EFFECTS OF BIOSTIMULANTS AND FERTILIZATION METHODS ON POTTED 'CHOCOLATE BEAUTY' BELL PEPPERS GROWN IN A PROTECTED STRUCTURE

by

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ABSTRACT

Three experiments were conducted in order to assess the effects of combining different post-transplant N fertilization methods (GUF, SOF, & SUF) with commercially available foliar biostimulants (AQC, VTZ, SPX, & WTR) on the growth, yield, and fruit quality of potted 'Chocolate Beauty' bell peppers grown in a Quonset-style greenhouse at the Finca Laboratorio Alzamora in Mayagüez, Puerto Rico. A broad mite infestation heavily affected the first experiment. In the first experiment, the combination of SOF + VTZ produced the greatest number of leaves, nodes, and buds at 71 DAS. The SOF method produced the greatest number of leaves, nodes and fruitlets at various stages during the crop cycle. The SOF method also produced the highest amount of total fruits per plant, and the greatest fresh and dry weight of shoots and roots. On the contrary, the SOF method produced unmarketable and total fruits with the shortest length. None of the biostimulants produced more marketable fruit weight per plant than the WTR (control). The highest amount of nitrate in fresh sap was obtained with the SOF method and the thickest pericarps of unmarketable and total fruits were achieved by combining SOF + AQC and SOF + VTZ. In the second experiment, the SUF method produced the greatest shoot dry weight and the most marketable and total fruits per plant. The highest marketable fruit weight per plant was achieved with the SOF + SPX combination. None of the biostimulants produced more total fruit weight per plant than the WTR (control). The greatest length and width of marketable fruits was obtained with the combination SOF + SPX. The thickest pericarps of marketable fruits were achieved with the SUF + WTR combination and the highest brix values of marketable fruits were obtained with the SUF + AQC, GUF + SPX, and SOF + VTZ combination. The earliest unmarketable and total fruit harvest times were obtained with the SUF + AQC and GUF + VTZ combinations while the latest were obtained with the SOF + SPX combination. The SUF and SOF methods produced the highest SPAD values. In the third experiment, the greatest number of leaves, nodes, and plant height is achieved with the SUF method. The SUF method also produced the highest amount of buds, flowers, and fruitlets during early development stages but the SOF method was not significantly different from the SUF on these variables after 47 DAT. Abscission of buds, flowers, and fruitlets was highest with the SUF method at 62 DAT but abscission of buds and fruitlets was highest with the SOF method at 70 DAT. The AQC biostimulant had the least amount of bud abscissions at 62 DAT also. In addition, the SUF method also produced the highest marketable fruit weight and quantity per plant, and the greatest fresh and dry weight of shoots and roots. The SOF + WTR combination produced marketable fruits with the greatest length but none of the biostimulants produced wider marketable fruits than the WTR (control). The earliest marketable and total fruit harvest times were obtained with the SUF method while the latest were obtained with the GUF and SOF methods.

RESUMEN

Se llevaron a cabo tres experimentos para evaluar los efectos de la combinación de diferentes métodos de fertilización de N post-trasplante (GUF, SOF y SUF) con bioestimulantes foliares comercialmente disponibles (AQC, VTZ, SPX y WTR) sobre el crecimiento, rendimiento y calidad del fruto del pimiento morrón "Chocolate Beauty" en tiesto bajo un invernadero estilo Quonset en la Finca Laboratorio Alzamora en Mayagüez, Puerto Rico. El primer experimento fue afectado por una infestación de ácaros. En el primer experimento, la combinación de SOF + VTZ produjo el mayor número de hojas, nudos y capullos a los 71 días después de la siembra. El método SOF produjo el mayor número de hojas, nudos y frutos en varias etapas durante el ciclo del cultivo. El método SOF también produjo la mayor cantidad de frutos totales por planta y el mayor peso fresco y seco de vástagos y raíces. Por el contrario, el método SOF produjo frutos no comercializables y totales de menor longitud. Ninguno de los bioestimulantes produjo más peso de frutos comercializables por planta que el WTR (control). La mayor cantidad de nitrato en savia se obtuvo con el método SOF y los pericarpios más gruesos de frutos no comercializables y totales se lograron combinando SOF + AQC y SOF + VTZ. En el segundo experimento, el método SUF produjo el mayor peso seco de vástagos y la mayor cantidad de frutos comercializables y totales por planta. El mayor peso de frutos comercializables por planta se logró con la combinación SOF + SPX. Ninguno de los bioestimulantes produjo más peso total de frutos por planta que el WTR (control). La mayor longitud y anchura de los frutos comercializables se obtuvo con la combinación SOF + SPX. Los pericarpios más gruesos de frutos comercializables se obtuvieron con la combinación SUF + WTR y los valores más altos de brix en frutos comercializables se obtuvieron con las combinaciones SUF + AQC, GUF + SPX y SOF + VTZ. Las cosechas más tempranas de frutos no comercializables y totales se obtuvieron con las combinaciones SUF + AQC y GUF + VTZ, mientras que las más tardías se obtuvieron con la combinación SOF + SPX. Los métodos SUF y SOF produjeron los valores más altos de SPAD. En el tercer experimento, el mayor número de hojas, nudos y altura de la planta se logró con el método SUF. El método SUF también produjo la mayor cantidad de capullos, flores y fructificación durante las etapas tempranas de desarrollo, sin embargo, el método SOF no fue significativamente diferente al SUF respecto a estas variables después de los 47 días después del trasplante. El aborto de capullos, flores y frutos fue mayor con el método SUF a los 62 días después del trasplante, pero el aborto de capullos y frutos fue mayor con el método SOF a los 70 días después del trasplante. También, el bioestimulante AQC tuvo la menor cantidad de abortos de capullos a los 62 días después del trasplante. Además, el método SUF produjo el mayor peso y cantidad de frutos comercializables por planta, y el mayor peso fresco y seco de vástagos y raíces. La combinación SOF + WTR produjo los frutos comercializables con la mayor longitud pero ninguno de los bioestimulantes produjo frutos comercializables más anchos que el WTR (control). El tiempo de cosecha más temprano de frutos comercializables y totales se obtuvo con el método SUF y el más tardío se obtuvo con los métodos GUF y SOF.

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DEDICATION

This thesis is dedicated to my parents Pedro E. Cruz González and Marie C. Carballo Lugo, and my sister, Claudette M. Cruz Carballo. Thank you for always providing me with unconditional support during the hardest and most challenging times during this research.

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General Introduction

Evidence suggests that greenhouse-like structures had been built by the Roman Empire ever since the first century AD. These structures probably originated by the human desire to produce certain crops in places where they normally wouldn't thrive without the extra protection. Also, by protecting crops and providing proper management, they could produce a fair and constant supply of fresh produce (Paris & Janick, 2008). People soon realized the many benefits of producing crops using protective structures. This relatively new technology proved to be an agricultural milestone because it enabled farmers to produce out-of-season crops and even exotic crops that are not even grown in that area. Greenhouses provided a different, alternative, and versatile way of producing, cultivating, researching, and preserving high-value plants and crops of economic, cultural, and/or medical importance (Elliot, 2008).

Today's greenhouses are able to provide farmers with many advantages depending on the level of technology and amount of resources that are used to build, run, and manage the protected structures. The top part of the greenhouse can shield crops against hail, precipitation, and solar radiation, while the walls can protect against strong winds and can keep out unwanted people, animals, insects, and other pests. Geographic location of the greenhouse dictates the kinds of microclimates that can be created and maintained inside the structures in order to extend the plants' growing season, optimize available light, manage water loss via transpiration and even help lower temperatures and control relative humidity (Lin & Saltveit, 2012). Most of the variables required to successfully grow healthy and high-yielding plants can be manipulated in the greenhouses without much effort. It’s generally easier to safely and reliably control the environment inside a greenhouse rather than trying to control the open-field agroecosystem and all of the variables that compose it. This opens the way for new, more precise research to be made in areas of agronomy and horticulture such as plant breeding, medicinal plants, endangered plant species, disease resistance, and direct relations between yields and fertilization rates (Elliot, 2008). All of these attributes that are brought forth by the use of greenhouses can be combined and turned into a profitable alternative for efficiently growing horticultural crops.

Horticulture is a diverse branch of agriculture whose main focus is growing plants used by people for food, medicine, and aesthetic gratification. On the other hand, olericulture is a branch of horticulture whose main objective is the production of herbs and vegetable crops. The USDA defines “crops” as any plant that is cultivated for the purpose of sale or subsistence. Vegetable crops are specifically described as herbaceous plants that are usually annuals, but some are perennials, and which provide a portion that can be consumed either raw, fresh, processed, or cooked during meals (Nösberger et al., 2001). According to Section 101 of the Specialty Crops Competitiveness Act of 2004 (7 U.S.C. §1621 note) and amended under section 10010 of the Agricultural Act of 2014, Public Law 113-79 (the Farm Bill) defines a specialty crop as “*fruits and vegetables, tree nuts, dried fruits and horticulture and nursery crops, including floriculture*” (Vilsack & Reilly, 2012).

Penn State Extension, (2015) specifies that “specialty vegetables” refer to a group of crops that fit into niche markets and may sometimes be called “exotic” or “alternative” because they represent new enterprises that are unlike the common, traditional, standard vegetables. They describe a specialty vegetable as "*new and unusual in the manner they are produced (organic, hydroponic); in the color, shape or flavor of the varieties grown (heirloom varieties); in their size (baby, miniature, micro); or in their ethnic origins and demand*". Growing specialty crops with the added protection and benefits of a greenhouse would be a strategic decision that must be based on estimated costs and expected profits in a given time margin. In greenhouses, some commonly cultivated vegetable species are usually cold sensitive. They are characterized by having thermal requirements that range from medium to slightly high (thermophilic, 17 to 28°C) and are grown in protected structures with the objective of extending the production season beyond the conventional growing season (Castilla, 2013).

Vegetables pertaining to the Solanaceae botanical family are being produced in greenhouses worldwide with great success and are a very important source of nutrition in the human diet. Solanaceous crops include potatoes (*Solanum tuberosum*), eggplant (*Solanum melongena*), tomato (*Solanum lycopersicum*), and peppers (*Capsicum* spp.), to name a few. Among these, the *Capsicum* genus holds one of the most widely used vegetables around the world. Peppers are very unique due to the fact that they can be used either as a spice or as a vegetable. Only five species of *Capsicum* have been domesticated to this day: *C. frutescens*, *C. chinense, C. baccatum*, *C. pubescens*, and most importantly, *C. annuum* (Bosland & Votava, 2012).

According to Shaw & Cantliffe (2002), consumption of high-quality colored bell peppers in the US has been steadily rising over the past decade. Countries like Mexico, Spain, the Netherlands, and Canada have seized the opportunity to export these high-quality greenhouse-grown bell peppers into the US in order to meet the consumers' demand. As a result, market prices tend to be above average, which in turn has encouraged greenhouse growers to invest in this specialty crop and also expand the total greenhouse-grown bell pepper area. Peppers grown in greenhouses are harvested when the fruit is ripe and fully colored. Plants can produce higher-quality pepper fruits with greater yields and can even fetch higher prices for off-season production (Jovicich et al., 2004). Many variables within the greenhouse have to be monitored, managed, and taken into consideration in order to achieve and obtain the highest possible marketable fruit yields. Greenhouse location, plant density, growing media, cultivar, weeds, temperature, air circulation, relative humidity, solar radiation, insect pests, irrigation timing, and fertilizer applications must always be taken into consideration prior to the establishment of plants.

The growth, yield, and fruit quality of pepper plants are heavily influenced by the fertilization methods used to deliver the required nutrients at an optimum rate (Ghoname et al., 2009). Information regarding the amounts of fertilizer required for an optimum plant nutrition in a greenhouse operation should be considered a suggestion instead of a rule because factors such as plant type, cultivar, temperature, medium composition, time and method of application, amount of water and nutrient solution, and day length can affect nutrient uptake and ultimately the efficiency of the materials used (Lin & Saltveit, 2012).

Fertilizers can provide plants with many nutrients that are essential for a proper and adequate development. Nutrients are divided into macro- and micronutrients. Manganese (Mn), chlorine (Cl), boron (B), copper (Cu), nickel (Ni), molybdenum (Mo), zinc (Zn), and iron (Fe) are called micronutrients or trace elements. Nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), sulfur (S), and magnesium (Mg) are considered macronutrients because plants consume these nutrients in larger quantities. N plays a major role in the plant's nutrition because of its many functions in plant physiology. It is found in the chlorophyll molecule, the nucleic acids, all plant proteins, enzymes, and cell walls. An insufficient amount of readily available N results in a poor fruit set, size, and number (Cook, 2013), while plants tend to be stunted, yellowish, and generally look sickly (Addiscott, 2005).

The Agricultural Experimental Station (AES) in the University of Puerto Rico (UPR) has devised a controlled fertilization program for field pepper production in order to minimize the potential risks of environmental contamination. Soil analysis is a highly recommended practice prior to fertilization in order to prevent excessive applications of N. An oversupply of N is detrimental to the crop because it over-stimulates vegetative growth and flower and fruit abortion (del Castillo et al., 2004), reduces fruit development and overall yield, increases the plant and fruits' vulnerability to diseases, and also turns into economic losses for farmers. Puerto Rico's AES recommends N fertilization rates between 173 and 231 $kg \cdot ha^{-1}$ for high and low total N content in soil, respectively. They suggest that 25% of the total N should be incorporated into the soil as a pre-plant fertilizer and the remainder can be applied as a solution in the irrigation line every 7, 10, or 14 days throughout the crop cycle, or as granular at the beginning of the flowering stage (Fornaris et al., 2005).

The N fertilization rates suggested by the AES of the UPR for field pepper production coincide with the findings of Hartz et al., (1993) at the University of California, Davis, in which drip-irrigated 'Capistrano' bell pepper fruit yield and mean fruit size peaked with N rates of 168 to 252 $kg \cdot ha^{-1}$. Castilla (2013) estimates that pepper plants have an approximate nutrient uptake of 180 $kg \cdot ha^{-1}$ of N. In addition, Hochmuth (1992) from the University of Florida also suggested a fertilization rate of 180 $kg \cdot ha^{-1}$ of N for drip-irrigated pepper production. Penn State Extension, (2015) recommends a similar total N application of 112 to 202 $kg \cdot ha^{-1}$ for commercial pepper production depending on the soil-test report. However, Fonseca-Fonseca & Piña-Antúnez (2006) at the "Jorge Dimitrov" Agricultural Station in Cuba concluded that only 120 $kg \cdot ha^{-1}$ of N were needed in order to produce the highest yield of pepper fruits, cv. 'Bouquet-50'. Acquaah, (2009) agrees that pepper plants do respond to N fertilizations, but contrary to these researchers, Bowen & Frey, (2002) did not find a positive yield response in 'Bell Boy' bell pepper to increasing N fertigation from 31.5 to 63 $kg \cdot ha^{-1}$ at British Columbia, Canada, and hence, stated that peppers require low N fertilizations rates for near maximum production.

In addition to an adequate fertilization program, many producers have incorporated the application of products called "biostimulants" in order to improve crop production (Ghoname et al., 2009). Biostimulants are being used in organic and conventional agriculture as well; they are mainly applied on high-value crops such as fruit trees, flowers, ornamentals, open-field vegetables, and greenhouse crops for the purpose of increasing yields and quality of products without causing a negative impact on the environment (Colla & Rouphael, 2015). The Biostimulant Coalition in the US defines a biostimulant as "*a material that, when applied to a plant, seed, soil or growing media – in conjunction with the established fertilization plans, enhances the plant's nutrient use efficiency, or provides other direct or indirect benefits to plant development or stress* *response*" (Beaudreau Jr., 2016). The European Biostimulants Industry Council (EBIC) in Europe defines a plant biostimulant as "*a material that contains substance(s) and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independent of its nutrient content*" (EBIC, 2015). The American Association of Plant Food Control Officials (AAPFCO) in the US does not have a definition for plant biostimulants but rather defines a "beneficial substance" as "*any substance or compound other than primary, secondary, and micro plant nutrients that can be demonstrated by scientific research to be beneficial to one or more species of plants, when applied to the plant or soil*" (AAPFCO, 2012).

Despite the recent and rapid increase in the amount of peer-reviewed articles regarding the use of plant biostimulants in agriculture (Colla & Rouphael, 2015), there is still no legal or regulatory definition that has been accepted at a worldwide level, and thus, du Jardin (2015) proposes that a plant biostimulant is "*any substance or microorganism applied to plants with the aim of enhancing nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. Plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms.*" Even though a proper and globally accepted definition for plant biostimulants has not yet been decided, there is agreement between scientists, regulators, and industry stakeholders that biostimulants can begin to be classified into recognizable categories. These proposed categories include but are not limited to: (1) humic & fulvic acids, (2) beneficial fungi, (3) beneficial bacteria, (4) chitosan & other biopolymers, (5) inorganic compounds, (6) protein hydrolysates & other N-containing compounds, and (7) seaweed extracts (SWE) & botanicals (du Jardin, 2015).

General Objective

The main objective of this study is to determine the effects of select biostimulants combined with different fertilization methods on the growth dynamics, yield parameters, and fruit quality of potted ‘Chocolate Beauty’ bell peppers (*Capsicum annuum* L.) grown in a Quonset-style greenhouse in Puerto Rico.

Chapter 1. Effects of foliar biostimulants and fertilization methods on ‘Chocolate Beauty’ bell peppers affected by biotic stressors in a passively ventilated greenhouse in Puerto Rico

1.1 Introduction

Bell peppers (*Capsicum annuum*) are one of the most important solanaceous crops in the vegetable production industry of Puerto Rico. More than 95% of the bell pepper production concentrates on the south region of Puerto Rico, specifically in the municipalities of Guánica, Juana Díaz, Lajas, and Santa Isabel (Fornaris et al., 2005). The production of bell peppers has been steadily rising during recent years. There was a 617 and 631% increase in production (kg) and value ($) respectively in the fiscal year 2013-2014 (Figure 1). During the fiscal year 2010-2011 through 2013-2014, bell pepper prices in Puerto Rico increased by 65%, with an average annual increase rate of 16% (Figure 2).

Bell peppers are sometimes called sweet bell peppers meaning that they are not pungent, spicy or hot. However, in Puerto Rico sweet peppers are typically known as “ají dulce” (*Capsicum chinense*). The word “sweet” will not be used during study in order to avoid confusion with *C. chinense*. The supply and availability of specialty bell peppers of uncommon colors in Puerto Rico is currently very limited or non-existent. The production season of peppers in Puerto Rico is usually during the dry season (December – March) to diminish risks of floods and pathogens that thrive during the wet season. The variety or cultivar used in production can directly influence yields and quality of pepper fruits. There is currently a lack of bell pepper varieties that have performed consistently well during the last two decades in Puerto Rico. Some promising hybrid bell pepper varieties for Puerto Rico include ‘Camelot’ and ‘Enterprise’. Open-pollinated bell pepper varieties like ‘Emerald Giant’, ‘Jupiter’, and ‘Yolo Wonder L’ have also been cultivated in Puerto Rico. The AES in the UPR developed an open-pollinated bell pepper variety called ‘Puerto Rico Wonder’ but it has not been cultivated for many decades. None of these varieties develop uncommon colors such as brown or purple when the fruits reach maturity.

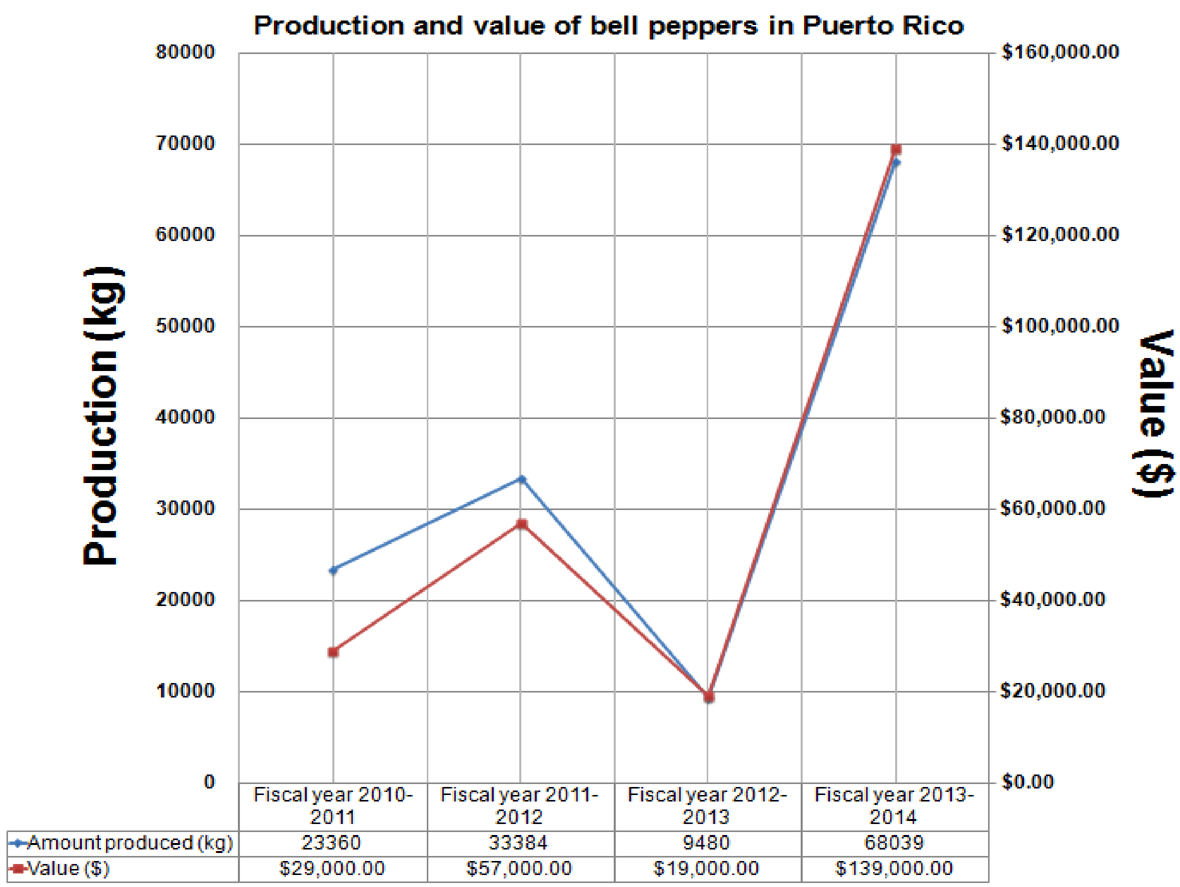


Figure 1. Recent market tendencies of bell pepper production (kg) and value ($) in Puerto Rico

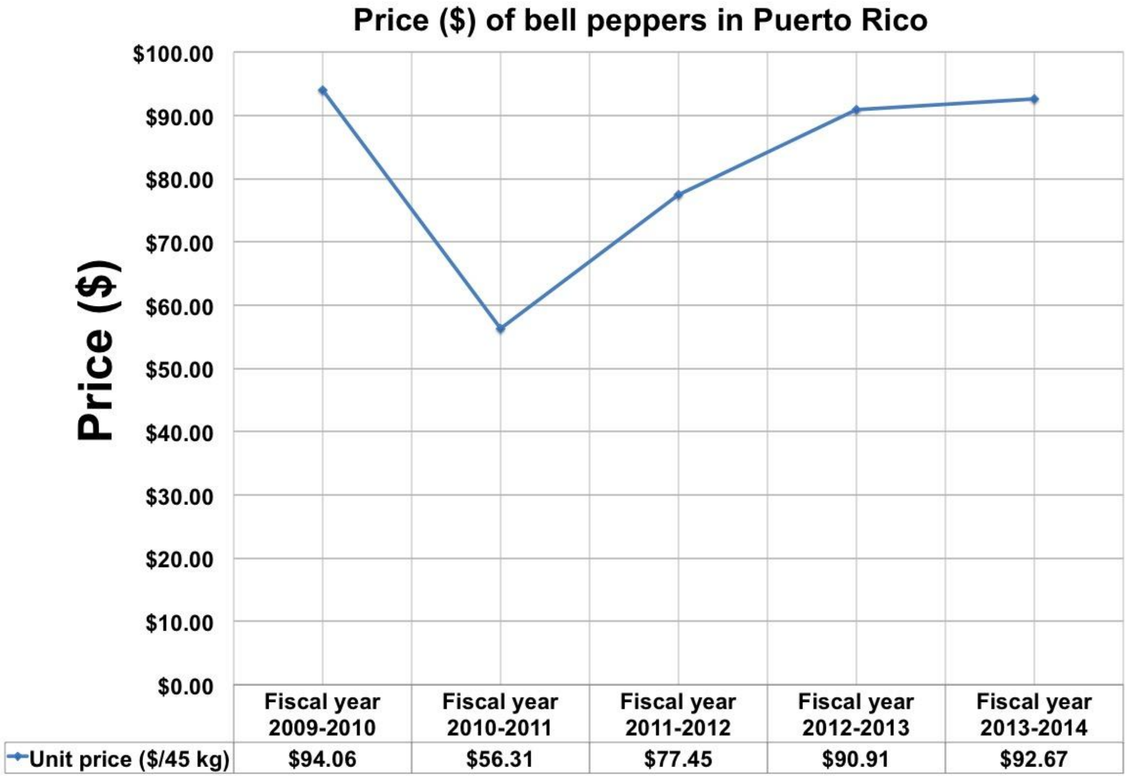


Figure 2. Recent price ($) per unit (quintal) tendencies of bell peppers in Puerto Rico

Bell peppers have high quantities of vitamins A & C and are usually consumed at the mature stage. Even though fruit production correlates negatively with the amount of fruits that are already developing, additional vegetative and reproductive growth occur while the fruits are being harvested (Fornaris et al., 2005). Bell peppers are warm season crops of herbaceous nature with woody stems and an erect arrangement. They show semi-indeterminate growth characteristics with plenty of ramifications and are usually cultivated as an annual crop. Leaves are simple and alternated, usually with an oval or lance shape and a pointy end. Even though root growth is vigorous and moderately deep, most of the root system will develop at depths of 30 - 45 cm. It is susceptible to competition by weeds due to its slow foliar development and root growth is limited by transplanting and poor irrigation.

Flowers measure approximately 1.3 cm across and are usually white, hermaphrodites with five stamens and one pistil in each flower. These tend to stay open for at least 24 hours and are self-pollinated but cross-pollination can also occur by insects and bees. Air temperature remains the most important factor during the flowering stage. In botanical terms, the fruit is classified as a non-fleshy berry with 2-5 lobules that are separated internally by incomplete walls that create a cavity when developed. The *C. annuum* species vary greatly in pungency, length, width, shape, color, and pericarp texture. Bell peppers are usually big, with varying lengths and widths from 7.6 to 12.7 cm. The shape is predominantly blocky with smooth and thick external walls. Fruits are commonly green while immature and can attain different colors when ripened (e.g. red, orange, yellow, purple, brown, etc.)

Optimum average monthly temperatures required for proper plant development range between 21 and 24°C. Average monthly temperatures under 18°C and over 27°C can become a limiting factor in production. Flowers will drop when night temperatures exceed 24°C and, even though the plant can tolerate day temperature of over 38°C, yields, pollination, and fruit development will be affected. Growth rates peak during the vegetative stage when day temperatures range from 25 to 27°C and night temperatures range from 18 to 20°C (Fornaris et al., 2005).

The supply and availability of specialty bell peppers of uncommon colors in Puerto Rico is currently very limited or non-existent. The production season of peppers in Puerto Rico is usually during the dry season (December – March) to diminish risks of floods and pathogens that thrive during the wet season. The variety or cultivar used in production can directly influence yields and quality of pepper fruits. There is currently a lack of bell pepper varieties that have performed consistently well during the last two decades in Puerto Rico. Some promising hybrid bell pepper varieties for Puerto Rico include ‘Camelot’ and ‘Enterprise’. Open-pollinated bell pepper varieties like ‘Emerald Giant’, ‘Jupiter’, and ‘Yolo Wonder L’ have also been cultivated in Puerto Rico. The AES in Puerto Rico developed an open-pollinated bell pepper variety called ‘Puerto Rico Wonder’ but it has not been cultivated for many decades.

None of these varieties develop uncommon colors such as brown or purple when the fruits reach maturity. ‘Chocolate Beauty’ bell pepper’s original breeder and vendor was a company called Petoseed founded in California in 1950. This company led the hybridization of hot peppers and tomatoes in the 1970s and 80s. The variety was developed in 1993 and is described as a non-pungent pepper with large, smooth bell type. Fruits turn from a medium-dark green to a chocolate brown color when fully mature and it is also resistant to Tobacco Mosaic Virus (Bosland & Wehner, 1999).

Once a crop has been established, the environment plays an important role in the presence or absence of abiotic stress factors. Such factors can severely impact yields, quality and productivity of crops. Some common abiotic factors include drought, salinity, heat, cold, light intensity and anaerobic stress. It is estimated that a combination of drought and heat stress in the United States would cause agricultural losses of $200 billion (Suzuki et al, 2014). Drought and heat are the worst combination of abiotic factors that could happen in terms of agricultural economic losses. Current climate prediction models indicate a combination of these two abiotic stress factors in the near future (Meehl et al., 2007). Recent studies in Puerto Rico show that precipitation is predicted to decline and that drought intensity will increase either gradually or linearly. At the same time, climate will continue getting warmer and will consequently increase energy demands as well. Drought and heat could push the animal production industry towards enclosed structures with climate control. Other climate-related interactions could emerge such as forest fires and increased insect pressure (Henareh Khalyani et al., 2016).

Biotic stress factors such as diseases, pests, nematodes, and other pathogens that are present under local conditions are another important environmental characteristic that must be considered when making critical decisions. These factors of biologic origin can be directly and/or indirectly influenced by abiotic factors. Climate change can alter the habitat range of pest and pathogens. It would facilitate the spread of pathogens and also weaken the plant's defense mechanisms, making them less resilient while increasing their susceptibilities and thus, the number of possible hosts (Acquaah, 2009). Crops cultivated in open fields will most likely face a greater amount of different stressors and will be exposed to an increased amount of diverse abiotic and biotic conditions, and their combinations also (Suzuki et al, 2014). Resistance to pests and pathogens could potentially reduce costs of insect control and disease treatments but, insect-resistant or insect-tolerant bell pepper varieties are very uncommon (Fornaris et al., 2005). This chapter will focus on the effects of select biostimulants of different categories on the growth dynamics and yield parameter of 'Chocolate Beauty' potted specialty bell peppers grown under a protected structure with biotic stressors.

1.2 Objectives

1. To determine the effects of select foliar biostimulants with different fertilization methods on ‘Chocolate Beauty’ bell peppers affected by biotic stressors.
2. To determine the effects of select foliar biostimulants with different fertilization methods on the morphological development of ‘Chocolate Beauty’ bell peppers.
3. To determine the effects of select foliar biostimulants with different fertilization methods on the yield and quality of ‘Chocolate Beauty’ bell peppers.

1.3 Methodology

1.3.1 Site description

The experiments were conducted in Greenhouse #5 (latitude: 18° 12’57” N, longitude: 67° 08’48” W, elevation: 15 meters above sea level) located in a Greenhouse Range of six freestanding, even-span Quonset style greenhouses arranged side by side and built upon a continuous concrete foundation at the Finca Laboratorio Alzamora in the UPR at Mayagüez (Figure 3, left). The entry road was towards the front of the greenhouse and a forest-like vegetation growth was towards the rear end. The adjacent greenhouse #6 was being used for an aquaponic system that grew tilapia (*Tilapia* spp.) and spearmint (*Mentha spicata*) and the other greenhouses #1 through #4 were used as nurseries for ornamental plants and vegetable seedlings. The experiment was replicated three times. The first experiment took place on December 2013 through April 2014.

1.3.2 Quonset style greenhouse

The experimental Quonset style greenhouse structure is based upon an arched roof (Figure 3, right) (Omid & Shafaei, 2005). It had a galvanized steel frame and support posts which were embedded into the concrete floors. The greenhouse measured 14.6 meters (m) long (L), 9.1 m wide (W), 4.6 m high (H), and had a bow length (B) of 15.2 m. The total floor surface area of the greenhouse was 133 m^2. On the inside, there were four floor areas with soil that measured 12.2 m long by 1.8 m wide. Each soil-floor surface area was 22 m^2. The total soil-floor surface area measured 88 m^2. This is equivalent to 66% of the total greenhouse area. Each of the soil-floor areas was covered with a black

polyethylene (PE) landscape fabric in order to suppress germination and growth of weeds (Figure 4, left).

Figure 3. (left) Satellite image of the Quonset style greenhouse range at the Finca Laboratorio Alzamora in the University of Puerto Rico, Mayagüez Campus and (right) experiments were done inside a Quonset style greenhouse without walls at the Finca Laboratorio Alzamora, Mayagüez, Puerto Rico.

1.3.3 Roof structure and material

The roof of the greenhouse was made from a nonporous single layer of transparent PE film without any sort of vents (Figure 3, right). This material is low in cost and lightweight. Light transmission is improved by using only one layer of PE film instead of two (Omid & Shafaei, 2005). Also, PE accelerates heat loss because this material allows easy passage of the reradiated heat energy emitted by the plants and soil inside the greenhouse (Worley, 2009) An accelerated heat loss rate was necessary due to Puerto Rico's year around warm tropical climate. Even though the roof was transparent, light transmission was decreased due to dirt, dust, debris, mold, leaf litter, and plant resin accumulation on top of the greenhouse (Figure 3, right). These impurities were probably originated by the forest-like growth at the rear end of the greenhouse (Figure 4, right).

Figure 4. (left) Black PE landscape fabric was placed over the soil-floor area under the raised benches to prevent growth of weeds and (right) forest-like growth located at the rear end of the experimental Quonset style greenhouse, Mayagüez, Puerto Rico.

Airborne dust, dirt and debris captured by the trees' leaves were probably deposited on the greenhouse roof by the rain. These impurities, in addition to the leaf litter that had already accumulated, stained the roof and gave way to mold growth (Figure 3, right). Black olive trees (*Bucida buceras*) in the forest-like growth at the rear of the greenhouse continuously exuded a dark, sticky, and staining, material (Francis, 1989; Gilman & Watson, 1993) that deposited on the roof and thus, diminished light transmission. It is also important to mention that these trees and other vegetation served as a habitat for the birds Red-legged thrush (*Turdus plumbeus*) and the Pearly-eyed Thrasher (*Margarops fuscatus*) (Figure 5, left) (Delannoy-Juliá & Mari-Mut, 2013), which heavily affected the first experiment due to the constant feeding on physiologically mature pepper fruits before they could be harvested (Figure 5, right).

Figure 5. (left) Pearly-eyed Thrasher bird feeding on mature 'Chocolate Beauty' bell peppers and (right) severe damage caused by birds feeding on 'Chocolate Beauty' bell peppers, Mayagüez, Puerto Rico.

1.3.4 Walls

The Quonset style greenhouse range was rather basic and low cost because none of the greenhouses had any materials covering the walls. The Quonset style greenhouse did not have walls on the front, rear, or sides of the structure (Figure 3, right), only the galvanized steel support posts that were embedded into the concrete and located at the sides of the greenhouses to provide a firm foundation. The design was basic and probably inexpensive to build because it had no climate control features, but the absence of walls could have possibly improved air movement. This could remove hot air, lower relative humidity and accelerate the structure's natural cooling capacity (Kessler, 1998). Even though the absence of walls allowed bees, beneficial insects and air to move freely within the greenhouses, it left the plants vulnerable to strong winds and the entrance of pests and other unwanted organisms (e.g. insects, mites, birds, cats, chickens, iguanas, unwanted people, etc.) that could ultimately result in damages to plants, yields and/or fruits.

1.3.5 Raised benches

Four longitudinal, galvanized steel raised benches (GSRB) that measured 12.4 m long by 1.5 m wide and 0.64 m high were placed directly above each of the soil-floor areas (Figure 4, left). The aisles between each GSRB were approximately 0.62 m wide. The individual GSRB had a surface area of 18.8 m^2 and their total surface area combined was 75.2 m^2. This meant that only 57% of the greenhouse area was devoted to growing plants. According to Kessler (1998), this is a rather low percentage. He states that with proper planning, 70-80% of the greenhouse's total surface area can be dedicated to growing plants. The top surface of the GSRB was made out of weld mesh with a rectangular grid and welded at each joint. The weld mesh facilitated air movement and allowed the growing medium to freely drain excess water through the drainage holes at the bottom of the containers (Kessler, 1998). The excess drainage water was then captured by the soil under each GSRB.

1.3.6 Containers

According to Acquaah (2009), the correct size of containers should be based on the plant's height. The plant's height should be approximately two times the container's height. Plastic containers were washed, cleaned, and disinfected using a 10% hypochlorite solution before being used. Tap water was used to give the containers a final rinse before placing them upside down to air dry on top of the raised benches. The standard sized, 5-gallon containers were lightweight and black color (Figure 4, left). Each container measured 30 cm long and had a 30 cm diameter (Nursery Supplies Inc., Chambersburg, Pennsylvania). The containers were arranged into longitudinal columns of 15 pots and horizontal rows of 4 pots for a total of 60 containers on each GSRB (Figure 3, right). The distance between the longitudinal columns of containers was 43.2 cm and the distance between the horizontal rows of containers was 76.2 cm. Containers were labeled on the outside with the corresponding treatment for easy identification during treatment applications.

1.3.7 Growing medium

Unsterilized alluvial soil (UAS) was collected from the "Quebrada de Oro" brook that runs across the Mayagüez campus (Figure 6). This brook directs the excess runoff water from precipitation events towards the Mayagüez bay. The water flow carries sediment and eroded soil from higher elevations that gradually accumulates and deposits at the bottom of the brook. The accumulated sediment and soil that gets scooped from the brook during regular maintenance events is transported to the Finca Laboratorio Alzamora. It is then left outdoors for a period of time until it eventually gets mixed with compost, sieved, and sold for income. The UAS used in the first experiment was not sieved or mixed with anything.

A soil analysis was made to the UAS and indicated a pH of 7.77 and a conductivity of 1013 μS/cm with 0.96% organic matter. Its structure was composed of 25.04% clay, 23.44% silt, and 51.52% sand. According to the soil textural triangle, this soil had a sandy clay loam texture (Appendix 1). For the first experiment, the pots were filled with UAS only, but plant growth seemed to be limited probably because it contained big soil aggregates, vast amounts of rocks, and many other impurities (e.g. broken glass, plastics, trash, etc.) that possibly interfered with water percolation and obstructed root exploration.

1.3.8 Irrigation

During the first experiment, three seeds were sown directly in the containers and were irrigated daily by hand. Approximately 100 mL of water were applied to seeds once or twice each day. An automated drip irrigation system was installed in January 26, 2014, or 44 days after sowing (DAS) in order to provide the plants with a steady supply of water on a timed basis. It consisted of a black, 13 mm polyethylene hose laid longitudinally along the center of each GSRB, between the second and third columns of pots. The hose was then punctured to insert plastic connectors (6mm) (RAINDRIP®. Fresno, California). Poly tubing (6.35 mm outer diameter x 4.32 mm inner diameter ± 2%) (RAINDRIP®. Fresno, California) was then attached to the connectors and pressure compensating drippers (2 L/hour) (RAINDRIP®. Fresno, California) were inserted at every end (Figure 7). Each dripper was placed near the plant's stem in each container.

The water source for irrigation events was a well located at a higher elevation in the Finca Laboratorio Alzamora. A 25-psi pressure regulator was added to the irrigation system to ensure an even water emission out of every dripper. A Steel Spin Clean® (Agricultural Products, Inc.) filter with a 150 mesh was used to remove debris and prevent clogging of drippers. The irrigation system was connected to an electric 12-Station Outdoor SST "Simple to Set" Irrigation Timer (RAINBIRD® model SST1200O).

Irrigation frequency was estimated and gradually increased according to visual assessments (e.g. wilting plants, dry substrate), plant growth stage (e.g. vegetative stage, flowering stage), air temperature, container size, and the minutes required for the growing medium to drain excess water.

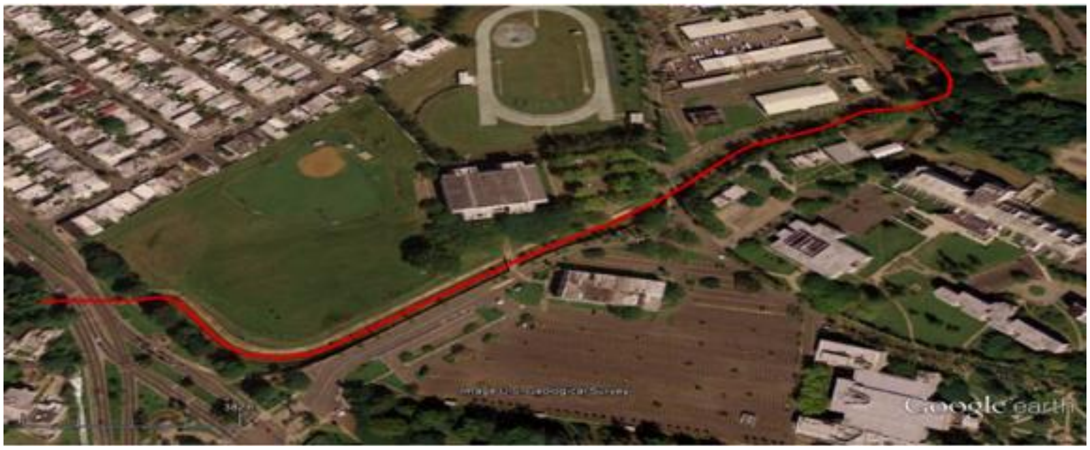


Figure 6. Aerial view of the Quebrada de Oro brook from where the alluvial soil was collected, Mayagüez, Puerto Rico (Piñero-Lugo, 2013).

Figure 7. Drip-irrigation system arrangement along the center of each galvanized steel raised bench.

1.3.9 Stakes

Pepper plants carry significant weight at fruit set and additional support is needed in order to prevent bending and breaking of stems and branches (Acquaah, 2009; Cánovas-Fernández, 2011). The use of stakes also maintains plants and branches in an upright position that allow light exposure to be optimized (Bowen & Frey, 2002). In addition, the absence of walls in the experimental Quonset style greenhouse left plants vulnerable to damage by strong winds. Dowels made from pinewood were purchased at a local hardware store and used at stakes. The 6 mm dowels measured 91 cm long and were inserted into the substrate approximately 8 cm apart from the stems when the plants reached a height of 30 cm. Additional dowels were used for extra support if needed. Stems and branches were attached to the dowels using 15 and 8 cm long plastic zip ties. About 50% of the dowels had to be replaced during harvesting because the irrigation water in the substrate rotted the dowels' wood and made them fragile. The weight of fruit set easily broke the already weakened dowels.

1.3.10 Pest management

Plant oils and extracts are used as environmentally friendly solutions to control pests and reduce the need for toxic agrochemicals. Neem™ extract is an oily substance derived from the margosa tree (*Azadirachta indica*), an evergreen tropical tree with insecticidal properties (Bader, 2012). Triple action neem oil (Southern Agricultural Insecticides, Inc.) is listed as a broad-spectrum fungicide, insecticide, and miticide for organic by the Organic Materials Review Institute (OMRI). The active ingredient is a clarified hydrophobic extract of neem oil and it is also approved for use on vegetables.

This product was used during the first experiment in an attempt to manage pest populations with organic solutions and without the use of chemicals. Liquid dish detergent was used as an emulsifier at a rate of 2 mL per liter of solution. Two neem oil applications were made on stems and leaves with a manual atomizer at 43 and 50 DAS, but had no effect on reducing damage caused by pests. A second attempt to control pest damage was made with the use of garlic. Some commercial pesticides use garlic extracts as their active ingredient (i.e. Garlic Barrier AG+), but homemade extracts are widely used as well. Two garlic cloves were crushed and mixed with 1L of water. The solution was left to sit for 24 hours and then strained to remove the garlic particles that could clog the manual atomizer. The garlic solution was applied six times on stems and leaves at 66, 68, 70, 77, 84, and 91 DAS.

1.3.11 'Chocolate Beauty' bell pepper

Seeds were ordered online from Mountain Valley Seed Co. in Salt Lake City, Utah. Seeds are affordable and are easily available for purchase. The seeds arrived guaranteed free of weeds and with a germination percentage of 99%. Only the most uniform, best-looking seeds were chosen first hand to maintain uniformity. Seeds that looked small, slim, or atrophied were not used.

For the first experiment, three seeds were sown directly into the containers at a depth of 1 cm, on December 13, 2013. Only one plant was selected in each pot and the other two were culled. Harvest began in March 29, 2014 (106 DAS) and ended in April 6, 2014 (114 DAS). Physiologically mature pepper fruits were harvested when they had at least 50% of their brown color developed. Severely damaged fruits that had been partially eaten by birds were also harvested as soon as they were seen.

1.3.12 Experimental design

This experiment was constructed as a completely randomized design. It consisted of a 3 x 4 factorial model arrangement with ten repetitions (one plant per repetition for a total of 120 plants). Three different fertilization methods were used to provide an equivalent of 116 $kg \cdot ha^{-1}$ of N as a post-transplant fertilizer: Granular Urea Fertilization (GUF), Spoon-fed Solubilized Urea Fertilization (SUF), and Spoon-fed Organic Fertilization (SOF). GUF was done with two applications, at the beginning of the flowering and fruiting stages (60 and 85 DAS, respectively). SUF and SOF were divided into ten equal applications every seven days for ten weeks beginning at 30 DAS. Four biostimulant treatments were used as foliar applications: Aminoquelant-Ca (AQC), Vitazyme (VTZ), Stimplex (SPX), and the water check (WTR). Biostimulant solutions were diluted according to the manufacturer's recommendations. Foliar applications of biostimulants began at 37 DAS and were sprayed on leaves, stems, buds, flowers, and immature fruits every 14 days for ten weeks.

1.3.12.1 The variables investigated to determine morphological development were:

Plant height (cm)
Number of leaves
Number of nodes

Number of buds

Number of flowers

Number of fruitlets

Abscission of buds

Abscission of flowers

Abscission of fruitlets

Shoot fresh weight (g)

Root fresh weight (g)

Shoot dry weight (g)

Root dry weight (g)

1.3.12.2 The variables investigated to determine the yield parameters were:

Number of marketable fruits per plant

Number of unmarketable fruits per plant

Number of total fruits per plant

Marketable fruit weight per plant (g)

Unmarketable fruit weight per plant (g)

Total fruit weight per plant (g)

Dry weight of total fruits per plant (g)

1.3.12.3 The variables investigated to determine the quality of fruits were:

Length of marketable fruits (cm)

Length of unmarketable fruits (cm)

Length of total fruits (cm)

Width of marketable fruits (cm)

Width of unmarketable fruits (cm)

Width of total fruits (cm)

Pericarp thickness of marketable fruits (mm)

Pericarp thickness of unmarketable fruits (mm)

Pericarp thickness of total fruits (mm)
Classification of marketable fruits

1.3.12.4 The variables investigated to determine changes in chlorophyll and N content:

SPAD index
Nitrate content in sap

1.3.12.5 The variables investigated to analyze the reasons for categorizing fruits as unmarketable were:

Percentage of unmarketable fruits damaged mites (scarring)
Percentage of unmarketable fruits with a diameter less than 5.6 cm
Percentage of unmarketable fruits damaged by sunburns
Percentage of unmarketable fruits damaged by birds

1.3.13 Fertilizer treatments

Fertilizer applications were determined by the recommendations found in the technological package for commercial pepper production in Puerto Rico (Fornaris et al., 2005). Calculations had to be made in order to determine the exact amounts of fertilizer needed for each individual plant. Current fertilization recommendations in Puerto Rico are focused on the open field, conventional agriculture and are meant for relatively big areas of land. Information regarding fertilizer management in greenhouse pepper production in Puerto Rico is currently very limited. This lack of information could slow Puerto Rico's agricultural transition to protected agriculture.

Bell peppers in Puerto Rico are cultivated on raised beds but most commonly in open fields. Raised beds are usually 1.5 to 1.8 meters apart with crops aligned in double rows. A distance of 30 to 45 cm separates each pair of rows and the distance of plants within the rows is 30cm. These planting distances can be used to estimate plant densities. Estimated plant densities range between 35,877 and 43,051 plants per hectare. A plant density of 36,890 plants per hectare (Equation 1) was used to calculate nutrient requirements of individual plants. The total amounts and requirements of N, phosphate and potash (K_2O) applied as a pre-plant fertilizer (Equation 2) and the N applied as post-transplant fertilizer (Equation 3) were calculated for 1 hectare based on plant density and local recommendations from Puerto Rico's commercial pepper production guide (Fornaris et al., 2005).

1.3.13.1 Granular Urea Fertilization (GUF)

Urea [$CO(NH_2)_2$] is the most widely used N source in the U.S. mainly due to its high N content and its high solubility in water. Manufacturing, handling, storage, and transportation logistics of granular urea are favorable and less dangerous (Havlin et al., 2005). It has a lower tendency to cake, stick, and/or explode than ammonium nitrate (NH_4NO_3), and it's also less corrosive to handling and equipment. In its current form, the N in urea is unavailable for plants. When urea is applied to the soil, urease enzymes hydrolyze it to ammonium (NH_4^+). Variations in soil pH may cause the NH_4^+ to form ammonia (NH_3), which can then be volatilized at the soil surface (Equation 4).

The urease enzyme that is needed to catalyze the hydrolysis of urea is usually very abundant in soils, especially in warm, moist soils that are high in organic matter content and also high in soil microbial populations. The application of urea to the soil surface is most efficient when washed into the soil and when there is a low volatilization potential. Deep incorporation of granular urea is advisable over surface applications or shallow tillage. This minimizes volatilization losses of NH_3 by increasing the volume of soil capable of retaining it. Many factors may interfere with the effectiveness of urea and can cause some degree of variability in the crop's response to this fertilizer. In order for N in the soil to be available for plant use, it has to be in one of two forms: ammonium or nitrate.

1.3.13.1.1 Pre-planting: synthetic granular fertilizer (10-10-10)

Only 16 grams of a synthetic 10-10-10 granular fertilizer were incorporated as a pre-plant fertilizer into the first 15 cm of each container. This amount of fertilizer per plant is equivalent to the application of 58 $kg \cdot ha^{-1}$ of N, phosphate, and K_2O recommended by local sources (Equation 5). A hand shovel was used to incorporate the pre-plant fertilizer on December 11, 2013.

1.3.13.1.2 Post-planting: synthetic granular urea fertilization (46-0-0)

Two post-transplant granular urea applications were made in order to apply a total N amount equivalent to 116 $kg \cdot ha^{-1}$. The first post-transplant granular urea application was made at the beginning of the flowering stage on February 11, 2014 (60 DAS). The second application was made at the beginning of the fruiting stage on March 8, 2014 (85 DAS). Amounts of granular urea needed for each plant were calculated based on the recommended rate for open field cultivation of bell peppers (Equation 6). A total of seven grams of urea were incorporated into the first 5 cm of soil in each container to avoid damaging the roots.

1.3.13.2 Spoon-fed Solubilized Urea Fertilization (SUF)

The adequate rates and the correct timing of fertilizer applications can maximize production of vegetable crops (Russo, 1991). By controlling the quantity and frequency of solubilized N applications, each growth stage can be supplied with specific and optimum nutrient rates that can increase the crop's N use efficiency (NUE).

1.3.13.2.1 Pre-planting: synthetic granular fertilizer (10-10-10)

The pre-plant fertilization rate for the SUF treatment was the same one used for the pre-plant fertilization rate in the GUF treatment that was previously described.

1.3.13.2.2 Post-planting: synthetic solubilized urea fertilization (46-0-0)

SUF was used to supply post-planting N in ten equal split-applications every 7 days for ten weeks. The first application was made 30 DAS, on January 12, 2014, when plants had an average length of 10 cm and 4-6 true leaves. The following nine applications were made on January 19, January 26, February 2, February 9, February 16, February 23, March 2, March 9, and March 16, 2014. Each individual application consisted of 0.7 grams of urea diluted in 100 mL of water. The total amount and source of post-planting N fertilization in the SUF treatment was the same as in the GUF treatment. The main difference is that the SUF treatment was applied through irrigation as ten equal split-applications for 10 weeks while the GUF treatment was incorporated into the soil as two equal applications at the beginning of the flowering and fruiting stages. The SUF was always applied directly to the soil, not the foliage. Foliar fertilizer application methods were not evaluated in this research.

1.3.13.3 Spoon-fed Solubilized Organic Fertilization (SOF)

The Green Revolution, climate change, and projected population growth in the near future have contributed to an increased demand for high quality, fresh agricultural produce (Negrete-Aveiga, 2013; Tilman et al., 2002). As a result, croplands are being cultivated intensively and depend on high fertilizer inputs in order to achieve and sustain economic profits. Misuse of fertilizers and inorganic salts can turn into an environmental hazard by polluting ecosystems or leaching into underground water supplies (Addiscott, 2005). Organic agriculture has recently been gaining popularity in Puerto Rico due to concerns about local fresh produce, costs of imports, and environmentally sustainable production systems. Organic production systems rely on different sources of N-containing materials that vary in costs, availability, nutrient content, mineralization rate, and environmental impact. In order to produce certified organic vegetables, the production system has to be verified and approved as organic by an independent party. Thus, this research will focus on the use of commercially available organic fertilizers rather than on the production of certified organic bell peppers.

1.3.13.3.1 Pre-planting: organic fertilizer Bioflora Dry Crumbles® (6-6-5+8% Ca) and organic liquid fertilizer Bioflora (0-0-15+1% Ca)

Bioflora Dry Crumbles® (6-6-5) (BDC) is an organic, dry granular fertilizer fortified with seaweed. This fertilizer is certified by the OMRI and it provides high quality organic nutrients for all types of plants. It contains balanced amounts of N, P, and K but also contains high levels of calcium (Ca) and trace elements. The carbon-based granules are characterized by a slow-release formula that provides plants with certain benefits like renewed vigor, root mass stimulation and reduced damage from disease (Global Organics Group, 2016). The nutrient content within the granules is derived from a blend of feather meal, dry composted poultry litter, sulfate of potash, and the seaweed *A. nodosum* (Negrete-Aveiga, 2013) (Appendix 2).

Each plant in the SOF treatment had 27 grams of granular BDC (6-6-5) fertilizer incorporated into the first 15 cm of each container as a pre-plant fertilizer on December 11, 2013. This amount of fertilizer per plant is equivalent to the application of 58 $kg \cdot ha^{-1}$ of N and phosphate recommended by local sources (Equation 7). Even though the required pre-plant amount of N and P was supplied with 27 grams of BDC, there was a very slight deficit in the amount of K applied due to the inherent nature and nutritional proportions in the BDC (6-6-5) fertilizer (Equation 8).

A deficit of 8 $kg \cdot ha^{-1}$of pre-plant K_2O could eventually bias conclusions on plant growth, development, or yield. In order to maintain the same amount of NPK in the pre-plant fertilization of all the treatments, Bioflora (0-0-15) liquid fertilizer (BLF) (Appendix 3) was applied at a rate of 2 mL per plant in combination with the BDC (6-6-5) fertilizer to correct the small deficit in the pre-plant K_2O (Equation 9). The BLF (0-0-15) is highly soluble in water and is derived from potassium carbonate and calcium EDTA. According to product label, when used as a pre-plant fertilizer, it should be applied to the ground as a concentrate. Both, the BDC (6-6-5) and the BLF (0-0-15) in the pre-plant SOF treatment, contained 8 and 1% Ca respectively. A total amount of 81 $kg \cdot ha^{-1}$ of pre-plant Ca was also included with the BDC and BLF in the SOF treatment (Equation 10 & 11). This extra amount of Ca was only applied in the SOF treatment because the commercially available products used in this study included this nutrient. There was no extra amount of Ca added to neither the GUF nor the SUF treatments.

1.3.13.3.2 Post-planting: organic liquid fertilizer Gluten-8 OLP™.

Gluten-8 OLP™ was originally used in the first experiment to supply post-planting N in ten equal split-applications every 7 days for ten weeks. The first application was made 30 DAS, on January 12, 2014, when plants had an average length of 10 cm and 4-6 true leaves. This is an organic liquid fertilizer with very low N content (1.5% N) that is derived from the enzymatic hydrolysis of corn gluten meal (Appendix 4). The amount of Gluten-8 OLP™ needed in order to meet the required N produced a thick fertilizer solution that caked on the soil surface and caused plant mortality. The use of this fertilizer was discontinued after the second application on January 19, 2014. A different organic liquid fertilizer (Bioflora 6-0-0+8% Ca) was used to finish the experiment and supply the remaining N during the last 7 weeks of treatment.

1.3.13.3.3 Post-planting: organic liquid fertilizer Bioflora (6-0-0+8% Ca)

Bioflora organic liquid fertilizer (6-0-0+8% Ca) (BLNF) was used to supply the remaining post-planting N in 7 equal split-applications every 7 days (Equation 12). This organic liquid fertilizer is derived from calcium nitrate (Appendix 5). There were no applications of this treatment during the third week in January 26, 2014 due to a delay in product shipping. The applications were resumed during the fourth week and the application dates were February 2, February 9, February 16, February 23, March 2, March 9, and March 16, 2014. The total amount of post-transplant N fertilization in the SOF treatment was the same as in the SUF and GUF treatments. The main difference is that the SUF and GUF treatments used synthetic urea fertilizer, while the SOF treatment used an organic fertilizer. The SOF was always applied directly to the soil, not the foliage.

1.3.14 Biostimulant treatments

All the biostimulant treatments were applied individually as a foliar spray with a plastic, hand held atomizer. Atomizers were always rinsed three times before and after the application of biostimulants. Every biostimulant treatment was applied five times during the crop cycle. The first biostimulant applications began in January 19, 2014 (37 DAS) and were repeated every 14 days. The four remaining applications events were done on February 2 (51 DAS), February 16 (65 DAS), March 2 (79 DAS), and March 16, 2014 (93 DAS). The dosage was determined according to each of the biostimulants' respective manufacturer. Leaves, stems, buds, flowers and immature fruits were covered evenly with the biostimulant solution until run-off. Foliar applications were always done during the cooler hours of the day (i.e., early morning or late afternoon) in order to prevent or diminish excessive evaporation from the plants' surface. Wind speed and wind direction were taken into consideration in order to diminish unintended drift potential by wind onto nearby plants. No surfactant was added in any of the biostimulant treatments.

Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

1.3.14.1 Aminoquelant®-Ca (AQC)

Aminoquelant®-Ca (BioIbérica, Barcelona, Spain) is a commercially available blend of amino acids for agricultural use. It combines Ca with L-α-amino acids from enzymatic hydrolysis that can improve Ca mobility in plants and can also mitigate the factors that lead to its deficiency. The amino acid blend contains 4.6% (w/w) biologically active free amino acids. It consists of aspartic acid (aspartate), serine, glutamic acid (glutamate), glycine, histidine, arginine, threonine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine, and tryptophan. This formulation includes 8% calcium oxide (CaO), 0.9% organic N, 4.9% total N, 0.2% boron, and 6.8% organic matter. It can be applied through the irrigation line or by foliar sprays and it is especially designed for crops that are prone to physiological alterations by Ca deficiencies such as peppers, tomatoes, lettuce, etc. (BioIbérica, 2016). The foliar spray solution was prepared by diluting 4 mL of Aminoquelant-Ca with 1 L of water. This is equivalent to a 0.4% Aminoquelant-Ca solution.

1.3.14.2 Vitazyme (VTZ)

Vitazyme® (Vital Earth Resources, Gladewater, Texas, USA) is a commercially available, OMRI and BCS certified organic biostimulant for agricultural use. It consists of certain biological activators that are created by an undisclosed, propietary fermentation process. The formulation contains Vitamin B1 (thiamin) (0.45 mg/100g), Vitamin B2 (riboflavin) (0.03 mg/100g), Vitamin B6 (pyridoxine) (0.19 mg/100g), K_2O (0.80%), Iron (Fe-EDTA) (0.2%), Copper (Cu-EDTA) (0.07%), Zinc (Zn-EDTA) (0.06%), brassinosteroids (i.e., homobrassinolide, dolicholide, homodolicholide, and brassinone) (0.03 mg/ml), 1-triacontanol (0.17 mg/ml), glycosides and water. This biostimulant accelerates and improves metabolic processes that can increases crop yields and profits. It improves crop quality, reduces N fertilizer inputs, and also hastens germination and maturity (Vital Earth Resources, 2016). In addition, it also acts on soils by improving their structure and infiltration capacity. According to the manufacturer's recommendations, seeds were pre-treated by soaking them directly in the Vitazyme concentrate for 90 seconds. This was the only biostimulant used to pre-treat seeds. The foliar spray solution was prepared by diluting 10 mL of Vitazyme with 1 L of water. This is equivalent to a 1% Vitazyme solution.

