Original Research Article

Gibberellins $A_{4/7}$, 6-Benzylaminopurine and Prohexadione Calcium Modify Growth, Development and Quality of Raspberry

ABSTRACT

Objectives: Currently, Mexico is the second-largest producer of red raspberries in the world. A steady increase in consumption, quality demands from the international market and its high value call for practices that can enhance production and quality of this crop. A viable option is the use of bioregulators, and this study evaluated the effects of prohexadione calcium (P-Ca), gibberellins $A_{4/7}$ ($GA_{4/7}$) and 6-benzylaminopurine (6-BAP) on the growth, development and quality of raspberry cv. UANC-2022.

Study Design: The study was set up under a completely randomized block design with a 4x3 factorial arrangement. The first factor was the bioregulators used (P-Ca, $GA_{4/7}$, 6-BAP and the combination of $GA_{4/7}$+6-BAP) and the second factor was the number of applications.

Location and Duration of the Study: Conducted at the Department of Horticulture, Universidad Autónoma Agraria Antonio Narro, in Saltillo, Mexico, during the period March to October 2022.

Methodology: Treatment applications were carried out at three specific times during the growing cycle, with a first application made one day after pruning (DAP), the second at 48 DAP and the third at 70 DAP. All treatments were applied to the foliage.

Results: P-Ca temporarily reduced lateral shoot growth (LSG), improved reproductive development and yield, and increased fruit quality. $GA_{4/7}$ promoted LSG, reproductive development, yield, and improved fruit quality. 6-BAP enhanced fruit quality. The combination of $GA_{4/7}$+6-BAP increased LSG in the early weeks and improved fruit quality.

Conclusion: The bioregulators $GA_{4/7}$, 6-BAP and P-Ca bring targeted benefits to growth, development and fruit quality, making them a viable management practice that can be implemented in the production of raspberries.

Keywords: Berries, bioregulators, cytokinins, growth retardants, Rubus idaeus

1. INTRODUCTION

Berries constitute a group of small fruits, among which the red raspberry (*Rubus* spp., Rosaceae family) is included [1]. In recent years this crop has generated numerous studies in the fields of agriculture, food and pharmaceuticals due to an interest in its high content of proteins, minerals, vitamins and polyphenolic compounds with significant antioxidant capacity, resulting in a healthy fruit for human consumption [2]. In recent years, raspberry cultivation in Mexico has grown in cultivation area and production, with a total yield of 165,677 t in 2021, making it the second-largest world producer with a 16.3% share of the global volume after Russia's 20.3% [3]. Mexican raspberries have tapped into 33 international markets, with the United States being the primary buyer with 2021 sales of $1,217 million dollars. The Mexican states of Jalisco, Michoacán and Baja California are the leading producers, contributing 70.2, 18.0 and 9.7% to national production, respectively [4]. Despite its economic importance, per capita consumption in Mexico is 315 grams, representing only 0.7 % of the national production [3,4].

Bioregulators are minerals, substances, plant hormones, synthetic hormone analogs and even microorganisms that cause an alteration in some physiological or metabolic processes of plants. Depending on their effect, these can be classified as promoters, inhibitors, or retardants [5,6]. Gibberellins (GAs) and cytokinins (Cyt) are endogenous plant growth-promoting hormones. They stimulate cell division, differentiation and elongation, and also influence developmental processes such as seed germination, stem elongation, bud dormancy, flowering, and leaf and fruit expansion [7,8]. Prohexadione calcium (P-Ca) is a plant growth retardant [9] with a mode of action that blocks the formation of GAs in their final stages of biosynthesis. This effect is temporary and is particularly beneficial in deciduous fruit trees as it does not affect the growth of subsequent biological cycles [10,11]. P-Ca has been primarily used to control vegetative growth in some crops by reducing growth and redirecting assimilates to other tissues, which has led to increased root, leaf, flower and fruit formation, as well as improved yield and quality in various horticultural crops [12,13,14].

The increasing demand for raspberries, a market's requirement for high-quality products and the significant social and economic impact of this berry, coupled with the adverse effects of climate change and seasonality, compel researchers to conduct studies that maximize the potential of this crop. In this regard, the use of bioregulators present a viable alternative, as these compounds are noted for their ability to temporarily modify gene expression, benefiting the physiology and metabolism of crops, resulting in increased yield, improved fruit quality and influence on harvesting periods. These aspects could help meet current market demands, which is why the present study was conducted with the aim of providing relevant information on the effects of P-Ca, $GA_{4/7}$ and 6-BAP on the growth, development and quality of a red raspberry crop.

2. MATERIALS AND METHODS

2.1. Establishment and Management of the Experiment

The study was conducted in a tunnel-type greenhouse at the Department of Horticulture of Universidad Autónoma Agraria Antonio Narro (UAAAN), in Buenavista Saltillo, Coahuila, Mexico (25.456056 N, 101.035139 W, at 1,742 meters above sea level). The experiment took place from March to October 2022. On March 12, 2022, 45-day old seedlings (average height of 10 cm) of the raspberry cultivar UANC-2022 were transplanted into 12 L polyethylene bags. The substrate used was a mix of coconut dust, coconut fiber and peat moss (1:2:2, v/v) with a 25% air-filled porosity [15]. The plants were spaced to a density of 6.2 plants/m^2. The plants were fertigated daily with a modified 75% Steiner solution [16]. Pests and diseases were managed using natural extracts (garlic, chili and orange) and chemical products (Imidacloprid and captan). A "V" shaped tutoring system was implemented [17] and the crop was managed to a single stem. At 82 days after transplant (DAT), when the plants reached a height of 65-70 cm, their apical apexes were removed. Subsequently, two lateral shoots were allowed to develop, carrying them through the entire production phase. A continuous defoliation of the senescing leaves of the main stem was also carried out. For pollination a bumblebee (*Bombus ephippiatus*) hive, supplied by Biobest (Zapopan, México) was used. A pruning was conducted at the end of the production cycle (217 DAT), leaving only the proximal 10-15 cm of each shoot.

