MARIA VITÓRIA BATISTA DUQUE GUTTIERREZ BAPTISTA

MONITORING PHYSIOLOGICAL CHANGES IN ALMOND TREES (*Prunus dulcis* (Mill.) D.A. Webb) UNDER ABIOTIC STRESS IN RESPONSE TO FOLIAR GAMMA-AMINOBUTYRIC ACID APPLICATION

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Monografia apresentada à Universidade Federal de Lavras, como parte das exigências do Curso de Agronomia, para a obtenção do título de Bacharel.

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MONITORAMENTO DE MUDANÇAS FISIOLÓGICAS NA AMENDOEIRA ((*Prunus dulcis* (Mill.) D.A. Webb) SOB CONDIÇÕES DE ESTRESSE ABIÓTICO EM RESPOSTA À APLICAÇÕES DE ÁCIDO GAMA AMINOBUTÍRICO

Monografia apresentada à Universidade Federal de Lavras, como parte das exigências do Curso de Agronomia, para a obtenção do título de Bacharel.

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ABSTRACT

Abiotic stresses such as water deficit and extreme temperatures are variables that directly affect yield and plant development of crops. Almond trees (*Prunus dulcis* (Mill.) D. A. Webb) while being originated from the Mediterranean climate, capable of handling high climate changes, has two critical periods during growth: flowering and early fruit set (0 – 30 days after full bloom DAFB) and mid-summer bud formation (100 – 140 DAFB). Unfavorable events at flowering and during mid-summer bud formation (June to August on the northern hemisphere) such as low temperatures, wind, water, and nutrient stress during either or both events, can affect the total number flowers that set fruits what can reduce the number of viable nuts. This research was conducted was to evaluate the almond tree's yield performance under stress conditions when sprayed with gamma-aminobutyric acid (GABA), an amino acid that accumulates rapidly in plant tissues in response to biotic and abiotic stress and acts as a growth regulator. The study was conducted in a 7-acre (2,83 ha) almond orchard from the University of California in Colusa County, northern California. This area is characterized by low winter temperatures, occasional spring frost and wet conditions during bloom and high summer temperatures. The main cultivar grown at the orchard is Nonpareil, which is also the most used cultivar in California, where plants are constantly exposed to unfavorable conditions during critical stages of development due to local climate conditions. The experiment was separated in four treatments: trees sprayed during pink bud stage (PB); tress sprayed during shell hardening stage (SH); trees sprayed during both pink bud and shell hardening stage (PB-SH); and trees that were not sprayed during any stage of plant development. From the orchard, were selected eighty trees based on yield and uniformity established from former years. From each tree were selected two branches that were tagged and evaluated throughout all stages of plant development. It was also collected data from Stem Water Potential (SWP), measured with a pressure chamber equipped with a manometer to evaluate the level of stress the plants were in, as well as the chlorophyll level measured with SPAD to ensure the plants were healthy. During harvest, 4 lb (1,8kg) were harvested separately from each evaluated tree and then taken to dry oven on 40° for a week, after dried, the final weight was measured, and each yield were calculated. Trees sprayed at PB stage during the first year, 2019, showed a decrease in stress levels and upgrades in both chlorophyll and final yield. This result was not seen for the subsequent year of 2020. New research must be developed to continue evaluating the effects of GABA application in Almond trees as a tool in abiotic stresses regulation.

Keywords: GABA. Growth regulator. Nonpareil.

RESUMO

Estresses abióticos como déficit hídrico e temperaturas extremas são fatores que afetam diretamente o desenvolvimento e produção das culturas em geral. A árvore de amêndoa (*Prunus dulcis* (Mill) D. A. Webb), apesar de ser uma planta rústica e de clima mediterrâneo, capaz de tolerar alta variação climática e baixa pluviosidade, possui dois períodos considerados críticos no seu desenvolvimento: florescimento e estabelecimento precoce de frutos (0-30 dias após florescimento total DAFT) e a formação de botões florais (100 – 140 DAFT). Sendo assim, caso afetada por fatores ambientais como frio, vento, água e falta de nutrientes durante algum desses períodos, as árvores podem ter seu número de flores drasticamente reduzido, bem como se atingida durante a formação de botões florais no verão no hemisfério norte, onde é amplamente cultivada (junho a agosto), que pode acarretar um menor número de nozes viáveis para a produção dos anos seguintes. O presente experimento foi realizado com intuito de avaliar a performance produtiva das amendoeiras, submetidas a essas condições de estresse, quando pulverizadas com ácido gama aminobutírico (GABA), um aminoácido que se acumula rapidamente nos tecidos vegetais das plantas como resposta a estresses bióticos e abióticos e atua como regulador de crescimento. O experimento foi montado em um pomar da Universidade da Califórnia, Davis no distrito de Colusa no norte da Califórnia, Estados Unidos, região caracterizada por baixas temperaturas no inverno, condições úmidas durante o florescimento da espécie e altas temperaturas no verão. As amendoeiras estudadas pertencem a cultivar Nonpareil, muito comum nesta região da Califórnia, onde estão frequentemente expostas à condições desfavoráveis durante os períodos críticos de desenvolvimento. O experimento foi dividido em quatro tratamentos: árvores pulverizadas em estádio de botão floral rosa ou “pink bud stage” (PB); estádio de endurecimento do epicarpo ou “shell hardening stage” (SH); em ambos os estádios PB e SH; e controle negativo, sem pulverização. Destes quatro tratamentos foram selecionadas oitenta árvores por uniformidade baseado no histórico de produção da área, onde duas ramificações do caule (galhos) de cada árvore foram marcadas para serem utilizados como base da contagem de flores, de número de frutos, posterior retenção dos mesmos e qualidade da noz determinada durante a colheita. Foram coletados dados de potencial hídrico da planta ou “stem water potential” (SWP) através de uma câmara de pressurização com manômetro para avaliar o estresse no qual as plantas se encontravam, assim como o índice SPAD de clorofila medido para assegurar que estavam saudáveis. Durante a colheita cerca de 1,8 kg (4 lb) foram coletadas das árvores avaliadas de cada tratamento e levadas para fornos de secagem a 40°C por uma semana. Após secas, foi registrado o peso final de cada uma e a produtividade individual de cada árvore também foi determinada por meio desta colheita individualizada. Ocorreu uma amenização de estresse, bem como aumento no teor de clorofila e rendimento e produtividade das plantas tratadas com pulverização em estádio “PB” quando comparado com o controle, no ano de 2019, sendo essa diferença não notada no ano seguinte, 2020. Novas pesquisas devem ser desenvolvidas visando avaliar os efeitos do GABA no controle dos danos por estresses abióticos na Amendoeira.