1.3.14.3 Stimplex (SPX)

Stimplex^{TM} (Acadian Seaplants, Nova Scotia, Canada) is an OMRI listed, commercially available SWE biostimulant for agricultural use. It is derived from the brown algae *Ascophyllum nodosum* L. Product label states that it contains an active ingredient concentration equivalent to 100 parts per million of kinetin (Appendix 6). It also contains zeatine, adenine, betaine, oligosaccharides, polysaccharides, organic acids, vitamins, gibberellins, auxins, micronutrients, and amino acids (Flores-Torres, 2013). It is used to improve resistance to biotic & abiotic stresses and enhance the crop's overall health. It is also used to increase yields and quality of crops, and also to increase root growth, early plant development, fruit set, fruit size, and nutrient levels (CDMS, 2016). The foliar spray solution was prepared by diluting 5 mL of Stimplex with 1 L of water. This is equivalent to a 0.5% Stimplex solution.

1.3.14.4 Water check (WTR)

In order to disregard the possible effect of spray vs. no spray, tap water was used as a control to spray the plants without biostimulants, rather than not spraying them at all. The water used as a control was always collected at the time of application in order to prevent the water from becoming stagnant over time. The source of the water used in the control check was from a well located at a higher elevation in the Finca Laboratorio Alzamora.

1.3.15 Morphological research

Morphological development was measured through the analysis of the following variables:

1.3.15.1 Plant height (cm)

A measuring tape (cm) was used to measure plant height every seven days for ten weeks, beginning on January 18, 2014 (36 DAS). Measurements were taken starting at the soil surface and extending to the apex.

1.3.15.2 Number of leaves and nodes

Leaves and nodes were counted on each plant every seven days for ten weeks, beginning on January 18, 2014 (36 DAS). Only the fully extended, mature leaves were counted.

1.3.15.3 Mean fresh weight of shoots and roots (g)

Plants were cut at the soil surface level using scissor-action pruners (Fiskars Brands Inc, Middleton, Wisconsin, USA). This was done after the last pepper was harvested in each plant or during the experiment's last day of harvest on April 6, 2014 (114 DAS). The top part of the plant, or everything above the soil surface level, was separated from its roots, segmented into smaller pieces, and placed in a paper bag. After the shoots were removed, the containers were emptied on a 15 mm sieve. The growing medium was carefully sieved until the roots were exposed. Roots were washed with running water and excess debris was rinsed off. The root ball and root fragments were then collected from the sieve, manually wringed, placed in individual paper bags, identified as shoots or roots, and labeled with the plant's treatment and location within the raised bench. Each paper bag was weighted with a digital scale (Fisher Science

Education^{TM}, USA) and data was named as fresh weight of shoots or fresh weight or roots (g).

1.3.15.4 Mean dry weight of shoots and roots (g)

After the fresh weight data was logged, paper bags with plant material were placed in a drying oven (Jisico Scientific Instruments, Seoul, Korea) for 96 hours at 75°C. Paper bags with the dehydrated plant material were weighted and the data was named as dry weight of shoots or dry weight of roots (g).

1.3.15.5 Mean number of buds, flowers, and fruitlets

Each plant was checked every seven days for seven weeks, beginning on February 1, 2014 (50 DAS). Buds, flowers, and fruitlets that were on the plant at the moment were counted.

1.3.15.6 Mean abscission of buds, flowers, and fruitlets

Abscission of reproductive organs and fruitlets was determined by counting the number of buds, flowers, and fruitlets that had fallen off of the plant on each container's soil surface area ($A \approx 0.6\ m^2$). Abscission of flowers refers to any flower that dropped prior to post-fertilization ovary enlargement and abscission of fruitlets refers to any ovary that dropped after post-fertilization enlargement (Bookman, 1983). After counting, aborted reproductive organs were removed in order to avoid counting them twice during the next data-logging event.

1.3.16 Yield and quality research

Physiologically mature pepper fruits were individually harvested when they had developed at least 50% of their chocolate brown color. The harvest period lasted for nine days, beginning in March 29 (106 DAS) and ending in April 6, 2014 (114 DAS). Each pepper was weighted, measured (i.e., length and width), and classified as marketable or unmarketable. Total fruits were the sum of marketable and unmarketable fruits. Marketable fruits had an equatorial diameter equal or greater than 5.6 cm and were free of decay or damages by abiotic or biotic stressors. These were then graded into categories according to two different grading methods: 1) the United States standards for grades of sweet peppers (2005) and 2) a diameter scale used for imported greenhouse-grown bell peppers (Jovicich et al., 2007).

The first grading system used was the United States standards for grades of sweet peppers (2005). These standards classify peppers into three categories: U.S. Fancy, U.S. No. 1, and U.S. No. 2. The criteria used to assign these categories are length, width, color, and damages. The second grading system is used for imported greenhouse-grown bell peppers and grades the pepper fruits based on size following a diameter scale: extra-large (diameter > 8.4 cm), large (7.6 – 8.39 cm), medium (6.4 – 7.59 cm), and small (5.6 – 6.39 cm) (Jovicich et al., 2007). Marketable fruit data was analyzed considering both grading systems. The grading system for imported greenhouse-grown bell peppers was modified and the categories of large and extra-large were eliminated. This modification of the grading system had to be made because there were insufficient extra-large and large pepper fruits. The newly modified grading system for imported greenhouse-grown bell peppers consisted of only two categories: regular (diameter > 6.4 cm), and small (5.6 – 6.39 cm).

The different reasons to categorize fruits as unmarketable fruits were also logged. After the pepper grading procedures, each pepper fruit was then sliced in half through the equator. Pericarp thickness was measured (mm) using a caliper (Vernier Callipers, Waukegan, IL, USA). Each pepper fruit was then segmented into smaller pieces and all the fruit contents (i.e. seeds, calyx, placenta, and fruit walls) were deposited in paper bags. Each paper bag was labeled with the plant's treatment, location within the raised bench, and also with the fruit's number according to the order of harvest. Paper bags with pepper fruit material were placed in a dehydrating oven (Jisico Scientific Instruments, Seoul, Korea) for 96 hours at 75°C and then weighted with a digital scale (Fisher Science Education™, USA). Dry weight of fruits was then organized and summed for each plant.

1.3.17 Physiological research

Physiological changes were measured through the analysis of the following variables:

1.3.17.1 Mean chlorophyll content

Chlorophyll content was measured using a SPAD-502Plus chlorophyll meter (Osaka, Japan) in March 8, 2014 (85 DAS) on fully expanded leaves.

1.3.17.2 Nitrate content in sap

Nitrate content was measured with a Cardy Nitrate NO_3^- meter (Spectrum Technologies, Inc., Plainfield, IL). The petioles of fully expanded leaves were separated and pressed to extract a few droplets of sap. The sap was then placed in the nitrate meter's sensor and the readings were logged. Nitrate content in sap was measured during pepper fruit harvest time (106 - 114 DAS).

1.3.18 Data analysis

Statistical analysis was conducted with Infostat version 2014 (Infostat software, National University of Córdoba, Argentina). The Shapiro-Wilks test was used to verify if the data followed a normal distribution and the Levene test was used to determine homoscedasticity of the data. Values were log-transformed or square root-transformed to homogenize variance or normalize distribution when needed. The non-parametric Kruskal-Wallis test was used when variance was not homogenous and/or distribution was not normal even after both transformations had been made. An analysis of variance (ANOVA) was conducted in order to evaluate the potential interactions of each factor. The interactions are described as combinations of the levels from each factor (Viggiano-Beltrocco, 2014). The levels were: 3 post-transplant N fertilization methods (GUF, SUF, and SOF) and 4 biostimulant treatments (AQC, VTZ, SPX, and WTR) that make a 3 x 4 factorial ANOVA model. Significant means were separated using the LSD statistical test ($\alpha < 0.05$).

1.4 Results

1.4.1 Mean plant height (cm)

There were no significant statistical differences in plant height (cm) at any time during the crop cycle (Appendix 7). Figure 8 shows the average height of all the plants in the experiment per measurement date. Plant height increased from between 36 and 78 DAS, remaining the same height afterwards.

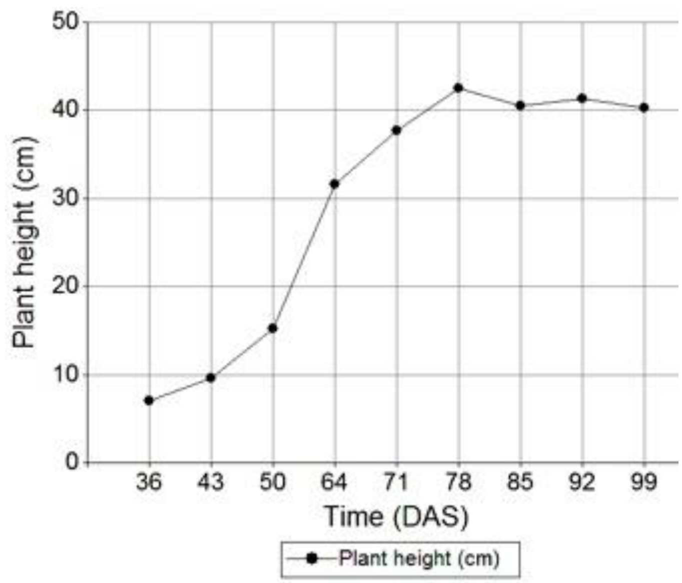


Figure 8. Mean plant height (cm) of potted 'Chocolate Beauty' bell pepper plants at various days after sowing (DAS). Mayagüez, Puerto Rico, 2014.

1.4.2 Mean number of leaves

None of the treatments significantly affected the mean number of leaves at 36, 43, and 50 DAS. In contrast, significant interaction effects were at 71 DAS. Individual treatment effects were seen on the fertilizing methods at 64, 78, 85, 92, and 99 DAS (Appendix 8).

1.4.2.1 Interaction effect on mean number of leaves

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean number of leaves at 71 DAS. At 71 DAS, the SOF + VTZ combination produced 55 and 124% more leaves than the GUF + VTZ and SUF + VTZ combinations, respectively. The SOF + AQC combination produced 44% more leaves than the GUF + AQC combination. Also, the SOF + SPX and SUF + SPX combinations produced 90 and 61% more leaves than the GUF + SPX combination (Figure 9).

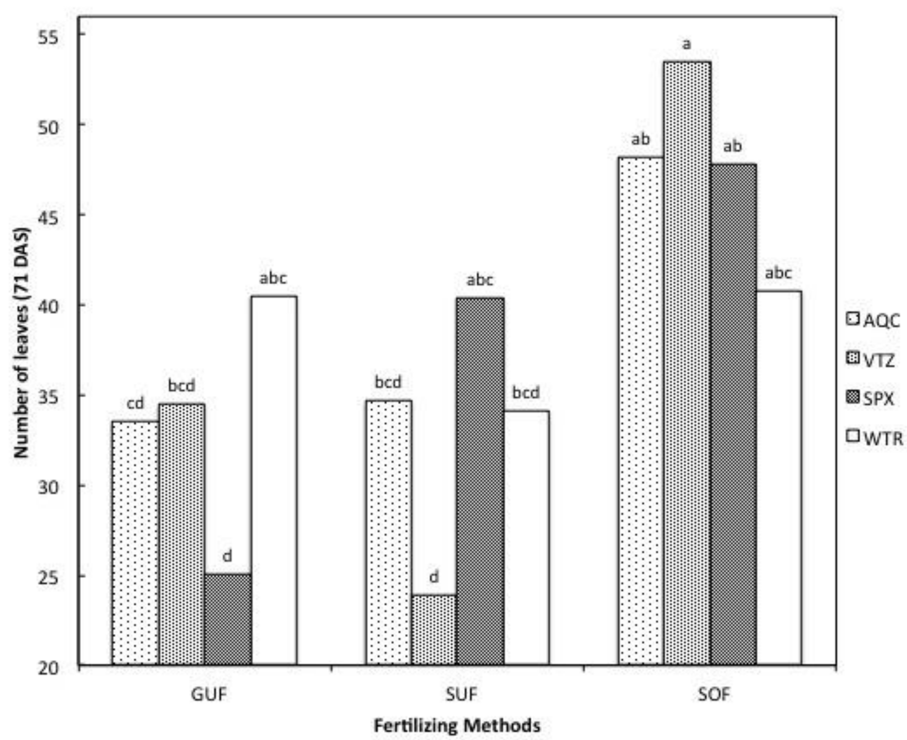


Figure 9. Combined effects of fertilizing methods and biostimulants on the mean number of leaves per plant at 71 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.2.2 Effect of fertilizing methods on the mean number of leaves

Fertilizing method treatments had significant effects on the mean number of leaves at 64, 78, 85, 92, and 99 DAS. At 64 DAS, the SOF method produced 17 and 21% more leaves than the GUF and SUF methods, respectively. At 78 DAS, the SOF method produced 43 and 45% more leaves than the GUF and SUF methods, respectively. At 85 DAS, the SOF method produced 67% more leaves than the GUF method and the SUF was not significantly different from either one. At 92 DAS, the SOF method produced 56 and 35% more leaves than the GUF and SUF methods, respectively. At 99 DAS, the SOF method produced 51 and 30% more leaves than the GUF and SUF methods, respectively (Figure 10).

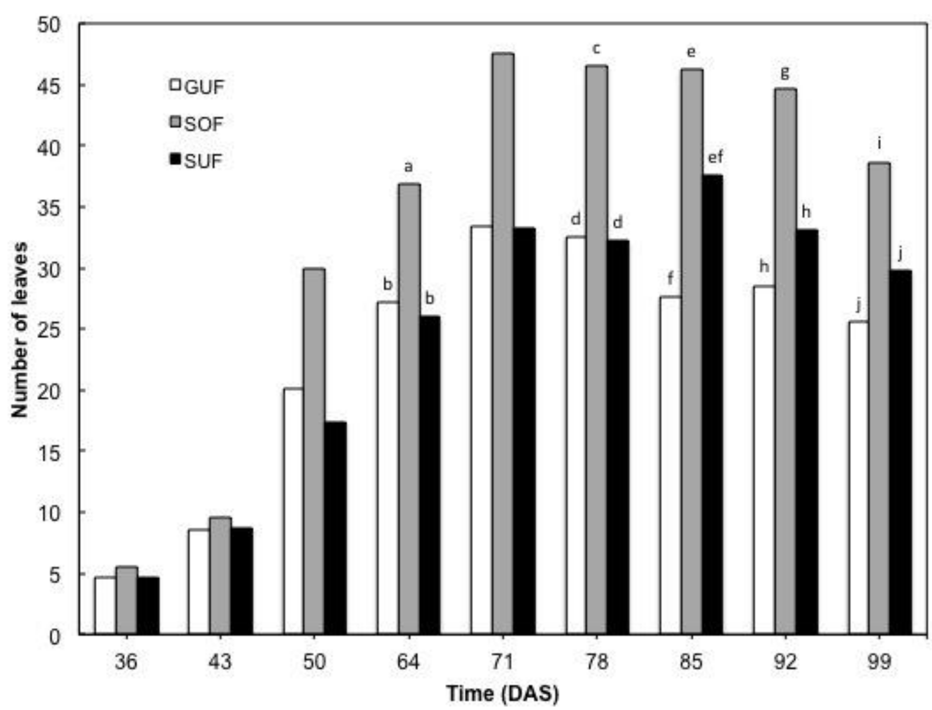


Figure 10. Individual treatment effects of fertilizing methods on the mean number of leaves at 64, 78, 85, 92 and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.3 Mean number of nodes

None of the treatments had any effect on the mean number of nodes at 36, 43, 64, and 85 DAS. In contrast, significant interaction effects were at 71 DAS. Individual treatment effects were seen on the fertilizing methods at 50, 78, 92, and 99 DAS (Appendix 9).

1.4.3.1 Interaction effect on mean number of nodes

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean number of nodes at 71 DAS. At 71 DAS, the SOF + VTZ combination produced 30 and 62% more nodes than the GUF + VTZ and SUF + VTZ combinations, respectively. The SOF + SPX and SUF + SPX combinations produced 31 and 16% more nodes than the GUF + SPX combination, respectively. The SOF + VTZ combination produced 30% more nodes than the SOF + WTR combination (Figure 11).

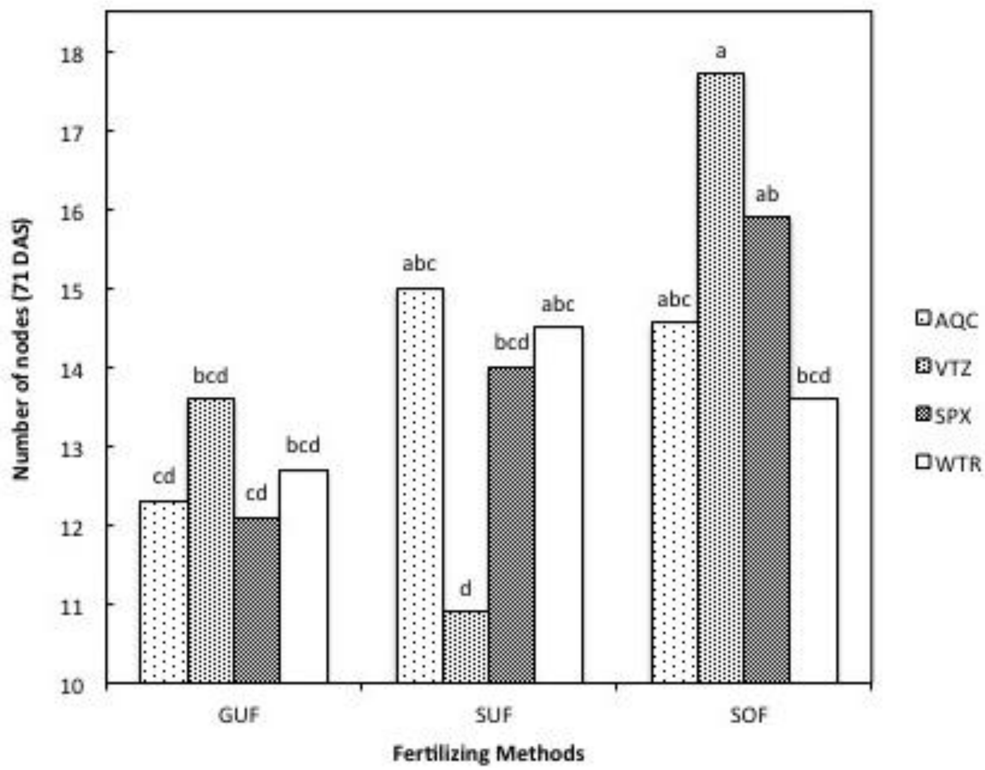


Figure 11. Combined effects of fertilizing methods and biostimulants on the mean number of nodes per plant at 71 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.3.2 Effect of fertilizing methods on the mean number of nodes

Fertilizing method treatments had a significant effect on the mean number of nodes at 50, 78, 92, and 99 DAS. At 50 DAS, the SOF method produced 22 and 35% more nodes than the GUF and SUF methods, respectively. At 78 DAS, the SOF method produced 28% more nodes than the GUF method, and the SUF method was not significantly different from either one. At 92 DAS, the SOF method produced 22% more nodes than the GUF method, and the SUF method was not significantly different from either one. At 99 DAS, the SOF method produced 23% more nodes than the GUF method, and the SUF method was not significantly different from either one (Figure 12).

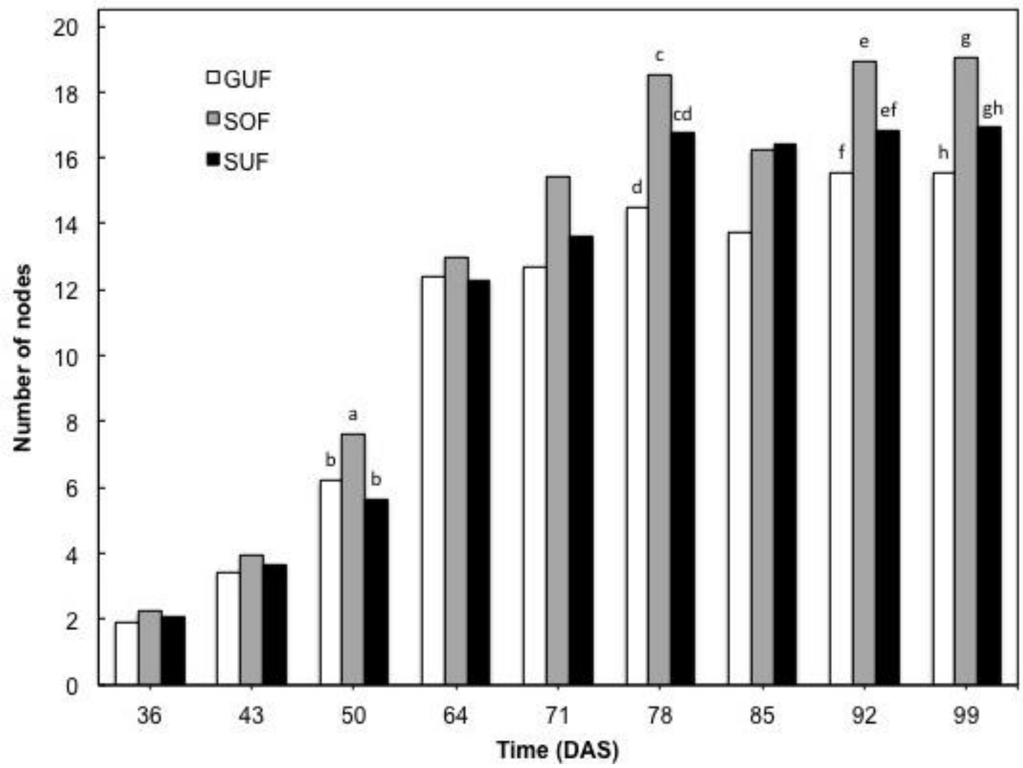


Figure 12. Individual treatment effects of fertilizing methods on the mean number of nodes at 50, 78, 92, and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.4 Mean number of buds

None of the treatments had any effect on the mean number of buds at 50, 64, 85, and 92 DAS. In contrast, significant interaction effects were at 71 DAS. Individual treatment effects were seen on the biostimulant treatments at 78 DAS (Appendix 10).

1.4.4.1 Interaction effect on mean number of buds

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean number of buds at 71 DAS. At 71 DAS, the SOF + VTZ combination produced 76 and 157% more buds than the GUF + VTZ and SUF + VTZ combinations, respectively. Also, the SOF + VTZ combination produced 73 and 54% more buds than the SOF + AQC and SOF + WTR combinations, respectively (Figure 13).

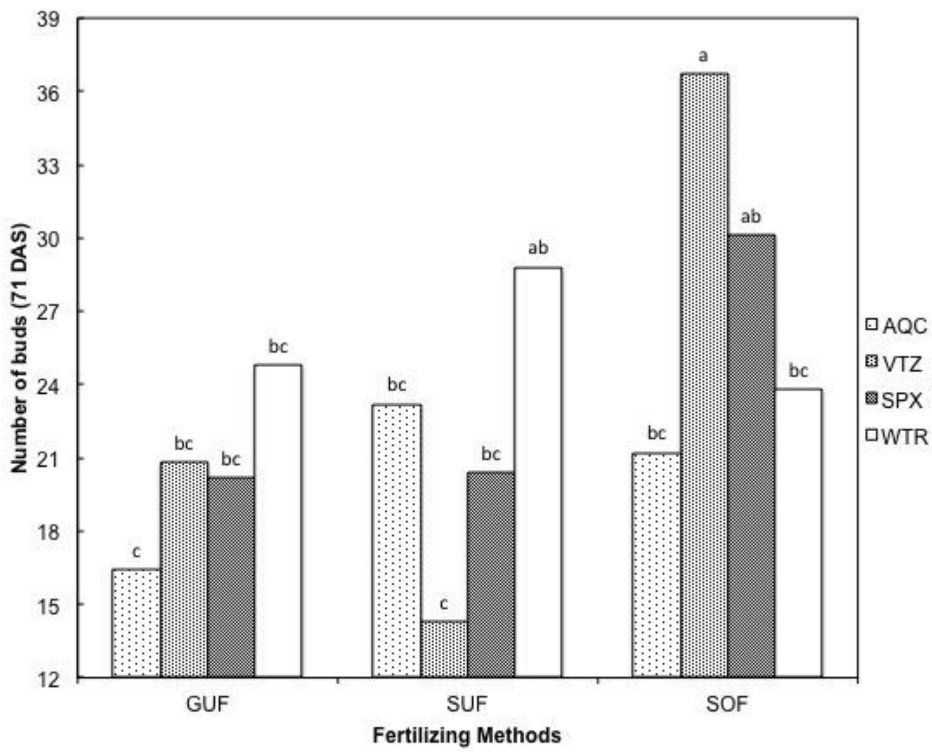


Figure 13. Combined effects of fertilizing methods and biostimulants on the mean number of buds per plant at 71 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05).

1.4.4.2 Effect of biostimulants on the mean number of buds

Biostimulant treatments had a significant effect on the mean number of buds at 78 DAS. At 78 DAS, the AQC biostimulant produced 45% more buds than the VTZ biostimulant. The VTZ biostimulant produced 30% less buds than the WTR. The SPX biostimulant was not significantly different from the rest (Figure 14).

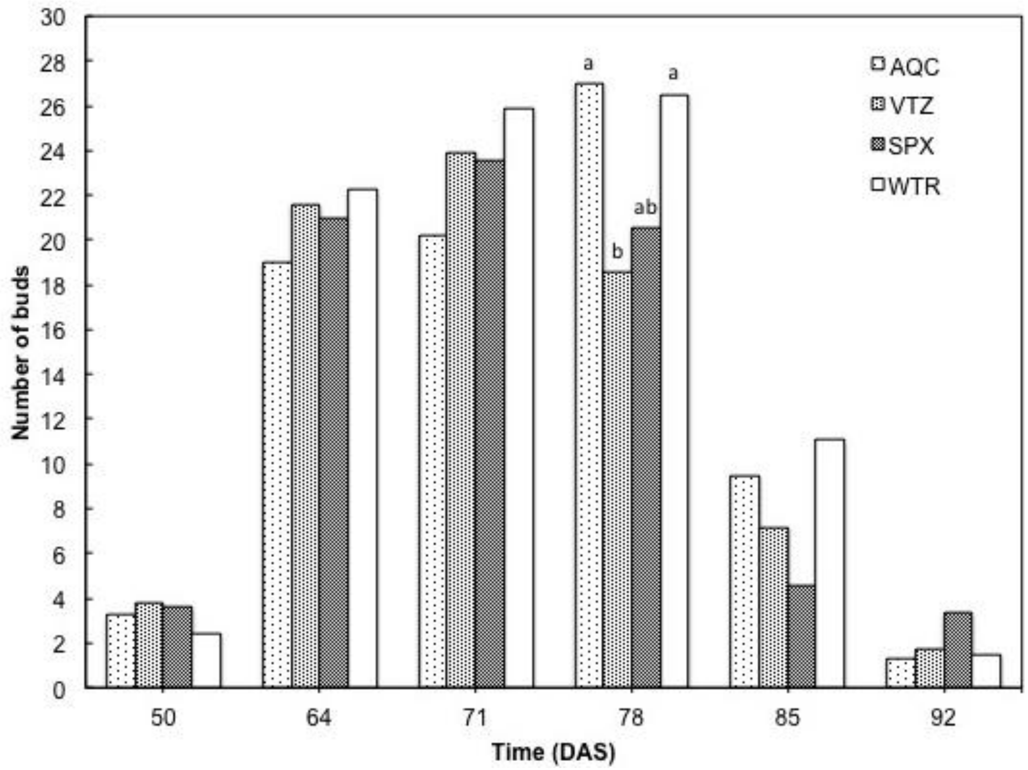


Figure 14. Individual treatment effects of biostimulants on the mean number of buds at 78 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.5 Mean number of flowers

There were no significant statistical differences in the number of flower at any time during the crop cycle (Appendix 11). Figure 15 shows the average number of flowers of all the plants in the experiment per counting date. Flowers began to bloom at 64 DAS and the highest number of flowers was seen at 78 DAS. There were practically no flowers present at 92 and 99 DAS.

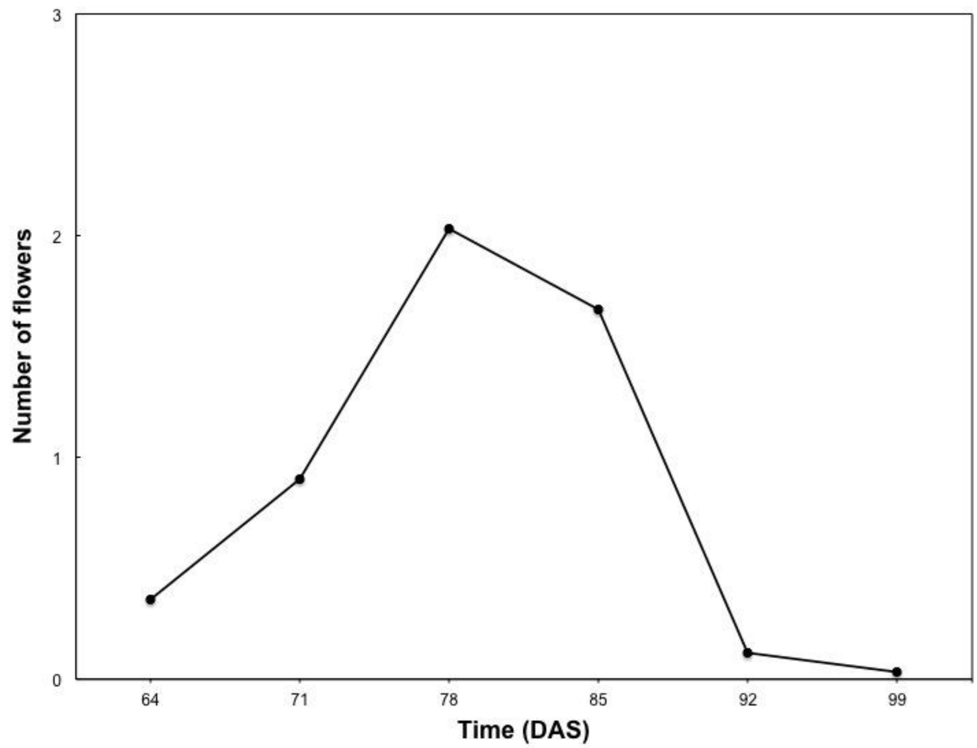


Figure 15. Mean number of flowers of 'Chocolate Beauty' bell pepper plants at 64, 71, 78, 85, 92, and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014.

1.4.6 Mean number of fruitlets

None of the treatments had any effect on the mean number of fruitlets at 64, 71, 78, and 85 DAS. Individual treatment effects were seen on the fertilizing methods at 92 and 99 DAS (Appendix 12).

1.4.6.1 Effect of fertilizing methods on the mean number of fruitlets

Fertilizing method treatments had a significant effect on the mean number of fruitlets at 92 and 99 DAS. At 92 DAS, the SOF method produced an average of 51% more fruitlets than both, the GUF and SUF methods, respectively. At 99 DAS, the SOF method produced an average of 55 and 58% more fruitlets than the GUF and SUF methods, respectively (Figure 16).

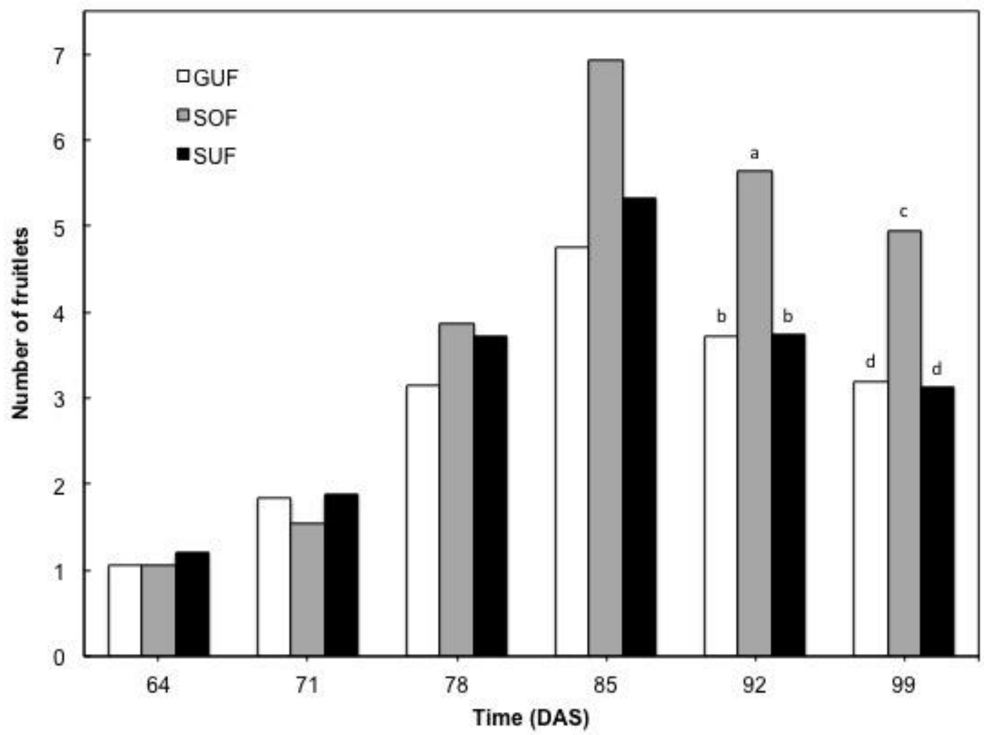


Figure 16. Individual treatment effects of fertilizing methods on the mean number of fruitlets at 92 and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.7 Mean abscission of buds

There were no significant statistical differences in the mean abscission of buds at any time during the crop cycle (Appendix 13). Figure 17 shows the average abscission of buds of all the plants in the experiment per counting date. Abscission of buds increased from 64 to 92 DAS and then rapidly decreased completely from 92 to 99 DAS.

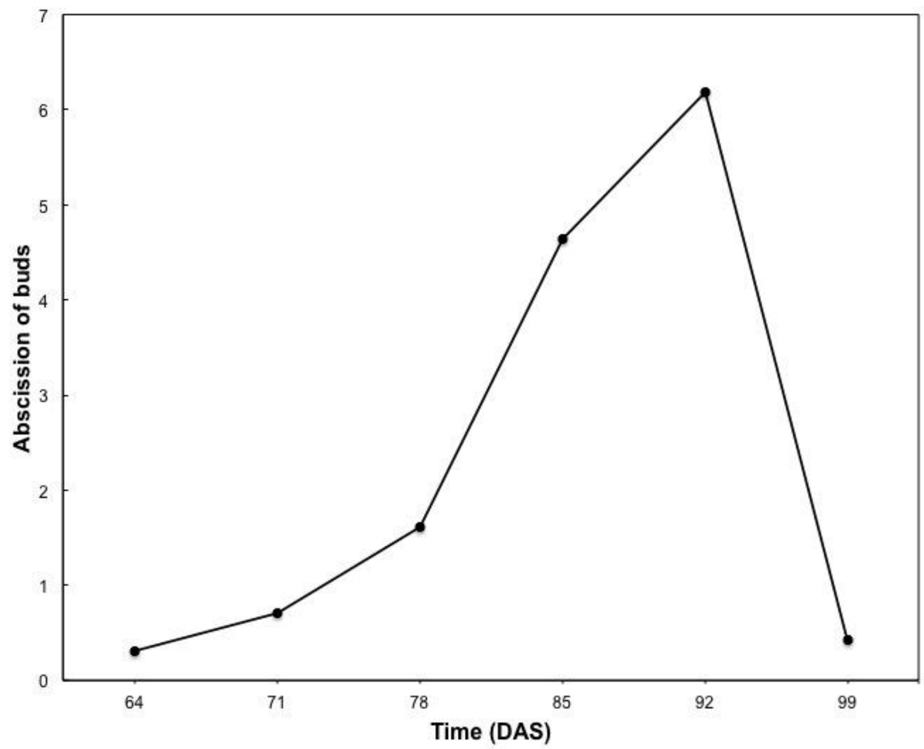


Figure 17. Mean bud abscission of 'Chocolate Beauty' bell pepper plants at 64, 71, 78, 85, 92, and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014.

1.4.8 Mean abscission of flowers

There were no significant statistical differences in the mean abscission of flowers at any time during the crop cycle (Appendix 14). Figure 18 shows the average abscission of flowers of all the plants in the experiment per counting date. Abscission of flowers increased from 71 to 85 DAS, rapidly decreased from 85 to 92 DAS, and remained the same from 92 to 99 DAS.

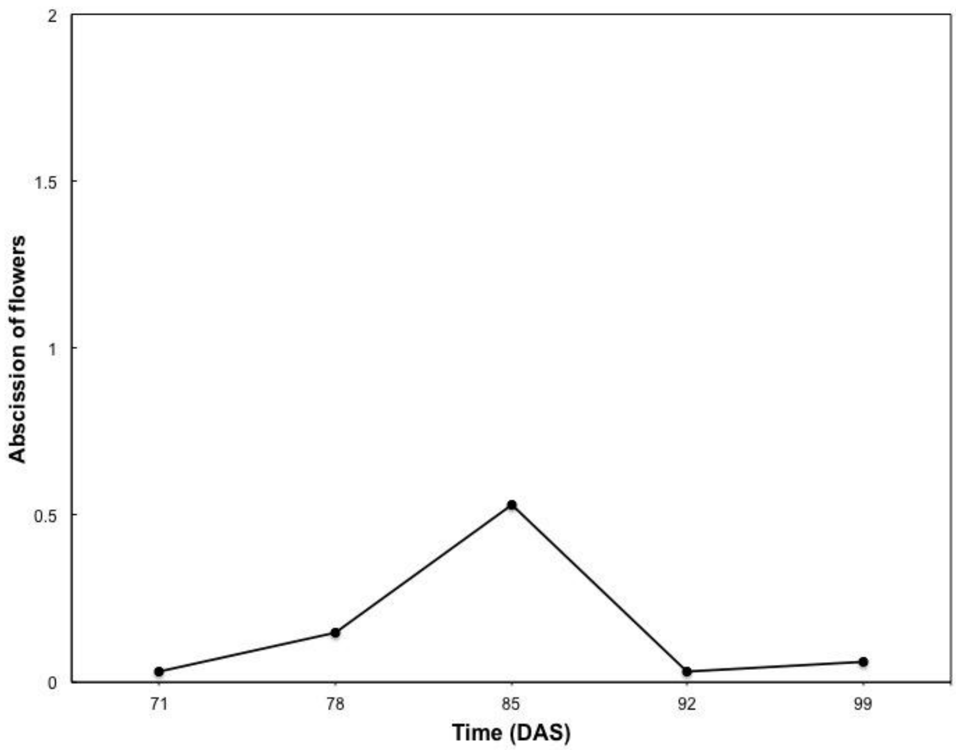


Figure 18. Mean flower abscission of ‘Chocolate Beauty’ bell pepper plants at 71, 78, 85, 92, and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014.

1.4.9 Mean abscission of fruitlets

There were no significant statistical differences in the mean abscission of fruitlets at any time during the crop cycle (Appendix 15). Figure 19 shows the average abscission of fruitlets of all the plants in the experiment per counting date. Abscission of fruitlets increased from 71 to 85 DAS, remained the same from 85 to 92 DAS, and then decreased rapidly from 92 to 99 DAS.

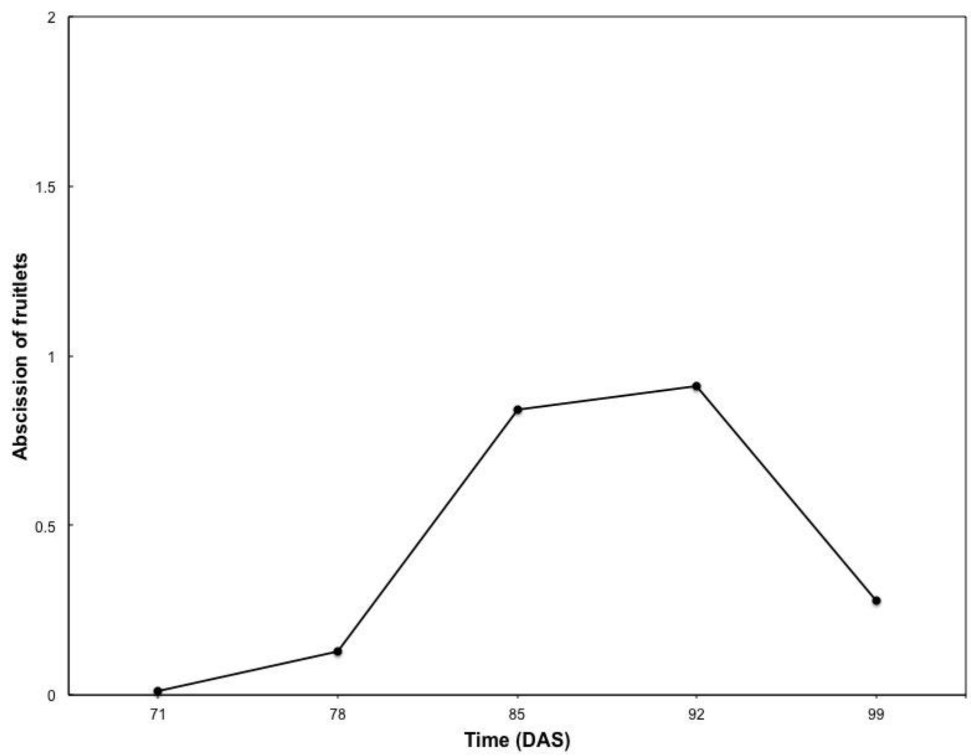


Figure 19. Mean fruitlet abscission of 'Chocolate Beauty' bell pepper plants at 71, 78, 85, 92, and 99 days after sowing (DAS). Mayagüez, Puerto Rico, 2014.

1.4.10 Mean fresh weight of shoots (g)

Treatments did not have interaction effects on the mean fresh weight of shoots. Individual treatment effects were seen with the fertilizing methods (Appendix 16).

1.4.10.1 Effect of fertilizing methods on the mean fresh weight of shoots (g)

Fertilizing method treatments had a significant effect on the mean fresh weight of shoots. The SOF method produced an average of 42 and 41% more fresh weight of shoots than the GUF and SUF methods, respectively (Figure 20).

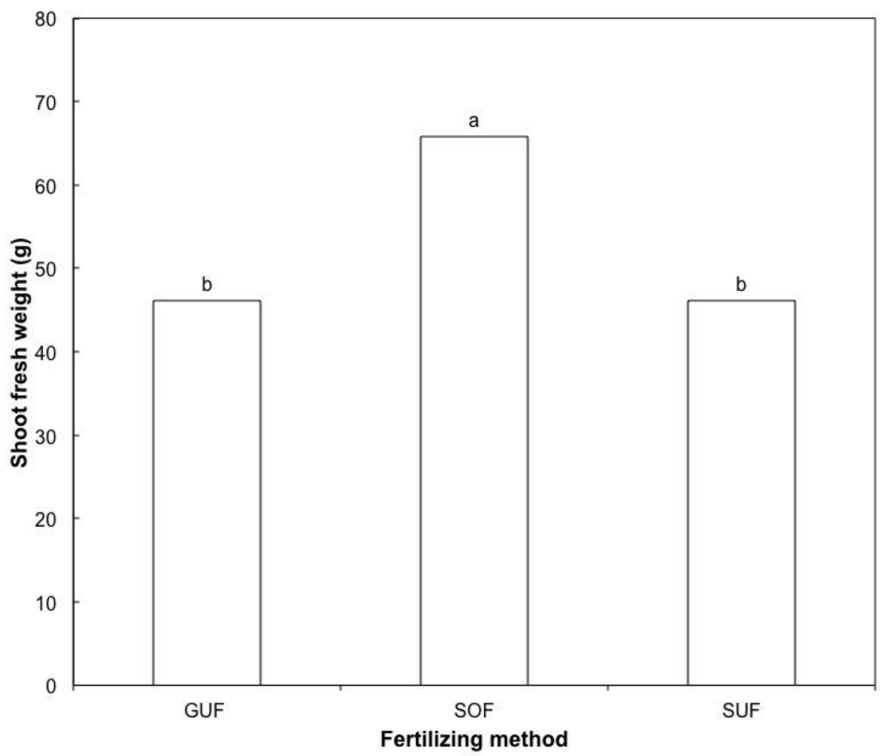


Figure 20. Effect of fertilizing methods on the mean fresh weight of shoots (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.11 Mean fresh weight of roots (g)

Treatments did not have interaction effects on the mean fresh weight of roots. Individual treatment effects were seen with the fertilizing methods (Appendix 16).

1.4.11.1 Effect of fertilizing methods on the mean fresh weight of roots (g)

Fertilizing method treatments had a significant effect on the mean fresh weight of roots. The SOF method produced an average of 90 and 117% more fresh weight of roots than the GUF and SUF methods, respectively (Figure 21).

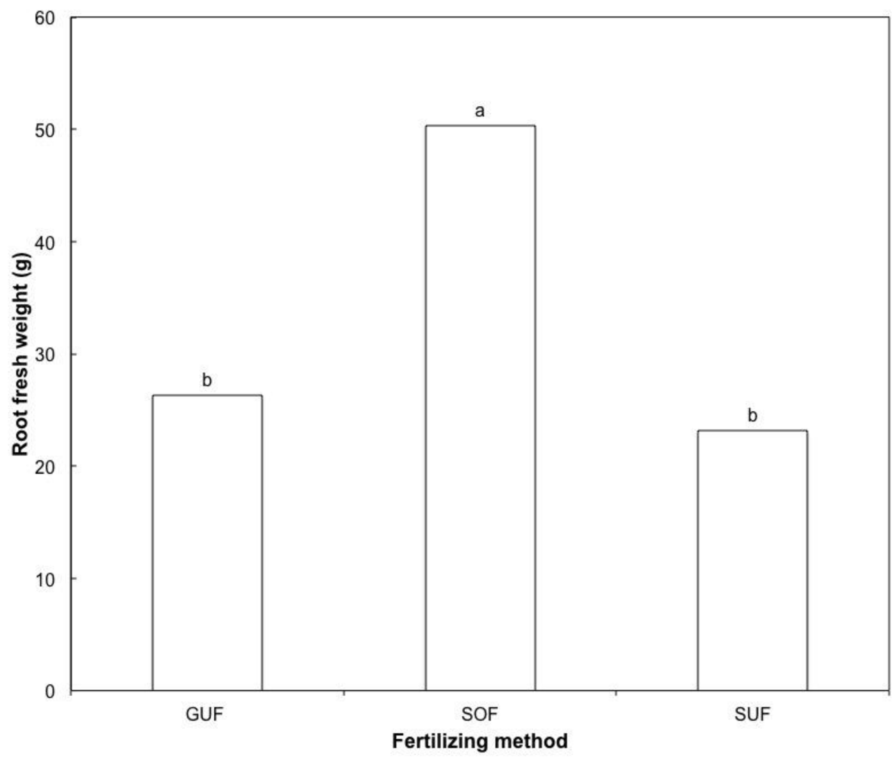


Figure 21. Effect of fertilizing methods on the mean fresh weight of roots (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.12 Mean dry weight of shoots (g)

Treatments did not have interaction effects on the mean dry weight of shoots. Individual treatment effects were seen with the fertilizing methods (Appendix 16).

1.4.12.1 Effect of fertilizing methods on the mean dry weight of shoots (g)

Fertilizing method treatments had a significant effect on the mean dry weight of shoots. The SOF method produced an average of 60% more dry weight of shoots than the GUF method. The SUF method was not significantly different from either one (Figure 22).

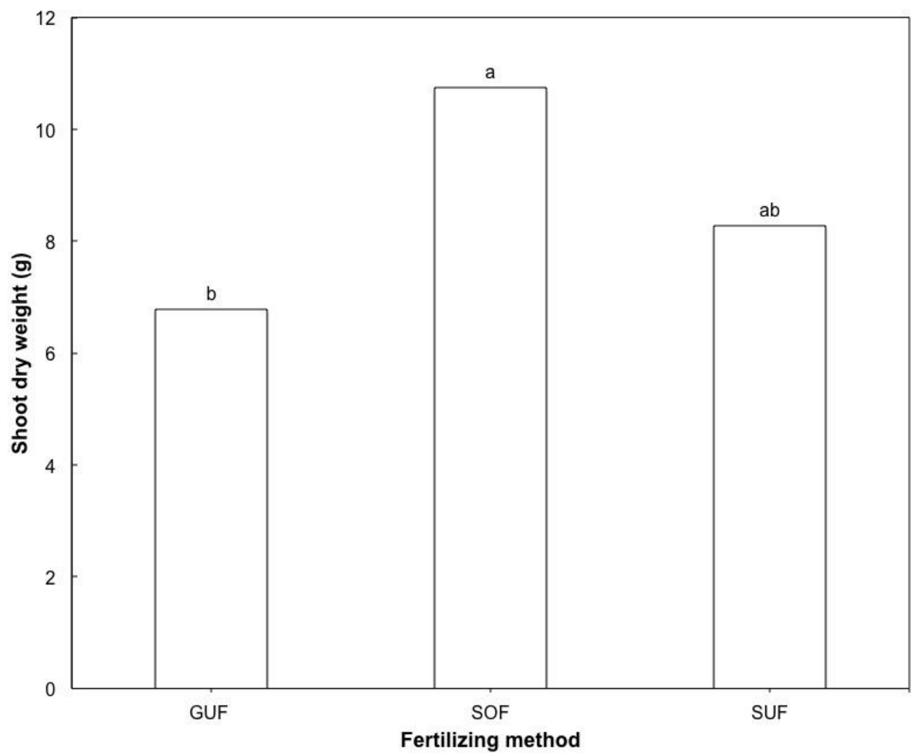


Figure 22. Effect of fertilizing methods on the mean dry weight of shoots (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.13 Mean dry weight of roots (g)

Treatments did not have interaction effects on the mean dry weight of roots. Individual treatment effects were seen with the fertilizing methods (Appendix 16).

1.4.13.1 Effect of fertilizing methods on the mean dry weight of roots (g)

Fertilizing method treatments had a significant effect on the mean dry weight of roots. The SOF method produced an average of 133 and 155% more dry weight of roots than the GUF and SUF methods, respectively (Figure 23).

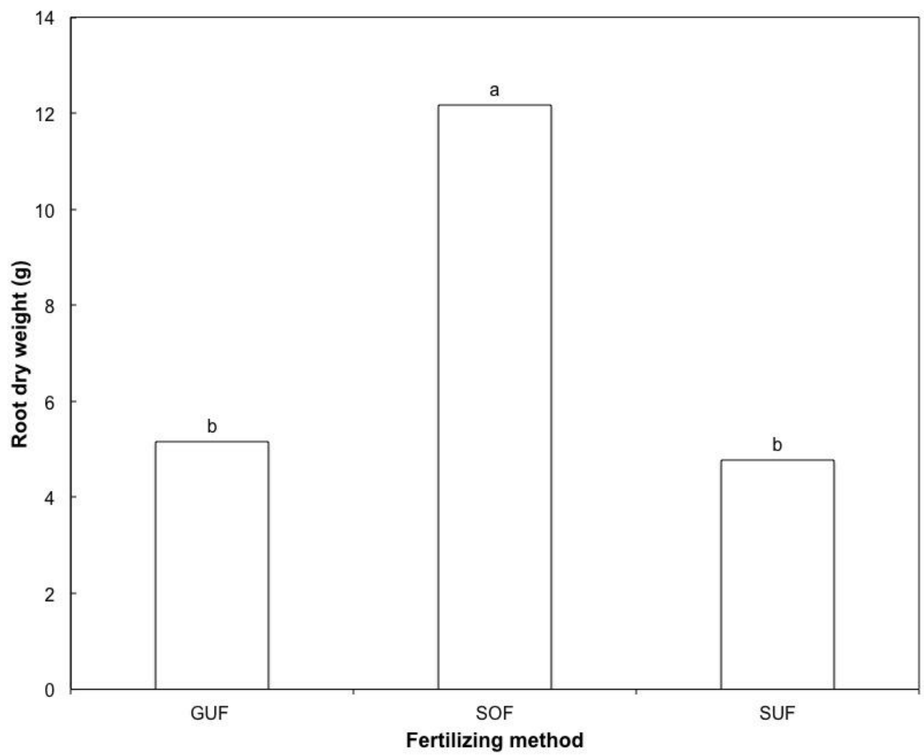


Figure 23. Effect of fertilizing methods on the mean dry weight of roots (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.14 Mean dry weight of fruits (g)

None of the treatments had any significant statistical differences on the mean dry weight of fruits (Appendix 16).

1.4.15 Mean number of marketable fruits per plant

None of the treatments had any significant statistical differences on the mean number of marketable fruits per plant (Appendix 17).

1.4.16 Mean number of unmarketable fruits per plant

None of the treatments had any significant statistical differences on the mean number of unmarketable fruits per plant (Appendix 17).

1.4.17 Mean number of total fruits per plant

Treatments did not have interaction effects on the mean number of total fruits per plant. Individual treatment effects were seen with the fertilizing methods (Appendix 17).

1.4.17.1 Effect of fertilizing methods on the mean number of total fruits per plant

Fertilizing method treatments had a significant effect on the mean number of total fruits per plant. The SOF method produced an average of 36% more total fruits per plant than the SUF method. The GUF method was not significantly different from either one (Figure 24).

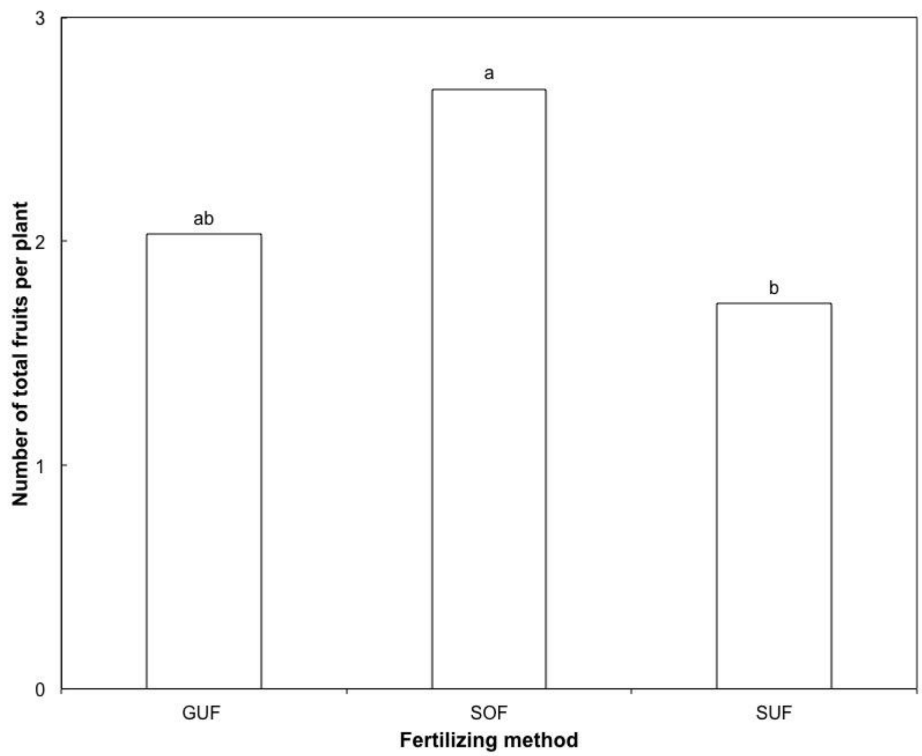


Figure 24. Effect of fertilizing methods on the mean number of total fruits per plant. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.18 Mean marketable fruit weight per plant (g)

Treatments did not have interaction effects on the mean marketable fruit weight per plant. Individual treatment effects were seen with the biostimulants (Appendix 17).

1.4.18.1 Effect of biostimulants on the mean marketable fruit weight per plant

Biostimulant treatments had a significant effect on the mean marketable fruit weight per plant. None of the biostimulants outperformed the WTR treatment. Plants treated with AQC, VTZ, and SPX produced 30, 40, and 51% less marketable fruit weight per plant than WTR, respectively (Figure 25).

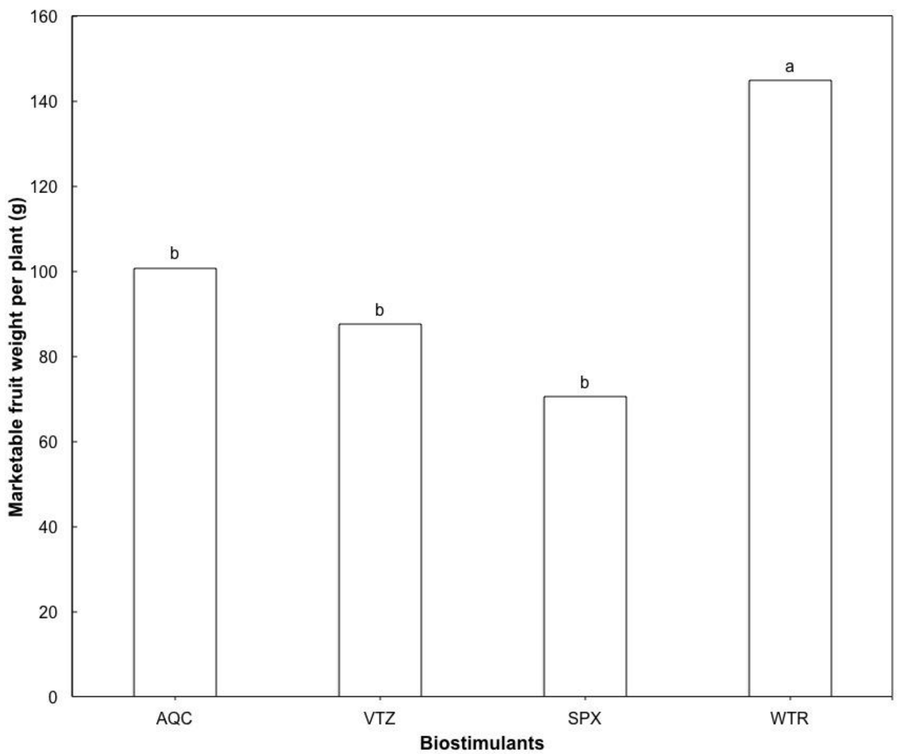


Figure 25. Effect of biostimulant treatments on the mean marketable fruit weight per plant (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.19 Mean unmarketable fruit weight per plant (g)

None of the treatments had any significant statistical differences on the mean unmarketable fruit weight per plant (Appendix 17).

1.4.20 Mean total fruit weight per plant (g)

None of the treatments had any significant statistical differences on the mean total fruit weight per plant (Appendix 17).

1.4.21 Mean length of marketable fruits (cm)

None of the treatments had any significant statistical differences on the mean length of marketable fruits (Appendix 18).

1.4.22 Mean length of unmarketable fruits (cm)

Treatments did not have interaction effects on the mean length of unmarketable fruits. Individual treatment effects were seen with the fertilizing methods (Appendix 18).

1.4.22.1 Effect of fertilizing methods on the mean length of unmarketable fruits

Fertilizing method treatments had a significant effect on the mean length of unmarketable fruits. The unmarketable fruits in the GUF and SUF methods were on average, 16 and 14% longer than the unmarketable fruits in the SOF method, respectively (Figure 26).