2.2. Treatments and Statistical Analysis

The study employed a randomized complete block design with a 4x3 factorial arrangement, where the first factor was the bioregulators and the second factor was the number of applications (Table 1), resulting in a total of 13 treatments with four replications. Each experimental unit consisted of a raspberry plant with a stem bifurcated into two lateral shoots. The results were subjected to an analysis of variance (ANOVA) and a means comparison test using Tukey's method ($P \leq 0.05$) using SAS System for Windows 9.0 software.

2.3. Treatment Application

Treatment applications were made at three specific times during the growing cycle. The first application was done one day after pruning (DAP), the second occurred when all plants showed their first flowers (48 DAP) and the third was conducted one week before the start of harvest (70 DAP). All three foliar applications were performed with a manual pump, ensuring a complete coverage of the foliage. These applications were carried out between 8:00 AM and 10:00 AM.

Table 1. Description of treatments evaluated in raspberry cv. UANC-2022.

Bioregulators	**Number of Applications (concentrations in mg L^{-1})**	**Abbreviation**

	1	2	3	
Prohexadione calcium	100	---	---	P-Ca1
Prohexadione calcium	100	25	---	P-Ca2
Prohexadione calcium	100	25	25	P-Ca3
Gibberellins$_{4/7}$	100	---	---	GA1
Gibberellins$_{4/7}$	100	25	---	GA2
Gibberellins$_{4/7}$	100	25	25	GA3
6-benzylaminopurine	100	---	---	6-BAP1
6-benzylaminopurine	100	25	---	6-BAP2
6-benzylaminopurine	100	25	25	6-BAP3
Gibberellins$_{4/7}$ + 6-benzylaminopurine	100	---	---	GA+6-BAP1
Gibberellins$_{4/7}$ + 6-benzylaminopurine	100	25	---	GA+6-BAP2
Gibberellins$_{4/7}$ + 6-benzylaminopurine	100	25	25	GA+6-BAP3
Control (distilled water)	---	---	---	Control

2.4. Vegetative Growth Evaluation

Lateral shoot growth (LSG) was periodically evaluated with a measurement tape (Foy Model 142124). Shoot diameters (SD) were taken with a digital caliper (Steren HER-411) from the base of each of the two shoots, and averaged at the end of the cycle (215 DAT). Also, the total number of leaves (LN) from both shoots was recorded at the end of the study. The fresh weight of the shoots (FWS) was obtained using a digital scale (Rhino BAR-7), and their dry weight (DWS) after drying at 65° C for 48 hours in an oven (MAPSA HDT-18).

2.5. Reproductive Development and Yield Evaluation

The number of inflorescences per plant (NIP) was determined by counting these on each shoot between 33 and 75 DAP. The number of flowers per plant (NFP) was recorded between 40 and 85 DAP. The number of harvested fruits (NHF) was recorded weekly during the 9 weeks of harvest. The weekly production (WP) was obtained by weighing the harvested fruits on a digital scale (Rhino BACI-5), and those values were subsequently summed to determine the total yield per plant.

2.6. Fruit Quality

The vitamin C content (VCC) was determined following the methodology of Padayatt *et al*. [18], with 3 samples per replicate. The values are reported in mg per 100 g using the following formula:

$$mg\ 100\ g^{-1}\ of\ VCC = \frac{(milliliters\ of\ Thielmann\ reagent\ used)(0.088)(total\ volume)(100)}{milliliters\ of\ titrated\ aliquot(grams\ of\ sample)}$$

Anthocyanin content (AC) was determined using a differential pH method [19] using a JENWAY 6320D spectrophotometer. Three measurements were taken per replication and reported in mg per 100 g. For the potassium content (KC) and total soluble solids (ºBrix), 10 samples were taken per replication, and evaluated at two, three, four, five and six weeks of production, starting from the first week of harvest for each treatment. The following was done to each sample immediately after harvest: two fruits were selected, mashed in a mortar to extract all their juice, then the juice was drawn with a syringe and deposited into the sensor of the portable potassium meter (HORIBA LAQUAtwin K-11), and into the refractometer (ATAGO ATC-1 Brix 0-32 %) cell to measure ºBrix.

3. RESULTS AND DISCUSSION

3.1. Vegetative Growth

Vegetative growth, measured as lateral shoot growth (LSG) (Figure 1), was significantly affected ($P \leq 0.05$) by the application of bioregulators. From 12 DAP until 40 DAP, the treatments P-Ca1, P-Ca2 and P-Ca3 showed reduced growth compared to the control. In contrast, treatments with $GA_{4/7}$ (GA1, GA2 and GA3), as well as in combination with 6-BAP (GA+6-BAP1, GA+6-BAP2 and GA+6-BAP3), resulted in greater shoot length compared to the control. However, plants treated with P-Ca basically resumed a growth comparable to the control from 47 DAP until the end of the study. Likewise, all other bioregulator treatments showed LSG similar to the control plants from 47 DAP onwards.

