



## AGRARIAN SCIENCES

# Plant growth regulators to increase fruit set and yield of 'Rocha' pear trees in Southern Brazil

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**Abstract:** The aim of this study was to evaluate the effect of different aminoethoxyvinylglycine (AVG), thidiazuron (TDZ) and prohexadione calcium (P-Ca) rates sprayed at different timings on fruit set, yield, and fruit quality of 'Rocha' pear trees in different climatic conditions of Southern Brazil. The study was performed in two commercial orchards located in São Joaquim, SC (2015/2016) and Antônio Prado, RS (2016/2017). Plant material consisted of 'Rocha' pear trees grafted onto *Pyrus calleryana* and quince rootstock 'BA29' in São Joaquim and Antônio Prado, respectively. Treatments consisted of AVG, TDZ and P-Ca sprayed at different rates and timings. Trunk cross-sectional area increase, fruit set, thinned fruit, fruit per tree, yield, average fruit weight, projected yield, yield efficiency, fruit length, fruit diameter, L/D ratio, seed number, flesh firmness, and soluble solids content were assessed. Fruit set and yield were consistently increased by AVG in all experiments. Fruit set was not affected by P-Ca and was significantly decreased by TDZ. However, yield was positively affected by P-Ca 100 mg L<sup>-1</sup> sprayed at full bloom + 7 days after full bloom and TDZ 10 mg L<sup>-1</sup> at full bloom. Fruit size was consistently increased by TDZ.

**Key words:** aminoethoxyvinylglycine, thidiazuron, fruitlet drop, fruit quality, prohexadione calcium, seed number.

## INTRODUCTION

Pear (*Pyrus* spp.) is widely cultivated in the world, with an estimated production of 27.4 million tons in 2016. However, pear in Brazil is still considered a minor crop (14,905 tons in 2016), representing no more than 10% of domestic demand which stands at about 200,000 tons a year (FAOSTAT 2018). Therefore, as roughly 90% of the domestic market is supplied by imported pears, this crop represents a potential opportunity for growers in Brazil. However, despite several attempts over the last decades, growers have lost interest on pears, because yields are usually low and inconsistent along the years.

The main factors leading to this scenario are poor flower bud development (Pasa et al.

2011), excessive vegetative growth (Carra et al. 2016) and low fruit set (Carra et al. 2018, Pasa et al. 2017a, b) of the main cultivars planted. Achieving high yields in pear orchards is dependent on the successful achievement of many sequential processes; those associated with floral induction, flower development, pollination, flower fertilization and fruitlet retention (fruit set), and fruit growth (Webster 2002). Among these factors, problems related to fruit set seems to be one of the most important, as it has been reported for some pear cultivars worldwide (Carra et al. 2018, Hawerth et al. 2011, Pasa et al. 2017a, b, Sánchez et al. 2011). Flowers are pre-programmed to abscise after anthesis unless they receive a new stimulus to continue growing, which is commonly associated with

pollination and fertilization. Furthermore, even if the first stimulus for fruit set is provided by pollination, the continued fruitlets growth and its attachment to the tree depends on its ability to compete with strong vegetative shoots growth for nutrients and carbohydrates (Jackson 2003). However, even when these factors are suitable, pear trees frequently fail to produce adequate yields (Webster 2002).

Pollination and fertilization are affected mainly by the presence of compatible pollen and pollination vectors, climatic conditions during flower period, and hormonal balance (Webster 2002). Climatic conditions play an important role on the fruit set process, mainly temperature and precipitation during the flowering, besides, temperature affects pollen germination, pollen tube growth rate and ovules longevity, resulting in a variation in the effective pollination period (EPP) from 1 to 9 days (Sanzol & Herrero 2001). Plant hormones are also involved on pear fruit set (Jackson 2003) as they are responsible for triggering and controlling critical processes in the trees.

Ethylene is a plant hormone that has shown to be partially responsible for low fruit set in pears (Carra et al. 2018, Einhorn & Wang 2016), as it is involved in the senescence and flowers abscission (Greene 1980, Martínez et al. 2013) and fruitlets (Webster 2002). The application of ethylene inhibitors such as aminoethoxyvinylglycine (AVG), may provide a potential tool to increase fruit set. AVG suppresses ethylene biosynthesis by inhibiting the enzymatic activity responsible for the conversion of S-adenosyl methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) (Yang & Hoffman 1984). Recent studies have shown that ethylene production rate was significantly and rate-dependently reduced by AVG and were associated with markedly higher fruit set and yield of 'D'Anjou', 'Comice' (Einhorn

& Wang 2016), and 'Rocha' (Carra et al. 2018) pears. In both trials, positive effects were only observed when AVG was sprayed between 7 and 14 DAFB. Pasa et al. (2017a) did not observe positive effect of AVG on 'Rocha' pears fruit set when sprayed at full bloom. Similar increase in fruit set and production in response to AVG were also observed in 'Packham's Triumph' and 'Abate Fetel' (Dussi et al. 2002, 2011, Sánchez et al. 2011).

The application of gibberellins (Hawerth et al. 2011, Vercammen & Gomand 2008) and thidiazuron (TDZ) (Bianchi et al. 2000, Pasa et al. 2017b, Petri et al. 2001) sprayed at full bloom showed positive effects on fruit set of apple and pear trees. Significant increase in fruit set was observed in 'Packham's Triumph' (Pasa et al. 2017b, Petri et al. 2001), 'Shinseiki' (Hawerth et al. 2011) and 'Hosui' (Pasa et al. 2017b) pears, respectively. The higher fruit set induced by these substances is usually due to a higher rate of parthenocarpy (Vercammen & Gomand 2008, Petri et al. 2001), which in some cases may lead to misshapen fruits (Bianchi et al. 2000), mainly in response to high rates of TDZ (Greene 1995).

Prohexadione calcium (P-Ca) is another plant growth regulator that could potentially improve fruit set of pear trees, by reducing the competition for carbohydrate between shoot growth and fruitlets (Carra et al. 2017a). In addition, P-Ca could potentially increase fruit set by interfering with the ethylene metabolism (Rademacher 2004), which carries essential role in fruit abscission (Gepstein & Kieber 2013).

The aim of this study was, therefore, to evaluate the effect of different AVG, TDZ and P-Ca rates sprayed at different timings on fruit set, yield, and fruit quality of 'Rocha' pear trees in different climatic conditions of Southern Brazil.