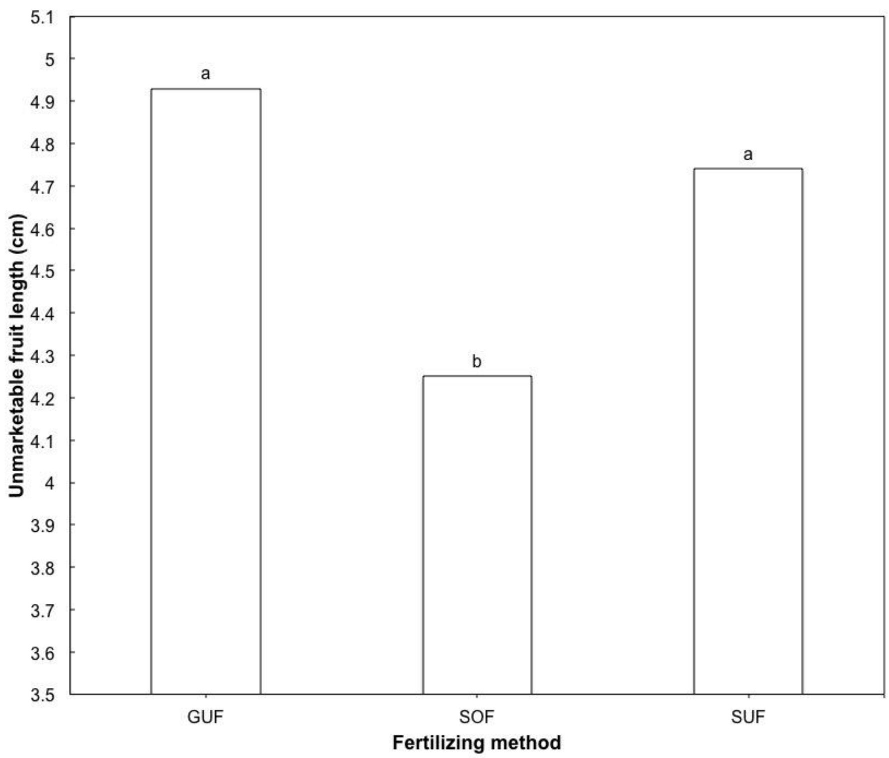


Figure 26. Effect of fertilizing methods on the mean length of unmarketable fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.23 Mean length of total fruits (cm)

Treatments did not have interaction effects on the mean length of total fruits. Individual treatment effects were seen with the fertilizing methods (Appendix 18).

1.4.23.1 Effect of fertilizing methods on the mean length of total fruits

Fertilizing method treatments had a significant effect on the mean length of total fruits. The total fruits in the GUF and SUF methods were on average, 15 and 14% longer than the total fruits in the SOF method, respectively (Figure 27).

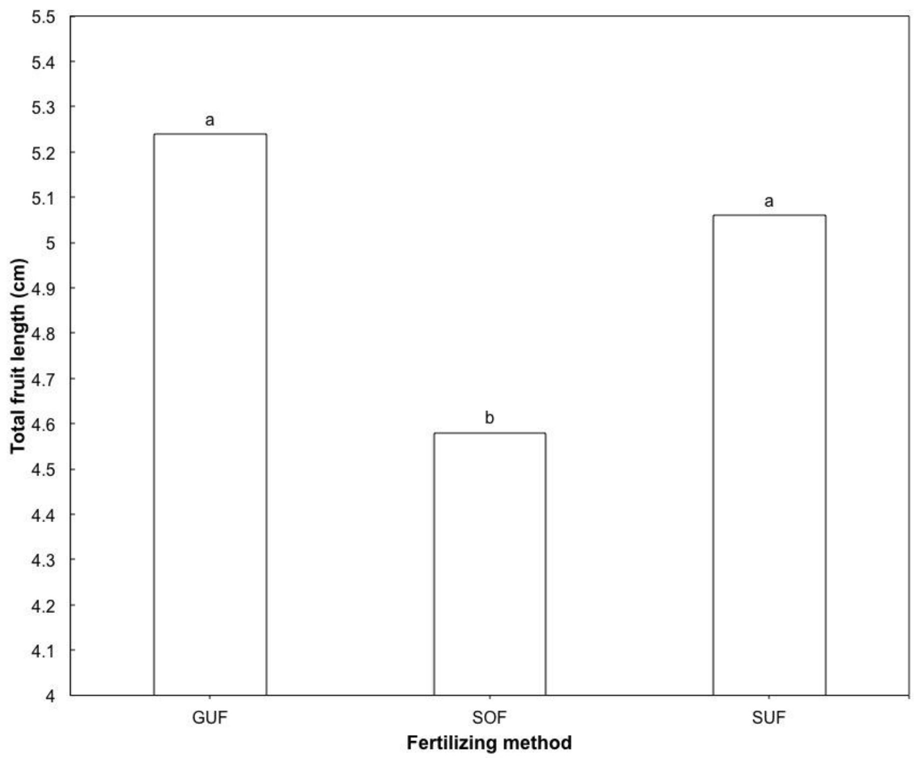


Figure 27. Effect of fertilizing methods on the mean length of total fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.24 Mean width of marketable fruits (cm)

None of the treatments had any significant statistical differences on the mean width of marketable fruits (Appendix 18).

1.4.25 Mean width of unmarketable fruits (cm)

None of the treatments had any significant statistical differences on the mean width of unmarketable fruits (Appendix 18).

1.4.26 Mean width of total fruits (cm)

None of the treatments had any significant statistical differences on the mean width of total fruits (Appendix 18).

1.4.27 Mean pericarp thickness of marketable fruits (mm)

None of the treatments had any significant statistical differences on the mean pericarp thickness of marketable fruits (Appendix 19).

1.4.28 Mean pericarp thickness of unmarketable fruits (mm)

Significant interaction effects were seen on the mean pericarp thickness of unmarketable fruits (Appendix 19).

1.4.28.1 Interaction effect on the mean pericarp thickness of unmarketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean pericarp thickness of unmarketable fruits. The SOF + VTZ combination produced unmarketable fruit pericarps that were 31% thicker than the unmarketable fruits in the SUF + VTZ combination. The SOF + AQC and SOF + VTZ combinations produced unmarketable fruit pericarps that were 29 and 27% thicker than the unmarketable fruits in the SOF + WTR combination, respectively (Figure 28).

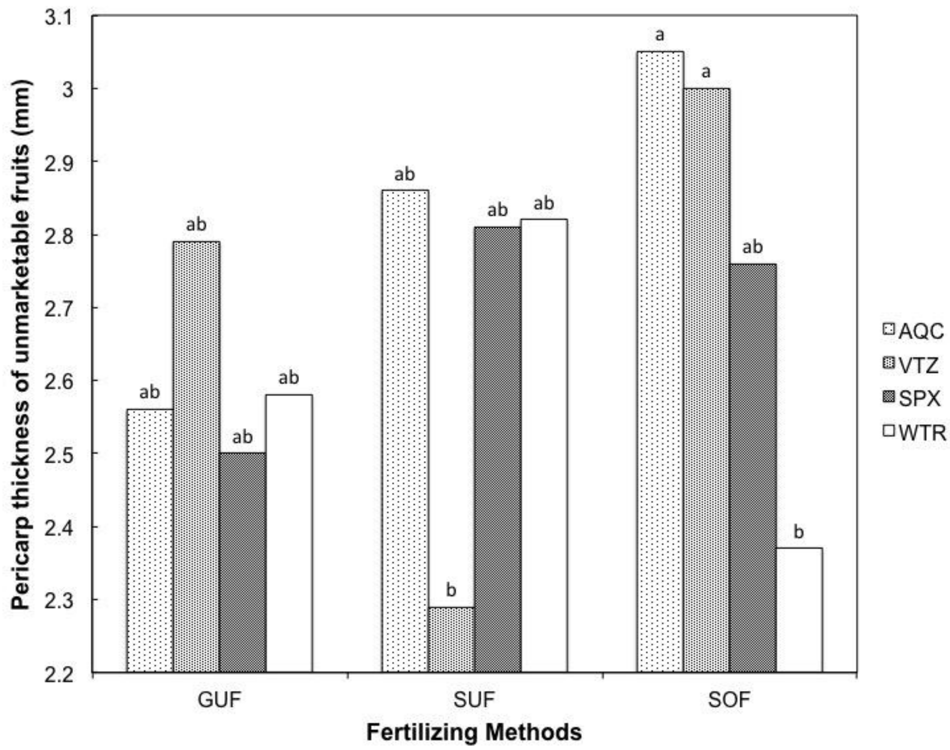


Figure 28. Combined effects of fertilizing methods and biostimulants on the mean pericarp thickness of unmarketable fruits. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.29 Mean pericarp thickness of total fruits (mm)

Significant interaction effects were seen on the mean pericarp thickness of total fruits (Appendix 19).

1.4.29.1 Interaction effect on the mean pericarp thickness of total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean pericarp thickness of total fruits. The SOF + AQC combination produced total fruit pericarps that were 24% thicker than the total fruits in the GUF + AQC combination. The SOF + VTZ combination produced total fruit pericarps that were 33% thicker than the total fruits in the SUF + VTZ combination. The SOF + AQC combination produced total fruit pericarps that were 37% thicker than the total fruits in the SUF + VTZ combination (Figure 29).

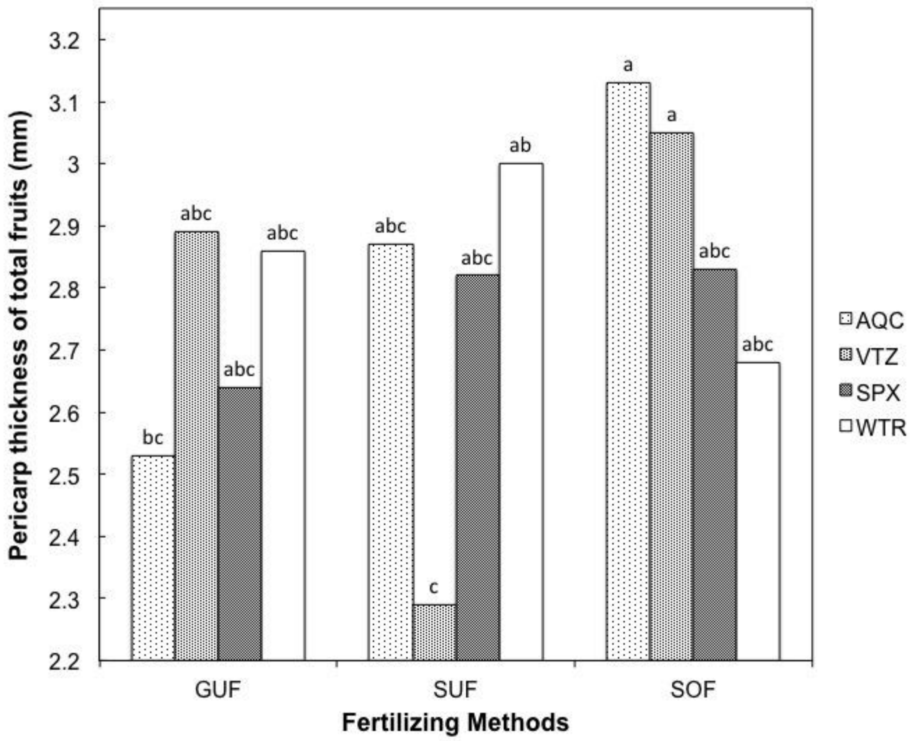


Figure 29. Combined effects of fertilizing methods and biostimulants on the mean pericarp thickness of total fruits. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.30 Mean SPAD readings

There were no significant statistical differences in the mean SPAD values at 85 DAS (Appendix 20). Readings averaged at a value of 66.14

1.4.31 Mean NO_3^--N content in fresh sap (ppm)

Treatments did not have interaction effects on the mean NO_3^--N content in fresh sap during fruit harvest time (106-114 DAS). Individual treatment effects were seen with the fertilizing methods (Appendix 21).

1.4.31.1 Effect of fertilizing methods on the mean NO_3^--N content in fresh sap (ppm)

Fertilizing method treatments had a significant effect on the mean NO_3^--N content in fresh sap. The SOF method produced an average of 73 and 97% more NO_3^--N content in fresh sap than the GUF and SUF methods, respectively (Figure 30).

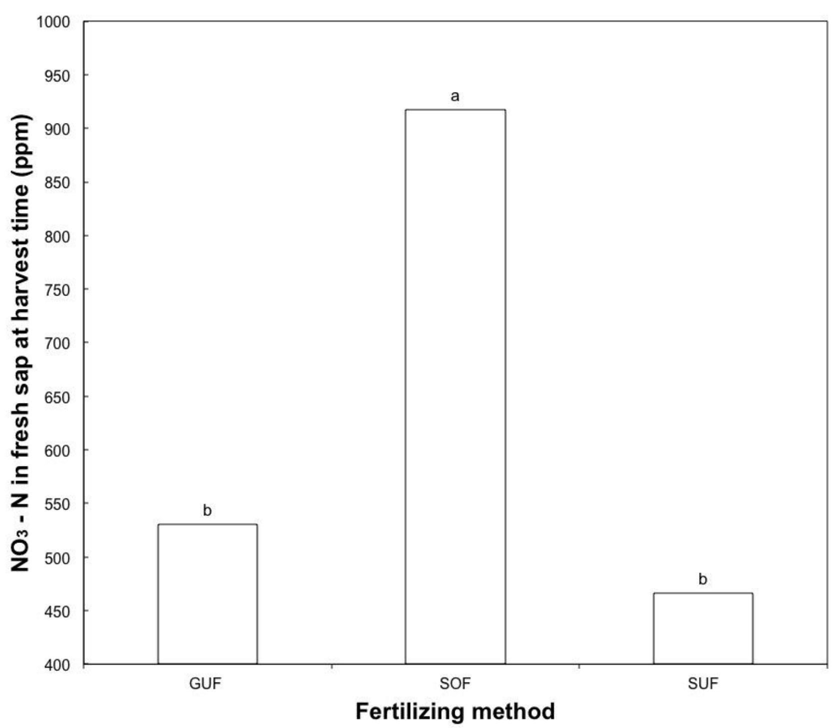


Figure 30. Effect of fertilizing methods on the mean NO_3^--N content in fresh sap during harvest time (ppm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

1.4.32 Analysis of reasons that categorize pepper fruits as unmarketable

Fruits were categorized as unmarketable when they had sunburns, scars, damage by birds, or when their width was less than 5.6 cm. In experiment 1, 86% of the fruits produced were categorized as unmarketable (Figure 31). Scarring and lack of minimum width were the most common reasons to categorize fruits as unmarketable, both occurring in 45% of the unmarketable fruits. Damage by birds and sunburns were observed in 4 and 6% of the unmarketable fruits, respectively (Figure 32).

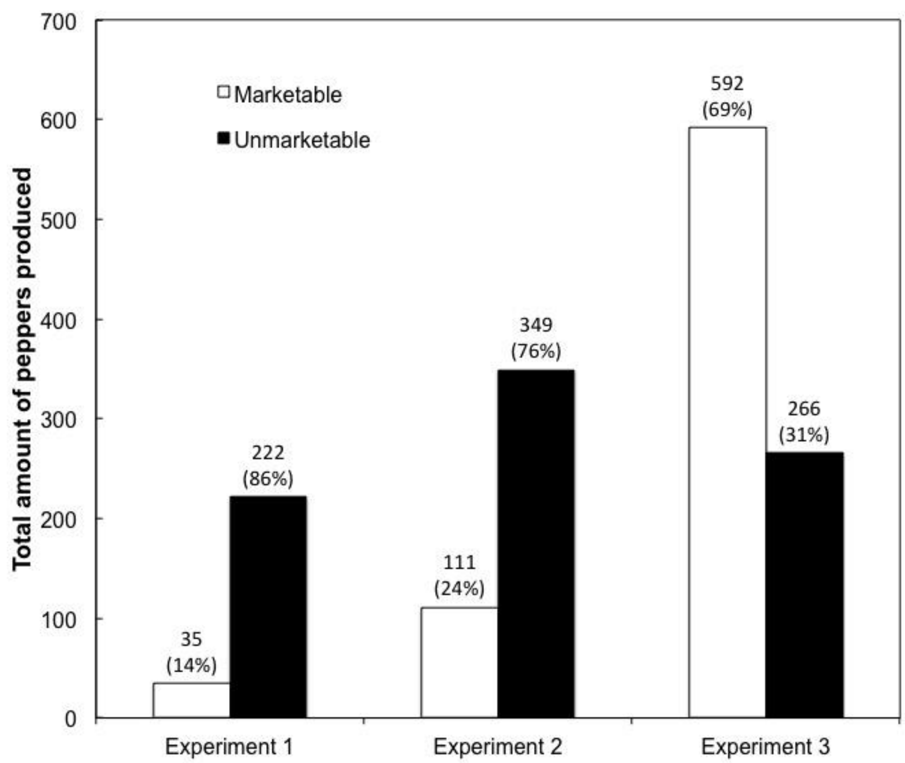


Figure 31. Marketable and unmarketable amounts of peppers produced in experiment 1 (2014), experiment 2 (2014), and experiment 3 (2015). Mayagüez, Puerto Rico.

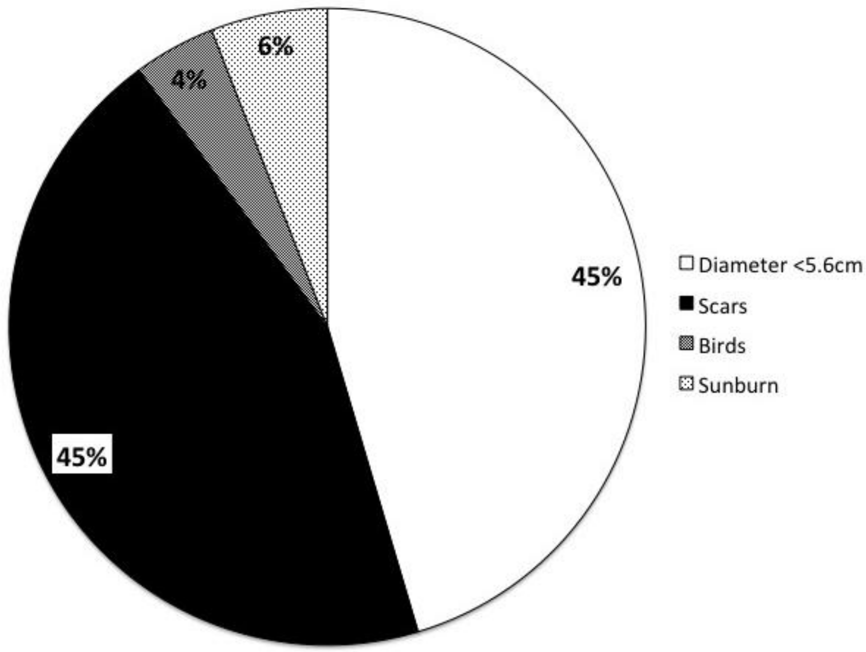


Figure 32. General distribution of different reasons to categorize pepper fruits as unmarketable during experiment 1. Mayagüez, Puerto Rico, 2014.

The most severe damages were caused by *Polyphagotarsonemus latus* (Banks), also called the broad mite (Figure 33, top left). Broad mites are considered a very destructive pest of high economic importance mainly because they cause malformations on leaves and flower buds (Figure 33, top right). The broad mite attacks the plant's fruitlets and new growth, causing shorter internodes, fragile lateral buds, abortions of blooms, and distorted growth which inevitably results in stunting of the plant when mite populations are at large (Fasulo, 2000), (Figure 33, middle left & middle right).

These result in drastic and severe yield losses because even if fruits ripen enough to reach maturity, they can still be unmarketable if mite populations are not controlled adequately (Figure 33, bottom left & bottom right). Also, even if the pepper fruits were unaffected by the mite's damage, the loss of foliage exposes the pepper fruits to direct sunlight, causing sunburns and thus reducing the marketable yield even more

Figure 33. (top left) Close up picture of two *Polyphagotarsonemus latus* (Banks) specimens; (top right) Top view of a pepper plant severely affected by broad mite damage; (middle left) Abortion of blooms, distorted growth, and loss of foliage caused by broad mites; (middle right) Stunted fruit development and severe scarring caused by broad mites; (bottom left) Mature pepper fruits are unmarketable due to aesthetic damages caused by broad mites; (bottom right) Mature pepper fruits that were unaffected by broad mites were still unmarketable due to sunburns or birds feeding on them.

1.4.32.1 Interaction effect on the distribution of reasons to categorize pepper fruits as unmarketable

Unmarketable reasons were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the unmarketable fruit damages. Combinations of fertilizing methods and biostimulant treatments were significantly ($p = 0.0012$) related to the observed damages in fruits. The damage distribution seen on the unmarketable fruits was arranged into percentages according to each combination of fertilizing method and biostimulant (Table 1).

Table 1. Distribution of reasons to categorize pepper fruits as unmarketable (fertilizing method*biostimulant) in the first experiment.

Fertilizing method	Biostimulant	Damage distribution (%)				
		Width < 5.6 cm	Scars	Bird	Sunburn	Total
GUF	AQC	42	48	0	10	100
GUF	VTZ	50	38	0	12	100
GUF	SPX	44	28	6	22	100
GUF	WTR	44	48	0	8	100
SUF	AQC	36	50	9	5	100
SUF	VTZ	42	37	21	0	100
SUF	SPX	37	41	11	11	100
SUF	WTR	32	47	21	0	100
SOF	AQC	53	45	0	2	100
SOF	VTZ	46	49	0	5	100
SOF	SPX	53	47	0	0	100
SOF	WTR	51	46	0	3	100

The total amounts of fruits affected by the different unmarketable reasons were graphed separately in order to better understand the effects of treatment combinations. The SOF method combined with any of the biostimulant treatments (including WTR), produced the highest amount of unmarketable fruits affected with scars or with a width less than 5.6

cm (Figure 34 and Figure 35). Damage by birds was highest on all the SUF method + biostimulants combinations but nonexistent on any of the SOF method + biostimulant combinations. The GUF + SPX was the only combination within this fertilizer method that had bird damage (Figure 36). Fruits affected by sunburns were observed mainly in the combination of the GUF method with biostimulant treatments (Figure 37). Pest populations might have been controlled more effectively if neem applications had been more frequent and had began earlier in the seedling stage. Also, the manual atomizer could have lacked the required pressure needed in order to effectively break down the solution's droplets and perform even applications.

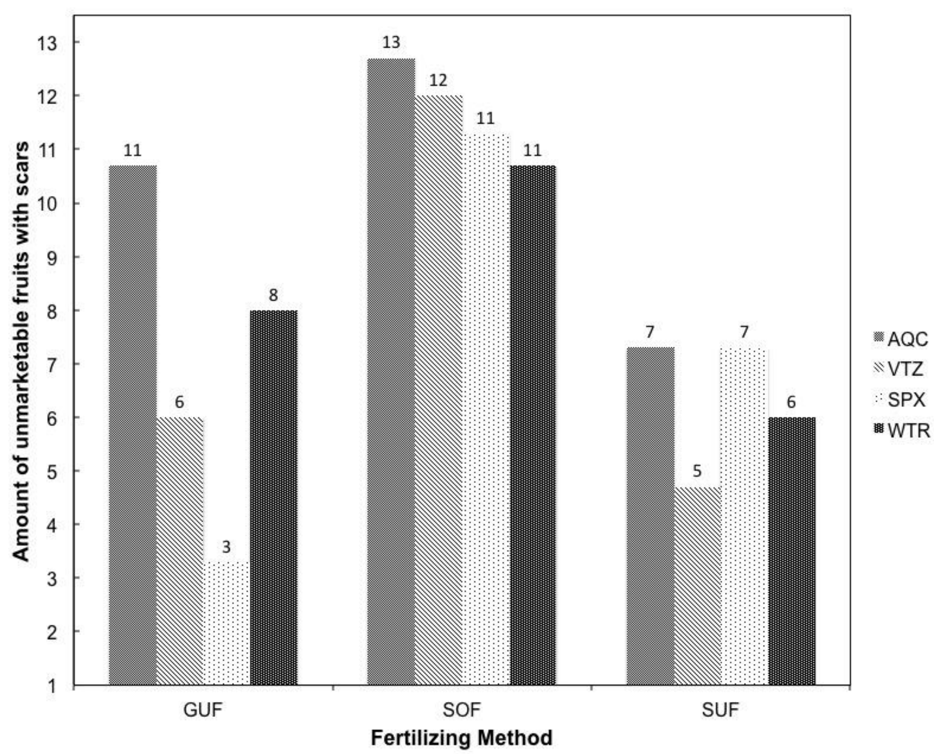


Figure 34. Interaction effects on the incidence of scar damage in unmarketable fruits during experiment 1. Mayagüez, Puerto Rico, 2014.

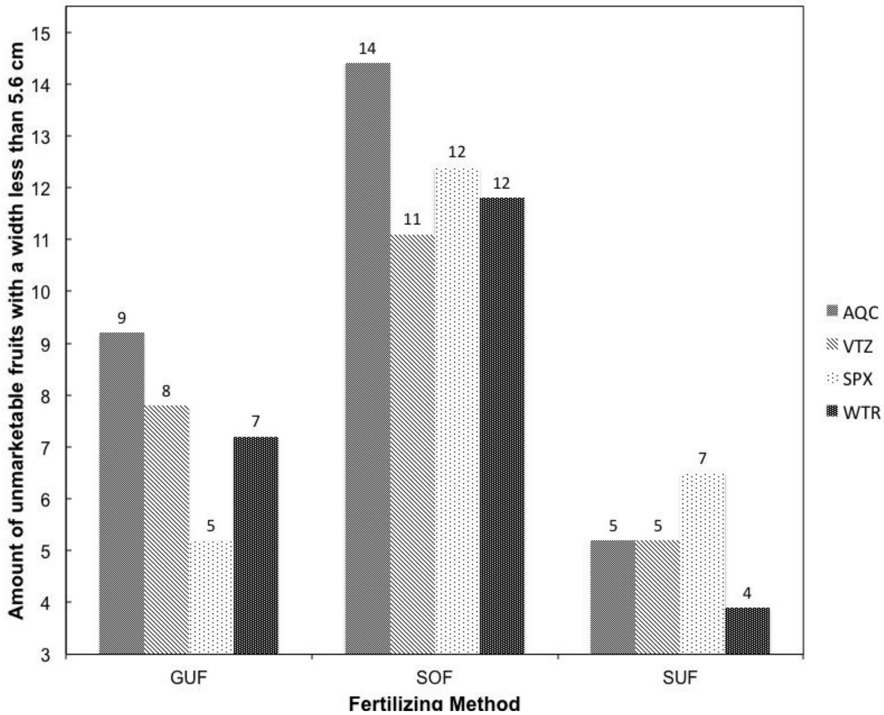


Figure 35. Interaction effects on the incidence of unmarketable fruits with a width of less than 5.6 cm during experiment 1. Mayagüez, Puerto Rico, 2014.

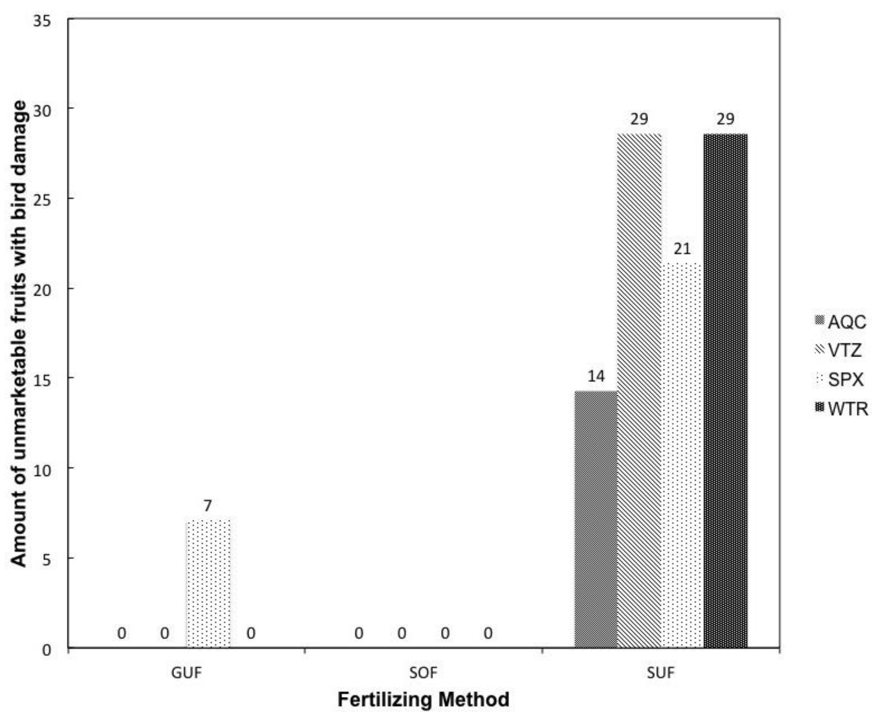


Figure 36. Interaction effects on the incidence of bird damage in unmarketable fruits during experiment 1. Mayagüez, Puerto Rico, 2014.

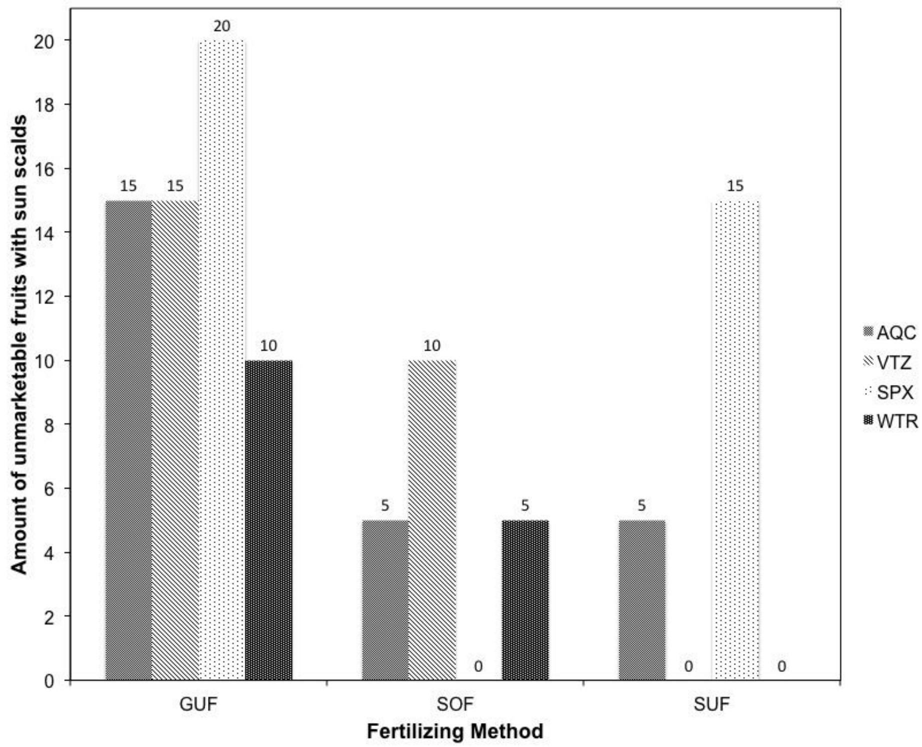


Figure 37. Interaction effects on the incidence of sunburn damage in unmarketable fruits during experiment 1. Mayagüez, Puerto Rico, 2014.

1.4.33 Marketable fruit grading analysis

Fruits categorized as marketable had to be free of decay. The marketable fruits were also free of injuries from scars, sunburn, disease, insects, mites, handling, or other means. An equatorial diameter greater or equal than 5.6 cm was also established to count pepper fruits as marketable. After the fruit was counted as marketable, it was further classified into categories according to two grading systems: 1)

the United States standards for grades of sweet peppers (2005) and 2) a diameter scale used for imported greenhouse-grown bell peppers (Jovicich et al., 2007). In experiment 1, only 14% of the fruits produced were categorized as marketable (Figure 31).

1.4.33.1 United States standards for grades of sweet peppers (2005)

Marketable fruits were classified into three categories: U.S. Fancy, U.S. No.1, or U.S. No.2. These categorizations were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the grading system. None of the treatments had any significant relationship with the grading system. In this experiment, only 3% of marketable fruits were U.S. Fancy, 43% were U.S. No.1, and 54% were U.S. No. 2 (Figure 38).

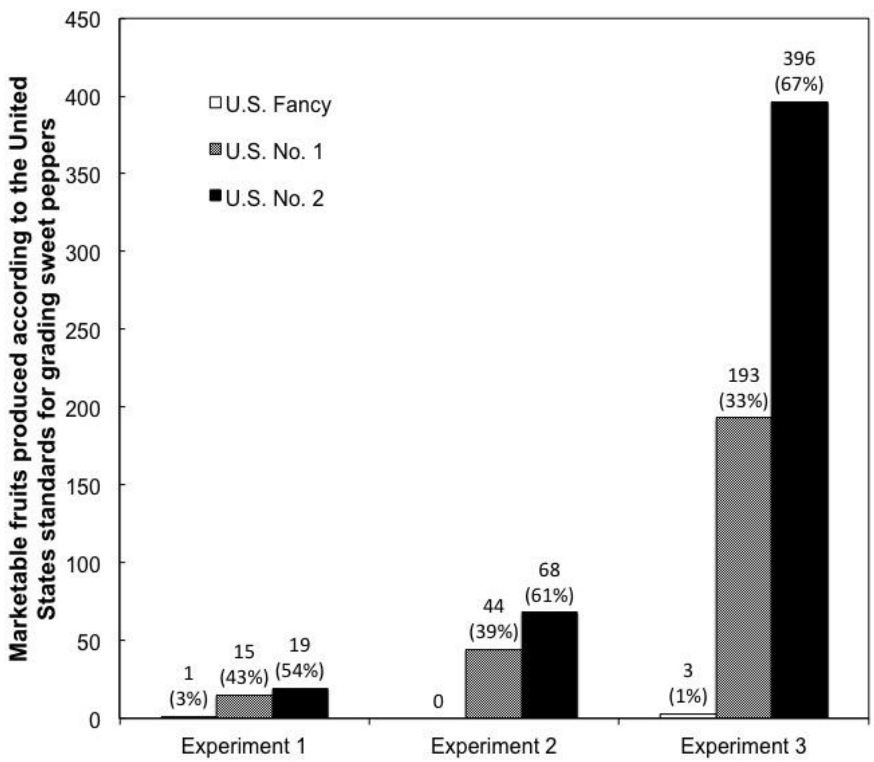


Figure 38. Marketable amounts of peppers produced in experiment 1 (2014), experiment 2 (2014), and experiment 3 (2015) according to the United States standards for grading sweet peppers. Mayagüez, Puerto Rico.

1.4.33.2 Grading system for imported greenhouse-grown bell peppers (modified from Jovicich et al., 2007)

Marketable fruits were classified into two categories according to a diameter scale: small (5.6 – 6.39 cm) and regular (diameter > 6.4 cm). None of the treatments had any significant relationship with the grading system. In this experiment, 43% of marketable fruits were classified as small and 57% were classified as regular (Figure 39).

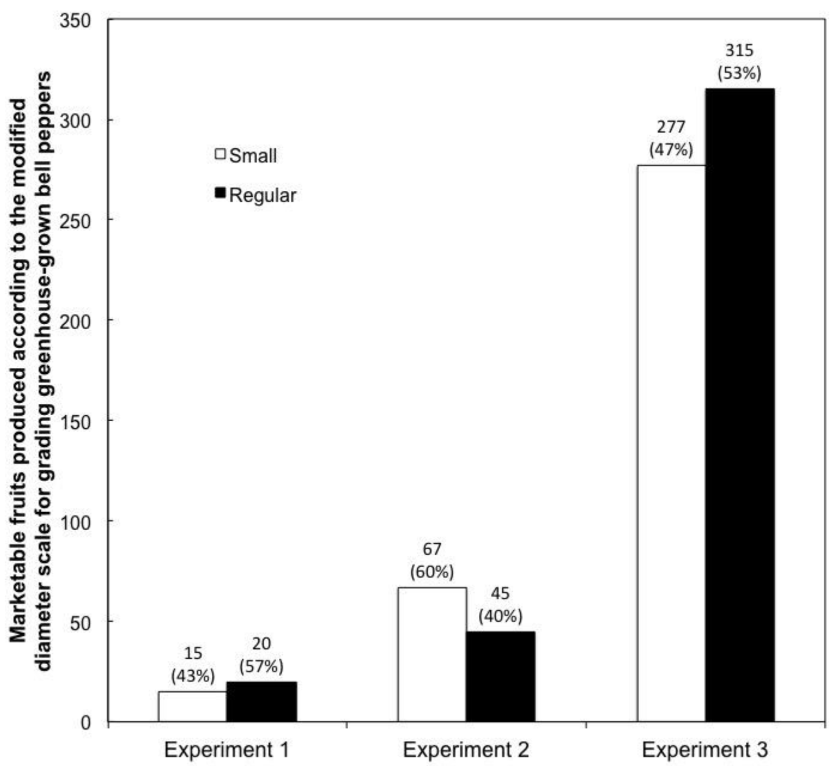


Figure 39. Marketable amounts of peppers produced in experiment 1 (2014), experiment 2 (2014), and experiment 3 (2015) according to the modified diameter scale for grading greenhouse-grown bell peppers. Mayagüez, Puerto Rico.

Chapter 2. Effects of foliar biostimulants and fertilization methods on potted 'Chocolate Beauty' bell peppers with a 3:1 growing medium mixture of alluvial soil and Promix®

2.1 Introduction

Protected agriculture has been recently introduced as a way of producing and extending the growing season of high quality fruits and vegetables that obtain peak market prices while minimizing external risks such as damages by abiotic and/or biotic factors (Zhang, 2003). A protective structure can be defined as any structure designed to alter or modify the crop's environmental factors (i.e. light, wind, temperature, air humidity, pest pressure, etc.) with the purpose of increasing yields and quality. The protective structures can be classified into greenhouses or screen/shade houses depending to their roof type. The main difference is that greenhouses have a nonporous roof structure and screen/shade houses have a porous roof structure (Santos et al., 2013).

The Quonset style greenhouses have a simple design and their construction is not expensive. They are usually covered with plastic and provide minimal shading to the interior. The curved sides may impose height limitations on plants unless the support posts are of sufficient length. As the height of the foundation increases, so does the risk of wind damage. Disadvantage of this type of greenhouse are that it may suffer from ventilation problems (Bucklin, 1988) and can be uplifted by winds if the foundation of the frame is not strong enough. Frames can be constructed from many different materials (e.g. wood, aluminum, galvanized steel, etc.) as long as they can withstand the uplifting forces produced by winds and the downward gravity loads of the structure.

The frames made from galvanized steel are usually cheaper than frames that are made from aluminum. Galvanized steel is capable of lasting a long time and it also exhibits high strength capabilities. Synthetic sheets and films (e.g. polyethylene film, polyvinyl fluoride films, fiberglass reinforced plastic, etc.) are the most widely used materials to cover greenhouses. Polyethylene film is the most common synthetic film used due to its low cost, lightweight, ease of use, varying thickness, and its high light transmittance, even though it has a short life of about two years (Kessler, 1998). Inexpensive, low maintenance greenhouses with minimal climate control are the most widely used in the tropical and subtropical areas. They usually enable plants to perform better than they do in open field conditions where protection is non-existent (Zhang, 2003).

Only in recent years Puerto Rico's agriculture has shifted to cultivating vegetable crops under protected structures. The protective structures could enable farmers to protect crop from adverse environmental tropical condition such as extreme heat, drought, floods, storms, and hurricanes. On a worldwide scale, the production of vegetable crops accounts for 65% of the total global area covered by greenhouses. Greenhouses provide the plants with a favorable and relatively stable environment in which they can flourish and provide sufficient economic yields but this environment is also favorable for pests, diseases, and pathogens that can rapidly develop and quickly increase their populations over short time periods.

'Chocolate Beauty' bell pepper's original breeder and vendor was a company called Petoseed founded in California in 1950. This company led the hybridization of hot peppers and tomatoes in the 1970s and 80s. The variety was developed in 1993 and is described as a non-pungent pepper with large, smooth bell type. Fruits turn from a medium-dark green to a chocolate brown color when fully mature and it is also resistant to Tobacco Mosaic Virus (Bosland & Wehner, 1999).

2.2 Objectives

1. To determine the effects of select foliar biostimulants with different fertilization methods on potted ‘Chocolate Beauty’ bell peppers with a specific growing medium.
2. To determine the effects of select foliar biostimulants with different fertilization methods on the physiological development of ‘Chocolate Beauty’ bell peppers.
3. To determine the effects of select foliar biostimulants with different fertilization methods on the yield and quality of ‘Chocolate Beauty’ bell peppers.

2.3 Methodology

2.3.1 Site description

The experiments were conducted in Greenhouse #5 (latitude: 18° 12’57” N, longitude: 67° 08’48” W, elevation: 15 meters above sea level) located in a Greenhouse Range of six freestanding, even-span Quonset style greenhouses arranged side by side and built upon a continuous concrete foundation at the Finca Laboratorio Alzamora in the UPR at Mayagüez (Figure 3, left). The entry road was towards the front of the greenhouse and a forest-like vegetation growth was towards the rear end. The adjacent greenhouse #6 was being used for an aquaponic system that grew tilapia (*Tilapia* spp.) and spearmint (*Mentha spicata*) and the other greenhouses #1 through #4 were used as nurseries for ornamental plants and vegetable seedlings. The experiment was replicated three times. The second experiment took place on July 2014 through November 2014.

2.3.2 Quonset style greenhouse

The experimental Quonset style greenhouse structure is based upon an arched roof (Figure 3, right) (Omid & Shafaei, 2005). It had a galvanized steel frame and support posts which were embedded into the concrete floors. The greenhouse measured 14.6 meters (m) long (L), 9.1 m wide (W), 4.6 m high (H), and had a bow length (B) of 15.2 m. The total floor surface area of the greenhouse was 133 m^2. On the inside, there were four floor areas with soil that measured 12.2 m long by 1.8 m wide. Each soil-floor surface area was 22 m^2. The total soil-floor surface area measured 88 m^2. This is equivalent to 66% of the total greenhouse area. Each of the soil-floor areas was covered with a black

polyethylene (PE) landscape fabric in order to suppress germination and growth of weeds (Figure 4, left).

2.3.3 Roof structure and material

The roof of the greenhouse was made from a nonporous single layer of transparent PE film without any sort of vents (Figure 3, right). This material is low in cost and lightweight. Light transmission is improved by using only one layer of PE film instead of two (Omid & Shafaei, 2005). Also, PE accelerates heat loss because this material allows easy passage of the reradiated heat energy emitted by the plants and soil inside the greenhouse (Worley, 2009) An accelerated heat loss rate was necessary due to Puerto Rico's year around warm tropical climate. Even though the roof was transparent, light transmission was decreased due to dirt, dust, debris, mold, leaf litter, and plant resin accumulation on top of the greenhouse (Figure 3, right). These impurities were probably originated by the forest-like growth at the rear end of the greenhouse (Figure 4, right).

Airborne dust, dirt and debris captured by the trees' leaves were probably deposited on the greenhouse roof by the rain. These impurities, in addition to the leaf litter that had already accumulated, stained the roof and gave way to mold growth (Figure 3, right). Black olive trees (*Bucida buceras*) in the forest-like growth at the rear of the greenhouse continuously exuded a dark, sticky, and staining, material (Francis, 1989; Gilman & Watson, 1993) that deposited on the roof and thus, diminished light transmission. It is also important to mention that these trees and other vegetation served as a habitat for the birds Red-legged thrush (*Turdus plumbeus*) and the Pearly-eyed Thrasher (*Margarops fuscatus*) (Figure 5, left) (Delannoy-Juliá & Mari-Mut, 2013), which heavily affected this second experiment due to the constant feeding on physiologically mature pepper fruits before they could be harvested (Figure 5, right).

2.3.4 Walls

The Quonset style greenhouse range was rather basic and low cost because none of the greenhouses had any materials covering the walls. The Quonset style greenhouse did not have walls on the front, rear, or sides of the structure (Figure 3, right), only the galvanized steel support posts that were embedded into the concrete and located at the sides of the greenhouses to provide a firm foundation. The design was basic and probably inexpensive to build because it had no climate control features, but the absence of walls could have possibly improved air movement. This could remove hot air, lower relative humidity and accelerate the structure's natural cooling capacity (Kessler, 1998). Even though the absence of walls allowed bees, beneficial insects and air to move freely within the greenhouses, it left the plants vulnerable to strong winds and the entrance of pests and other unwanted organisms (e.g. insects, mites, birds, cats, chickens, iguanas, unwanted people, etc.) that could ultimately result in damages to plants, yields and/or fruits.

2.3.5 Raised benches

Four longitudinal, galvanized steel raised benches (GSRB) that measured 12.4 m long by 1.5 m wide and 0.64 m high were placed directly above each of the soil-floor areas (Figure 4, left). The aisles between each GSRB were approximately 0.62 m wide. The individual GSRB had a surface area of 18.8 m^2 and their total surface area combined was 75.2 m^2. This meant that only 57% of the greenhouse area was devoted to growing plants. According to Kessler (1998), this is a rather low percentage. He states that with proper planning, 70-80% of the greenhouse's total surface area can be dedicated to growing plants. The top surface of the GSRB was made out of weld mesh with a rectangular grid and welded at each joint. The weld mesh facilitated air movement and allowed the growing medium to freely drain excess water through the drainage holes at the bottom of the containers (Kessler, 1998). The excess drainage water was then captured by the soil under each GSRB.

2.3.6 Containers

According to Acquaah (2009), the correct size of containers should be based on the plant's height. The plant's height should be approximately two times the container's height. Plastic containers were washed, cleaned, and disinfected using a 10% hypochlorite solution before being used. Tap water was used to give the containers a final rinse before placing them upside down to air dry on top of the raised benches. The standard sized, 5-gallon containers were lightweight and black color (Figure 4, left). Each container measured 30 cm long and had a 30 cm diameter (Nursery Supplies Inc., Chambersburg, Pennsylvania). The containers were arranged into longitudinal columns of 15 pots and horizontal rows of 4 pots for a total of 60 containers on each GSRB (Figure 3, right). The distance between the longitudinal columns of containers was 43.2 cm and the distance between the horizontal rows of containers was 76.2 cm. Containers were labeled on the outside with the corresponding treatment for easy identification during treatment applications.

2.3.7 Growing medium

Unsterilized alluvial soil (UAS) was collected from the “Quebrada de Oro” brook that runs across the Mayagüez campus (Figure 6). This brook directs the excess runoff water from precipitation events towards the Mayagüez bay. The water flow carries sediment and eroded soil from higher elevations that gradually accumulates and deposits at the bottom of the brook. The accumulated sediment and soil that gets scooped from the brook during regular maintenance events is transported to the Finca Laboratorio Alzamora. It is then left outdoors for a period of time until it eventually gets mixed with compost, sieved, and sold for income.

A soil analysis was made to the UAS and indicated a pH of 7.77 and a conductivity of 1013 μS/cm with 0.96% organic matter. Its structure was composed of 25.04% clay, 23.44% silt, and 51.52% sand. According to the soil textural triangle, this soil had a sandy clay loam texture (Appendix 1). For the second experiment, the UAS was sieved with a 2.54 cm mesh in order to break the soil aggregates and also remove rocks and other impurities (e.g. broken glass, plastics, trash, etc.). The sieved UAS was then mixed with a general-purpose sphagnum + perlite mix (Promix®) at a ratio of 3:1. This growing medium mixture was equivalent to having 25% Promix® mixed with 75% sieved UAS in each container.

2.3.8 Irrigation

During the second experiment, the growing medium was saturated with water 24 hours before transplanting the seedlings. The same automated drip irrigation system that was used in the first experiment was also used in the second experiment. It consisted of a black, 13 mm polyethylene hose laid longitudinally along the center of each GSRB, between the second and third columns of pots. The hose was then punctured to insert plastic connectors (6mm) (RAINDRIP®. Fresno, California). Poly tubing (6.35 mm outer diameter x 4.32 mm inner diameter ± 2%) (RAINDRIP®. Fresno, California) was then attached to the connectors and pressure compensating drippers (2 L/hour) (RAINDRIP®. Fresno, California) were inserted at every end (Figure 7). Each dripper was placed near the plant's stem in each container.

The water source for irrigation events was a well located at a higher elevation in the Finca Laboratorio Alzamora. A 25-psi pressure regulator was added to the irrigation system to ensure an even water emission out of every dripper. A Steel Spin Clean® (Agricultural Products, Inc.) filter with a 150 mesh was used to remove debris and prevent clogging of drippers. The irrigation system was connected to an electric 12-Station Outdoor SST "Simple to Set" Irrigation Timer (RAINBIRD® model SST1200O). Irrigation frequency was estimated and gradually increased according to visual assessments (e.g. wilting plants, dry substrate), plant growth stage (e.g. vegetative stage, flowering stage), air temperature, container size, and the minutes required for the growing medium to drain excess water.

2.3.9 Stakes

Pepper plants carry significant weight at fruit set and additional support is needed in order to prevent bending and breaking of stems and branches (Acquaah, 2009; Cánovas-Fernández, 2011). The use of stakes also maintains plants and branches in an upright position that allow light exposure to be optimized (Bowen & Frey, 2002). In addition, the absence of walls in the experimental Quonset style greenhouse left plants vulnerable to damage by strong winds. Dowels made from pinewood were purchased at a local hardware store and used at stakes. The 6 mm dowels measured 91 cm long and were inserted into the substrate approximately 8 cm apart from the stems when the plants reached a height of 30 cm. Additional dowels were used for extra support if needed. Stems and branches were attached to the dowels using 15 and 8 cm long plastic zip ties. About 50% of the dowels had to be replaced during harvesting because the irrigation water in the substrate rotted the dowels' wood and made them fragile. The weight of fruit set easily broke the already weakened dowels.

2.3.10 Pest management

Pest populations were controlled with agrochemicals during the second experiment because the organic pest control strategies used during the first experiment were ineffective. Oberon® 2 SC (spiromesifen; 23.1% A.I.) (Appendix 22) was applied at a dose of 0.6 L ha^{-1} with a manual atomizer to control broad mites on August 12 and September 11, 2014 (30 and 60 days after transplant (DAT), respectively).

2.3.11 'Chocolate Beauty' bell pepper

Seeds were ordered online from Mountain Valley Seed Co. in Salt Lake City, Utah. The seeds arrived guaranteed free of weeds and with a germination percentage of 99%. Only the most uniform, best-looking seeds were chosen first hand to maintain uniformity. Seeds that looked small, slim, or atrophied were not used.

Germination trays were used for the second experiment due to an early mite infestation in the greenhouse during the first experiment. The germination trays eased watering in order to keep the medium saturated and stimulate sprouting. Also, they facilitated an even application of pesticide to every seedling before transplanting to the containers. Germination trays were soaked, cleaned and disinfected with a brush using a 10% hypochlorite solution. The germination trays were then rinsed with tap water and left upside down to air dry. They were lightweight, plastic, and black color (Figure 40). Each germination tray measured 52 cm long, 27 cm wide, and had 72 cells. The cells were filled with a general-purpose sphagnum + perlite mix (Promix® BX) to be used as a germination medium. A single seed was superficially sown into each cell and only the most vigorous seedlings were used for transplant on July 13, 2014. Harvest began in October 4, 2014 (83 DAT) and ended in November 2, 2014 (112 DAT). Physiologically mature pepper fruits were harvested when they had at least 50% of their brown color developed. Severely damaged fruits that had been partially eaten by birds were also harvested as soon as they were seen.

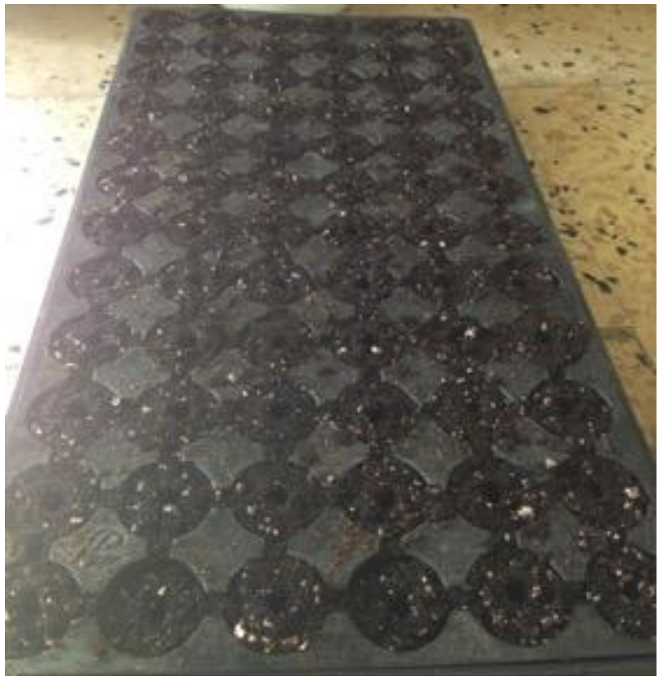


Figure 40. Plastic germination trays were used for the second and third experiments

2.3.12 Experimental design

This experiment was constructed as a completely randomized design. It consisted of a 3 x 4 factorial model arrangement with ten repetitions (one plant per repetition for a total of 120 plants). Three different fertilization methods were used to provide an equivalent of 116 $kg \cdot ha^{-1}$ of N as a post-transplant fertilizer: Granular Urea Fertilization (GUF), Spoon-fed Solubilized Urea Fertilization (SUF), and Spoon-fed Organic Fertilization (SOF). GUF was done with only one application at the beginning of the fruiting stage (70 DAT). SUF and SOF were divided into ten equal applications every seven days for ten weeks beginning at 14 DAT. Four biostimulant treatments were used as foliar applications: Aminoquelant-Ca (AQC), Vitazyme (VTZ), Stimplex (SPX), and the water check (WTR). Biostimulant solutions were diluted according to the manufacturer's recommendations. Foliar applications of biostimulants began at 14 DAT and were sprayed on leaves, stems, buds, flowers, and immature fruits every 14 days for ten weeks.

2.3.12.1 The variables investigated to determine morphological development were:

Shoot fresh weight (g)
Root fresh weight (g)
Shoot dry weight (g)
Root dry weight (g)

2.3.12.2 The variables investigated to determine the yield parameters were:

Number of marketable fruits per plant
Number of unmarketable fruits per plant
Number of total fruits per plant
Marketable fruit weight per plant (g)
Unmarketable fruit weight per plant (g)
Total fruit weight per plant (g)
Harvest time of marketable fruits (time as days after transplant, DAT)
Harvest time of unmarketable fruits (time as days after transplant, DAT)
Harvest time of total fruits (time as days after transplant, DAT)

2.3.12.3 The variables investigated to determine the quality of fruits were:

Length of marketable fruits (cm)
Length of unmarketable fruits (cm)
Length of total fruits (cm)
Width of marketable fruits (cm)

Width of unmarketable fruits (cm)
Width of total fruits (cm)
Pericarp thickness of marketable fruits (mm)
Pericarp thickness of unmarketable fruits (mm)
Pericarp thickness of total fruits (mm)
Total soluble solids content in marketable fruits (Brix %)
Total soluble solids content in unmarketable fruits (Brix %)
Total soluble solids content in total fruits (Brix %)
Classification of marketable fruits

2.3.12.4 The variables investigated to determine changes in chlorophyll and N content:

SPAD index
Nitrate content in sap

2.3.12.5 The variables investigated to analyze the reasons for categorizing fruits as unmarketable were:

Percentage of unmarketable fruits damaged mites (scarring)
Percentage of unmarketable fruits with a diameter less than 5.6 cm
Percentage of unmarketable fruits damaged by sunburns
Percentage of unmarketable fruits damaged by birds

2.3.13 Fertilizer treatments

Fertilizer applications were determined by the recommendations found in the technological package for commercial pepper production in Puerto Rico (Fornaris et al., 2005). Calculations had to be made in order to determine the exact amounts of fertilizer needed for each individual plant. Current fertilization recommendations in Puerto Rico are focused on the open field, conventional agriculture and are meant for relatively big areas of land. Information regarding fertilizer management in greenhouse pepper production in Puerto Rico is currently very limited. This lack of information could slow Puerto Rico's agricultural transition to protected agriculture.

Bell peppers in Puerto Rico are cultivated on raised beds but most commonly in open fields. Raised beds are usually 1.5 to 1.8 meters apart with crops aligned in double rows. A distance of 30 to 45 cm separates each pair of rows and the distance of plants within the rows is 30cm. These planting distances can be used to estimate plant densities. Estimated plant densities range between 35,877 and 43,051 plants per hectare. A plant density of 36,890 plants per hectare (Equation 1) was used to calculate nutrient requirements of individual plants. The total amounts and requirements of N, phosphate and potash (K_2O) applied as a pre-plant fertilizer (Equation 2) and the N applied as post-transplant fertilizer (Equation 3) were calculated for 1 hectare based on plant density and local recommendations from Puerto Rico's commercial pepper production guide (Fornaris et al., 2005).

2.3.13.1 Granular Urea Fertilization (GUF)

Urea $[CO(NH_2)_2]$ is the most widely used N source in the U.S. mainly due to its high N content and its high solubility in water. Manufacturing, handling, storage, and transportation logistics of granular urea are favorable and less dangerous (Havlin et al., 2005). It has a lower tendency to cake, stick, and/or explode than ammonium nitrate

(NH_4NO_3), and it's also less corrosive to handling and equipment. In its current form, the N in urea is unavailable for plants. When urea is applied to the soil, urease enzymes hydrolyze it to ammonium (NH_4^+). Variations in soil pH may cause the NH_4^+ to form ammonia (NH_3), which can then be volatilized at the soil surface (Equation 4).

The urease enzyme that is needed to catalyze the hydrolysis of urea is usually very abundant in soils, especially in warm, moist soils that are high in organic matter content and also high in soil microbial populations. The application of urea to the soil surface is most efficient when washed into the soil and when there is a low volatilization potential. Deep incorporation of granular urea is advisable over surface applications or shallow tillage. This minimizes volatilization losses of NH_3 by increasing the volume of soil capable of retaining it. Many factors may interfere with the effectiveness of urea and can cause some degree of variability in the crop's response to this fertilizer. In order for N in the soil to be available for plant use, it has to be in one of two forms: ammonium or nitrate.

2.3.13.1.1 Pre-planting: synthetic granular fertilizer (10-10-10)

Only 16 grams of a synthetic 10-10-10 granular fertilizer were incorporated as a pre-plant fertilizer into the first 15 cm of each container. This amount of fertilizer per plant is equivalent to the application of 58 $kg \cdot ha^{-1}$ of N, phosphate, and K_2O recommended by local sources (Equation 5). A hand shovel was used to incorporate the pre-plant fertilizer on July 12, 2014.

2.3.13.1.2 Post-transplanting: synthetic granular urea fertilization (46-0-0)

One post-transplant granular urea application was made in order to apply a total N amount equivalent to 116 $kg \cdot ha^{-1}$. The post-transplant granular urea application was made at the beginning of the fruiting stage on September 21, 2014 (70 DAT). Amounts of granular urea needed for each plant were calculated based on the recommended rate for open field cultivation of bell peppers (Equation 6). A total of seven grams of urea were incorporated into the first 5 cm of soil in each container to avoid damaging the roots.

2.3.13.2 Spoon-fed Solubilized Urea Fertilization (SUF)

The adequate rates and the correct timing of fertilizer applications can maximize production of vegetable crops (Russo, 1991). By controlling the quantity and frequency of solubilized N applications, each growth stage can be supplied with specific and optimum nutrient rates that can increase the crop’s NUE.

2.3.13.2.1 Pre-planting: synthetic granular fertilizer (10-10-10)

The pre-plant fertilization rate for the SUF treatment was the same one used for the pre-plant fertilization rate in the GUF treatment that was previously described.

2.3.13.2.2 Post-transplanting: synthetic solubilized urea fertilization (46-0-0)

SUF was used to supply post-transplant N in ten equal split-applications every 7 days for ten weeks. The first application was made 14 DAT, on July 27, 2014, when plants had an average length of 10 cm and 4-6 true leaves. The following nine applications were made on August 3, August 10, August 17, August 24, August 31, September 7, September 14, September 21, and September 28. Each individual application consisted of 0.7 grams of urea diluted in 100 mL of water. The total amount and source of post-transplant N fertilization in the SUF treatment was the same as in the GUF treatment. The main difference is that the SUF treatment was applied through irrigation as ten equal split-applications for 10 weeks while the GUF treatment was incorporated into the soil one application at the beginning of the fruiting stage. The SUF was always applied directly to the soil, not the foliage. Foliar fertilizer application methods were not evaluated in this research.

