SCIENTIFIC NOTE

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SIDE EFFECTS OF FUNGICIDES USED IN CUCURBITACEOUS CROP ON *Trichogramma atopovirilia* OATMAN & PLATNER (HYMENOPTERA: TRICHOGRAMATIDAE)

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ABSTRACT

Trichogramma spp. (Hymenoptera: Trichogrammatidae) can control *Diaphania hyalinata* Linnaeus (Lepidoptera: Pyralidae). On the other hand, pesticides may reduce the efficiency of natural enemies. The objective was to evaluate the side-effects of fungicides used in the production of cucurbitaceous crops on *Trichogramma atopovirilia* Oatman & Platner parasitizing *D. hyalinata* eggs. The fungicides used in bioassays were: azoxystrobin (0.08 g active ingredient [ai] L^{-1}), chlorothalonil (2.00 g ai L^{-1}), mancozeb (1.60 g ai L^{-1}), tebuconazole (0.25 g ai L^{-1}) and thiophanate-methyl (0.49 g ai L^{-1}). Cardboards with 30 *D. hyalinata* eggs previously immersed in fungicide solutions and distilled water (control) were offered separately to 20 newly emerged *T. atopovirilia* females in glass tubes. Parasitism, parasitism reduction, emergence, sex ratio, and number of individuals per egg were evaluated. The fungicides chlorothalonil, thiophanate-methyl and tebuconazole reduced parasitism of *T. atopovirilia* by 43.37, 27.64 and 18.51%, respectively. However, parasitism with azoxystrobin (79.21%) was higher than the control (67.37%) ($P \leq 0.05$). Chlorothalonil, thiophanate-methyl and tebuconazole reduced emergence by 73.77, 75.62 and 79.35% ($P \leq 0.05$), respectively. Azoxystrobin and thiophanate-methyl reduced the sex ratio by 0.77 and 0.76 ($P \leq 0.05$), respectively. Fungicides did not reduce the number of individuals per egg. The fungicides azoxystrobin and mancozeb were selective for *T. atopovirilia* for most studied parameters suggesting that these products must have the priority in crop disease management to allow efficient biological control of *T. atopovirilia* against *D. hyalinata*.

Key words: Cucurbit borer, pesticides, egg parasitoid, selectivity.

INTRODUCTION

Diaphania spp. (Lepidoptera: Pyralidae) outbreaks may cause 100% of loss at production of cucurbitaceous crop, due to reduction of photosynthetic area. Its caterpillar feeds on leaves, flowers, stems and fruits (Sobrinho *et al.*, 2003; Arcaya *et al.*, 2004).

The Integrated Pest Management (IPM) suggests the use of tactics based on a cost-benefit analyses and lower operational impact on agroecosystem (Kogan, 1998). Thus, the egg parasitoids *Trichogramma* may be an alternative to minimize the use of insecticides to control *Diaphania* spp. These parasitoids are highly efficient in the control of lepidopteran pests (Hassan, 1994; Pratissoli *et al.*, 2005a). In consequence, the management of insect pests can be optimized by methods of control not antagonistic to the beneficial organisms (Degrande *et al.*, 2002; Devotto *et al.*, 2008).

The mass rearing of this parasitoid in *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechidae) or *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) eggs has allowed its use in programs of IPM (Parra, 1997; Pratissoli *et al.*, 2005b). On other hand, pesticides may limit the efficiency of biological control agents. Fungicides, herbicides and plant growth regulators can have deleterious effects on parasitoids *Trichogramma,* although pesticides not have a specific site of action in arthropods, but these products can induce sublethal effects on reproduction and other biological function (Manzoni *et al.*, 2006; Stefanello Júnior *et al.*, 2008). Studies of selectivity generate information that helps the decision in IPM and maintenance of beneficial organisms in the agroecosystem (Degrande *et al.*, 2002; Moura *et al.*, 2005).

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The objective of this study was to evaluate the selectivity of fungicides used to control diseases in cucurbitaceous crop on the species *Trichogramma atopovirilia* Oatman & Platner (Hymenoptera: Trichogrammatidae) parasitizing *D. hyalinata* eggs.

