

# Effects of Thidiazuron and Pollinator visits on Fruit Set and Quality of 'Maxigala' Apple Trees

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## Abstract

The objective of this study was to assess the effects of thidiazuron (TDZ) application and pollinator visits on fruit set and quality in apple trees of the cultivar Maxigala. The experiment was conducted at the Temperate Fruit Farming Experimental Station of the Brazilian Agricultural Research Corporation (EMBRAPA Grape and Wine), in Vacaria, RS, Brazil. The evaluated treatments were: TDZ application; floral visitors combined with TDZ application; floral visitors; and control (no floral visitors with no TDZ application). The highest mean final fruit set was found for the treatment with only TDZ application, followed by that with floral visitors and TDZ application.

**Keywords:** bees, fruiting, complementary action

## Introduction

Bees (*Apis mellifera*; Hymenoptera: Apidae) are extensively utilized for pollination apple orchards in Brazil. The species *A. mellifera* has characteristics that make it the preferred pollinator in apple orchards (Santos et al., 2013; Sheffield, 2014). However, the disappearance of numerous hives in North America and Europe has raised concerns in the fruit production sector (Message et al., 1995; Deprá et al., 2018).

Colony Collapse Disorder (CCD) is part of a global phenomenon known as pollinator decline. Typically, decreases in *A. mellifera* populations is connected to health issues (diseases and parasites), nutritional deficiencies (lack of adequate food), and use of pesticides in agricultural crops.

Reductions in beehive availability for apple orchard pollination services can jeopardize fruit production and result in financial, social, and ecological

losses. Apple production is economically and socially important in Brazil, with an annual production value of US\$440.8 million (Giannini et al., 2015), generating numerous direct and indirect jobs.

The use of thidiazuron (TDZ), a plant growth regulator, has proven to be an effective alternative for successful fruiting in several fruit species, including apple (Ayub, et al., 2019). TDZ is a phenylurea compound with cytokinin activity. Cytokinins, combined with auxins, stimulate cell division in plants, promoting growth and defining fruit shape in some temperate fruit species. TDZ mimics the action of cytokinins and auxins in plants, which are hormones directly involved in fruit set and development. Cytokinins and auxins are typically produced by seeds in fruits; an inefficient pollination reduces seed production, resulting in fruit abortion or deformation.

Therefore, studies focused on developing

techniques to enhance pollination and fruit set to increase fruit yield and quality in apple orchards are necessary.

Considering the lack of scientific information on effects of TDZ application combined with pollination services typically carried out by bees (*A. mellifera*), the objective of this study was to assess the effects of TDZ application during full flowering stage, combined with pollination by *A. mellifera* and other natural pollinators, on fruit set and quality in apple trees of the cultivar Maxigala.

## Material and Methods

The experiment was conducted in the 2017-2018 and 2018-2019 growing seasons in an apple orchard at the Temperate Fruit Farming Experimental Station of the Brazilian Agricultural Research Corporation (EMBRAPA Grape and Wine), in Vacaria, RS, Brazil. The orchard has in an area of 0.4 hectares and was established in 2011 with scions of the apple cultivars Fuji Suprema and Maxigala grafted onto M-9 rootstocks. The spacing used was 1.20 m (Fuji Suprema) and 1.0 m (Maxigala) between plants in the rows, totaling populations of 325 and 385 plants, respectively, arranged in blocks of five rows for each cultivar.

Disease control treatments were carried out following guidelines for integrated apple production, except for insecticide, which was not allowed in the experimental area because it is used for entomological studies. Colonies of *A. mellifera* with good population conditions were introduced into the orchard at a density of two hives per hectare during the flowering stage.

The effect of applying the phytohormone thidiazuron (TDZ) to 'Maxigala' apple trees, combined or not with pollination by bees, was evaluated in a completely randomized experimental design with four treatments (**Table 1**) and eight replications. Randomly selected branches on the plants were considered as replications. Each apple tree had up to two marked branches randomly distributed in groups of two plants, with a distance corresponding to two plants between the treatments; the branches were identified using striped tape and labels with information on each treatment.

