Are you a Citrus Grower?

Improving Citrus Yield and Quality through Biostimulant Use

In terms of international trade and importance, citrus fruits are the highest-value fruit crop, with more than 140 countries worldwide contributing to citrus production. As the market competition strengthens fruit values decrease in relation to overall supply; to maintain economic sustainability, citrus producers are continuously evaluating and implementing management strategies to reduce input costs to maximise their revenue. In production areas facing water deficits such as Australia, the use of biostimulants in crop management is becoming a popular way to improve yield and quality.

Citrus Production in Australia

The Australian citrus industry contributes over $200 million annually to the country's economy, with citrus fruits claiming the largest percentage of fresh fruit exports (NSW Department of Primary Industries). Currently, over 28,000 hectares of citrus are planted with approximately 78% attributed to oranges, 12% to mandarins, and smaller areas of lemons, grapefruit, and limes.

Challenges facing the Australian Citrus Industry

- Increasing levels of global competition, facilitating a need for reduced inputs while maintaining high yields and quality.
- Rising cost of production has escalated due to increases in the price of water, fertiliser, labour cost, various quarantine requirements, etc.

One of the most harrowing challenges facing the citrus industry right now is the water deficit due to drought conditions and the resulting sky-rocketing prices of irrigation water. Producers are frantically searching for ways to improve yield and quality while reducing their water usage.

This is where the application of biostimulants to citrus crops becomes beneficial, providing a feasible way to sustainably produce citrus under suboptimal growing conditions.

Ensuring citrus trees and their fruit have all their required needs met will boost overall yield and fruit quality.

Our products help farmers to meet the increasing agricultural demands they are facing while simultaneously increasing sustainability.

Benefits of Using Biostimulants in Citrus Management Practices

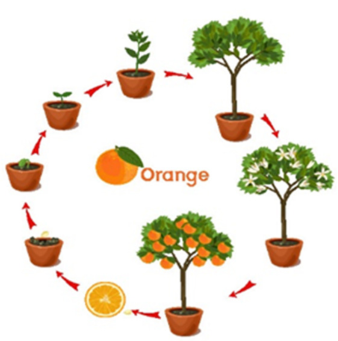
GrowGreen products are microbially active fertilisers, classified as packed with vital amino acids and growth hormones, that also nourish and feed plants with a full spectrum of macro and micronutrients.
The inclusion of both amino acids and plant growth hormones increases the efficiency of the products exponentially, as the combination works together symbiotically, providing heightened benefits compared to supplementation of either component alone. Evidence based research and field trials present farmers with a solid understanding of how these products improve their returns whilst utilising environmentally friendly methods.

An application of biostimulant products will improve the overall health and strength of trees, help increase the number of fruit set and fruit size by providing complete nutrition to citrus trees.

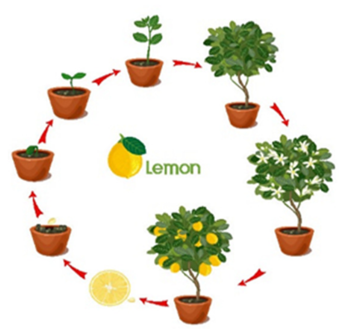
Benefits of biostimulant use

- **Improving** the efficiency of the plant's metabolism to induce yield increases and enhanced crop quality;
- **Enhancing** soil fertility, particularly by fostering the development of complementary soil microorganisms
- **Increasing** plant tolerance to and recovery from abiotic stresses;
- **Facilitating** nutrient assimilation, translocation, and use;
- **Boosting** quality attributes of produce, including sugar content, colour, fruit setting, etc;
- **Rendering** water use more efficient;
- **Enriching** soil fertility, particularly by fostering the development of complementary soil microorganisms.

Citrus fruit production is a complex system, with many abiotic and biotic factors affecting a grove, or a single tree. Added complexity comes in as the citrus season is broken down into four major growth stages that have carrying nutritional and other physiological needs at each stage to promote better fruit size, quality and overall yield.



