Colegio de Postgraduados

Effect of aminoethoxyvinylglycine addition on the floral bud opening of *Ranunculus asiaticus* L. cultivars

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ABSTRACT

Objective: To evaluate the effect of aminoethoxyvinylglycine (AVG) additions on several growth variables of *R. asiaticus* in a greenhouse under a typically dry environment of the Mexicali valley.

Design/methodology/approach: The variables determined were, bud opening rate, flower diameter, height of stem and bud growing rate and completely randomized block experimental design under a 2 by 4 factorial arrangement and three replicates was used for setting up the treatments with rates AVG of 0,100,150 and 200 mg L^{-1} over La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) *R. asiaticus* cultivars.

Results: The optimal dose of AVG for reducing flower bud diameter on both cultivars were those of 200 and 100 mg L^{-1} respectively. Also, AVG addition had a significant effect in reducing stem height and growing rate for both cultivars.

Limitations on study/implications: The use of AVG has shown good results as inhibitor of ethylene synthesis and extension of shelf life in carnations, snap dragons (Antirrhinum), ripening of fruits, and to reduce abortion of female flowers in certain species of walnuts, so, based on the above and considering the lack of information regarding the use of AVG inhibitor in the opening of Persian Buttercup flower buds was used AVG in *R. asiaticus*.

Findings/conclusions: AVG addition on plants of *R. asiaticus* had a significant effect reducing growth, both in diameter and height in the La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) varieties.

Keywords: *R. asiaticus*, flower bud growing, stem height, aminoethoxyvinylglycine, diameter ethylene.

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INTRODUCTION

In the last decades, the demand for fresh flowers has consistently increased in Europe, EEUU and Japan. The offer comes mainly from countries such as Holland, Italy, Colombia, Kenya, Ethiopia, Turkey, Morocco, China and India. European Union imports account for 60% of world's total production, while EEUU flower imports made up 20%

being Colombia and Ecuador its main suppliers. In 2021 global flower exports reached a total value of 11,000 million dollars. 57.9% of those exports accounted for European union, 24.8% accounted for Latin-American (excluding Mexico but including the Caribbean), Africa participation accounted for 10.4% and finally Asia participation was that of 5.3% (Workman, 2022).

Even though Mexico is not one of the main world flower exporters, since most of its market is local (80%). What is left is exported to EEUU (96.6%) and Canada (3%) (Workman, 2022). In 2021, gross value of Mexico's flower exports overcame 44 million dollars (Exports of cut flowers by country México, 2019). Nowadays, flower industry in Mexico includes an annual production over 96 million dozen of roses, over 12 million dozens of gerbera, over 4 million dozens of sunflower, over 12 million dozens of orchids and almost 187 000 tulips. The State of Mexico concentrates 90% of the country's total production being the only state in Mexico able to export. On the other hand, the state of Baja California exports to EEUU 85% of its flower production (SADER, 2018). One source of economic losses during harvest time is due to the maturation process of the flower which is commonly very fast. Thus, the purchase price is reduced.

Persian Buttercup (*Ranunculus asiaticus* L.) belong to the Ranunculus family of plants. These flowers have been cultivated since the 18th century and have undergone various improvements, both in the number of petals and in the length and consistency of the stems, which has made it possible to increase their availability for marketing as cut flowers, but potentially also for a pot (Scariot *et al.*, 2014). This ornamental plant has the potential to be used more widely in the floral industry, although growers unfortunately face various challenges in growing these plants from their tuberous roots. These challenges include proper root storage conditions, low seed germination, non-uniform growth of crop, and disease susceptibility (Cerveny, 2011).

Crop growth of Persin Buttercup can also be done from seed, although the tuberous roots offer faster and more prolific crops. Tissue culture has also been used for its propagation, although it is an expensive process (Beruto and Debergh, 2004). Life span of cut flowers is highly affected by field production practices, as well as postharvest management, such as the use of preservatives, which may contain biocides and/or ethylene biosynthesis inhibitors to prevent senescence or abscission of leaves and flowers. Since after cutting and before the sale process flowers are exposed to low temperatures to suppress opening and aging process, the size of the bud during cutting has an important impact on its marketing, since the flowers of the Ranunculus family are generally highly sensitive to ethylene additions (Scariot *et al.*, 2009).