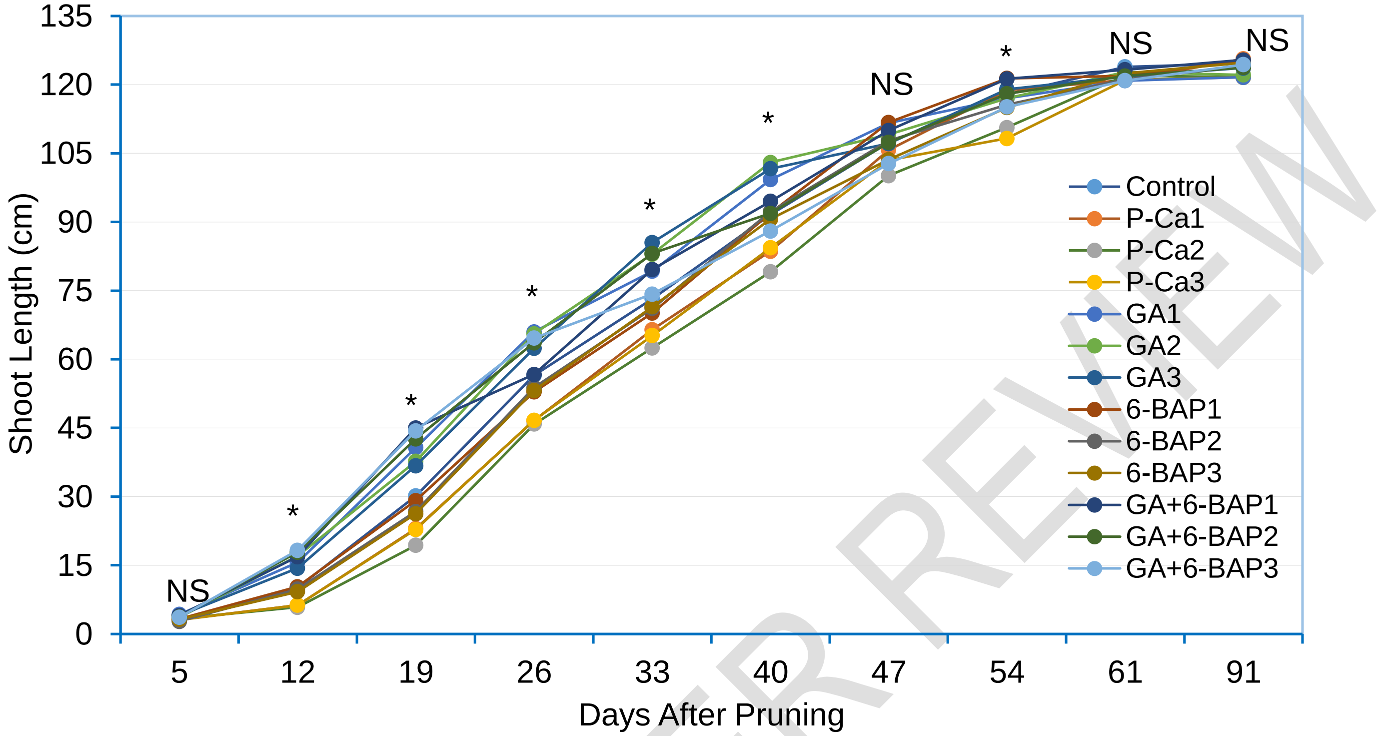

Figure 1. Influence of bioregulators on the growth of lateral shoots in raspberry cv. UANC-2022.
NS: not significant. *: Significant difference (Tukey $P \leq 0.05$).

Regarding shoot diameter (SD, Table 2), the treatments with P-Ca (P-Ca1, P-Ca2 and P-Ca3) showed the highest values ($P \leq 0.05$), with the P-Ca3 treatment being the highest at 11.0 mm, while the control grew to 9.8 mm. Leaf numbers experienced significant changes as a result of the application of bioregulators (Table 2), with P-Ca2 producing the highest number of leaves per plant, totaling 56, compared to the 51.5 counted in the control plants. On the other hand, treatments with $GA_{4/7}$ (GA1, GA2 and GA3), as well as in combination with 6-BAP (GA+6-BAP1, GA+6-BAP2 and GA+6-BAP3) showed lower leaf numbers. No significant differences in shoot fresh weight were observed among treatments. Conversely, significant differences were found for shoot dry weights (Table 2), where P-Ca3 had the highest value of 156.2 g/plant, 22.8% larger than the 127.2 g/plant observed in the control plants.

Similar results on vegetative growth have been reported in various studies. Juárez *et al*. [20] found increased stem length in corn plants treated with Gas, and in strawberries [21] the number and length of lateral shoots were increased. Kalra and Bhatla [8] reported that the exogenous application of GAs and Cyt promoted bud initiation, longitudinal elongation and consequently an increase in the biomass of different plant species. Conversely, the modification of vegetative growth in raspberry plants with the use of P-Ca (Table 2 and Figure 1) aligns with similar studies on vegetables such as okra [12], sweet potato [13] and the mirador [22], habanero [23] and jalapeño [24] chili peppers, where P-Ca influences longitudinal growth and stem diameter, number of leaves and total biomass. In fruit trees, vegetative growth has been modified under the influence of P-Ca, as reported in passion fruit [25], pear [26], cherry [27] and mango [28]. The eventual restoration of vegetative growth suggests that the physiological effect of P-Ca is temporary, which would be considered of great utility in fruit crops.

Treatments	**SD (mm)**	**LN (leaves/plant)**	**FWS (g/plant)**	**DWS (g/plant)**

Table 2. Influence of bioregulator application on shoot diameter, number of leaves, fresh and dry weight of shoots in raspberry cv. UANC-2022.

