6.49	<0.0001
Error	7895.33	50	157.91		
Total	17120.73	59			

**Test:LSD Fisher Alfa=0.05 DMS=14.57217**

Error: 157.9067 gl: 50

trat peso	Medias	n	E.E.					
S	98.00	6	5.13	A				
A II	102.67	6	5.13	A	B			
C	108.33	6	5.13	A	B			
F	108.67	6	5.13	A	B			
F+N	110.67	6	5.13	A	B	C		
AI	116.00	6	5.13		B	C		
V	123.33	6	5.13			C	D	
H	123.33	6	5.13			C	D	
B	133.00	6	5.13				D	E
N	138.33	6	5.13					E

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia peso	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
64	peso	60	0.48	0.39	12.12

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	7196.94	9	799.66	5.13	0.0001
trat peso	7196.94	9	799.66	5.13	0.0001
Error	7790.75	50	155.81		
Total	14987.69	59			

**Test:LSD Fisher Alfa=0.05 DMS=14.47533**

Error: 155.8149 gl: 50

trat peso	Medias	n	E.E.				
S	85.84	6	5.10	A			
A II	89.60	6	5.10	A	B		
C	97.05	6	5.10	A	B	C	
F	97.49	6	5.10	A	B	C	
F+N	98.87	6	5.10	A	B	C	
AI	102.82	6	5.10		B	C	
H	109.10	6	5.10			C	D
V	109.86	6	5.10			C	D
B	117.45	6	5.10				D
N	121.70	6	5.10				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

dia peso	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
1	peso	57	0.38	0.27	11.10

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	4297.60	9	477.51	3.26	0.0037
trat peso	4297.60	9	477.51	3.26	0.0037
Error	6889.03	47	146.58		
Total	11186.64	56			

**Test:LSD Fisher Alfa=0.05 DMS=14.45169**

Error: 146.5752 gl: 47

trat peso	Medias	n	E.E.				
AII	96.52	6	4.94	A			
F	97.97	6	4.94	A	B		
N	99.44	5	5.41	A	B		
V	105.68	5	5.41	A	B	C	
AI	107.90	6	4.94	A	B	C	
H	110.47	6	4.94	A	B	C	D
F+N	112.58	5	5.41		B	C	D
C	116.47	6	4.94			C	D
B	119.68	6	4.94			C	D
S	122.02	6	4.94				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia peso	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
28	peso	57	0.41	0.30	11.08

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	4600.48	9	511.16	3.69	0.0015
trat peso	4600.48	9	511.16	3.69	0.0015
Error	6511.12	47	138.53		
Total	11111.60	56			

**Test:LSD Fisher Alfa=0.05 DMS=14.04972**

Error: 138.5346 gl: 47

trat peso	Medias	n	E.E.			
AII	94.60	6	4.81	A		
F	95.75	6	4.81	A	B	
N	96.44	5	5.26	A	B	
V	97.86	5	5.26	A	B	
AI	105.88	6	4.81	A	B	C
H	108.15	6	4.81	A	B	C
F+N	110.04	5	5.26		B	C
C	114.38	6	4.81			C
B	117.72	6	4.81			C
S	119.23	6	4.81			C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia peso	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
59	peso	55	0.42	0.31	11.09

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	4568.01	9	507.56	3.66	0.0017
trat peso	4568.01	9	507.56	3.66	0.0017
Error	6237.36	45	138.61		
Total	10805.37	54			

#### Test:LSD Fisher Alfa=0.05 DMS=14.38743

Error: 138.6080 gl: 45

trat peso	Medias	n	E.E.				
N	93.33	4	5.89	A			
AII	94.27	6	4.81	A			
F	95.37	6	4.81	A	B		
V	99.65	4	5.89	A	B	C	
AI	105.65	6	4.81	A	B	C	D
H	107.82	6	4.81	A	B	C	D
F+N	109.72	5	5.27		B	C	D
C	114.00	6	4.81			C	D
B	117.42	6	4.81				D
S	118.95	6	4.81				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

#### Análisis de la varianza

##### dia 1 tamaño

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 1 tamaño	60	0.32	0.19	5.23

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	210.18	9	23.35	2.57	0.0163
trat tamaño	210.18	9	23.35	2.57	0.0163
Error	454.50	50	9.09		
Total	664.68	59			