## MATERIALS AND METHODS

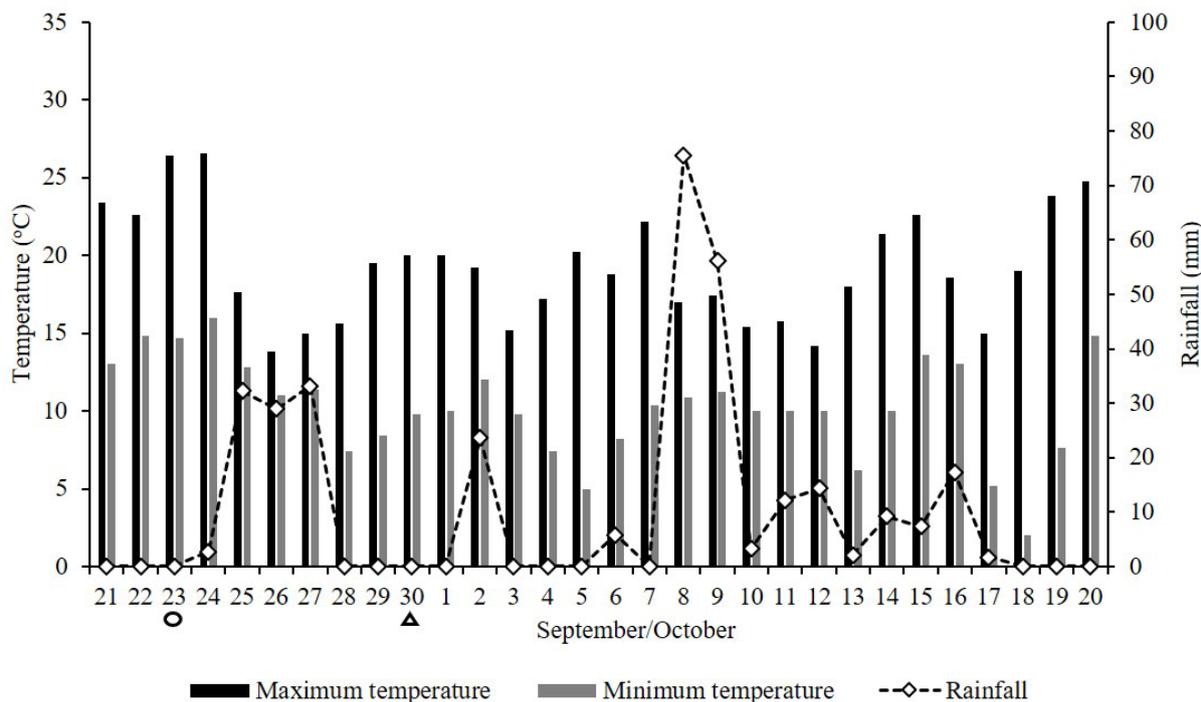
The study was conducted at two commercial orchards at different locations in Southern Brazil as described below.

Experiment 1. This experiment was set up at a commercial orchard located in São Joaquim, Santa Catarina, Brazil (Latitude 28° 07' 29.68" S, Longitude 49° 48' 52.65" W Greenwich, at 1231m of altitude), during the 2015/2016 growing season. According to Köppen-Geiger classification, this region is defined as humid mesothermal (Cfb) temperate climate, constantly humid, without a dry season, and cool summer. The average chill hour accumulation (below 7.2 °C) is around 800 hours. Plant material consisted of 11-year-old 'Rocha' pear trees grafted on *Pyrus calleryana*, trained in a central-leader system. Trees were spaced at 4 m between rows and 2 m within rows, totalizing 1,250 trees per hectare. Climatic

conditions before and following treatments application are shown in Figure 1.

The experiment was arranged in a randomized complete block design with four single-tree replications. For each replication, two surrounding trees were used as guard, to avoid drift to adjacent treatments. All trees were selected by size (canopy volume) and then grouped into blocks based on bloom density (number of flower clusters per tree at full bloom).

Treatments consisted on: 1) UTC (untreated control trees); 2) AVG 60 mg L<sup>-1</sup> at full bloom (FB); 3) AVG 60 mg L<sup>-1</sup> at FB + 7 days after full bloom (DAFB); 4) AVG 60 mg L<sup>-1</sup> at 7 DAFB; 5) AVG 30 mg L<sup>-1</sup> at FB + 7 DAFB; 6) P-Ca 200 mg L<sup>-1</sup> at FB; 7) P-Ca 200 mg L<sup>-1</sup> at 7 DAFB; 8) P-Ca 200 mg L<sup>-1</sup> at FB + 7 DAFB; 9) P-Ca 100 mg L<sup>-1</sup> at FB + 7 DAFB; 10) TDZ 20 mg L<sup>-1</sup> at FB; 11) TDZ 40 mg L<sup>-1</sup> at FB. The source of AVG, P-Ca and TDZ were ReTain® [15%



**Figure 1.** Climatic conditions before and following treatments applications in September and October 2015/2016 growing season in São Joaquim, SC. Application dates are indicated by a circle (full bloom) and a triangle (7 days after full bloom – DAFB) below the “x” axis. Source: INMET/ BDMEP (São Joaquim, SC).

of active ingredient (a.i.)], Viviful® (27.5% a.i.) and Dropp® (50% a.i.), respectively. All solutions were supplemented with 0.05% of a nonionic silicone surfactant (Break-Thru®). Treatments were sprayed using a motorized hand-gun backpack sprayer (Stihl SR 450) with a flow rate of 2.64 L min<sup>-1</sup>. Spraying volume was approximately 1500 L ha<sup>-1</sup>. The pH of the water used to prepare the solutions was 6.95. Trees were sprayed to runoff during the morning, with temperature ranging from 20 to 25 °C, relative humidity of 85 to 95% and wind speed not exceeding 5 km h<sup>-1</sup>.

Trunk diameter was measured at 20 cm above the graft union and then trunk cross-sectional area (TCSA) was calculated according to Carra et al. (2017a) and expressed in cm<sup>2</sup> to calculate crop load and yield efficiency. Fruit set was determined by counting all flower clusters per tree at full bloom (FB) and then the remaining number of fruit per tree after natural fruit drop (~40 DAFB), and expressed as number of fruit per flower cluster. In the ensuing year, return bloom was determined by counting the number of flower clusters per tree at FB. Full bloom dates were September 23, 2015 and September 07, 2016.