There are different products available on the market capable of suppressing or controlling the production of ethylene in different fruits. One of the most effective to ensure that the state of the bud lasts is 1-MCP (1-Methyl cyclopropene), which delays the maturation of the flower and increases its shelf life; unfortunately, this product can only be used in storage conditions, therefore, it is a very highly expensive agricultural supply. A feasible solution is using field ethylene inhibitors to increase the bud stage of the flower during and after cutting (Gamrasni *et al.*, 2017). It has been reported that AGV gives good output when used in the field and sometimes in storage conditions. Ethylene inhibitors

act on the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC) to reduce flower ripening (Yang and Hoffman, 1984; Schaller and Binder, 2017; Katayose *et al.*, 2021). There is no background information of the use AVG in Persian Buttercup. However, the use of AVG has shown good results as inhibitor of ethylene synthesis and extension of shelf life in carnations (Baker *et al.*, 1977), snap dragons (*Antirrhinum*) (Wang *et al.*, 1977), ripening of fruits (Khan and Ali, 2018) and to reduce abortion of female flowers in certain species of walnuts (Depaepe and Van Der Straeten, 2017). The application of 1mM AGV solution doubled the life cutting span of *lisianthus* flowers, with respect to the control (Shimizu-Yumoto and Ichimura, 2010). AVG doses of 375 mg/L proved to be optimal to reduce the abscission of *Corymbia torelliana* and *Corymbia citriodora* leaves, caused by the generation of ethylene (Trueman and Adkins, 2013). Based on the above and considering the lack of information regarding the use of AVG inhibitor in the opening of Persian Buttercup flower buds, the objective of this study was to evaluate the effect of three doses of AVG on several growth variables of Persian Buttercup such as: bud opening rate, flower diameter, height of stem and bud growing rate over two *R. asiaticus* flower varieties (La Belle Deep Rose (DER) and La Belle Dark Orange (DAR), during the agricultural cycle of 2018-2019.

MATERIALS AND METHODS

Study Site

The experiment was carried out in the autumn-winter 2018-2019 agricultural cycle on land owned by the company Agroproductos y Servicios del Golfo de California SPR de RL, in Colonia Luis Romero Lot 1, Valle de Mexicali, Baja California, Mexico (32° 38' 22.9" N, 114° 57' 28" O). The climate in this area is desert type and the summer is characterized by being hot, very dry, with average maximum and minimum temperatures of 49 °C and 16 °C respectively (García, 1988).

Agronomic Practices

Planting was done on 1m by 40 m seed beds with a plant density of 3 bulbs per foot, placed 3 inches deep and 4 inches apart. Prior to sowing, a soil pH analysis was carried out at a depth of 30 cm in three sites of each plot. The results showed an average pH value of 8.2. Before planting, bulbs were treated for 5 min with a 1L ha^{-1} solution of Metalaxil against fungal diseases, following crop guidelines for rose (*Rosa* sp.) cultivation (Álvarez *et al.*, 2013). To measure the diameter of the button, a digital Vernier D-200w brand Caliper with an accuracy of ±0.2 mm was used, while height of the stem, from top to bottom was measured with a Truper® tape measure.

During the growing season, the varieties were attacked by Thrips (*Frankliniella occidentalis*), an insect capable of causing premature wilting, retarding leaf development and distorting shoots, in a very similar fashion to the damage caused in avocado (*Persea americana*) (Castresana *et al.*, 2008). Three applications of Metamidos were made at a dose of 500 mL ha^{-1} in the months of December, January, and February for the control of thrips. Also, three applications of cytokinins (0.204%) 1L ha^{-1} (AGRIMIL plus:Quinival Agroindustrias, Mexico) were added in the months of December and January.

Treatments

A completely randomized block experimental design under a 2 by 4 factorial arrangement and three replicates was used for setting up the treatments. The Combination of the AVG doses of 0 mg L^{-1}, 100 mg L^{-1}, 150 mg L^{-1} and 200 mg L^{-1}, respectively and the varieties La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) formed the different treatments under evaluation.

