Journal of Economic Entomology, 113(4), 2020, 1694–1701
doi: 10.1093/jee/toaa119
Advance Access Publication Date: 15 June 2020
Research

Side Effects of Organic Products on *Telenomus podisi* (Hymenoptera: Platygastridae)

Lucas Battisti,[1] Jheniffer V. Warmling,[1] Claudinei F. Vieira,[1] Darlin H. R. Oliveira,[1] Yuri R. A. Lima,[1] Michele Potrich,[1,] Adeney F. Bueno,[2] and Everton R. Lozano[1,3,]

[1]Laboratório de Controle Biológico, Coordenação de Ciências Biológicas, Universidade Tecnológica Federal do Paraná Câmpus Dois Vizinhos (UTFPR-DV), Estrada para Boa Esperança, Km 04, Comunidade São Cristóvão, ZIP: 86660-000, Dois Vizinhos, PR, Brasil, [2]Laboratório de Entomologia, Empresa Brasileira de Pesquisa Agropecuária – Rodovia Carlos João Strass, s/nº Acesso Orlando Amaral, Distrito de Warta Caixa Postal: 231 ZIP: 86001–970, Londrina, PR, Brasil, and [3]Corresponding author, e-mail: evertonloz@gmail.com and evertonlricardi@utfpr.edu.br

Subject Editor: Mary Cornelius

Received 1 March 2020; Editorial decision 9 May 2020

Abstract

Telenomus podisi Ashmead, 1893 is an important biocontrol agent, both in conventional and organic production systems. It can be used in association with other control strategies, such as natural botanical products and biological insecticides. Studies of selectivity and side effects are fundamental for proper management of insect control strategies because the interaction between different control strategies may negatively affect *T. podisi*. In this context, the present study evaluated the side effects of commercial natural products on *T. podisi* under laboratory conditions. Five natural products (insecticide, fungicide, and leaf fertilizer) allowed in organic farming were evaluated at concentrations recommended by the manufacturer in three bioassays. First bioassay (free-choice test), the preference of *T. podisi* parasitism between treated and non-treated *E. heros* eggs was assessed. In the second and third bioassay (no-choice tests) the treatments were applied to *E. heros* eggs, repectively before and after *T. podisi* parasitism (pre- and post-parasitism) and parasitism, emergence, offspring sex ratio, developmental time, and adult longevity were assessed. The products formulated with *Metarhizium* anisopliae (Metsch.) Sorok. (Hypocreales), *Beauveria bassiana* (Bals.) Vuill. (Hypocreales), orange oil fertilizer, and the fungicide copper oxychloride did not have side effects on *T. podisi* because they did not affect most of the evaluated characteristics. In contrast, azadirachtin A/B had a sublethal effect due to the reduced parasitism in all tests performed and, although it did not affect other aspects, this could compromise the performance of the parasitoid.

Key words: complementary strategies control, biological control, egg parasitoid, natural enemy

In recent decades, organic farming has been gaining ground in agricultural production systems, reaching an area of 50.9 million hectares worldwide in more than 120 countries (FIBL-IFOAM 2009, FIBL and IFOAM – Organics International 2017). The increased demand for food without chemical residues has promoted the production and commercialization of organic products. In addition, organic agriculture reduces the negative impact of conventional agriculture on agroecosystems, such as water and soil contamination, organic matter reduction, and the loss of biodiversity of beneficial organisms (Mazzoleni and Nogueira 2006, Lairon 2010, Cavigelli et al. 2013). However, pest management is one of the major challenges of organic systems since synthetic insecticides are not allowed.

Among the pest management tools available, augmentative biological control (ABC) stands out, a method that has been applied for over 100 yr (Cock et al. 2010) on more than 30 million ha worldwide (van Lenteren et al. 2018) and acceptable in organic farming. ABC includes the use of natural enemies of insects, such as microorganisms, parasitoids, and predators, to reduce the pest population (van Lenteren et al. 2018). In ABC programs, egg parasitoids have been widely used can be considered the most important stink bug biocontrol agent (Koppel et al. 2009). Although this strategy has gained increasing global interest in recent years, it can be useful to use different pest management tools within the context of integrated pest management (IPM), because it can be more efficient to use different types of biocontrol agents (Parra 2014, Siegwart et al. 2015). Biological control is a highly relevant and fundamental component in IPM programs because it constitutes a sustainable practice of pest management that can help reduce the unnecessary overuse of insecticides (Nava 2007, van Lenteren et al. 2018).

In South America, seed feeders are among the major insect pests of soybean, one of the biggest crops grown worldwide. Noteworthy for feeding directly on the pods, these pests severely affect crop yields by lowering the physiological and sanitary quality of the

seeds (Panizzi and Slansky Jr 1985, Corrêa-Ferreira and De Azevedo 2002). The egg parasitoid, *Telenomus podisi* Ashmead, 1893, is an effective biocontrol agent of one of the major and most abundant soybean pests *Euschistus heros* Fabricius, 1798 (Hemiptera: Pentatomidae) (Panizzi and Corrêa-Ferreira 1997) owing to its generalist nature. This species parasitizes eggs of several pests and so stands out as an important natural enemy of stink bugs of the Pentatomidae family (Koppel et al. 2009), including the exotic invasive brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Cornelius et al. 2016, Herlihy et al. 2016). These characteristics make it a potential candidate for use in (ABC) programs (Pacheco and Corrêa-Ferreira 2000, Tognon et al. 2013). Together with egg parasitoids, such as *T. podisi*, products based on entomopathogenic fungi are already widely used and important for IPM as well (Agüero and Neves 2014).