#### Test:LSD Fisher Alfa=0.05 DMS=3.49628

Error: 9.0900 gl: 50

trat tamaño	Medias	n	E.E.				
S	54.67	6	1.23	A			
AII	55.92	6	1.23	A	B		
F	56.17	6	1.23	A	B	C	
AI	56.75	6	1.23	A	B	C	
F+N	56.83	6	1.23	A	B	C	
C	57.42	6	1.23	A	B	C	
B	58.50	6	1.23		B	C	D
V	59.17	6	1.23		B	C	D
H	59.50	6	1.23			C	D
N	61.25	6	1.23				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**dia 64 tamano**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
dia 64 tamano	60	0.41	0.31	4.38

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	211.02	9	23.45	3.92	0.0008
trat tamano	211.02	9	23.45	3.92	0.0008
Error	299.17	50	5.98		
Total	510.18	59			

**Test:LSD Fisher Alfa=0.05 DMS=2.83658**

Error: 5.9833 gl: 50

trat tamano	Medias	n	E.E.					
S	52.83	6	1.00	A				
F	54.17	6	1.00	A	B			
AII	54.17	6	1.00	A	B			
F+N	54.50	6	1.00	A	B	C		
C	55.17	6	1.00	A	B	C		
AI	55.67	6	1.00	A	B	C	D	
H	57.00	6	1.00		B	C	D	E
V	57.17	6	1.00			C	D	E
B	58.33	6	1.00				D	E
N	58.83	6	1.00					E

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )**Análisis de la varianza****tamano inicial**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
tamano inicial	57	0.37	0.25	3.97

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	144.07	9	16.01	3.10	0.0052
trat tamano	144.07	9	16.01	3.10	0.0052
Error	242.47	47	5.16		
Total	386.54	56			

**Test:LSD Fisher Alfa=0.05 DMS=2.71122**

Error: 5.1589 gl: 47

trat tamano	Medias	n	E.E.					
AII	55.00	6	0.93	A				
F	55.50	6	0.93	A	B			
N	55.60	5	1.02	A	B			
V	56.20	5	1.02	A	B			
AI	56.67	6	0.93	A	B	C		
F+N	57.20	5	1.02	A	B	C	D	
H	58.00	6	0.93		B	C	D	
C	59.00	6	0.93			C	D	
S	59.17	6	0.93			C	D	
B	59.50	6	0.93				D	

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**peso inicial**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso inicial	57	0.38	0.27	11.10

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	4297.60	9	477.51	3.26	0.0037
trat tamano	4297.60	9	477.51	3.26	0.0037
Error	6889.03	47	146.58		
Total	11186.64	56			

**Test:LSD Fisher Alfa=0.05 DMS=14.45169**

Error: 146.5752 gl: 47

trat tamano	Medias	n	E.E.				
AII	96.52	6	4.94	A			
F	97.97	6	4.94	A	B		
N	99.44	5	5.41	A	B		
V	105.68	5	5.41	A	B	C	
AI	107.90	6	4.94	A	B	C	
H	110.47	6	4.94	A	B	C	D
F+N	112.58	5	5.41		B	C	D
C	116.47	6	4.94			C	D
B	119.68	6	4.94			C	D
S	122.02	6	4.94				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
1 color a	60	0.29	0.16	9.55

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	12.16	9	1.35	2.25	0.0333
trat color a	12.16	9	1.35	2.25	0.0333
Error	29.97	50	0.60		
Total	42.14	59			

**Test:LSD Fisher Alfa=0.05 DMS=0.89783**

Error: 0.5994 gl: 50

trat color a	Medias	n	E.E.				
F	-8.69	6	0.32	A			
V	-8.49	6	0.32	A			
B	-8.44	6	0.32	A			
H	-8.42	6	0.32	A			
AI	-8.30	6	0.32	A			
C	-8.28	6	0.32	A	B		
S	-7.93	6	0.32	A	B	C	
AII	-7.87	6	0.32	A	B	C	
F+N	-7.39	6	0.32		B	C	
N	-7.29	6	0.32				C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
13 color a	60	0.19	0.05	14.48

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	14.06	9	1.56	1.33	0.2479
trat color a	14.06	9	1.56	1.33	0.2479
Error	58.93	50	1.18		
Total	72.99	59			