1. **Floral Initiation, Flowering and Initial Fruit Set (Jun-Oct)**
2. **Stage I – Fruit Growth (Nov-Dec)**
3. **Stage II – Fruit Growth (Jan-Apr)**
4. **Stage III – Ripening/Maturation (May onward)**



Application of biostimulant products improves the overall health and strength of trees and can help increase the number of fruit set and size by providing complete nutrition.

Providing key phytohormones reduces energy expenditure on phytohormone biosynthesis, allowing the tree to direct that energy toward fruiting.

Parameters Measured in Citrus Production

To gauge a citrus operation's yield, and the resulting fruit quality the following parameters are looked at.

- ### Fruit number and size

Fruit size has become almost as important as total plantation yield due to a rising preference for large citrus fruits by the consumer. Therefore, producers strive to manage groves in a way to produce a high amount of fruit, while encouraging the largest size possible, to maximise their revenue.

The number and size of fruit on a tree are inversely correlated, with both being hormone driven and affected by water availability during each life stage. Fewer fruits mean a larger percentage of resources from the tree resulting in larger fruit; a higher amount of fruit on one tree will result in them being smaller overall as the pool of plant resources is shared across a great number.

At the basis of fruit numbers and size are the rates of flower induction, fruit setting, and fruit abortion, all of which are affected by phytohormone relationships, nutrient availability, and water availability.

The final fruit size results from the accumulation of dry matter and water within the fruit; determined by the sink strength - the potential capacity to accumulate assimilates - of the fruit and the metabolites supplied during the bulking stage of fruit development.

An increased immune system response can also occur. Less incidence of disease of disease infestation, pest problems and plant defence against other stressors can help trees achieve better yields.

Phytohormone relationships

There are five primary phytohormones that work to regulate cellular development and gene expression controlling plant growth and plant responses to stressors: auxins, cytokinins, gibberellins, abscisic acid, and ethylene. Through complex metabolic processes, they may function independently, symbiotically, and sometimes counterintuitively depending upon the concentration of the hormone, its sites of action, and the developmental stage the plant is in.

Due to the complex nature of the phytohormone interactions, it is the ratio at which all five phytohormones are present that signal certain processes to occur. As flower initiation and set occur, gibberellins and cytokinins play the most important roles in flower numbers and the resulting fruit numbers.

Gibberellins are thought to be a pivotal effector responsible for the set and development of citrus fruits. They activate cell division and cell enlargement processes in vegetative organs *(Talon et al., 1991)*, triggering the ovary-fruit transition *(Ben-Cheik et al., 1997)* and initiating growth. Several studies report exogenous applications of GA3 improves parthenocarpic fruit (seedless fruits as they are produced without fertilisation of ovules) set and growth in genotypes that show negligible fruit set in the absence of cross-pollination *(Soost, & Burnett, 1961)*.

Optimal nutrition provides plants with the nutrients they need immediately and minimises yield reductions correlated to deficiencies.

Depending on the concentration, and the plants developmental stage, auxins either encourage fruit/flower senescence or cellular elongation.

Cytokinins also stimulate cell division, with increasing levels found in developing ovaries at anthesis, the period when the flower is fully open and functional. In relation to auxins concentrations, cytokinins stimulate cell division and differentiation in actively growing plant parts. If cytokinin is present alone it has no effect on generalised, parenchyma cells. When these generalised cells are exposed to auxin without cytokinin, they elongate and grow large but do not divide. The addition of cytokinin may cause cells to expand and differentiate, depending on the hormone ratio.

Thinning trees to reduce fruit numbers and increase fruit size is a common practice in commercial orchards through auxin-induced ethylene synthesis and induced abscission, an interplay between the two hormones. When auxins are applied at the time of flowering up to 6-weeks after full bloom, fruitlet thinning occurs, increasing fruit size through the inverse relationship.

Recent studies have determined the pivotal application of auxins should be performed after the physiological fruitlet drop triggered naturally by the tree when the fruit is less sensitive to the abscission. There is less overall yield loss when thinning is done via exogenous auxin application as the smaller fruits are selectively thinned from the tree, increasing both fruit size and overall crop yield *(Guardiola, 1981; Guardiola, & Lazaro, 1987; Vanniere et al., 1987)*.