Fertilization rates of 20-20-20 kg ha^{-1} (NPK) were applied every other day during the first six months of the cycle. On March 4, 2019, the AVG was applied in the flowering stage, when the bud opening reached a diameter between 2 to 3 cm, which was considered the initial measurement. The growth of the bud diameter and stem height were measured every 24 h during a period of three days (0, 0-24 and 0-48 h), which formed the variables: diameter 1, 2 and 3 (DM1, DM2 and DM3), as well as the height of stem 1, 2 and 3 (AT1, AT2 and AT3, respectively). The button diameter growth percentages were analyzed in order to eliminate the effect of different button sizes at the beginning, the growth percentages were analyzed during the first and second day, as well as the overall percentage. During the growth period from 0 to 24 hours (Figure 1), (PD1, PD2), height growth percentage (PA1, PA2) and the total growth percentage for both variables (PDT3 and PAT3). From data of diameter and height of the stem of the plant the percentages of growth in each stage as were calculated as follow:

$$\%\ Growth\ 0-24h = \left(Diameter\ at\ 24h - Initial\ Diameter\right) / \left(Initial\ Diameter\right) \times 100$$

(PD1, PAT1)

$$\%\ Growth\ 24-48h = \left(Diameter\ at\ 48\ h - Diameter\ at\ 24h\right) / \left(Diameter\ at\ 24h\right) \times 100$$

(PD2, PAT2)

$$\%\ Growth\ 0-48h = Diameter\ at\ 48h - Initial\ Diameter) / \left(Initial\ Diameter\right) \times 100$$

(PDT3, PAT3)

Measurements

The following measurements were performed: growth of the bud diameter (DM, cm), stem height (AT, cm), percentage growth of the bud diameter (PD, cm) and percentage growth of the stem height (PAT, %). In a similar fashion, the number of buttons per plant was measured for both varieties in the first cut.

Statistical Analysis

The response variables were analyzed under a 2 by 4 factorial arrangement of treatments under a completely randomized experimental block design. Means treatments were compared using the least significant difference test, also, orthogonal contrasts were performed to determine the trend through VFA doses. The error level used was 5% and the trend was considered the probability between 0.05 and 0.10. The analyzes were performed using the SAS statistical program version 9.4 (SAS Institute, 2020).

RESULTS AND DISCUSSION

Opening of bud diameter

A significant statistical difference (P=0.6) for the interaction AVG × variety was found (Table 1). These results suggest that the presence of aminoethoxyvinylglycine can inhibit the growth process of the uncut flower.

Several studies have found that flowers of other member plants of the Ranunculus family have shown sensitivity to ethylene, including *Aconitum napellus* L., *Anemone* × *hybrida*, *Delphinium ajacis*, and *Nigella damascena* L. (Cerveny, 2011). Additions with AVG has shown similar results on carnations (Baker *et al.*, 1977) and snapdragon flowers (*Antirrhinum*) (Wang *et al.*, 1977). According to the Yang cycle for the synthesis of ethylene (Yang and Hoffman, 1984), the addition of AVG inhibits the enzyme ACC-synthetase, whose half-life is very short and is found in low concentrations in cells (Li *et al.*, 2020). When comparing the effects of the AVG treatments on the diameter of the flower bud reached at 48 h, it was found that for the DAR variety there were no significant differences between the effects of the doses of 100 and 150 mg L^{-1}, finding the smallest diameter with a reduction of 29% and 33%, respectively, with respect to the control (Table 2, Figure 1); this could be related to a phenomenon observed with the growth of various rose crops (Van Doorn and Kamdee, 2014); where it was found that ethylene inhibited bud opening in various crops, but promoted it in others.

A relatively small dose of ethylene promoted bud opening, while a relatively highly one inhibited it, this is known to be due to differences in the expression of a family of 'DELLA (RhGAI1)' growth repressor genes.

Table 1. Mean values of the effect of the AVG doses on the growth of bud diameter, height, growth percentage, in varieties of *Ranunculus asiaticus* L. during the 2018-2019 cycle.

Variables	mg L^{-1}					Variety			P value		
	0	100	150	200	SE	DER	DAR	SE	Dose	Variety	Dose × Variety
DM1	2.83	3.22	2.61	2.66	0.22	2.72	2.94	0.16	0.246	0.335	0.253
DM2	3.71	3.59	3.11	2.92	0.26	3.27	3.40	0.19	0.145	0.627	0.252
DM3	5.02	4.78	3.85	3.74	0.34	4.20	4.48	0.24	0.039*	0.418	0.060*
PD1	31.65	12.47	18.66	10.85	4.23	20.53	16.29	2.99	0.013*	0.330	0.179
PD2	35.11	33.00	25.96	29.44	9.57	28.55	33.20	6.77	0.910	0.634	0.185
PD3	77.57	50.60	48.00	45.59	13.11	54.71	56.17	9.27	0.312	0.913	0.096
AT1	45.07	51.22	50.58	52.27	1.37	48.57	51	0.97	0.009*	0.096	0.772
AT2	48.48	53.97	52.63	53.75	1.43	51.40	53.01	1.01	0.053*	0.280	0.132
AT3	49.48	55.48	54.18	56.95	1.57	52.30	55.75	1.11	0.022*	0.043*	0.399
PA1	8.12	5.62	3.93	2.87	3.01	6.25	4.02	2.13	0.636	0.469	0.370
PA2	2.15	2.74	3.06	5.93	1.62	1.72	5.22	1.14	0.384	0.046*	0.115
PA3	10.32	8.45	7.05	8.93	3.22	8.03	9.35	2.27	0.9116	0.6875	0.4105