Palavras-chave: GABA. Regulador de crescimento. Nonpareil

SUMMARY

1. INTRODUCTION ... 6
2. LITERATURE REVIEW ... 9
2.1 Almonds ... 9
2.2 Market ... 9
2.3 Almonds physiology ... 10
2.4 Nonpareil ... 10
2.5 GABA ... 11
3. OBJECTIVE ... 11
4. MATHERIALS AND METHODS ... 11
4.1 Experimental site ... 11
4.2 Stem Water Potential and Total Chlorophyll Content ... 14
5. RESULTS AND DISCUSSION ... 14
5.1 Chlorophyll and Stem-water Potential ... 14
5.2 Fruit Set ... 17
5.3 Yield ... 19
5.4 Fruit Quality ... 21
6. CONCLUSION ... 22
REFERENCES ... 23

1. INTRODUCTION

The Almond tree (*Prunus dulcis* (Mill.) D.A. Webb) is a crop from the Rosaceae family, originated from Central Asia and widely consumed all around the world. The edible part of the almond is the nut's kernel, with a high nutritional and medical value that can be consumed raw, cooked and often as a substitute for milk. One of the benefits from the almond nut consumption is that the high lipid content is used by the human body as a source of calories and energy but does not contribute to cholesterol formation due to the presence of mainly unsaturated fatty acids.

The state of California, in the United States of America is the main Almond producer in the world, being responsible for more than half of the world's production. California has a Mediterranean climate, characterized by high summer temperatures and low winter temperatures, similar to where the Almonds are originated from. Even though Almonds are known to be drought tolerant, producers are still highly dependent on light, nutrients and even water to improve yield and nut quality. Both these factors plus the nut's oil condition must be of high quality to fulfill the industry's requirements.

Under stress conditions, especially during flowering and mid-summer bud formation, almonds to accumulate a non-protein amino acid, the Gamma-aminobutyric acid (GABA), in plant tissues and such stresses interfere on a final product's quality, in this case the nut production. Some examples of stresses are drought and excessive watering, extreme temperatures, salinity, and mineral toxicity.

The function of GABA in plants has attracted renewed attention in the last decade following the discovery of GABA accumulation due to stress, however, the effects of foliar applied GABA on the physiological characteristics and productivity of almond plants are not well documented. GABA was first identified in potato tubers almost a century ago, and today motivates various researches on its function in plants, as seen on this paper.

2. LITERATURE REVIEW

2.1 Almonds

The Almond [*Prunus dulcis* (Miller) D.A. Webb] is originated in Central Asia (KESTER et al., 1990) and belongs to the genus *Prunus*, in the Rosaceae family (SOCIAS I COMPANY; GRADZIEL, 2017) and it was first introduced in California during the end of the

19th century (KERSTER; GRADZIEL; GRASSELY, 1989). California's Mediterranean climate allows it to be one of the few places on Earth where almonds can grow (CALIFORNIA, 2013) and was also made possible largely because of the genetic and associated developmental/physiological diversity promoted by this genus (SOCIAS I COMPANY; GRADZIEL, 2017).

Almond growing in California became successful after the selection of adapted cultivars and recognition of this self-incompatible characteristic of the California germplasm (KERSTER; GRADZIEL; GRASSELY, 1989). Today, California accounts for almost 80% of the world's production, and nearly 70% of California almond farms are 100 acres or less, (ALMOND ALMANAC, 2020) driven mostly by family farmers owned and operated by generations who live there and plan to pass it on to their children (USDA, 2019).

2.2 Market

The United States is the world's largest almond producer, having produced 1.936.840 tons of almonds in 2019 (FAOSTAT, 2019) and exports worldwide, having exported the amount of almost U$ 5 million dollars in 2019 (CDFA, 2020).

The world's almond consumption in 2013/14 was 1.049.880 tons and in 2019/20 was 1.346.220 tons (STATISTA, 2020), showing significant growth during the years. The maximum productivity for a mature and healthy orchard is 5,000 lbs. (2268 kg) though this is rarely achieved. In 2019 almond crops totaled $6,09 billion dollars (CDFA, 2019) with an average orchard production of 2,600 lbs. per acre (around 2914 kg/ha). Environmental stresses are the main cause for the fluctuations in yield and failure to reach full yield potential (SAA et al., 2013).

Almond nuts are marketed in a variety of ways depending on the cultivar differences (ROSENGARTEN, 1989). These may include "in-shell", "shelled natural", "blanched", "roasted in oil", "dry roasted" and "paste", being also consumed fresh in some countries.