MATERIAL AND METHODS

This research was carried out at Núcleo de Desenvolvimento Científico e Tecnológico em Manejo Fitossanitário (NUDEMAFI) of the Centro de Ciências Agrárias da Universidade Federal do Espírito Santo, Alegre, Espírito Santo, Brazil, in an acclimated chamber (25 ± 1 °C, 70 ± 10% RH and 14:10 h L:D photoperiod). The maintenance and multiplication of the egg parasitoid *T. atopovirilia* in factitious host *A. kuehniella* was outlined according to Parra (1997).

Diaphania hyalinata caterpillars were reared in plastic containers (35 x 20 cm) containing pieces (10 x 4 x 2 cm) of squash *Cucurbita moschata* (Cucurbitaceae), until the last larval stage. The edges of the container were involved with sheets of paper folded for last stage and pupa molt. The sheets of paper were removed and the pupae collected and sexed by characteristics of the genitalia, which was performed taking the eighth and ninth abdominal segments of pupa under a stereomicroscope (SZ40, Olympus, Tokyo, Japan) (Butt and Cantu, 1962). Thirty couples of *D. hyalinata* were formed, which received a 10% of honey solution as food. These insects were kept in cages made of PVC tubes (20 x 40 cm), with a screen of veil on top and a sheet of styrofoam at the bottom. Females were induced to oviposite on discs of paper moistened with extract of cucumber (250 mL of distilled water and 50 g of cucumber), which replaced during the scotophase, after the 3rd day of transfer of couples in cages.

The methodology for the implementation of this work was outline by Hassan *et al*. (2000) and Vianna *et al*. (2009).

The fungicides azoxystrobin (methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate)(0.08 g active ingredient [ai] L^{-1}) (Amistar, 500 g kg^{-1}, Syngenta Proteção de Cultivos Ltda., São Paulo, São Paulo, Brazil), chlorothalonil (tetrachloroisophthalonitrile)(2.00 g ai L^{-1}) (Dacostar 500, 500 g L^{-1}, Hokko do Brasil Indústria Química Agropecuária Ltda., São Paulo, São Paulo, Brazil), tebuconazole ((*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol)(0.25 g ai L^{-1}) (Folicur PM, 250 g kg^{-1}, Bayer CropScience Ltda., São Paulo, São Paulo, Brazil), thiophanate-methyl (dimethyl 4,4′-(*o*-phenylene)bis(3-thioallophanate))(0.49 g ai L^{-1}) (Cercobin 700, PM 700 g kg^{-1}, Iharabrás S/A Indústrias Químicas, São Paulo, São Paulo, Brazil) and mancozeb (manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt)(1.60 g ai L^{-1}) (Manzate 800, 800 g kg^{-1}, Du Pont do Brasil S.A, Barueri, São Paulo, Brazil) were used in bioassays and chosen according to the diseases that commonly occur in Brazil (anthracnose, alternaria leaf spot, net spot, sclerotinia rot, powdery mildew and downy mildew). Twenty females of *T. atopovirilia* population up to 24 h after emergence were individualized in tubes (3.0 × 0.5 cm) with a honey droplet (as food) in its internal wall. Thirty *D. hyalinata* eggs from paper where the oviposition occurs were glued on a cardboard strip (2.5 × 0.5 cm) and immersed for 5 s in solutions of each fungicide at the commercial concentrations indicated for the control of plant diseases to cucurbitaceous crop and maintained at acclimatized room (25 ± 1 °C, 70 ± 10% RH) to allow drying for 1 h. Control eggs were immersed in distilled water. The treatment cardboard strips were then exposed to parasitism by *T. atopovirilia* in tubes (3.0 × 0.5 cm) for 24 h. After this period, the cardboards were transferred to glasses tubes (2.5 × 10 cm) and kept in an acclimatized chamber until emergence of adults.

Parasitism, reduction in parasitism, emergence, sex ratio and number of individuals per egg were evaluated. Parasitism was determined by counting the number of parasitized eggs per cardboard under stereomicroscope. The reduction in parasitism (RP) was determined for each fungicide by the equation:

$$RP = \left(1 - f/t\right) \times 100$$

where f = average number of parasitized eggs in the fungicide treatment and t = average number of parasitized eggs in the control treatment (Hassan *et al*., 2000).

The emergence was determined by the rate between the eggs with parasitoid emergence hole and parasitized eggs. The obtained value was converted into percentage. The sex ratio was obtained by the rate between the number female and the total emerged individuals. The number of individuals/egg was determined by the ratio of number of total emerged parasitoids and total of eggs with holes.