2.3.13.3 Spoon-fed Solubilized Organic Fertilization (SOF)

The Green Revolution, climate change, and projected population growth in the near future have contributed to an increased demand for high quality, fresh agricultural produce (Negrete-Aveiga, 2013; Tilman et al., 2002). As a result, croplands are being cultivated intensively and depend on high fertilizer inputs in order to achieve and sustain economic profits. Misuse of fertilizers and inorganic salts can turn into an environmental hazard by polluting ecosystems or leaching into underground water supplies (Addiscott, 2005). Organic agriculture has recently been gaining popularity in Puerto Rico due to concerns about local fresh produce, costs of imports, and environmentally sustainable production systems. Organic production systems rely on different sources of N-containing materials that vary in costs, availability, nutrient content, mineralization rate, and environmental impact. In order to produce certified organic vegetables, the production system has to be verified and approved as organic by an independent party.

Thus, this research will focus on the use of commercially available organic fertilizers rather than on the production of certified organic bell peppers.

2.3.13.3.1 Pre-planting: organic fertilizer Bioflora Dry Crumbles® (6-6-5+8% Ca) and organic liquid fertilizer Bioflora (0-0-15+1% Ca)

Bioflora Dry Crumbles® (6-6-5) (BDC) is an organic, dry granular fertilizer fortified with seaweed. This fertilizer is certified by the OMRI and it provides high quality organic nutrients for all types of plants. It contains balanced amounts of N, P, and K but also contains high levels of calcium (Ca) and trace elements. The carbon-based granules are characterized by a slow-release formula that provides plants with certain benefits like renewed vigor, root mass stimulation and reduced damage from disease (Global Organics Group, 2016). The nutrient content within the granules is derived from a blend of feather meal, dry composted poultry litter, sulfate of potash, and the seaweed *A. nodosum* (Negrete-Aveiga, 2013) (Appendix 2).

Each plant in the SOF treatment had 27 grams of granular BDC (6-6-5) fertilizer incorporated into the first 15 cm of each container as a pre-plant fertilizer on July 12, 2014. This amount of fertilizer per plant is equivalent to the application of 58 $kg \cdot ha^{-1}$ of N and phosphate recommended by local sources (Equation 7). Even though the required pre-plant amount of N and P was supplied with 27 grams of BDC, there was a very slight deficit in the amount of K applied due to the inherent nature and nutritional proportions in the BDC (6-6-5) fertilizer (Equation 8).

A deficit of 8 $kg \cdot ha^{-1}$of pre-plant K_2O could eventually bias conclusions on plant growth, development, or yield. In order to maintain the same amount of NPK in the pre-plant fertilization of all the treatments, Bioflora (0-0-15) liquid fertilizer (BLF)

(Appendix 3) was applied at a rate of 2 mL per plant in combination with the BDC (6-6-5) fertilizer to correct the small deficit in the pre-plant K_2O (Equation 9). The BLF (0-0-15) is highly soluble in water and is derived from potassium carbonate and calcium EDTA. According to product label, when used as a pre-plant fertilizer, it should be applied to the ground as a concentrate. Both, the BDC (6-6-5) and the BLF (0-0-15) in the pre-plant SOF treatment, contained 8 and 1% Ca respectively. A total amount of 81 $kg \cdot ha^{-1}$ of pre-plant Ca was also included with the BDC and BLF in the SOF treatment (Equation 10 & 11). This extra amount of Ca was only applied in the SOF treatment because the commercially available products used in this study included this nutrient. There was no extra amount of Ca added to neither the GUF nor the SUF treatments.

2.3.13.3.2 Post-transplanting: organic liquid fertilizer Bioflora (6-0-0+8% Ca)

Bioflora organic liquid fertilizer (6-0-0+8% Ca) (BLNF) was used to supply post-transplant N in ten equal split-applications every 7 days for ten weeks in the SOF treatment (Equation 12). This organic liquid fertilizer is derived from calcium nitrate (Appendix 5). The first application was made 14 DAT, on July 27, 2014, when plants had an average length of 10 cm and 4-6 true leaves. The following nine applications were made on August 3, August 10, August 17, August 24, August 31, September 7, September 14, September 21, and September 28. The total amount of post-transplant N fertilization in the SOF treatment was the same as in the SUF and GUF treatments. The main difference is that the SUF and GUF treatments used synthetic urea fertilizer, while the SOF treatment used an organic fertilizer. The SOF was always applied directly to the soil, not the foliage.

2.3.14 Biostimulant treatments

All the biostimulant treatments were applied individually as a foliar spray with a plastic, hand held atomizer. Atomizers were always rinsed three times before and after the application of biostimulants. Every biostimulant treatment was applied five times during the crop cycle. The first biostimulant applications began in July 27, 2014 (14 DAT) and were repeated every 14 days. The four remaining applications events were done on August 10 (28 DAT), August 24 (42 DAT), September 7 (56 DAT), and September 21, 2014 (70 DAT). The dosage was determined according to each of the biostimulants' respective manufacturer. Leaves, stems, buds, flowers and immature fruits were covered evenly with the biostimulant solution until run-off. Foliar applications were always done during the cooler hours of the day (i.e., early morning or late afternoon) in order to prevent or diminish excessive evaporation from the plants' surface. Wind speed and wind direction were taken into consideration in order to diminish unintended drift potential by wind onto nearby plants. No surfactant was added in any of the biostimulant treatments.

Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

2.3.14.1 Aminoquelant®-Ca (AQC)

Aminoquelant®-Ca (BioIbérica, Barcelona, Spain) is a commercially available blend of amino acids for agricultural use. It combines Ca with L-α-amino acids from enzymatic hydrolysis that can improve Ca mobility in plants and can also mitigate the factors that lead to its deficiency. The amino acid blend contains 4.6% (w/w) biologically active free amino acids. It consists of aspartic acid (aspartate), serine, glutamic acid (glutamate), glycine, histidine, arginine, threonine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine, and tryptophan. This formulation includes 8% calcium oxide (CaO), 0.9% organic N, 4.9% total N, 0.2% boron, and 6.8% organic matter. It can be applied through the irrigation line or by foliar sprays and it is especially designed for crops that are prone to physiological alterations by Ca deficiencies such as peppers, tomatoes, lettuce, etc. (BioIbérica, 2016). The foliar spray solution was prepared by diluting 4 mL of Aminoquelant-Ca with 1 L of water. This is equivalent to a 0.4% Aminoquelant-Ca solution.

2.3.14.2 Vitazyme (VTZ)

Vitazyme® (Vital Earth Resources, Gladewater, Texas, USA) is a commercially available, OMRI and BCS certified organic biostimulant for agricultural use. It consists of certain biological activators that are created by an undisclosed, propietary fermentation process. The formulation contains Vitamin B1 (thiamin) (0.45 mg/100g), Vitamin B2 (riboflavin) (0.03 mg/100g), Vitamin B6 (pyridoxine) (0.19 mg/100g), K_2O (0.80%), Iron (Fe-EDTA) (0.2%), Copper (Cu-EDTA) (0.07%), Zinc (Zn-EDTA) (0.06%), brassinosteroids (i.e., homobrassinolide, dolicholide, homodolicholide, and brassinone) (0.03 mg/ml), 1-triacontanol (0.17 mg/ml), glycosides and water. This biostimulant accelerates and improves metabolic processes that can increases crop yields and profits. It improves crop quality, reduces N fertilizer inputs, and also hastens germination and maturity. In addition, it also acts on soils by improving their structure and infiltration capacity (Vital Earth Resources, 2016). According to the manufacturer's recommendations, seeds were pre-treated by soaking them directly in the Vitazyme concentrate for 90 seconds. This was the only biostimulant used to pre-treat seeds. The foliar spray solution was prepared by diluting 10 mL of Vitazyme with 1 L of water. This is equivalent to a 1% Vitazyme solution.

2.3.14.3 Stimplex (SPX)

Stimplex™ (Acadian Seaplants, Nova Scotia, Canada) is an OMRI listed, commercially available SWE biostimulant for agricultural use. It is derived from the brown algae *Ascophyllum nodosum* L. Product label states that it contains an active ingredient concentration equivalent to 100 parts per million of kinetin (Appendix 6). It also contains zeatine, adenine, betaine, oligosaccharides, polysaccharides, organic acids, vitamins, gibberellins, auxins, micronutrients, and amino acids (Flores-Torres, 2013). It is used to improve resistance to biotic & abiotic stresses and enhance the crop's overall health. It is also used to increase yields and quality of crops, and also to increase root growth, early plant development, fruit set, fruit size, and nutrient levels (CDMS, 2016). The foliar spray solution was prepared by diluting 5 mL of Stimplex with 1 L of water. This is equivalent to a 0.5% Stimplex solution.

2.3.14.4 Water check (WTR)

In order to disregard the possible effect of spray vs. no spray, tap water was used as a control to spray the plants without biostimulants, rather than not spraying them at all. The water used as a control was always collected at the time of application in order to prevent the water from becoming stagnant over time. The source of the water used in the control check was from a well located at a higher elevation in the Finca Laboratorio Alzamora.

2.3.15 Morphological research

Morphological development was measured through the analysis of the following variables:

2.3.15.1 Mean fresh weight of shoots and roots (g)

Plants were cut at the soil surface level using scissor-action pruners (Fiskars Brands Inc, Middleton, Wisconsin, USA). This was done after the last pepper was harvested in each plant or during the experiment's last day of harvest on November 2, 2014 (112 DAT). The top part of the plant, or everything above the soil surface level, was separated from its roots, segmented into smaller pieces, and placed in a paper bag. After the shoots were removed, the containers were emptied on a 15 mm sieve. The growing medium was carefully sieved until the roots were exposed. Roots were washed with running water and excess debris was rinsed off. The root ball and root fragments were then collected from the sieve, manually wringed, placed in individual paper bags, identified as shoots or roots, and labeled with the plant's treatment and location within the raised bench. Each paper bag was weighted with a digital scale (Fisher Science Education™, USA) and data was named as fresh weight of shoots or fresh weight or roots (g).

2.3.15.2 Mean dry weight of shoots and roots (g)

After the fresh weight data was logged, paper bags with plant material were placed in a drying oven (Jisico Scientific Instruments, Seoul, Korea) for 96 hours at 75°C. Paper bags with the dehydrated plant material were weighted and the data was named as dry weight of shoots or dry weight of roots (g).

2.3.16 Yield and quality research

Physiologically mature pepper fruits were individually harvested when they had developed at least 50% of their chocolate brown color. The harvest period lasted for thirty days, beginning in October 4 (83 DAT) and ending in November 2, 2014 (112 DAT). Each pepper was weighted, measured (i.e., length and width), and classified as marketable or unmarketable. Total fruits were the sum of marketable and unmarketable fruits. Marketable fruits had an equatorial diameter equal or greater than 5.6 cm and were free of decay or damages by abiotic or biotic stressors. The marketable fruits were then classified into categories according to two different grading systems: 1) the United States standards for grades of sweet peppers (2005) and 2) a diameter scale used for imported greenhouse-grown bell peppers (Jovicich et al., 2007).

The first grading system used was the United States standards for grades of sweet peppers (2005). These standards classify peppers into three categories: U.S. Fancy, U.S. No. 1, and U.S. No. 2. The criteria used to assign these categories are length, width, color, and damages. The second grading system is used for imported greenhouse-grown bell peppers and grades the pepper fruits based on size following a diameter scale: extra-large (diameter > 8.4 cm), large (7.6 – 8.39 cm), medium (6.4 – 7.59 cm), and small (5.6 – 6.39 cm) (Jovicich et al., 2007). Marketable fruit data was analyzed considering both grading systems. The grading system for imported greenhouse-grown bell peppers was modified and the categories of large and extra-large were eliminated. This modification of the grading system had to be made because there were insufficient extra-large and large pepper fruits. The newly modified grading system for imported greenhouse-grown bell peppers consisted of only two categories: regular (diameter > 6.4 cm), and small (5.6 – 6.39 cm).

The different reasons to categorize fruits as unmarketable fruits were also logged. Each pepper fruit was then sliced in half through the equator. Pericarp thickness was measured (mm) using a caliper (Vernier Callipers, Waukegan, IL, USA). Each pepper fruit was then segmented into smaller pieces in order to extract drops of juice from the pericarp and measure the concentration of soluble solids using a digital hand-held refractometer ATAGO® PAL-1 Pocket (ATAGO, Tokyo, Japan). Pepper fruit contents (i.e. seeds, calyx, placenta, and fruit walls) were deposited in paper bags. Each paper bag was labeled with the plant's treatment, location within the raised bench, and also with the fruit's number according to the order of harvest. Paper bags with pepper fruit material were placed in a dehydrating oven (Jisico Scientific Instruments, Seoul, Korea) for 96 hours at 75°C and then weighted with a digital scale (Fisher Science Education™, USA). Dry weight of fruits was then organized and summed for each plant.

2.3.17 Physiological research

Physiological changes were measured through the analysis of the following variables:

2.3.17.1 Mean chlorophyll content

Chlorophyll content was measured using a SPAD-502Plus chlorophyll meter (Osaka, Japan) on October 11, 2014 (90 DAT) on fully expanded leaves.

2.3.18 Data analysis

Statistical analysis was conducted with Infostat version 2014 (Infostat software, National University of Córdoba, Argentina). The Shapiro-Wilks test was used to verify if the data followed a normal distribution and the Levene test was used to determine homoscedasticity of the data. Values were log-transformed or square root-transformed to homogenize variance or normalize distribution when needed. The non-parametric Kruskal-Wallis test was used when variance was not homogenous and/or distribution was not normal even after both transformations had been made. An analysis of variance (ANOVA) was conducted in order to evaluate the potential interactions of each factor. The interactions are described as combinations of the levels from each factor (Viggiano-Beltrocco, 2014). The levels were: 3 post-transplant N fertilization methods (GUF, SUF, and SOF) and 4 biostimulant treatments (AQC, VTZ, SPX, and WTR) that make a 3 x 4 factorial ANOVA model. Significant means were separated using the LSD statistical test ($\alpha < 0.05$).

2.4 Results

2.4.1 Mean fresh weight of shoots (g)

None of the treatments had any significant statistical differences on the mean fresh weight of shoots (Appendix 23).

2.4.2 Mean fresh weight of roots (g)

None of the treatments had any significant statistical differences on the mean fresh weight of roots (Appendix 23).

2.4.3 Mean dry weight of shoots (g)

Treatments did not have interaction effects on the mean dry weight of shoots. Individual treatment effects were seen with the fertilizing methods (Appendix 23).

2.4.3.1 Effect of fertilizing methods on the mean dry weight of shoots (g)

Fertilizing method treatments had a significant effect on the mean dry weight of shoots. The SUF method produced an average of 41% more dry weight of shoots than the GUF method. The SOF method was not statistically different from either one (Figure 41).

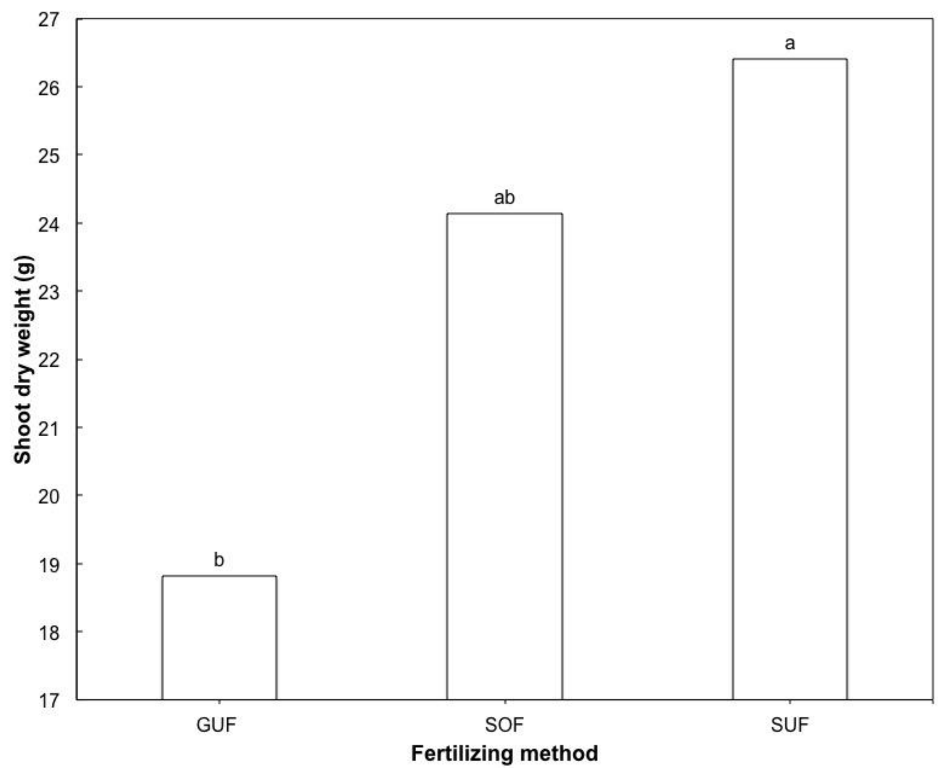


Figure 41. Effect of fertilizing methods on the mean dry weight of shoots (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.4 Mean dry weight of roots (g)

None of the treatments had any significant statistical differences on the mean dry weight of roots (Appendix 23).

2.4.5 Mean number of marketable fruits per plant

Treatments did not have interaction effects on the mean number of marketable fruits per plant. Individual treatment effects were seen with the fertilizing methods (Appendix 24).

2.4.5.1 Effect of fertilizing methods on the mean number of marketable fruits per plant

Fertilizing method treatments had a significant effect on the mean number of marketable fruits per plant. The SUF and SOF methods produced an average of 82 and 53% more marketable fruits per plant than the GUF method, respectively (Figure 42).

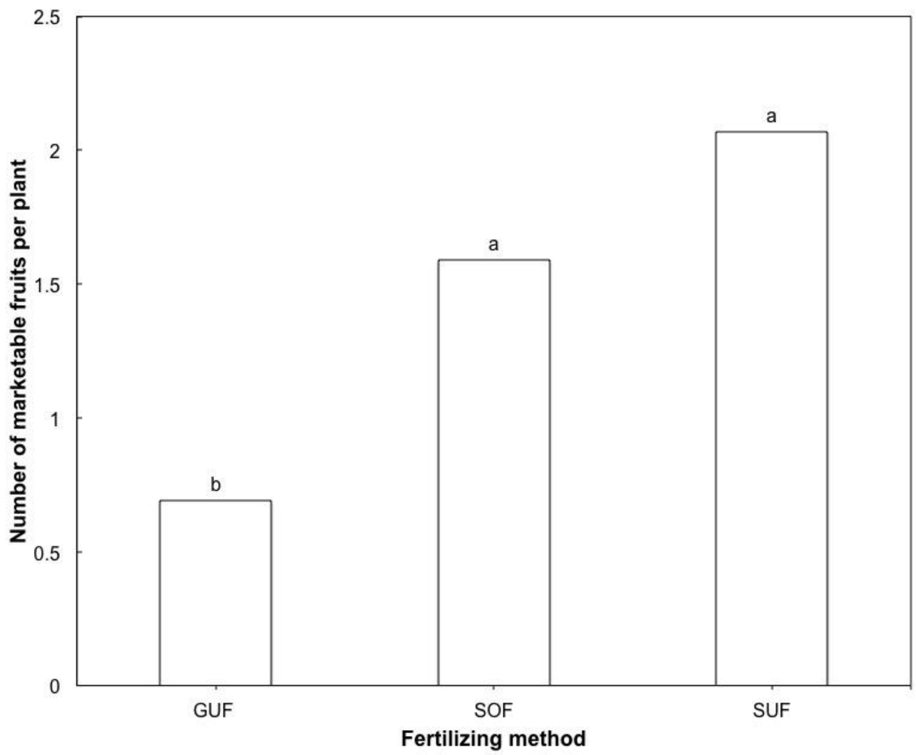


Figure 42. Effect of fertilizing methods on the mean number of marketable fruits per plant. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.6 Mean number of unmarketable fruits per plant

None of the treatments had any significant statistical differences on the mean number of unmarketable fruits per plant (Appendix 24).

2.4.7 Mean number of total fruits per plant

Treatments did not have interaction effects on the mean number of total fruits per plant. Individual treatment effects were seen with the fertilizing methods (Appendix 24).

2.4.7.1 Effect of fertilizing methods on the mean number of total fruits per plant

Fertilizing method treatments had significant effects on the mean number of total fruits per plant. The SUF method produced an average of 58% more total fruits per plant than the GUF method. The SOF method was not statistically different from either one (Figure 43).

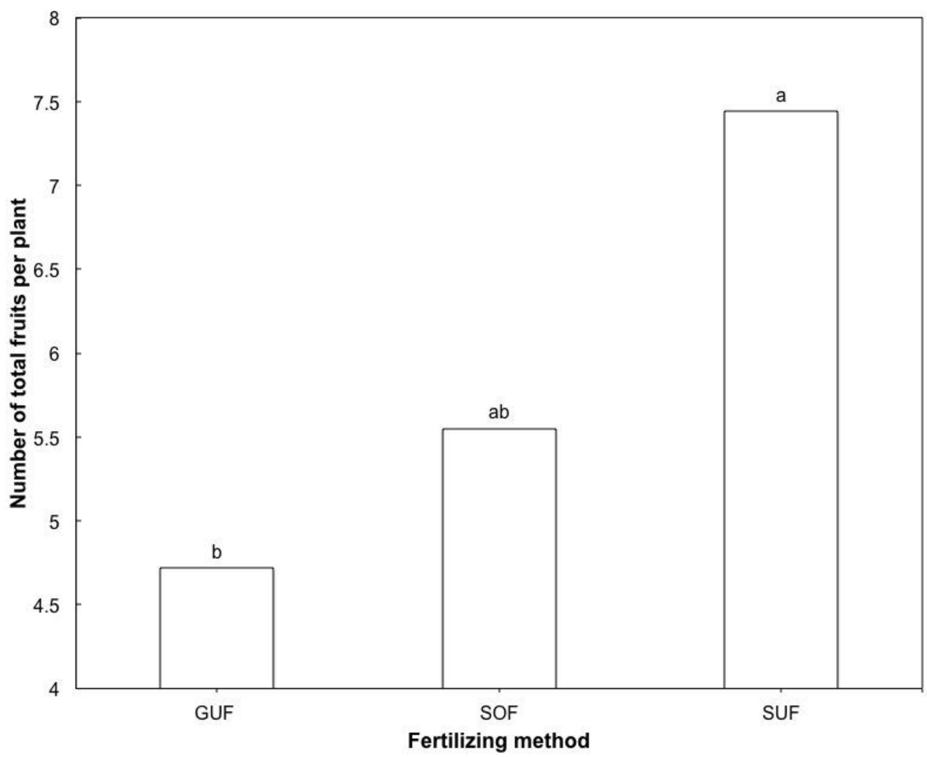


Figure 43. Effect of fertilizing methods on the mean number of total fruits per plant. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.8 Mean marketable fruit weight per plant (g)

Significant interaction effects were seen on the mean marketable fruit weight per plant (Appendix 24).

2.4.8.1 Interaction effect on the mean marketable fruit weight per plant

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean marketable fruit weight per plant. The SOF + SPX combination produced 63 and 39% more marketable fruit weight per plant than the GUF + SPX and SUF + SPX combinations, respectively. The SUF + AQC and SUF + VTZ combinations produced 25 and 17% less marketable fruit weight per plant than the SUF + WTR combination, respectively. The SOF + SPX combination produced 58, 51 and 41% more marketable fruit weight per plant than the SOF + AQC, SOF + VTZ, and SOF + WTR combinations, respectively (Figure 44).

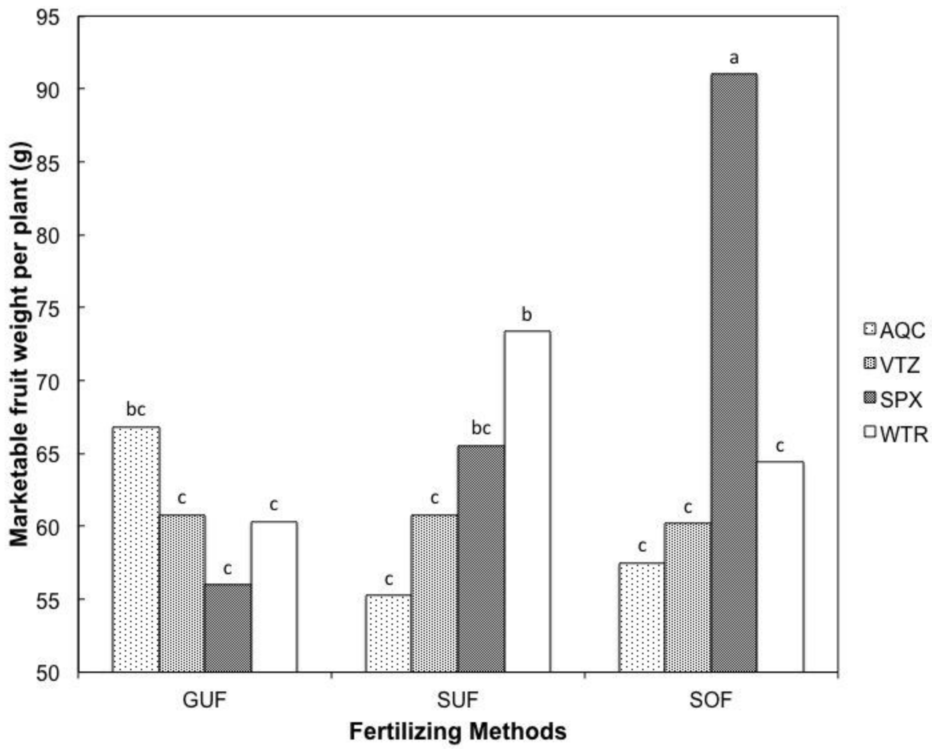


Figure 44. Combined effects of fertilizing methods and biostimulants on the mean marketable fruit weight per plant (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.9 Mean unmarketable fruit weight per plant (g)

None of the treatments had any significant statistical differences on the mean unmarketable fruit weight per plant (Appendix 24).

2.4.10 Mean total fruit weight per plant (g)

Treatments did not have interaction effects on the mean total fruit weight per plant. Individual treatment effects were seen with the biostimulants (Appendix 24).

2.4.10.1 Effect of biostimulants on the mean total fruit weight per plant

Biostimulant treatments had a significant effect on the mean total fruit weight per plant. None of the biostimulants outperformed the WTR treatment. Plants treated with AQC and VTZ produced 16 and 18% less total fruit weight per plant than the WTR, respectively. SPX was not significantly different from any biostimulant treatment (Figure 45).

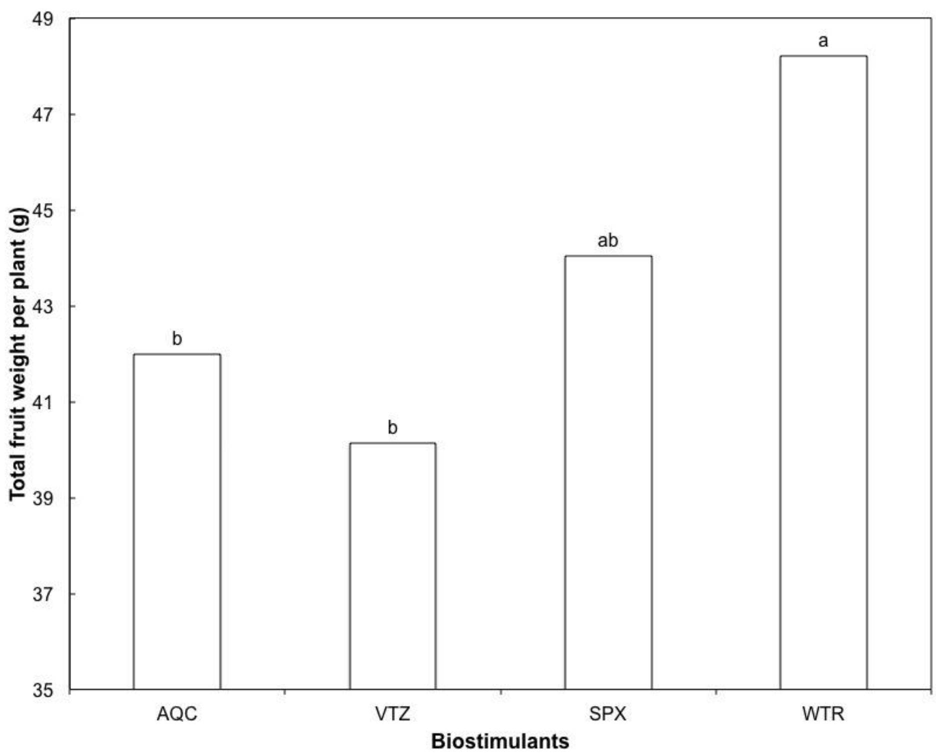


Figure 45. Effect of biostimulant treatments on the mean total fruit weight per plant (g). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.11 Mean length of marketable fruits (cm)

Significant interaction effects were seen on the mean length of marketable fruits (Appendix 25).

2.4.11.1 Interaction effect on the mean length of marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean length of marketable fruits. The SOF + SPX combination produced marketable fruits that were 34% longer than the GUF + SPX combination. The SUF + WTR combination produced marketable fruits that were 23% longer than the SOF + WTR combination. The SOF + VTZ and the SOF + SPX treatment combinations produced marketable fruits that were 14 and 38% longer than the SOF + WTR combination, respectively (Figure 46).

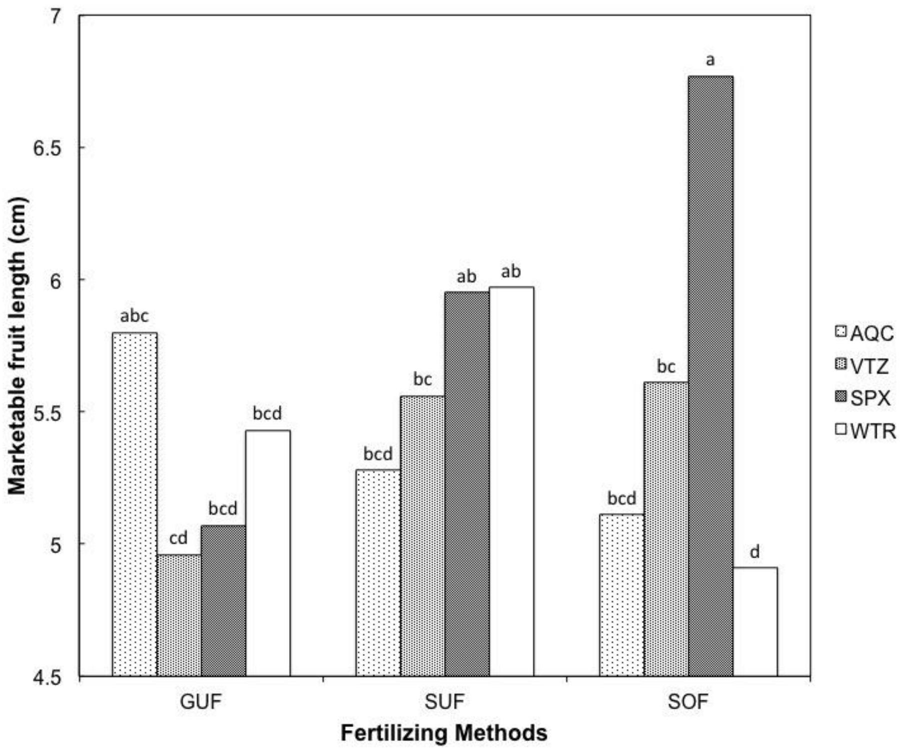


Figure 46. Combined effects of fertilizing methods and biostimulants on the mean length of marketable fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.12 Mean length of unmarketable fruits (cm)

Treatments did not have interaction effects on the mean length of unmarketable fruits. Individual treatment effects were seen with the fertilizing methods (Appendix 25).

2.4.12.1 Effect of fertilizing methods on the mean length of unmarketable fruits

Fertilizing method treatments had a significant effect on the mean length of unmarketable fruits. The unmarketable fruits in the GUF method were 13 and 9% longer than the unmarketable fruits in the SOF and SUF methods, respectively (Figure 47).

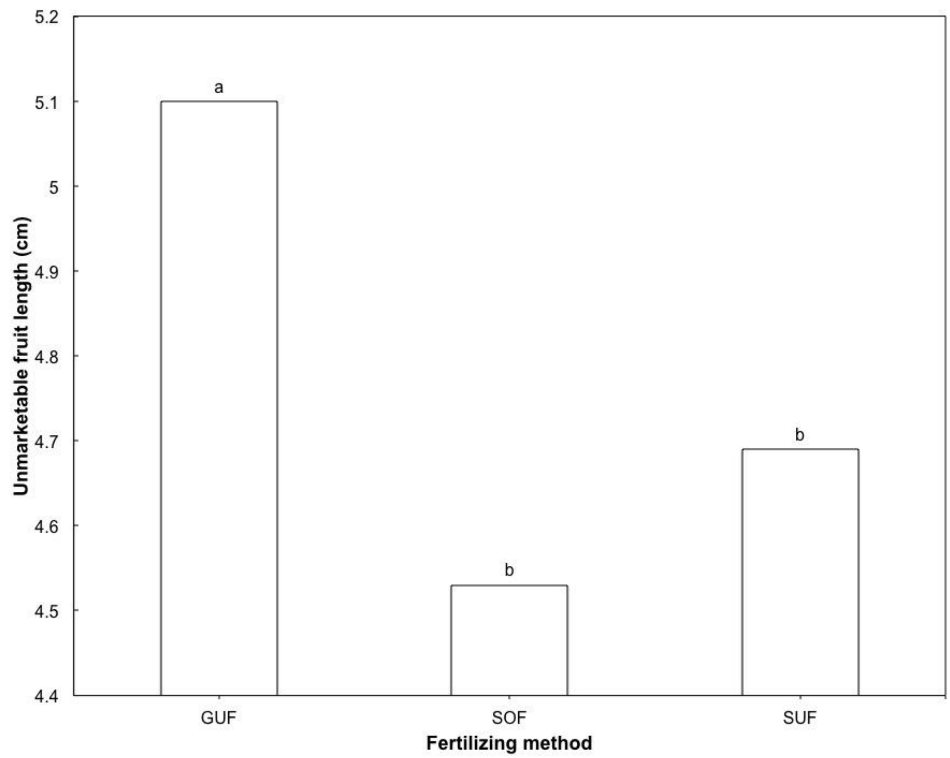


Figure 47. Effect of fertilizing methods on the mean length of unmarketable fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.13 Mean length of total fruits (cm)

Significant interaction effects were seen on the mean length of total fruits (Appendix 25).

2.4.13.1 Interaction effect on the mean length of total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean length of total fruits. None of the treatments performed better than the GUF + WTR combination. Both, the GUF + AQC and the GUF + VTZ treatment combinations produced total fruits that were 10% shorter than the GUF + WTR combination. Both, the SUF + AQC and the SUF + VTZ treatment combinations produced total fruits that were 12% shorter than the SUF + WTR combination. The SOF + AQC and the SOF + SPX treatment combinations produced total fruits that were 15 and 17% longer than the SOF + WTR combination, respectively. The GUF + WTR and the SUF + WTR treatment combinations produced total fruits that were 25 and 23% longer than the SOF + WTR combination, respectively (Figure 48).

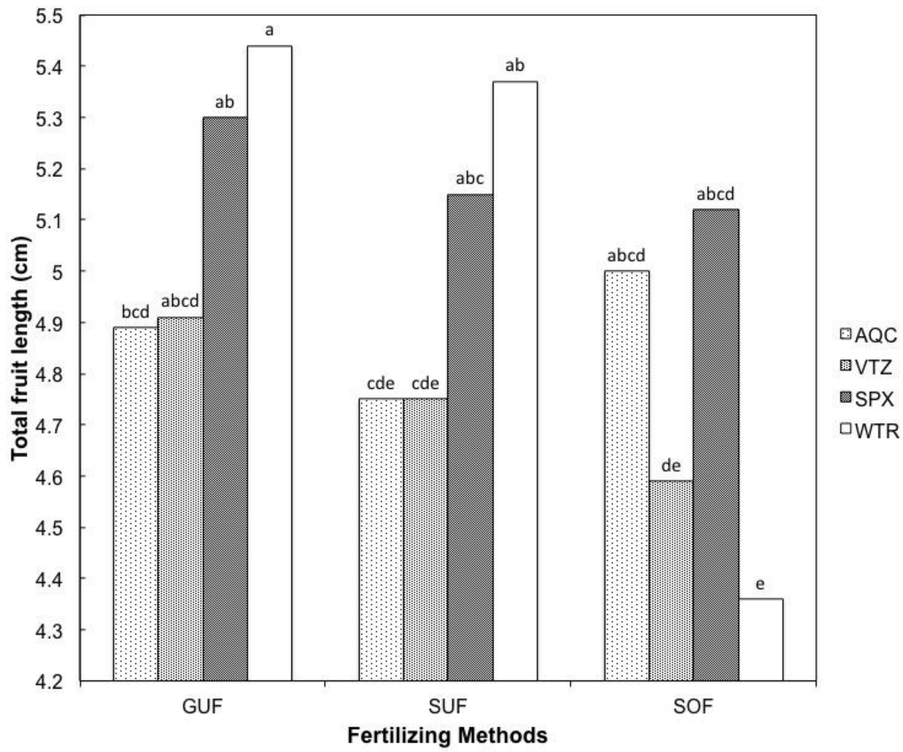


Figure 48. Combined effects of fertilizing methods and biostimulants on the mean length of total fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.14 Mean width of marketable fruits (cm)

Significant interaction effects were seen on the mean width of marketable fruits (Appendix 25).

2.4.14.1 Interaction effect on the mean width of marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean width of marketable fruits. The SOF + SPX treatment combination produced marketable fruits that were 17 and 13% wider than the GUF + SPX and SUF + SPX combinations, respectively. The SUF + WTR combination produced marketable fruits that were 8% wider than the GUF + WTR combination. The SUF + AQC, SUF + VTZ, and SUF + SPX treatment combinations produced marketable fruits that were 10, 6, and 7% less wide than marketable fruits in the SUF + WTR combination, respectively. The SOF + SPX treatment combination produced marketable fruits that were 12, 15, and 10% wider than the SOF + AQC, SOF + VTZ, and SOF + WTR combinations, respectively (Figure 49).

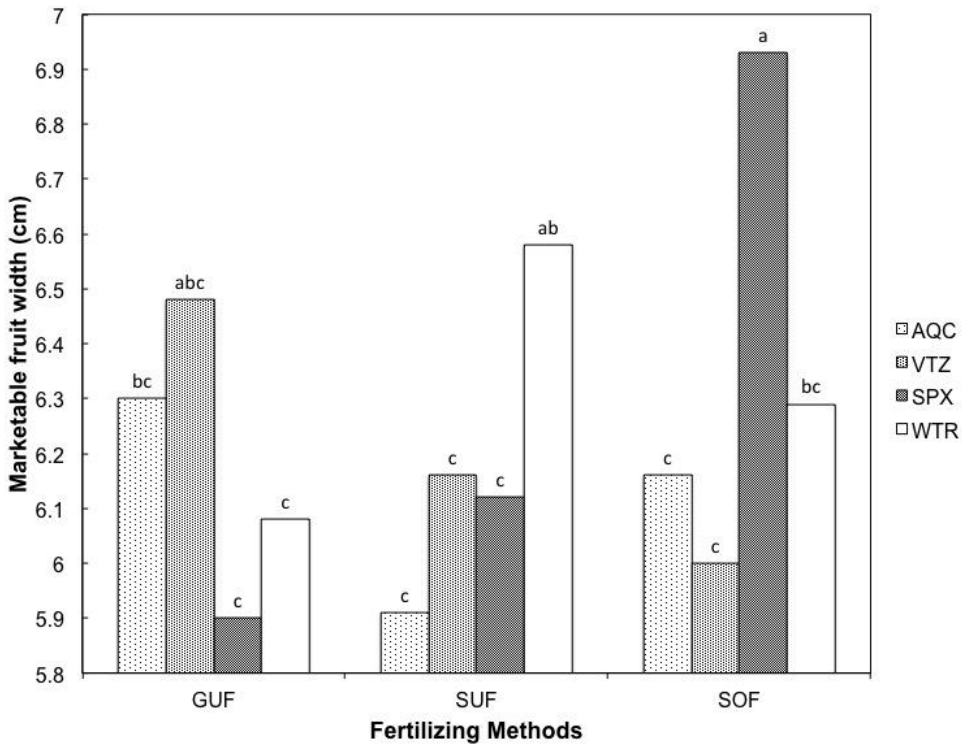


Figure 49. Combined effects of fertilizing methods and biostimulants on the mean width of marketable fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.15 Mean width of unmarketable fruits (cm)

None of the treatments had any significant statistical differences on the mean width of unmarketable fruits (Appendix 25).

2.4.16 Mean width of total fruits (cm)

Significant interaction effects were seen on the mean width of total fruits (Appendix 25).

2.4.16.1 Interaction effect on the mean width of total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean width of total fruits. The SUF + WTR combination produced total fruits that were 12% wider than the GUF + WTR combination. The SUF + AQC, SUF + VTZ, and SUF + SPX treatment combinations produced total fruits that were 11, 11, and 9% less wide than total fruits in the SUF + WTR combination, respectively. The SOF + AQC combination produced total fruits that were 11% wider than the GUF + SPX combination (Figure 50).

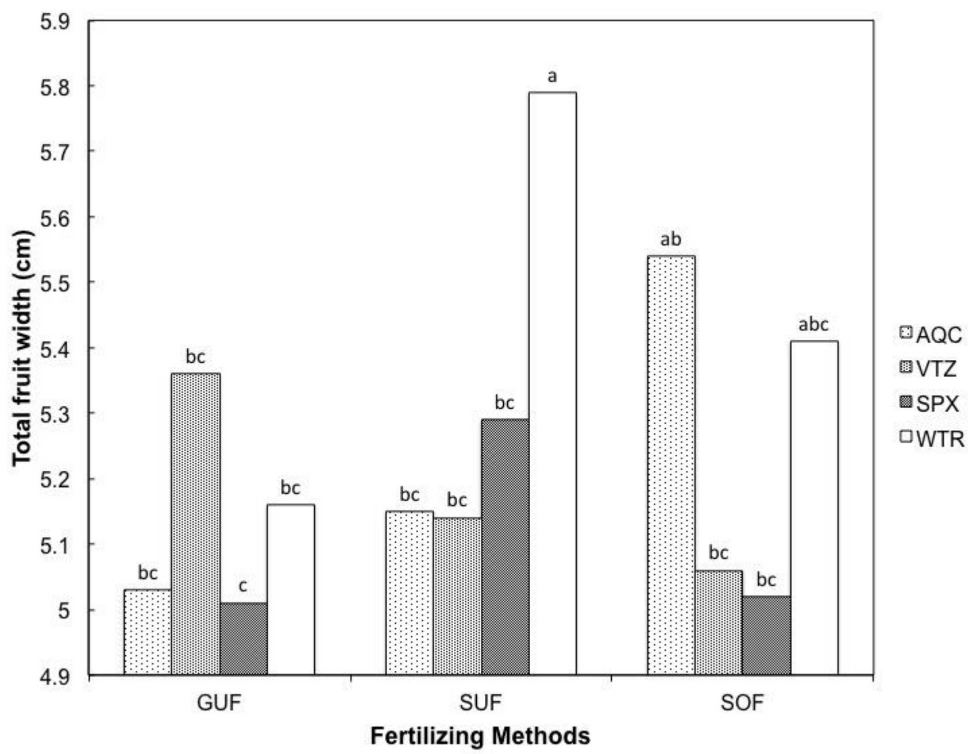


Figure 50. Combined effects of fertilizing methods and biostimulants on the mean width of total fruits (cm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.17 Mean pericarp thickness of marketable fruits (mm)

Significant interaction effects were seen on the mean pericarp thickness of marketable fruits (Appendix 26).

2.4.17.1 Interaction effect on the mean pericarp thickness of marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean pericarp thickness of marketable fruits. None of the treatments performed better than the SUF + WTR combination. The SUF + WTR combination produced marketable fruit pericarps that were 19% thicker than the GUF + WTR combination. The SUF + AQC and SUF + SPX treatment combinations produced marketable fruit pericarps that were 20 and 15% thinner than the pericarps in the SUF + WTR combination, respectively (Figure 51).

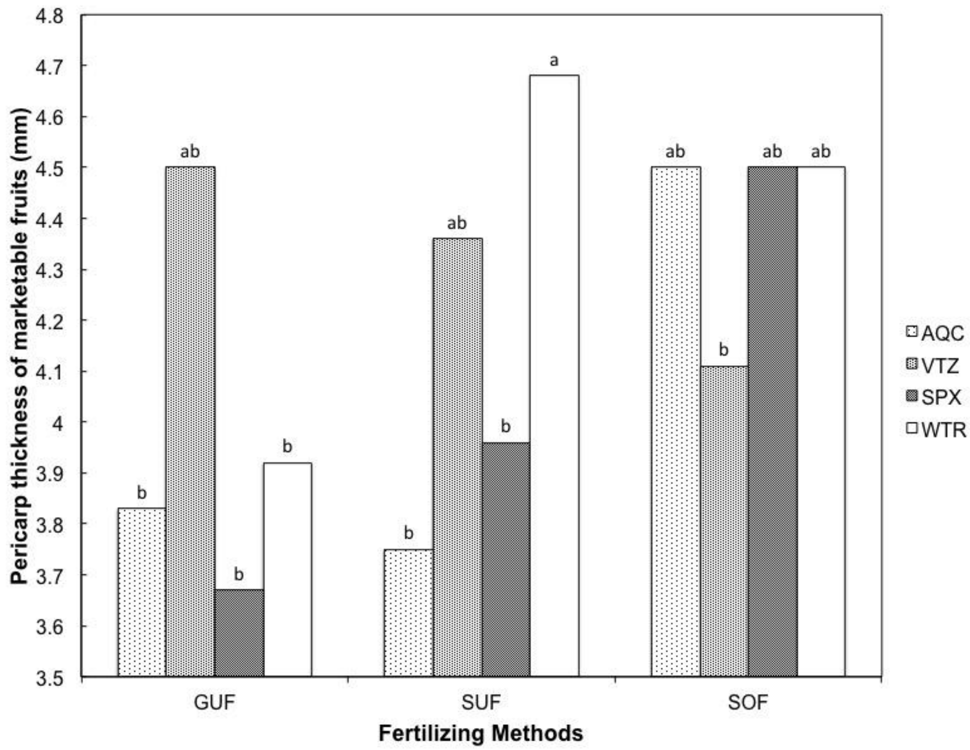


Figure 51. Combined effects of fertilizing methods and biostimulants on the mean pericarp thickness of marketable fruits (mm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.18 Mean pericarp thickness of unmarketable fruits (mm)

None of the treatments had any significant statistical differences on the mean pericarp thickness of unmarketable fruits (Appendix 26).

2.4.19 Mean pericarp thickness of total fruits (mm)

Significant interaction effects were seen on the mean pericarp thickness of total fruits (Appendix 26).

2.4.19.1 Interaction effect on the mean pericarp thickness of total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean pericarp thickness of total fruits. None of the treatments performed better than the SUF + WTR combination. The SUF + WTR combination produced total fruit pericarps that were 17% thicker than the GUF + WTR combination. The SUF + AQC treatment combination produced total fruit pericarps that were 14% thinner than the pericarps in the SUF + WTR combination. The GUF + VTZ treatment combination produced total fruit pericarps that were 13% thicker than the pericarps in the GUF + SPX combination (Figure 52).

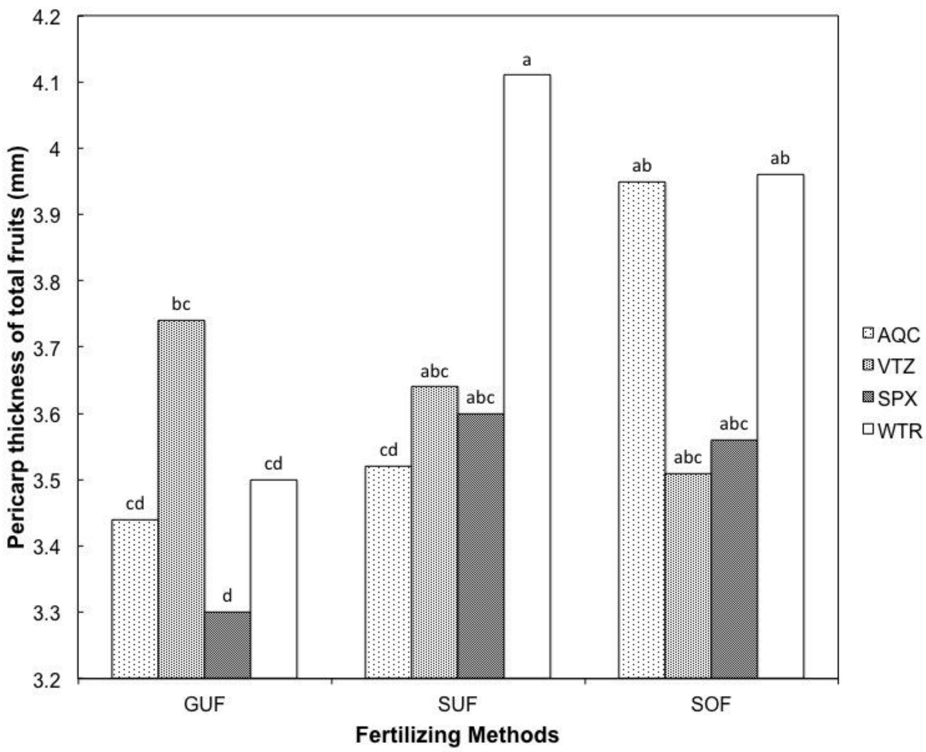


Figure 52. Combined effects of fertilizing methods and biostimulants on the mean pericarp thickness of total fruits (mm). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.20 Total soluble solids content in marketable fruits

Significant interaction effects were seen on the mean Brix readings of marketable fruits (Appendix 27).

2.4.20.1 Interaction effect on the mean content of the total soluble solids in marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean Brix readings of marketable fruits. The SUF + AQC combination produced brix readings that were 41% higher than the SUF + WTR combination. The SUF + AQC combination produced brix readings that were 32% higher than the SOF + AQC combination. The SOF + VTZ combination produced brix readings that were 31% higher than the SOF + AQC combination (Figure 53).

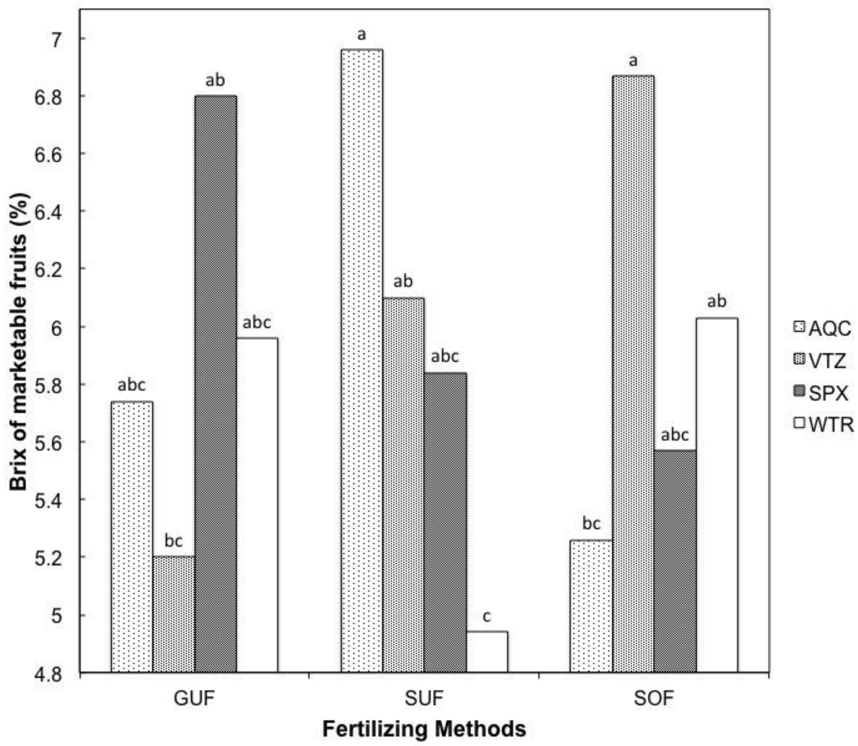


Figure 53. Combined effects of fertilizing methods and biostimulants on the mean Brix readings of marketable fruits. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.21 Total soluble solids content in unmarketable fruits

Significant interaction effects were seen on the mean Brix readings of unmarketable fruits (Appendix 27).

2.4.21.1 Interaction effect on the mean content of the total soluble solids in unmarketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean Brix readings of unmarketable fruits. None of the treatments performed better than the SOF + AQC combination. The SOF + AQC combination produced brix readings in unmarketable fruits that were 22 and 25% higher than the GUF + AQC and the SUF + AQC treatment combinations, respectively. The SOF + AQC combination produced brix readings in unmarketable fruits that were 18, 32, and 25% higher than the SOF + VTZ, SOF + SPX, and the SOF + WTR treatment combinations, respectively (Figure 54).

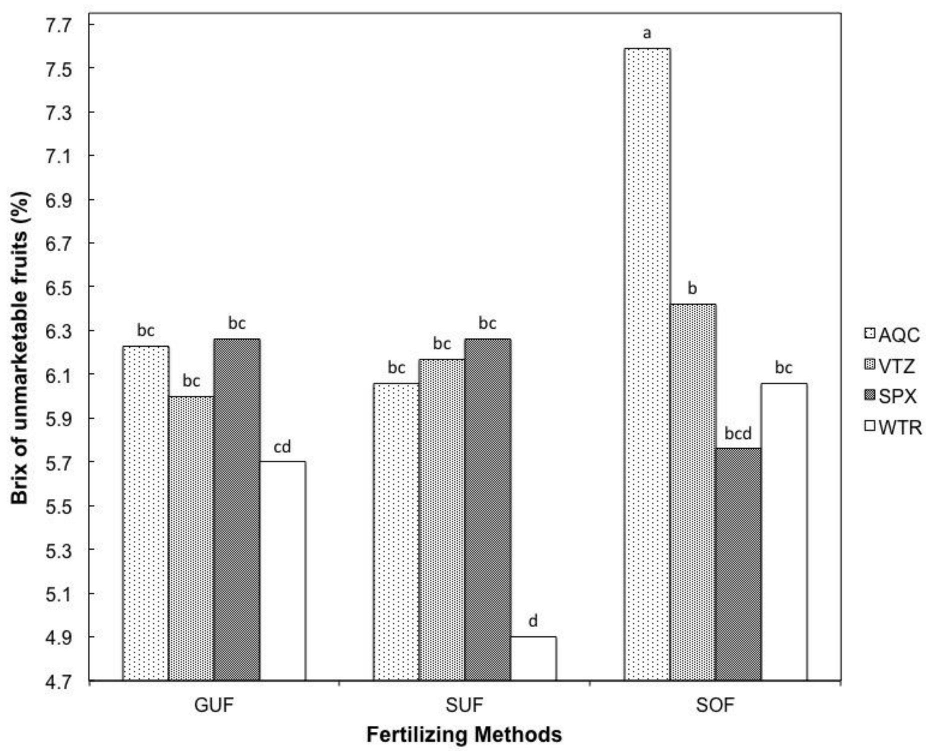


Figure 54. Combined effects of fertilizing methods and biostimulants on the mean Brix readings of unmarketable fruits. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.22 Total soluble solids content in total fruits

Significant interaction effects were seen on the mean Brix readings of total fruits (Appendix 27).

2.4.22.1 Interaction effect on the mean content of the total soluble solids in total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean Brix readings of total fruits. The SOF + VTZ combination produced brix readings in total fruits that were 14 and 32% higher than the SOF + SPX and the SUF + WTR treatment combinations, respectively (Figure 55).

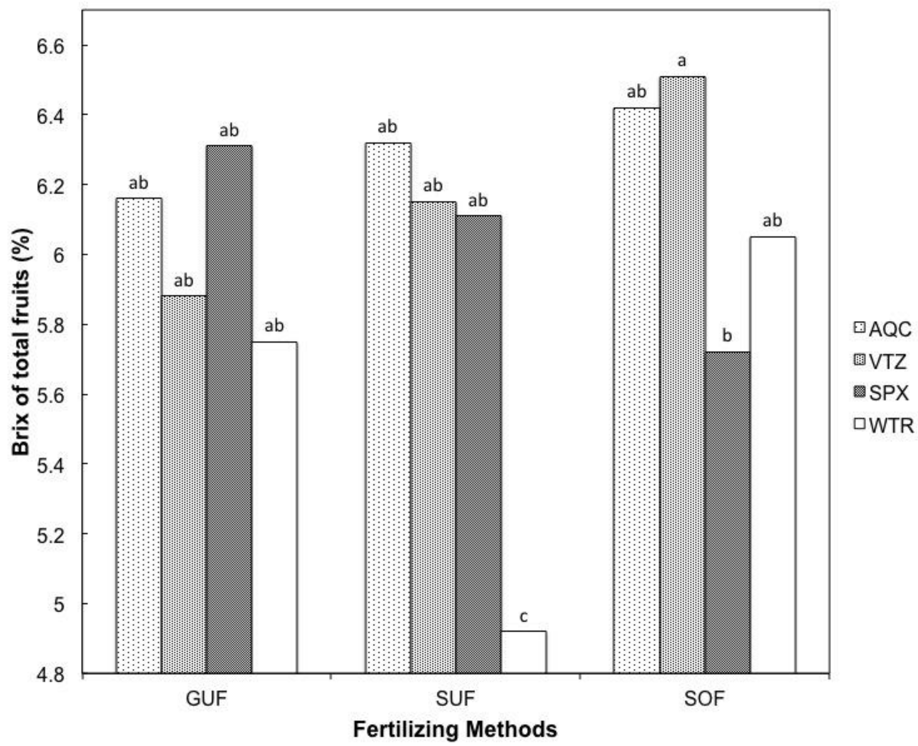


Figure 55. Combined effects of fertilizing methods and biostimulants on the mean Brix readings of total fruits. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.23 Harvest time of marketable fruits

None of the treatments had any significant statistical differences on the mean time of harvest (DAT) of marketable fruits (Appendix 28).

2.4.24 Harvest time of unmarketable fruits

Significant interaction effects were seen on the mean time of harvest (DAT) of unmarketable fruits (Appendix 28).

2.4.24.1 Interaction effect on the mean harvest time of unmarketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean harvest time of unmarketable fruits (DAT). Unmarketable fruits that were treated with the SUF + AQC and SUF + VTZ combinations took 11 and 7% less time to be harvested than the SUF + WTR combination, respectively. Unmarketable fruits that were treated with the SOF + VTZ combination took 8 and 5% less time to be harvested than the SOF + SPX and SOF + WTR combinations, respectively. Unmarketable fruits that were treated with the GUF + WTR combination took 7 and 6% less time to be harvested than the SUF + WTR and SOF + WTR combinations, respectively (Figure 56).