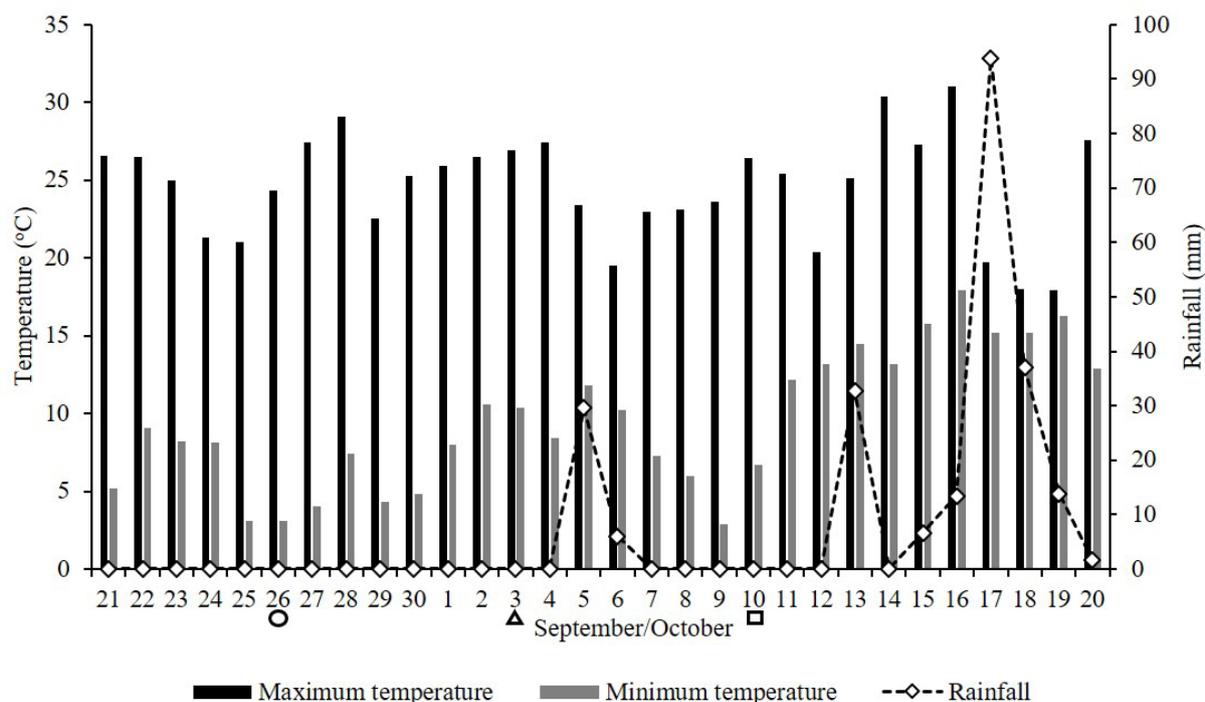
Trees were harvested at commercial maturity on February 03, 2016 (134 DAFB) and all fruit per tree were counted and weighed (kg). From these data, the following parameters were calculated: crop load (number of fruit cm<sup>-2</sup>), calculated as the number of fruit per trunk cross-sectional area (TCSA); yield (kg tree<sup>-1</sup>); average fruit weight (g); estimated yield (Mg ha<sup>-1</sup>), obtained from yield and number of trees per hectare (1,250) and; yield efficiency (Kg cm<sup>-2</sup>) calculated as the yield per TCSA.

At harvest, 15 fruits per replicate (tree) were sampled for fruit quality analysis. Flesh firmness (FF) was measured with a digital firmness tester (Fruit Texture Analyzer, Güss Manufacturing, Strand, South Africa), using an 8mm diameter

probe, and expressed in Newtons. Sections of skin (2 cm in diameter) were removed at the fruit widest point on opposite sides prior to the FF determination. A composite sample of fruit flesh per replicate was juiced, and 0.5 mL of juice was placed onto a digital refractometer (model PR-32, Atago Co., Tokyo, Japan) to determine soluble solids content (SSC), expressed as °Brix. From these samples, fruit diameter (at the widest point) and length were also measured with a digital caliper, expressed in millimeters. From these data, the length/diameter fruit ratio was calculated as the reason between length and diameter. The number of viable seeds per fruit was assessed by cutting the fruit in two halves and manually removing and counting the viable seeds of each fruit.

Experiment 2. This experiment was set up at a commercial orchard in Antônio Prado, Rio Grande do Sul, Brazil (Latitude 28° 49' 15.81" S, Longitude 51° 18' 41.45" W Greenwich, at 772m of altitude), during the 2016/2017 growing season. According to Köppen-Geiger classification, this region is defined as humid mesothermal (Cfb), marine climate, constantly humid, without a dry season. The average chill hour accumulation (below 7.2 °C) is around 410 hours. Plant material consisted of 5-year-old 'Rocha' pear trees grafted onto quince rootstock 'BA29', trained in a central-leader system. Trees were spaced at 3.5 m between rows and 0.7 m within rows, totalizing 4082 trees ha<sup>-1</sup>. Climatic conditions before and following treatments application are shown in Figure 2.

The experiment was arranged similarly as in experiment 1, except for the number of replicates used in this trial, which was five. Treatments consisted on: 1) UTC (untreated control trees); 2) AVG 60 mg L<sup>-1</sup> at 7 DAFB; 3) AVG 80 mg L<sup>-1</sup> at 7 DAFB; 4) AVG 100 mg L<sup>-1</sup> at 7 DAFB; 5) AVG 60 mg L<sup>-1</sup> at 14 DAFB; 6) AVG 80 mg L<sup>-1</sup> at 14 DAFB; 7) AVG 100 mg L<sup>-1</sup> at 14 DAFB; 8) TDZ 10 mg L<sup>-1</sup> at FB;



**Figure 2.** Climatic conditions before and following treatments application in September and October 2016/2017 growing season in Antônio Prado, RS. Application dates are indicated by circle (full bloom), triangle (7 days after full bloom – DAFB) and a square (14 DAFB) below the “x” axis. Source: FieldClimate - Antônio Prado Weather Station (Antônio Prado, RS).

9) TDZ 20 mg L<sup>-1</sup> at FB; and, 10) TDZ 30 mg L<sup>-1</sup> at FB. The source of plant growth regulators and surfactant, surfactant rate, spray application, climatic conditions during application were similar to experiment 1. Spraying volume was approximately 1000 L ha<sup>-1</sup>.

Fruit set, number of fruits per tree, crop load, yield, average fruit weight, estimated yield, fruit length, fruit diameter, L/D ratio, seed number, flesh firmness and soluble solids content were assessed similarly as in experiment 1. In experiment 2, after counting the number of fruits per tree to obtain fruit set, trees were hand-thinned, and the number of fruit removed was recorded. The total number of fruit per tree was also calculated by adding the number of fruit thinned to the number of fruit harvested. Full bloom occurred on September 26, 2016.

Trees were harvest at commercial maturity on February 06, 2017 (134 DAFB).

Statistical analyses were performed using the R software (R Core Team 2017), with the package ExpDes (Ferreira et al. 2013). Analysis of variance (ANOVA) was performed by *F* test and when significant the data were submitted to mean comparison by Duncan’s test at 5% of significance. Linear and polynomial regression were performed to determine the effect of AVG and TDZ rates when applicable.

## RESULTS

Experiment 1. The greatest fruit set was observed with 60 mg L<sup>-1</sup> of AVG (FB + 7 DAFB) followed by 60 mg L<sup>-1</sup> of AVG (7 DAFB) and AVG 60 mg L<sup>-1</sup> (FB). These two treatments and P-Ca 100 mg L<sup>-1</sup> (FB + 7 DAFB) also showed greater number of fruit

per tree, yield and estimated yield compared to control trees. Trees sprayed with 60 mg L<sup>-1</sup> of AVG (FB + 7 DAFB) and 60 mg L<sup>-1</sup> of AVG (7 DAFB) showed greater crop load compared to control trees. Fruit weight was significantly increased by AVG 60 mg L<sup>-1</sup> (FB + 7DAFB), relative to P-Ca 200 mg L<sup>-1</sup> (FB), P-Ca 100 mg L<sup>-1</sup> (FB + 7 DAFB, and TDZ 40 mg L<sup>-1</sup> (FB). TDZ 40 mg L<sup>-1</sup> (FB) was the only treatment that negatively affected fruit size when compared to untreated control trees (Table I). Fruit length, fruit diameter, L/D ratio, number of seeds per fruit, flesh firmness, soluble solids content and flower clusters per tree were not affected by treatments (Table II).