2.3 Almonds physiology

Even though almond trees are usually considered to be drought tolerant (TORRECILLAS et al., 1996), the species *Prunus dulcis* is highly dependent on light, soil nutrients, and water to improve fruit set and yield (KLEIN et al., 2015; BRITTAIN et al., 2014) and growers are still dependent on irrigation and nutrient inputs to produce high yields (CASTEL; FERERES, 1982; MICKE, 1996). The content of the leaf pigments is also affected by plant exposure to various stresses (GOMES et al, 2017). Thus, abiotic stresses can negatively impact on top quality results.

In almond two periods have been established as critical to tree productivity in the current year and in the subsequent year. These critical periods are flowering and early fruit set (0-30 days after full bloom, DAFB), and mid-summer bud formation (100-140 DAFB). Unfavorable events at flowering (cold, wind, water, or nutrient stress) can reduce the number of flowers that set fruit and the number of fruits that are retained by the tree, while unfavorable conditions (water stress, drought stress, within tree competition for carbon or nutrients) during bud formation in late June-early August can reduce the number of viable cuds for the subsequent years fruit production (VALDEBENITO et al., 2017).

The 2019 almond crop experienced unusual weather in the early part of the season. Significant rainfall during the bloom hindered pollination. However, an extended bloom period provided the opportunity to compensate for disruptions and allow for more overlap between blooming varieties.

2.4 Nonpareil

The Nonpareil cultivar is the standard cultivar used in California, for both production and marketing. Its kernel's qualities include a smooth, uniform, bright and attractive appearance, and unique versatility for processing (KESTER et al., 1990). This cultivar has also early harvest, good tree structure, the potential for high yield, and some frost resistance in the pre-bloom stage and became the dominant cultivar because of these good qualities (TAYLOR, 1916). Its paper shell is vulnerable to worm damage and bird and rodent loss and is also self-incompatible. Nonpareil normally have a single flower in each bud, and flowers are pentamerous (five petals and five sepals) with various number of stamens and one pistil.

2.5 GABA

Gamma-aminobutyric acid (GABA) is a non-protein amino acid and a significant component of the free amino acid pool in most organisms, (RABESH et al., 2015). GABA is highly soluble in water: structurally it is a flexible molecule that can assume several conformations in solution (SHELP et al., 2007). GABA concentration in plants is low, ranging from 0.03 to 2.00 mmol g^{-1} fresh weight – FW, and accumulates rapidly in plant tissues in response to biotic and abiotic stress (RHODES et al., 1986). Its accumulation in agriproducts has been attractive because it processes the physiological function of hypotension induction, and it is naturally caused in plants by environmental stress conditions such as hypoxia, temperature shock, and mechanical damage (UENO et al., 2011).

GABA is mostly known as a metabolite and it is not yet proven if this accumulation happens through the regulation of carbon metabolism or via an unidentified signaling pathway, however, GABA gradients are required during the female reproductive cycle to guide pollen tubes to the ovaries, helping to ensure a successful fertilization.

There are a few methodologies used to quantify GABA, one being by Scholz et al, 2015, where multiple leaves of a plant are collected, cut according to the age stage, homogenized in a Geno/Grinder® 2010 equipped with aluminum racks. These racks are cooled in liquid nitrogen prior to usage to prevent a thawing of leaf material during the homogenization process and then the amino acids (GABA) is extracted twice with a total of 2ml of methanol on ice.

3. OBJECTIVE

The need to work and study new technologies to assist plants to cope with these adverse environmental conditions through biochemical and physiological manifestations, such as GABA production under stress conditions, and considering the importance of Almond production to the state of California, there is the need of developing tools to work towards better productivity.

In this study we hypothesize that the well-timed foliar application of the GABA, may reduce plant stress levels at critical developmental phases and that this reduction in plant stress will result in greater fruit set, yield, and bud retention for return bloom.

In order to confirm or not this hypothesis the goal of this study was to monitor physiological changes in almonds under abiotic stress and GABA application, to determine whether GABA application can reduce the detrimental effects of abiotic stress during flowering or mid-summer bud formation.

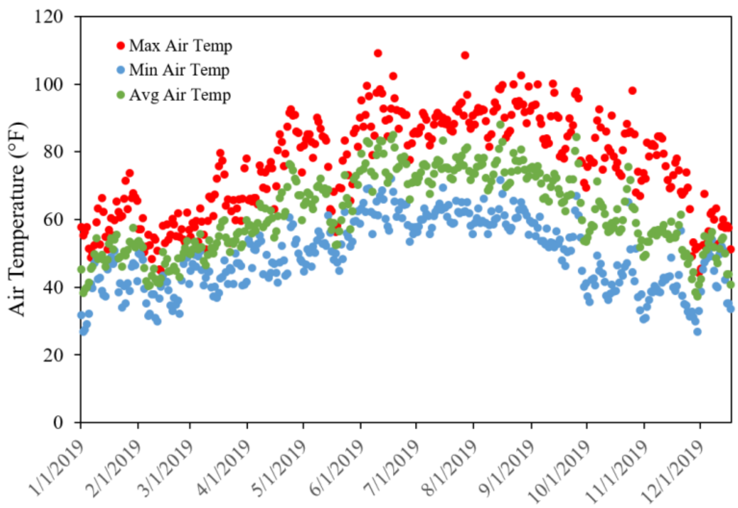
4. MATHERIALS AND METHODS

4.1 Experimental site

The present experiment was conducted on a University of California managed research station in Colusa County, California, located at 38°58’12” N/122°04’10” W in both the 2018/19 and 2019/20 crop.