DM1:DM2:DM3=Diameter 1, 2 y 3, respectively. DER=Deep Rose variety, DAR= Dark Orange variety. PDB1:PDB2:PDB3=Bud diameter growth percentage 1, 2, 3 respectively. PAT1:PAT2:PAT3=Stem height growth percentage 1, 2 y 3, respectively. SE=Standard error. AVG=aminoetoxiviniglicina; * P≤0.05.

Table 2. Mean values of the effect of the interaction among AVG doses × variety upon flower bud diameter after 48 h during the 2018-2019 agricultural cycle.

Variety	AVG Dose (mg L^{-1})	Flower bud diameter Mean value	Statistical
Deep Rose	0	4.17	a
	100	5.37	b
	150	3.8	a
	200	3.47	a
Dark Orange	0	5.87	b
	100	4.18	a
	150	3.89	ab
	200	4.01	ab

Means with the same letter are not statistically different ($P \leq 0.05$).

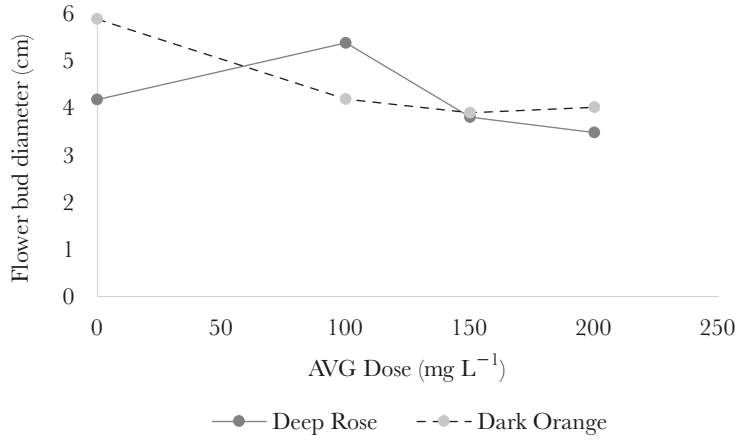

Figure 1. Mean values of the effect of the interaction among AVG doses × variety upon flower bud diameter after 48 h during the 2018-2019 agricultural cycle.

That is, due to the differences in the amount of ethylene generated by various *R. asiaticus* cultures (Scariot *et al*, 2009), the same doses used in this study can generate different degrees of inhibition of bud growth. The differences in sensitivity, as well as in the promoter-inhibitor role of bud growth, have also been related to the variation between crops in the concentrations of other phytohormones, mainly cytokinins, gibberellins, abscisic acid (ABA) and auxins (Iqbal *et al.*, 2017).

For the DER variety, the smallest diameter of the bud was found at a concentration of 200 mg L^{-1} (17%) lower than the control ($p<0.05$) (Table 2 and Figure 1). The addition of aminoethoxyvinylglycine showed a positive effect for both varieties from the lowest dose, although for the DAR variety there was no further decrease with higher doses, which did occur with the Deep Rose variety; such a difference was observed at twice the dose, that is, at 200 mg L^{-1} (Figure 1).