Experiment 2. Fruit set was increased by AVG compared to control, regardless the rate or spraying timing. However, trees receiving AVG

at 7 DAFB showed greater fruit set than those sprayed at 14 DAFB. TDZ significantly reduced fruit set compared to all treatments and was linearly reduced by increasing TDZ rates (Table III). Higher fruit set in response to AVG rates resulted in greater number of fruit thinned, while the opposite was observed with TDZ treated trees. Number of fruits per tree and crop load were significantly increased by AVG 60, 80 and 100 mg L<sup>-1</sup> at 7 DAFB and 100 mg L<sup>-1</sup> at 14 DAFB, followed by 60 and 80 mg L<sup>-1</sup> sprayed 14 DAFB. TDZ 10 mg L<sup>-1</sup> did not differ from control trees, but 20 and 30 mg L<sup>-1</sup> significantly decreased the number of fruit and crop load (Table III). All AVG rates sprayed 7 DAFB significantly increased number of fruit per tree, crop load, yield and estimated yield compared to control trees,

**Table I. Fruit set, crop load, number of fruit per tree, yield, average fruit weight, estimated yield and yield efficiency of 'Rocha' pear trees treated with different rates and timings of aminoethoxyvinilglycine (AVG), prohexadione calcium (P-Ca) and thidiazuron (TDZ) in São Joaquim, SC, in the 2015/2016 growing season.<sup>1</sup>**

Treatment (mg L <sup>-1</sup> )	Fruit set <sup>3</sup>	Fruit tree <sup>-1</sup>	Crop load (fruit cm <sup>-2</sup> of TCSA)	Yield (Kg tree <sup>-1</sup> )	Average fruit weight (g)	Estimated yield (Mg ha <sup>-1</sup> )	Yield efficiency (Kg cm <sup>-2</sup> )
Untreated control	0.212 c	22.3 d	0.260 c	3.20 c	141.0 ab	4.00 c	0.033 c
AVG 60 (FB <sup>4</sup> )	0.383 bc	30.3 d	0.377 c	3.75 c	123.8 abc	4.69 c	0.044 c
AVG 60 (FB + 7 DAFB <sup>5</sup> )	0.827 a	68.0 ab	0.865 ab	9.97 a	148.0 a	12.47 a	0.116 ab
AVG 60 (7 DAFB)	0.567 b	76.8 a	1.086 a	9.51 a	124.7 abc	11.89 a	0.126 a
AVG 30 (FB + 7 DAFB)	0.263 c	39.3 cd	0.406 c	5.55 bc	139.8 ab	6.93 bc	0.054 c
P-Ca 200 (FB)	0.237 c	33.1 d	0.465 bc	4.03 c	121.8 bc	5.04 c	0.053 c
P-Ca 200 (7 DAFB)	0.203 c	28.7 d	0.377 c	3.54 c	124.0 abc	4.42 c	0.044 c
P-Ca 200 (FB + 7 DAFB)	0.162 c	30.8 d	0.398 c	4.21 c	135.0 ab	5.27 c	0.050 c
P-Ca 100 (FB + 7 DAFB)	0.307 c	62.7 abc	0.685 bc	7.55 ab	119.1 bc	9.43 ab	0.079 bc
TDZ 20 (FB)	0.197 c	44.7 bcd	0.505 bc	4.90 bc	124.3 abc	6.13 bc	0.050 c
TDZ 40 (FB)	0.241 c	35.5 d	0.434 c	3.26 c	100.5 c	4.08 c	0.038 c
p-value	<0.0001	0.0004	0.0025	<0.0001	0.0115	<0.0001	<0.0001

<sup>1</sup> Mean separation within columns by Duncan's test at  $p < 0.05$ ; means followed by different letters are significantly different.

<sup>2</sup> TCSA=trunk cross-sectional area.

<sup>3</sup> Number of fruits per flower cluster.

<sup>4</sup> FB=full bloom.

<sup>5</sup> DAFB=days after full bloom.

following a quadratic curve response (Figure 3b). However, when AVG was sprayed at 14 DAFB a reduction in the efficiency was observed compared to 7 DAFB, but still crop load and yield were linearly increased by AVG rate (Figure 3d). On the contrary, TDZ linearly decreased crop load and yield as increasing rate, but yield of trees sprayed with 10 mg L<sup>-1</sup> at FB was still greater than untreated control trees (Table III and Figure 3f).

In order to test AVG performance isolated, a variance analysis was performed considering rate and time as factors, having three levels for rate (60, 80 and 100 mg L<sup>-1</sup>) and two levels for time (7 and 14 DAFB). The interaction among the factors was not significant ( $p$ -value > 0.05) for all variables. Then, the main factors were analyzed, which for the variables fruit set ( $p$ -value < 0.01),

total number of fruit per tree ( $p$ -value = 0.027), number of thinned fruit per tree ( $p$ -value = 0.023), and number of fruit per tree ( $p$ -value = 0.039), time factor was significant, and it was higher in all cases when sprayed at 7 DAFB. These results confirmed that the best AVG effect was reached when sprayed at 7 DAFB (between the two application times tested) at concentrations of 60 to 100 mg L<sup>-1</sup>.

Fruits were smaller when AVG 80 and 100 mg L<sup>-1</sup> at 7 DAFB and 80 mg L<sup>-1</sup> at 14 DAFB were applied. A negative linear effect of AVG rate on fruit weight was observed when sprayed at 7 DAFB (Figure 3a). Whereas, when AVG was applied at 14 DAFB a quadratic curve response was observed; fruit weight reached its minimum at 80 mg L<sup>-1</sup> (Figure 3c). On the other hand, all

**Table II. Fruit length, fruit diameter, fruit length/diameter ratio, number of seed per fruit, flesh firmness (FF), solids soluble contents (SSC) and number of flower clusters on 2015/2016 and 2016/2017 growing season of 'Rocha' pear trees treated with different rates and timings of aminoethoxyvinylglycine (AVG), prohexadione calcium (P-Ca) and thidiazuron (TDZ) in São Joaquim, SC, in the 2015/2016 growing season.**