**Test:LSD Fisher Alfa=0.05 DMS=1.25896**

Error: 1.1786 gl: 50

trat color a	Medias	n	E.E.		
B	-8.18	6	0.44	A	
C	-8.05	6	0.44	A	
F	-8.02	6	0.44	A	
V	-7.74	6	0.44	A	B
H	-7.70	6	0.44	A	B
F+N	-7.31	6	0.44	A	B
AI	-7.31	6	0.44	A	B
AII	-6.98	6	0.44	A	B
N	-6.97	6	0.44	A	B
S	-6.73	6	0.44		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**% jugo**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
% jugo	54	0.38	0.25	10.67

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	361.26	9	40.14	2.95	0.0078
trat jugo	361.26	9	40.14	2.95	0.0078
Error	597.92	44	13.59		
Total	959.17	53			

**Test:LSD Fisher Alfa=0.05 DMS=4.53971**

Error: 13.5890 gl: 44

trat jugo	Medias	n	E.E.		
H	31.79	6	1.50	A	
B	32.13	5	1.65	A	
C	32.51	6	1.50	A	
S	33.61	5	1.65	A	
V	34.02	6	1.50	A	B
AII	34.40	5	1.65	A	B
F	34.49	6	1.50	A	B
AI	34.86	5	1.65	A	B
N	38.16	6	1.50		B C
F+N	41.19	4	1.84		C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza**

dia jugo	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
1	% jugo	52	0.24	0.08	11.76

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	211.24	9	23.47	1.46	0.1927
trat jugo	211.24	9	23.47	1.46	0.1927
Error	672.95	42	16.02		
Total	884.19	51			

**Test:LSD Fisher Alfa=0.05 DMS=5.04752**

Error: 16.0227 gl: 42

trat jugo	Medias	n	E.E.			
V	30.43	6	1.63	A		
C	30.69	5	1.79	A	B	
H	33.69	6	1.63	A	B	C
F+N	33.76	4	2.00	A	B	C
A I	34.02	6	1.63	A	B	C
N	35.21	6	1.63	A	B	C
B	35.29	4	2.00	A	B	C
AII	35.37	5	1.79		B	C
F	35.77	4	2.00		B	C
S	36.64	6	1.63			C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia jugo	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
28	% jugo	52	0.43	0.31	9.44

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	342.05	9	38.01	3.52	0.0025
trat jugo	342.05	9	38.01	3.52	0.0025
Error	453.81	42	10.80		
Total	795.86	51			

**Test:LSD Fisher Alfa=0.05 DMS=4.14496**

Error: 10.8049 gl: 42

trat jugo	Medias	n	E.E.			
C	30.65	5	1.47	A		
V	31.07	6	1.34	A		
N	33.14	6	1.34	A	B	
H	34.49	6	1.34	A	B	C
F	34.61	4	1.64	A	B	C
S	35.41	6	1.34		B	C
B	37.01	4	1.64		B	C
AII	37.30	5	1.47			C
F+N	37.49	4	1.64			C
A I	38.29	6	1.34			C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia jugo	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
56	% jugo	60	0.61	0.54	10.54

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	996.15	9	110.68	8.64	<0.0001
trat jugo	996.15	9	110.68	8.64	<0.0001
Error	640.86	50	12.82		
Total	1637.00	59			

#### Test:LSD Fisher Alfa=0.05 DMS=4.15163

Error: 12.8171 gl: 50

trat jugo	Medias	n	E.E.			
S	28.78	6	1.46	A		
F	29.00	6	1.46	A		
N	29.78	6	1.46	A		
H	31.65	6	1.46	A	B	
V	31.85	6	1.46	A	B	
C	34.94	6	1.46		B	C
F+N	36.39	6	1.46			C D
A I	38.56	6	1.46			C D
B	38.59	6	1.46			C D
AII	40.11	6	1.46			D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

#### Análisis de la varianza

dia jugo	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
1	% jugo	54	0.37	0.24	9.03

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	281.86	9	31.32	2.87	0.0093
trat jugo	281.86	9	31.32	2.87	0.0093
Error	479.85	44	10.91		
Total	761.71	53			

#### Test:LSD Fisher Alfa=0.05 DMS=4.06689

Error: 10.9058 gl: 44

trat jugo	Medias	n	E.E.			
V	32.67	5	1.48	A		
N	32.67	4	1.65	A	B	
C	35.61	6	1.35	A	B	C
AI	36.33	5	1.48	A	B	C
F	36.48	6	1.35	A	B	C
S	36.76	5	1.48	A	B	C
AII	36.94	6	1.35		B	C
H	37.07	6	1.35			C
F+N	38.45	5	1.48			C D
B	41.03	6	1.35			D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

dia jugo	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
28	% jugo	45	0.29	0.16	11.27

