PREHARVEST SPRAYING WITH THIDIAZURON REDUCES CALCIUM CONTENT AND INCREASES THE CALIX-END OPENING AND THE INCIDENCE OF MOLDY CORE ON APPLE FRUITS

A PULVERIZAÇÃO PRÉ-COLHEITA COM TIDIAZURON REDUZ O TEOR DE CÁLCIO E AUMENTA A ABERTURA CARPELAR E A INCIDÊNCIA DE PODRIDÃO CARPELAR EM FRUTOS DE MAÇÃ

Cassandro Vidal Talamini do Amarante[1]; Luiz Eduardo Bassay Blum[2]; Paulo Roberto Ernani[1]; Clarice Aparecida Megguer[3]; Juliana Golin Krammes[3]; Amauri Bogo[1]

SUMMARY

Thidiazuron (TDZ; N-phenyl-N'-1,2,3-thiadiazol-5-ylureia) is a substituted phenylurea that shows strong cytokinin-like activity in plant tissues. The product is sprayed at full bloom on apple trees to increase fruit set and improve fruit growth. Besides affecting tree physiology and fruit size, TDZ might influence other aspects related to fruit quality. Treated plants normally bear malformed fruits with a more protruded distal end. This work was carried out to investigate the effects of TDZ on fruit carpel aperture, fruit shape, seed number, fruit calcium content, and moldy core (caused by several pathogens) of apples. Apple trees, cultivars Gala and Fuji, were sprayed at full bloom with TDZ at doses of 0, 5, 10, or 20 g (a.i) ha^{-1}. TDZ caused fruit malformation and an increment of carpel aperture, reduced the concentration of calcium in the fruit skin, and increased the incidence of moldy core in 'Gala' (from 0 to 4%) and in 'Fuji' (from 29 to 42%). The increase of moldy core by TDZ in apples may be related to decreases on fruit calcium content and increases on carpel aperture.

KEY WORDS: *Malus domestica* Borkh., cytokinin, diphenylurea derivative, nutrition, core rot, fruit quality.

RESUMO

O tidiazuron (TDZ; N-phenil-N'-1,2,3-thiadiazol-5-ilureia) é uma feniluréia substituida que apresenta forte ação citocinínica em tecidos de plantas. O produto é pulverizado em macieiras na plena floração para aumentar a frutificação efetiva e promover crescimento de frutos. Além de afetar a fisiologia das plantas e crescimento de frutos, o TDZ interfere em outros aspectos relacionados com a qualidade dos frutos. As plantas tratadas normalmente apresentam frutos deformados, com a parte distal protrusa. Este trabalho foi conduzido com o objetivo de avaliar os efeitos do TDZ na abertura carpelar, no formato dos frutos, no número de sementes, nos teores de cálcio e na incidência de podridão carpelar (causada por diversos patógenos) em maçãs. Macieiras, cultivares Gala e Fuji, foram pulverizadas na plena floração com TDZ nas doses de 0, 5, 10 e 20 g (i.a.) ha^{-1}. O TDZ ocasionou deformação e aumento na abertura carpelar, reduziu a concentração de cálcio na película e aumentou a incidência de podridão carpelar em 'Gala' (de 0 para 4%) e 'Fuji' (de 29 para 42%). O aumento na incidência de podridão carpelar em macieiras pulverizadas com TDZ pode estar relacionado à redução nos teores de cálcio e o aumento na abertura carpelar nos frutos.

PALAVRAS-CHAVE: *Malus domestica* Borkh., citocinina, feniluréia substituída, nutrição, podridões, qualidade de fruto.

INTRODUCTION

[1] Ph.D., Professor da Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Cx. Postal 281, Lages, SC, CEP 88.502-970. E-mail: amarante@cav.udesc.br.

[2] Ph.D., Professor da Universidade de Brasília, Departamento de Fitopatologia, Campus Universitário, Asa Norte, Brasília, DF, CEP 70910-900. E-mail: luizblum@unb.br.

[3] Bolsista de Iniciação Científica do CNPq, Acadêmica do Curso de Agronomia, Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Cx. Postal 281, Lages, SC, CEP 88.502-970.