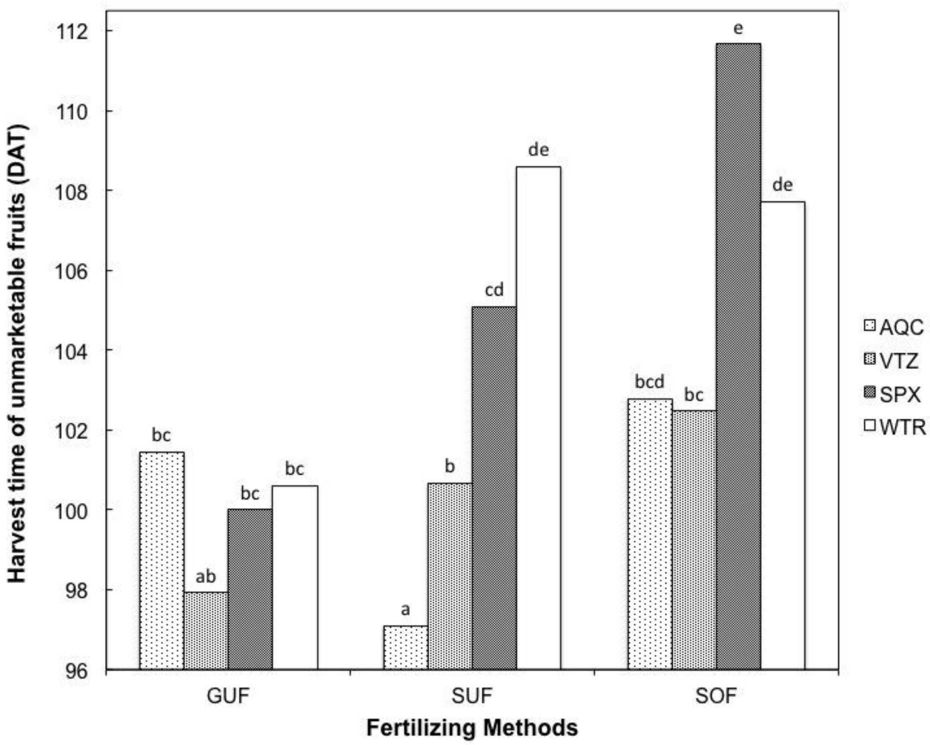


Figure 56. Combined effects of fertilizing methods and biostimulants on the mean harvest time of unmarketable fruits (days after transplant). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.25 Harvest time of total fruits

Significant interaction effects were seen on the mean time of harvest (DAT) of total fruits (Appendix 28).

2.4.25.1 Interaction effect on the mean harvest time of total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean harvest time of total fruits (DAT). Total fruits that were treated with the SUF + AQC and SUF + VTZ combinations took 6 and 4% less time to be harvested than the SUF + WTR combination, respectively. Total fruits that were treated with the SOF + VTZ combination took 7 and 3% less time to be harvested than the SOF + SPX and SOF + WTR combinations, respectively. Total fruits that were treated with the GUF + WTR combination took 5% less time to be harvested than the SOF + WTR combination (Figure 57).

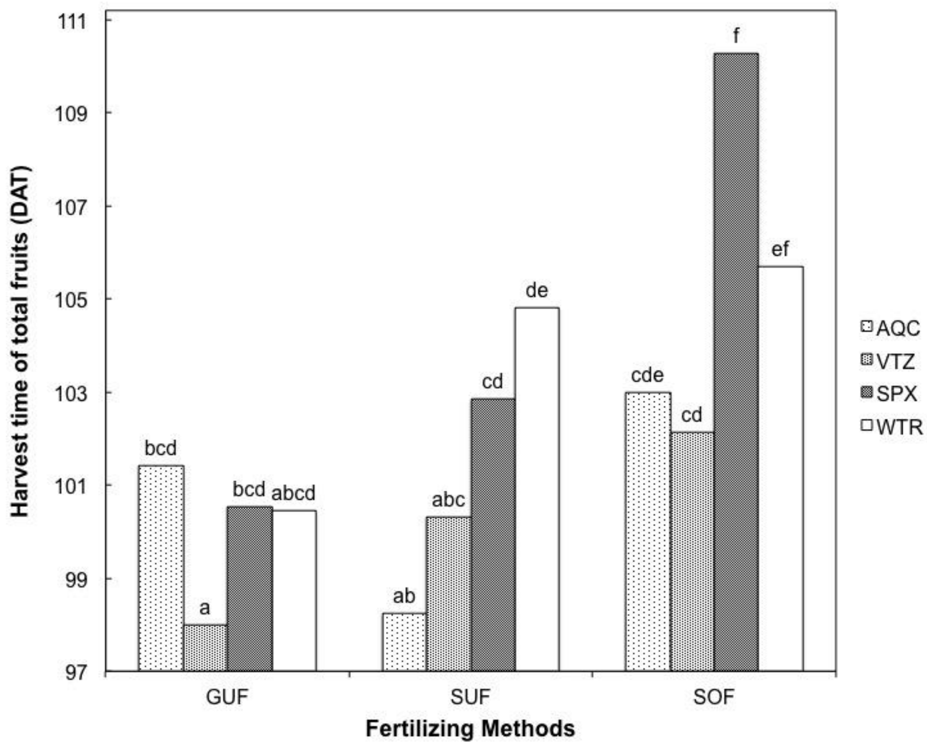


Figure 57. Combined effects of fertilizing methods and biostimulants on the mean harvest time of total fruits (days after transplant). Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.26 Mean SPAD readings

Treatments did not have interaction effects on the mean SPAD readings at 90 DAT. Individual treatment effects were seen with the fertilizing methods (Appendix 20).

2.4.26.1 Effect of fertilizing methods on the mean SPAD readings

Fertilizing method treatments had a significant effect on the mean mean SPAD readings at 90 DAT. The plants treated with the SOF and SUF methods increased their SPAD index by 24 and 19% respectively, when compared to the GUF method (Figure 58).

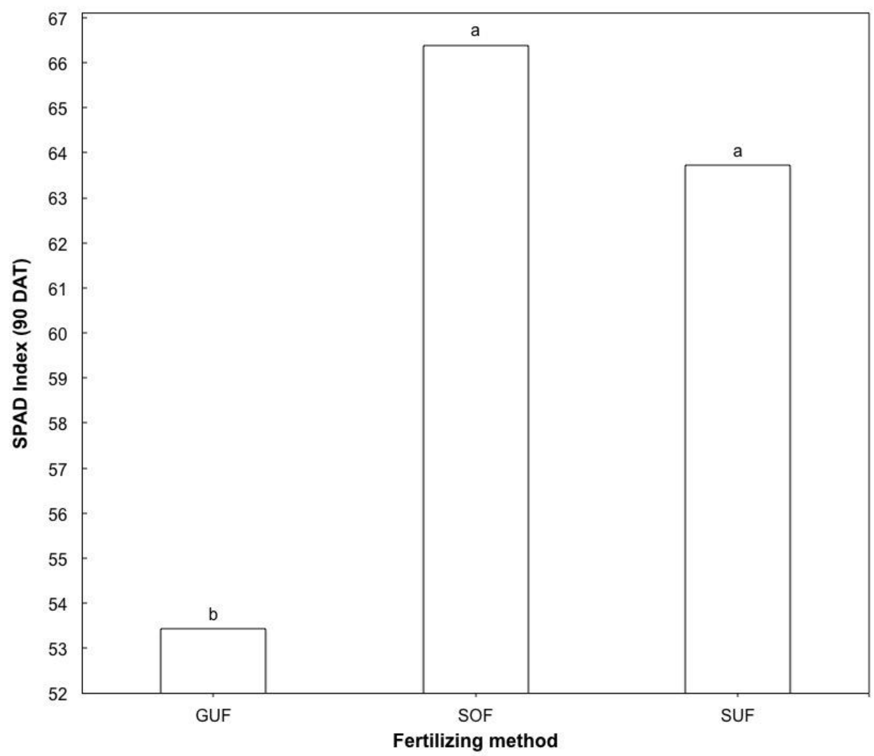


Figure 58. Effect of fertilizing methods on the mean SPAD readings at 90 days after transplant. Mayagüez, Puerto Rico, 2014. Means with the same letter are not significantly different (α = 0.05)

2.4.27 Analysis of reasons that categorize pepper fruits as unmarketable

Fruits were categorized as unmarketable when they had sunburns, scars, damage by birds, or when their width was less than 5.6 cm. In experiment 2, 76% of the fruits produced were categorized as unmarketable (Figure 31). 63% of the unmarketable fruits had a diameter less than 5.6 cm. Damages by birds, sunburns, and scarring occurred in 20, 10, and 7% of unmarketable fruits, respectively (Figure 59).

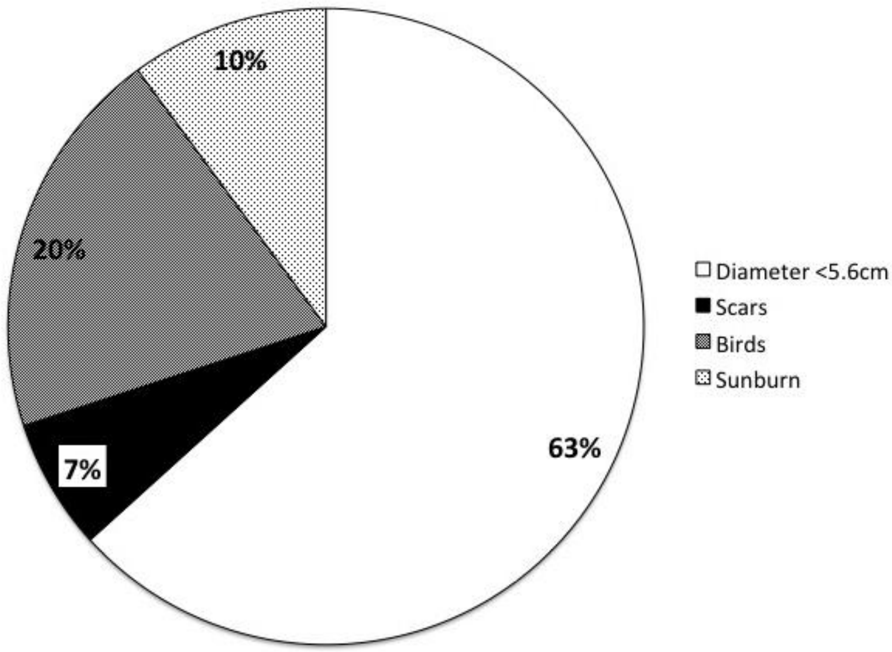


Figure 59. General distribution of different reasons to categorize pepper fruits as unmarketable during experiment 2. Mayagüez, Puerto Rico, 2014.

2.4.27.1 Effect of fertilizing methods on the distribution of reasons to categorize pepper fruits as unmarketable

Unmarketable reasons were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the reasons that categorized pepper fruits as unmarketable. Treatments did not have interaction effects on the observed reasons that categorize fruits as unmarketable. Individual treatment effects of the fertilizing methods were significantly ($p = 0.0079$) related to the observed reasons that categorize fruits as unmarketable. The most common unmarketable reason in all the fertilizing methods was an insufficient width of fruits. Damage by scars was lowest in the SUF method and damage by birds was highest in the GUF method. Sunburn damage did not vary much between fertilizing method treatments (Figure 60).

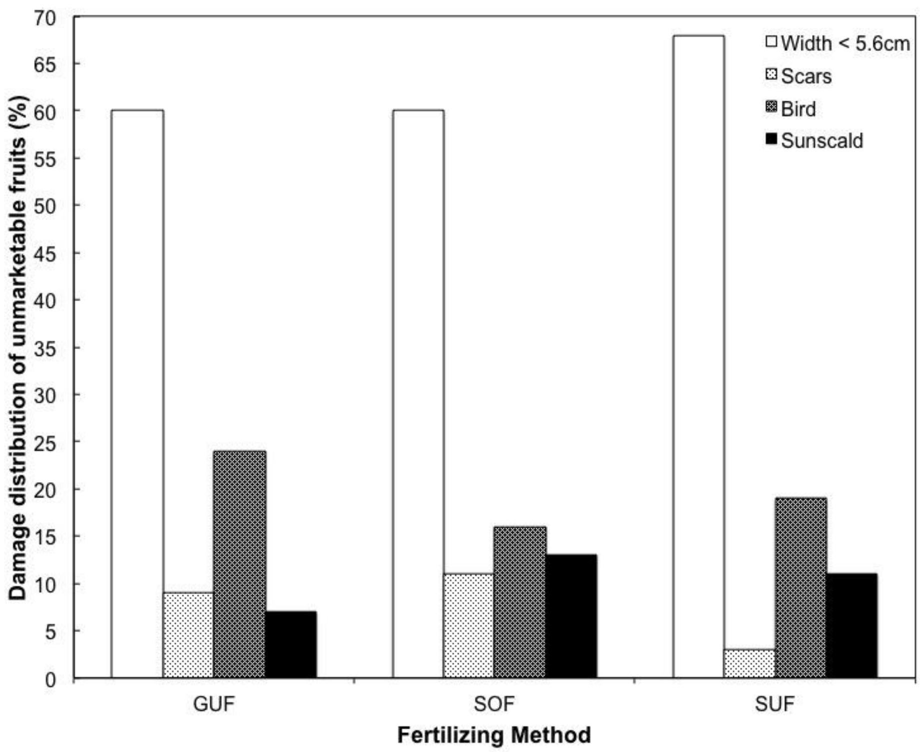


Figure 60. Effects of fertilizing methods on the incidence of reasons to categorize pepper fruits as unmarketable during experiment 2. Mayagüez, Puerto Rico, 2014.

2.4.28 Marketable fruit grading analysis

Fruits categorized as marketable had to be free of decay. The marketable fruits were also free of injuries from scars, sunburn, disease, insects, mites, handling, or other means. An equatorial diameter greater or equal than 5.6 cm was also established to count pepper fruits as marketable. After the fruit was counted as marketable, it was further classified into categories according to two grading systems: 1) the United States standards for grades of sweet peppers (2005) and 2) a diameter scale used for imported greenhouse-grown bell peppers (Jovicich et al., 2007). In experiment 2, only 24% of the fruits produced were categorized as marketable (Figure 31).

2.4.28.1 United States standards for grades of sweet peppers (2005)

Marketable fruits were classified into three categories. None of the fruits were graded as U.S. Fancy, 39 % of fruits were graded as U.S. No.1, and 61% of fruits were graded as U.S. No.2 (Figure 38). These categorizations were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the grading system. Treatment interactions were significantly (p = 0.0213) related to the grading system. The grades of marketable fruits were arranged into percentages according to each combination of fertilizing method and biostimulant (Table 2).

Table 2. Grading distribution of marketable fruits (fertilizing method*biostimulant) in the second experiment using the United States standards for grades of sweet peppers.

Fertilizing method	Biostimulant	Grading distribution (%)			
		U.S. Fancy	U.S. No. 1	U.S. No. 2	Total
GUF	AQC	0	33	67	100
GUF	VTZ	0	50	50	100
GUF	SPX	0	0	100	100
GUF	WTR	0	33	67	100
SUF	AQC	0	8	92	100
SUF	VTZ	0	29	71	100
SUF	SPX	0	31	69	100
SUF	WTR	0	71	29	100
SOF	AQC	0	40	60	100
SOF	VTZ	0	22	78	100
SOF	SPX	0	100	0	100
SOF	WTR	0	54	46	100

None of the treatments produced any U.S. Fancy peppers. The SUF method combined with AQC, VTZ, or SPX produced higher amounts of peppers graded as U.S. No. 2, but when combined with WTR it produced higher amount of U.S. No. 1 peppers (Figure 61). The combination of GUF + SPX did not produce any peppers graded as U.S. No.1 and the combination of SOF + SPX did not produce any peppers graded as U.S. No.2 (Figure 62).

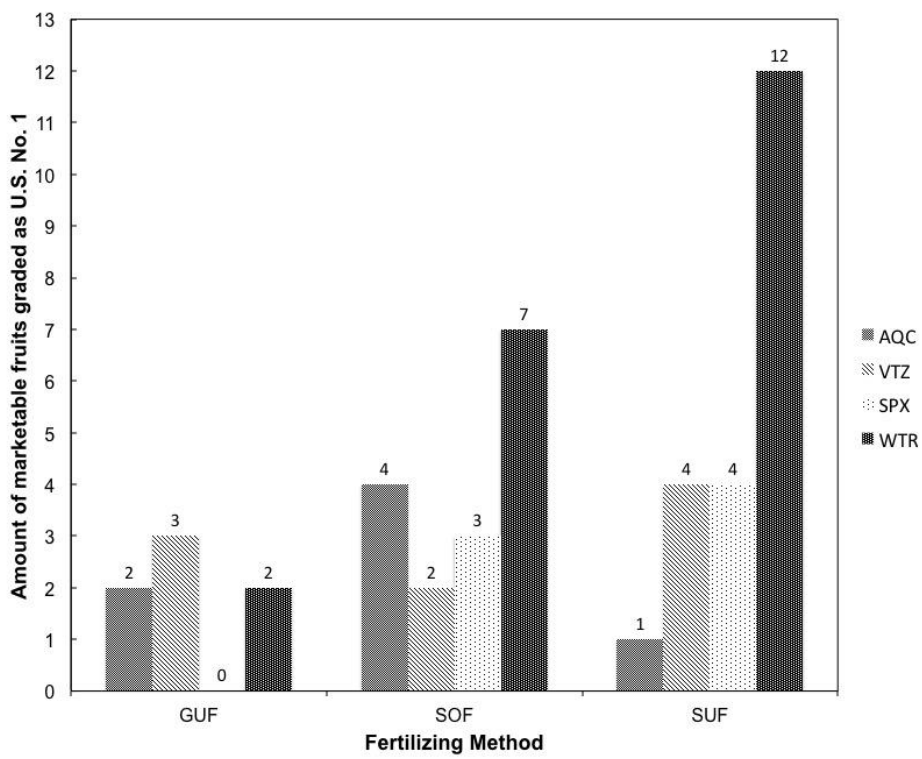


Figure 61. Interaction effects on the amount of marketable fruits graded as U.S. No. 1 during the second experiment. Mayagüez, Puerto Rico, 2014.

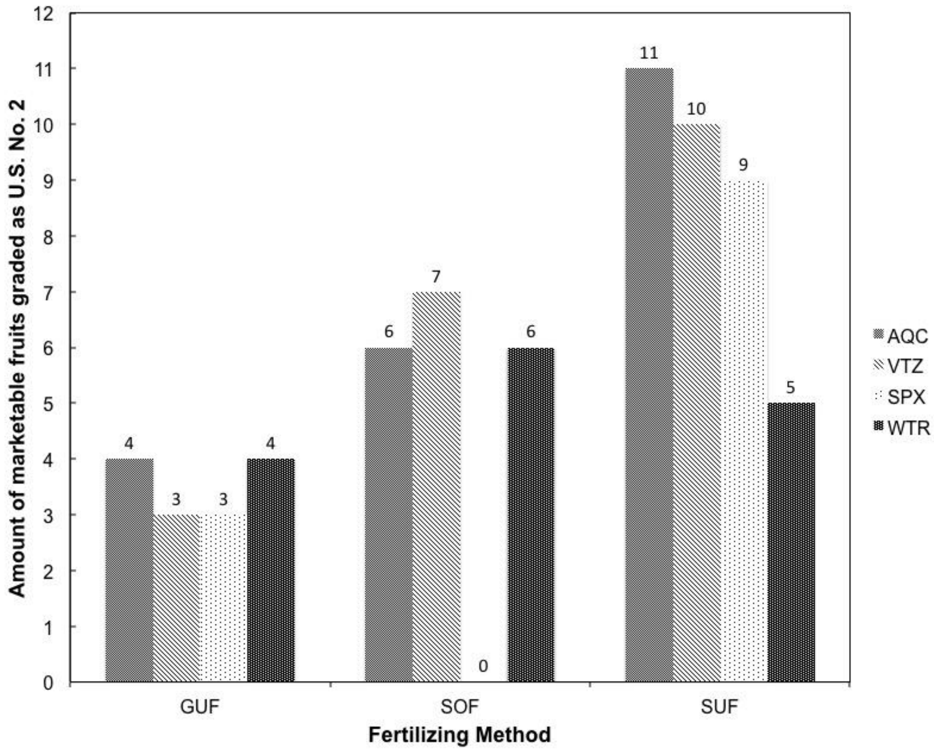


Figure 62. Interaction effects on the amount of marketable fruits graded as U.S. No. 2 during the second experiment. Mayagüez, Puerto Rico, 2014.

2.4.28.2 Grading system for imported greenhouse-grown bell peppers (modified from Jovicich et al., 2007)

Marketable fruits were classified into two categories according to a diameter scale. Small fruits (diameter 5.6 – 6.39 cm) accounted for 60% of the marketable fruits and regular fruits (diameter > 6.4 cm) accounted for 40% of the marketable fruits in the second experiment (Figure 39). These categorizations were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the grading system. Treatment interactions were significantly ($p = 0.0297$) related to the grading system. The grades of marketable fruits were arranged into percentages according to each combination of fertilizing method and biostimulant (Table 3).

Table 3. Grading distribution of marketable fruits (fertilizing method*biostimulant) in the second experiment using the modified diameter scale for imported greenhouse-grown bell peppers

Fertilizing method	Biostimulant	Grading distribution (%)		
		Small	Regular	Total
GUF	AQC	67	33	100
GUF	VTZ	50	50	100
GUF	SPX	100	0	100
GUF	WTR	67	33	100
SUF	AQC	92	8	100
SUF	VTZ	64	36	100
SUF	SPX	69	31	100
SUF	WTR	29	71	100
SOF	AQC	60	40	100
SOF	VTZ	78	22	100
SOF	SPX	0	100	100
SOF	WTR	46	54	100

The SUF method combined with AQC, VTZ, or SPX produced higher amounts of peppers graded as small, but when combined with WTR it produced higher amounts of regular peppers. The combination of GUF + SPX did not produce any peppers graded as regular, and the combination of SOF + SPX did not produce any small peppers (Figure 63 and Figure 64).

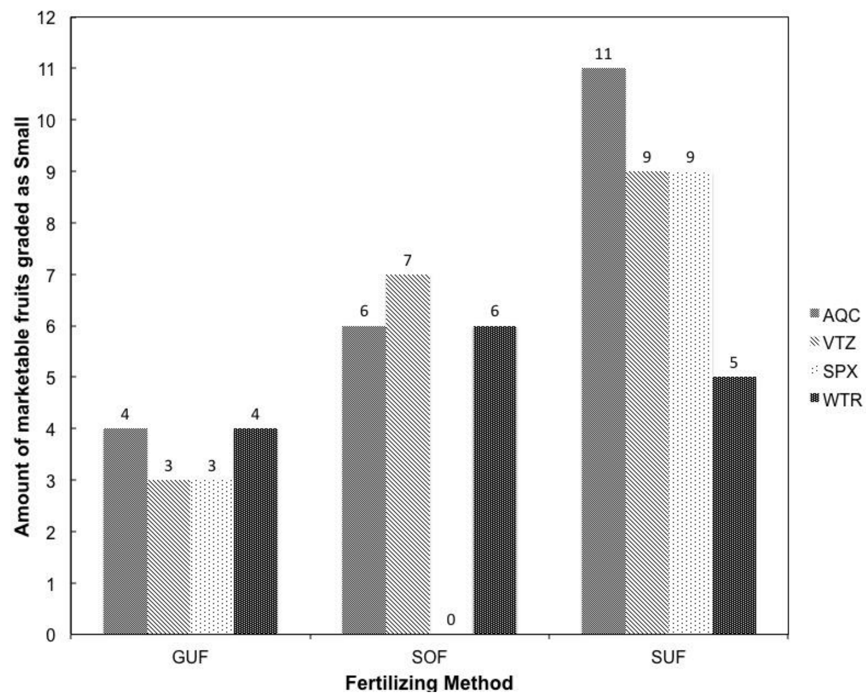


Figure 63. Interaction effects on the amount of marketable fruits graded as Small during the second experiment. Mayagüez, Puerto Rico, 2014.

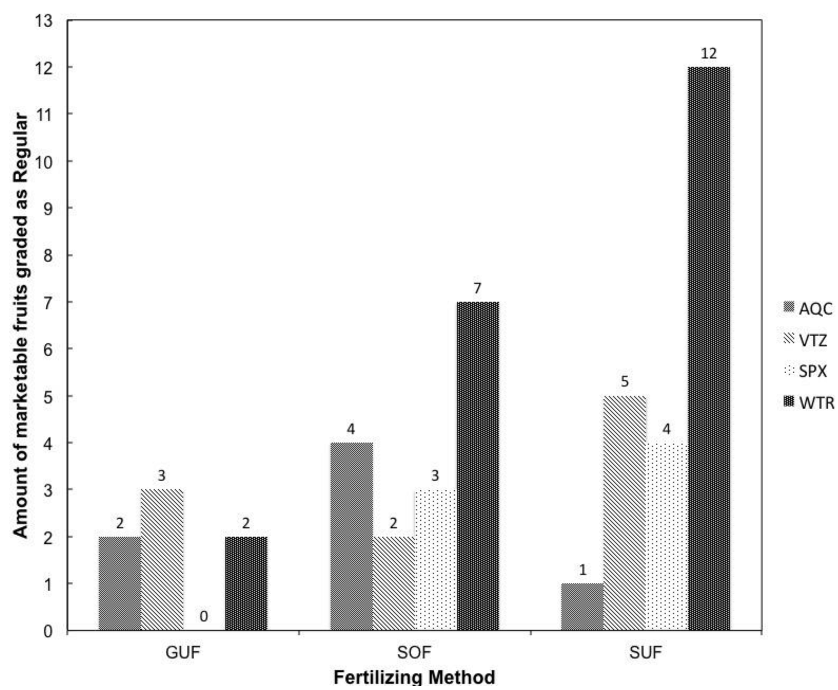


Figure 64. Interaction effects on the amount of marketable fruits graded as Regular during the second experiment. Mayagüez, Puerto Rico, 2014.

Chapter 3. Effects of foliar biostimulants and fertilization methods on potted and drip-irrigated ‘Chocolate Beauty’ bell peppers in a passively ventilated greenhouse

3.1 Introduction

Biostimulants are environmentally friendly, natural substances that when applied in low quantities, can promote vegetative growth, enhance mineral nutrient uptake, and can also increase the plant’s tolerance to abiotic stresses (Chojnacka, 2015). New advances in biotechnology and recent scientific breakthroughs have now enabled the conventional agriculture mindset to shift towards more eco-friendly, less polluting natural substances that can substitute the use of synthetic, potentially harmful chemicals in agriculture without affecting yields or income. Some substances have the capacity to modify the plant’s physiology when applied to the crops or the growing substrate (du Jardin, 2015). Seaweed extracts (SWE) are a very popular source of biofertilizer and are also used to suppress effects of pathogens. SWE can be exploited as an organic method to control diseases, increase plant’s tolerance levels, and protect the environment from dangerous agro-chemicals (Sultana et al., 2011).

Fresh seaweeds have been used in agriculture throughout history because they serve as a source of organic matter and they also carry a fair supply of macro- and micronutrients that benefit crop growth and development (Craige, 2011; du Jardin, 2015; Battacharyya et al., 2015). Over 9,000 species of macroalgae have been identified and classified into three phylum according to their pigmentation: (1) Phaeophyta- brown algae, (2) Rhodophyta- red algae, and (3) Chlorophyta- green algae (Khan et al., 2009).

Ascophyllum nodosum (L.) Le Jolis is a very abundant species of brown algae in the North Atlantic and has been widely researched mainly because of its chemical content, nutritional value, its uses in industrial products and processes, and its uses as human food, animal fodder, and fertilizer properties (Sharp, 1987). In 1947, a biochemist named Dr. Reginald F. Milton was the first who succeeded in patenting a process that liquefied kelp in order to use it as a liquid fertilizer in agriculture (Milton, 1952). It wasn't until recently that the biostimulant effects of SWE have been recognized (du Jardin, 2015), probably due to the presence of a wide range of organic and inorganic plant-growth-promoting and resistance-inducing (Stadnik & Freitas, 2014) constituents such as polysaccharides (e.g. alginate, laminaran, fucoidan), phytohormones (e.g. cytokinin, auxins), sterols (e.g. fucosterols), betaines (Khan et al., 2009), enzymes, proteins, amino acids (e.g. aspartic acid, glutamic acid, glycine, alanine, methionine, tryptophan), vitamins (e.g. niacin, biotin), macronutrients (e.g. S, Mg, Ca, P, K), and trace elements (e.g. Fe, Mn, Zn, Mo, etc.) (Baardseth, 1970).

More than 60% of commercial seaweed products that are currently used in agriculture and horticulture utilize the brown algae group of *Ascophyllum nodosum* (L.) as their main ingredient (Khan et al., 2009), most likely due to its high abundance, high biomass yields, its plant-growth-stimulation properties (Stadnik & Freitas, 2014), and also its wide range of beneficial effects on plants. In avocado trees (*Persea americana*), the applications of commercial SWE have been proven to suppress insect and pest populations (Holden & Ross, 2012). In strawberry plants [*Fragaria ananassa* (cv Queen Elisa)], SWE have also been proven to increase vegetative growth, leaf chlorophyll content, plant biomass, rate of photosynthesis, yield, and weight of berries (Spinelli et al., 2010). The application of a commercial SWE as a spray treatment in camellia plants (*Camellia japonica* L.) grown under nursery conditions improved the rooting percentage of cuttings by 70% and also speeded up growth when compared to the control (Ferrante et al., 2013). In peach trees (*Prunus persica*), significantly higher yields (40%) were achieved through the application of a SWE biostimulant containing micronutrients + humic substances, mainly due to an increase on the quantity of marketable fruits (Waldo, 2014). Also, a SWE derived from the marine algae *Ascophyllum nodosum* was applied to organically grown mango trees (*Mangifera indica*) and resulted in the production of marketable fruits that were 13% larger than the control (Morales-Payan, 2014). In addition, the polysacharides found in SWE (e.g. carrageenans, fucans, laminarans, ulvans) have high potential as elicitors of plant resistance to pathogens (e.g. bacteria, fungi, virus) by triggering a wide array of signaling events that ultimately activate various plant defense mechanisms such as production of hydrolytic enzimes (degrade microbial cell walls), phytoalexins (antimicrobial activity), and ligning and callose (strengthen cell wall) (Burketová et al., 2015; Stadnik & Freitas, 2014). Despite all of these positive attributes of biostimulants based on SWE, some studies have found inconsistent or no results regarding the use of these products on certain crops of economic importance (Diaz-Perez & Bautista, 2014)

Protein hydrolysates are another important plant-biostimulant category that has been widely researched in recent years mainly because of their positive effects on physiological and metabolic processes, N-use efficiency, and stimulation of plant defenses to biotic and abiotic stress (du Jardin, 2012). This category of plant biostimulant is usually produced through the enzymatic and/or chemical hydrolysis of proteins derived from animal or plant byproducts of agro-industries. Their composition is mainly based on mixtures of amino acids and peptides of varying lengths but also include the presence of phytohormones, phenols, carbohydrates, fats, mineral elements, and other organic compounds (Colla et al., 2015).

A plant-derived protein hydrolysate applied to the roots of corn plants (*Zea mays*) improved the salinity tolerance of the crop. This could have been achieved by enhancement of N metabolism and proline accumulation in leaves (Ertani et al., 2013). In papaya plants (*Carica papaya* L.), foliar applications of organic amino acid and peptide complexes at the beginning of the flowering stage increased yields by ~20%, mainly due to a higher fruit number and better fruit set (Morales-Payan & Stall, 2003). Studies done by Colla et al. (2014) observed phytohormone-like effects on corn (i.e. coleoptile elongation), pea plants (*Pisum sativum* L.) (i.e. shoot length), and tomato cuttings (i.e. growth, root production, chlorophyll content, leaf N content) when treated with a plant-derived protein hydrolysate. Positive results with the use of plant-derived biostimulants were also observed in the yield of lettuce (Lactuca sativa L.) and height, number of leaves, fruit quality, and fruit weight of pepper plants as well (Baldoquin-Hernandez et al., 2015; Cabrera-Medina et al., 2012; Rodríguez-Fajardo et al., 2011).

The products called biostimulants have such a wide diversity of ingredients that research often cannot attribute a specific result to a single molecule. Some effects on plants can be caused by a diverse group of molecules, a specific concentration a single molecule, or even a change in the environment. After research on many of the ingredients commonly found in biostimulants, a definitive positive effect has been observed in the growth and health of plants (Karnak, 2000). 'Chocolate Beauty' bell pepper's original breeder and vendor was a company called Petoseed founded in California in 1950. This company led the hybridization of hot peppers and tomatoes in the 1970s and 80s. The variety was developed in 1993 and is described as a non-pungent pepper with large, smooth bell type. Fruits turn from a medium-dark green to a chocolate brown color when fully mature and it is also resistant to Tobacco Mosaic Virus (Bosland & Wehner, 1999). This chapter will focus on the effects of select biostimulants of different categories on the growth dynamics, yield parameters, and fruit quality of potted and drip-irrigated 'Chocolate Beauty' specialty bell peppers grown under a protected structure in Puerto Rico.

3.2 Objectives

1. To determine the effects of select foliar biostimulants with different fertilization methods on ‘Chocolate Beauty’ bell peppers.
2. To determine the effects of select foliar biostimulants with different fertilization methods on the morphological development of ‘Chocolate Beauty’ bell peppers.
3. To determine the effects of select foliar biostimulants with different fertilization methods on the yield and fruit quality of ‘Chocolate Beauty’ bell peppers.

3.3 Methodology

3.3.1 Site description

The experiments were conducted in Greenhouse #5 (latitude: 18° 12’57” N, longitude: 67° 08’48” W, elevation: 15 meters above sea level) located in a Greenhouse Range of six freestanding, even-span Quonset style greenhouses arranged side by side and built upon a continuous concrete foundation at the Finca Laboratorio Alzamora in the University of Puerto Rico at Mayagüez (Figure 3, left). The entry road was towards the front of the greenhouse and a forest-like vegetation growth was towards the rear end. The adjacent greenhouse #6 was being used for an aquaponic system that grew tilapia (*Tilapia* spp.) and spearmint (*Mentha spicata*) and the other greenhouses #1 through #4 were used as nurseries for ornamental plants and vegetable seedlings. The experiment was replicated three times. The third experiment took place on March 2015 through June 2015.

3.3.2 Quonset style greenhouse

The experimental Quonset style greenhouse structure is based upon an arched roof (Figure 3, right) (Omid & Shafaei, 2005). It had a galvanized steel frame and support posts which were embedded into the concrete floors. The greenhouse measured 14.6 meters (m) long (L), 9.1 m wide (W), 4.6 m high (H), and had a bow length (B) of 15.2 m. The total floor surface area of the greenhouse was 133 m^2. On the inside, there were four floor areas with soil that measured 12.2 m long by 1.8 m wide. Each soil-floor surface area was 22 m^2. The total soil-floor surface area measured 88 m^2. This is equivalent to 66% of the total greenhouse area. Each of the soil-floor areas was covered with a black

polyethylene (PE) landscape fabric in order to suppress germination and growth of weeds (Figure 4, left).

3.3.3 Roof structure and material

The roof of the greenhouse was made from a nonporous single layer of transparent PE film without any sort of vents (Figure 3, right). This material is low in cost and lightweight. Light transmission is improved by using only one layer of PE film instead of two (Omid & Shafaei, 2005). Also, PE accelerates heat loss because this material allows easy passage of the reradiated heat energy emitted by the plants and soil inside the greenhouse (Worley, 2009) An accelerated heat loss rate was necessary due to Puerto Rico's year around warm tropical climate. Even though the roof was transparent, light transmission was decreased due to dirt, dust, debris, mold, leaf litter, and plant resin accumulation on top of the greenhouse (Figure 3, right). These impurities were probably originated by the forest-like growth at the rear end of the greenhouse (Figure 4, right).

Airborne dust, dirt and debris captured by the trees' leaves were probably deposited on the greenhouse roof by the rain. These impurities, in addition to the leaf litter that had already accumulated, stained the roof and gave way to mold growth (Figure 3, right). Black olive trees (*Bucida buceras*) in the forest-like growth at the rear of the greenhouse continuously exuded a dark, sticky, and staining, material (Francis, 1989; Gilman & Watson, 1993) that deposited on the roof and thus, diminished light transmission

3.3.4 Walls

The Quonset style greenhouse range was rather basic and low cost because none of the greenhouses had any materials covering the walls. The Quonset style greenhouse did not have walls on the front, rear, or sides of the structure (Figure 3, right), only the galvanized steel support posts that were embedded into the concrete and located at the sides of the greenhouses to provide a firm foundation. The design was basic and probably inexpensive to build because it had no climate control features, but the absence of walls could have possibly improved air movement. This could remove hot air, lower relative humidity and accelerate the structure's natural cooling capacity (Kessler, 1998).

Walls had to be erected for the third experiment in order to prevent the entry of birds that constantly fed on pepper fruits during the harvest time of the previous first and second experiments. A weld mesh with a rectangular grid was attached to the galvanized steel support posts at both sides on the greenhouse using rope and zip-ties, a door was incorporated to allow entry (Figure 65, left), and a polypropylene black shade cloth was hanged from roof to floor and served as a wall at the front and rear ends of the greenhouse (Figure 65, right). The shade cloth had a weave density equivalent to 25% light reduction and did not affect the plant's normal growth rate. The weld mesh on the sides of the greenhouse allowed bees, beneficial insects, and air to move freely within the greenhouses while keeping out the birds that fed on pepper fruits.

Figure 65. (left) A weld mesh with a rectangular grid attached to the support posts was used as side walls; (right) Black shade cloth hanging from the roof was used as a wall for the front and rear ends of the greenhouse

3.3.5 Raised benches

Four longitudinal, galvanized steel raised benches (GSRB) that measured 12.4 m long by 1.5 m wide and 0.64 m high were placed directly above each of the soil-floor areas (Figure 4, left). The aisles between each GSRB were approximately 0.62 m wide. The individual GSRB had a surface area of 18.8 m^2 and their total surface area combined was 75.2 m^2. This meant that only 57% of the greenhouse area was devoted to growing plants. According to Kessler (1998), this is a rather low percentage. He states that with proper planning, 70-80% of the greenhouse's total surface area can be dedicated to growing plants. The top surface of the GSRB was made out of weld mesh with a rectangular grid and welded at each joint. The weld mesh facilitated air movement and allowed the growing medium to freely drain excess water through the drainage holes at the bottom of the containers (Kessler, 1998). The excess drainage water was then captured by the soil under each GSRB.

3.3.6 Containers

According to Acquaah (2009), the correct size of containers should be based on the plant's height. The plant's height should be approximately two times the container's height. Plastic containers were washed, cleaned, and disinfected using a 10% hypochlorite solution before being used. Tap water was used to give the containers a final rinse before placing them upside down to air dry on top of the raised benches. The standard sized, 5-gallon containers were lightweight and black color (Figure 4, left). Each container measured 30 cm long and had a 30 cm diameter (Nursery Supplies Inc., Chambersburg, Pennsylvania). The containers were arranged into longitudinal columns of 15 pots and horizontal rows of 4 pots for a total of 60 containers on each GSRB (Figure 3, right). The distance between the longitudinal columns of containers was 43.2 cm and the distance between the horizontal rows of containers was 76.2 cm. Containers were labeled on the outside with the corresponding treatment for easy identification during treatment applications.

3.3.7 Growing medium

Unsterilized alluvial soil (UAS) was collected from the "Quebrada de Oro" brook that runs across the Mayagüez campus (Figure 6). This brook directs the excess runoff water from precipitation events towards the Mayagüez bay. The water flow carries sediment and eroded soil from higher elevations that gradually accumulates and deposits at the bottom of the brook. The accumulated sediment and soil that gets scooped from the brook during regular maintenance events is transported to the Finca Laboratorio

Alzamora. It is then left outdoors for a period of time until it eventually gets mixed with compost, sieved, and sold for income.

A soil analysis was made to the UAS and indicated a pH of 7.77 and a conductivity of 1013 μS/cm with 0.96% organic matter. Its structure was composed of 25.04% clay, 23.44% silt, and 51.52% sand. According to the soil textural triangle, this soil had a sandy clay loam texture (Appendix 1). For the third experiment, the UAS was sieved with a 2.54 cm mesh in order to break the soil aggregates and also remove rocks and other impurities (e.g. broken glass, plastics, trash, etc.). The sieved UAS was then mixed with a general-purpose sphagnum + perlite mix (Promix®) at a ratio of 3:1. This growing medium mixture was equivalent to having 25% Promix® mixed with 75% sieved UAS in each container.

3.3.8 Irrigation

During the third experiment, the growing medium was saturated with water 24 hours before transplanting the seedlings. The same automated drip irrigation system that was used in the first and second experiments was also used in the third experiment. It consisted of a black, 13 mm polyethylene hose laid longitudinally along the center of each GSRB, between the second and third columns of pots. The hose was then punctured to insert plastic connectors (6mm) (RAINDRIP®. Fresno, California). Poly tubing (6.35 mm outer diameter x 4.32 mm inner diameter ± 2%) (RAINDRIP®. Fresno, California) was then attached to the connectors and pressure compensating drippers (2 L/hour) (RAINDRIP®. Fresno, California) were inserted at every end (Figure 7). Each dripper was placed near the plant's stem in each container.

The water source for irrigation events was a well located at a higher elevation in the Finca Laboratorio Alzamora. A 25-psi pressure regulator was added to the irrigation system to ensure an even water emission out of every dripper. A Steel Spin Clean® (Agricultural Products, Inc.) filter with a 150 mesh was used to remove debris and prevent clogging of drippers. The irrigation system was connected to an electric 12-Station Outdoor SST "Simple to Set" Irrigation Timer (RAINBIRD® model SST1200O). Irrigation frequency was estimated and gradually increased according to visual assessments (e.g. wilting plants, dry substrate), plant growth stage (e.g. vegetative stage, flowering stage), air temperature, container size, and the minutes required for the growing medium to drain excess water.

3.3.9 Stakes

Pepper plants carry significant weight at fruit set and additional support is needed in order to prevent bending and breaking of stems and branches (Acquaah, 2009; Cánovas-Fernández, 2011). The use of stakes also maintains plants and branches in an upright position that allow light exposure to be optimized (Bowen & Frey, 2002). Dowels made from pinewood were purchased at a local hardware store and used at stakes. The 6 mm dowels measured 91 cm long and were inserted into the substrate approximately 8 cm apart from the stems when the plants reached a height of 30 cm. Additional dowels were used for extra support if needed. Stems and branches were attached to the dowels using 15 and 8 cm long plastic zip ties. About 50% of the dowels had to be replaced during harvesting because the irrigation water in the substrate rotted the dowels' wood and made them fragile. The weight of fruit set easily broke the already weakened dowels.

3.3.10 Pest management

Pest populations were controlled with agrochemicals during the third experiment because the organic pest control strategies used during the first experiment were ineffective. Oberon ® 2 SC (spiromesifen; 23.1% A.I.) (Appendix 22) was applied at a dose of 0.6 L ha^{-1} with a manual atomizer to control broad mites on March 20, April 12, and May 12, 2015 (7, 30, and 60 DAT, respectively).

3.3.11 'Chocolate Beauty' bell pepper

Seeds were ordered online from Mountain Valley Seed Co. in Salt Lake City, Utah. The seeds arrived guaranteed free of weeds and with a germination percentage of 99%. Only the most uniform, best-looking seeds were chosen first hand to maintain uniformity. Seeds that looked small, slim, or atrophied were not used.

Germination trays were used for the third experiment due to an early mite infestation in the greenhouse during the first experiment. The germination trays eased watering in order to keep the medium saturated and stimulate sprouting. Also, they facilitated an even application of pesticide to every seedling before transplanting to the containers. Germination trays were soaked, cleaned and disinfected with a brush using a 10% hypochlorite solution. The germination trays were then rinsed with tap water and left upside down to air dry. They were lightweight, plastic, and black color (Figure 40). Each germination tray measured 52 cm long, 27 cm wide, and had 72 cells. The cells were filled with a general-purpose sphagnum + perlite mix (Promix® BX) to be used as a germination medium. A single seed was superficially sown into each cell and only the most vigorous seedlings were used for transplant on March 13, 2015. Harvest began in

May 28, 2015 (76 DAT) and ended in June 28, 2015 (107 DAT). Physiologically mature pepper fruits were harvested when they had at least 50% of their brown color developed.

3.3.12 Experimental design

This experiment was constructed as a completely randomized design. It consisted of a 3 x 4 factorial model arrangement with ten repetitions (one plant per repetition for a total of 120 plants). Three different fertilization methods were used to provide an equivalent of 116 kg·ha^{-1} of N as a post-transplant fertilizer: Granular Urea Fertilization (GUF), Spoon-fed Solubilized Urea Fertilization (SUF), and Spoon-fed Organic Fertilization (SOF). GUF was done with two applications, at the beginning of the flowering and fruiting stages (30 and 50 DAT, respectively). SUF and SOF were divided into ten equal applications every seven days for ten weeks beginning at 7 DAT. Four biostimulant treatments were used as foliar applications: Aminoquelant-Ca (AQC), Vitazyme (VTZ), Stimplex (SPX), and the water check (WTR). Biostimulant solutions were diluted according to the manufacturer's recommendations. Foliar applications of biostimulants began at 10 DAT and were sprayed on leaves, stems, buds, flowers, and immature fruits every 14 days for ten weeks.

3.3.12.1 The variables investigated to determine morphological development were:

Plant height (cm)
Number of leaves
Number of nodes
Number of buds
Number of flowers

Number of fruitlets

Abscission of buds

Abscission of flowers

Abscission of fruitlets

Shoot fresh weight (g)

Root fresh weight (g)

Shoot dry weight (g)

Root dry weight (g)

3.3.12.2 The variables investigated to determine the yield parameters were:

Number of marketable fruits per plant

Number of unmarketable fruits per plant

Number of total fruits per plant

Marketable fruit weight per plant (g)

Unmarketable fruit weight per plant (g)

Total fruit weight per plant (g)

Harvest time of marketable fruits (time as days after transplant, DAT)

Harvest time of unmarketable fruits (time as days after transplant, DAT)

Harvest time of total fruits (time as days after transplant, DAT)

3.3.12.3 The variables investigated to determine the quality of fruits were:

Length of marketable fruits (cm)

Length of unmarketable fruits (cm)

Length of total fruits (cm)

Width of marketable fruits (cm)

Width of unmarketable fruits (cm)

Width of total fruits (cm)

Pericarp thickness of marketable fruits (mm)

Pericarp thickness of unmarketable fruits (mm)
Pericarp thickness of total fruits (mm)
Total soluble solids content in marketable fruits (Brix %)
Total soluble solids content in unmarketable fruits (Brix %)
Total soluble solids content in total fruits (Brix %)
Classification of marketable fruits

3.3.12.4 The variables investigated to determine changes in chlorophyll and N content:

SPAD index
Nitrate content in sap

3.3.12.5 The variables investigated to analyze the reasons for categorizing fruits as unmarketable were:

Percentage of unmarketable fruits damaged mites (scarring)
Percentage of unmarketable fruits with a diameter less than 5.6 cm
Percentage of unmarketable fruits damaged by sunburns

3.3.13 Fertilizer treatments

Fertilizer applications were determined by the recommendations found in the technological package for commercial pepper production in Puerto Rico (Fornaris et al., 2005). Calculations had to be made in order to determine the exact amounts of fertilizer needed for each individual plant. Current fertilization recommendations in Puerto Rico are focused on the open field, conventional agriculture and are meant for relatively big areas of land. Information regarding fertilizer management in greenhouse pepper production in Puerto Rico is currently very limited. This lack of information could slow Puerto Rico's agricultural transition to protected agriculture.

Bell peppers in Puerto Rico are cultivated on raised beds but most commonly in open fields. Raised beds are usually 1.5 to 1.8 meters apart with crops aligned in double rows. A distance of 30 to 45 cm separates each pair of rows and the distance of plants within the rows is 30cm. These planting distances can be used to estimate plant densities. Estimated plant densities range between 35,877 and 43,051 plants per hectare. A plant density of 36,890 plants per hectare (Equation 1) was used to calculate nutrient requirements of individual plants. The total amounts and requirements of N, phosphate and potash (K_2O) applied as a pre-plant fertilizer (Equation 2) and the N applied as post-transplant fertilizer (Equation 3) were calculated for 1 hectare based on plant density and local recommendations from Puerto Rico's commercial pepper production guide (Fornaris et al., 2005).

3.3.13.1 Granular Urea Fertilization (GUF)

Urea [$CO(NH_2)_2$] is the most widely used N source in the U.S. mainly due to its high N content and its high solubility in water. Manufacturing, handling, storage, and transportation logistics of granular urea are favorable and less dangerous (Havlin et al., 2005). It has a lower tendency to cake, stick, and/or explode than ammonium nitrate (NH_4NO_3), and it's also less corrosive to handling and equipment. In its current form, the N in urea is unavailable for plants. When urea is applied to the soil, urease enzymes hydrolyze it to ammonium (NH_4^+). Variations in soil pH may cause the NH_4^+ to form ammonia (NH_3), which can then be volatilized at the soil surface (Equation 4).

The urease enzyme that is needed to catalyze the hydrolysis of urea is usually very abundant in soils, especially in warm, moist soils that are high in organic matter content and also high in soil microbial populations. The application of urea to the soil surface is most efficient when washed into the soil and when there is a low volatilization potential. Deep incorporation of granular urea is advisable over surface applications or shallow tillage. This minimizes volatilization losses of NH_3 by increasing the volume of soil capable of retaining it. Many factors may interfere with the effectiveness of urea and can cause some degree of variability in the crop’s response to this fertilizer. In order for N in the soil to be available for plant use, it has to be in one of two forms: ammonium or nitrate.

3.3.13.1.1 Pre-planting: synthetic granular fertilizer (10-10-10)

Only 16 grams of a synthetic 10-10-10 granular fertilizer were incorporated as a pre-plant fertilizer into the first 15 cm of each container. This amount of fertilizer per plant is equivalent to the application of 58 $kg \cdot ha^{-1}$ of N, phosphate, and K_2O recommended by local sources (Equation 5). A hand shovel was used to incorporate the pre-plant fertilizer on March 12, 2015.

3.3.13.1.2 Post-transplanting: synthetic granular urea fertilization (46-0-0)

Two post-transplant granular urea applications were made in order to apply a total N amount equivalent to 116 $kg \cdot ha^{-1}$. The first post-transplant granular urea application was made at the beginning of the flowering stage on April 12, 2015 (30 DAT). The second application was made at the beginning of the fruiting stage on May 2, 2015 (50 DAT). Amounts of granular urea needed for each plant were calculated based on the recommended rate for open field cultivation of bell peppers (Equation 6). A total of seven grams of urea were incorporated into the first 5 cm of soil in each container to avoid damaging the roots.

3.3.13.2 Spoon-fed Solubilized Urea Fertilization (SUF)

The adequate rates and the correct timing of fertilizer applications can maximize production of vegetable crops (Russo, 1991). By controlling the quantity and frequency of solubilized N applications, each growth stage can be supplied with specific and optimum nutrient rates that can increase the crop's NUE.

3.3.13.2.1 Pre-planting: synthetic granular fertilizer (10-10-10)

The pre-plant fertilization rate for the SUF treatment was the same one used for the pre-plant fertilization rate in the GUF treatment that was previously described.

3.3.13.2.2 Post-transplanting: synthetic solubilized urea fertilization (46-0-0)

SUF was used to supply post-transplant N in ten equal split-applications every 7 days for ten weeks. The first application was made 7 DAT, on March 20, 2015, when plants had an average length of 10 cm and 4-6 true leaves. The following nine applications were made on March 27, April 3, April 10, April 17, April 24, May 1, May 8, May 15, and May 22, 2015. Each individual application consisted of 0.7 grams of urea diluted in 100 mL of water. The total amount and source of post-transplant N fertilization in the SUF treatment was the same as in the GUF treatment. The main difference is that the SUF treatment was applied through irrigation as ten equal split-applications for 10 weeks while the GUF treatment was incorporated into the soil as two equal applications at the beginning of the flowering and fruiting stages. The SUF was always applied directly to the soil, not the foliage. Foliar fertilizer application methods were not evaluated in this research.

3.3.13.3 Spoon-fed Solubilized Organic Fertilization (SOF)

The Green Revolution, climate change, and projected population growth in the near future have contributed to an increased demand for high quality, fresh agricultural produce (Negrete-Aveiga, 2013; Tilman et al., 2002). As a result, croplands are being cultivated intensively and depend on high fertilizer inputs in order to achieve and sustain economic profits. Misuse of fertilizers and inorganic salts can turn into an environmental hazard by polluting ecosystems or leaching into underground water supplies (Addiscott, 2005). Organic agriculture has recently been gaining popularity in Puerto Rico due to concerns about local fresh produce, costs of imports, and environmentally sustainable production systems. Organic production systems rely on different sources of N-containing materials that vary in costs, availability, nutrient content, mineralization rate, and environmental impact. In order to produce certified organic vegetables, the production system has to be verified and approved as organic by an independent party. Thus, this research will focus on the use of commercially available organic fertilizers rather than on the production of certified organic bell peppers.

3.3.13.3.1 Pre-planting: organic fertilizer Bioflora Dry Crumbles® (6-6-5+8% Ca) and organic liquid fertilizer Bioflora (0-0-15+1% Ca)

Bioflora Dry Crumbles® (6-6-5) (BDC) is an organic, dry granular fertilizer fortified with seaweed. This fertilizer is certified by the OMRI and it provides high quality organic nutrients for all types of plants. It contains balanced amounts of N, P, and K but also contains high levels of calcium (Ca) and trace elements. The carbon-based granules are characterized by a slow-release formula that provides plants with certain benefits like renewed vigor, root mass stimulation and reduced damage from disease (Global Organics Group, 2016). The nutrient content within the granules is derived from a blend of feather meal, dry composted poultry litter, sulfate of potash, and the seaweed *A. nodosum* (Negrete-Aveiga, 2013) (Appendix 2).

Each plant in the SOF treatment had 27 grams of granular BDC (6-6-5) fertilizer incorporated into the first 15 cm of each container as a pre-plant fertilizer on March 12, 2015. This amount of fertilizer per plant is equivalent to the application of 58 $kg \cdot ha^{-1}$ of N and phosphate recommended by local sources (Equation 7). Even though the required pre-plant amount of N and P was supplied with 27 grams of BDC, there was a very slight deficit in the amount of K applied due to the inherent nature and nutritional proportions in the BDC (6-6-5) fertilizer (Equation 8).

A deficit of 8 $kg \cdot ha^{-1}$of pre-plant K_2O could eventually bias conclusions on plant growth, development, or yield. In order to maintain the same amount of NPK in the pre-plant fertilization of all the treatments, Bioflora (0-0-15) liquid fertilizer (BLF) (Appendix 3) was applied at a rate of 2 mL per plant in combination with the BDC (6-6-5) fertilizer to correct the small deficit in the pre-plant K_2O (Equation 9). The BLF (0-0-15) is highly soluble in water and is derived from potassium carbonate and calcium EDTA.

According to product label, when used as a pre-plant fertilizer, it should be applied to the ground as a concentrate. Both, the BDC (6-6-5) and the BLF (0-0-15) in the pre-plant SOF treatment, contained 8 and 1% Ca respectively. A total amount of 81 $kg \cdot ha^{-1}$ of pre-plant Ca was also included with the BDC and BLF in the SOF treatment (Equation 10 & 11). This extra amount of Ca was only applied in the SOF treatment because the commercially available products used in this study included this nutrient. There was no extra amount of Ca added to neither the GUF nor the SUF treatments.

3.3.13.3.2 Post-transplanting: organic liquid fertilizer Bioflora (6-0-0+8% Ca)

Bioflora organic liquid fertilizer (6-0-0+8% Ca) (BLNF) was used to supply post-transplant N in ten equal split-applications every 7 days for ten weeks in the SOF treatment (Equation 12). This organic liquid fertilizer is derived from calcium nitrate (Appendix 5). The first application was made 7 DAT, on March 20, 2015, when plants had an average length of 10 cm and 4-6 true leaves. The following nine applications were made on March 27, April 3, April 10, April 17, April 24, May 1, May 8, May 15, and May 22. The total amount of post-transplant N fertilization in the SOF treatment was the same as in the SUF and GUF treatments. The main difference is that the SUF and GUF treatments used synthetic urea fertilizer, while the SOF treatment used an organic fertilizer. The SOF was always applied directly to the soil, not the foliage.

3.3.14 Biostimulant treatments

All the biostimulant treatments were applied individually as a foliar spray with a plastic, hand held atomizer. Atomizers were always rinsed three times before and after the application of biostimulants. Every biostimulant treatment was applied five times during the crop cycle. The first biostimulant applications began in March 23, 2015 (10 DAT) and were repeated every 14 days. The four remaining applications events were done on April 6 (24 DAT), April 20 (38 DAT), May 4 (52 DAT), and May 18, 2015 (66 DAT). The dosage was determined according to each of the biostimulants' respective manufacturer. Leaves, stems, buds, flowers and immature fruits were covered evenly with the biostimulant solution until run-off. Foliar applications were always done during the cooler hours of the day (i.e., early morning or late afternoon) in order to prevent or diminish excessive evaporation from the plants' surface. Wind speed and wind direction were taken into consideration in order to diminish unintended drift potential by wind onto nearby plants. No surfactant was added in any of the biostimulant treatments.

Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

3.3.14.1 Aminoquelant®-Ca (AQC)

Aminoquelant®-Ca (BioIbérica, Barcelona, Spain) is a commercially available blend of amino acids for agricultural use. It combines Ca with L-α-amino acids from enzymatic hydrolysis that can improve Ca mobility in plants and can also mitigate the factors that lead to its deficiency. The amino acid blend contains 4.6% (w/w) biologically active free amino acids. It consists of aspartic acid (aspartate), serine, glutamic acid (glutamate), glycine, histidine, arginine, threonine, alanine, proline, cysteine, tyrosine, valine, methionine, lysine, isoleucine, leucine, phenylalanine, and tryptophan. This formulation includes 8% calcium oxide (CaO), 0.9% organic N, 4.9% total N, 0.2% boron, and 6.8% organic matter. It can be applied through the irrigation line or by foliar sprays and it is especially designed for crops that are prone to physiological alterations by Ca deficiencies such as peppers, tomatoes, lettuce, etc. (BioIbérica, 2016). The foliar spray solution was prepared by diluting 4 mL of Aminoquelant-Ca with 1 L of water. This is equivalent to a 0.4% Aminoquelant-Ca solution.

3.3.14.2 Vitazyme (VTZ)

Vitazyme® (Vital Earth Resources, Gladewater, Texas, USA) is a commercially available, OMRI and BCS certified organic biostimulant for agricultural use. It consists of certain biological activators that are created by an undisclosed, propietary fermentation process. The formulation contains Vitamin B1 (thiamin) (0.45 mg/100g), Vitamin B2 (riboflavin) (0.03 mg/100g), Vitamin B6 (pyridoxine) (0.19 mg/100g), K_2O (0.80%), Iron (Fe-EDTA) (0.2%), Copper (Cu-EDTA) (0.07%), Zinc (Zn-EDTA) (0.06%), brassinosteroids (i.e., homobrassinolide, dolicholide, homodolicholide, and brassinone) (0.03 mg/ml), 1-triacontanol (0.17 mg/ml), glycosides and water. This biostimulant accelerates and improves metabolic processes that can increases crop yields and profits. It improves crop quality, reduces N fertilizer inputs, and also hastens germination and maturity. In addition, it also acts on soils by improving their structure and infiltration capacity (Vital Earth Resources, 2016). According to the manufacturer's recommendations, seeds were pre-treated by soaking them directly in the Vitazyme concentrate for 90 seconds. This was the only biostimulant used to pre-treat seeds. The foliar spray solution was prepared by diluting 10 mL of Vitazyme with 1 L of water. This is equivalent to a 1% Vitazyme solution.

3.3.14.3 Stimplex (SPX)

Stimplex^{TM} (Acadian Seaplants, Nova Scotia, Canada) is an OMRI listed, commercially available SWE biostimulant for agricultural use. It is derived from the brown algae *Ascophyllum nodosum* L. Product label states that it contains an active ingredient concentration equivalent to 100 parts per million of kinetin (Appendix 6). It also contains zeatine, adenine, betaine, oligosaccharides, polysaccharides, organic acids, vitamins, gibberellins, auxins, micronutrients, and amino acids (Flores-Torres, 2013). It is used to improve resistance to biotic & abiotic stresses and enhance the crop's overall health. It is also used to increase yields and quality of crops, and also to increase root growth, early plant development, fruit set, fruit size, and nutrient levels (CDMS, 2016). The foliar spray solution was prepared by diluting 5 mL of Stimplex with 1 L of water. This is equivalent to a 0.5% Stimplex solution.

3.3.14.4 Water check (WTR)

In order to disregard the possible effect of spray vs. no spray, tap water was used as a control to spray the plants without biostimulants, rather than not spraying them at all. The water used as a control was always collected at the time of application in order to prevent the water from becoming stagnant over time. The source of the water used in the control check was from a well located at a higher elevation in the Finca Laboratorio Alzamora.

3.3.15 Morphological research

Morphological development was measured through the analysis of the following variables:

3.3.15.1 Plant height (cm)

A measuring tape (cm) was used to measure plant height every seven days for ten weeks, beginning on March 17, 2015 (4 DAT). Measurements were taken starting at the soil surface and extending to the apex.