*Datos desbalanceados en celdas.  
Para otra descomposición de la SC  
especifique los contrastes apropiados.. !!*

#### Cuadro de Análisis de la Varianza (SC tipo I)

F.V.	SC	gl	CM	F	p-valor
Modelo.	282.87	7	40.41	2.21	0.0558
trat jugo	282.87	7	40.41	2.21	0.0558
Error	676.75	37	18.29		
Total	959.62	44			

#### Test:LSD Fisher Alfa=0.05 DMS=5.19026

*Error: 18.2906 gl: 37*

trat jugo	Medias	n	E.E.					
F+N	sd	0	sd	A				
AI	sd	0	sd		B			
H	34.23	6	1.75			C		
V	35.68	5	1.91			C	D	
C	35.79	6	1.75			C	D	
N	37.63	4	2.14			C	D	E
F	38.75	6	1.75			C	D	E
S	39.07	6	1.75			C	D	E
AII	39.47	6	1.75				D	E
B	42.43	6	1.75					E

*Medias con una letra común no son significativamente diferentes (p > 0.05)*

dia jugo	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
56	% jugo	55	0.38	0.25	7.92

#### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	206.52	9	22.95	3.03	0.0064
trat jugo	206.52	9	22.95	3.03	0.0064
Error	340.53	45	7.57		
Total	547.05	54			

#### Test:LSD Fisher Alfa=0.05 DMS=3.36171

*Error: 7.5673 gl: 45*

trat jugo	Medias	n	E.E.				
F	30.58	6	1.12	A			
N	31.75	4	1.38	A	B		
V	34.14	4	1.38	A	B	C	
AI	34.28	6	1.12		B	C	
H	35.12	6	1.12		B	C	
F+N	35.57	5	1.23			C	
S	35.71	6	1.12			C	
AII	36.06	6	1.12			C	
C	36.12	6	1.12			C	
B	37.07	6	1.12			C	

*Medias con una letra común no son significativamente diferentes (p > 0.05)*

**BAR**

cosecha	Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
II	BAR	30	0.50	0.28	3.51

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	0.05	9	0.01	2.26	0.0621
trat	0.05	9	0.01	2.26	0.0621
Error	0.05	20	2.4E-03		
Total	0.10	29			

**Test:LSD Fisher Alfa=0.05 DMS=0.08429**

Error: 0.0024 gl: 20

trat	Medias	n	E.E.		
F	1.35	3	0.03	A	
AI	1.35	3	0.03	A	
V	1.40	3	0.03	A	
C	1.41	3	0.03	A	
F+N	1.41	3	0.03	A	
B	1.41	3	0.03	A	
S	1.41	3	0.03	A	
H	1.43	3	0.03	A	B
AII	1.43	3	0.03	A	B
N	1.50	3	0.03		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Tamaño y peso EI****Análisis de la varianza****tamaño EI**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
tamaño EI	92	0.10	0.03	4.74

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	85.32	7	12.19	1.40	0.2167
Trat EI	85.32	7	12.19	1.40	0.2167
Error	731.93	84	8.71		
Total	817.25	91			

**Test:LSD Fisher Alfa=0.05 DMS=2.45170**

Error: 8.7135 gl: 84

Trat EI	Medias	n	E.E.		
GA3	60.50	10	0.93	A	
Check	61.00	12	0.85	A	B
TB	61.75	12	0.85	A	B
AI	62.33	12	0.85	A	B
GA4/7	62.75	12	0.85	A	B
AN	63.00	12	0.85	A	B
GA mix	63.20	10	0.93		B
AHS	63.33	12	0.85		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**peso EI**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso EI	92	0.15	0.08	13.80

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	5562.34	7	794.62	2.13	0.0489
Trat EI	5562.34	7	794.62	2.13	0.0489
Error	31314.95	84	372.80		
Total	36877.29	91			

**Test:LSD Fisher Alfa=0.05 DMS=16.03643**

Error: 372.7970 gl: 84

Trat EI	Medias	n	E.E.		
GA3	121.01	10	6.11	A	
AN	133.37	12	5.57	A	B
AI	138.05	12	5.57		B
AHS	144.06	12	5.57		B
Check	144.07	12	5.57		B
TB	145.01	12	5.57		B
GA mix	145.42	10	6.11		B
GA4/7	145.73	12	5.57		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Tamaño y peso EII**