Auxins also promote cellular elongation instead of cellular division triggered by gibberellins or cytokinins, controlling fruit size during the phase of rapid fruit growth. The number of cells within citrus fruits doesn't increase due to auxin levels but rather the cells elongate, leading to a marked increase in fruit size.

The synergistic and sometimes counter-intuitive relationships between the plant hormone types are adversely affected when any of the types of hormones are lacking; this adverse effect isn't seen when all five phytohormones are present through natural biosynthesis or exogenous application.

Using AminoKelp™ for tree thinning, provides a balanced response of initial fruitlet reduction and later season growth stimulation related to the inclusion of all five classic phytohormones in the product. The interplay between the hormones isn't negatively affected.

Amino acid biosynthesis is a very energy-intensive process. By providing high-quality amino acids through foliar or soil applications the amount of plant energy expended on amino acid synthesis decreases, allowing the plant to utilise the energy elsewhere.

Inclusion of all five classical phytohormones in GrowGreen products extends the benefits seen from either soil or foliar application.

Both high and low levels of specific nutrients impact fruit number and size, depending on the growth stage of the tree and fruit.

Nutrient availability

Crop management must consider the seasonal fluctuations in nutrients needs, carefully monitoring plant concentrations and adjusting management practices accordingly. Higher contents of nitrogen, especially ammonia in the buds and leaves increase the number of flowers initiated on the tree in the spring *(Lovatt et al., 1988)* leading to a potentially higher amount of fruit depending on the percentage of pollination and fruit set.

Phosphorus is also an essential element for flower and fruit formation; deficient trees exhibit limited flower development with reduced fruit set and fruit yield. Due to interactions with other ions in the soil, phosphorus can easily be "locked up", demanding well-timed application of plant-available phosphorus for improved flower and fruit formation.

Later in the growing season, as fruits are bulking, nitrogen levels must drop, or a negative effect will be observed on fruit size. High nitrogen levels encourage vegetative growth instead of accumulating assimilates in fruit halting the increase in fruit size.

At this stage in growth low potassium, iron, manganese, and zinc are also known to negatively affect fruit size, producing smaller fruit. Zinc deficiency is more prominent in alkaline soils or extremely wet soil conditions and shows symptomatically as small, elongated fruit that is pale and coarse.

Nutrient levels must be closely monitored through leaf analysis and quickly ameliorated with applications of plant-available nutrients to maintain optimum fruit size. Biostimulant use is beneficial when quick nutrient uptake needs to occur to adjust plant tissue concentrations with minimal yield reduction.

- **Nitrogen** supplied via AminoElite™, AminoKelp™, and Microbe Plus® Citrus is held in the root zone via "sticky" substances (organic acids, vitamins, auxins) that bind nutrients to particles in the soil stratum, reducing leaching and facilitating plant uptake. Better nutrient retention reduces fertiliser needs.
- **Potassium** applications are more efficient through foliar application of a product such as AminoKelp™, Microbe Plus® Potassium, and Microbe Plus® Citrus due to potassium's propensity to leach through the soil profile and out of the root zone. Leaching occurs at a much faster, or higher rate in sandy soils containing fewer charge sites than soils higher in clay content. However, the attraction between potassium cations and negatively charged particles on either organic matter or clay particles is relatively weak compared to other ionic attractions.
- **Applying** Microbe Plus® PhosCal via foliar spray or soil drench during high demand times such as fruit development provide plant-available calcium for quick uptake.
- With the wide range of trace elements in AminoKelp™ (including plant essential nutrients such as iron, manganese, molybdenum, boron, and cobalt) helps to reduce the phenomenon known as "hidden hunger" with plants -- the area on a yield curve where deficiency symptoms are not yet visible but yield reductions are occurring. High levels of the AA glycine act as a natural chelating agent of the trace minerals, binding to calcium, iron, manganese, zinc, and cobalt ions making them more available and easily absorbed by plants.