3.3.15.2 Number of leaves and nodes

Leaves and nodes were counted on each plant every seven days for ten weeks, beginning on March 17, 2015 (4 DAT). Only the fully extended, mature leaves were counted.

3.3.15.3 Mean fresh weight of shoots and roots (g)

Plants were cut at the soil surface level using scissor-action pruners (Fiskars Brands Inc, Middleton, Wisconsin, USA). This was done after the last pepper was harvested in each plant or during the experiment's last day of harvest on June 28, 2015 (107 DAT). The top part of the plant, or everything above the soil surface level, was separated from its roots, segmented into smaller pieces, and placed in a paper bag. After the shoots were removed, the containers were emptied on a 15 mm sieve. The growing medium was carefully sieved until the roots were exposed. Roots were washed with running water and excess debris was rinsed off. The root ball and root fragments were then collected from the sieve, manually wringed, placed in individual paper bags, identified as shoots or roots, and labeled with the plant's treatment and location within the raised bench. Each paper bag was weighted with a digital scale (Fisher Science Education™, USA) and data was named as fresh weight of shoots or fresh weight or roots (g).

3.3.15.4 Mean dry weight of shoots and roots (g)

After the fresh weight data was logged, paper bags with plant material were placed in a drying oven (Jisico Scientific Instruments, Seoul, Korea) for 96 hours at 75°C. Paper bags with the dehydrated plant material were weighted and the data was named as dry weight of shoots or dry weight of roots (g).

3.3.15.5 Mean number of buds, flowers, and fruitlets

Each plant was checked every seven days for seven weeks, beginning on April 2, 2015 (20 DAT). Buds, flowers, and fruitlets that were on the plant at the moment were counted.

3.3.15.6 Mean abscission of buds, flowers, and immature fruits

Abscission of reproductive organs and fruitlets was determined by counting the number of buds, flowers, and fruitlets that had fallen off of the plant on each container's soil surface area ($A \approx 0.6\ m^2$). Abscission of flowers refers to any flower that dropped prior to post-fertilization ovary enlargement and abscission of fruitlets refers to any ovary that dropped after post-fertilization enlargement (Bookman, 1983). After counting, aborted reproductive organs were removed in order to avoid counting them twice during the next data-logging event.

3.3.16 Yield and quality research

Physiologically mature pepper fruits were individually harvested when they had developed at least 50% of their chocolate brown color. The harvest period lasted for thirty-two days, beginning in May 28 (76 DAT) and ending in June 28, 2015 (107 DAT). Each pepper was weighted, measured (i.e., length and width), and classified as marketable or unmarketable. Total fruits were the sum of marketable and unmarketable fruits. Marketable fruits had an equatorial diameter equal or greater than 5.6 cm and were free of rot or damages by abiotic or biotic stressors. These were then graded into categories according to two different grading methods: 1) the United States standards for grades of sweet peppers (2005) and 2) a diameter scale used for imported greenhouse-grown bell peppers (Jovicich et al., 2007).

The first grading system used was the United States standards for grades of sweet peppers (2005). These standards classify peppers into three categories: U.S. Fancy, U.S. No. 1, and U.S. No. 2. The criteria used to assign these categories are length, width, color, and damages. The second grading system is used for imported greenhouse-grown bell peppers and grades the pepper fruits based on size following a diameter scale: extra-large (diameter > 8.4 cm), large (7.6 – 8.39 cm), medium (6.4 – 7.59 cm), and small (5.6 – 6.39 cm) (Jovicich et al., 2007). Marketable fruit data was analyzed considering both grading systems. The grading system for imported greenhouse-grown bell peppers was modified and the categories of large and extra-large were eliminated. This modification of the grading system had to be made because there were insufficient extra-large and large pepper fruits. The newly modified grading system for imported greenhouse-grown bell peppers consisted of only two categories: regular (diameter > 6.4 cm), and small (5.6 – 6.39 cm).

The different reasons to categorize fruits as unmarketable fruits were also logged. Each pepper fruit was then sliced in half through the equator. Pericarp thickness was measured (mm) using a caliper (Vernier Callipers, Waukegan, IL, USA). Each pepper fruit was then segmented into smaller pieces in order to extract drops of juice from the pericarp and measure the concentration of soluble solids using a digital hand-held refractometer ATAGO® PAL-1 Pocket (ATAGO, Tokyo, Japan). Pepper fruit contents (i.e. seeds, calyx, placenta, and fruit walls) were deposited in paper bags. Each paper bag was labeled with the plant's treatment, location within the raised bench, and also with the fruit's number according to the order of harvest. Paper bags with pepper fruit material were placed in a dehydrating oven (Jisico Scientific Instruments, Seoul, Korea) for 96 hours at 75°C and then weighted with a digital scale (Fisher Science Education™, USA). Dry weight of fruits was then organized and summed for each plant.

3.3.17 Physiological research

Physiological changes were measured through the analysis of the following variables:

3.3.17.1 Mean chlorophyll content

Chlorophyll content was measured using a SPAD-502Plus chlorophyll meter (Osaka, Japan) in April 23, 2015 (41 DAT) on fully expanded leaves.

3.3.17.2 Nitrate content in sap

Nitrate content was measured with a LAQUA Twin Nitrate NO_3^- meter (Spectrum Technologies, Inc., Aurora, IL). The petioles of fully expanded leaves were separated and pressed to extract a few droplets of sap. The sap was then placed in the nitrate meter's sensor and the readings were logged. Nitrate content in sap was measured during pepper fruit harvest time (80-107 DAT).

3.3.18 Data analysis

Statistical analysis was conducted with Infostat version 2014 (Infostat software, National University of Córdoba, Argentina). The Shapiro-Wilks test was used to verify if the data followed a normal distribution and the Levene test was used to determine homoscedasticity of the data. Values were log-transformed or square root-transformed to homogenize variance or normalize distribution when needed. The non-parametric Kruskal-Wallis test was used when variance was not homogenous and/or distribution was not normal even after both transformations had been made. An analysis of variance (ANOVA) was conducted in order to evaluate the potential interactions of each factor. The interactions are described as combinations of the levels from each factor (Viggiano-Beltrocco, 2014). The levels were: 3 post-transplant N fertilization methods (GUF, SUF, and SOF) and 4 biostimulant treatments (AQC, VTZ, SPX, and WTR) that make a 3 x 4 factorial ANOVA model. Significant means were separated using the LSD statistical test ($\alpha < 0.05$).

3.4 Results

3.4.1 Mean plant height (cm)

Treatments did not have interaction effects on the mean plant height at any time during the crop cycle. Individual treatment effects were seen on the fertilizing methods during the entire crop cycle (Appendix 29).

3.4.1.1 Effect of fertilizing methods on the mean plant height (cm)

Fertilizing method treatments had significant effects during the entire crop cycle. The SUF method resulted in significantly taller plants when compared with the GUF or SOF methods. The GUF method produced more plant height than the SOF until 41 DAT. Plant height from GUF and SOF was not significantly different from 47 to 70 DAT (Figure 66).

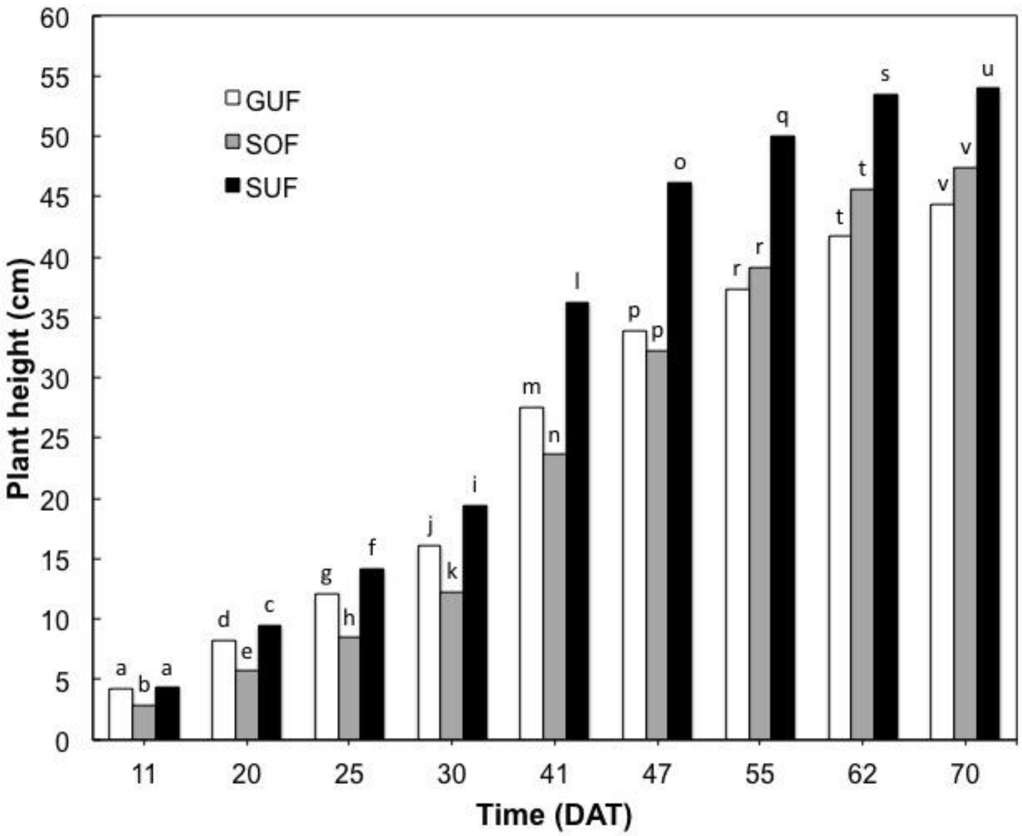


Figure 66. Individual treatment effects of fertilizing methods on plant height (cm) at various days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.2 Mean number of leaves

Treatments did not have interaction effects on the mean number of leaves at any time during the crop cycle. Individual treatment effects were seen on the fertilizing methods during the entire crop cycle (Appendix 30).

3.4.2.1 Effect of fertilizing methods on the mean number of leaves

Fertilizing method treatments had significant effects during the entire crop cycle. The SUF method resulted in significantly more leaves when compared with the GUF or SOF methods. The GUF method produced more leaves than the SOF until from 20 to 41 DAT. The number of leaves from GUF and SOF was not significantly different from at 47 DAT. The SOF method produced significantly more leaves than the GUF method from 55 to 70 DAT (Figure 67).

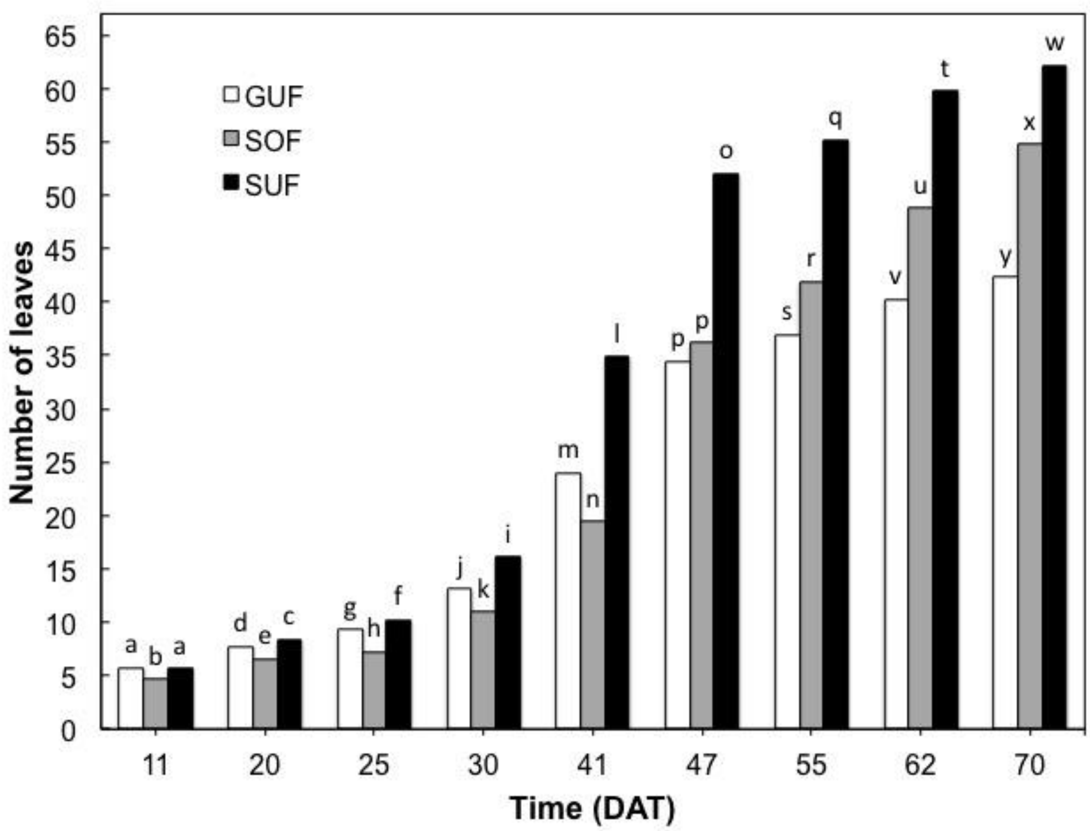


Figure 67. Individual treatment effects of fertilizing methods on the mean number of leaves at various days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05).

3.4.3 Mean number of nodes

Treatment combinations did not have interaction effects on the mean number of nodes at any time during the crop cycle. Individual treatment effects were seen on the fertilizing methods during the entire crop cycle. Biostimulant treatments did not have significant effects on the mean number of nodes (Appendix 31).

3.4.3.1 Effect of fertilizing methods on the mean number of nodes

Fertilizing method treatments had significant effects during the entire crop cycle. The SUF method resulted in significantly more nodes when compared with the GUF or SOF methods starting at 11 DAT. The GUF method produced more nodes than the SOF until at 11, 20, 25, and 41 DAT. The number of leaves from GUF and SOF was not significantly different at 30, 47, 55, and DAT. The SOF method produced significantly more nodes than the GUF method from at 70 DAT (Figure 68).

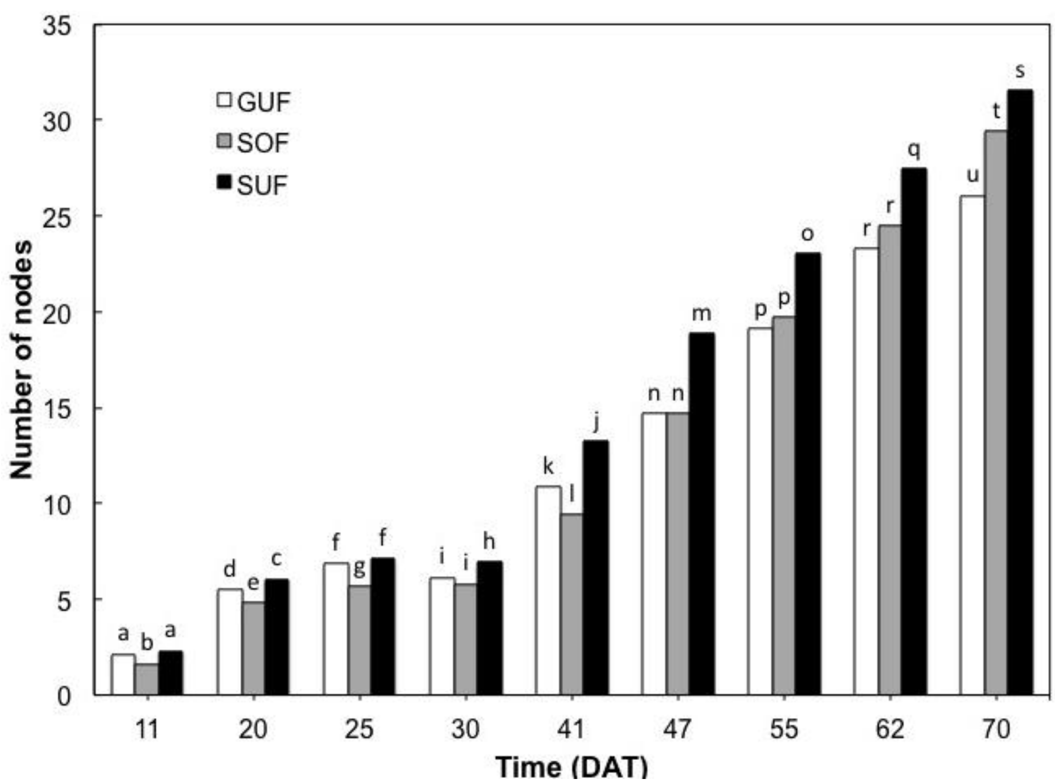


Figure 68. Individual treatment effects of fertilizing methods on the mean number of nodes at various days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05).

3.4.4 Mean number of buds

Treatment did not have interaction effects on the mean number of buds at any time during the crop cycle. Individual treatment effects were seen on the fertilizing methods at 20, 25, 30, 41, 47, 62, and 70 DAT (Appendix 32).

3.4.4.1 Effect of fertilizing methods on the mean number of buds

Fertilizing method treatments had significant effects during the entire crop cycle. The SUF method resulted in significantly more buds when compared with the GUF or SOF methods from 20 to 47 DAT. The GUF method produced more buds than the SOF from 20 to 30 DAT. The number of buds from GUF and SOF was not significantly different at 41 DAT. The SOF method resulted in significantly more buds when compared with the GUF or SUF methods at 62 and 70 DAT (Figure 69).

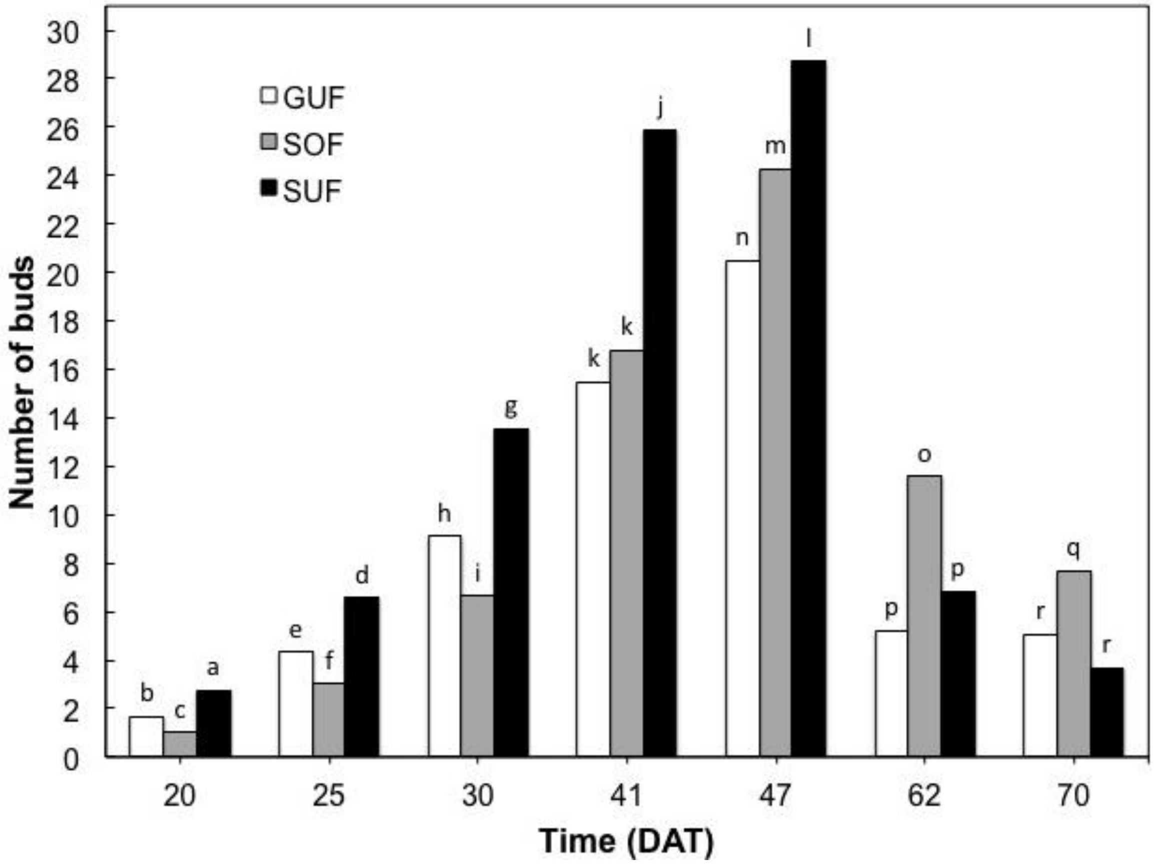


Figure 69. Individual treatment effects of fertilizing methods on the mean number of buds at various days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05).

3.4.5 Mean number of flowers

Treatment combinations did not have interaction effects on the mean number of flowers at any time during the crop cycle. None of the treatments had any significant statistical differences on the mean number of flowers at 55 and 70 DAT. Individual treatment effects were seen on the fertilizing methods at 41, 47, and 62 DAT. Biostimulant treatments did not have significant effects on the mean number of flowers (Appendix 33).

3.4.5.1 Effect of fertilizing methods on the mean number of flowers

Fertilizing method treatments had significant effects on the mean number of flowers at 41, 47, and 62 DAT but not at 55 nor at 70 DAT. The SUF method produced an average of 54 and 40% more flowers than the GUF and SOF methods at 41 DAT, respectively. Also, the SUF method produced an average of 99 and 63% more flowers than the GUF and SOF methods at 47 DAT, respectively. At 62 DAT, the SOF method produced 65 and 12% more flowers than the GUF and SUF methods, respectively (Figure 70).

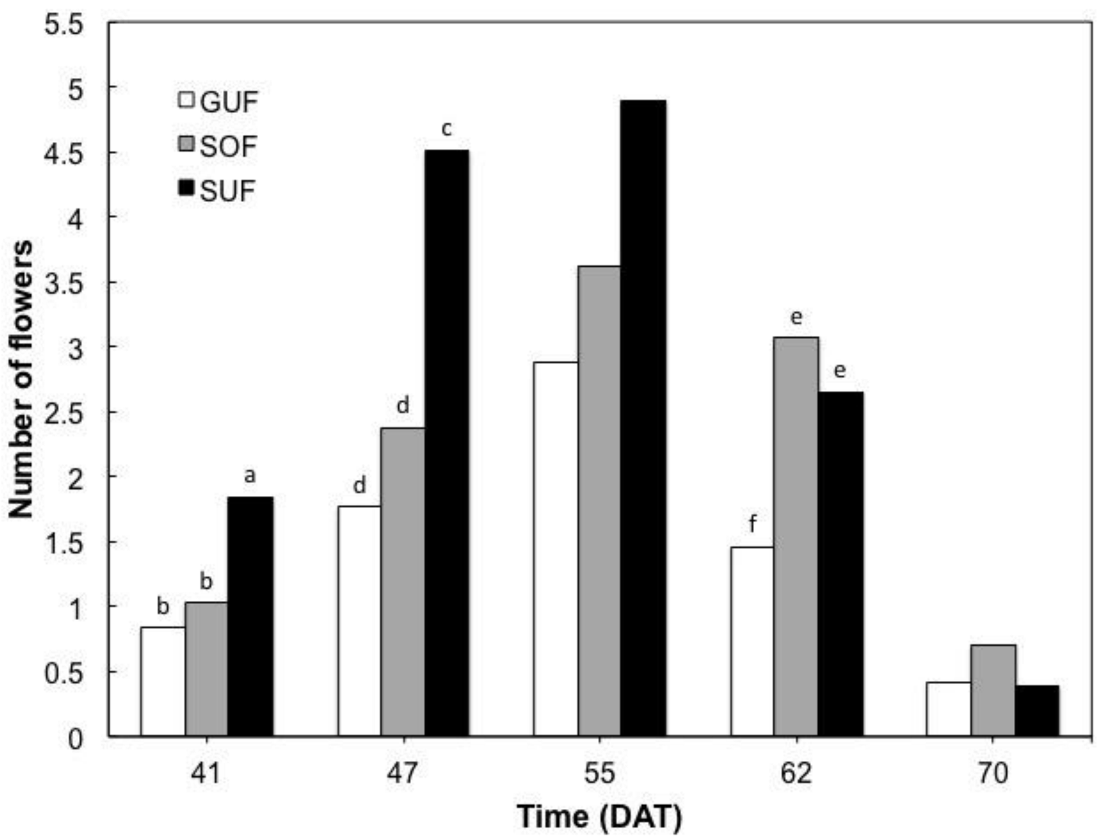


Figure 70. Individual treatment effects of fertilizing methods on the number of flowers at 41, 47, and 62 days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05).

3.4.6 Mean number of fruitlets

Treatments did not have interaction effects on the mean number of fruitlets at any time during the crop cycle. None of the treatments had any significant statistical differences on the mean number of fruitlets at 55 DAT. Individual treatment effects were seen on the fertilizing methods at 41, 47, 62, and 70 DAT (Appendix 34).

3.4.6.1 Effect of fertilizing methods on the mean number of fruitlets

Fertilizing method treatments had a significant effect on the mean number of fruitlets at 41, 47, 62, and 70 DAT. The GUF and SOF methods produced 37 and 55% more fruitlets than the SOF method at 41 DAT, respectively. The SUF method produced 46 and 79% more fruitlets than the GUF and SOF methods at 47 DAT, respectively. The SUF and SOF methods produced 66 and 51% more fruitlets than the GUF method at 62 DAT, respectively. At 70 DAT, the SUF method produced 95 and 27% more fruitlets than the GUF and SOF methods, respectively (Figure 71).

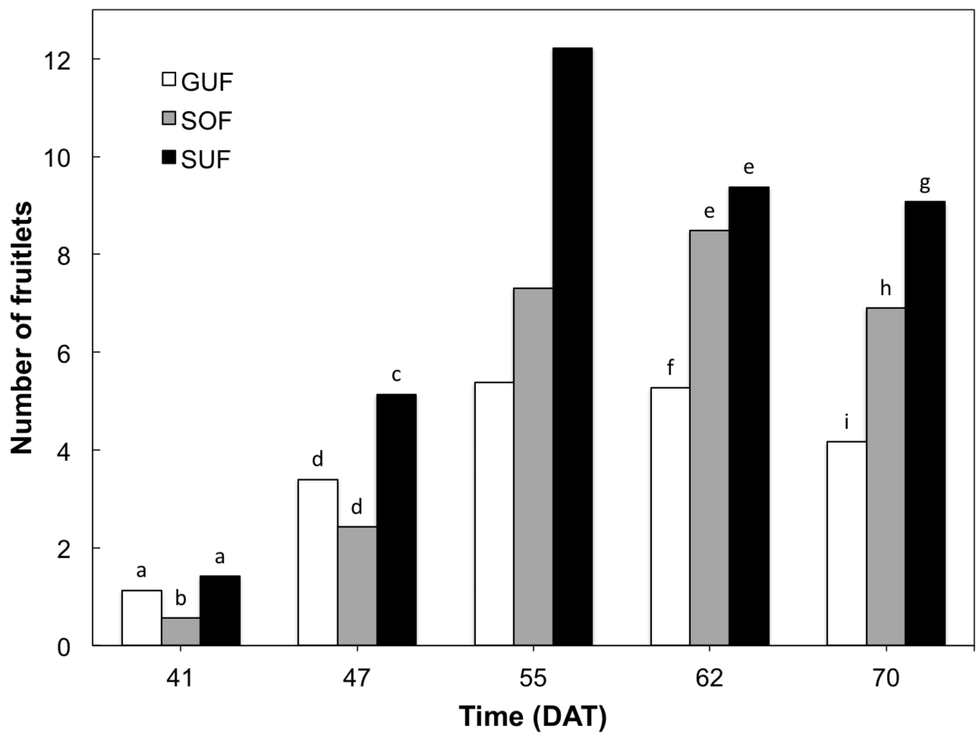


Figure 71. Individual treatment effects of fertilizing methods on the number of fruitlets at 41, 47, 62, and 70 days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05).

3.4.7 Mean abscission of buds

Treatments did not have interaction effects on the mean abscission of buds at any time during the crop cycle. None of the treatments had any significant statistical differences on the mean abscission of buds at 47 nor 55 DAT. Individual treatment effects were seen on the fertilizing methods at 62 and 70 DAT. Individual treatment effects were seen on biostimulants at 62 DAT (Appendix 35).

3.4.7.1 Effect of fertilizing methods on the mean abscission of buds

Fertilizing method treatments had a significant effect on the mean abscission of buds at 62 and 70 DAT. At 62 DAT, the SOF and GUF methods produced 40 and 43% less abscission of buds than the SUF method, respectively. At 70 DAT, the GUF and SUF methods produced 49 and 35% less abscission of buds than the SOF method, respectively (Figure 72).

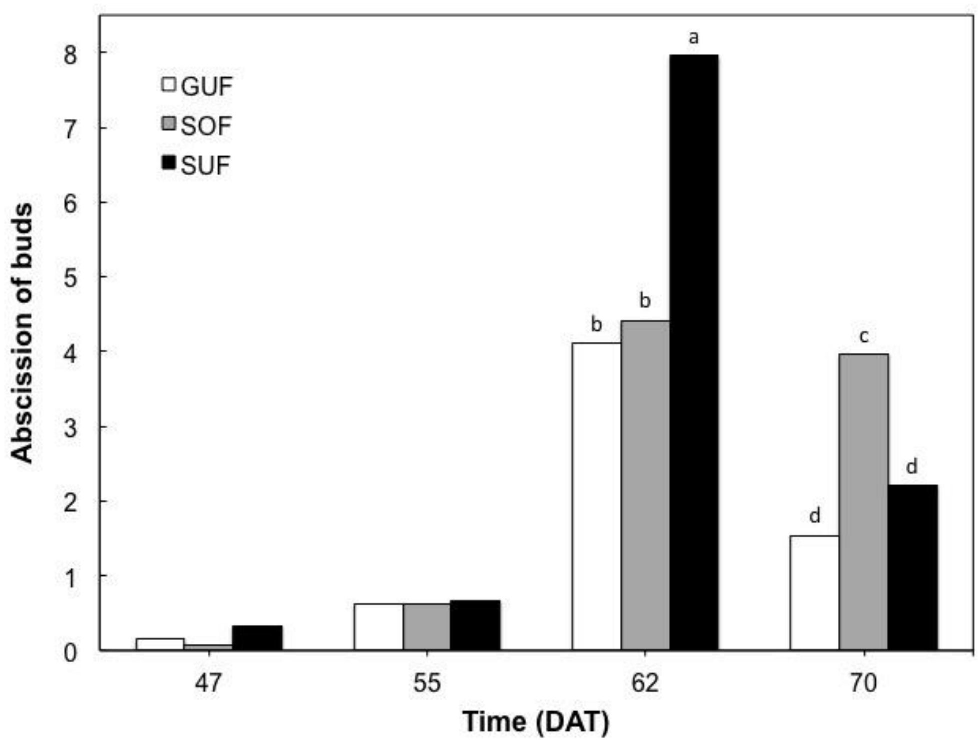


Figure 72. Individual treatment effects of fertilizing methods on the abscission of buds at 62 and 70 days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different ($\alpha = 0.05$).

3.4.7.2 Effect of biostimulants on the mean abscission of buds

Biostimulant treatments had a significant effect on the mean abscission of buds at 62 DAT. The AQC biostimulant produced 44 and 35% less abscission of buds than VTZ and SPX, respectively. None of the biostimulant treatments were significantly different than the WTR (Figure 73).

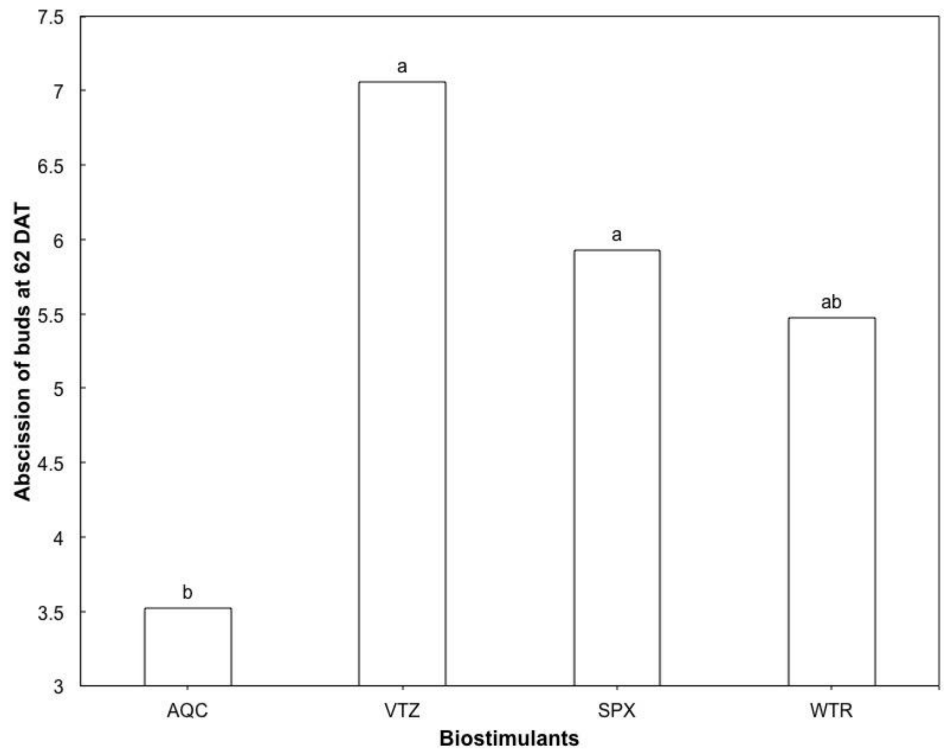


Figure 73. Individual treatment effects of biostimulants on the abscission of buds at 62 days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.8 Mean abscission of flowers

Treatments did not have interaction effects on the mean abscission of flowers at any time during the crop cycle. None of the treatments had any significant statistical differences on the mean abscission of flowers at 55 nor 70 DAT. Individual treatment effects were seen on the fertilizing methods at 62 DAT (Appendix 36).

3.4.8.1 Effect of fertilizing methods on the mean abscission of flowers

Fertilizing method treatments had a significant effect on the mean abscission of flowers at 62 DAT. The GUF method produced 25% less abscission of flowers than the SUF method. The SOF method was not significantly different from either the GUF nor the SUF methods (Figure 74).

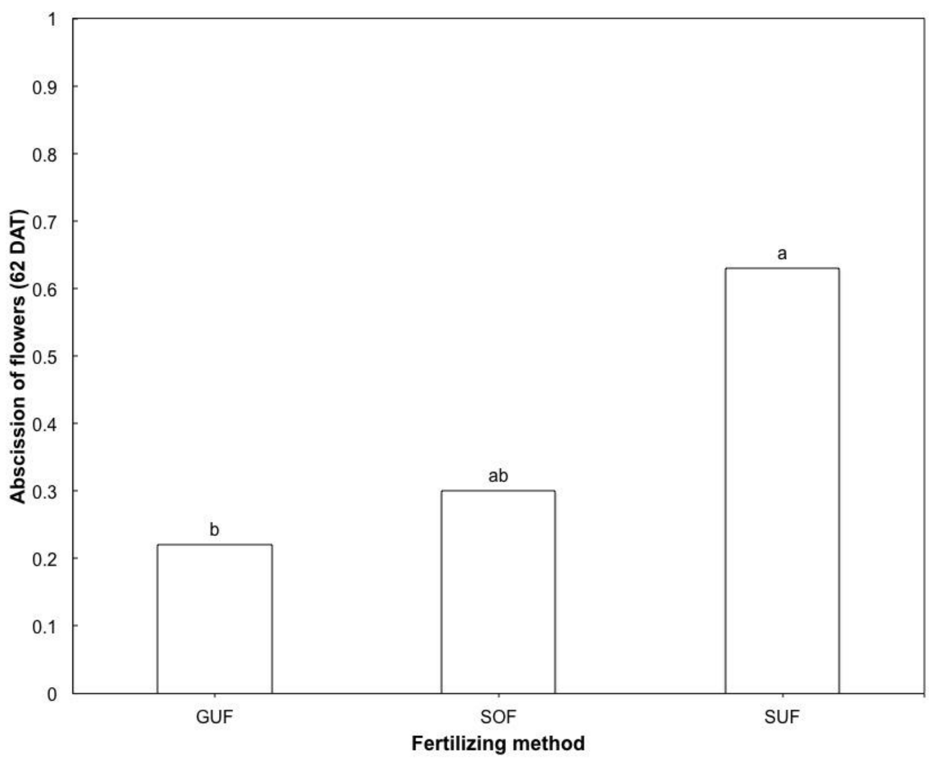


Figure 74. Individual treatment effects of fertilizing methods on the abscission of flowers at 62 days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.9 Mean abscission of fruitlets

Treatments did not have interaction effects on the mean abscission of fruitlets at any time during the crop cycle. None of the treatments had any significant statistical differences on the mean abscission of fruitlets at 55 DAT. Individual treatment effects were seen on the fertilizing methods at 41, 47, 62, and 70 DAT (Appendix 37).

3.4.9.1 Effect of fertilizing methods on the mean abscission of fruitlets

Fertilizing method treatments had a significant effect on the mean abscission of fruitlets at 62 and 70 DAT. The GUF method produced 38 and 51% less abscission of fruitlets than the SOF and SUF methods at 62 DAT, respectively. The GUF and SUF methods produced 29 and 9% less abscission of fruitlets than the SOF method at 70 DAT, respectively (Figure 75).

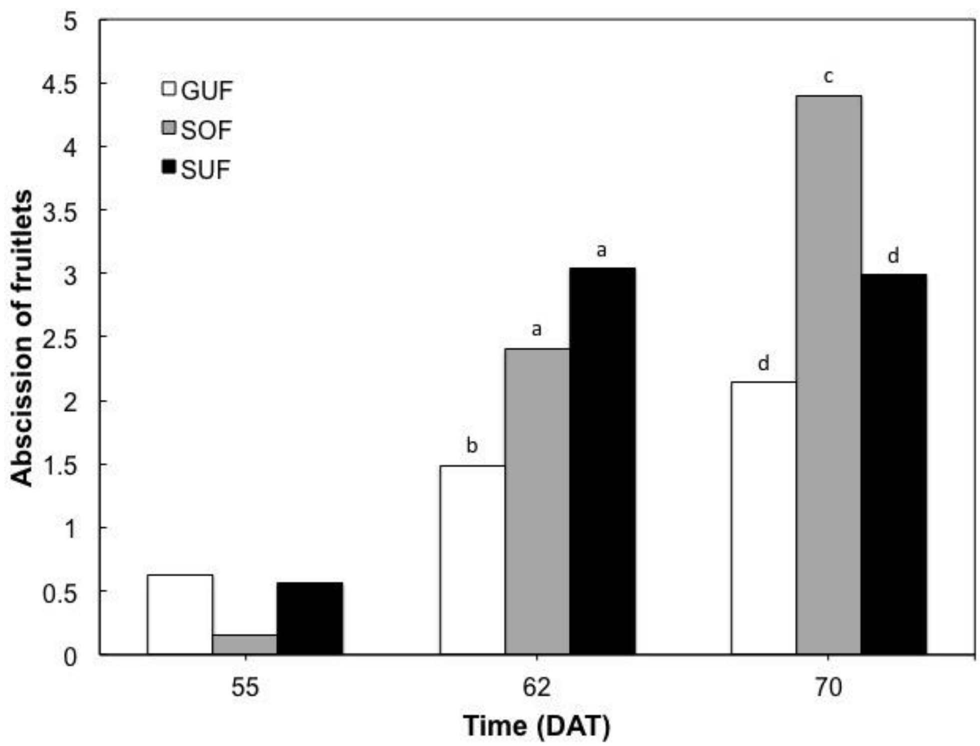


Figure 75. Individual treatment effects of fertilizing methods on the abscission of fruitlets at 62 and 70 days after transplant (DAT). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.10 Mean fresh weight of shoots (g)

Treatments did not have interaction effects on the mean fresh weight of shoots. Individual treatment effects were seen with the fertilizing methods (Appendix 38).

3.4.10.1 Effect of fertilizing methods on the mean fresh weight of shoots (g)

Fertilizing method treatments had a significant effect on the mean fresh weight of shoots. The SUF method produced an average of 20 and 44% more fresh weight of shoots than the SOF and GUF methods, respectively (Figure 76).

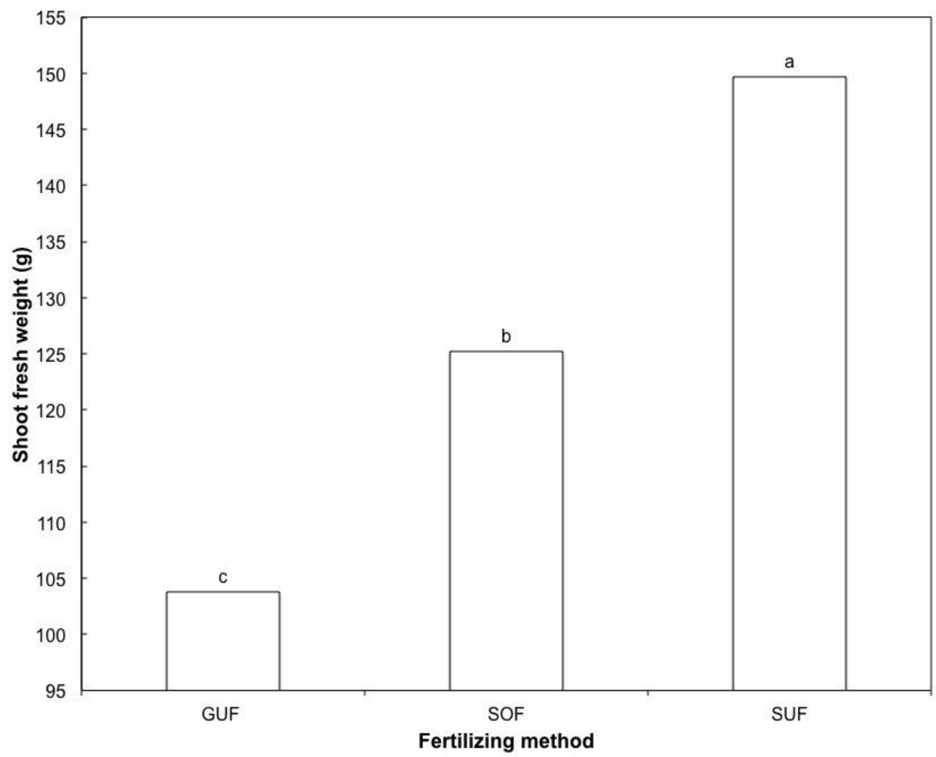


Figure 76. Effect of fertilizing methods on the fresh weight of shoots (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.11 Mean fresh weight of roots (g)

Treatments did not have interaction effects on the mean fresh weight of roots. Individual treatment effects were seen only with the fertilizing methods (Appendix 38).

3.4.11.1 Effect of fertilizing methods on the mean fresh weight of roots (g)

Fertilizing method treatments had a significant effect on the mean fresh weight of roots. The SUF method produced an average of 34 and 30% more fresh weight of roots than the SOF and GUF methods, respectively (Figure 77).

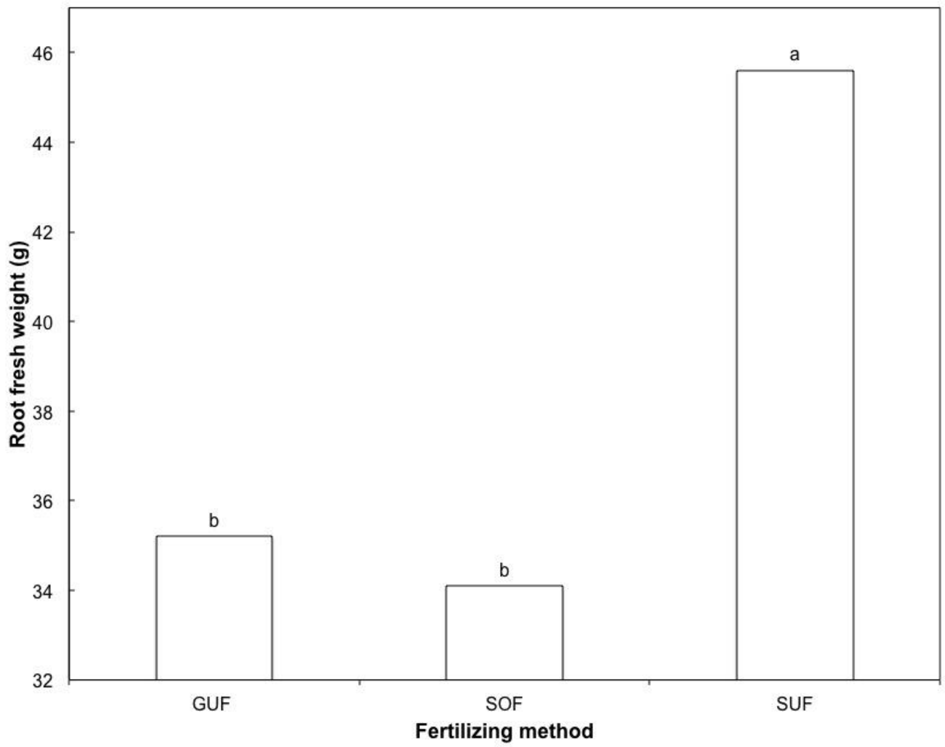


Figure 77. Effect of fertilizing methods on the fresh weight of roots (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.12 Mean dry weight of shoots (g)

Treatments did not have interaction effects on the mean dry weight of shoots. Individual treatment effects were seen with the fertilizing methods (Appendix 38).

3.4.12.1 Effect of fertilizing methods on the mean dry weight of shoots (g)

Fertilizing method treatments had a significant effect on the mean dry weight of shoots. The SUF method produced an average of 21 and 49% more dry weight of shoots than the SOF and GUF methods, respectively (Figure 78).

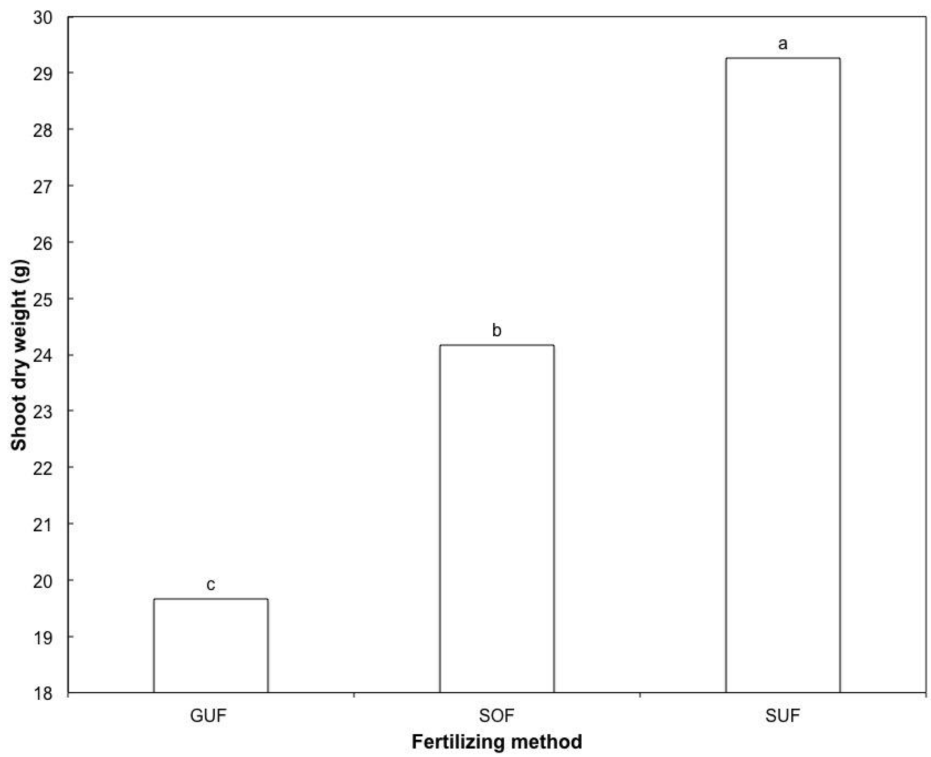


Figure 78. Effect of fertilizing methods on the dry weight of shoots (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.13 Mean dry weight of roots (g)

Treatments did not have interaction effects on the mean dry weight of roots. Individual treatment effects were seen with the fertilizing methods (Appendix 38).

3.4.13.1 Effect of fertilizing methods on the mean dry weight of roots (g)

Fertilizing method treatments had a significant effect on the mean dry weight of roots. The SUF method produced an average of 36 and 42% more dry weight of roots than the SOF and GUF methods, respectively (Figure 79).

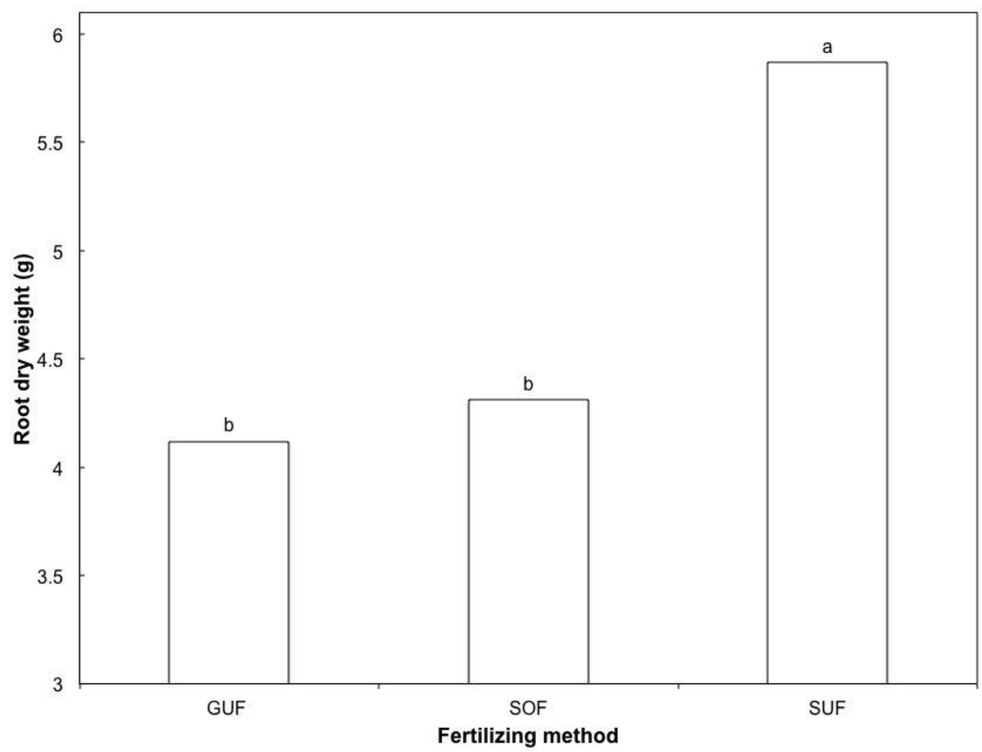


Figure 79. Effect of fertilizing methods on the dry weight of roots (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.14 Mean number of marketable fruits per plant

Treatments did not have interaction effects on the mean number of marketable fruits per plant. Individual treatment effects were seen with the fertilizing methods (Appendix 39).

3.4.14.1 Effect of fertilizing methods on the mean number of marketable fruits per plant

Fertilizing method treatments had a significant effect on the mean number of marketable fruits per plant. The SUF method produced an average of 28 and 62% more marketable fruits per plant than the SOF and GUF methods, respectively (Figure 80).

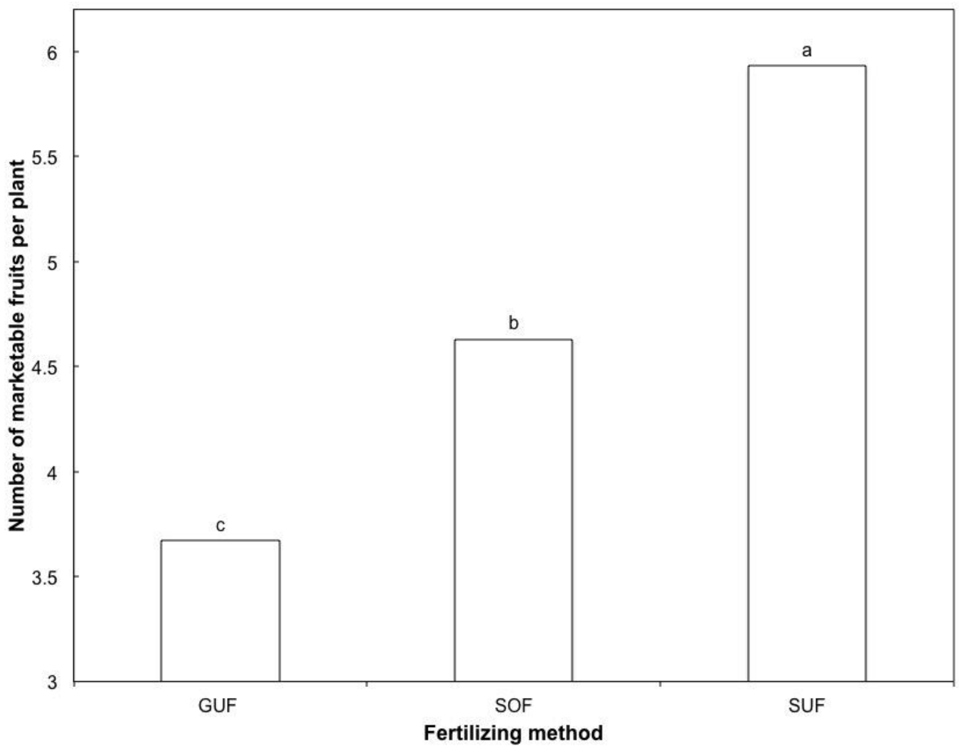


Figure 80. Effect of fertilizing methods on the number of marketable fruits per plant. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.15 Mean number of unmarketable fruits per plant

None of the treatments had any significant statistical differences on the mean number of unmarketable fruits per plant (Appendix 39).

3.4.16 Mean number of total fruits per plant

Treatments did not have interaction effects on the mean number of total fruits per plant. Individual treatment effects were seen with the fertilizing methods (Appendix 39).

3.4.16.1 Effect of fertilizing methods on the mean number of total fruits per plant

Fertilizing method treatments had a significant effect on the mean number of total fruits per plant. The SUF and SOF methods produced an average of 48 and 24% more total fruits per plant than the GUF method, respectively (Figure 81).

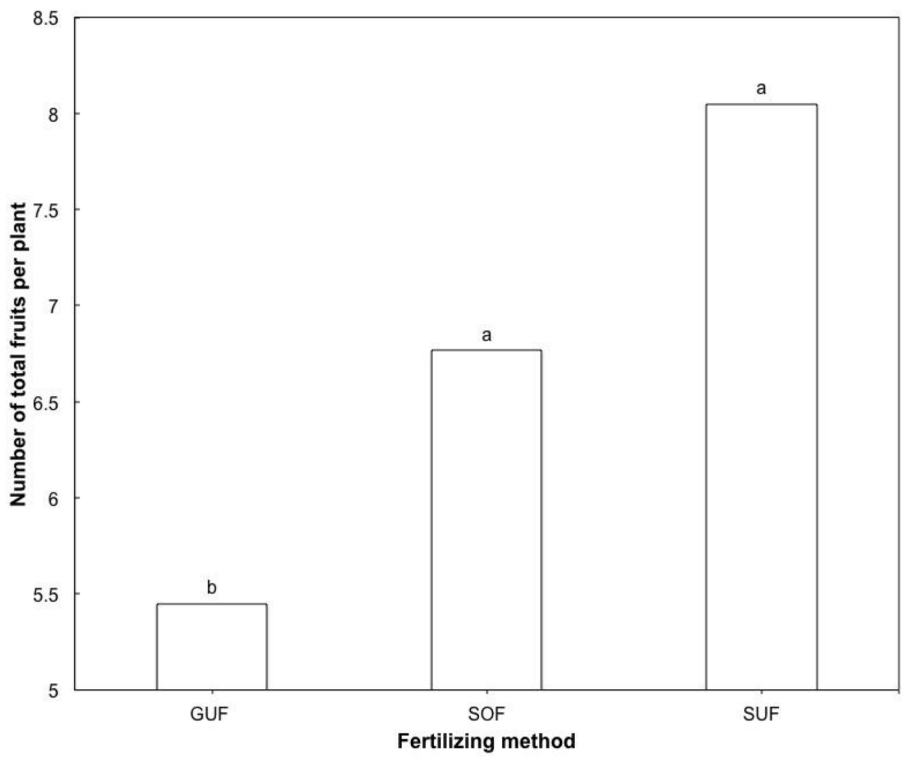


Figure 81. Effect of fertilizing methods on the number of total fruits per plant. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.17 Mean marketable fruit weight per plant (g)

Treatments did not have interaction effects on the mean marketable fruit weight per plant. Individual treatment effects were seen on the fertilizing methods and also on the biostimulants (Appendix 39).

3.4.17.1 Effect of fertilizing methods on the mean marketable fruit weight per plant

Fertilizing method treatments had a significant effect on the mean marketable fruit weight per plant. The SUF and SOF methods produced an average of 62 and 29% more marketable fruit weight per plant than the GUF method, respectively (Figure 82).

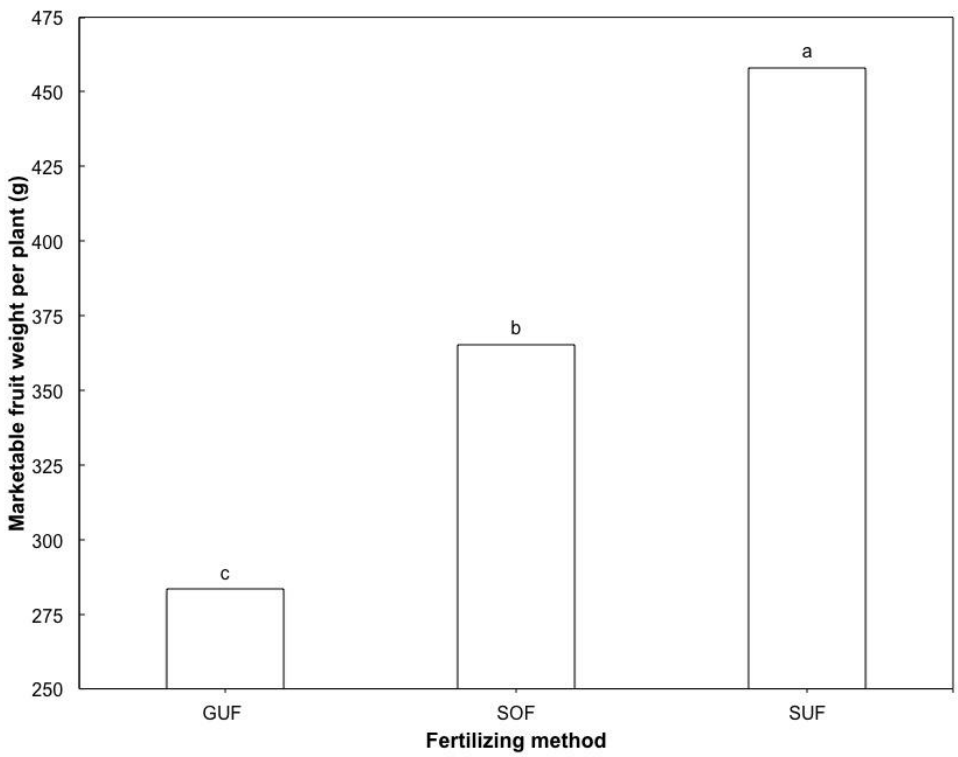


Figure 82. Effect of fertilizing methods on the marketable fruit weight per plant (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.17.2 Effect of biostimulants on the mean marketable fruit weight per plant

Biostimulant treatments had a significant effect on the mean marketable fruit weight per plant. None of the biostimulants outperformed the WTR treatment. Plants treated with

AQC, SPX, and VTZ produced 23, 35, and 11% less marketable fruit weight per plant than the WTR method, respectively (Figure 83).