**Table 1.** Treatments evaluated in the experiment with thidiazuron (TDZ) application to 'Maxigala' apple trees at the flowering stage, combined or not with floral visitors, in the 2017-2018 and 2018-2019 growing seasons. Vacaria, RS, Brazil

Identification	Treatment
T1	Application of TDZ and branch protection until petal fall
T2	Application of TDZ with no branch protection
T3	No TDZ application and no branch protection
T4	No TDZ application with branch protection until petal fall (control)

TDZ was applied at a rate of 20 g ha<sup>-1</sup>, using a backpack sprayer; the application volume corresponded to 1,000 L ha<sup>-1</sup>. A single application was performed to branches at the full flowering stage. The branches were then protected using wire cages covered with nonwoven fabric to prevent pollinators from visiting the flowers, or left unprotected, according to the treatments. The branches remained protected by the cages until the petals had completely fallen.

The analyzed variables included final fruit set, number of fruits, and number of seeds per fruit. Final fruit set was determined by counting the number of flowers per floral cluster at full flowering, and number of fruits set 30 days after flowering. The percentage of final fruit set was calculated by dividing the number of set fruits by the initial number of flowers, and multiplying the result by 100 (Lopes et al., 2012). Additionally, fruit length (FL) and diameter (FD), FL to FD ratio, distance between lobes, and length and diameter of the calyx tube were measured in the 2018-2019 growing season.

Ten fruits were randomly collected in each treatment and evaluated for pulp firmness and total soluble solids (TSS; °Brix) in both seasons. The analyses were conducted at the Plant Production Laboratory of the Temperate Fruit Farming Experimental Station of Embrapa Grape and Wine.

The data were tabulated and the means of each variable were compared using the Tukey's test at 5% significance level, using the SASM-Agri program (Canterri et al., 2001).

## Results and discussion

The application of Thidiazuron (TDZ) to apple trees at full flowering, with flower protection from pollinator visits until petal fall, resulted in final fruit sets of 22.8% in 2017-2018 and 22.9% in the 2018/2019 growing season, significantly differing from the other treatments in both growing seasons (**Table 2**). This result confirms the efficiency of TDZ in fruit formation and set in apple trees. Similarly, Petri et al. (2001) found a final fruit set of 40%, and Fagundes et al. (2017) found increases of 25% and 30% compared to control treatments and reported that these percentages are sufficient for financial return in apple production.

The final fruit set in branches with application of TDZ and pollinator visits significantly decreased compared to those treated with TDZ and with flower protection (Table 2). The decrease of approximately 50% in final fruit set in branches with TDZ application and pollinator visits was not expected. However, the final fruit set obtained only by the action of pollinators was lower than that

**Table 2.** Mean final fruit set percentages in branches of 'Maxigala' apple trees subjected to different treatments at the flowering stage in the 2017-2018 and 2018-2019 growing seasons. Vacaria, RS, Brazil

Treatment	Growing season	
	2017/2018	2018/2019
Thidiazuron - TDZ	22.8a	22.9a
TDZ + floral visitors	9.4b	11.6b
Only floral visitors	3.5c	0.2c
Control	-	-

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at 5% significance level.

in treatments with TDZ application (Table 2). Thus, the results indicate a positive interaction increasing final fruit set when using a management with phytohormone application compared to natural pollination.

The production of 'Maxigala' apples using only TDZ resulted in lower final fruit set percentages compared to the treatment with TDZ and pollinator visits. Thus, the visits of bees and other pollinators to flowers in apple trees treated with TDZ resulted in a higher flower abortion, probably due to mechanic action and levels of hormones, such as ethylene. According to Fung & Thomson (2017), there is an increase in the lifespan of flowers without pollinator visits. Thus, the treatment with application of TDZ without pollinator visits showed a greater number of flowers capable of generating fruits than those with pollinator visits. Despite this negative effect, the use of TDZ in 'Maxigala' apple trees, with or without pollinator visits resulted in higher final fruit set than that obtained with only pollinator visits. However, without pollinator visits or TDZ application, there is no formation of apple fruits, as reported by Petri et al. (2001)

The application of growth regulators such as TDZ to flowers increases fruit set, as also reported by Vieira et al. (2016). However, the quality of fruits is lower, presenting lower quantity, or absence of seeds. According to Vieira et al. (2016) TDZ inhibits the development of seeds in the fruits, leading to seed abortion. Seed formation in the treatments with TDZ application was significantly lower than in the treatment with only pollinator visits in both growing seasons (Table 3). The use of TDZ without pollinator visits resulted in absence of seeds (Table 3). Fagundes et al. (2017) found a small number of seeds in fruits produced with the use of TDZ, with 3.8 to 4.1 for the control and 2.3 to 2.4 for TDZ applications.