Treatment (mg L <sup>-1</sup> )	Fruit length (mm)	Fruit diameter (mm)	L/D ratio	Seeds fruit <sup>-1</sup>	FF (N)	SSC (°Brix)	Flower clusters tree <sup>-1</sup>	
							15/16	16/17 <sup>2</sup>
Untreated control	74.9	61.2	1.222	0.253	63.65	12.25	78.0	54.3
AVG 60 (FB <sup>3</sup> )	72.9	60.2	1.211	0.290	63.05	12.10	74.8	48.8
AVG 60 (FB + 7 DAFB <sup>4</sup> )	74.6	63.6	1.173	0.556	62.56	12.48	115.3	66.0
AVG 60 (7 DAFB)	71.9	60.7	1.186	0.256	62.85	11.65	80.8	44.2
AVG 30 (FB + 7 DAFB)	74.5	62.3	1.196	0.106	61.70	12.50	77.5	41.5
P-Ca 200 (FB)	70.1	60.1	1.166	0.504	66.61	11.93	66.5	34.6
P-Ca 200 (7 DAFB)	67.6	58.2	1.161	0.225	66.73	12.05	100.5	31.0
P-Ca 200 (FB + 7 DAFB)	69.6	61.8	1.126	0.139	65.59	12.00	80.5	33.6
P-Ca 100 (FB + 7 DAFB)	70.1	60.3	1.162	0.266	66.78	12.00	96.8	46.0
TDZ 20 (FB)	69.6	62.6	1.113	0.028	65.75	12.35	114.5	34.0
TDZ 40 (FB)	67.7	58.1	1.166	0.208	63.98	12.05	104.0	32.8
$p$ -value	0.2302	0.1844	0.1389	0.8235	0.2474	0.2922	0.2648	0.1472

<sup>1</sup> Means were analysed by Duncan's test at  $p < 0.05$ .

<sup>2</sup> Return bloom.

<sup>3</sup> FB=full bloom.

<sup>4</sup> DAFB=days after full bloom.

TDZ treatments significantly increased fruit size compared to all treatments; the higher the rate the larger the fruit (Table III and Figure 3e). FF was significantly decreased by AVG 100 mg L<sup>-1</sup> (7 DAFB) compared to control trees and SSC was not affected by AVG treatments (Table IV).

TDZ applications slightly induced fruit elongation, being significant longer at the lowest rate tested (10 mg L<sup>-1</sup>). Furthermore, fruit diameter was also significantly increased at all TDZ rates compared to all other treatments. TDZ treated fruit were considerable firmer and slightly sweeter than the untreated fruit (Table IV).

L/D ratio was significantly decreased by TDZ rates (10, 20 and 30 mg L<sup>-1</sup>) compared to control trees. Number of seeds per fruit was significantly increased by AVG, showing a positive quadratic (7 DAFB) and linear (14 DAFB) rate effect, while fruit length, fruit diameter, L/D ratio, FF and SSC were not affected by AVG sprayed at 14 DAFB (Table IV). As for TDZ, seed number per fruit was reduced as the rate increased, showing a negative linear rate effect, while fruit length and SSC were not affected (Table IV).

## DISCUSSION

We have tested the effect of AVG, TDZ and P-Ca on fruit set and yield of 'Rocha' pear trees. The results we have found show that AVG significantly increased fruitlet retention in both places and growing seasons, ultimately resulting in greater yields. Similar results regarding increased fruit set and yield after AVG applications were previously reported (Carra et al. 2018, Dussi 2011, Dussi et al. 2011, Einhorn & Wang 2016, Pasa et al. 2017a, c, Sánchez et al. 2011). Carra et al. (2018) observed a reduction in flowers ethylene production rate following AVG applications at 7 DAFB, which resulted higher in yields. Einhorn &

Wang (2016) also observed a marked reduction in the ethylene production rate following AVG applications between 7 and 14 DAFB, when ethylene production of fruitlets was higher. AVG sprayed at 14 DAFB has been reported to induce the greatest response on increasing pear fruit set (Dussi et al. 2002, Sánchez et al. 2011). According to Carra et al. (2018) and Pasa et al. (2017a), the first peak of ethylene production of 'Rocha' pears, in the climatic conditions of Southern Brazil, is around 7 DAFB.

Trees sprayed with AVG at 7 DAFB in experiment 2 usually showed better results to fruit set and yields compared to applications at 14 DAFB. This confirm previous work conducted in Southern Brazil that showed great increase of fruit set in 'Rocha' when AVG was applied 7 DAFB (Carra et al. 2018, Pasa et al. 2017a). The results suggest that tree responses to AVG on reducing fruit drop is related to climatic conditions, since the best results were observed when AVG was sprayed at 14 DAFB in typical winter conditions (Dussi et al. 2002, Einhorn et al. 2013, Sánchez et al. 2011) and at 7 DAFB in warm winter conditions (Carra et al. 2018, Pasa et al. 2017a). No differences in fruit set and yield among AVG rate when rates ranged from 60 to 100 mg L<sup>-1</sup> sprayed at 7 DAFB in experiment 2 were observed. Therefore, the lowest AVG rate would be recommended by economic reasons. On the other hand, when AVG was sprayed at 14 DAFB, the best results were observed with rates ranging from 80 to 100 mg L<sup>-1</sup>. Based on the equations, the maximum yields when AVG was sprayed at 7 DAFB were obtained with ~80 mg L<sup>-1</sup>, confirming previously results where similar rates had the maximum yield and projected yield of 'Rocha' pear trees (Carra et al. 2018).

P-Ca did not affect fruit set of 'Rocha' pear trees in experiment 1, however, increased the number of fruit per tree, yield and estimated yield when 100 mg L<sup>-1</sup> was sprayed at FB + 7

**Table III.** Fruit set, total number of fruit per tree, number of thinned fruit, number of fruit per tree, crop load, yield, average fruit weight and estimated yield of 'Rocha' pear trees treated with aminoethoxyvinylglycine (AVG) and thidiazuron (TDZ) in Antônio Prado, RS in the 2016/2017 growing season.<sup>1</sup>