Nitrogen fertilisation has also been shown to improve canopy width & flower yield

(Menino et al., 2004)

A reduction in fruit size over the course of the growing season is related to drought conditions and a resulting decrease in potassium levels within citrus trees.

Water availability

Drought conditions lead to leaf drop and/or a decrease in transpiration (due to stomatal closure). Potassium helps to sync stomatal opening/closing under drought stress through the regulation of cell water potential and turgor pressure *(Jordan et al., 2008; Peiter 2011)* to direct respiration and transpiration rates minimising water loss from tissues. Both cause a reduction in photosynthesis, resulting in a slowing of the fruit growth rate.

Plant response to drought is also related to low water potential and the fundamental role osmotic pressure plays in water stress responses and the resulting growth. To prevent movement of water and nutrients from inside the plant back into the root zone when low potential occurs in the soil from drought, amino acids modulate membrane permeability and ion uptake. This dehydration avoidance occurs via solute accumulation in the tissue. By accumulating proline in plant tissues *(Gzik, 1996)*, lower water potential is created within the cells without experiencing any water loss *(Verslues et al., 2006)*.

When under drought stress, silica aids in plant response by decreasing leaf transpiration *(Yoshida, 1965; Wong et al, 1972; Matoh et al, 1991)*, increasing stomatal conductance *(Chen et al, 2011)*, and maintaining chlorophyll concentration *(Lobato et al, 2009)* to sustain photosynthesis rates. Some studies have shown it even enhances root growth and in turn nutrient uptake through more root surface area *(Barber, 1984)* allowing plants to take in more potassium for the aforementioned functions.

Applying a product containing amino acids, potassium, and silica, such as AminoElite™ or AminoKelp™ before trees experience water stress, or even under drought conditions maintains photosynthesis rates, reduces fruit drop, and protects fruit tissues from dehydration and the resulting movement of water and nutrients back into the soil, preventing a reduction in fruit size.

Studies in pecan trees demonstrate that even under K-sufficient conditions, additional supplementation of potassium reduces Stage II fruit drop to improve yield *(Wood, Wells, & Funderburke, 2010)*.

- <u>Fruit number and size</u>

Another component of citrus yield is individual fruit weight, which is directly related to calcium nutrition within the fruit. Calcium influences cellular water/ionic transport mechanisms; higher concentrations of tissue calcium lead to increased water and assimilate movement into the fruit, increase the size *(Franceschi, & Nakata, 2005; Saure, 2005; Gilliham et al., 2011)*.

Calcium is generally an immobile nutrient via phloem transport so mobilising calcium within the plant must, therefore, be dependent upon other chelating substances to facilitate transport.

Phloem loading of calcium happens primarily through the apoplastic pathway *(Saeedi et al., 2015)*, compared to most other nutrients that load via the symplastic pathway and cytoplasmic cells. When calcium cations are taken into the plant, they easily enter the apoplast and interchangeably adhere to cell walls and the outer surface of cytoplasm membrane *(White, & Broadley, 2003)*, preventing phloem loading. The use of amino chelates prevents the binding of calcium with the anionic region of plant cell membranes *(Saftner et al., 2003)*.

Tissue calcium levels have been found to have a positive relationship with fruit weight.

Seaweed extracts stimulate cell division of the root cells, encouraging lateral root growth, and an overall increase in root mass.

As a result, it is possible to hypothesise these foliar applications of amino acids has converted the cationic calcium into a chelated form, allowing the calcium to easily pass through the phloem cell wall pores. This in turn improves calcium phloem mobility, and positively influences fruit weight.

Foliar-applied nutrients mainly enter the plant through the leaf stomata and the cuticle. Although the quantity of nutrients absorbed by the leaf during foliar application is small, it is compensated by a higher efficiency of nutrient uptake as opposed to applying the same quantity of nutrients through the roots. Most soils are generally unbalanced in terms of ions; proper cation/anion exchange does not allow for nutrients to enter plants easily.

Application of amino acid containing biostimulants like AminoElite™ or AminoKelp™ via foliar applications may chelate calcium cations, mobilising them within the plant tissues, and increasing fruit quality.