P-Ca1	10.5 ab	55.5 ab	275.5 a	145.6 ab
P-Ca2	10.6 ab	56.0 a	279.5 a	144.0 ab
P-Ca3	11.0 a	55.2 abc	279.7 a	156.2 a
GA1	9.1 c	48.6 bcd	258.0 a	129.0 b
GA2	9.1 c	46.2 d	237.5 a	130.2 b
GA3	9.2 c	49.4 a-d	261.0 a	130.6 b
6-BAP1	9.6 bc	50.2 a-d	258.0 a	141.6 ab
6-BAP2	9.9 bc	51.6 a-d	273.5 a	140.4 ab
6-BAP3	9.8 bc	47.6 bcd	279.5 a	146.4 ab
GA+6-BAP1	9.4 bc	47.7 bcd	158.5 a	135.4 ab
GA+6-BAP2	9.5 bc	49.0 a-d	249.5 a	128.4 b
GA+6-BAP3	9.1 c	47.4 cd	224.5 a	127.0 b
Control	9.8 bc	51.5 a-d	196.5 a	127.2 b
***P* ≤**	<0.0001	0.0008	0.178	<0.0001
CV %	5.15	6.18	13.48	11.88

SD: Shoot Diameter. LN: Number of Leaves. FWS: Fresh Weight of Shoots. DWS: Dry Weight of Shoots. *P*: Probability of the F-value. CV: Coefficient of Variation. Values with the same letter are statistically equal according to Tukey's test ($P \leq 0.05$).

These results are mainly attributed to the physiological functions of GAs and Cyt, which stimulate lateral bud initiation and elongation, properties that contributed to an increase in shoot growth (Figure 1). The reason for this lies in the active participation of GAs and Cyt in the processes of cell division and elongation. These hormones trigger the activation of enzymes that play a crucial role in the cell interface, thus accelerating the transition from the G1 phase to the S phase [29]. Additionally, it has been observed that they enhance the elasticity of the cell wall, thereby fostering the longitudinal growth of plants [30], which in this case was reflected in LSG values. On the other hand, P-Ca caused a temporary decrease in LSG (Figure 1), a phenomenon explained by Evans *et al*. [10]. According to these authors, this growth retardant acts by inhibiting the synthesis of active endogenous gibberellins, behaving as a mimic of the chemical structure of 2-oxoglutarate. Thus, it blocks the formation of 3β-hydroxylase dioxigenase, which catalyzes hydroxylations at the 3β position of biologically inactive GAs, preventing their transformation into biologically active GAs. This modification in plant physiology leads to a compacted plant growth, an effect that has been corroborated in previous research. In a study conducted on saladette tomatoes and bell peppers, Ramírez *et al*. [31] reported that in plants treated with P-Ca, only biologically inactive GAs (GA_{20} and GA_{53}) were present, while control plants had biologically active GAs (GA_1, GA_4 and GA_7). A similar pattern was observed in jalapeño chili pepper [24], where applications of P-Ca inhibited the synthesis of biologically active GAs in the apexes of treated plants.

3.2. Reproductive Development and Fruit Yield

The hormones $GA_{4/7}$, 6-BAP and GA+6-BAP, with any number of applications, advanced inflorescence development by seven days compared to the control ($P \leq 0.05$) (Figure 2). This was evident at 33 DAP, when the GA2 treatment showed the highest value of 7.75 inflorescences per plant, while the control and P-Ca2 treatments did not show their first inflorescences until 40 DAP.