Plant growth regulators have been used to improve fruit set and fruit growth in temperate fruit trees (ELFVING & CLINE, 1993; FAMIANI et al., 1999; GREENE, 1995). Cell division and elongation, which affect the final fruit size, are promoted by the growth substances auxin, cytokinin, and gibberellin (TAIZ & ZAIGER, 1998). A synthetic substituted phenylurea, thidiazuron (TDZ; N-phenyl-N'-1,2,3-thiadiazol-5-ylureia), shows cytokinin-like activity in plant tissues (ELFVING & CLINE, 1993; GREENE, 1995) and strong fruit growth promoting effect in apple, kiwifruit, grape, and persimmon (ELFVING & CLINE, 1993; FAMIANI et al., 1999; GREENE, 1995; ITAI et al., 1995; PETRI et al., 2001; REYNOLDS et al., 1992; SCHUCK & PETRI, 1992). GREENE (1995) reported increases in fruit weight greater than 30% for 'McIntosh' and 'Empire' apples sprayed at full bloom with TDZ at doses of 50 mg L^{-1} and 15 mg L^{-1}, respectively.

However, on apples, treatments with TDZ might affect vegetative growth, flowering behavior, and mineral content of leaves and fruits, depending on product concentration, time of application, and cultivar (ELFVING & CLINE, 1993; GREENE, 1995). ELFVING & CLINE (1993) reported higher K and lower N, Ca, and Mg contents in the leaves of 'Empire' apples sprayed with TDZ at full bloom at doses of 65 and 125 mg L^{-1}. TDZ effects on fruit-flesh nutrient concentration were associated with higher content of K and slightly lower content of Ca. However, the authors did not assess the incidence of decay and physiological disorders and postharvest quality of the fruit. GREENE (1995) reported a small increase of bitter pit incidence at harvest when apple trees were sprayed 18 days after full bloom with 15 mg L^{-1} of TDZ.

Besides affecting tree physiology and fruit growth, TDZ also influences other aspects of fruit quality. The chemical increased the fruit length : diameter (L : D) ratio in apple (GREENE, 1995) and reduced it in kiwifruit (FAMIANI et al., 1999). TDZ increased the number of malformed fruit in apple (GREENE, 1995) and the number of fruit with distal protuberance in kiwifruit (FAMIANI et al., 1999; SCHUCK & PETRI, 1992). At doses of 10 or 50 mg L^{-1}, TDZ reduced seed count in 'McIntosh' apples (GREENE, 1995). On apples, the product also delayed fruit maturity and ripening (FAMIANI et al., 1999; GREENE, 1995; ITAI et al., 1995; MEGGUER et al., 2001; PETRI et al., 2001; REYNOLDS et al., 1992).

On apples, TDZ sprayed at full bloom to increase fruit set also increased the incidence of moldy core (MEGGUER et al., 2001). Moldy core, also called core rot, is caused by several pathogens, such as, *Alternaria* Ness., *Fusarium* Link., *Penicillium* Link., *Botrytis* Pers., *Phoma* Desm., *Phomopsis* Sacc., *Mucor* Link., *Pestalotia* de Not., *Aspergillus* Link., *Candida* Berkhout., *Cladosporium* Link., *Gloeosporium* Desm. & Mont., *Epicoccus* Link., and *Rhizopus* Corda (JONES & ALDWINCKLE, 1990; BONETI et al., 1999). These fungi infect carpel and core regions of the fruit and cause internal dry or soft rots (JONES & ALDWINCKLE, 1990; BONETI et al., 1999). Apple cultivars with larger carpel aperture are more susceptible to moldy core (SPOTS, 1990). Since apple trees treated with TDZ bear malformed fruits with a more protruded distal end (MEGGUER et al., 2001) and, possibly, a larger carpel aperture, this product might also increase the incidence of moldy core.

Published information shows that TDZ has a diversity of physiological effects on treated plants, which might have substantial impacts on fruit quality. The product effects on fruit shape (with a more protruded distal end) and nutrition (reduced calcium content) might affect fruit susceptibility to decay. In recent years, the increasing concern of apple growers in Southern Brazil with the high losses caused by moldy core might reflect the intensive use of TDZ to improve fruit set and fruit growth. The objective of this work was to evaluate the effects of different doses of TDZ, sprayed at full bloom, on seed

number, calcium content, shape and moldy core of 'Gala' and 'Fuji' apples.