- Sugar Content

The most significant chemical component of fruit production is the sugar content, which is measured by degrees Brix, approximating the total dissolved solids. In citrus production, it gives an indication of how nutrient-dense and sweet the fruit is.

Fruit with a higher Brix level will have higher specific gravity, weighing more, and in turn increase the grove yield. The fruit will also be less prone to frost damage because high brix plant sap has a lower freezing point than low brix sap due to the solution concentration *(Jie, Lite, & Yang, 2003)*. Fruit with a higher Brix value also experiences less disease and insect infestations.

The nutritional status of the plant can significantly affect Brix values, demanding growers keep a close eye on leaf analysis to ensure high sugar levels in their fruit.

- Sufficient potassium is needed at fruit maturation for sugar translocation within the plant *(Conti, & Geiger, 1982)*. When potassium concentrations are low, fewer carbohydrates are moved to the fruit, and the Brix will be lower.
- High nitrate levels in the leaf resulting in a reduced Brix value. Nitrate can only be taken into the roots with a corresponding water molecule *(Ding et al., 2018)*. More water within the cells of the plant, the fruit included, means a diluted sugar concentration.
- The micronutrients boron, iron, manganese and zinc, when used effectively, increase sugar levels in the fruit.

The more efficiently citrus trees take up water and nutrients, the higher the Brix value. As biostimulants have substantiated research to document their enhancement of yield attributes of crops when applied exogenously (Panda, Pramanik, & Nayak, 2012), the use of AminoElite™ or AminoKelp™ can help improve Brix.

A greater root mass corresponds with an increased surface area for more efficient uptake of water and nutrients and a higher Brix level.

The marketability or saleability of citrus fruits is highly dependent upon its peel quality, especially good colour, proper thickness and a lack of or minimal blemishes.

- <u>Peel Quality</u>

Consumers "buy with their eyes" and desire fruit that looks pleasing. This makes peel quality an essential component of citrus management.

Colour

The peel colour in citrus fruits is an important factor in customer/consumer satisfaction. When buying fruit, a customer looks for citrus that has properly ripened to brilliant, glossy colour, with little to no green left on the peel (except in the case of limes).

Peels start off as green, full of chlorophyll to protect the fruit from sun damage during development. During ripening, citrus fruit-peel undergoes a process known as "colour break" where chloroplasts are converted to chromoplasts *(Iglesias, 2001)*. Chlorophyll is broken down by the plant, biosynthesis of additional carotenoids occurs, and then the gene expression of both existing and new carotenoids triggers to change the peel colour from green to yellow or orange.

For this colour break to occur, phytohormones and nutrients need to be in proper balance.

At the beginning of ripening, ethylene production increases and induces an increase of autocatalytic biosynthesis. Ethylene promotes an increase in the activity of chlorophyllase and oxidase enzymes *(Rodrigo, & Zacarias, 2007)*, which are responsible for chlorophyll degradation and green colour disappearance, and stimulates carotenogenesis, which promotes yellowing or oranging of fruit peel *(Porat, 2008)*. Increased levels of gibberellin retard external ripening *(Alos et al., 2006; Porat et al., 2001)*.

The crosstalk between phytohormones during ripening is still misunderstood, with more research needed. Yet again, the application of a product such as AminoKelp™ with the inclusion of all five classical phytohormones ensures proper gene expression controlling the ripening process in citrus.

Thickness

The peel of citrus fruit not only protects the flesh from insect and microbial damage, while limiting water and gas exchange, it also acts as a natural package maintaining its integrity in storage and transport.

Peel thickness requirements of fruit depend on the specific citrus type and the associated market needs. Thicker peels provide higher levels of oil for extraction, offer greater resistance against pests, and improve shelf life. Consumers in the fresh fruit market desire a thicker peel that is regarded as being easier to peel, however, an excessively thick peel is undesirable.

Research in Florida demonstrates a direct correlation between peel potassium concentrations and peel thickness *(Morgan et al., 2005)* which could be attributed to a thickening of cell walls due to potassium levels *(Datnoff, 2007)*.