Treatment (mg L <sup>-1</sup> )	Fruit set <sup>2</sup>	Total of fruit (fruit tree <sup>-1</sup> ) <sup>3</sup>	Thinned fruit tree <sup>-1</sup>	Fruit tree <sup>-1</sup>	Crop load (fruit cm <sup>-2</sup> of TCSA)	Yield (Kg tree <sup>-1</sup> )	Average fruit weight (g)	Estimated yield (Mg ha <sup>-1</sup> )
Untreated control	1.03 d	34.6 c	6.6 d	28.0 d	2.175 d	3.77 d	134.8 c	15.40 d
AVG 60 (7 DAFB <sup>4</sup> )	2.03 ab	73.6 a	20.6 a	53.0 ab	4.063 ab	6.85 a	130.4 cd	27.97 a
AVG 80 (7 DAFB)	2.02 ab	72.8 a	16.8 ab	56.0 a	4.242 a	6.41 ab	115.0 e	26.15 ab
AVG 100 (7 DAFB)	2.12 a	75.0 a	20.6 a	54.4 ab	4.246 a	6.66 a	123.0 de	27.19 a
AVG 60 (14 DAFB)	1.72 c	61.5 b	19.0 a	42.5 c	3.315 c	5.40 c	127.0 cd	22.02 c
AVG 80 (14 DAFB)	1.72 c	58.5 b	11.8 c	46.8 bc	3.613 bc	5.64 bc	120.6 de	23.00 bc
AVG 100 (14 DAFB)	1.79 bc	65.6 ab	14.7 bc	50.9 ab	3.929 ab	6.60 ab	129.3 cd	26.95 ab
TDZ 10 (FB <sup>5</sup> )	0.77 e	31.8 c	2.5 e	29.3 d	2.255 d	4.73 c	162.2 b	19.32 c
TDZ 20 (FB)	0.56 ef	18.4 d	0.8 e	17.6 e	1.343 e	2.94 de	167.6 ab	11.99 de
TDZ 30 (FB)	0.41 f	15.8 d	1.3 e	14.5 e	1.109 e	2.52 e	174.1 a	10.30 e
<i>p</i> -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<b><i>p</i>-value (AVG rate effect 7 DAFB)</b>								
Linear effect	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.0001	- <sup>6</sup>	-
Quadratic effect	0.0238	0.0238	0.0457	0.0296	0.0440	0.0253	-	-
<b><i>p</i>-value (AVG rate effect 14 DAFB)</b>								
Linear effect	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	-	-
Quadratic effect	0.0421	0.0746	0.0007	0.7380	0.6386	0.5526	-	-
<b><i>p</i>-value (TDZ rate effect)</b>								
Linear effect	0.0010	0.0001	0.0009	0.0001	0.0001	0.0027	-	-
Quadratic effect	0.6015	0.9699	0.0270	0.3174	0.3546	0.0498	-	-

<sup>1</sup> Mean separation within columns by Duncan's test at  $p < 0.05$ ; means followed by different letters are significantly different. Significant regression associated with: AVG 7 DAFB: fruit set,  $y = -0.0001x^2 + 0.0237x + 1.0347$  ( $R^2 = 0.9901$ ); total of fruits,  $y = -0.0057x^2 + 0.961x + 34.764$  ( $R^2 = 0.9916$ ); thinned fruits,  $y = -0.0018x^2 + 0.304x + 6.7961$  ( $R^2 = 0.8939$ ); fruits,  $y = -0.0039x^2 + 0.657x + 27.968$  ( $R^2 = 0.9993$ ); and yield,  $y = -0.0005x^2 + 0.0766x + 3.7936$  ( $R^2 = 0.9679$ ). AVG 14 DAFB: fruit set,  $y = -9E-05x^2 + 0.0163x + 1.0331$  ( $R^2 = 0.9912$ ); total of fruits,  $y = 0.3068x + 36.643$  ( $R^2 = 0.904$ ); thinned fruits,  $y = -0.0024x^2 + 0.3028x + 6.8613$  ( $R^2 = 0.6959$ ); fruits,  $y = 0.2307x + 28.207$  ( $R^2 = 0.9986$ ); and yield,  $y = 0.0269x + 3.7389$  ( $R^2 = 0.977$ ). TDZ: fruit set,  $y = -0.0207x + 1.003$  ( $R^2 = 0.9861$ ); total of fruits,  $y = -0.698x + 35.62$  ( $R^2 = 0.914$ ); thinned fruits,  $y = 0.0114x^2 - 0.5188x + 6.5875$  ( $R^2 = 0.9999$ ); fruits,  $y = -0.522x + 30.18$  ( $R^2 = 0.8287$ ); and yield,  $y = -0.0034x^2 + 0.0481x + 3.976$  ( $R^2 = 0.7032$ ).

<sup>2</sup> Number of fruits per flower cluster.

<sup>3</sup> Thinned fruits + fruits per tree.

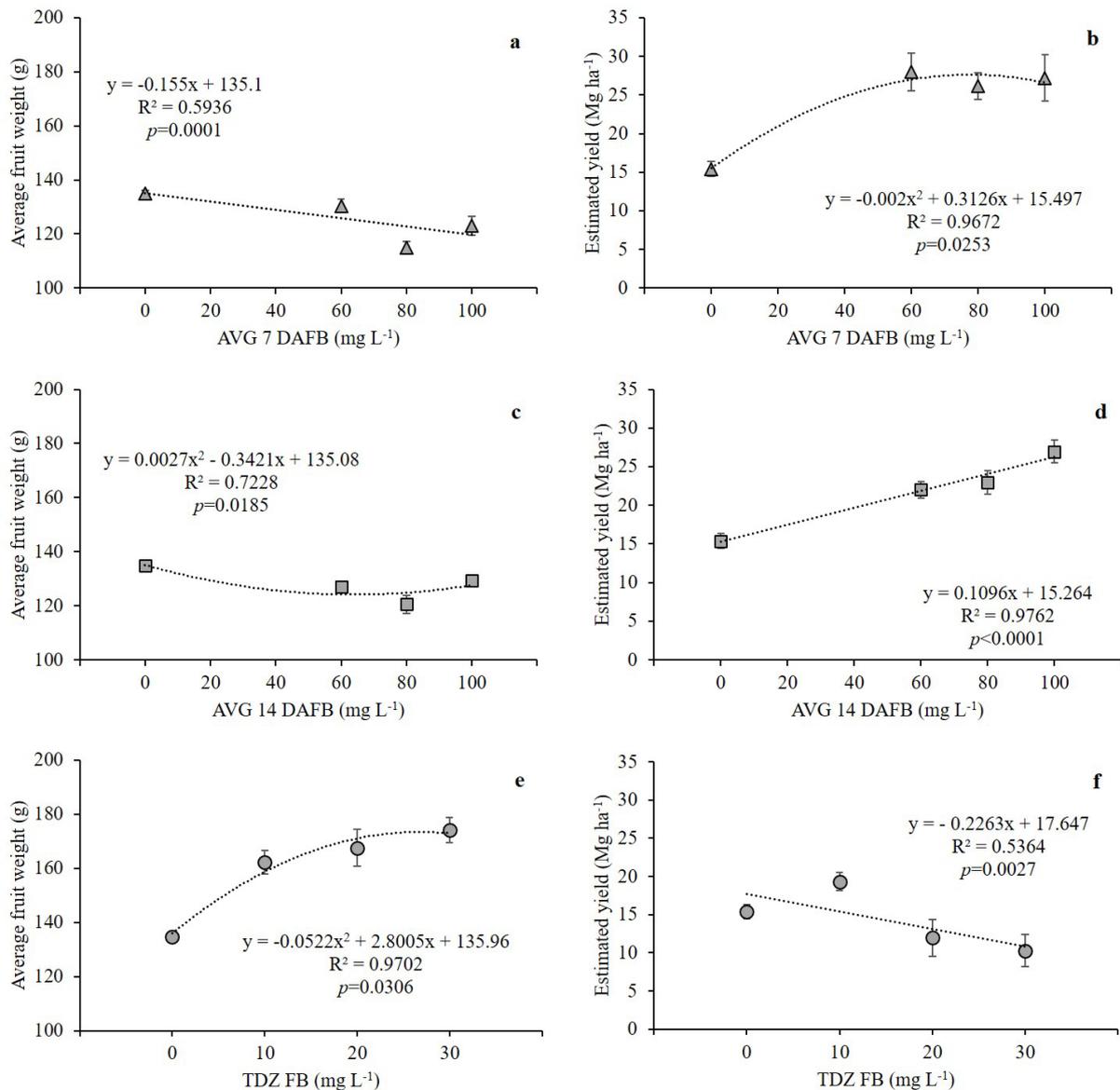
<sup>4</sup> DAFB=days after full bloom.