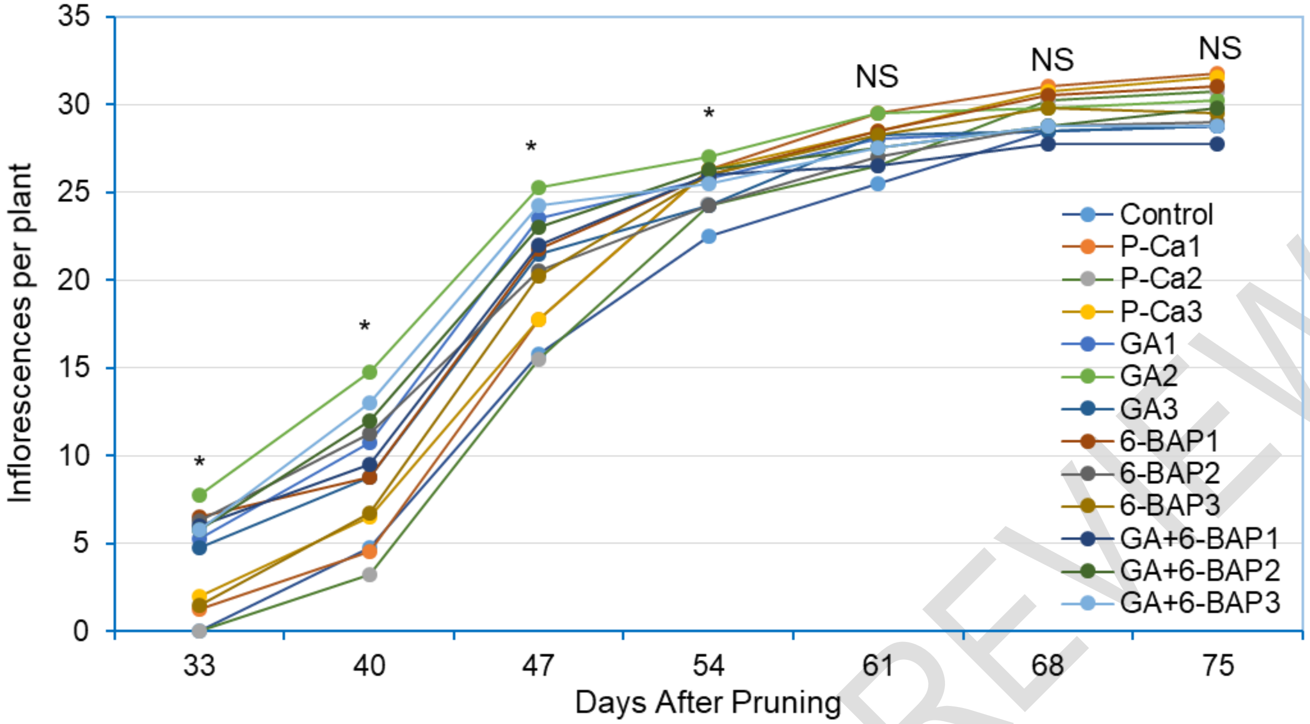

Figure 2. Influence of bioregulators on the number of inflorescences per plant in raspberry cv. UANC-2022
NS: not significant. *: Significant difference (Tukey $P \leq 0.05$).

Bioregulators significantly influenced ($P \leq 0.05$) the timing of floral differentiation and the number of flowers per plant (NFP) (Figure 3). This effect became evident from 40 DAP, when the GA2 treatment produced the highest NFP (4.65). Treatments that included 6-BAP showed their first flowers at 45 DAP, while plants treated with P-Ca and the control showed their first flowers later (55 DAP). At 85 DAP, the GA2 treatment exhibited the highest NFP with 154.5 flowers per plant, being 22.6% greater than the control with 126 (Figure 3).

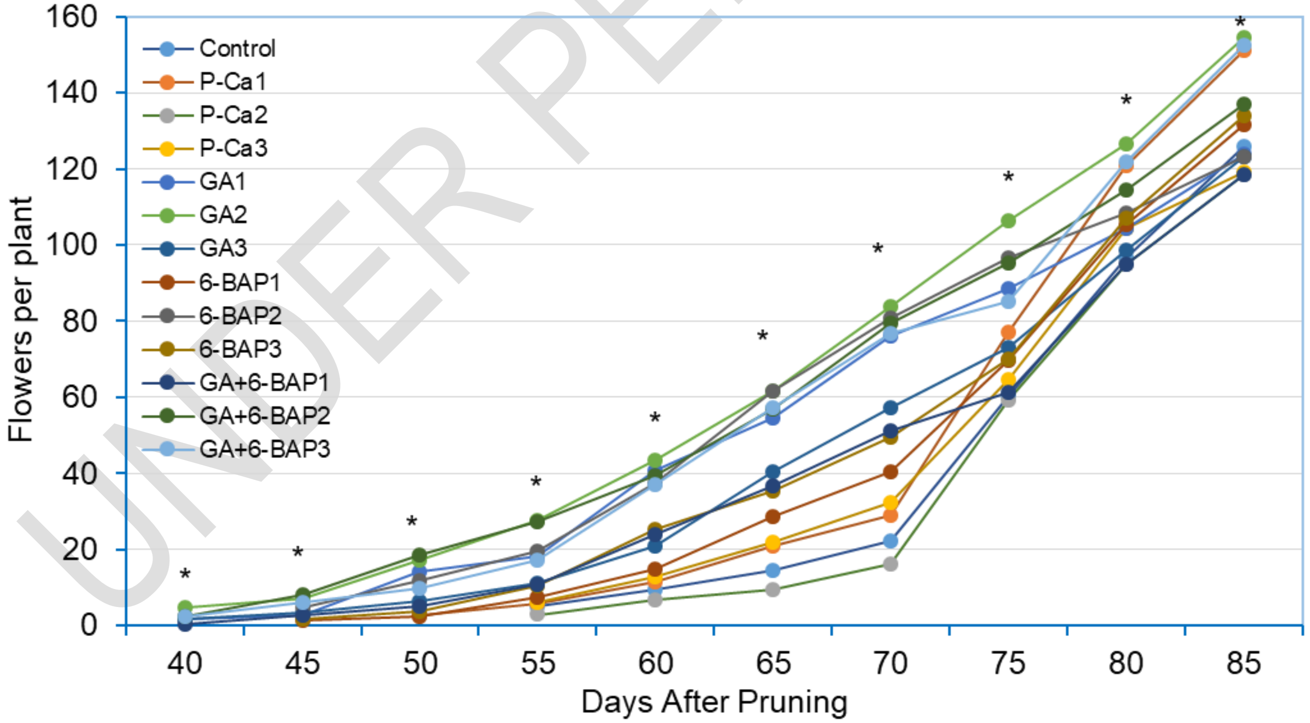

Figure 3. Influence of bioregulators on the total number of flowers per plant in raspberry cv. UANC-2022.
*: Significant difference (Tukey $P \leq 0.05$).

The treatments significantly modified the number of harvested fruit (NHF) ($P \leq 0.05$) and the harvest stage (Figure 4A). In the first week of harvesting, the GA1 treatment stood out with 12.5 fruits per plant, while the P-Ca1 treatment and the control showed their first fruits until the second week, and the P-Ca2 and P-Ca3 treatments not until the third week. Starting from the sixth week, the P-Ca1 treatment began to surpass all others with higher NHF, producing a total of 128.4 fruits per plant by week 9, being 16.8 % higher than the control´s 109.9 fruits per plant.