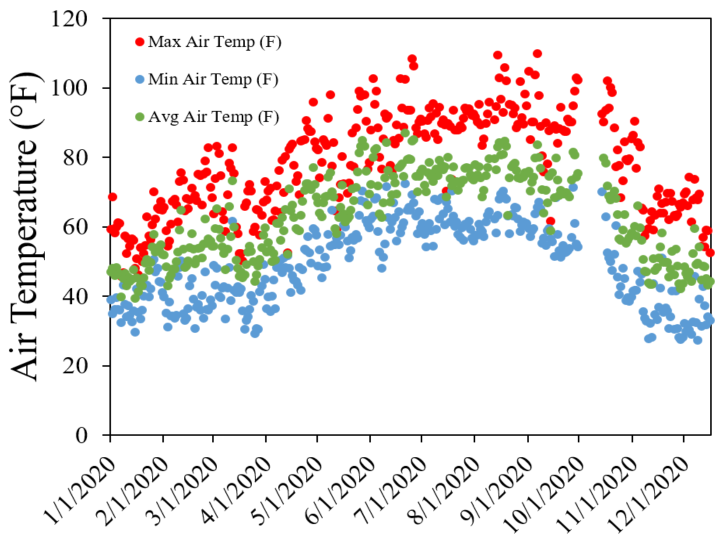
The site is extensively instrumented for environmental monitoring and has been monitored for 14 years and the yield history of each tree is known. This site’s location is classified as Csa according to the Köppen-Geiger climate classification (PEEL et al., 2007), which is characterized by low temperatures, occasional spring frost, and wet conditions during bloom – March to May - and high summer temperatures – June to August (Fig). The orchard occupies a 7-acre area (2,83 ha) and has several cultivars and rootstocks including cultivar Nonpareil, the most widely cultivated rootstock/scion combination in California and the one evaluated in this study.

Figure 4.1 – Daily air temperature at the experimental site in Colusa County.



Source: University of California, Davis (2019).

Figure 4.2 – Daily air temperature at the experimental site in Colusa County.



Source: University of California, Davis (2020).

The experiment was started in the crop of 2018/19 when eighty individual trees were selected for evaluation based on known yield history. The trees were sprayed with GABA at two different stages: pink bud stage and Shell Hardening stage. Four treatments were established according to Table 1. Two limbs (around 15-30% of total tree volume) were labeled and used for flower count, nut set and retention and nut quality. A total of one hundred and sixty branches were monitored (two limbs per tree, forty limbs per treatment and four treatments) across multiple sample dates during that year's crop (2018/19) and the next 2019/20.

Table 4.1 – Treatment's description.

Treatment	**Description**
PB Spray	Sprayed at Pink Bud Stage
SH Spray	Sprayed at Shel Hardening Stage
PB-SH Spray	Sprayed at both Pink Bud and Shell Hardening stage
Control	Not sprayed

Source: University of California, Davis (2019).

During the harvest 4 lb. (1,8kg) of fruit samples were collected from the trees in each treatment and weighed at the field site. These samples were then dried in air driers at 40°C for a week and were later cleaned and weighted again for record. The yield of each individual tree was also determined by separately harvesting the trees. Final fruit yield was adjusted for moisture loss by multiplying the field weight of individual tree with the weight loss from the started 4lb. sample.

4.2 Stem Water Potential and Total Chlorophyll Content

A pressure chamber equipped with a manometer (MCCUTCHAN; SHACKEL, 2019) was used to measure the Stem-water Potential (SWP). SWP is a plant-based indicator or orchard water status and integrates root health, soil moisture and weather conditions into each measurement. The measurements were made at midday from the beginning of May to mid-June. First it was selected shaded leaves from lower canopy branches, from the trees selected for the experiment, and they were covered in opaque bags for 15 minutes prior to evaluation to simulate a more stable condition for all leaves and guarantee that they were all in a similar state before measuring. After the 15 minutes the leaves were measured with the pressure chamber with a manometer. The results on this study varied from -6 to -16 bars being the highest (-6) the least pressure it was needed to extract the water from the leaf therefore the least water stress. Measurements of midday stem water potential (SWP) were made on lower canopy, shaded leaves which had equilibrated for at least 15 minutes.

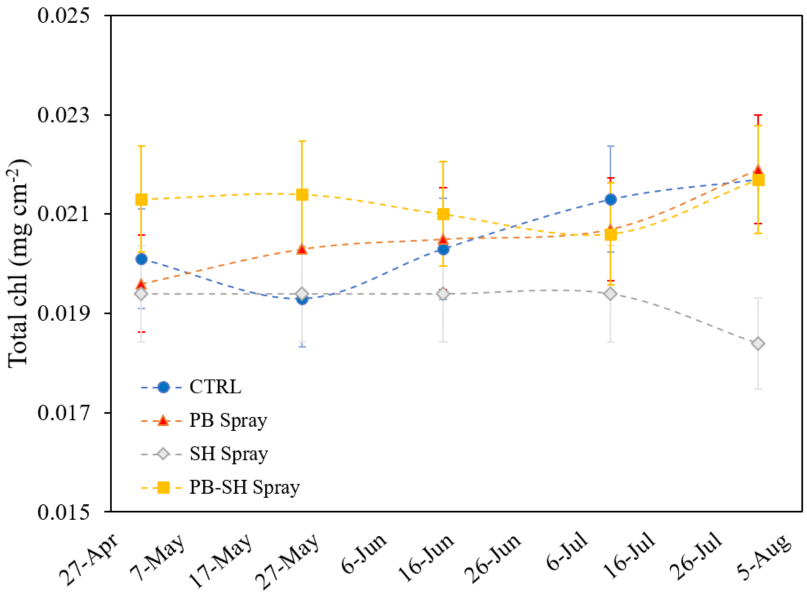
Total chlorophyll content (Chlorophyll a plus Chlorophyll b) of leaves was also measured using an atLEAF chlorophyll meter (atLEAF CHL BLUE, FT Green LLC). Values were obtained by converting the atLEAF CHL values in SPAD and considering the relationship among chlorophyll content and SPAD units. SPAD is a widely used device for accurate and non-destructive measurement of leaf chlorophyll concentration. Measurement with the SPAD produce relative values that are proportional to the amount of chlorophyll and later there is the need to convert these values into absolute units (LING; HUANG; JARVIS; 2010).