<sup>5</sup> FB=full bloom.

<sup>6</sup> *p*-value,  $R^2$  value and equations showed in Figure 2.

DAFB. Similar results where fruit set was not significantly affected by P-Ca applications were observed in 'Le Conte' (Carra et al. 2016), 'Shinseiki' (Carra et al. 2017b) and 'Spadona' pear trees (Asín et al. 2007). The opposite was observed in one out of three growing seasons in 'D'Anjou' (Einhorn et al. 2014), and one of two growing seasons in 'Smith' pear trees (Carra et

al. 2017a), where P-Ca significantly increased fruit set and number of fruit compared to control trees. Fruit set response to P-Ca application is not consistent among studies, which indicates the complexity of the process and various factors that modulate it, such as genotypic responses/sensitivity to ethylene, hormonal balance, production of previous years, previous



**Figure 3.** Average fruit weight (a, c and e) and estimated yield (b, d and f) of 'Rocha' pear trees subjected to aminoethoxyvinylglycine (AVG) at 7 days after full bloom (DAFB) (a and b), 14 DAFB (c and d) and thidiazuron (TDZ) at full bloom (FB) (e and f) in Antônio Prado, RS on 2016/2017 growing season. Vertical bars represent standard error.

P-Ca applications and environmental conditions before, during and after application (Stover & Greene 2005).

TDZ did not affect fruit set, yield and projected yield of 'Rocha' pear trees in experiment 1 and significantly reduced fruit set, fruit number, yield and projected yield in experiment 2. Greene (1995) reported similar effects in 'Empire' apple trees treated with TDZ 15 mg L<sup>-1</sup>, where TDZ significantly reduced fruit set, working as a fruit thinner. The same author also observed that TDZ (10 and 50 mg L<sup>-1</sup>) sprayed at full bloom, showed no effect on fruit set of 'McIntosh' apples. On the other hand, Petri et al. (2001) found that TDZ 10 mg L<sup>-1</sup> significantly reduced fruit drop and increased fruitlet retention, ultimately resulting in greater yield in 'Packham's Triumph' pears. Pasa et al. (2017b) observed the same responses in 'Hosui' and 'Packham's Triumph' when TDZ was applied in the range of 20 to 60 mg L<sup>-1</sup> at FB. Pasa et al. (2017b) also observed that TDZ 60 mg L<sup>-1</sup> resulted in the highest number of fruits per tree and yields compared to other treatments. In the present study, TDZ reduced fruit set even at the lowest rate tested (10 mg L<sup>-1</sup>) but significantly increased yield compared to control. This increase in yield was associated with higher average fruit weight of trees sprayed with TDZ 10 mg L<sup>-1</sup>, since no differences on the number of fruits per tree were observed when comparing to the untreated control treatment. Likewise, Stern et al. (2003) observed a slight thinning effect and increased fruit size of 'Spadona' and 'Coscia' pears sprayed with TDZ 20–30 mg L<sup>-1</sup>. Collectively, these results suggest TDZ effect on fruit set is rate and cultivar dependent, therefore, it should be tested for each cultivar and species separately, as means to adjust the rate according the expected results, i.e., fruitlet retention or thinning.

Higher fruit set and yields responses of AVG-treated trees are probably a direct effect

of fruit drop reduction in response to ethylene synthesis inhibition, as observed by Pasa et al. (2017a) and not an effect on EPP as suggested and observed in other studies (Lombard & Richardson 1982, Crisosto et al. 1986, Carra et al. 2018) since at 7 DAFB most flowers should be opened, and fertilization process ended. However, the increased number of seeds per fruit of AVG treated trees in experiment 2 (Table IV) is intriguing and further investigation is needed. A hypothesis to the increase of seed number per fruit may be related to the prevention of seeds abortion in AVG-treated trees by the reduction in the ethylene production in seeds. Several studies with embryo and seed abortion in plants have been carried out during the years, attributing the abortion to genetic load (Bawa 1989, Kärkkäinen et al. 1999) and the amount of growth inhibitors, abscisic acid and ethylene increase (Stephenson 1981, Hays et al. 2007). Hays et al. (2007) in a study with heat stress in wheat (*Triticum aestivum* L.) found that a cultivar susceptible to heat shows increased ethylene production rate when exposed to 38°C during early kernel development, causing kernel abortion. Collectively, ethylene inhibition by AVG in the present study may have increased seed longevity after pollination and fertilization, then increasing the number of viable seeds.

Average fruit weight of 'Rocha' pears was significantly reduced in some AVG-treated trees, which was likely a direct effect of higher crop load. Similar results were reported by Dussi et al. (2002, 2011) in AVG treated 'Packham's Triumph' and 'Abate Fetel' and also by Carra et al. (2018) in 'Rocha' pear trees. On the other hand, Pasa et al. (2017a) observed no differences in average fruit weight of 'Rocha' pears treated with AVG, probably because the crop load was below the maximum crop load capacity of trees, even with AVG-enhanced fruit set. Fruit weight was significantly increased by TDZ in all treatments in experiment 2. Increase

in fruit weight is commercially desirable since 'Rocha' pears usually yield small fruits, and larger fruits usually achieve better prices. The increase in fruit weight in experiment 2 in response to TDZ could be partially attributed to a crop load effect, since the higher rates significantly reduced crop load, but not when trees were sprayed with TDZ 10 mg L<sup>-1</sup>, since number of fruit per tree was

similar to control. Several studies suggest that endogenous cytokinin levels play a major role on cell division and fruit growth (Shargal et al. 2006, Stern et al. 2003). TDZ is a phenylurea compound, which shows cytokinin-like activity (Greene 1995), then a positive effect on fruit growth should be expected. Indeed, exogenous application of TDZ increased fruit size of 'Spadona', 'Coscia' (Stern

**Table IV.** Fruit length, fruit diameter, fruit length/diameter ratio, number of seeds per fruit, flesh firmness (FF) and solids soluble contents (SSC) of 'Rocha' pear trees subjected to aminoethoxyvinilglycine (AVG) and thidiazuron (TDZ) at different times in Antônio Prado, RS on 2016/2017 growing season.<sup>1</sup>