Nueva tabla: 4/8/2013 - 3:54:36 AM

**Análisis de la varianza****tamaño EII**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
tamaño EII	117	0.16	0.09	5.02

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	165.34	9	18.37	2.21	0.0269
trat EII	165.34	9	18.37	2.21	0.0269
Error	890.29	107	8.32		
Total	1055.63	116			

**Test:LSD Fisher Alfa=0.05 DMS=2.36513**

Error: 8.3205 gl: 107

trat EII	Medias	n	E.E.		
AII	55.46	12	0.83	A	
GA3	55.83	12	0.83	A	
AI	56.71	12	0.83	A	B
AN	56.92	12	0.83	A	B
GA mix	57.00	11	0.87	A	B
TB	57.82	11	0.87	A	B
BR	58.21	12	0.83		B
GA4/7	58.68	11	0.87		B
AHS	58.75	12	0.83		B
Check	59.00	12	0.83		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Peso EII**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso EII	117	0.22	0.15	13.11

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6443.83	9	715.98	3.28	0.0015
trat EII	6443.83	9	715.98	3.28	0.0015
Error	23381.08	107	218.51		
Total	29824.91	116			

**Test:LSD Fisher Alfa=0.05 DMS=12.12050**

Error: 218.5147 gl: 107

trat EII	Medias	n	E.E.				
AII	99.59	12	4.27	A			
GA3	103.32	12	4.27	A	B		
AN	110.01	12	4.27	A	B	C	
GA mix	111.54	11	4.46	A	B	C	
AI	111.95	12	4.27		B	C	
BR	112.40	12	4.27		B	C	
TB	115.31	11	4.46		B	C	D
AHS	116.90	12	4.27			C	D
GA4/7	120.65	11	4.46			C	D
Check	126.34	12	4.27				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

Jugo

**Análisis de la varianza****peso fruto EII**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso fruto EII	107	0.16	0.08	14.96

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	4447.35	9	494.15	2.01	0.0460
trat EII	4447.35	9	494.15	2.01	0.0460
Error	23840.11	97	245.77		
Total	28287.46	106			

**Test:LSD Fisher Alfa=0.05 DMS=13.47641**

Error: 245.7743 gl: 97

trat EII	Medias	n	E.E.			
AII	92.84	11	4.73	A		
TB	95.18	11	4.73	A	B	
GA4/7	101.63	10	4.96	A	B	C
GA3	103.37	10	4.96	A	B	C
GA mix	104.64	9	5.23	A	B	C
BR	106.38	11	4.73		B	C
Check	108.67	10	4.96		B	C
AHS	109.35	12	4.53			C
AI	111.96	11	4.73			C
AN	112.68	12	4.53			C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**peso jugo EII**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso jugo EII	106	0.17	0.09	22.01

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	1333.36	9	148.15	2.20	0.0283
trat EII	1333.36	9	148.15	2.20	0.0283
Error	6461.82	96	67.31		
Total	7795.19	105			

**Test:LSD Fisher Alfa=0.05 DMS=7.08426**

Error: 67.3107 gl: 96

trat EII	Medias	n	E.E.				
TB	30.31	11	2.47	A			
AII	33.66	11	2.47	A	B		
GA4/7	34.94	10	2.59	A	B	C	
BR	35.71	11	2.47	A	B	C	D
GA3	37.70	10	2.59		B	C	D
GA mix	38.71	9	2.73		B	C	D
AHS	38.79	12	2.37		B	C	D
AI	39.24	11	2.47		B	C	D
AN	41.88	11	2.47			C	D
Check	42.28	10	2.59				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**% jugo EII**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
% jugo EII	106	0.21	0.14	10.94

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	383.77	9	42.64	2.86	0.0050
trat EII	383.77	9	42.64	2.86	0.0050
Error	1432.55	96	14.92		
Total	1816.32	105			