5. RESULTS AND DISCUSSION

5.1 Chlorophyll and Stem-water Potential

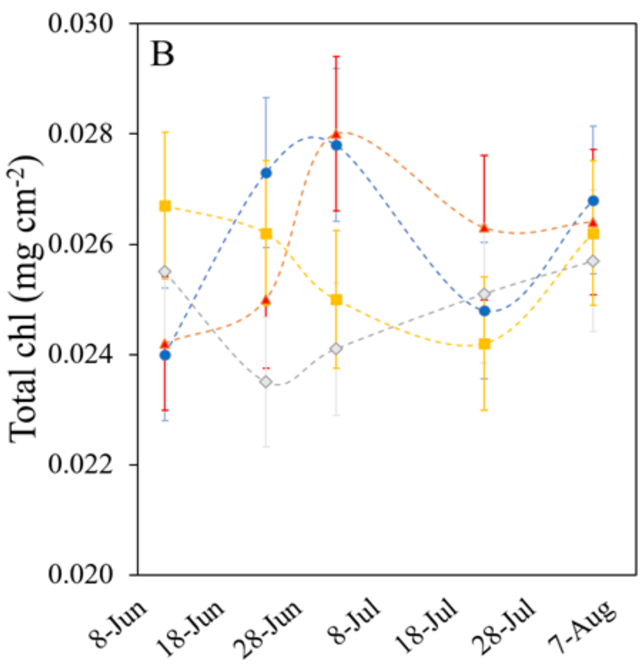
In both the 2019 and the 2020 studies, it was not observed any significant changes in chlorophyll content throughout the season, indicating that all monitored trees were healthy and showing no evident signs of stress during the trial.

Figure 5.1 – Total chlorophyll content in leaves of mature almond trees treated with different doliar fertilizers. Data are means of eight measurements. Error bars indicate standard error.



Source: University of California, Davis (2019)

Figure 5.2 – Total chlorophyll content in leaves of mature almond trees treated with different foliar fertilizers. Data are means of eight measurements. Error bars indicate standard error.



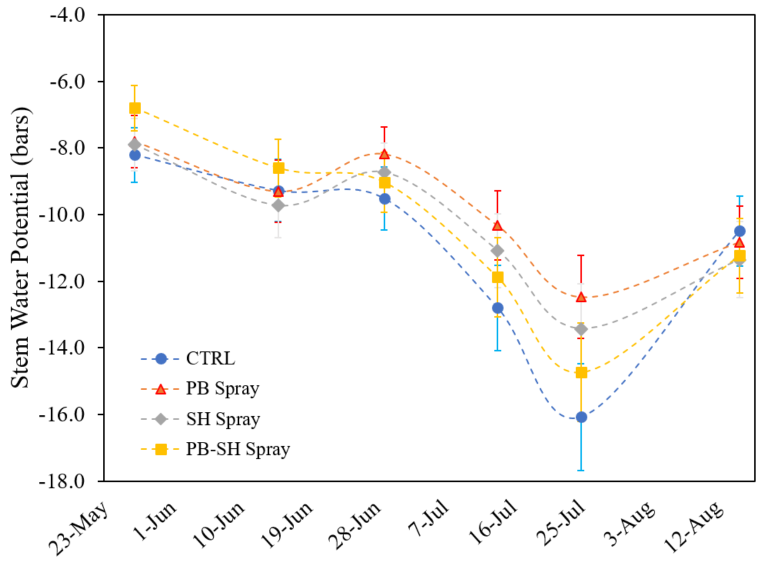
Source: University of California, Davis (2020)

The estimated level of Chlorophyll from the treatments did not suffer any statistic significant changes throughout the study under abiotic stresses, which indicates that the stresses did not affect expressively the photosynthetic cells.

GABA is shown to improve the significantly promote photosynthetic rate and chlorophyll content (SALAH *et al.*, 2019), and to enhance the Chlorophyll A content and reduce the Chlorophyll B content, but not the total Chlorophyll content compared to control plants (JIN *et al.*, 2019).

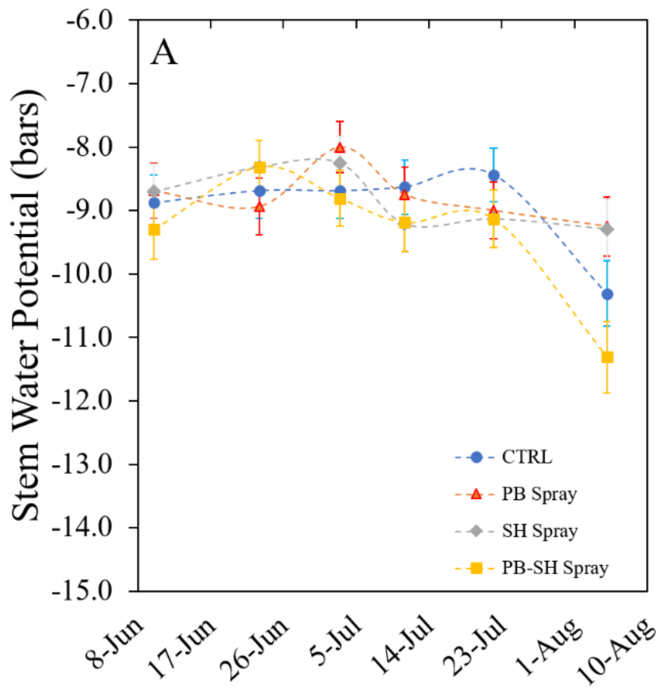
In addition to evaluating the chlorophyll content, it was also monitored the water status (SWP) of the trees, as seen below.