The action of pollinators facilitates the fertilization of flower ovules, which develop into seeds, by aiding in the transfer of pollen between compatible cultivars (Petri et al., 2001).

The application of TDZ in apple trees contributes to fruit set, but results in fruits of low quality, with smaller

**Table 3.** Mean number of seeds (± Standard Error) in fruits and locules of 'Maxigala' apple trees subjected to different treatments at the flowering stage in the 2017/18 and 2018/19 growing seasons. Vacaria, RS, Brazil

Treatment	Number of seeds per fruit		Number of seeds per locule	
	Growing season		Growing season	
	2017/2018	2018/2019	2017/2018	2018/2019
Thidiazuron - TDZ	0c	0c	0c	0c
TDZ + floral visitors	2.0±0.69b	1.8±0.29b	0.36±0.129b	0.3±0.5b
Only floral visitors	4.1±0.74a	5.4±0.78a	0.82±0.148a	1.1±0.15a
Control	-	-	-	-

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at 5% significance level.

diameter and deformities, mainly due to the small number of developed seeds (Vieira et al., 2016).

The number of seeds affects fruit size and weight and, consequently, results in a higher fruit yield in the orchard. The treatment with only TDZ application resulted in significantly lower fruit weight than those with floral visitors in the 2017/2018 growing season (Table 4).

**Table 4.** Mean fruit weight (± Standard Error) of 'Maxigala' apple trees subjected to different treatments at the flowering stage in the 2017/2018 and 2018/2019 growing seasons. Vacaria, RS, Brazil

Treatment	Mean fruit weight (g)	
	Growing season	
	2017/2018	2018/2019
Thidiazuron - TDZ	76.1±2.90b	85.1±2.10b
TDZ + floral visitors	98.7±5.69a	98.7±3.3a
Only floral visitors	100.8±5.56a	103.1±5.3a
Control	-	-

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at 5% significance level.

The lower fruit weight found in the treatment with only TDZ application may be connected to the higher number of set fruits, which can lead to increased competition for photoassimilates (Fagundes et al., 2017).

The treatment with only TDZ application resulted in longer fruits (Table 5). According to Vieira et al. (2016), lighter fruits in apple trees treated with TDZ are correlated with higher fruit production. Tavares et al. (2002), reported a higher degree of deformity in fruits produced under TDZ application. These are elongated fruits that do not stand upright on a flat surface, lacking the characteristic apple shape. These deformities may be connected to the number of seeds in the fruits; parthenocarpic fruits present irregular cytokinin distribution, which is not found in seeded fruits (Podesta, 2007).

Fruit diameters were similar among the treatments; however, the fruit length to diameter ratio found for the treatment with only TDZ application differed significantly from those found for the other treatments (Table 5).

In addition to fruit length and diameter, other aspects are important for assessing fruit quality, including

the distance between lobes and the length and diameter of the calyx tube. The distance between lobes did not vary significantly among treatments (Table 6).

The calyx tube length and diameter varied among treatments. Fruits from apple trees treated with only TDZ application had longer calyx tube than those in the other treatments. Fruits in the treatment with TDZ application and floral visitors presented the second highest mean, differing from those in the treatment with only floral visitors (Table 6).