**Test:LSD Fisher Alfa=0.05 DMS=3.33559**

Error: 14.9224 gl: 96

trat EII	Medias	n	E.E.				
TB	31.45	11	1.16	A			
BR	33.37	11	1.16	A	B		
GA4/7	34.20	10	1.22	A	B	C	
AI	35.07	11	1.16		B	C	
AHS	35.38	12	1.12		B	C	
GA3	36.20	10	1.22		B	C	D
AII	36.22	11	1.16		B	C	D
GA mix	36.36	9	1.29		B	C	D
AN	36.69	11	1.16			C	D
Check	38.73	10	1.22				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

## Análisis de la varianza

### Peso fruto EI d1

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso fruto EI d1	48	0.37	0.26	13.83

### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	7078.59	7	1011.23	3.36	0.0065
trat EI	7078.59	7	1011.23	3.36	0.0065
Error	12036.64	40	300.92		
Total	19115.23	47			

### Test:LSD Fisher Alfa=0.05 DMS=20.24158

Error: 300.9161 gl: 40

trat EI	Medias	n	E.E.		
GA3	103.88	6	7.08	A	
AI	118.32	6	7.08	A	B
AHS	118.68	6	7.08	A	B
GA4/7	119.43	6	7.08	A	B
GA mix	129.18	6	7.08		B C
TB	129.83	6	7.08		B C
AN	139.82	6	7.08		C
Check	143.95	6	7.08		C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

### peso jugo EI d1

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso jugo EI d1	48	0.20	0.06	23.29

### Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	1334.31	7	190.62	1.41	0.2288
trat EI	1334.31	7	190.62	1.41	0.2288
Error	5412.50	40	135.31		
Total	6746.81	47			

### Test:LSD Fisher Alfa=0.05 DMS=13.57347

Error: 135.3125 gl: 40

trat EI	Medias	n	E.E.		
GA3	39.67	6	4.75	A	
AHS	44.50	6	4.75	A	B
TB	47.33	6	4.75	A	B
GA4/7	51.33	6	4.75	A	B
GA mix	52.67	6	4.75	A	B
Check	53.17	6	4.75	A	B
AN	54.83	6	4.75		B
AI	56.00	6	4.75		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**% jugo EI d1**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
% jugo EI d1	48	0.28	0.15	14.98

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	545.69	7	77.96	2.20	0.0551
trat EI	545.69	7	77.96	2.20	0.0551
Error	1419.74	40	35.49		
Total	1965.44	47			

**Test:LSD Fisher Alfa=0.05 DMS=6.95179**

Error: 35.4935 gl: 40

trat EI	Medias	n	E.E.		
TB	36.61	6	2.43	A	
Check	36.70	6	2.43	A	
AHS	37.43	6	2.43	A	
GA3	38.43	6	2.43	A	
AN	38.93	6	2.43	A	
GA mix	40.37	6	2.43	A	B
GA4/7	42.34	6	2.43	A	B
AI	47.30	6	2.43		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**peso fruto EI d28**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso fruto EI d28	48	0.28	0.16	17.01

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6033.22	7	861.89	2.26	0.0486
trat EI	6033.22	7	861.89	2.26	0.0486
Error	15228.22	40	380.71		
Total	21261.44	47			

**Test:LSD Fisher Alfa=0.05 DMS=22.76755**

Error: 380.7055 gl: 40

trat EI	Medias	n	E.E.			
GA3	95.50	6	7.97	A		
Check	99.98	6	7.97	A	B	
AN	112.82	6	7.97	A	B	C
AHS	114.72	6	7.97	A	B	C
GA mix	117.12	6	7.97	A	B	C
TB	121.13	6	7.97		B	C
AI	127.77	6	7.97			C
GA4/7	128.80	6	7.97			C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**peso jugo EI d28**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
peso jugo EI d28	48	0.37	0.26	28.20

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	1939.81	7	277.12	3.33	0.0069
trat EI	1939.81	7	277.12	3.33	0.0069
Error	3329.17	40	83.23		
Total	5268.98	47			

**Test:LSD Fisher Alfa=0.05 DMS=10.64534**

Error: 83.2292 gl: 40

trat EI	Medias	n	E.E.				
GA3	21.00	6	3.72	A			
Check	25.00	6	3.72	A	B		
AHS	30.67	6	3.72	A	B	C	
TB	32.83	6	3.72		B	C	D
AN	34.33	6	3.72		B	C	D
GA mix	34.50	6	3.72		B	C	D
GA4/7	38.83	6	3.72			C	D
AI	41.67	6	3.72				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**% jugo EI d28**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
% jugo EI d28	48	0.47	0.37	13.70