Treatment (mg L <sup>-1</sup> )	Fruit length (mm)	Fruit diameter (mm)	L/D ratio	Seeds fruit <sup>-1</sup>	FF (N)	SSC (°Brix)
Untreated control	77.7 bcd	62.5 bc	1.242 a	5.3 cd	57.31 ab	12.47 b
AVG 60 (7 DAFB <sup>2</sup> )	76.0 cd	62.8 bc	1.211 abc	6.1 ab	54.59 abc	12.57 b
AVG 80 (7 DAFB)	77.7 bcd	62.0 bc	1.255 a	6.6 a	54.00 bc	12.96 ab
AVG 100 (7 DAFB)	75.6 cd	61.5 bc	1.230 ab	6.5 ab	52.72 c	12.69 ab
AVG 60 (14 DAFB)	78.1 bcd	63.3 bc	1.234 ab	6.3 ab	57.00 ab	12.63 b
AVG 80 (14 DAFB)	75.3 d	60.7 c	1.241 a	6.7 a	58.79 a	12.60 b
AVG 100 (14 DAFB)	76.9 cd	63.7 b	1.208 abc	5.8 bc	57.50 ab	12.65 b
TDZ 10 (FB <sup>3</sup> )	82.8 a	70.1 a	1.181 bcd	4.7 de	50.77 c	13.36 a
TDZ 20 (FB)	79.5 abc	70.2 a	1.134 d	4.2 e	50.66 c	13.05 ab
TDZ 30 (FB)	81.3 ab	69.4 a	1.171 cd	3.5 f	52.12 c	13.35 a
<i>p</i> -value	0.0014	<0.0001	0.0006	<0.0001	0.0012	0.0323
<b><i>p</i>-value (AVG rate effect 7 DAFB)</b>						
Linear effect	0.3919	0.5045	0.8911	0.0023	0.0430	0.2735
Quadratic effect	0.9187	0.5250	0.6387	0.4383	0.9471	0.7996
<b><i>p</i>-value (AVG rate effect 14 DAFB)</b>						
Linear effect	0.1965	0.7752	0.1407	0.0015	0.7418	0.5111
Quadratic effect	0.7947	0.1638	0.2326	0.0012	0.9878	0.8558
<b><i>p</i>-value (TDZ rate effect)</b>						
Linear effect	0.1118	0.0008	0.0007	0.0002	0.0555	0.0521
Quadratic effect	0.1142	0.0018	0.0024	0.6546	0.0325	0.2511

<sup>1</sup>Mean separation within columns by Duncan's test at  $p < 0.05$ ; means followed by different letters are significantly different. Significant regression associated with: AVG 7 DAFB: number of seeds per fruit,  $y = 0.0132x + 5.3321$  ( $R^2 = 0.9335$ ); flesh firmness,  $y = -0.0446x + 57.331$  ( $R^2 = 0.9926$ ); AVG 14 DAFB: number of seeds per fruit,  $y = -0.0004x^2 + 0.042x + 5.2779$  ( $R^2 = 0.8404$ ); TDZ: fruit diameter,  $y = -0.021x^2 + 0.838x + 62.83$  ( $R^2 = 0.9475$ ); L/D ratio,  $y = 0.0002x^2 - 0.01x + 1.2455$  ( $R^2 = 0.9593$ ); number of seed per fruit,  $y = -0.059x + 5.31$  ( $R^2 = 0.996$ ); flesh firmness,  $y = 0.02x^2 - 0.7568x + 57.067$  ( $R^2 = 0.9599$ ).

<sup>2</sup> DAFB=days after full bloom.

<sup>3</sup> FB=full bloom.

et al. 2003), 'Shinseiki' (Hawerth et al. 2011) and 'Hosui' pears (Pasa et al. 2017b).

Flesh firmness (FF) and soluble solids content (SSC) at harvest were not influenced by AVG, P-Ca and TDZ in experiment 1 (Table II). However, in experiment 2, FF was significantly reduced, and SSC increased by TDZ. Similar results were observed in pears, where FF and SSC were not significantly affected by P-Ca (Pasa et al. 2017d) and AVG (Pasa et al. 2017a) application. SSC increase by TDZ was probably a direct effect of low crop load. Similar results were observed in 'Jonagold' apples, where low-cropping trees had significantly higher soluble solids than high-cropping trees (Stopar et al. 2002) and in 'Red Fuji' apples, where medium and low-cropping load treatments significantly improved fruit quality (Ding et al. 2017). Other authors did not observe effect of TDZ sprayed at full bloom on FF and SSC of 'McIntosh' and 'Empire' apples (Greene 1995) and 'Hosui' and 'Packham's Triumph' pears (Pasa et al. 2017b). Based on our results and on the literature, it seems that when AVG, P-Ca and TDZ were sprayed near and/or at bloom there was little effect on fruit quality attributes of both pears and apples.

Return bloom was not affected by AVG, P-Ca and TDZ in experiment 1 (Table II). Similar results were observed when the rates of 60 and 80 mg L<sup>-1</sup> of AVG had similar return bloom compared to untreated trees (Carra et al. 2018). Pasa et al. (2017) also observed no significant difference between untreated trees and trees treated with 60 and 80 mg L<sup>-1</sup> of AVG at 7 or 14 DAFB.

## CONCLUSION

Fruit set and yield of 'Rocha' pear trees increased with AVG at rates ranging from 60 to 100 mg L<sup>-1</sup>, with optimum rate indicated by regression analysis around 80 mg L<sup>-1</sup>. No differences in

fruit set and yield between rates were observed when AVG as sprayed at 7 DAFB, indicating that the lowest AVG rate would be recommended by economic reasons. P-Ca 100 mg L<sup>-1</sup> increased yield when sprayed at FB + 7 DAFB, but similar results are not observed with increasing rates. Despite of several studies reporting increased fruit set in response to TDZ, in our study TDZ decreased fruit set in a rate-responsive manner, showing a thinning effect. Fruit weight is reduced by some AVG treatments, but most probably as a direct effect of increased crop load. Fruit quality attributes were little affected by AVG and P-Ca treatments, but TDZ reduced flesh firmness at harvest. Return bloom was not influenced by any treatment. Collectively, the results we have found show that use of AVG is a potential tool to improve fruit set of 'Rocha' pear orchards, increasing yield and orchard efficiency. Further studies testing TDZ rates lower than 10 mg L<sup>-1</sup> on fruit set are necessary, as well as lower rates of P-Ca.

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### Author contributions

B Carra collected the data, made most of the analyses, and wrote the first draft of the manuscript. MS Pasa set the main lines of the experiments, contributed with data collection, analyses, interpretation of results, manuscript writing, and financial acquisition. ES Abreu, M Dini and CP Pasa contributed with data collection and analyses. MN Ciotta contributed to the interpretation of results and discussion. FG Herter and P Mello-Farias contributed on setting the main line of the experiments, financial acquisition and manuscript review.