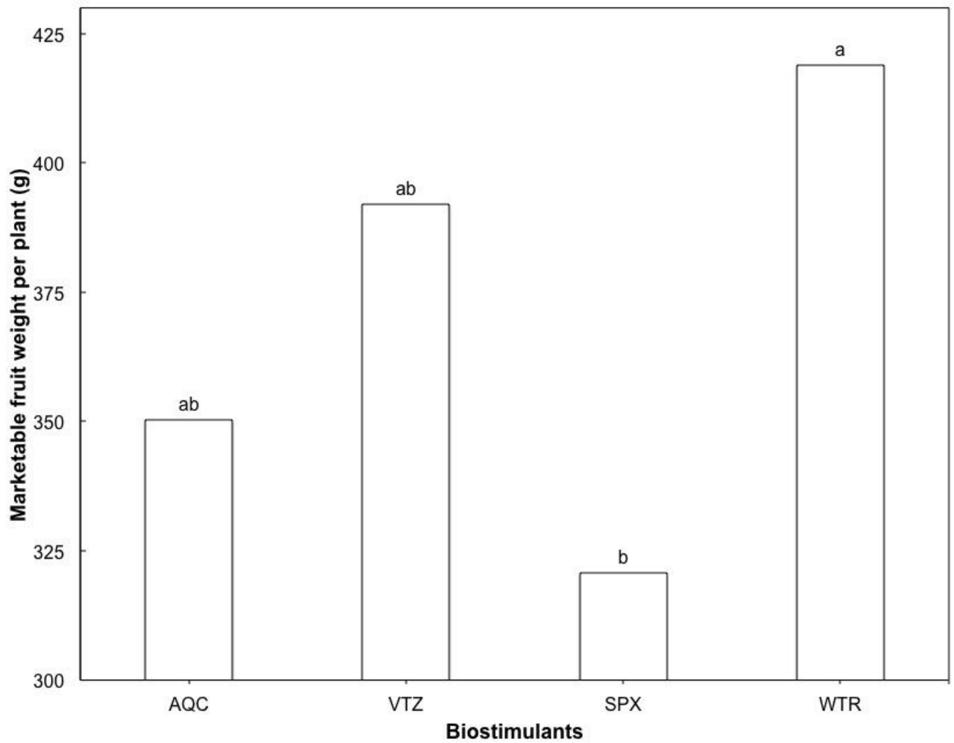


Figure 83. Effect of biostimulant treatments on the marketable fruit weight per plant (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.18 Mean unmarketable fruit weight per plant (g)

Treatments did not have interaction effects on the mean unmarketable fruit weight per plant. Individual treatment effects were seen with the fertilizing methods (Appendix 39).

3.4.18.1 Effect of fertilizing methods on the mean unmarketable fruit weight per plant

Fertilizing method treatments had a significant effect on the mean unmarketable fruit weight per plant. The SOF and GUF methods produced an average of 43 and 34% less unmarketable fruit weight per plant than the SUF method, respectively (Figure 84).

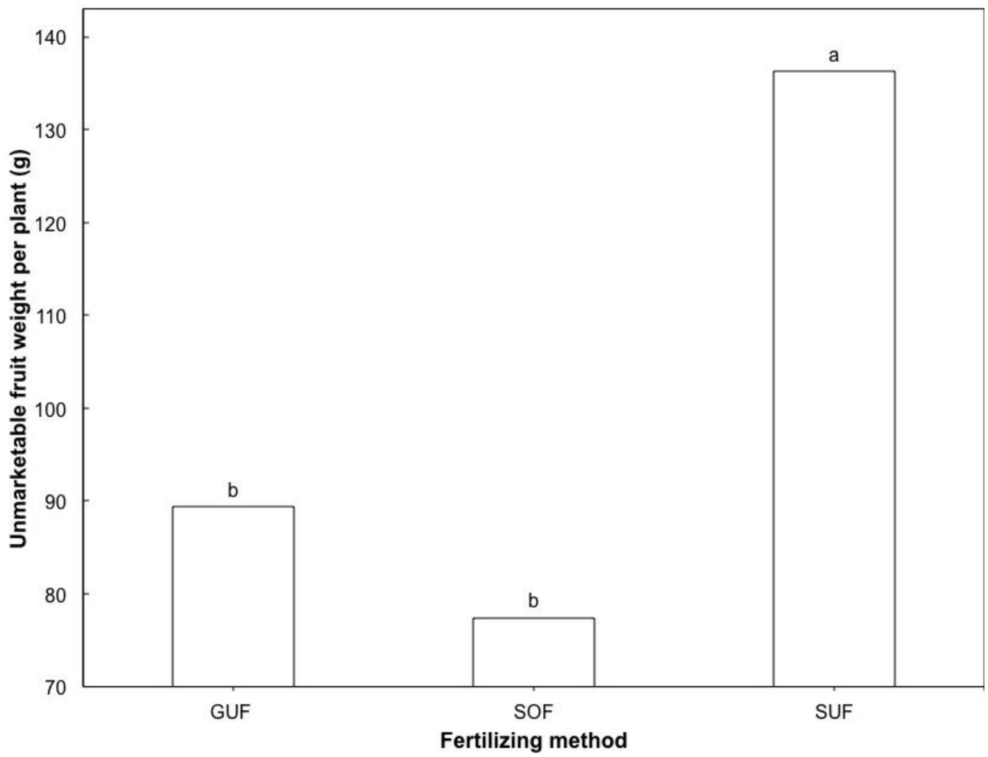


Figure 84. Effect of fertilizing methods on the unmarketable fruit weight per plant (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different ($\alpha = 0.05$)

3.4.19 Mean weight of total fruits per plant (g)

Treatments did not have interaction effects on the mean total fruit weight per plant. Individual treatment effects were seen on the fertilizing methods and also on the biostimulants (Appendix 39).

3.4.19.1 Effect of fertilizing methods on the mean total fruit weight per plant

Fertilizing method treatments had a significant effect on the mean total fruit weight per plant. The SUF method produced an average of 26 and 62% more total fruit weight per plant than the SOF and GUF methods, respectively (Figure 85).

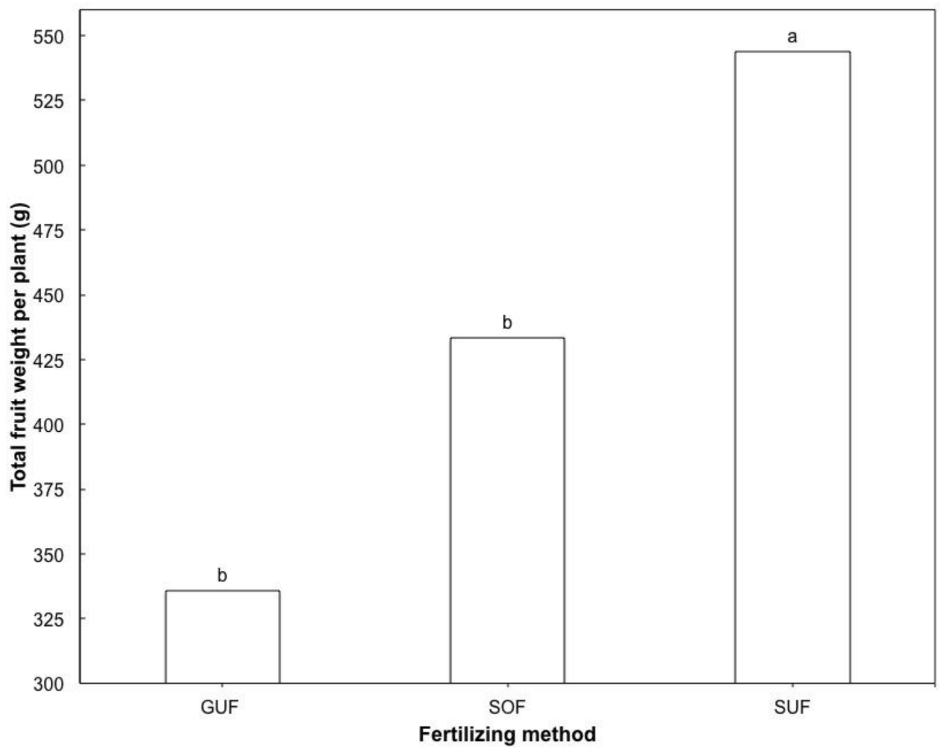


Figure 85. Effect of fertilizing methods on the total fruit weight per plant (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.19.2 Effect of biostimulants on the mean total fruit weight per plant

Biostimulant treatments had a significant effect on the mean total fruit weight per plant. Plants treated with VTZ produced 17 and 18% more total fruit weight per plant than the AQC and SPX biostimulants, respectively. Plants treated with WTR produced 15 and 17%

more total fruit weight per plant than the AQC and SPX biostimulants, respectively (Figure 86).

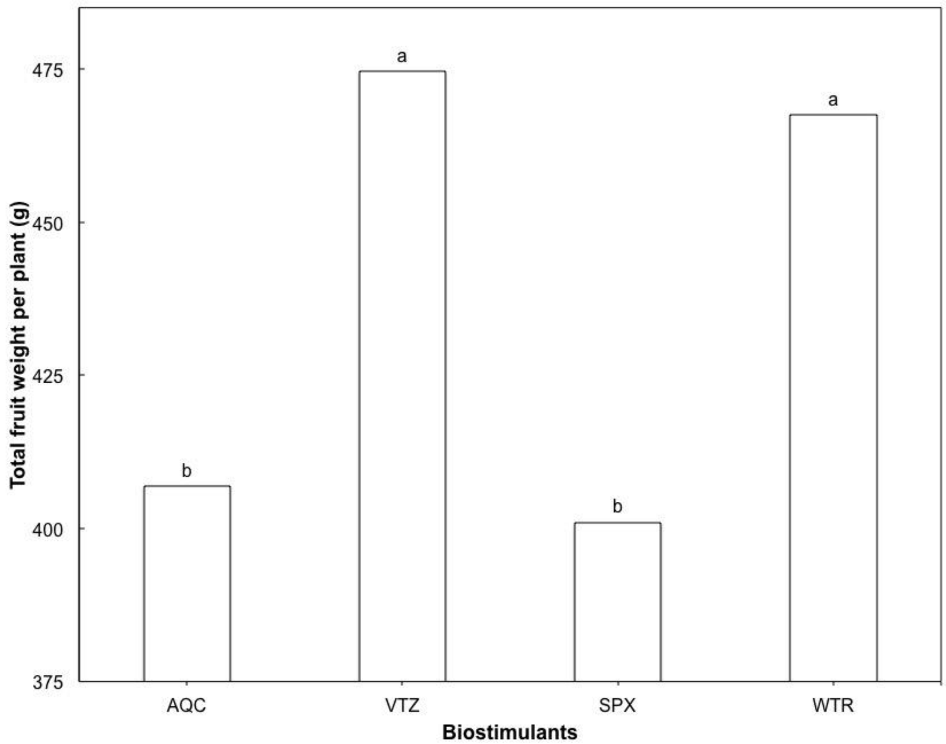


Figure 86. Effect of biostimulant treatments on the total fruit weight per plant (g). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.20 Mean length of marketable fruits (cm)

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean length of marketable fruits (Appendix 40).

3.4.20.1 Interaction effect on the mean length of marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean length of marketable fruits. None of the treatment outperformed the SOF + WTR combination. The SOF + WTR and the GUF + WTR treatment combinations produced marketable fruits that were 14 and 11% longer than the SUF + WTR combination, respectively. The SUF + AQC combination produced marketable fruits that were 7% longer than the SUF + WTR combination. Also, the GUF + SPX combination produced marketable fruits that were 11% longer than the SUF + WTR combination (Figure 87).

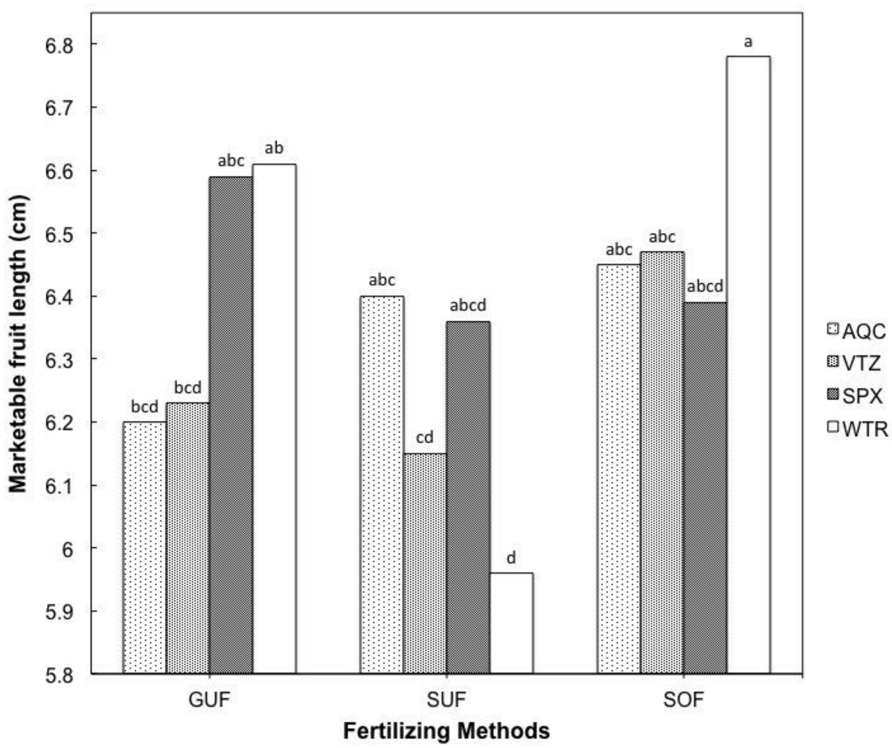


Figure 87. Combined effects of fertilizing methods and biostimulants on the mean length of marketable fruits (cm). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.21 Mean length of unmarketable fruits (cm)

Treatments did not have interaction effects on the mean length of unmarketable fruits. Individual treatment effects were seen with the biostimulants (Appendix 40).

3.4.21.1 Effect of biostimulants on the mean length of unmarketable fruits

Biostimulant treatments had significant effects on the mean length of unmarketable fruits. The SPX biostimulant produced unmarketable fruits that were 11 and 10% longer than the AQC and WTR, respectively. VTZ was not significantly different from any other biostimulant treatment (Figure 88).

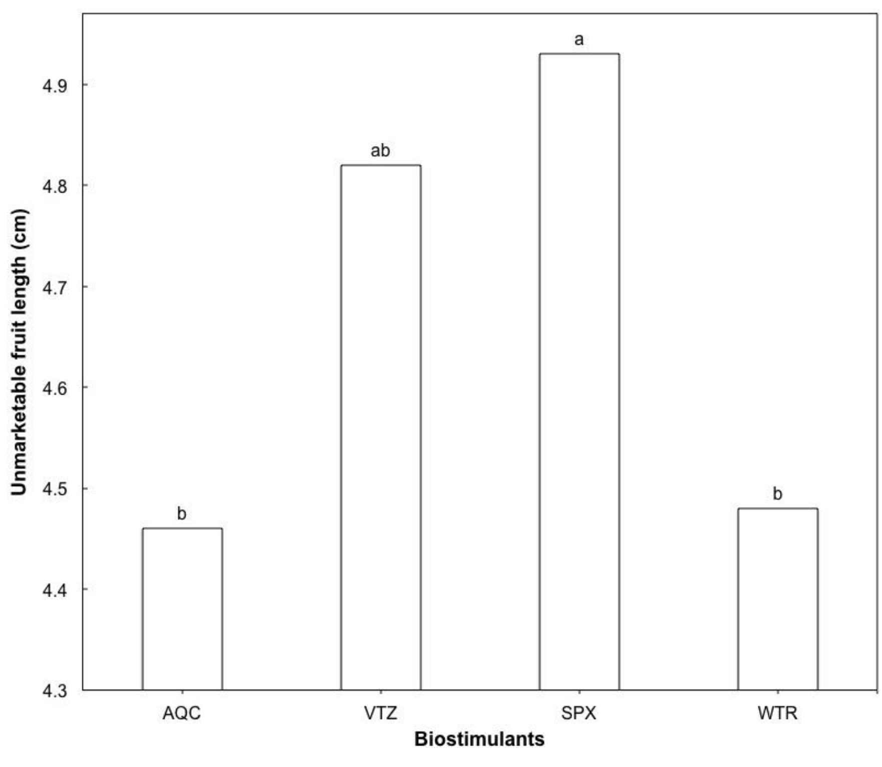


Figure 88. Effect of biostimulant treatments on the length of unmarketable fruits. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.22 Mean length of total fruits (cm)

None of the treatments had any significant statistical differences on the mean length of total fruits (Appendix 40).

3.4.23 Mean width of marketable fruits (cm)

Treatments did not have interaction effects on the mean width of marketable fruits. Individual treatment effects were seen with the biostimulant treatments (Appendix 40).

3.4.23.1 Effect of biostimulants on the mean width of marketable fruits

Biostimulant treatments had a significant effect on the mean width of marketable fruits. None of the biostimulants outperformed the WTR treatment. The WTR produced marketable fruits that were 3% wider than the marketable fruits treated with the SPX biostimulant (Figure 89).

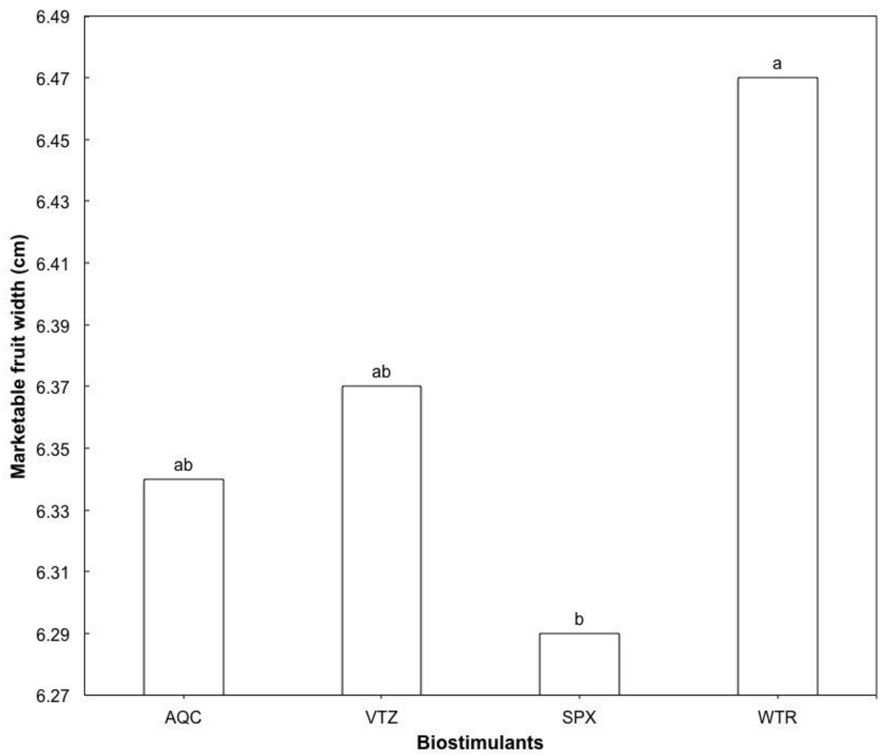


Figure 89. Effect of biostimulant treatments on the width of marketable fruits. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.24 Mean width of unmarketable fruits (cm)

None of the treatments had any significant statistical differences on the mean width of unmarketable fruits (Appendix 40).

3.4.25 Mean width of total fruits (cm)

Treatments did not have interaction effects on the mean width of total fruits. Individual treatment effects were seen with the biostimulant treatments (Appendix 40).

3.4.25.1 Effect of biostimulants on the mean width of total fruits

Biostimulant treatments had a significant effect on the mean width of marketable fruits. None of the biostimulants outperformed the WTR treatment. The WTR produced total fruits that were 6 and 4% wider than the total fruits treated with the AQC and SPX biostimulants, respectively. The VTZ biostimulant produced total fruits that were 4% wider than the total fruits treated with the AQC biostimulant (Figure 90).

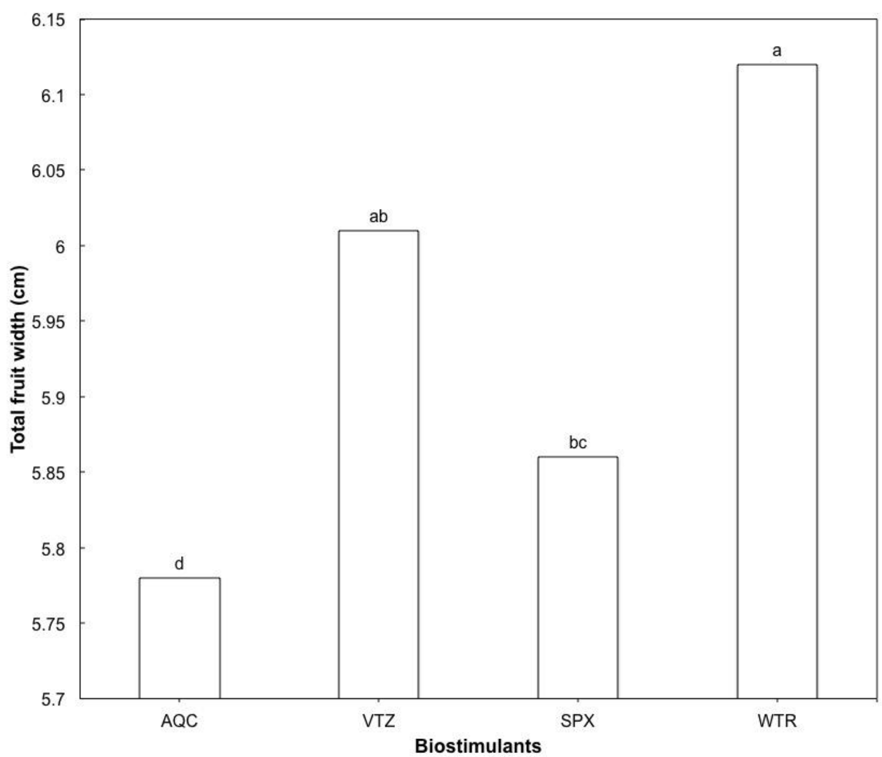


Figure 90. Effect of biostimulant treatments on the width of total fruits. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.26 Mean pericarp thickness of marketable fruits (mm)

Significant interaction effects were seen on the mean pericarp thickness of marketable fruits (Appendix 41).

3.4.26.1 Interaction effect on the mean pericarp thickness of marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean pericarp thickness of marketable fruits. The SOF + VTZ and SUF + AQC treatment combinations produced the thickest pericarps of marketable fruits. The SOF + VTZ produced marketable fruit pericarps that were 9 and 12% thicker than the combinations of SUF + VTZ and GUF + VTZ, respectively. The SUF + AQC produced marketable fruit pericarps that were 8% thicker than both combinations of SUF + VTZ and SUF + WTR. The SUF + AQC combination also produced marketable fruit pericarps that were 10% thicker than the marketable fruit pericarps of GUF + AQC (Figure 91).

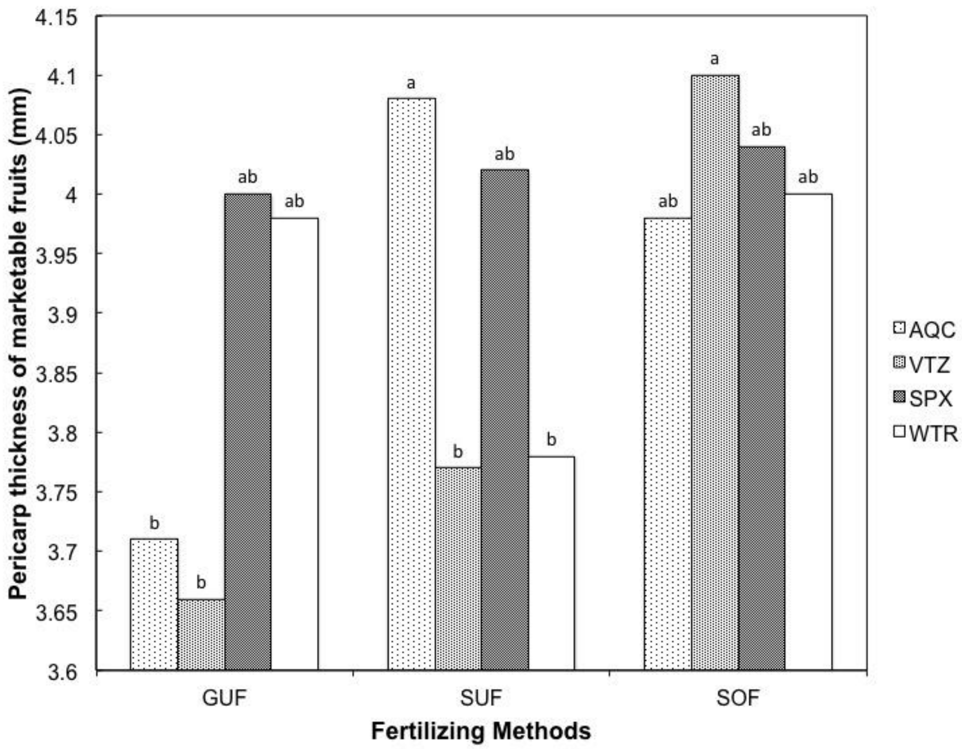


Figure 91. Interaction effects of fertilizing methods and biostimulants on the mean pericarp thickness of marketable fruits (mm). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different ($\alpha = 0.05$)

3.4.27 Mean pericarp thickness of unmarketable fruits (mm)

None of the treatments had any significant statistical differences on the mean pericarp thickness of unmarketable fruits (Appendix 41).

3.4.28 Mean pericarp thickness of total fruits (mm)

Significant interaction effects were seen on the mean pericarp thickness of total fruits (Appendix 41).

3.4.28.1 Interaction effect on the mean pericarp thickness of total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean pericarp thickness of total fruits. The SOF + VTZ combination produced the thickest pericarps of total fruits. The SOF + VTZ produced total fruit pericarps that were 7 and 10% thicker than the combinations of SUF + VTZ and GUF + VTZ, respectively. The SOF + VTZ combination also produced total fruit pericarps that were 7% thicker than the SOF + AQC combination (Figure 92).

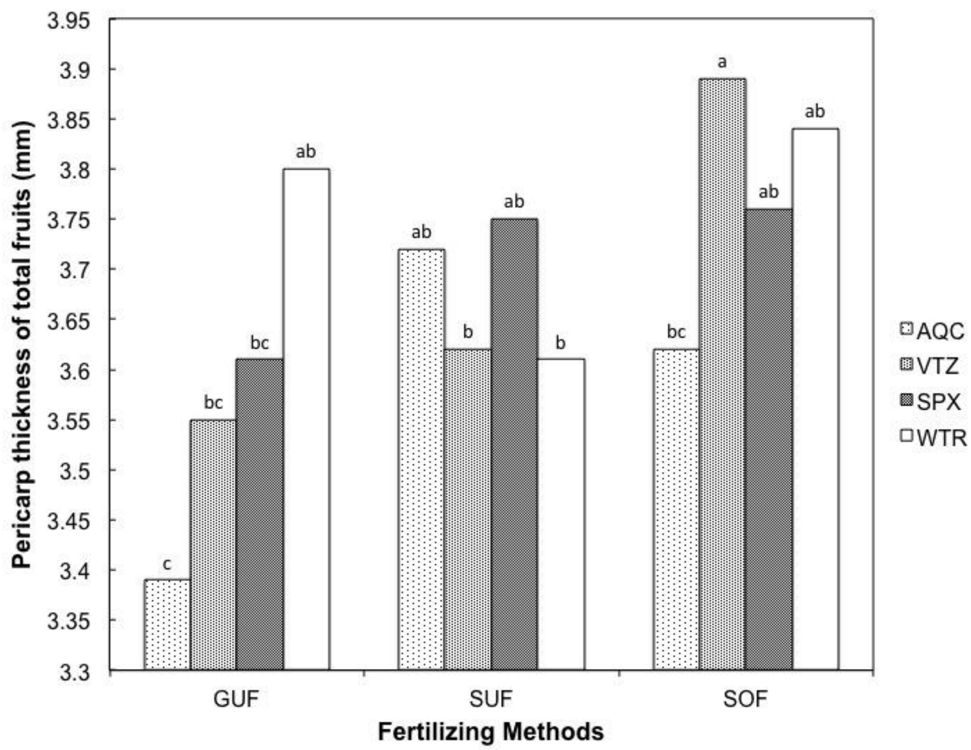


Figure 92. Interaction effects of fertilizing methods and biostimulants on the mean pericarp thickness of total fruits (mm). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.29 Total soluble solids content in marketable fruits

Significant interaction effects were seen on the mean Brix readings of marketable fruits (Appendix 42).

3.4.29.1 Interaction effect on the mean content of the total soluble solids in marketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean Brix readings of marketable fruits. The GUF + VTZ combination produced marketable fruits with 12 and 37% higher brix readings than the SUF + VTZ and SOF + VTZ combinations, respectively. The GUF + VTZ combination also produced marketable fruits with 17 and 18% higher brix readings than the GUF + AQC and GUF + SPX combinations, respectively. The GUF + WTR combination produced marketable fruits with 12 and 16% higher brix readings than the SUF + WTR and SOF + WTR combinations, respectively (Figure 93).

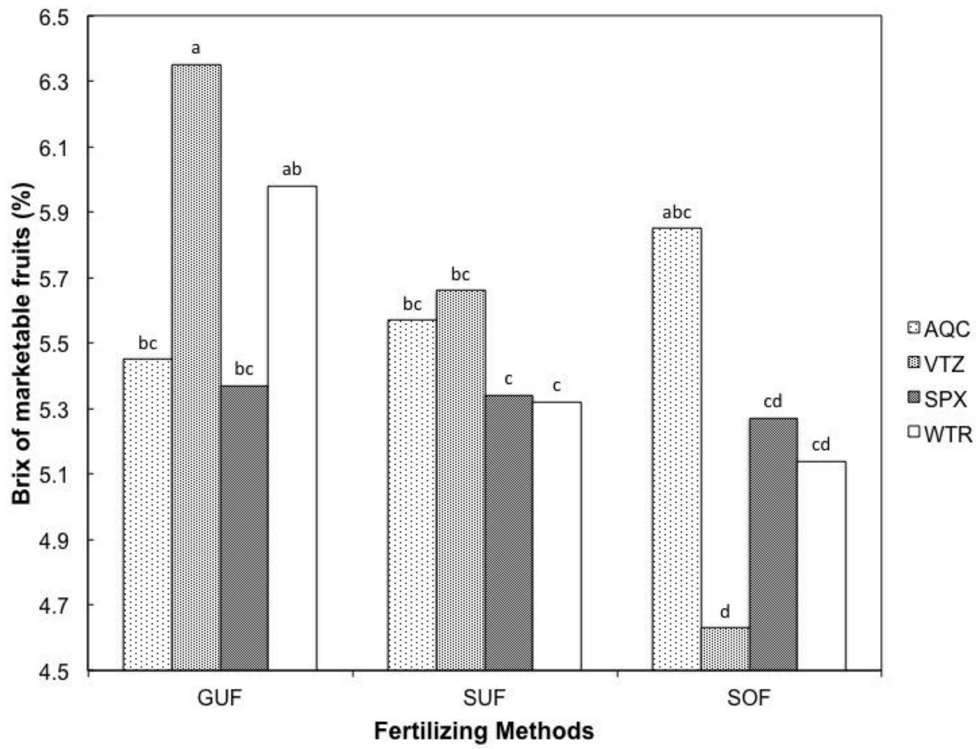


Figure 93. Interaction effects of fertilizing methods and biostimulants on the mean Brix readings of marketable fruits. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.30 Total soluble solids content in unmarketable fruits

Significant interaction effects were seen on the mean Brix readings of unmarketable fruits (Appendix 42).

3.4.30.1 Interaction effect on the mean content of the total soluble solids in unmarketable fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean Brix readings of unmarketable fruits. The GUF + AQC and SOF + AQC combinations produced unmarketable fruits with 15 and 20% higher brix readings than the SUF + AQC, respectively. The GUF + SPX and SOF + SPX combinations both produced unmarketable fruits with 32% higher brix readings than the SUF + SPX. The GUF + AQC and GUF + VTZ combinations produced unmarketable fruits with 20 and 26% higher brix readings than the GUF + WTR, respectively. The SUF + AQC, SUF + VTZ, and SUF + WTR combinations produced unmarketable fruits with 22, 40 and 38% higher brix readings than the SUF + SPX, respectively (Figure 94).

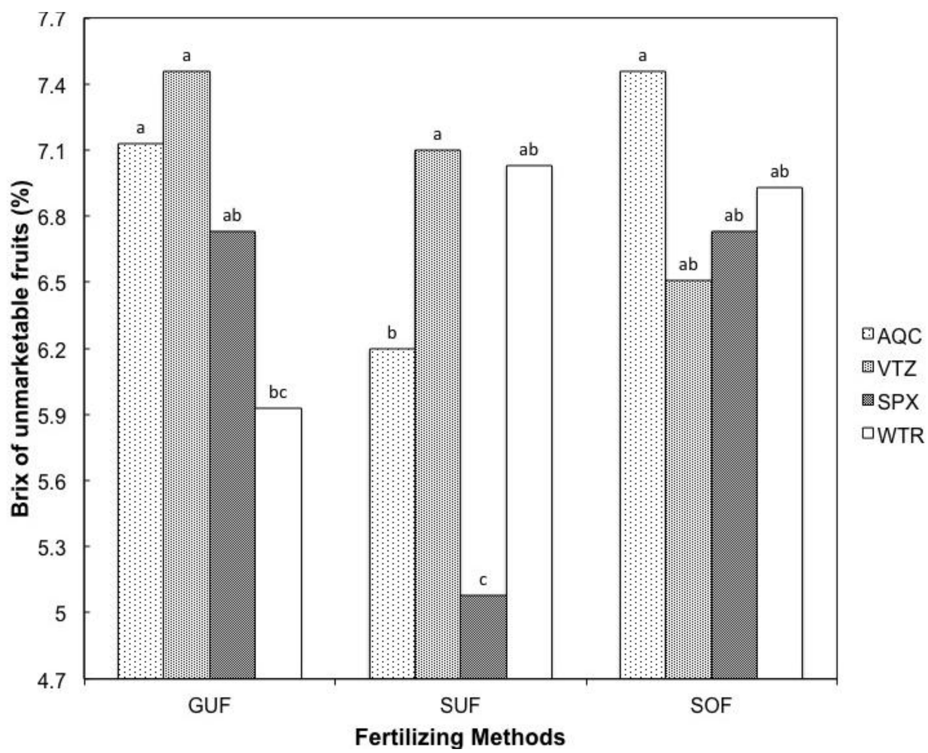


Figure 94. Interaction effects of fertilizing methods and biostimulants on the mean Brix readings of unmarketable fruits. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.31 Total soluble solids content in total fruits

Significant interaction effects were seen on the mean Brix readings of total fruits (Appendix 42).

3.4.31.1 Interaction effect on the mean content of the total soluble solids in total fruits

A significant interaction effect was observed between fertilizing methods and biostimulants on the mean Brix readings of total fruits. The GUF + AQC and SOF + AQC combinations produced total fruits with 7 and 11% higher brix readings than the SUF + AQC, respectively. The GUF + VTZ combination produced total fruits with 9 and 28% higher brix readings than the SUF + VTZ and SOF + VTZ combinations, respectively. The GUF + SPX combination produced total fruits with 13% higher brix readings than the SUF + SPX combination. The GUF + VTZ combination produced total fruits with 10% higher brix readings than the GUF + WTR combination. The SOF + AQC combination produced total fruits with 26, 13, and 17% higher brix readings than the SOF + VTZ, SOF + SPX, and SOF + WTR combinations, respectively (Figure 95).

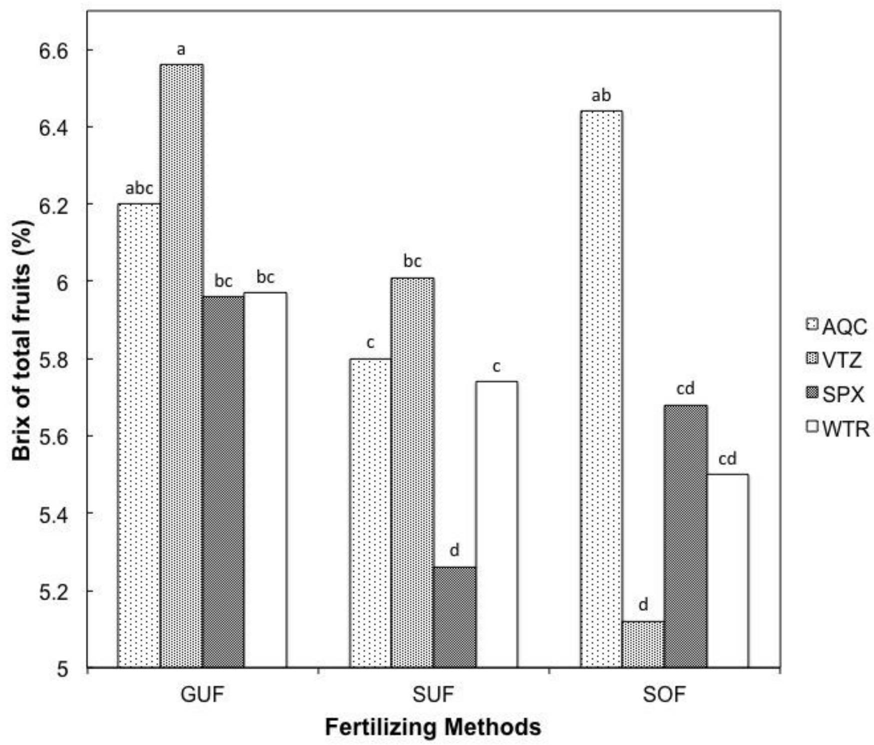


Figure 95. Interaction effects of fertilizing methods and biostimulants on the mean Brix readings of total fruits. Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.32 Harvest time of marketable fruits

Treatments did not have interaction effects on the mean harvest time (DAT) of marketable fruits. Individual treatment effects were seen with the fertilizing methods (Appendix 43).

3.4.32.1 Effect of fertilizing methods on the mean harvest time of marketable fruits

Fertilizing method treatments had a significant effect on the mean harvest time of marketable fruits. The marketable fruits in both, the GUF and SOF methods, took on average 6% more time to harvest than the marketable fruits in the SUF method (Figure 96).

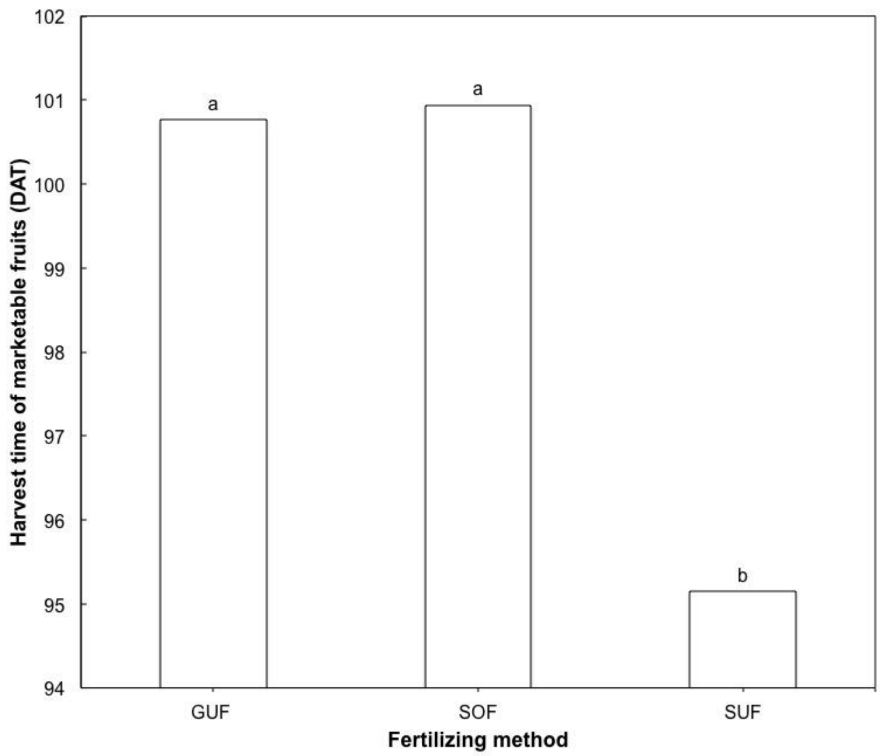


Figure 96. Interaction effects of fertilizing methods and biostimulants on the mean harvest time of marketable fruits (days after transplant). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.33 Harvest time of unmarketable fruits

None of the treatments had any significant statistical differences on the mean harvest time of unmarketable fruits (Appendix 43).

3.4.34 Harvest time of total fruits

Treatments did not have interaction effects on the mean harvest time (DAT) of total fruits. Individual treatment effects were seen with the fertilizing methods (Appendix 43).

3.4.34.1 Effect of fertilizing methods on the mean harvest time of total fruits

Fertilizing method treatments had a significant effect on the mean harvest time of total fruits. The total fruits in the GUF and SOF methods took 6 and 4% more time to harvest than the total fruits in the SUF method, respectively (Figure 97).

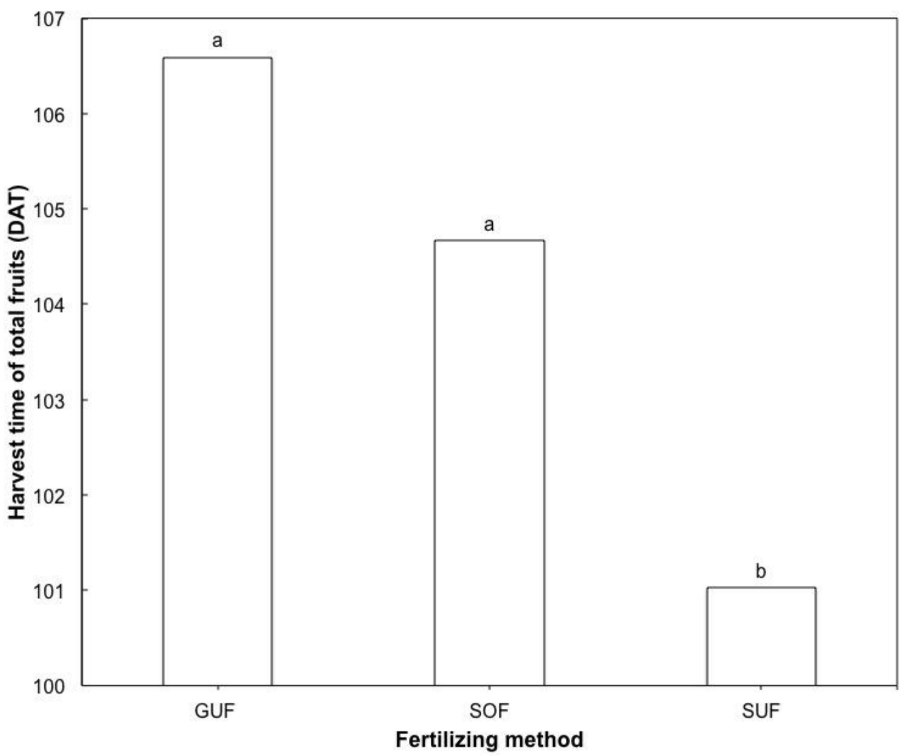


Figure 97. Interaction effects of fertilizing methods and biostimulants on the mean harvest time of total fruits (days after transplant). Mayagüez, Puerto Rico, 2015. Means with the same letter are not significantly different (α = 0.05)

3.4.35 Mean SPAD readings

There were no significant statistical differences in the mean SPAD values at 41 DAT (Appendix 20). Readings averaged at a value of 49.72

3.4.36 Mean NO_3^--N content in fresh sap (ppm)

There were no significant statistical differences in the mean NO_3^--N content in fresh sap during the harvest time (80-107 DAT) (Appendix 21). NO_3^--N content in fresh sap averaged at 690 ppm.

3.4.36 Analysis of reasons that categorize pepper fruits as unmarketable

Fruits were categorized as unmarketable when they had sunburns, scars, or when their width was less than 5.6 cm. In experiment 3, 31% of the fruits produced were categorized as unmarketable (Figure 31). A lack of minimum width was the most common reasons to categorize fruits as unmarketable, occurring in 90% of the unmarketable fruits. Sunburns and scarring were both observed in 5% of the unmarketable fruits (Figure 98). Damage by birds was nonexistent because the walls prevented their entry into the greenhouse. Unmarketable reasons were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the unmarketable fruit damages. Combinations of fertilizing methods and biostimulant treatments were significantly ($p = 0.0019$) related to the observed damages in fruits.

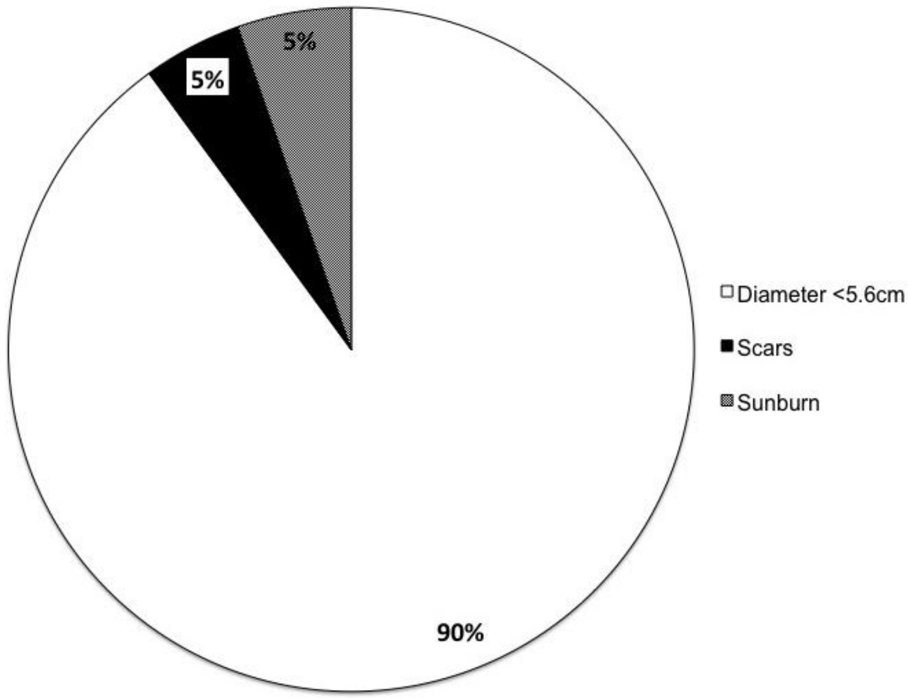


Figure 98. General distribution of different reasons to categorize pepper fruits as unmarketable during experiment 3. Mayagüez, Puerto Rico, 2015.

3.4.36.1 Interaction effect on the distribution of reasons to categorize pepper fruits as unmarketable

Unmarketable reasons were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the reasons that categorized pepper fruits as unmarketable. Combinations of fertilizing methods and biostimulant treatments were significantly ($p = 0.0019$) related to the observed reasons that categorize fruits as unmarketable. The distribution of reasons that categorize fruits as unmarketable was arranged into percentages according to each combination of fertilizing method and biostimulant (Table 4).

Table 4. Distribution of reasons to categorize pepper fruits as unmarketable (fertilizing method*biostimulant) in the third experiment.

Fertilizing method	Biostimulant	Damage distribution (%)			
		Width < 5.6 cm	Scars	Sunburn	Total
GUF	AQC	96	0	4	100
GUF	VTZ	94	6	0	100
GUF	SPX	75	0	25	100
GUF	WTR	94	0	6	100
SUF	AQC	93	7	0	100
SUF	VTZ	90	7	3	100
SUF	SPX	97	0	3	100
SUF	WTR	88	6	6	100
SOF	AQC	87	13	0	100
SOF	VTZ	100	0	0	100
SOF	SPX	85	15	0	100
SOF	WTR	80	0	20	100

The total amounts of fruits affected by the different unmarketable reasons were graphed separately in order to better understand the effects of interactions. The most common unmarketable reason was fruits with a width less than 5.6 cm (Figure 99). Damage by scars was highest in the SOF + AQC and SOF + SPX combinations but was lowest in the combinations with the GUF method (Figure 100). The only treatment combinations that produced unmarketable fruits with sunburns were the GUF + SPX and SOF + WTR. There were no unmarketable fruits affected by sunburns when the SOF was combined with AQC, VTZ, or SPX (Figure 101).

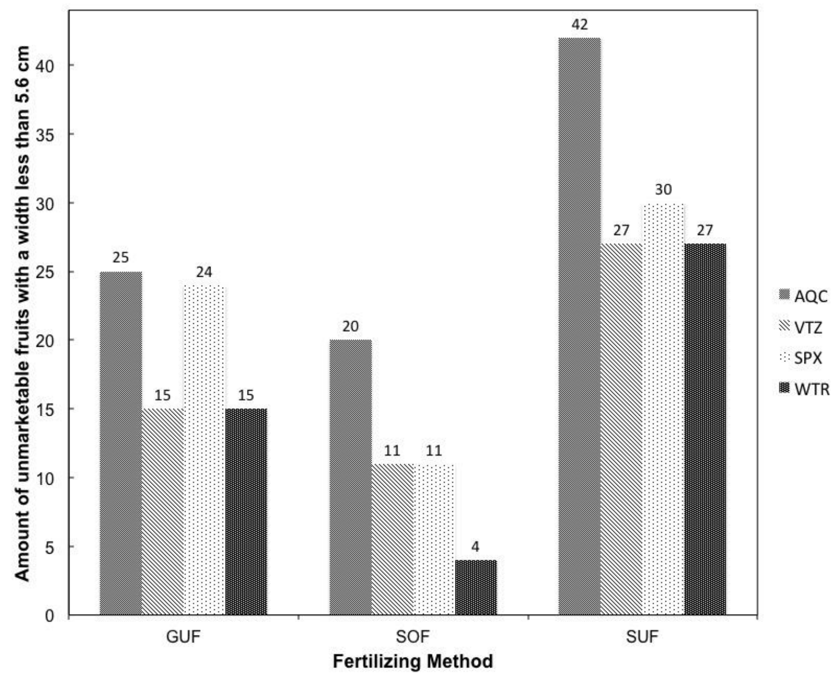


Figure 99. Interaction effects on the incidence of unmarketable fruits with a width of less than 5.6 cm during experiment 3. Mayagüez, Puerto Rico, 2015.

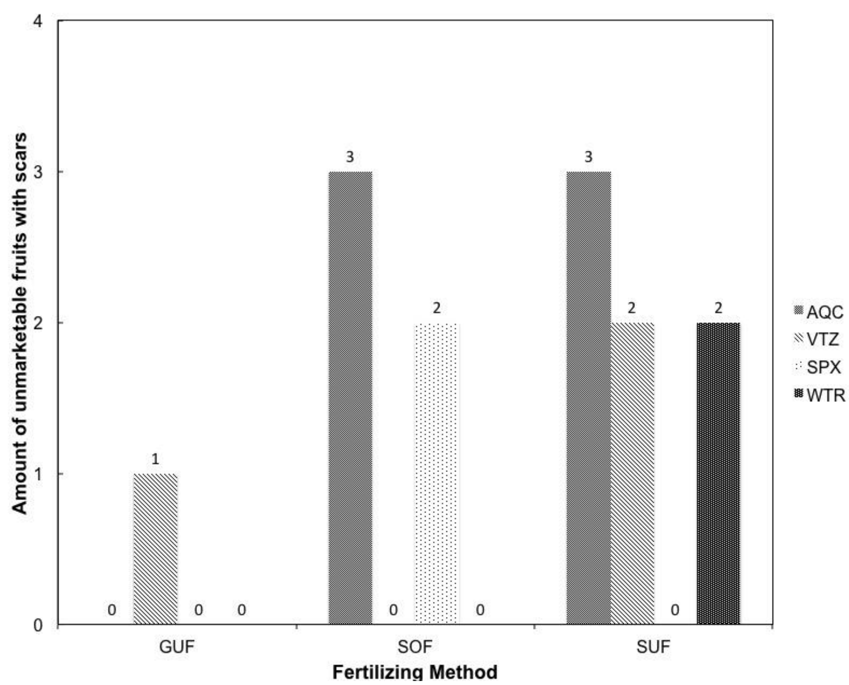


Figure 100. Interaction effects on the incidence of scar damage in unmarketable fruits during experiment 3. Mayagüez, Puerto Rico, 2015.

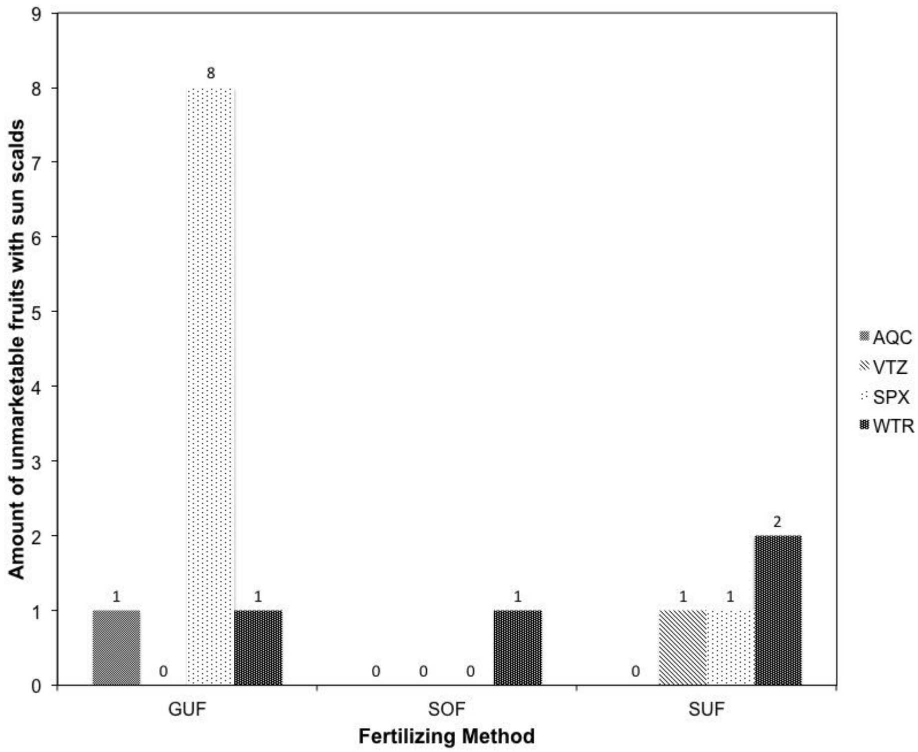


Figure 101. Interaction effects on the incidence of sun scald damage in unmarketable fruits during experiment 3. Mayagüez, Puerto Rico, 2015.

3.4.37 Marketable fruit grading analysis

Fruits categorized as marketable had to be free of decay. The marketable fruits were also free of injuries from scars, sunburn, disease, insects, mites, handling, or other means. An equatorial diameter greater or equal than 5.6 cm was also established to count pepper fruits as marketable. After the fruit was counted as marketable, it was further classified into categories according to two grading systems: 1)

the United States standards for grades of sweet peppers (2005) and 2) a diameter scale used for imported greenhouse-grown bell peppers (Jovicich et al., 2007). In experiment 3, 69% of the fruits produced were categorized as marketable (Figure 31).

3.4.37.1 United States standards for grades of sweet peppers (2005)

Marketable fruits were classified into three categories. Only 1% of marketable fruits were graded as U.S. Fancy. Marketable fruits graded as U.S. No.1 and U.S. No.2 accounted for the remaining 33 and 67% of marketable fruits, respectively (Figure 38). These categorizations were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the grading system. Combinations of fertilizing methods and biostimulant treatments were significantly ($p = 0.0415$) related to the grading system. The SOF method combined with AQC, VTZ, or SPX produced higher amounts of peppers graded as U.S. No. 2, but when combined with WTR it produced higher amount of U.S. No. 1 peppers. The GUF method combined with VTZ, SPX, or WTR produced higher amounts of peppers graded as U.S. No. 1, but when combined with AQC it produced higher amount of U.S. No. 2 peppers (Table 5).

Table 5. Grading distribution of marketable fruits (fertilizing method*biostimulant) in the second experiment using the United States standards for grades of sweet peppers.

Fertilizing method	Biostimulant	Grading distribution (%)			
		U.S. Fancy	U.S. No. 1	U.S. No. 2	Total
GUF	AQC	0	11	89	100
GUF	VTZ	2	31	67	100
GUF	SPX	0	47	53	100
GUF	WTR	0	40	60	100
SUF	AQC	0	32	68	100
SUF	VTZ	1	29	70	100
SUF	SPX	0	33	67	100
SUF	WTR	1	24	75	100
SOF	AQC	0	28	72	100
SOF	VTZ	0	41	59	100
SOF	SPX	0	29	71	100
SOF	WTR	0	67	33	100

3.4.37.2 Grading system for imported greenhouse-grown bell peppers (modified from Jovicich et al., 2007)

Marketable fruits were classified into two categories according to a diameter scale. Small fruits (diameter 5.6 – 6.39 cm) accounted for 47% of the marketable fruits and regular fruits (diameter > 6.4 cm) accounted for the remaining 53% of the marketable fruits in the third experiment (Figure 39). These categorizations were organized into a contingency table and a Pearson's chi-square test was used to determine if treatments had any relationship with the grading system. None of the treatments had any significant relationship with the grading system.

Chapter 4. Discussion

The demand for high-yielding and high-quality crops will keep increasing as global population keeps rising. Fertilizer applications will shift towards increasing nutrient use efficiency and diminishing productions costs while reducing pollution and conserving natural resources at the same time. Production of fresh vegetables requires adequate nutrient management practices in order to achieve the highest marketable yields and avoid physiological disorders. Bell peppers are low-calorie, nutrient-dense vegetables that form an integral part of the global human diet. Foliar biostimulants have the potential to increase yields, quality, growth, or stress tolerance levels in certain crops when applied at very low concentrations. If fertilizer applications can be modified to achieve a plant's maximum nutrient use efficiency, biostimulant applications could potentially be combined to produce added or synergistic effects on the crop's economically important characteristics.

4.1 Morphological development

Significant interaction effects were seen in the number of leaves, nodes, and buds but only at 71 DAS and only during the first experiment. At 71 DAS, the combination of SOF + VTZ produced at least 11, 11, and 22% more leaves, nodes, and buds than the rest of treatment combinations, respectively. The consistent effects of SOF + VTZ in the growth of plants during the first experiment only, may suggest that pests can be selective depending on amount or form of fertilizer applied (Brust, 2008) and/or that that a brassinosteroid-based biostimulant may play a role at mitigating this type of stress. Research suggests that positive effects of brassinosteroids are more distinguishable when plants are undergoing some type of stress rather than when grown in optimal conditions. Brassinosteroids are a class of plant polyhydroxy steroids that can dictate or influence a wide array of plant responses. They have been known to act on cell division, cell expansion, reproductive and vascular development, source/sink relationships, vegetative growth, apical dominance, photosynthesis, and stress modulation (Clouse & Sasse, 1998; Unterholzner et al, 2015). Studies on brassinosteroids have found that when applied exogenously, these compounds can help mitigate abiotic/biotic stresses such as unfavorable temperatures, drought, salinity, heavy metals, and pathogen attack (Kagale et al., 2007; Aghdam et al., 2016). Arora et al, (2008) found that pre-sowing treatments of 28-homobrassinolide can alleviate salt stress in maize plants by enhancing anti-oxidative enzyme activity, lowering lipid peroxidation, and increasing protein concentrations. Specific brassinosteroid compounds have been shown to enhance plant resistance to various fungal and viral diseases (Khripach et al., 2000), but protective effects against herbivory by pests have also been reported (Campos et al., 2009).

Individual effects by fertilizers were also seen during the first experiment on 1) the number of leaves at 64, 78, 85, 92, and 99 DAS; 2) the number of nodes at 50, 78, 92, and 99 DAS; 3) the number of fruitlets at 92 and 99DAS; and 4) the fresh and dry weight of shoots and roots. On all of these variables, the SOF method always performed significantly better than the SUF or GUF methods. This could be explained by the slow release characteristic of the pre-planting fertilizer used in the SOF method combined with the predominantly sandy texture of the UAS used during the first experiment. A 51%

sandy texture (Appendix 1) implies that the synthetic 10-10-10 pre-planting fertilizer used in the GUF and SUF methods was probably easily leached during irrigation events but due to the inherent nature of the slow-release BDC (6-6-5) used in the SOF method, the nutrients were available for a longer period of time and the plant had more time to assimilate them. This is further supported by the fact that the SOF method produced an average of 73 and 97% more NO_3^--N content in fresh sap than the GUF and SUF methods, respectively (Figure 30).