Finally, Figure 2 shows the global average outputs for both growing periods, it was confirmed that the lowest growth percentage of *R. asiaticus* buds was achieved at an aminoethoxyvinylglycine concentration of 100 mg L^{-1} (24%) for the DAR variety, while

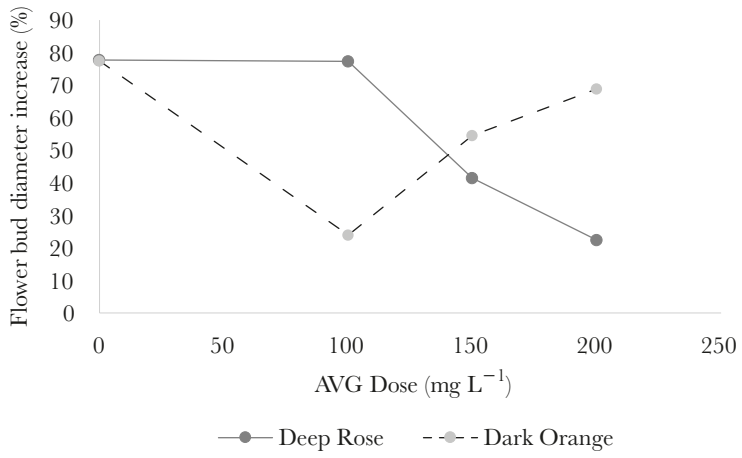

Figure 2. Mean values of the effect of the interaction among AVG doses × variety upon flower bud diameter increase after 48 h during the 2018-2019 agricultural cycle.

for the DER variety, the lowest percentage of growth is obtained with 200 mg L^{-1} (22%). In some cases, even when the differences do not reach the level of significance established in the analysis; there are important results from the point of view of quality control during the harvest, since the reduction in the speed of the opening of the button allows to reduce the percentage of buttons that exceed the diameter specifications established by the customers.

In addition to the differences in the sensitivity of different *R. asiaticus* cultivars to ethylene generated from methionine, it is necessary to evaluate the effect of exogenous ethylene (Scariot *et al*, 2009). The reduction in the diameter of the bud could be related to environmental ethylene coming from the cutting of contiguous *R. asiaticus* buds, however, it is necessary to carry out tests under isolation conditions to evaluate this hypothesis. Some researchers have found that for *R. asiaticus* cut flowers, the addition of ACC synthetase inhibitors, such as aminooxyacetic acid (AOA) and sodium thiosulfate (STS) at a maximum concentration of 48 mg L^{-1}, had no effect in their senescence (Kenza *et al.*, 2000), (Belding and Lokaj, 2002). Other authors found that the use of STS and AOA in concentrations of 300 to 350 mg L^{-1} increased the post-harvest life of 2 and 4 different types of *R. asiaticus*, respectively; although they also found two types of cultivars of the same species that were insensitive to ethylene (Scariot *et al.*, 2009).

The results obtained in this study showed that the addition of AVG in doses of 100 to 200 mg L^{-1} does have an effect on the reduction of ethylene generated during the bud opening process in live flowers, also, considering that ethylene is a multifunctional phytohormone, in the sense that is capable of regulating growth and senescence processes (Iqbal *et al.*, 2017). In another study, it has been reported that by applying AVG to pears during a two weeks period at early ripening and cold temperature, high fruit firmness was obtained due to the reduction in respiration rate and ethylene production (Khan and Ali, 2018).

On the other hand, in a study conducted by Doerflinger *et al.* (2019) AVG and 1-MCP were used to evaluate their effect on delaying apple ripening and therefore reducing fruit dropping, thus, facilitating harvest. It was found that AVG acted as an intermediate in

the internal production of ethylene, between week 1 and 4, while it did not affect neither softening nor the rate of loss of ethylene concentration starch during the storage period. Moreover, the use of AVG and 1-MCP before harvest affected the reliability of the starch concentration since a harvest index cannot be satisfactorily met. The effects of both inhibitors on starch hydrolysis depend on factors such as cultivar and growing season (Doerflinger *et al.*, 2019).

Plant Height

As far as plant height, either AVG doses and variety were statistically significant for AT1 ($P=0.009$), AT2 ($P=0.053$), AT3 ($P=0.022$), and in AT3 both effects ($P=0.022$ and $P=0.043$) respectively (Table 1).

The effect of AVG treatments at 48 h showed that for the Dark Orange and Deep Rose varieties, the minimum percentage of growth was at a dose of 100 mg L^{-1} (7%) and 150 mg L^{-1} (6%), with respect to the control ($p<0.05$) (Table 3). Accordingly, an increase in the AVG dose would imply a greater growth as expressed in the height of the plant compared to the control. However, it has been found that the development of leaves and flower stems were affected by the presence of ethylene and other phytohormones, such as auxins, gibberellins and cytokinins (Iqbal *et al.*, 2017). Several studies have shown that in *Arabidopsis* plants treated with AVG, ethylene promoted cell division, increasing stem mass, except for plants exposed to environmental stress, where ethylene had a negative effect on the cell cycle (Barry and Giovannoni, 2007; Dubois *et al.*, 2018).