Figure 5.3 - Seasonal midday stem water potential of almond leaves treated with different foliar fertilizers. Data are means of eight measurements. Error bars indicate standard error. Unit of measurement: 1 bar = 100 kPa.



Source: University of California, Davis

Figure 5.4 Seasonal midday stem water potential of almond leaves treated with different foliar fertilizers. Data are means of eight measurements. Error bars indicate standard error. Unit of measurement: 1 bar = 100 kPa.



Source: University of California, Davis (2020)

For the first dates of sampling in both 2019 and 2020 (early-May to mid-June), SWP measurements of each treatment were not different compared to the control treatment. Readings varied from -6 to -10 bars indicating low plant stress early in the season, if any. However, during the phase of growth just before the onset of hull split (late June) and during hull split the measurements varied from -10 to -16 bars in 2019 and from -9 to -15 bars in 2020 indicating mild to moderate stress.

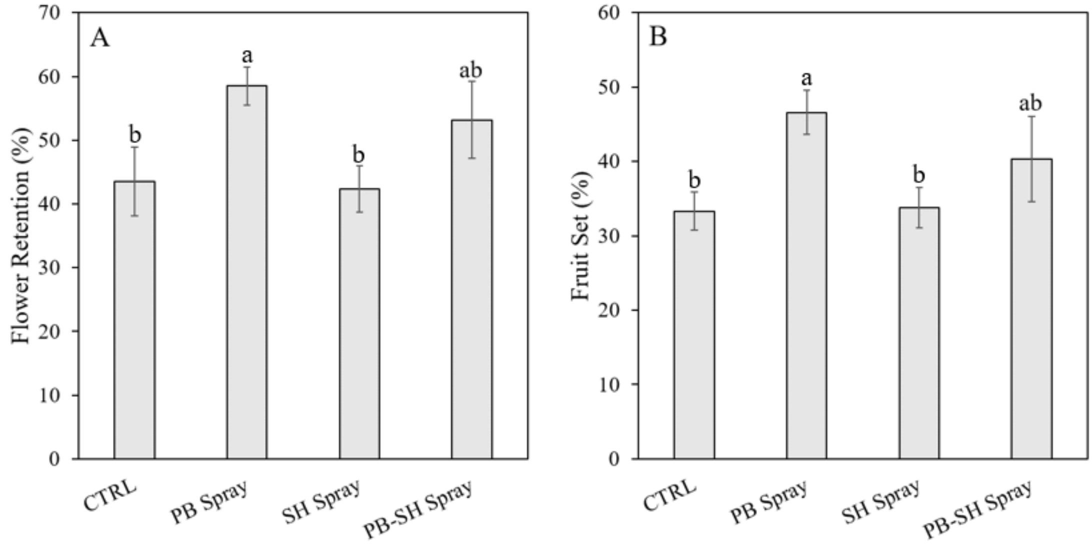
For both year measurements PB treatment showed the highest SWP (least water stress), and the control treatment showed the lowest SWP (most water stress). Moderate to high stress may affect shoot growth and fruit development. In general, all treatments alleviated water stress in treated trees compared to the control trees. Nayyar et al. (2014) found that exogenous application of GABA to stressed plants of rice (*Oriza sativa* L.) significantly improved growth and survival, showing that GABA appears to provide partial protection from the heat to rice plants. Accordingly, Li et al., (2016) found that GABA improved heat tolerance also in creeping bentgrass (*Agrotis stolonifera* L.).

5.2 Fruit Set

For the 2019 crop, while the amount of flower retention and fruit set varied depending on application timing (pink bud or shell hardening), the pattern of flower retention and fruit set over the season was generally consistent with early treatments (PB and PB-SH treatments) showing increased flower retention and fruit set compared to late (SH treatment) and control treatments. Flower retention percentages ranged from 38 to 51%, 52 to 62%, 33 to 51%, and 38 to 67% for control, PB, SH, and PB-SH treatments, respectively. Fruit set percentages ranged from 29 to 41%, 42 to 55%, 32 to 41%, and 32 to 55% for control, PB, SH, and PB-SH treatments, respectively. Flower retention was measured both years after first (defective flowers) and second (unfertilized flowers) drop.

It must be emphasized that the biggest flower retention and fruit retention happened with the PB Spray treatment in comparison to the control treatment, that was similar to PB-SH Spray treatment. The PB-SH Spray allowed greater values in both variables evaluated in this case (flower retention and fruit set), being the recommended one.

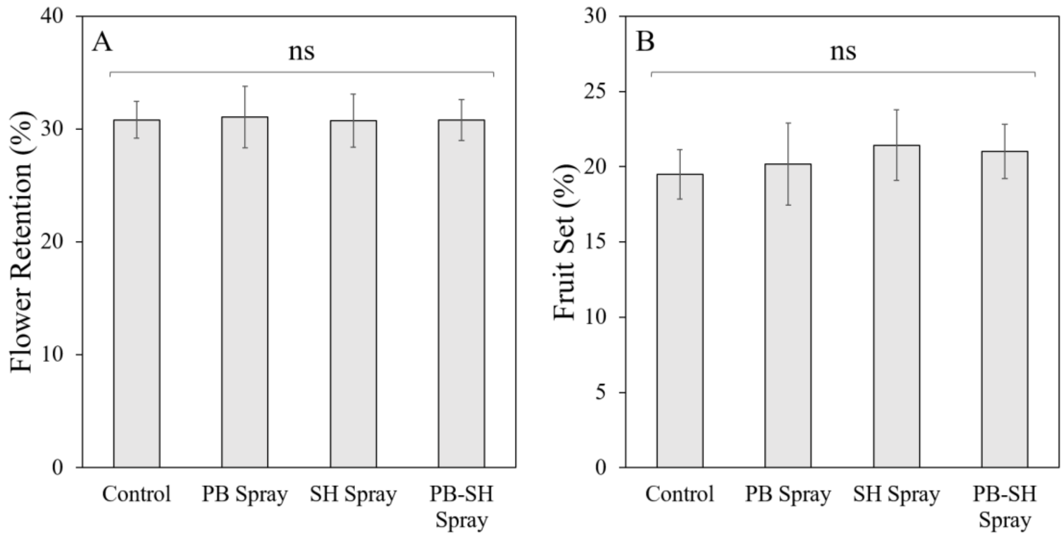
Figure 5.5 - Percentage of flower retention after first second drop (A); and (B) fruit percentage post June drop (6-7 weeks after bloom). Each bar is the average ± standard deviation (n = 4). Different letters denote significant differences (P<0.05)