There were significant individual effects caused by fertilizers on every growth variable investigated during the third experiment. These included plant height and number of leaves, nodes, buds, flowers, and fruitlets. Abortion of buds, flowers, and fruitlets also had significant effects by fertilizing methods, along with the fresh and dry weight of shoots and roots. Results indicated that plants achieved the tallest weekly height and greatest weekly number of leaves, nodes, and fruitlets when fertilized with the 10 post-transplant N split applications of solubilized urea (SUF) throughout the crop cycle. The SUF method also resulted in the highest number of total buds and weekly buds until 47 DAT. At 62 and 70 DAT the SOF maintained the highest amounts of weekly buds. The SUF method also resulted in the highest number of total flowers and weekly flowers at 41 and 47 DAT. At 62 DAT the SOF maintained the highest amounts of weekly flowers. Fresh and dry weight of shoots and roots was also achieved with the SUF method.

All of these consistent results on the plant's growth and development indicate that NUE can be increased when urea is dissolved and applied in 10 split applications rather than applying it in granular for with only 2 split applications. Muirhead et al., (1985) found that water-run urea increased yields on maize by 27% when compared to band applications and that nitrate was the least efficient carrier of N. The split applications of post-transplant N allowed this nutrient to be readily available during the period of most growth, where N deficiency stress was most likely to occur. Another reason why the SUF method performed significantly better than the GUF method might be because the pH in the vicinity of the urea granule can be substantially higher than the surrounding soil due to the removal of hydrogen ions (H+) from the soil solution (Jones et al., 2007). This change in pH near the urea granules might have affected the plant's growth and development due to their proximity to the roots in the containers. Also, N losses by volatilization were diminished when urea was applied dissolved, more frequently and less concentrated.

4.2 Yield parameters

The highest number of marketable fruits per plant was produced in the third experiment and the lowest in the first experiment. Marketable yields averaged at 0.2, 1.4, and 4.8 marketable fruits per plant in the first, second, and third experiments, respectively. None of the treatments had interaction effects on any experiment. Biostimulant treatments did not cause any significant effects on any of the experiments either. Individual effects were caused by the fertilizing methods on marketable number of fruits per plant during the second and third experiments only.

4.2.1 Number of fruits per plant

None of the treatments had a significant effect in the marketable yield of the first experiment. Marketable yield in the first experiment was practically nonexistent probably because of biotic stress caused by broad mites. The marketable yield in the first experiment coincides with the findings of de Coss-Romero & Peña (1998) where they state that yields of ‘Early Calwonder’ pepper were reduced to 0.6 fruits per plant as a result of high stress levels induced by broad mite feeding. Studies made by Gómez et al, (2009) revealed that broad mite infestations are the most important biotic stress factor in pepper production, followed by insects and nematodes. Broad mites are a common problem in tropical areas and can affect a wide range of field-grown and greenhouse-grown crops, including ornamental plants also (Baker, 1997; Fasulo, 2000). The presence of ornamental plants and other vegetable seedlings in the neighboring greenhouses at the experimental site might have been the original hosts for the broad mites. An active broad mite population could have already been established in nearby plants and this would explain the severe damage seen on seedlings only a few weeks after emergence (Figure 102).

Figure 102. Twisted, hardened, and distorted growth caused by the broad mite's saliva at the terminal part of young plants. Mayagüez, Puerto Rico, 2014.

No interaction effects were seen on the number of marketable fruits per plant during the second or third experiments, but individual effects were caused by the fertilizing methods on both cases. Experiments 2 and 3 showed that the number of marketable fruits per plant could be significantly increased when post-transplant N is applied in solubilized split applications rather than in granular form. The number of marketable fruits per plant increased by an average of 70% when post-transplant N was applied in 10 solubilized split applications throughout the crop cycle rather than with one granular application at the beginning of the fruiting stage in experiment 2. The number of marketable per plant fruits increased by an average of 44% when post-transplant N was applied in 10 solubilized split applications rather than with two granular applications at the beginning of the flowering and fruiting stages in experiment 3.

Generally, the post-transplant solubilized N split applications (statistically superior to SUF over SOF) produced more marketable fruits per plant than the granular applications in experiments 2 and 3. In addition, the SUF produced 28% more marketable fruits per plant than the SOF in experiment 3. This may indicate that the source of the N [nitrate (NO_3^-) vs. urea (NH_2)] used for post-transplant N fertilizations could also have an effect on the number of marketable fruits per plant (Ghoname et al., 2009; Heeb et al., 2005[a]; Heeb et al., 2005[b]). Also, the granular application of urea could have increased salt stress to the rhizosphere and thus, affected the marketable yield (Bryla & Machado, 2011).

None of the treatments had interaction or individual effects on the number of unmarketable fruits per plant during any of the experiments. The highest number of unmarketable fruits per plant was produced in the second experiment. Production of unmarketable fruits per plant averaged at 1.8, 4.5, and 1.9 in the first, second, and third experiments, respectively.

None of the treatments caused any interaction or individual biostimulant effects on the number of total fruits per plant during any of the experiments. Only the fertilizing methods caused significant individual effects in the number of total fruits per plant in all three experiments. The highest number of total fruits per plant was produced in the third experiment. An average of number of 2.1, 5.9 and 6.8 total fruits per plant were produced in the first, second, and third experiment, respectively.

In the first experiment, the SOF method produced 21 and 36% more total fruits per plant than GUF and SUF methods, respectively. In combination, the urea fertilizers produced an average of 22% less total fruits per plant than the fertilizer derived from calcium nitrate (SOF). These results are similar to the findings of Ghoname et al., (2009) where ‘Mansoura Hybrid F1’ bell peppers produced 46% less fruits per plant when N fertilization was supplied with urea instead of calcium nitrate. Perhaps the additional amount of Ca applied with the SOF in this case, increased the plant’s resilience and thus played a role at retaining more fruitlets (Figure 26) that in turn developed into mature fruits rather than actually increasing production (Buczkowska et al., 2015[a]; Buczkowska et al., 2015[b]; Kazemi, M., 2014). Another explanation for these increases in total fruit production during the first experiment might be the slow-release BDC (6-6-5) formulations used as a pre-planting fertilizer. Instead of leaching easily with irrigation, it released nutrients slowly and allowed the plant to make better use of the N supplied.

In the second experiment, the SUF and SOF methods produced 48 and 15% more total fruits per plant than GUF method, respectively. In combination, the 10 post-transplant N split applications produced an average of 31% more total fruits per plant than the single granular urea fertilizer application (GUF). In the third experiment, the SUF and SOF methods produced 48 and 24% more total fruits per plant than GUF method, respectively. In combination, the 10 post-transplant N split applications produced an average of 36% more total fruits per plant than the single granular urea fertilizer application (GUF).

The results of the second and third experiments suggest that total fruit yields per plant can be increased by an average of 48% when the post-transplant N fertilizations are applied as and urea solution in 10 equally split applications instead of 1 or 2 applications of granular urea (GUF). These results also suggest that even the organic fertilizer derived from calcium nitrate (SOF) can produce an average of 19% more total fruits per plant if the post-transplant N is applied as a solution in 10 equally split applications instead of 1 or 2 applications of granular urea (GUF). It seems that total fruit yield per plant in potted 'Chocolate Beauty' bell peppers will increase significantly when broad mites are controlled and also when the post-transplant N is administered as a solution in split applications rather than in granular form, irrespective of N source (Coss-Romero & Peña, 1998; Fasulo, 2000; Jovicich et al., 2008; Zhang, 2003).

Future research should focus on selecting adequate post-transplant N fertilizer according to the plant's preference of nitrate or ammonium. The optimum amount of split-applications should also be established for each greenhouse-grown crop as well as the rate of solubilized N fertilizer in each individual application. Post-transplant N fertilization can be manipulated as long as economic feasibility is maintained in order to optimize NUE at specific phonologic stages during the crop cycle.

4.2.2 Fruit weight (g)

The highest marketable fruit weight per plant was produced in the third experiment. Yields averaged at 108.4, 63.74, and 371 grams of marketable fruits per plant in the first, second, and third experiments, respectively. No interaction effects were seen on the marketable fruit weight per plant during the first experiment, but individual effects were seen with biostimulant treatments. The third experiment also had individual effects caused by biostimulant treatment but in both cases, none of the biostimulants produced significantly more marketable fruit weight per plant than the WTR. The WTR produced an average of 68 and 18% more marketable fruit weight than the rest of the biostimulants in experiments 1 and 3, respectively.

There was an interaction effect on the marketable fruit weight per plant in experiment 2. The combination of SOF + SPX averaged at 91.0 grams of marketable fruit weight per plant. This is equivalent to at least 24% more marketable fruit weight per plant than any other treatment combination. Given the fact that the same SOF + SPX combination produced the longest and also the widest marketable fruits (Figure 55 and Figure 58), we can assume that the increase in marketable fruit weight per plant was probably due to the production of bigger fruits, not more quantity of fruits. These positive interactions between SOF and SPX might have been achieved due to the fact that both products were derived from the same raw material: *A. nodosum*. This could have produced some sort of synergistic effect on the plants ability to tolerate environmental stresses such as high temperature or high light intensity. Biostimulants based on seaweeds have been shown to increase the size of vegetables (Papadopoulos et al., 2006), but these effects could possibly be amplified by combining them with certain fertilizers such as the ones used in the SOF method.

4.2.3 Harvest time

Significant interaction effects were seen on the harvest time of unmarketable and total fruits during the second experiment. Effects of treatment interactions had very similar behavior in unmarketable and total fruits. Results indicate that plants treated with the combination of SOF + SPX produced fruits that took approximately 12 more days to be harvested GUF + VTZ and SUF + AQC combinations. This data suggests that biostimulant and fertilizer combination can be used to accelerate the harvest time of fruits by more than a week (Olivera-Olivera, 2015).

Individual fertilizing method effects were seen on the harvest time of marketable and total fruits during the third experiment. The SUF method quickened the harvest time of marketable and total fruits by approximately 5 days. This effect was probably a result of a better performance at the growth and development stage previously mentioned. Fruit development had an early advantage due to better growth dynamics seen on the plants and as a result, reached maturity faster.

4.3 Fruit quality

4.3.1 Fruit length

Lengths of marketable fruits averaged at 6.9, 5.5, and 6.3 cm in the first, second and third experiment, respectively. None of the treatments had a significant effect on the length of marketable fruits in the first experiment, but significant interaction effects were seen on the length of marketable fruits in the second and third experiment. These results suggest that broad mites must be controlled in order to better appreciate the effects of treatment interactions on the length of marketable fruits (Jovicich et al., 2004).

In the second experiment, the SOF + SPX combination produced marketable fruits that were at least 13% longer than any other treatment combination. It seems that under the second experiment's conditions, the marketable fruits are approximately 25% longer when SPX is combined with split application of post-transplant N instead of with a single application of granular urea (GUF). Other studies have found that kinetin foliar sprays at 2.5 or 10ppm significantly increased the size of 'Bodega' cucumbers and also the yield of extra-large fruits in 'Rapsodie' tomatoes and in '4-Ever' peppers (Papadopoulos et al., 2006). Since kinetin is the active ingredient in SPX (Appendix 6), it might explain why it produced larger marketable fruits when combined with SOF during the second experiment.

In the third experiment, none of the treatments produced longer marketable fruits than the SOF + WTR combination. Other studies regarding the effects of N forms and biostimulant combinations found no effect on pepper fruit length. This result was attributed to the fact that fruit length is a characteristic more closely related to the plant's genotype than to environmental effects (Ghoname et al., 2009). Nevertheless, tallest fruits were achieved with SOF combinations on both cases, indicating that perhaps the post-transplant split applications of this organic fertilizer may play a role at elongating 'Chocolate Beauty' bell peppers.

Fertilizer methods had significant effects over the length of unmarketable and total fruits in the first experiment. Fertilizing with urea produced unmarketable and total fruits that were on average 15% longer than when fertilized with the organic fertilizer

(SOF). In the second experiment, fertilizer and interaction effects were seen on the unmarketable and total fruit length, respectively. Granular application of post-transplant N (GUF) produced an average of 11% longer unmarketable fruits than split applications of N solution (SOF and SUF). Interaction effects were seen on the length of total fruits, where GUF and SUF produced the longest total fruits when combined with WTR. It is also worth to mention that combinations of SPX with all fertilizing methods produced the second best results in the interaction effects of the second experiment, and also, that the SPX produced individual biostimulant effects on the length of unmarketable fruits in the third experiment. When compared to WTR, the SPX biostimulant produced unmarketable fruits that were 10% longer in the third experiment.

The combined results from the experiments seem to suggest that SPX would be the biostimulant of choice for lengthening fruits, but mainly under stress conditions. SPX is a plant biostimulant derived from the brown algae *Ascophyllum nodosum* L. and contains 100 parts per million of kinetin among other substances. Studies conducted with biostimulants derived from the same seaweed also produced larger fruit sized tomatoes but only in early yields (Csizinszky, 1994; Papadopoulos et al., 2006).

4.3.2 Fruit width

None of the treatments had significant effects on the width of fruits during the first experiment. Significant interaction effects were seen on the width of marketable and total fruits in the second experiment. Significant individual biostimulant effects were seen on the width of marketable and total fruits in the third experiment. The widest marketable fruits were produced with the combination of SOF + SPX in the second experiment. This combination also produced the longest marketable fruits in the second experiment too, suggesting that SPX plays an important role in fruit size, possibly by the effects of kinetin (Papadopoulos et al., 2006). The WTR treatment produced the widest marketable and total fruits in the third experiment. The lack of biostimulants effects on horticultural crops is not uncommon (de la Cruz-Rodríguez) and future research of biostimulants on fruit elongation should be conducted in order to possibly compensate lower yields with bigger fruits.

4.3.3 Total soluble solids in fruits

Significant interaction effects were seen on brix readings of marketable, unmarketable, ant total fruits in the second and third experiments but the results are puzzling. The highest marketable fruit brix readings in the second experiment were obtained with the SUF + AQC, SOF + VTZ, and GUF + SPX. These results suggest the possibility that biostimulants based on amino acids, brassinosteroids and enzymes, and seaweed extracts play an important role in the nutritional quality of ‘Chocolate Beauty’ bell peppers (Bulgari et al., 2015). The type, form, and time of application of post-transplant N also have an effect on this characteristic. Other chemical analysis should be made in order to shed more light on the combined effects of different types of biostimulants with different kinds of fertilizing methods.

4.4 Chlorophyll and N content

4.4.1 SPAD

Individual fertilizing methods caused significant effects on SPAD values at 90 DAT during the second experiment. SUF and SOF methods had significantly higher SPAD values than the GUF method. The 10 split applications of post-transplant N produced an average of 22% higher SPAD values than when plants were fertilized with a single application of granular urea. Irrespective of the N form, it seem that split applications allow for a better assimilation of the nutrient, and this in turn is reflected on better production of chlorophyll (Addiscott, 2005).

4.4.2 N content in sap

Fertilizing methods had significant individual effects on the N content in fresh sap during the first experiment only. In this experiment, the SOF method produced significantly more NO_3^--N content in fresh sap than the GUF and SUF methods (Figure 30). This difference is probably due to the fact that the organic pre-planting fertilizer used in SOF had a slow-release effect while the synthetic pre-transplant fertilizer used in SUF and GUF was easily diluted and leached with irrigation events early in the crop cycle. Also, since the seeds were sown directly in the containers, the recently emerged roots might have encountered salt stress if they happened to be in proximity to the synthetic 10-10-10 granules used as a pre-planting fertilizer in the GUF and SUF methods (Bryla & Machado, 2011). This would also mean that the roots in the SOF method had a more favorable environment to explore further and develop more root hairs that play an important role during nutrient acquisition (Figure 21 and Figure 23). It is less likely that the difference of N content in sap was due to the difference of organic vs. synthetic or to the difference of direct sowing vs. transplanting.

4.5 Unmarketable peppers

Data on unmarketable fruits is important because yields were categorized as marketable assuming a market where the bell peppers are sold as fresh vegetables. This information might be useful if the bell pepper producer wants to sell the unmarketable produce to a processing plant. Unmarketable reasons vary in the experiments due in part to the different strategies and management practices implemented during the crop cycle, such as the use of agrochemicals for pest control and the construction of walls for the greenhouse (Table 6).

Table 6. Distribution of reasons to categorize pepper fruits as unmarketable in all experiments.

Unmarketable reasons	Scars or broad mite damage	Damage by sunburns	Damage by birds	Fruit diameter <5.6 cm
Experiment 1	45%	6%	4%	45%
Experiment 2	7%	10%	20%	63%
Experiment 3	5%	5%	0%	90%

According to these results, scarring damage done by broad mites can be lowered to less than 10% when using Oberon® at a rate of 0.6 L ha^{-1} either two or three times during the crop cycle, especially during the early stages when shoots are actively growing and buds are starting to appear (Coss-Romero & Peña, 1998). Organic control of pests might have worked better if the application had been implemented before the pests arrived, as a preventive measure. Damage by sunburns never occurred in more than 10% of fruits. Damage by birds was nonexistent in the third experiment because the walls prevented the entry of birds and had a significant impact on marketable yield. Bird damage was highest during the second experiment probably because pepper fruits were less damaged by broad mites. This could have turned the fruits more palatable and more attractive to birds. The scarring damage caused by broad mites during the first experiment turned the bell pepper's skin harder and opaque (Figure 33, middle left & middle right), thus making it harder for the birds to eat it.

A fruit diameter less than 5.6 cm classified bell peppers as unmarketable, even if the fruit did not have any other visible damage. These fruits are still useful and carry the same nutrition even if they are not categorized as marketable by market standards. In the third experiment, 90% of fruits were categorized as unmarketable because the diameter did not meet the market's standard. This means that these fruits could have been sold at a lower price to a processing plant or that many fruits were still developing when the experiment concluded.

Chapter 5. Conclusions and Recommendations

The three greenhouse experiments on potted specialty 'Chocolate Beauty' bell peppers revealed that:

Post-transplant N should be applied as solubilized or dissolved split applications instead of granular applications in order to increase marketable yields by consistently providing the nutrients during critical stages of growth and development.

The effects of biostimulants were inconsistent across the experiments, which may be associated to management practices and environmental factors being different in each experiment. Future research should focus on which types of biostimulants have the most significant effects on specific phenological stages considering time of applications, dosage, and environmental conditions.

An adequate control of broad mites in the greenhouse is critical for achieving higher marketable crop yields. Other crops and ornamental plants that serve as hosts for broad mites should be avoided in order to decrease the chance of infestations.

Appendices

Appendix 1. Soil analysis details of the UAS used during the experiments.

pH	7.77
Conductivity µS/cm	1013
Organic Matter %OM	0.96
Available Phosphorus mgP•□□□/Kg	143
Calcium mgCa/Kg	5510
Magnesium mgMg/Kg	709
Potassium mgK/Kg	672
Sodium mgNa/Kg	65
Aluminum mgAl/Kg	N/A
ECEC meq/100g	35
Texture %Clay	25.04
%Silt	23.44
%Sand	51.52
Copper mgCu/Kg	1
Iron mgFe/Kg	28
Manganese mgMn/Kg	7
Zinc mgZn/Kg	6
Boron mgB/Kg	N/A
Cadmium mgCd/Kg	N/A
Chromium mgCr/Kg	N/A
Nickel mgNi/Kg	N/A

Appendix 2. Bioflora dry crumbles® (6 – 6 – 5 + 8% Ca) fertilizer label.


BIOFLORA DRY CRUMBLES® 6 - 6 - 5 + 8% Ca

GUARANTEED ANALYSIS:

Total Nitrogen (N)	6.00%
0.22% Ammoniacal Nitrogen	
0.50% Water Soluble Nitrogen	
5.28% Water Insoluble Nitrogen	
Available Phosphate (P_2O_5)	6.00%
Soluble Potash (K_2O)	5.00%
Calcium (Ca)	8.00%

NUTRIENTS DERIVED FROM:
Feather meal, dry composted poultry litter, sulfate of potash and seaweed (Ascophyllum nodosum).

DIRECTIONS FOR USE:
Apply material with banding applicator or broadcast spreader. It is recommended for best results to be applied with ***Humega***® at a rate of 5 to 10 gallons per acre.

KEEP AWAY FROM CHILDREN. NOT FOR HUMAN CONSUMPTION.
Note: Buyer assumes all risks of use, storage and handling of this material. Neither seller nor its agent make any warranty, expressed or implied, concerning this product, except in conformity with statements on the label.

APPLICATION RECOMMENDATIONS:
We recommend the following general application rates of this ***plant food***. Rates may need to be modified depending on local conditions.

Turf - Apply 7 lbs to 14 lbs. per 1,000 sq. ft. or 0.42 lbs to 0.84 lbs. actual N per 1,000 sq. ft. of turf area.

Shrubs, Orchards & Vineyards - Apply one cup of fertilizer for every one inch diameter of the tree or shrub. Apply to drip line and work into the soil. Water thoroughly. Apply every two months during the growing season.

Agriculture - 200 lbs. to 500 lbs. per acre broadcast or sidedress.

PRECAUTIONARY STATEMENT:
May be hazardous to humans and domestic animals. Harmful if swallowed or inhaled. Call physician at any sign of problems from using the contents of this package. Store in a dry place away from children and pets. Wash hands after use.

Information regarding the contents and levels of metals in this product is available on the internet at http://www.aapfco.org/metals.htm

BIOFLORA® SYSTEMS
Manufactured & Guaranteed by:
GLOBAL ORGANICS, LLC
16121 West Eddie Albert Way
Goodyear, AZ 85338
TEL: (623) 932-1522

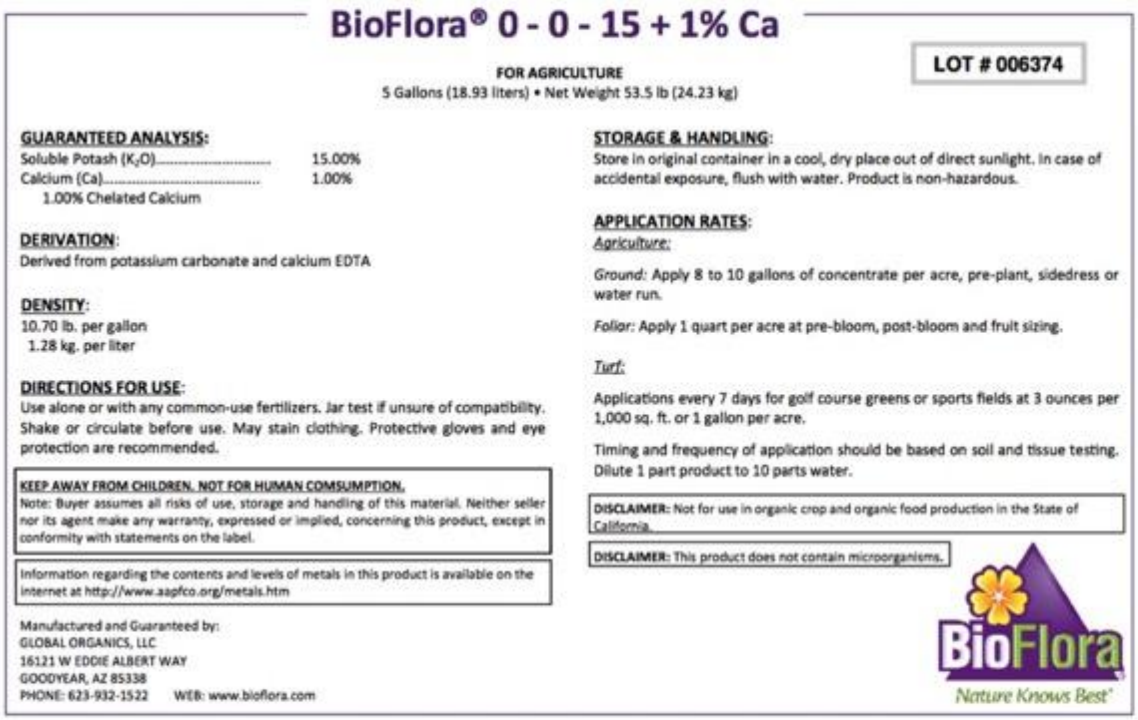
OMRI Listed

Net Weight 40 lb. (18.14 kg)

BioFlora

Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

Appendix 3. Bioflora® (0 – 0 – 15 + 1% Ca) fertilizer label.



BioFlora® 0 - 0 - 15 + 1% Ca

LOT # 006374

FOR AGRICULTURE

5 Gallons (18.93 liters) • Net Weight 53.5 lb (24.23 kg)

GUARANTEED ANALYSIS:

Soluble Potash (K_2O)............................ 15.00%

Calcium (Ca).. 1.00%

1.00% Chelated Calcium

DERIVATION:

Derived from potassium carbonate and calcium EDTA

DENSITY:

10.70 lb. per gallon

1.28 kg. per liter

DIRECTIONS FOR USE:

Use alone or with any common-use fertilizers. Jar test if unsure of compatibility. Shake or circulate before use. May stain clothing. Protective gloves and eye protection are recommended.

KEEP AWAY FROM CHILDREN. NOT FOR HUMAN COMSUMPTION.

Note: Buyer assumes all risks of use, storage and handling of this material. Neither seller nor its agent make any warranty, expressed or implied, concerning this product, except in conformity with statements on the label.

Information regarding the contents and levels of metals in this product is available on the internet at http://www.aapfco.org/metals.htm

Manufactured and Guaranteed by:
GLOBAL ORGANICS, LLC
16121 W EDDIE ALBERT WAY
GOODYEAR, AZ 85338
PHONE: 623-932-1522 WEB: www.bioflora.com

STORAGE & HANDLING:

Store in original container in a cool, dry place out of direct sunlight. In case of accidental exposure, flush with water. Product is non-hazardous.

APPLICATION RATES:

Agriculture:

Ground: Apply 8 to 10 gallons of concentrate per acre, pre-plant, sidedress or water run.

Foliar: Apply 1 quart per acre at pre-bloom, post-bloom and fruit sizing.

Turf:

Applications every 7 days for golf course greens or sports fields at 3 ounces per 1,000 sq. ft. or 1 gallon per acre.

Timing and frequency of application should be based on soil and tissue testing. Dilute 1 part product to 10 parts water.

DISCLAIMER: Not for use in organic crop and organic food production in the State of California.

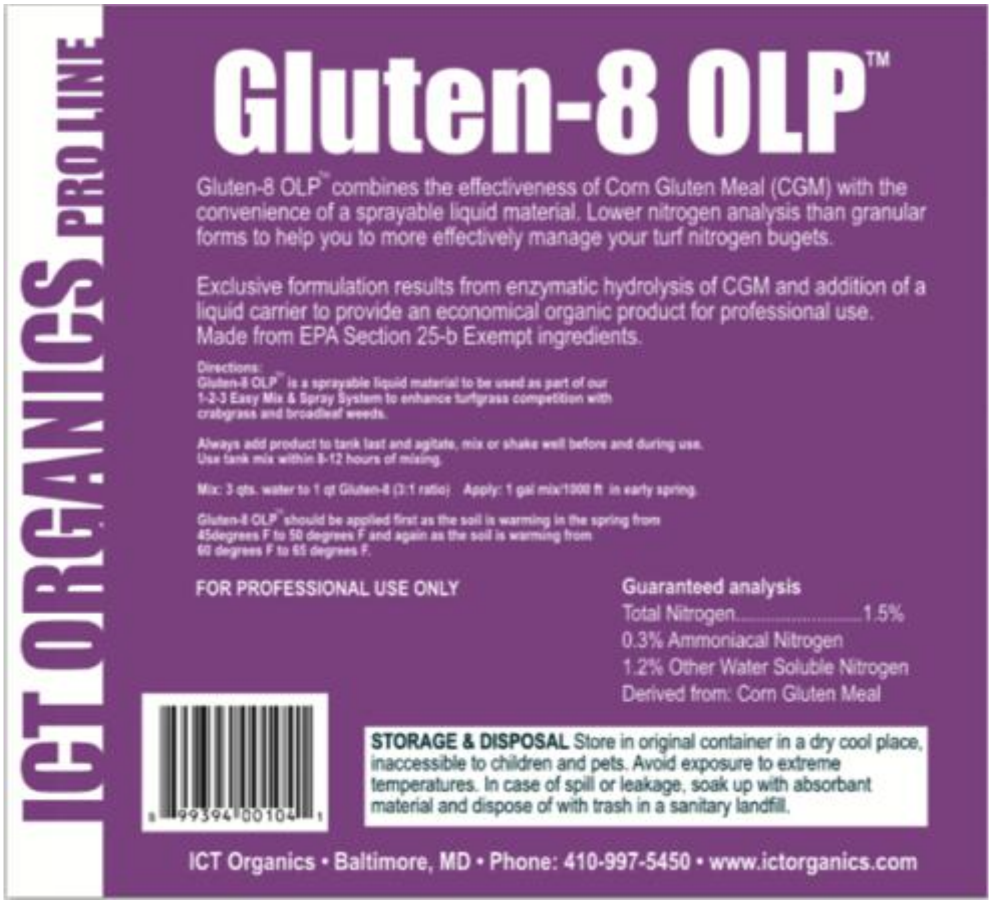
DISCLAIMER: This product does not contain microorganisms.

BioFlora

Nature Knows Best

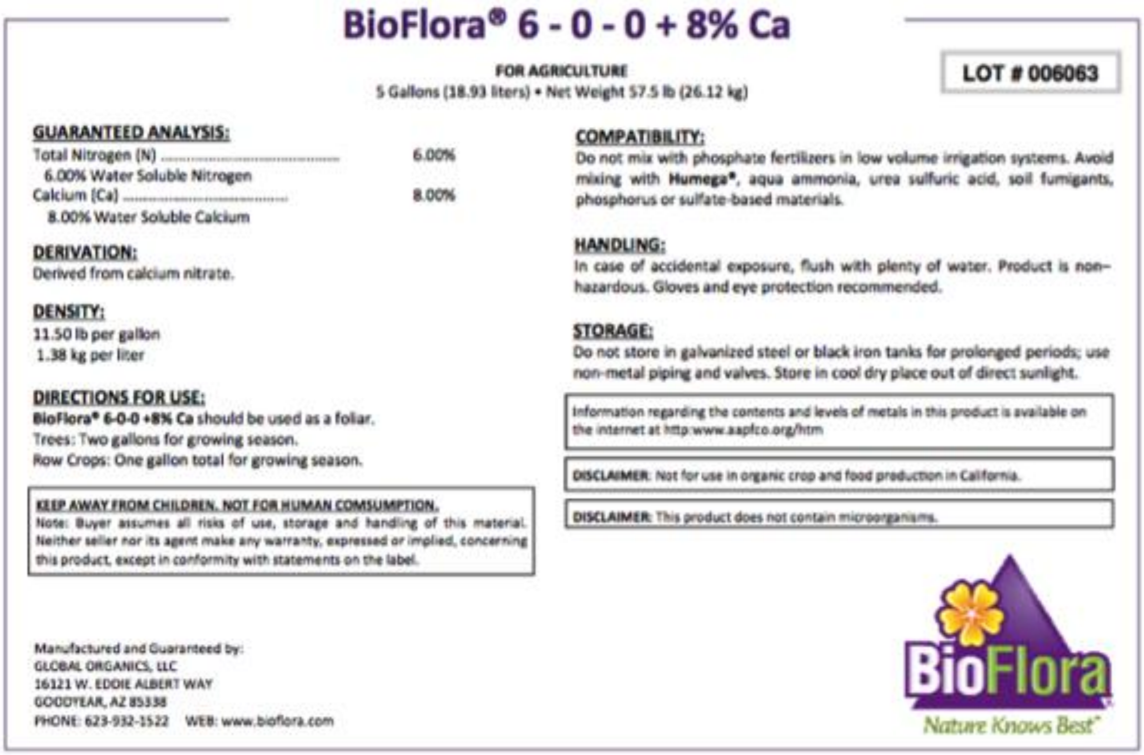
Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

Appendix 4. Gluten-8 OLP™ (1.5 – 0 – 0) fertilizer label.



Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

Appendix 5. Bioflora® (6 – 0 – 0 + 8% Ca) fertilizer label.



BioFlora® 6 - 0 - 0 + 8% Ca

FOR AGRICULTURE

5 Gallons (18.93 liters) • Net Weight 57.5 lb (26.12 kg)

LOT # 006063

GUARANTEED ANALYSIS:

Total Nitrogen (N) 6.00%

6.00% Water Soluble Nitrogen

Calcium (Ca) 8.00%

8.00% Water Soluble Calcium

DERIVATION:

Derived from calcium nitrate.

DENSITY:

11.50 lb per gallon

1.38 kg per liter

DIRECTIONS FOR USE:

BioFlora® 6-0-0 +8% Ca should be used as a foliar.

Trees: Two gallons for growing season.

Row Crops: One gallon total for growing season.

KEEP AWAY FROM CHILDREN. NOT FOR HUMAN COMSUMPTION.

Note: Buyer assumes all risks of use, storage and handling of this material. Neither seller nor its agent make any warranty, expressed or implied, concerning this product, except in conformity with statements on the label.

COMPATIBILITY:

Do not mix with phosphate fertilizers in low volume irrigation systems. Avoid mixing with **Humega®**, aqua ammonia, urea sulfuric acid, soil fumigants, phosphorus or sulfate-based materials.

HANDLING:

In case of accidental exposure, flush with plenty of water. Product is non-hazardous. Gloves and eye protection recommended.

STORAGE:

Do not store in galvanized steel or black iron tanks for prolonged periods; use non-metal piping and valves. Store in cool dry place out of direct sunlight.

Information regarding the contents and levels of metals in this product is available on the internet at http:www.aapfco.org/htm

DISCLAIMER: Not for use in organic crop and food production in California.

DISCLAIMER: This product does not contain microorganisms.

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Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

Appendix 6. Stimplex® crop biostimulant label.

Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

Appendix 7. Analysis of variance results on mean plant height (cm) through out the crop cycle in experiment 1.

Source of variation	PLANT HEIGHT (cm)								
	36 DAS	43 DAS	50 DAS	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.2938	0.7708	0.5976	0.9248	0.3816	0.0697	0.3126	0.1323	0.2528
Biostimulant	0.8444	0.9762	0.9092	0.6167	0.9626	0.6076	0.4503	0.9157	0.9257
Fertilizer method *Biostimulant	0.9444	0.8923	0.4473	0.1286	0.0731	0.3092	0.4049	0.2886	0.3921

Alpha < 0.05.

Appendix 8. Analysis of variance results on mean number of leaves through out the crop cycle in experiment 1.

Source of variation	NUMBER OF LEAVES								
	36 DAS	43 DAS	50 DAS	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.1639	0.6508	0.0522	<0.0001*	0.0001	0.0001*	0.0478*	0.0011*	0.0117*
Biostimulant	0.8894	0.8095	0.9296	0.9471	0.9839	0.6077	0.3434	0.3963	0.5005
Fertilizer method *Biostimulant	0.9316	0.6198	0.7355	0.1870	0.0499*	0.2277	0.7315	0.5273	0.6948

***α = 0.05. Mean differences will be analyzed**

Appendix 9. Analysis of variance results on mean number of nodes through out the crop cycle in experiment 1.

Source of variation	NUMBER OF NODES								
	36 DAS	43 DAS	50 DAS	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.1906	0.2114	0.0122*	0.5176	0.0049	0.0027*	0.1059	0.0144*	0.0108*
Biostimulant	0.7019	0.9917	0.9899	0.6079	0.9634	0.1788	0.0762	0.7601	0.6911
Fertilizer method *Biostimulant	0.9419	0.3786	0.5449	0.4795	0.0286*	0.4224	0.1584	0.2706	0.2730

***α = 0.05. Mean differences will be analyzed**

Appendix 10. Analysis of variance results on mean number of buds through out the crop cycle in experiment 1.

Source of variation	NUMBER OF BUDS					
	50 DAS	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS
Fertilizer method	0.4502	0.2497	0.0265	0.0174	0.6689	0.2869
Biostimulant	0.8479	0.7133	0.4269	0.0391*	0.3924	0.3175
Fertilizer method *Biostimulant	0.6593	0.1879	0.0326*	0.5777	0.3867	0.3441

***α = 0.05. Mean differences will be analyzed**

Appendix 11. Analysis of variance results on mean number of flowers through out the crop cycle in experiment 1.

Source of variation	NUMBER OF FLOWERS					
	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.3607	0.5859	0.1281	0.3897	0.2509	0.4065
Biostimulant	0.4291	0.5548	0.5394	0.4283	0.4433	0.4354
Fertilizer method *Biostimulant	0.4066	0.2001	0.1501	0.5206	0.7728	0.4784

Alpha < 0.05.

Appendix 12. Analysis of variance results on mean number of fruitlets through out the crop cycle in experiment 1.

Source of variation	NUMBER OF FRUITLETS					
	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.3374	0.5057	0.3255	0.2068	0.0033*	0.0004*
Biostimulant	0.7588	0.4164	0.9328	0.5138	0.7564	0.4315
Fertilizer method *Biostimulant	0.5744	0.7628	0.6134	0.7280	0.9488	0.8695

***α = 0.05. Mean differences will be analyzed**

Appendix 13. Analysis of variance results on mean abortion of buds through out the crop cycle in experiment 1.

Source of variation	ABSCISSION OF BUDS					
	64 DAS	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.1009	0.4282	0.5977	0.5272	0.1540	0.6927
Biostimulant	0.6424	0.4892	0.8562	0.9888	0.2764	0.9052
Fertilizer method *Biostimulant	0.8843	0.4716	0.4775	0.6170	0.2292	0.2350

Alpha < 0.05.

Appendix 14. Analysis of variance results on mean abortion of flowers through out the crop cycle in experiment 1.

Source of variation	ABSCISSION OF FLOWERS				
	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.7819	0.5965	0.0725	0.2798	0.4719
Biostimulant	0.2753	0.7238	0.1587	0.4208	0.4884
Fertilizer method *Biostimulant	0.7475	0.5473	0.0175	0.5770	0.4408

Alpha < 0.05.

Appendix 15. Analysis of variance results on mean abortion of fruitlets through out the crop cycle in experiment 1.

Source of variation	ABSCISSION OF FRUITLETS				
	71 DAS	78 DAS	85 DAS	92 DAS	99 DAS
Fertilizer method	0.3763	0.6035	0.5507	0.0720	0.4168
Biostimulant	0.4015	0.2076	0.2667	0.4104	0.0923
Fertilizer method *Biostimulant	0.4363	0.1531	0.4913	0.2097	0.6994

Alpha < 0.05.

Appendix 16. Analysis of variance results on fresh and dry weight of shoot, roots and fruits (g) in experiment 1.

Source of variation	FRESH WEIGHT (g)		DRY WEIGHT (g)		
	Shoots	Roots	Shoots	Roots	Fruits
Fertilizer method	0.0048*	<0.0001*	0.0012*	<0.0001*	0.2137
Biostimulant	0.7922	0.5413	0.6953	0.9249	0.1793
Fertilizer method *Biostimulant	0.4793	0.8895	0.6894	0.7263	0.0370

***α = 0.05. Mean differences will be analyzed**

Appendix 17. Analysis of variance results on the number and weight (g) of marketable, unmarketable, and total fruits per plant in experiment 1.

Source of variation	FRUIT NUMBER PER PLANT			FRUIT WEIGHT PER PLANT (g)		
	Marketable	Unmarketable	Total	Marketable	Unmarketable	Total
Fertilizer method	0.2131	0.3667	0.0150*	0.0862	0.0897	0.0849
Biostimulant	0.2696	0.7318	0.9001	0.0079*	0.4980	0.3232
Fertilizer method *Biostimulant	0.5899	0.8473	0.9798	0.3502	0.2734	0.5560

***α = 0.05. Mean differences will be analyzed**

Appendix 18. Analysis of variance results on the length and width (cm) of marketable, unmarketable, and total fruits in experiment 1.

Source of variation	FRUIT LENGTH (cm)			FRUIT WIDTH (cm)		
	Marketable	Unmarketable	Total	Marketable	Unmarketable	Total
Fertilizer method	0.0657	0.0079*	0.0099*	0.7390	0.7529	0.9141
Biostimulant	0.5814	0.6853	0.3134	0.7282	0.4336	0.0888
Fertilizer method *Biostimulant	0.3470	0.4098	0.1019	0.1573	0.5157	0.4109

***α = 0.05. Mean differences will be analyzed**

Appendix 19. Analysis of variance results on the pericarp thickness (mm) of marketable, unmarketable, and total fruits in experiment 1.

Source of variation	PERICARP THICKNESS (mm)		
	Marketable	Unmarketable	Total
Fertilizer method	0.7828	0.2230	0.1099
Biostimulant	0.1436	0.3489	0.8061
Fertilizer method *Biostimulant	0.4524	0.0226*	0.0136*

***α = 0.05. Mean differences will be analyzed**

Appendix 20. Analysis of variance results on the SPAD index of each experiment.

Source of variation	SPAD Index		
	Experiment 1 (85 DAS)	Experiment 2 (90 DAT)	Experiment 3 (41 DAT)
Fertilizer method	0.5284	<0.0001*	0.6087
Biostimulant	0.3120	0.5309	0.8967
Fertilizer method *Biostimulant	0.2308	0.7595	0.0826

***α = 0.05. Mean differences will be analyzed**

Appendix 21. Analysis of variance results on the SPAD index of each experiment.

Source of variation	NO_3^--N (ppm)	
	Experiment 1 (106-114 DAS)	Experiment 3 (80-107 DAT)
Fertilizer method	0.0010*	0.0492
Biostimulant	0.9940	0.0733
Fertilizer method *Biostimulant	0.1714	0.5433

***α = 0.05. Mean differences will be analyzed**

Appendix 22. Label of Oberon 2 SC used to control broadmites

Disclaimer: Mentioning market names does not imply a brand endorsement from the authors or from the University, and is done solely to specify the materials used in this research.

Appendix 23. Analysis of variance results on fresh weight and dry weight of shoots and roots (g) in experiment 2.

Source of variation	FRESH WEIGHT (g)		DRY WEIGHT (g)	
	Shoots	Roots	Shoots	Roots
Fertilizer method	0.1385	0.1551	0.0183*	0.2832
Biostimulant	0.9677	0.5838	0.9065	0.1429
Fertilizer method *Biostimulant	0.2482	0.3318	0.4614	0.4040

***α = 0.05. Mean differences will be analyzed**

Appendix 24. Analysis of variance results on the number and weight (g) of marketable, unmarketable, and total fruits per plant in experiment 2.

Source of variation	FRUIT NUMBER PER PLANT			FRUIT WEIGHT PER PLANT (g)		
	Marketable	Unmarketable	Total	Marketable	Unmarketable	Total
Fertilizer method	0.0056*	0.1874	0.0079*	0.1258	0.1477	0.5708
Biostimulant	0.3119	0.3731	0.8370	0.0297	0.5130	0.0112*
Fertilizer method *Biostimulant	0.7922	0.3018	0.6575	0.0011*	0.5902	0.0473

***α = 0.05. Mean differences will be analyzed**

Appendix 25. Analysis of variance results on the length and width (cm) of marketable, unmarketable, and total fruits in experiment 2.

Source of variation	FRUIT LENGTH (cm)			FRUIT WIDTH (cm)		
	Marketable	Unmarketable	Total	Marketable	Unmarketable	Total
Fertilizer method	0.2182	0.0011*	0.0341	0.3046	0.4954	0.1396
Biostimulant	0.2143	0.1564	0.0192	0.3706	0.5844	0.0471
Fertilizer method *Biostimulant	0.0037*	0.0971	0.0256*	0.0012*	0.6715	0.0297*

***α = 0.05. Mean differences will be analyzed**

Appendix 26. Analysis of variance results on the pericarp thickness (mm) of marketable, unmarketable, and total fruits in experiment 2.

Source of variation	PERICARP THICKNESS (mm)		
	Marketable	Unmarketable	Total
Fertilizer method	0.0211	0.6206	0.0063
Biostimulant	0.0347	0.2632	0.0043
Fertilizer method *Biostimulant	0.0012*	0.5405	0.0059*

***α = 0.05. Mean differences will be analyzed**

Appendix 27. Analysis of variance results on the Brix readings of marketable, unmarketable, and total fruits in experiment 2.

Source of variation	BRIX (%)		
	Marketable	Unmarketable	Total
Fertilizer method	0.9925	0.0203	0.2058
Biostimulant	0.5824	0.0004	0.0009
Fertilizer method *Biostimulant	0.0013*	0.0365*	0.0227*

***α = 0.05. Mean differences will be analyzed**

Appendix 28. Analysis of variance results on the harvest time (DAT) of marketable, unmarketable, and total fruits in experiment 2.

Source of variation	HARVEST TIME (DAT)		
	Marketable	Unmarketable	Total
Fertilizer method	0.3463	<0.0001	<0.0001
Biostimulant	0.3684	<0.0001	<0.0001
Fertilizer method *Biostimulant	0.6661	<0.0001*	0.0025*

***α = 0.05. Mean differences will be analyzed**

Appendix 29. Analysis of variance results on mean plant height (cm) through out the crop cycle in experiment 3.

Source of variation	PLANT HEIGHT (cm)								
	11 DAT	20 DAT	25 DAT	30 DAT	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0003*
Biostimulant	0.9769	0.9441	0.3767	0.2628	0.5115	0.2172	0.1435	0.3387	0.5426
Fertilizer method *Biostimulant	0.1241	0.5530	0.7295	0.3856	0.5472	0.6181	0.4283	0.7715	0.8460

***α = 0.05. Mean differences will be analyzed**

Appendix 30. Analysis of variance results on mean number of leaves through out the crop cycle in experiment 3.

Source of variation	NUMBER OF LEAVES								
	11 DAT	20 DAT	25 DAT	30 DAT	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Biostimulant	0.9432	0.1846	0.5498	0.5426	0.5884	0.5746	0.2676	0.3969	0.5407
Fertilizer method *Biostimulant	0.1630	0.3973	0.5722	0.7673	0.6270	0.6897	0.6674	0.5704	0.2923

***α = 0.05. Mean differences will be analyzed**

Appendix 31. Analysis of variance results on mean number of nodes through out the crop cycle in experiment 3.

Source of variation	NUMBER OF NODES								
	11 DAT	20 DAT	25 DAT	30 DAT	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	<0.0001*	0.0006*	<0.0001*	0.0305*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Biostimulant	0.5165	0.3713	0.6846	0.4804	0.4735	0.3315	0.2347	0.5558	0.6325
Fertilizer method *Biostimulant	0.1656	0.4966	0.0847	0.6639	0.9552	0.8959	0.9101	0.4579	0.2109

***α = 0.05. Mean differences will be analyzed**

Appendix 32. Analysis of variance results on mean number of buds through out the crop cycle in experiment 3.

Source of variation	NUMBER OF BUDS							
	20 DAT	25 DAT	30 DAT	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	0.0752	0.0018*	0.0393*
Biostimulant	0.3017	0.1774	0.1457	0.1467	0.2581	0.0257	0.3226	0.2252
Fertilizer method *Biostimulant	0.7249	0.9263	0.9615	0.6793	0.2216	0.1336	0.3332	0.2208

***α = 0.05. Mean differences will be analyzed**

Appendix 33. Analysis of variance results on mean number of flowers through out the crop cycle in experiment 3.

Source of variation	NUMBER OF FLOWERS				
	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	0.0001*	<0.0001*	0.4936	0.0420*	0.4619
Biostimulant	0.1312	0.3436	0.9663	0.7608	0.1226
Fertilizer method *Biostimulant	0.8539	0.9950	0.6675	0.8934	0.9798

***α = 0.05. Mean differences will be analyzed**

Appendix 34. Analysis of variance results on mean number of fruitlets through out the crop cycle in experiment 3.

Source of variation	NUMBER OF FRUITLETS				
	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	0.0144*	<0.0001*	0.0623	0.0002*	<0.0001*
Biostimulant	0.6412	0.1681	0.2941	0.7466	0.1212
Fertilizer method *Biostimulant	0.5040	0.5965	0.3812	0.7440	0.8428

***α = 0.05. Mean differences will be analyzed**

Appendix 35. Analysis of variance results on mean abortion of buds through out the crop cycle in experiment 3.

Source of variation	ABSCISSION OF BUDS			
	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	0.0964	0.9727	0.0002*	0.0001*
Biostimulant	0.7744	0.2318	0.0378*	0.9524
Fertilizer method *Biostimulant	0.1174	0.7952	0.2365	0.8193

***α = 0.05. Mean differences will be analyzed**

Appendix 36. Analysis of variance results on mean abortion of flowers through out the crop cycle in experiment 3.

Source of variation	ABSCISSION OF FLOWERS		
	55 DAT	62 DAT	70 DAT
Fertilizer method	0.2158	0.0161*	0.0989
Biostimulant	0.4950	0.7488	0.1359
Fertilizer method *Biostimulant	0.2650	0.5653	0.4006

***α = 0.05. Mean differences will be analyzed**

Appendix 37. Analysis of variance results on mean abortion of fruitlets through out the crop cycle in experiment 3.

Source of variation	ABSCISSION OF FRUITLETS				
	41 DAT	47 DAT	55 DAT	62 DAT	70 DAT
Fertilizer method	0.0144*	<0.0001*	0.0623	0.0002*	<0.0001*
Biostimulant	0.6412	0.1681	0.2941	0.7466	0.1212
Fertilizer method *Biostimulant	0.5040	0.5965	0.3812	0.7440	0.8428

***α = 0.05. Mean differences will be analyzed**

Appendix 38. Analysis of variance results on fresh and dry weight of shoots and roots (g) in experiment 3.

Source of variation	FRESH WEIGHT (g)		DRY WEIGHT (g)	
	Shoots	Roots	Shoots	Roots
Fertilizer method	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Biostimulant	0.6567	0.9104	0.7883	0.9646
Fertilizer method *Biostimulant	0.8232	0.5969	0.8394	0.5546

***α = 0.05. Mean differences will be analyzed**

Appendix 39. Analysis of variance results on the number and weight (g) of marketable, unmarketable, and total fruits per plant in experiment 3.

Source of variation	FRUIT NUMBER PER PLANT			FRUIT WEIGHT PER PLANT (g)		
	Marketable	Unmarketable	Total	Marketable	Unmarketable	Total
Fertilizer method	<0.0001*	0.9808	<0.0001*	<0.0001*	0.0182*	<0.0001*
Biostimulant	0.0961	0.2595	0.4358	0.0030*	0.8859	0.0318*
Fertilizer method *Biostimulant	0.8879	0.9526	0.5961	0.7518	0.9815	0.3643

***α = 0.05. Mean differences will be analyzed**

Appendix 40. Analysis of variance results on the length and width (cm) of marketable, unmarketable, and total fruits in experiment 3.

Source of variation	FRUIT LENGTH (cm)			FRUIT WIDTH (cm)		
	Marketable	Unmarketable	Total	Marketable	Unmarketable	Total
Fertilizer method	0.0163	0.4339	0.4460	0.7563	0.5726	0.2775
Biostimulant	0.5292	0.0466*	0.1891	0.0335*	0.3612	<0.0001*
Fertilizer method *Biostimulant	0.0452*	0.9778	0.2251	0.7344	0.6538	0.9924

***α = 0.05. Mean differences will be analyzed**

Appendix 41. Analysis of variance results on the pericarp thickness (mm) of marketable, unmarketable, and total fruits in experiment 3.

Source of variation	PERICARP THICKNESS (mm)		
	Marketable	Unmarketable	Total
Fertilizer method	0.0510	0.0256	0.0202
Biostimulant	0.1759	0.6702	0.0709
Fertilizer method *Biostimulant	0.0200*	0.0618	0.0130*

***α = 0.05. Mean differences will be analyzed**

Appendix 42. Analysis of variance results on the Brix readings of marketable, unmarketable, and total fruits in experiment 3.

Source of variation	BRIX (%)		
	Marketable	Unmarketable	Total
Fertilizer method	0.0044	0.1175	0.0010
Biostimulant	0.4190	0.0686	0.0171
Fertilizer method *Biostimulant	0.0005*	0.0051*	0.0016*

***α = 0.05. Mean differences will be analyzed**

Appendix 43. Analysis of variance results on the harvest time (DAT) of marketable, unmarketable, and total fruits in experiment 3.

Source of variation	HARVEST TIME (DAT)		
	Marketable	Unmarketable	Total
Fertilizer method	<0.0001*	0.5837	0.0003*
Biostimulant	0.9505	0.6350	0.3293
Fertilizer method *Biostimulant	0.8063	0.9683	0.9820

***α = 0.05. Mean differences will be analyzed**

Equation 1. Conversion of plants·cuerda^{-1} to plants·ha^{-1}.

$$\frac{14{,}500\ plants}{1\ \cancel{cuerda}} \rightarrow \frac{1\ \cancel{cuerda}}{0.971\ \cancel{acre}} \rightarrow \frac{2.47\ \cancel{acre}}{1\ ha} \Rightarrow \frac{\mathbf{36{,}890\ plants}}{\mathbf{1\ ha}}$$

Equation 2. Nitrogen, phosphate (P_2O_5), and potash (K_2O) recommendations were applied as a pre-plant fertilizer (lbs·cuerda^{-1} to kg·ha^{-1}).

$$\frac{50\ \cancel{lbs\ NPK}}{1\ \cancel{cuerda}} \rightarrow \frac{1\ kg\ NPK}{2.2\ \cancel{lbs\ NPK}} \rightarrow \frac{1\ \cancel{cuerda}}{0.971\ \cancel{acre}} \rightarrow \frac{2.47\ \cancel{acre}}{1\ ha} \Rightarrow \frac{\mathbf{58\ kg\ NPK}}{\mathbf{ha}}$$

Equation 3. Nitrogen recommendation was applied as a post-transplant fertilizer (lbs·cuerda^{-1} to kg·ha^{-1}).

$$\frac{100\ \cancel{lbs}\ Nitrogen}{1\ \cancel{cuerda}} \rightarrow \frac{1\ kg\ Nitrogen}{2.2\ \cancel{lbs}\ Nitrogen} \rightarrow \frac{1\ \cancel{cuerda}}{0.971\ \cancel{acre}} \rightarrow \frac{2.47\ \cancel{acre}}{1\ ha} \Rightarrow \frac{\mathbf{116\ kg\ Nitrogen}}{\mathbf{ha}}$$

Equation 4. Urea hydrolysis in soil by the enzyme urease (Havlin et al., 2005).

$$CO(NH_2)_2 + H^+ + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + HCO_3^-$$

$$NH_4^+ \longrightarrow NH_3 + H^+$$

Equation 5. Conversion of 10-10-10 pre-plant synthetic fertilizer amounts for each plant.

$$\frac{58\ \cancel{kg\ NPK}}{1\ \cancel{ha}} \rightarrow \frac{1\ \cancel{ha}}{36{,}890\ plants} \rightarrow \frac{1\ \cancel{kg\ fert.(10-10-10)}}{0.1\ \cancel{kg\ NPK}} \rightarrow \frac{1{,}000\ g\ fert.(10-10-10)}{1\ \cancel{kg\ fert.(10-10-10)}} \Rightarrow \frac{\mathbf{16\ g\ fert.(10-10-10)}}{\mathbf{1\ plant}}$$

Equation 6. Conversion of post-transplant granular urea to amount needed per plant.

$$\frac{116\ \cancel{kg\ N}}{1\ \cancel{ha}} \rightarrow \frac{1\ \cancel{ha}}{36{,}890\ plants} \rightarrow \frac{1\ \cancel{kg\ urea}}{0.46\ \cancel{kg\ N}} \rightarrow \frac{1{,}000\ g\ urea}{1\ \cancel{kg\ urea}} \Rightarrow \frac{\mathbf{7\ g\ urea}}{\mathbf{1\ plant}}$$

Equation 7. Conversion of BDC (6-6-5) kg·ha^{-1} pre-plant organic fertilizer into amount needed for each plant in terms of N and P.

$$\frac{58\ \cancel{kg\ NP}}{1\ \cancel{ha}} \rightarrow \frac{1\ \cancel{ha}}{36{,}890\ plants} \rightarrow \frac{1\ \cancel{kg\ BDC\ (6-6-5)}}{0.06\ \cancel{kg\ NP}} \rightarrow \frac{1{,}000\ g\ BDC\ (6-6-5)}{1\ \cancel{kg\ BDC\ (6-6-5)}} \Rightarrow \frac{\mathbf{27\ g\ BDC\ (6-6-5)}}{\mathbf{1\ plant}}$$

Equation 8. Amount of potash applied in kg·ha^{-1} by the application of 27 grams of BDC (6-6-5) fertilizer per plant.

$$\frac{27\ \cancel{g\ BDC\ (6-6-5)}}{1\ \cancel{plant}} \rightarrow \frac{0.05\ \cancel{g\ K}}{1\ \cancel{g\ BDC\ (6-6-5)}} \rightarrow \frac{1\ kg\ K}{1{,}000\ \cancel{g\ K}} \rightarrow \frac{36{,}890\ \cancel{plants}}{1\ ha} \Rightarrow \frac{\mathbf{50\ kg\ K}}{\mathbf{1\ ha}}$$

Equation 9. Application of 8 kg·ha^{-1} of pre-plant K_2O using BLF (0-0-15) in terms of amount needed per plant.

$$\frac{8\ \cancel{kg\ K}}{1\ \cancel{ha}} \rightarrow \frac{1\ \cancel{kg\ BLF\ (0-0-15)}}{0.15\ \cancel{kg\ K}} \rightarrow \frac{1\ \cancel{L\ BLF (0-0-15)}}{1.28\ \cancel{kg\ BLF\ (0-0-15)}} \rightarrow \frac{1{,}000\ mL\ BLF\ (0-0-15)}{1\ \cancel{L\ BLF\ (0-0-15)}} \rightarrow \frac{1\ \cancel{ha}}{36{,}890\ plants} \Rightarrow \frac{\mathbf{2\ mL\ BLF\ (0-0-15)}}{\mathbf{1\ plant}}$$

Equation 10. Amount of calcium applied in kg·ha^{-1} by the application of 27 grams of BDC (6-6-5) fertilizer per plant.

$$\frac{27\ \cancel{g\ BDC\ (6-6-5)}}{1\ \cancel{plant}} \rightarrow \frac{0.08\ \cancel{g\ Ca}}{1\ \cancel{g\ BDC\ (6-6-5)}} \rightarrow \frac{1\ kg\ Ca}{1{,}000\ \cancel{g\ Ca}} \rightarrow \frac{36{,}890\ \cancel{plants}}{1\ ha} \Longrightarrow \frac{\mathbf{80\ kg\ Ca}}{\mathbf{1\ ha}}$$

Equation 11. Amount of calcium applied in kg·ha^{-1} by the application of 2 mL of BLF (0-0-15) per plant.

$$\frac{2\ \cancel{mL\ BLF\ (0-0-15)}}{1\ \cancel{plant}} \rightarrow \frac{1\ \cancel{L\ BLF\ (0-0-15)}}{1{,}000\ \cancel{mL\ BLF\ (0-0-15)}} \rightarrow \frac{1.28\ \cancel{kg\ BLF\ (0-0-15)}}{1\ \cancel{L\ BLF\ (0-0-15)}} \rightarrow \frac{0.01\ kg\ Ca}{1\ \cancel{kg\ BLF\ (0-0-15)}} \rightarrow \frac{36{,}890\ \cancel{plants}}{1\ ha} \Longrightarrow \frac{\mathbf{1\ kg\ Ca}}{\mathbf{1\ ha}}$$

Equation 12. Conversion of post-transplant BLNF to amount needed per plant.

$$\frac{116\ \cancel{kg\ N}}{1\ \cancel{ha}} \rightarrow \frac{1\ \cancel{ha}}{36{,}890\ plants} \rightarrow \frac{1\ \cancel{kgBLNF}}{0.06\ \cancel{kg\ N}} \rightarrow \frac{1{,}000\ mL\ BLNF}{1.38\ \cancel{kg\ BLNF}} \Longrightarrow \frac{\mathbf{38\ mL\ BLNF}}{\mathbf{1\ plant}}$$

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