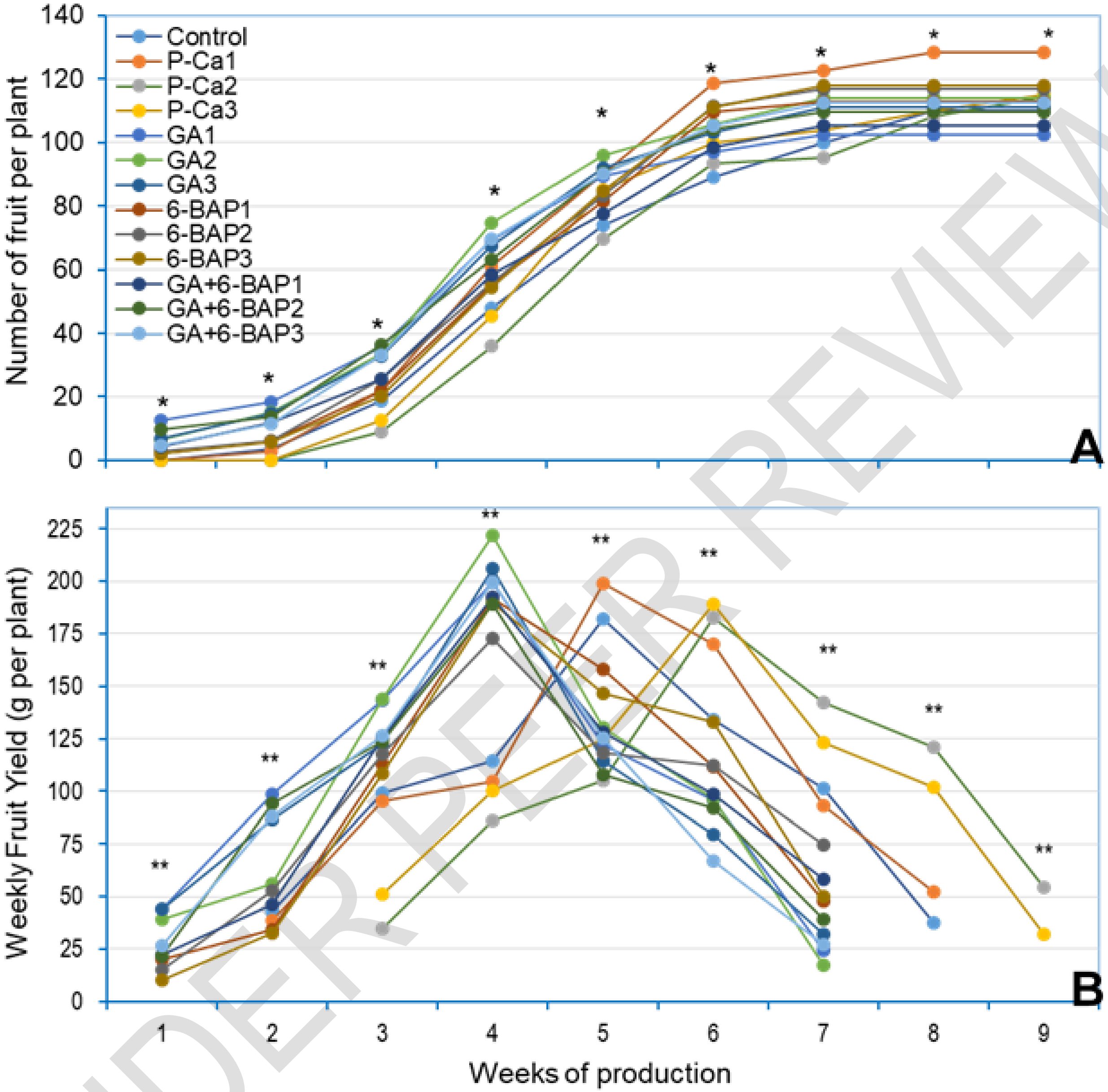

Figure 4. Influence of bioregulators on the cumulative number of harvested fruits in raspberry cv. UANC-2022 (A) and on weekly fruit production (B) in raspberry cv. UANC-2022.
*: Significant difference (Tukey $P \leq 0.05$). **: Highly significant differences (Tukey $P \leq 0.01$).

Fruit production showed highly significant differences ($P \leq 0.01$) in yields and earliness among treatments (Figure 4B). Plants treated with gibberellins (GA), benzylaminopurine (6-BAP) and their combinations (GA+6-BAP) produced the first harvested fruit, with GA1 and GA3 treatments yielding the most (44.2 and 43.9 g per plant, respectively) on the first week. Harvest on P-Ca1 and control plants began a week later, and it took one additional week to harvest fruit on plants treated with P-Ca2 and P-Ca3. Fruit production lasted a total of 7 weeks for all treatments. However, this period ended first for plants treated with GA, 6-BAP and their combinations, whereas it was displaced by one week for control and P-Ca1, and two weeks for treatments P-Ca2 and P-Ca3.

Total (cumulative) yields were significantly different among treatments ($P \leq 0.05$; Table 3), with P-Ca1 plants having the highest yields of 795.8 g per plant, being 17% higher than the control plants. The rest of the treatments had cumulative yields that were statistically similar to the control plants.

Table 3. Influence of bioregulator applications on fruit yield and quality in raspberry cv. UANC-2022.