MATERIAL AND METHODS

The study was conducted in a commercial orchard in Lages, Santa Catarina State, Southern Brazil, in 1999/2000. Ten-year-old apple trees, cultivars Gala and Fuji, grafted on 'Marubakaido' rootstock, were sprayed at full bloom, with TDZ at doses of 0, 5, 10, or 20 g (a.i.) ha^{-1}, with a total spray volume of 1,000 L ha^{-1}. The trees were treated with a tractor-mounted airblast sprayer. Spray barriers, made of polyethylene mounted on wood poles, were used to prevent spray drift between experimental plots. The experiment followed a completely randomized block design with four replicates. Each experimental unity was composed by five rows of eight plants. Only four plants in the center of the middle row had the fruit harvested at commercial maturity and assessed for number of viable seed, calcium content, shape and moldy core.

Ten fruits per plant were collected at commercial harvesting and assessed for calcium content in the skin. The fresh fruit samples were digested with a mixture of sulfuric acid and hydrogen peroxide, as described by ADLER & WILCOX (1985). Calcium was determined with an inductive coupled plasma spectrophotometer (ICP).

Lots of 30 fruit per replicate were selected for count 110-135 and assessed for number of viable seeds per fruit, percentage of asymmetric fruit, carpel aperture (with a caliper), and incidence of moldy core (independent of the pathogen).

All data were subjected to statistical analysis using SAS (1990). Percentage data were transformed to arc sin $[(x+5)/100]^{1/2}$ before being submitted to the ANOVA. The effect of TDZ dose on each attribute assessed was analyzed by orthogonal polynomial contrasts.

RESULTS AND DISCUSSION

Since the experimental area had high presence of bees, and the weather conditions were favorable for the entomophilic pollination, yield was not affected by TDZ treatments in any cultivar (data not shown). Average fruit weight did not increase with increases on TDZ dose (data not shown), that disagrees with previous published studies, which show substantial increase on fruit size with increases on TDZ doses (FAMIANI et al., 1999; GREENE, 1995; ITAI et al., 1995; PETRI et al., 2001; REYNOLDS et al., 1992). However, those studies were performed mainly by dipping the fruit in a solution of TDZ, or by spraying some trees or some limbs per tree with the product to the drip point. This might result in a much higher dose of TDZ than those applied in our study, where the plants were treated with a tractor-mounted airblast sprayer, as done commercially. This might have reduced the product effectiveness in promoting fruit growth. REYNOLDS et al. (1992) also emphasized the importance of field trials to determine the effective concentration of TDZ when the product is sprayed commercially in grapes to promote fruit growth.

The number of viable seeds per fruit was not affected by TDZ in both cultivars (Table 1), probably due to the use of small doses (up to 20 g ha^{-1}, corresponding to ~20 mg L^{-1}) and the application time (at full bloom). This might also indicate that 'Gala' and 'Fuji' are less prone to seed abortion by TDZ than other cultivars. In addition, since the experimental area had high presence of honeybees, and the weather conditions were favorable for the entomophilic pollination, this might have prevented any effect of TDZ on seed count. The number of seeds per fruit was not also affected by TDZ sprayed at full bloom at the doses of 5 or 10 mg L^{-1} in 'Double Red Delicious' apples (GREENE, 1995), and at the dose of 20 mg L^{-1} in kiwifruit (FAMIANI et al., 1999). However, in 'Empire' apples, TDZ sprayed 22 days after full bloom to promote fruit thinning, at doses of 62 or 125 mg L^{-1}, nearly eliminated seed development (ELFVING & CLINE, 1993). Nevertheless, the reduced seed count did not influence fruit shape, fruit size and

fruit-fresh nutrient concentration (N, P, K, Ca, and Mg). At doses of 10 or 50 mg L^{-1}, TDZ reduced seed count in 'McIntosh' apples when the product was sprayed at full bloom or 22 days after full bloom; the highest concentration and latter application caused the greatest reduction of seed count (GREENE, 1995).

TDZ at doses of 10-20 g ha^{-1} reduced calcium content ~ 17-20% in the fruit skin in both cultivars (Table 1). This might have also increased fruit susceptibility to moldy core infection during fruit growth on the tree. Apples with low calcium content are more susceptible to decay (CONWAY et al., 1999). The reduction of calcium concentration in 'Gala' and 'Fuji' by TDZ was not related to seed abortion. Instead, it might have been the result of an intense growth of vegetative tissues (terminal buds and growing leaves) caused by TDZ that increases calcium accumulation into these organs in detriment of growing fruit (AMARANTE et al., 2002).