The experimental design was completely randomized. Data were submitted to ANOVA and averages compared by Scott-Knott test ($P \leq 0.05$) because this test discriminates better the effect of treatment by group of fungicides.

RESULTS

The fungicides affected the parasitism of *T. atopovirilia*. Mancozeb, tebuconazole, thiophanate-methyl and chlorothalonil reduced the parasitism of *T. atopovirilia* in *D. hyalinata* eggs at 7.38, 18.51, 27.64 and 43.37% respectively. Parasitism of *T. atopovirilia* was 62.40%

in eggs with mancozeb, which was similar to control (67.37%). However, parasitism in eggs with azoxystrobin was superior to control with 79.21%. Chlorothalonil was the most harmful fungicide with parasitism of 38.15% (Table 1).

Chlorothalonil, thiophanate-methyl and tebuconazole reduced the emergence of *T. atopovirilia* on *D. hyalinata* eggs of 85.49% (control) to 73.77, 75.62 and 79.35% respectively. The percentage of emergence of *T. atopovirilia* in *D. hyalinata* eggs with mancozeb was 96.75%, which was higher than the control (Table 1).

Azoxystrobin and thiophanate-methyl reduced sex ratio with 0.76 and 0.77 respectively. The other fungicides were similar (Table 1).

The fungicides did not reduce the parasitoids offspring per egg. However, eggs treated with azoxystrobin and tebuconazole showed more progeny of *T. atopovirilia*, with 1:31 and 1:34 parasitoids/egg, respectively (Table 1).

DISCUSSION

Fungicides affected *T. atopovirilia* fitness. Thiophanate-methyl, tebuconazole and chlorothalonil had negative effect on the parasitoid. On other hand, mancozeb not affect the parasitism of *T. atopovirilia* on *D. hyalinata* eggs. Similar reduction in the parasitism was observed with *Trichogramma pretiosum* in *A. kuehniella* eggs with mancozeb and tebuconazole (Manzoni *et al*., 2006). However, mancozeb was harmless to *T. pretiosum* in *A. kuehniella* eggs (Giolo *et al*., 2007), such as *T. atopovirilia* in *D. hyalinata* eggs. These results may be due to storage of the substances in the insect fat body, its excretion and selective metabolism (Foerster, 2002) or, its degradation by the enzyme system (Croft, 1990; Rigitano and Carvalho, 2001). The repellency was probably another factor that may have contributed to reduction of the parasitism of *T. atopovirilia*. This effect was report to *T. pretiosum* on factitious host with pyrethroids (Vianna *et al*., 2009).

Parasitism of *T. atopovirilia* increased in eggs with azoxystrobin, which is known as hormesis, i.e. generally-favorable biological responses to sub-lethal exposures to pesticide or other stressors (Calabrese, 1999; Forbes, 2000; Desneux *et al*., 2003). However, confirmation of this phenomenon requires major advances in chemical analysis to biological organisms with greater complexity such as parasitoids.

The lower emergence induced by chlorothalonil, tebuconazole and thiophanate-methyl may be due to consumption of extra-embryonic fluid containing pesticide residues by parasitoids (Cônsoli *et al*., 2001; Moura *et al*., 2005). Some products reached the stages of pre-mature parasitoid diffuse through the shell (Guifen and Hirai, 1997; Schuld and Schmuck, 2000; Carvalho *et al*., 2003).

The effect on the sex ratio by azoxystrobin and thiophanate-methyl can be due to the ability of females of some *Trichogramma* species to control the fertilization of their eggs exposed to unsuitable conditions (Cook, 1993; Greeff, 1996; Flanagan *et al*., 1998; Delpuech and Meyet, 2003).

The increase in the number of individuals per egg in treatments with azoxystrobin and tebuconazole is probably due to hormesis as reported previously. Studies support the idea that hormesis contributes to increasing the performance of beneficial organisms (Calabrese, 1999; Guedes *et al*., 2009; Zanuncio *et al*., 2003).

Parasitoids are exposed to chemical residues of plant protection products in laboratory conditions every time. However, the behavior of parasitoids can avoid contaminated sites in field level, what can reduce its side effect (Rocha and Carvalho, 2004). However, the success of insect pest control can be lower at such sites than another without these chemical products.