Treatments	Yield (g/plant)	VCC (mg/100 g)	AC (mg/100 g)	KC (mg/100 g)	Brix (%)
P-Ca1	795.8 a	48.9 ab	251.2 d	165.4 bcd	10.1 a-d
P-Ca2	663.5 bc	52.3 ab	352.6 bcd	156.9 cd	8.0 cd
P-Ca3	705.2 bc	54.5 a	584.3 a	187.7 ab	10.6 abc
GA1	735.4 b	50.9 ab	398.7 a-d	143.1 cd	9.3 a-d
GA2	731.1 b	47.0 ab	517.2 ab	180.0 abc	10.9 ab
GA3	753.0 ab	54.9 a	447.8 abc	182.5 abc	10.8 ab
6-BAP1	669.9 bc	50.9 ab	399.0 a-d	216.2 a	11.3 a
6-BAP2	687.7 bc	49.0 ab	388.2 bcd	190.8 ab	9.8 a-d
6-BAP3	732.0 b	48.7 ab	295.7 cd	163.3 bcd	9.8 a-d
GA+6-BAP1	685.7 bc	48.5 ab	337.3 bcd	140.7 d	8.2 bcd
GA+6-BAP2	677.9 bc	49.4 ab	492.6 abc	145.8 cd	9.3 a-d
GA+6-BAP3	670.2 bc	49.1 ab	409.6 a-d	209.5 ab	10.2 a-d
Control	679.9 bc	45.3 b	230.4 d	151.6 cd	7.4 d
***P* ≤**	0.0348	0.0091	<0.0001	0.0013	<0.0001
CV %	7.78	7.52	20.19	13.36	12.0

VCC: Vitamin C Content; AC: Anthocyanin Content; KC: Potassium Content; *P* ≤: Probability of the F-value; CV: Coefficient of Variation. Values with the same letter are statistically equal according to Tukey's test (*P* ≤ 0.05).

The application of GAs and Cyt has been extensively documented across various crops, including vegetables, fruits and ornamental plants, with effects like those observed in the present study. In the case of strawberries, Viasus-Quintero *et al*. [21] reported an increase in both the number of flowers per plant and yields when applying GAs in combination with Cyt. In mango, Pérez-Barraza *et al*. [32] noted that the exogenous application of GAs can have various effects, such as inhibition, delay, or advancement of flowering, depending on the timing of the application. In jalapeño chili, Pichardo-González *et al*. [33] observed an increase in yield with the application of 50 mg L^{-1} of GAs, while in potatoes, Lizarazo-Peña *et al*. [34] found that GAs favored lateral bud initiation. In *Solidago* X *luteus*, GAs stimulated floral formation, while Cyt accelerated anthesis [35]. Similarly, P-Ca has been evaluated in other crops, such as in habanero chili, where a dose of 150 mg L^{-1} resulted in a significant increase in the number of fruits, while two applications of 50 mg L^{-1} stimulated a greater number of flowers [23]. In mirador chili, according to Ramírez *et al*. [22], P-Ca increased the number of flowers and the percentage of fruit set, leading to higher yields. Similar phenomena have been documented in jalapeño chili [24]. In passion fruit cultivation it has been observed that P-Ca causes an increase in the number of floral buds [25], and Einhorn *et al*. [26] also found that it increases the percentage of fruit set and improves yield in pears.

The combination of GA_4 with GA_7 is used in agriculture to induce early flowering in some crops, as it was confirmed in this study with an early presence of inflorescences and flowers (Figure 2). However, it is crucial to highlight that this effect cannot be generalized across all species [36]. An example of this is seen in blueberries, where Lindberg *et al*. [37] reported that the application of GAs during the juvenile stage inhibited the formation of floral buds. It is important to note that the impact of any type of bioregulator is conditioned by various factors, such as dosage, physiological stage, absorption, translocation, assimilation, response capacity and genetics of each species [11]. The increase in flowers (Figure 3) could be attributed to the ability of GAs and Cyt to stimulate the expression of genes related to floral identity. These hormones play an essential role in the normal development of flowers, while also driving key processes such as pollen germination and pollen tube growth [38].

Regarding the reproductive development attributes exhibited by plants treated with P-Ca, Rademacher *et al*. [9] mention that one of the side effects of this retardant is its influence on various metabolic processes, such as the reduction of ethylene synthesis. This effect could decrease the drop of flowers and fruits, resulting in a higher number of harvested fruit (Table 2). Additionally, P-Ca can intervene in enzymes related to the synthesis of other endogenous hormones, such as cytokinins [9, 24], which could explain the observed increase in the number of raspberry fruits per plant (Figure 4A). It is important to highlight that P-Ca has a short lifespan within the plant [10] and therefore its effects are temporary. Consequently, the proper selection of the phenological stage and dosage in applications is crucial to achieve the desired agronomic results [39].

3.3. Fruit Quality

There were significant differences between treatments ($P \leq 0.05$) for vitamin C content (VCC; Table 3), with GA_3 and P-Ca3 showing the highest values at 54.9 and 54.5 mg 100 g^{-1}, respectively. These values were 21.1 and 20.3 % higher than the control (45.3 mg 100 g^{-1}). Anthocyanin content (AC) was affected by the application of bioregulators ($P \leq 0.05$; Table 3), with the treatments P-Ca3 (584.3 mg 100 g^{-1}), GA2 (517.2 mg 100 g^{-1}), GA+6-BAP2 (492.6 mg 100 g^{-1}) and GA3 (447.8 mg 100 g^{-1}) showing the highest values, being 154, 124, 114 and 94 % respectively, above the control treatment (230.4 mg 100 g^{-1}). Significant differences were observed for potassium content (KC; Table 3), with the treatments P-Ca3, 6-BAP1, 6-BAP2 and GA+6-BAP3 having contents that were 24 to 46 % higher than the control. The content of total soluble solids, expressed as ºBrix were also affected by the bioregulators (Table 3), with P-Ca3, GA2, GA3 and 6-BAP1 having the highest values, being 43 % to 53 % larger than in the control plants.