A correct balance of these three major nutrients is essential.

A nutrient imbalance is a leading factor in contributing to undesirable, thicker peels. Nitrogen and potassium increase peel thickness whilst phosphorous reduces it.

The occurrence of blemishes on citrus fruit peels is related to a physical injury incurred whilst on the tree, insect damage or fungal/bacterial diseases.

Blemishes

A high rate of blemishes renders fruit unmarketable. Applications of gibberellins, specific nutrients, and amino acids can help mitigate blemish occurrence.

Watermarking occurs on citrus fruit peels when the bottom of the fruit is wet for prolonged periods, such as during wet weather. When applied at the correct rate and timing, improved quality is seen in Imperial mandarins through the application of gibberellic acid. Orchards in Bindoon, Chittering, and Harvey were studied in 2001 and 2002, demonstrating the incidence of watermark was reduced by up to 80% without any negative effects *(Department of Primary Industries and Regional Development, 2019)*.

When citrus undergoes water stress, the overall health of the plant is affected, impairing its defences against other abiotic stressors making it more susceptible to infestation of fungal infections, diseases, insects, and other pathogens.

Potassium and silica provided via foliar applications of AminoElite™ and AminoKelp™ can also help to strengthen plant defences against both abiotic and biotic stressors in citrus.

- Potassium thickens cell walls to guard against diseases and pathogens.
- Silica strengthens cell walls by increasing the production of cellulose and hemicellulose *(Van Bockhaven et al., 2013)* to protect against insects and fungal infections.

Diseases such as anthracnose, black spot, citrus canker, citrus powdery mildew, citrus tristeza virus, collar rot, melanose, Phytophthora fruit rot, Phytophthora root rot, and stem-end rot are common in Australian citrus production. Major pest problems also occur due to African citrus psyllid, Asiatic citrus psyllid, citrus fruit borer, mealybug, navel orangeworm, Queensland fruit fly, red scale, rust mites, wax scale, and white louse.

Another major problem in citrus orchards is citrus greening disease, also known as HLB (huanglongbing). It is the most destructive disease for citrus worldwide, caused by a bacterial infection. Once infected it is fatal for a tree as no efficient management measures exist for its treatment *(Canales et al., 2016)*.

There is increasing evidence that citrus trees prefer the nitrate form of nitrogen and can experience toxicity to ammonium forms of N. These increasing ammonium concentrations in the soil can also reduce uptake of potassium, calcium and magnesium and trees can test positive for, and exhibit, HLB greening symptoms.

Strengthening plant defences by potassium and silica application may reduce peel blemishes, maintaining the marketability of citrus fruit.

Applying GrowGreen products to citrus can help to boost plant defence responses against pests through various components.

Field observations and greenhouse studies demonstrate some citrus genotypes are more resistant to the pathogenic bacteria *Candidatus Liberibacter asiaticus* (CLas) than others. While the mechanisms triggering tolerance are yet to be understood, the levels of most amino acids, especially those implicated in plant defence to pathogens such as phenylalanine, tyrosine, tryptophan, lysine, and asparagine were higher in the tolerant citrus varieties *(Killiny, & Hijaz, 2016)*.

GrowGreen have a range of products that provide supplementation of amino acids, strengthening of the overall health of the citrus tree and preventing other management problems. Increasing membrane rigidity by silica/potassium/amino acid supplementation and strengthening the tree's immune system through the addition of beneficial microbes in the soil can also help to increase the overall resistance of a citrus tree against bacterial or fungal infections.

Conclusion

Proper management practices are important in citrus production to garner the highest yields and quality fruit. This is increasingly important in Australian citrus production as growers face market competition and concerning water shortages. Groves must be carefully managed through the different growing stages to ensure optimal production. The use of GrowGreen products such as AminoElite, AminoKelp™, Microbe Plus® Potassium, Microbe Plus® PhosCal, and Microbe Plus® Citrus can provide producers with sustainable options to improve citrus yield and quality.

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