Table 1. Parasitism, emergence, sex ratio, individuals emerged by egg (± SE) and reduction in parasitism of *Trichogramma atopovirilia* on *Diaphania hyalinata* eggs treated with fungicides. Temperature: 25 ± 1 °C, relative humidity: 70 ± 10% and photoperiod 14:10 h.

Active ingredient	Parasitism	Emergence	Sexual ratio	Individuals/egg	RP*
	%				%
Azoxystrobin	79.21 ± 2.83a	90.46 ± 2.72b	0.76 ± 0.01b	1.31 ± 0.04a	(17.51)
Chlorothalonil	38.15 ± 3.32d	73.77 ± 2.48c	0.79 ± 0.02a	1.16 ± 0.27b	43.37
Mancozeb	62.40 ± 4.37b	96.75 ± 0.70a	0.82 ± 0.01a	1.12 ± 0.01b	7.38
Tebuconazole	54.90 ± 5.40c	79.35 ± 2.70c	0.79 ± 0.02a	1.34 ± 0.03a	18.51
Thiophanate-methyl	48.75 ± 4.16c	75.62 ± 1.41c	0.77 ± 0.01b	1.14 ± 0.01b	27.64
Control	67.37 ± 3.32b	85.49 ± 2.62b	0.86 ± 0.03a	1.13 ± 0.03b	–

Averages followed by the same letter in column do not differ statistically by Scott-Knott test (P ≤ 0.05). *Reduction in parasitism. Value in parenthesis characterizes increase in parasitism.

CONCLUSIONS

The results show that fungicides evaluated can affect parasitism, emergence and sex ratio of *T. atopovirilia*, although there is no site of action known in arthropods similar to insecticides.

The fungicides azoxystrobin and mancozeb were selective to the parasitoid *T. atopovirilia* and can be prioritized in integrated management of diseases in cucurbitaceous crop to ensure high level *D. hyalinata* control by *T. atopovirilia*.

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RESUMEN

Efectos secundarios sobre *Trichogramma atopovirilia* Oatman & Platner (Hymenoptera: Trichogramatidae) de fungicidas usados en cucurbitáceas. *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) pueden controlar *Diaphania hyalinata* Linnaeus (Lepidoptera: Pyralidae). Sin embargo, los plaguicidas pueden reducir la eficiencia de los enemigos naturales. El objetivo de este trabajo fue evaluar la influencia de los fungicidas utilizados en la producción de cucurbitáceas en *Trichogramma atopovirilia* Oatman & Platner sobre huevos de *D. hyalinata*. Se evaluaron los fungicidas azoxystrobina (0.08 g ingrediente activo [ia] L^{-1}), clorotalonil (2.00 g ia L^{-1}), mancozeb (1.60 g ia L^{-1}), tebuconazole (0.25 g ia L^{-1}) and tiofanato-metil (0.49 g ia L^{-1}). Tarjetas con 30 huevos de *D. hyalinata* previamente sumergidos en los fungicidas y en agua destilada (control) fueron ofrecidas a 20 hembras de *T. atopovirilia* con hasta 24 h de emergencia individualizadas en tubos de vidrio. Se evaluó parasitismo, reducción de parasitismo, emergencia, proporción sexual en la población y el número de individuos emergidos por huevo. Los fungicidas clorotalonil, tiofanato-metil y tebuconazol redujeron el parasitismo de *T. atopovirilia* en 43,37; 27,64 y 18,51%, respectivamente. Sin embargo, el parasitismo con azoxistrobina (79,21%) fue mayor que el control (67,37%) ($P \leq 0{,}05$). Clorotalonil, tiofanato-metilo y el tebuconazol redujo la aparición de 73,77, 75,62 y 79,35% ($P \leq 0{,}05$), respectivamente. Azoxistrobina y tiofanato-metil reduciran la proporción de sexos de 0,77 y 0,76 ($P \leq 0{,}05$), respectivamente. Ninguno de los fungicidas redujo el número de individuos por cada huevo. Los fungicidas azoxistrobina y mancozeb fueron selectivos para *T. atopovirilia* en la mayoría de los parámetros estudiados, por lo tanto, deberían tener prioridad en el manejo de enfermedades de cucurbitáceas para permitir el uso eficiente de los enemigos naturales de *D. hyalinata*.

Palabras clave: Barrenador de las cucurbitáceas, plaguicidas, parasitoide de huevos, selectividad.

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