Previous studies have shown that P-Ca, GAs and Cyt provoke increases in the content of phytochemicals in various horticultural crops. In habanero chili [23], the application of 150 mg L^{-1} of P-Ca increased the content of capsaicin, total carotenoids and vitamin C. In jalapeño chili [24], P-Ca increased the content of lutein, capsaicin, total carotenoids and vitamin C, whereas in grapevines [40,41] it favored the content of monoterpenes, polyphenols and anthocyanins. The influence of P-Ca on potassium content in fruit has not been previously reported. However, Ramírez *et al*. [14] reported an increase in potassium in tomato leaves and nitrogen and calcium in their fruits with applications of 50 mg L^{-1} of P-Ca and 6-BAP. Additionally, GAs increase acidity percentage in passion fruit [42], and 100 mg L^{-1} of $GA_{4/7}$ combined with 50 mg L^{-1} of 6-BAP increasing the content of vitamin C and lycopene in tomato fruits [31]. In *Capsicum annum* the application of BAP increased the content of capsaicin and vitamin C [43], and in black habanero chili a cytokinin-based hormonal complex applied to the foliage increases the content of alkaloids in fruits [44].

The increase in vitamin C in raspberry fruit (Table 3) is of great importance, as this vitamin plays fundamental roles in plants, is essential in plant-environment interactions, acting as a defense against free radicals, ultraviolet radiation and pathogen attacks [45]. Additionally, it participates in optimizing photosynthesis and in the processes of growth and development. Vitamin C also has an important role as a cofactor for enzymes involved in the synthesis of polysaccharides and some hormones, such as gibberellins, ethylene and abscisic acid [46]. Similarly, the increase in the content of anthocyanins, as observed in this study (Table 3), is of great importance. In the case of raspberry, anthocyanins gives it its characteristic red-pink color. By presenting a higher content of anthocyanins, the fruits have a more intense, brighter color, making them more attractive to consumers. In addition, this pigment stimulates greater tolerance to cold stress, ultraviolet radiation and pathogen attacks [47,48]. Red raspberry has a high antioxidant capacity in human nutrition, with anthocyanins being one of the main phytochemicals responsible for this property. On the other hand, the content of total soluble solids (°Brix) is directly related to the sugar content. This characteristic is important for defining fruit quality, as it reflects its sweetness [49,50,51].

Endogenous hormones and nutrients maintain a close and synergistic relationship. The production and effects of hormones depend on the presence or absence of nutrients, while hormones regulate nutrient content in the plant through homeostasis. This interaction can explain the higher potassium content in raspberry fruits (Table 3) caused by the application of 6-BAP and in combination with GAs [52].

Regarding the increase in phytochemicals caused by P-Ca (Table 3), it is hypothesized that this growth retardant affects the biosynthesis of anthocyanins and other flavonoids, such as flavone 3-hydroxylase [9]. Additionally, by inhibiting growth, photosynthates may be directed toward the root, causing greater formation of the root system. This has been proven in sweet potato, where the application of 810 mg L^{-1} of P-Ca inhibited vegetative growth and generated greater root formation, which translated into higher yields [13]. A more developed root system leads to greater absorption and translocation of water, nutrients and even hormones [53]. These elements are transported to the aerial part, which promotes greater accumulation of nutrients in the different organs of the plants. It even can promote the formation of molecules involved in the phytochemicals of the fruit [54].

The production of raspberries with a higher content of phytochemicals in the fruit is of great utility, particularly in the case of fruit destined to export markets, like Mexico's raspberries to the United States. In United States the consumption of raspberries has experienced an exponential increase since 2020, due to the benefits found for the immune system in preventing COVID-19 [55]. Therefore, the current challenge is to produce highly nutritious berries, improving their phytochemical properties to promote human health.

The results from this study and the reports of other authors suggest that bioregulators are a feasible tool of great utility in raspberry cultivation. The bioregulators used in this study showed physiological and morphological effects that led to modifications in the architecture and production habit of raspberry cv. UANC-2022. This bioregulator alternative is a flexible tool for producers due to its temporary effect, allowing for crop management strategies that could accelerate, inhibit or delay physiological processes that permit a better control of plant architecture, and help schedule fruit harvest cycles.

4. CONCLUSIONS

Based on the bioregulators and doses used in raspberry cv. UANC-2022, the following conclusions are drawn:
P-Ca temporarily reduces the growth of lateral shoots, increases their diameter, number of leaves and dry weight; promotes a higher number of flowers, harvested fruits and total yield; and increases the content of vitamin C, anthocyanins, potassium and Brix in fruit. P-Ca with two or three applications delays harvest by one week. The gibberellins $GA_{4/7}$ stimulate the growth of lateral shoots, increases the number of flowers and enhances the content of vitamin C, anthocyanins and ºBrix, plus advancing the harvest period by one week. The cytokinin 6-BAP promotes a higher number of flowers, increases the content of potassium and Brix in fruit and anticipates the formation of inflorescences and start of harvest. The combination of GA+6-BAP promotes the growth of lateral shoots; increases the number of flowers; increases the content of potassium and ºBrix in fruit; and advances the flowering and harvest by one week.

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