Source: University of California, Davis (2019)

For the 2020 study the flower retention percentages ranged from 26 to 33%, 26 to 36% and 23 to 36% and 29 to 32% for control, PB, SH, and PB-SH treatments, respectively. Fruit set percentages ranged from 17to 21%, 19 to 22%, 20 to 26%, and 21 to 24% for control, PB, SH, and PB-SH treatments, respectively. No significant differences were observed compared to the control.

Figure 5.6 – Percentage of flower retention (A); and (B) fruit set percentage post June drop (6-7 weeks after bloom). Each bar is the average ± standard deviation (n = 4). *ns = no significant difference (P < 0,1)



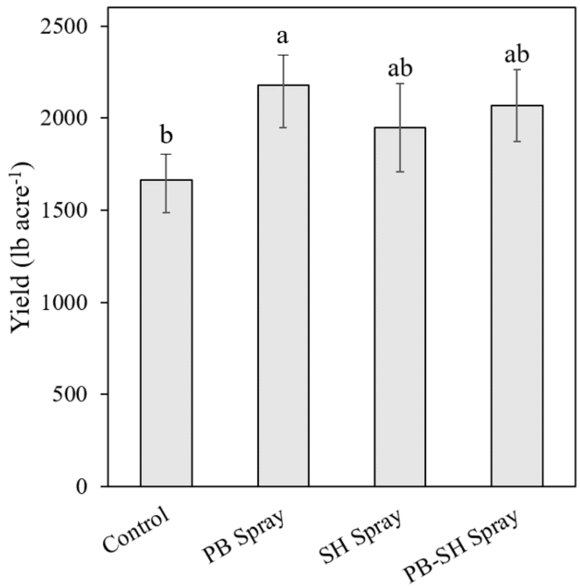
Source: University of California, Davis (2020)

GABA treatments helps to mitigate injuries in flowers (RAMOS-RUIZ; MARTINEZ & GERTRUDE KNAUF-BEITER, 2017) and reportedly interacts with the fruit's capacity to resist adverse conditions (CEKIC, 2018).

5.3 Yield

For yield, in 2019 all treatments positively affected yield, which ranged from 1,300 to 2,800 lbs./acre (1160 to 2500 kg/ha). Total tree yield was lowest for the control trees, while yield for trees treated with foliar gamma-aminobutyric acid was generally higher. Moreover, relative to non-treated trees, PB-treated trees significantly increased yield by 30%. Additionally, SH and PB-SH treatments increased yield by 17 and 25%, respectively, which indicates the fact that PB Spray treatments had a statistically superior results in comparison to the other treatments.

Figure 5.7 – Total fruit yield of almonds treated with different foliar fertilizers.

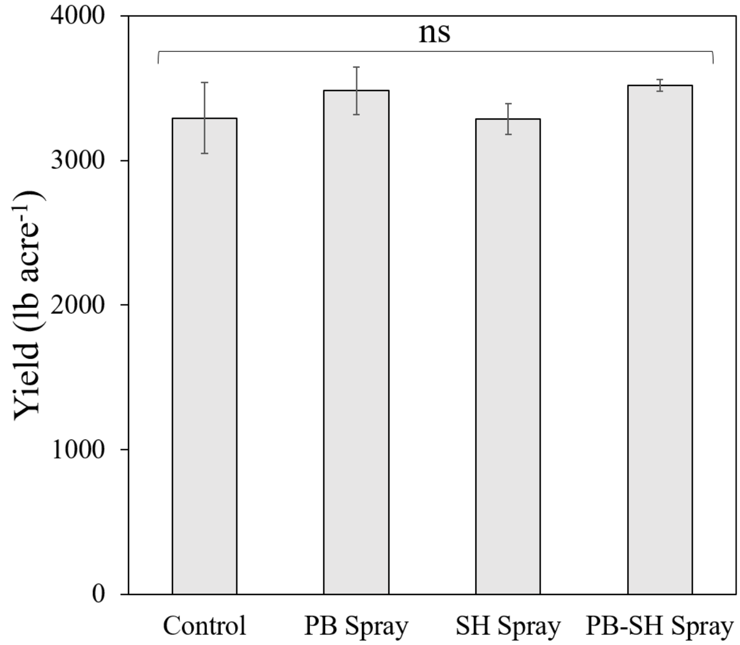


Each bar is the average ± deviation *n = 4). Different letters denote significant differences (P < 0.1)

Source: University of California, Davis (2019)

For the 2020 crop, the same measurements of yield ranged from 2700 to 3700 lbs./acre (2410 to 3300 kg/ha). Total tree yield was lowest for the control trees and SH spray, while yield for trees treated early (PB and PB-SH sprays) was moderately higher but were not statistically divergent. On average, relative to non-treated trees, PB and PB-SH treatments increased yield by 6 and 7%, respectively. Overall, biostimulants application under drought conditions are shown to improve the almond yield response, varying from cultivar according to Gutiérrez-Gordillo et al.(2019).