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	501.62	7	71.66	5.03	0.0004
trat EI	501.62	7	71.66	5.03	0.0004
Error	570.19	40	14.25		
Total	1071.81	47			

**Test:LSD Fisher Alfa=0.05 DMS=4.40556**

Error: 14.2547 gl: 40

trat EI	Medias	n	E.E.				
GA3	21.26	6	1.54	A			
Check	24.78	6	1.54	A	B		
AHS	26.48	6	1.54		B	C	
TB	26.80	6	1.54		B	C	
GA mix	28.99	6	1.54		B	C	D
GA4/7	29.87	6	1.54			C	D
AN	30.32	6	1.54			C	D
AI	31.96	6	1.54				D

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

## Cascara

## Análisis de la varianza

## gr. flv.

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
gr. flv.	18	0.51	0.45	16.69

## Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	0.95	2	0.47	7.82	0.0047
fruto grosor	0.95	2	0.47	7.82	0.0047
Error	0.91	15	0.06		
Total	1.85	17			

## Test:LSD Fisher Alfa=0.05 DMS=0.30274

Error: 0.0605 gl: 15

fruto grosor	Medias	n	E.E.		
M	1.19	6	0.10	A	
P	1.47	6	0.10	A	B
G	1.76	6	0.10		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

## gr. alb.

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
gr.alb.	18	0.52	0.46	20.10

## Cuadro de Análisis de la Varianza (SC tipo III)

F.V.	SC	gl	CM	F	p-valor
Modelo.	3.41	2	1.71	8.13	0.0041
fruto grosor	3.41	2	1.71	8.13	0.0041
Error	3.15	15	0.21		
Total	6.56	17			

## Test:LSD Fisher Alfa=0.05 DMS=0.56394

Error: 0.2100 gl: 15

fruto grosor	Medias	n	E.E.		
M	1.75	6	0.19	A	
G	2.28	6	0.19	A	B
P	2.81	6	0.19		B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**g cascara**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
gr. cascara	18	0.59	0.53	14.27

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	6.15	2	3.07	10.71	0.0013
fruto grosor	6.15	2	3.07	10.71	0.0013
Error	4.30	15	0.29		
Total	10.45	17			

**Test:LSD Fisher Alfa=0.05 DMS=0.65916**

Error: 0.2869 gl: 15

fruto grosor	Medias	n	E.E.	
M	2.94	6	0.22	A
G	4.03	6	0.22	B
P	4.29	6	0.22	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**prof. gland.**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
prof. gland.	17	0.26	0.16	18.85

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	0.24	2	0.12	2.50	0.1181
fruto grosor	0.24	2	0.12	2.50	0.1181
Error	0.68	14	0.05		
Total	0.92	16			

**Test:LSD Fisher Alfa=0.05 DMS=0.28131**

Error: 0.0486 gl: 14

fruto grosor	Medias	n	E.E.	
M	0.99	5	0.10	A
G	1.21	6	0.09	A B
P	1.28	6	0.09	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**Análisis de la varianza****numero de gland.**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
numero de gland.	53	0.73	0.72	20.89

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	101851.76	2	50925.88	67.12	<0.0001
fruto gland.	101851.76	2	50925.88	67.12	<0.0001
Error	37937.56	50	758.75		
Total	139789.32	52			

**Test:LSD Fisher Alfa=0.05 DMS=18.61874**

Error: 758.7512 gl: 50

fruto gland.	Medias	n	E.E.	
G	88.33	18	6.49	A
M	117.50	18	6.49	B
P	193.24	17	6.68	C

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )

**tamano glandulas**

Variable	N	R <sup>2</sup>	R <sup>2</sup> Aj	CV
tamano glandulas	53	0.54	0.52	19.29

**Cuadro de Análisis de la Varianza (SC tipo III)**

F.V.	SC	gl	CM	F	p-valor
Modelo.	2.30	2	1.15	28.90	<0.0001
fruto gland.	2.30	2	1.15	28.90	<0.0001
Error	1.99	50	0.04		
Total	4.29	52			

**Test:LSD Fisher Alfa=0.05 DMS=0.13491**

Error: 0.0398 gl: 50

fruto gland.	Medias	n	E.E.	
P	0.74	17	0.05	A
M	1.11	18	0.05	B
G	1.24	18	0.05	B

Medias con una letra común no son significativamente diferentes ( $p > 0.05$ )