TDZ increased fruit L : D ratio (data not shown), caused fruit malformation, increased the carpel aperture and the incidence of moldy core in both cultivars (Table 1). In 'Gala', the percentage of asymmetrical fruit varied from 4% for the control treatment to higher that 50% for TDZ at doses of 10 and 20 g ha^{-1} (Table 1). In 'Fuji', the percentage of asymmetrical fruit varied from about 30% for the control treatment to about 55% for TDZ at doses higher than 5 g ha^{-1} (Table 1). These increases indicate that TDZ, by its cytokinin-like effect, affects the direction of cell division or cell expansion resulting in fruit elongation and opening of the carpel aperture of apples (ELFVING & CLINE, 1993; GREENE, 1995). In apple, GREENE (1995) observed increases on fruit malformation with increases of TDZ dose especially when treatments were made later after full bloom (18 days after full bloom). In kiwifruit, high doses of TDZ increased the number of fruit with distal protuberance (FAMIANI et al., 1999; SCHUCK & PETRI, 1992). The asymmetry in fruits treated with phenylureas seems to be the result of extremely limited mobility and redistribution of the product from the application sites, causing uneven fruit growth and misshapen (BIASI et al., 1993). The asymmetry may be overcome by more uniform distribution over the fruit surface, using uniform application of small droplets or multiple sprays at low concentration (BIASI et al., 1993), and by avoiding treatments later after full bloom (GREENE, 1995).

Despite of a lower increase of carpel aperture in 'Fuji' than in 'Gala' with increments of TDZ dose, 'Fuji' had higher incidence of moldy core than 'Gala' (Table 1). The higher incidence of moldy core in 'Fuji' than in 'Gala' might reflect differences in susceptibility to fungal infection and/or the differences of fruit harvesting dates between these cultivars. 'Fuji' had slightly higher calcium content than 'Gala', but since 'Fuji' is harvested later than 'Gala' and, therefore, exposed to a longer period of fungal infection in the orchard, this might increase the incidence of moldy core in 'Fuji'.

TABLE 1 - Number of viable seeds, concentration of calcium (Ca) in the fruit skin, asymmetrical fruit, carpel aperture, and moldy core (mean ± SE) of apples from trees sprayed with different doses of thidiazuron (TDZ). The significance of linear and quadratic relationships between doses of TDZ and fruit quality attributes were analyzed by means of polynomial orthogonal contrasts (*, **, and *** for 5, 1, and 0.1 % of significance, respectively; ns: non significant).

TDZ	'GALA'					'FUJI'				
(g ha^{-1})	Seeds/ fruit	Ca in the skin (mg kg^{-1})	Asymmetrical fruit (%)	Carpel aperture (mm)	Moldy core (%)	Seeds/ fruit	Ca in the skin (mg kg^{-1})	Asymmetrical fruit (%)	Carpel aperture (mm)	Moldy core (%)
0	6.1 ± 0.2	305.4 ± 20.8	4.2 ± 2.5	5.4 ± 0.1	0.0 ± 0.0	7.2 ± 0.2	329.7 ± 3.6	34.2 ± 4.4	5.0 ± 0.1	29.2 ± 1.6
5	5.7 ± 0.2	257.3 ± 23.5	30.8 ± 3.9	6.0 ± 0.1	1.7 ± 1.0	7.3 ± 0.2	284.3 ± 22.9	55.0 ± 6.2	5.2 ± 0.1	35.0 ± 5.5
10	6.0 ± 0.2	240.7 ± 9.3	53.3 ± 3.6	5.8 ± 0.1	0.8 ± 0.8	7.1 ± 0.2	261.5 ± 17.8	55.8 ± 2.8	5.3 ± 0.1	41.5 ± 7.8
20	5.9 ± 0.2	253.5 ± 10.8	56.7 ± 8.5	6.8 ± 0.1	4.2 ± 1.6	7.3 ± 0.2	272.2 ± 11.7	55.0 ± 6.4	5.3 ± 0.1	41.7 ± 5.5
Linear	ns	*	***	***	*	ns	**	*	*	*
Quadratic	ns	*	***	ns	ns	ns	*	*	ns	ns

In conclusion, TDZ increased carpel aperture and reduced fruit calcium content in both apple cultivars. This might contribute to increase fruit susceptibility to moldy core infection during fruit growth on the tree. In recent years, the increasing concern of apple growers in Southern Brazil with the high losses caused by moldy core might reflect the intensive used of TDZ to improve fruit set.