Figure 5.8 - Total fruit yield of almonds treated with different foliar fertilizers.



Each bar is the average ± deviation *n = 4). Different letters denote significant differences (P < 0.1)

Source: University of California, Davis (2020)

5.4 Fruit Quality

In general, for both years of study of the fruit quality parameters analyzed, none were significantly affected by the treatments compared to the control. However, it is important to note that all treatments had a positive effect on fruit weight which might have contributed to higher yields observed for the treated trees.

First year results varied on average from 57 to 66g, 67 to 70g, 65 to 69g, and 66 to 71g for the control, PB, SH, and PB-SH treatments, respectively. Four-pound dry weight and fruit quality, Hull, in-shell, and kernel average weight per 50 fruits, and Defects per 50 fruits (TABLE 5.4.1).

Table 5.4.1 – Four-pound (lb.) dry weight (DW) and fruit quantity.

Treatment	*4 lb. DW*	*4 lb. Count*
Control	3.89±0.02	269±5.7
PB Spray	3.89±0.03	253±12.4
SH Spray	3.87±0.03	261±16.1
PB-SH Spray	3.89±0.02	270±19.7

Values represent means of four different replications (n = 4) and ± represents standard deviation

Source: University of California, Davis

Table 2. Hull, in-shell, and kernel average weight (g) per 50 fruits.

Treatment	*Hull*	*In-shell*	*Kernel*
	g per 50 fruits		
Control	188.9±22.4	99.2±4.8	64.4±4.1
PB Spray	152.3±9.4	103.5±1.6	68.1±1.0
SH Spray	161.9±17.2	93.9±5.6	65.7±1.5
PB-SH Spray	170.3±14.1	106.9±2.2	68.0±1.3

Values represent means of four different replications (n = 4) and ± represents standard deviation

Source: University of California, Davis

Table 3. Defects per 50 fruits.

Treatment	*Double*	*Twin*	*NOW*	*Blank*
Control	4.7±2.6	2.0±1.5	2.3±0.9	2.7±1.5
PB Spray	2.3±0.9	1.8±0.7	1.2±1.0	2.0±0.1
SH Spray	2.0±0.6	5.7±1.6	3.4±0.3	3.0±0.6
PB-SH Spray	7.3±3.0	3.7±0.9	1.3±0.9	3.0±0.6

Values represent means of four different replications (n = 4) and ± represents standard deviation. NOW = navel orange worm

Source: University of California, Davis

Second year (2020) four-pound dry wight and fruit quality, Hull, in-shell and kernel average weight per 50 fruits, and Defects per 50 fruits are represented results represented on tables 4-6.

Table 4. Four-pound (lb.) dry weight (DW) and fruit quantity.

Treatment	*4 lb. DW*	*4 lb. Count*
Control	3.74±0.03	451±1.0
PB Spray	3.74±0.03	395±8.8
SH Spray	3.72±0.03	370±16.5
PB-SH Spray	3.74±0.02	423±13.0

Values represent means of four different replications (n = 4) and ± represents standard deviation

Source: University of California, Davis

Table 5. Hull, in-shell, and kernel average weight (g) per 50 fruits.

Treatment	Hull	In-shell	Kernel
	g per 50 fruits		
Control	105±1.2	90±1.0	56±0.6
PB Spray	120±0.7	96±0.7	63±0.9
SH Spray	119±2.3	93±2.1	60±1.0
PB-SH Spray	105±2.6	86±1.8	57±0.2

Values represent means of four different replications (n = 4) and ± represents standard deviation

Source: University of California, Davis

Table 6. Defects per 50 fruits.

Treatment	*Double*	*Twin*	*NOW*	*Blank*
Control	1±0.1	2±0.3	1±0.1	2±0.2
PB Spray	2±0.3	2±0.4	1±0.1	2±0.2
SH Spray	2±0.3	2±0.4	1±0.3	1±0.1
PB-SH Spray	1±0.1	3±0.1	1±0.1	1±0.1

Values represent means of four different replications (n = 4) and ± represents standard deviation. NOW = navel orange worm

Source: University of California, Davis

On average, kernel weight (50 fruits) varied from 57 to 66g, 67 to 70g, 65 to 69g, and 66 to 71g for the control, PB, SH, and PB-SH treatments, respectively. According to Gutiérrez-Gordillo et al. (2019) the application of biostimulants on almond trees can affect in nut weight, but it varies from different cultivars.

5.5 Pollination and Biannuality

The definition of pollination is defined as the transport of pollen from the anthers to the stigma (GRAZIEL, 2017), and many horticultural crops benefit from it, including Almonds (KLEIN et al, 2007) since they depend exclusively on honeybees to pollinate the orchards as its flowers are self-incompatible. Considering the importance of Almond production in California and the pollination factor, in further studies it could also be evaluated the effect of pollination on final production amongst the factors already shown in this paper.

Another factor that can also be further evaluated, is the possibility of a biannuality effect on Almond’s productivity, since the results show one year’s production was superior to the next one. This biannuality phenomenon is seen in other crops such as coffee (*Coffea arabica* L.) (SILVA et al 2013) and could be a motivation for further studies in Almonds.

6. CONCLUSION

In the first year of research, 2019, GABA application during the pink bud stage (PB Spray) is the one treatment that presented the best performance under stress by drought and higher chlorophyll levels in comparison to the control treatment, as well as yield, flower retention and set fruit.

In the second year of research, 2020, the same result was not observed considering the same treatments and methods of evaluation. The results did not vary nor had a statistic difference within all four treatments. Hence, there is a need to continue studying and evaluating these factors in following year’s crops.

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