Furthermore, despite the fact that height percentage increase of the stem was not significantly affected by the treatments ($P>0.05$); the observed changes for each dose were similar to those found in the button diameter percentage increase. In other words, the growth regulation processes mediated by ethylene for the crops examined in this study were similar both in the stem and the flower bud. Apparently, the analysis carried out on growth percentages showed similarities in the growth patterns of both parts of the flower, as opposed to the analysis of the absolute value of the diameter of the flower bud or the length of the stem (Figure 3).

Table 3. Mean values of the effect of AVG doses upon plant height after 48 hours during the 2018-2019 agricultural cycle.

Variety	AVG Dose (mg L^{-1})	Plant height Mean value (cm)	Statistical
Deep Rose	0	47.84	a
	100	55.88	a
	150	50.51	a
	200	54.61	b
Dark Orange	0	51.11	a
	100	55.02	a
	150	57.57	b
	200	59.26	ab

1) means with the same letter are not statistically different ($P\leq0.05$).

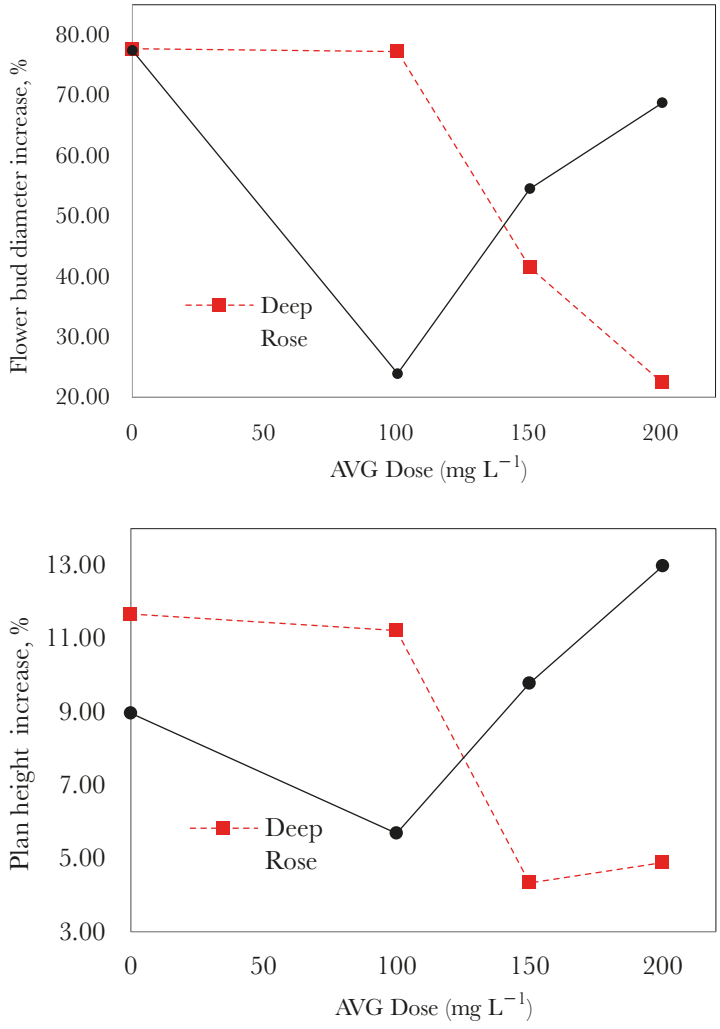

Figure 3. Mean values of the effect of AVG doses upon plant height and flower bud diameter after 48 hours during the 2018-2019 agricultural cycle.

CONCLUSIONS

AVG addition on plants of *R. asiaticus* had a significant effect reducing growth, both in diameter and height in the La Belle Deep Rose (DER) and La Belle Dark Orange (DAR) varieties. The optimal AVG dose to reduce bud diameter growth for the Deep Rose variety was 200 mg L^{-1}, while for the Dark Orange variety was 100 mg L^{-1}. In addition, plant height of both varieties increased as AVG doses increased. Reduction in the diameter of the flower bud could be related to environmental ethylene from the cutting of contiguous *R. asiaticus* flower buttons, in any event, it is necessary to carry out further tests under isolation conditions.

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