REFERENCES

ADLER, P. R. & WILCOX, G. E. Rapid perchloric acid digestion methods for analysis of major elements in plant tissue. **Communications in Soil Science and Plant Analysis**, Athens, v. 16, n. 11, p. 1153-1163, 1985.

AMARANTE, C. V. T. do, ERNANI, P.R., BLUM, L. E. B. et al. Thidiazuron effects on shoot growth, return bloom, fruit set and nutrition os apples. **Pesquisa Agropecuária Brasileira**, Brasília, v.37, n.10, p.1365-1371, 2002.

BIASI, R., NERI, D., SUGIYAMA, N. et al. ^{14}C-CCPU uptake and distribution in developing kiwifruits and apples. **Acta Horticulturae, Wageningen**, n. 329, p. 101-104, 1993.

BONETI, J. I. S., RIBEIRO, L. G., KATSURAYAMA, Y. **Manual de Identificação de Doenças e Pragas da Macieira.** Florianópolis: Epagri, 1999. 210 p.

CONWAY, W. S., JANISIEWICZ, W. J., KLEIN, J. D. et al. Strategy for combining heat treatment, calcium infiltration, and biological control to reduce postharvest decay of 'Gala' apples. **HortScience**, Alexandria, v. 34, n. 4, p. 700-704, 1999.

ELFVING, D.C. & CLINE, R.A. Cytokinin and ethephon affects crop load, shoot growth, and nutrient concentration of 'Empire' apple trees. **HortScience**, Alexandria, v. 28, n. 10, p. 1011-1014, 1993.

FAMIANI, F., BATTISTELLI, A., MOSCATELLO, S. et al. Thidiazuron affects growth, ripening and quality of *Actinidia deliciosa*. **Journal of Horticultural Science & Biotechnology**, Kent, v. 74, n. 3, p. 375-380, 1999.

GREENE, D. W. Thidiazuron effects on fruit set, fruit quality, and return bloom of apples. **HortScience**, Alexandria, v. 30, n. 6, p. 1238-1240, 1995.

ITAI, A., TANABE, K., TAMURA, F. et al. Synthetic cytokinins control persimmon fruit shape, size and quality. **Journal of Horticultural Science**, Kent, v. 70, n. 6, p. 867-873, 1995.

JONES, A. L. & ALDWINCKLE, H. S. **Compendium of Apple and Pear Diseases**. St. Paul: APS Press, 1990, 213 p.

MEGGUER, C. A., AMARANTE, C. V. T. do, BLUM, L. E. B. et al. Postharvest physiology and quality of apples is affected by preharvest spraying of thidiazuron. In: CONGRESSO BRASILEIRO DE FISIOLOGIA VEGETAL, 8, 2001, Ilhéus, BA. **Resumos... Ilhéus**, BA, 2001. p. 138.

PETRI, J. L., SCHUCK, E., LEITE, G. B. Efeito do thidiazuron (TDZ) na frutificação de fruteiras de clima temperado. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 23, n. 3, p. 513-517, 2001.

REYNOLDS, A. G., WARDLE, D. A., ZUROWSKI, C. et al. Phenylureas CPPU and thidiazuron affects yield components, fruit composition, and storage potential of four seedless grape selections. **Journal of the American Society for Horticultural Science**, Alexandria, v. 117, n. 1, p. 85-89, 1992. SAS. Cary: SAS institute Inc. 1990. 789 p.

SCHUCK, E. & PETRI, J. L. Efeitos do thidiazuron no peso médio dos frutos de quivi.

Revista Brasileira de Fruticultura, Cruz das Almas, v. 14, n. 2, p. 185-188, 1992.

SPOTS, R. A. Moldy core and core rot. In: Jones, A. L. & Aldwinckle, H. S. (Ed). **Compendium of Apple and Pear Diseases**. St. Paul: APS Press, 1990. p. 29-30.

TAIZ, L. & ZEIGER, E. **Plant Physiology**. 2 ed. Sunderland: Sinauer Associates, Inc., Publishers, 1998. 792 p.