**Table 5.** Mean fruit length (FL), fruit diameter (FD), and FL to FD ratio (FL/FD) ( $\pm$  Standard Error) of apple trees of the cultivar Maxigala subjected to different treatments at the flowering stage in the 2018/2019 growing season. Vacaria, RS, Brazil

Treatment	Mean $\pm$ SE (mm)		
	FL	FD	FL/FD
Thidiazuron - TDZ	61.24 $\pm$ 0.54a	58.23 $\pm$ 0.45a	1.05 $\pm$ 0.011a
TDZ + floral visitors	59.24 $\pm$ 0.52b	65.28 $\pm$ 6.77a	1.00 $\pm$ 0.013b
Only floral visitors	57.35 $\pm$ 0.46c	61.31 $\pm$ 1.04a	0.93 $\pm$ 0.005c
Control	-	-	-

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at 5% significance level.

**Table 6.** Mean distance between lobes and calyx tube length and diameter ( $\pm$  Standard Error) in fruits of 'Maxigala' apple trees subjected to different treatments at the flowering stage in the 2018/2019 growing season. Vacaria, RS, Brazil

Treatment	Mean $\pm$ SE (mm)		
	Distance between lobes	Calyx tube length	Calyx tube diameter
Thidiazuron - TDZ	26.04 $\pm$ 0.43a	18.55 $\pm$ 0.43a	22.31 $\pm$ 12.85a
TDZ + floral visitors	25.48 $\pm$ 0.25a	14.89 $\pm$ 0.23b	9.08 $\pm$ 0.12ab
Only floral visitors	26.71 $\pm$ 0.43a	13.50 $\pm$ 0.43c	6.95 $\pm$ 0.09b
Control	-	-	-

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at 5% significance level.

Kretschmar et al. (2007) evaluated apples of the cultivar Fuji and found no differences in calyx tube opening; however, this factor may be cultivar-dependent. Another factor that may be related to greater calyx tube opening and fruit length is product distribution, as multiple applications are recommended throughout fruit development (Petri et al., 2001).

The calyx tube diameter showed similar results; fruits in the treatment with only TDZ application had higher means than those in the other treatments. The treatment with TDZ application and floral visitors presented intermediate means, while the treatment with only floral visitors presented the lowest mean (Table 6). However, a larger calyx tube opening can favor the entry of pests and diseases into the fruits. According to Silveira et al. (2013), fruits with wide calyx tube opening present a higher incidence of carpel rot.

According to Vieira et al., (2016), some physical and chemical aspects of the fruits at harvest may be affected by TDZ application. Thus, fruits from branches

subjected only to TDZ application presented lower pulp firmness compared to those in the treatment with TDZ application and floral visitors (Table 7). Similar results were reported by Pasa et al. (2017), who found no differences in fruit pulp firmness due to the action of TDZ and floral visitors. Additionally, Petri et al. (2001) reported a higher pulp firmness in fruits from plants subjected to TDZ application, which is related to a delay in fruit maturation.

Total soluble solids contents (TSS; °Brix) in fruits in the treatment with only TDZ were significantly lower than those in the treatment with only pollinator visits (Table 7).

**Table 7.** Mean pulp firmness and total soluble solids (TSS) ( $\pm$  Standard Error) in fruits of 'Maxigala' apple trees subjected to different treatments at the flowering stage in the 2018/2019 growing season. Vacaria, RS, Brazil

Treatment	Mean $\pm$ SE	
	Pulp firmness	TSS (°Brix)
Thidiazuron - TDZ	22.16 $\pm$ 0.18b	9.66 $\pm$ 0.22b
TDZ + floral visitors	22.99 $\pm$ 0.17a	10.13 $\pm$ 0.15ab
Only floral visitors	21.5 $\pm$ 0.18c	10.52 $\pm$ 0.14a
Control	-	-

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test at 5% significance level.

This result may be attributed to differences in the degree of maturation between the TDZ treatment and the control (Petri et al., 2001). Similar results were found by Vieira et al. (2016), who reported lower pulp firmness and Brix° in fruits from apple trees treated with TDZ application.

### Conclusions

The use of thidiazuron (TDZ) alone was shown to be efficient in fruit formation and set in 'Maxigala' apple trees.

The application of TDZ combined with pollinator visits was shown to be efficient in increasing the number of developed and set fruits compared to the control.

Fruits from apple trees treated with only TDZ application and with TDZ combined with pollinator visits presented smaller numbers of seeds.

The treatment with only TDZ application resulted in lighter and more elongated fruits compared to the other treatments.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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