Organic botanical products (biocontrol agents, syrups, extracts, and essential oils) tend to have a less pronounced negative impact on the environment (Siegwart et al. 2015) as compared with synthetic chemical products and offer a viable option for pest control in organic cultivation. However, some natural products may exert negative impacts on the parasitoid due to lethal (less selectivity) or sublethal effects. Sublethal effects are physiological or behavioral effects observed in individuals that survive exposure to an insecticide (at either sublethal or lethal dose). Examples include reduced parasitism and fecundity, and interference with oviposition, emergence, the longevity of offspring, and courtship (Desneux et al. 2007). The choice of these products in IPM systems tends to depend on their selectively (Desneux et al. 2007, Amaro et al. 2015), yet, given the potential importance of sublethal effects on natural enemies (as mentioned above), the choice should also prioritize those that interact harmoniously with natural enemies with minimal sublethal effects (Desneux et al. 2007).

It is important to highlight that the effects (lethal or sublethal) of these products on natural enemies vary according to the organism and other factors, such as the crop, form of application, dosage, among others abiotic factors. Therefore, studies on this subject are fundamental to the success of IPM, as they provide the necessary information to make decisions regarding the control of pests. Silva and Bueno (2014) have previously studied selectivity of different organic products above *T. podisi*, but as far as we know, this is the first study to report sub-lethal effects of those organic products such as repellence, among others on this biocontrol agent. Therefore, in this context, the current investigation evaluated the side effects of commercial natural products on *T. podisi* under laboratory conditions.

Material and Methods

The experiments were performed under controlled conditions (26 ± 2°C, 75% relative humidity [RH], photoperiod of 12:12 [L:D] h) at the Biological Control Laboratory I of the Federal Technological University of Paraná, Campus Dois Vizinhos, Paraná, Brazil (UTFPR-DV). Three bioassays were performed: preference of *T. podisi* parasitism between treated and non-treated pest eggs (Bioassay 1); effect on *T. podisi* parasitism with no-choice test (preparasitism; Bioassay 2); selectivity for *T. podisi* larval stage (postparasitism; Bioassay 3).

Organic Products, Parasitoid, and Host

To perform the bioassays, *E. heros* eggs (up to 3 d old), nonparasitized and parasitized by *T. podisi*, were purchased from a company that specializes in insect rearing and commercialization (Bug Agentes de Controle Biológico Ltda, Piracicaba, São Paulo, Brazil). The non-parasitized eggs were stored under refrigeration at 4°C for a maximum of 24 h until further experimentation. However, the eggs parasitized by *T. podisi* were stored in 1000-ml plastic flasks kept in a room under controlled conditions (26 ± 2°C, 75% RH, photoperiod of 12:12 [L:D] h) until adult's emergence, to be used in further experimentation. Pure honey drops were provided as food on the wall of the plastic container. The commercial natural products evaluated are used in the production of organic soybean as insecticide, fungicide, or leaf fertilizer (Table 1), and were purchased from agricultural supply stores: azadirachtin A/B (Azamax, UPL from Brazil Industry and Commerce of Agricultural Inputs S.A, Ituverava, SP, Brazil); *Beauveria bassiana* (Bals.) Vuill. (Hypocreales) (Boveril, Koppert from Brazil Biological Systems Ltda, Piracicaba, SP, Brazil); orange oil fertilizer (Orobor, Oro Agri, Astorga, PR, Brazil), copper oxychloride (Difere, Oxiquímica Agrociência, Jaboticabal, SP, Brazil); *Metarhizium anisopliae* (Metsch.) Sorok. (Hypocreales) (Metarril, Koppert from Brazil Biological Systems Ltda).

Side Effect Bioassays

For all bioassays, 25 non-parasitized *E. heros* eggs were fixed onto cards (1.5 × 1.5 cm sulfite paper) via a non-toxic glue made of wheat flour and distilled water. The natural products were prepared in Becker flasks containing 100 ml of an aqueous solution of 0.01% Tween 80, according to the concentrations recommended by the manufacturer (Table 1). In bioassays 2 and 3, the products were

Table 1. Formulation, trademark, manufacturer localization, recommended concentration, active ingredient concentration and cultures of the products evaluated in the selectivity bioassays at *Telenomus podisi*

Formulation	Trademark	Manufacturer	Localization	Recommended concentration[a]	Active ingredient concentration	Crops
Metarhizium anisopliae	Metarril	Koppert from Brazil Biological Systems Ltda	Piracicaba – SP, Brazil	0.5 kg/ha	1.39×10^8 conídios/g	Pasture
Beauveria bassiana	Boveril			0.5 kg/ha	1×10^8 conídios/g	Soy
Copper Oxychloride	Difere	Oxiquímica Agrociência	Jaboticabal – SP, Brazil	0.5–1.0 liter/ha	58,800 g/liter (58,800% m/v)	Soy, wheat
Azadirachtin A/B	Azamax	UPL from Brazil Industry and Commerce of Agricultural Inputs S.A	Ituverava – SP, Brazil	0.4–0.6 liter/ha	12 g/liter (1.2% m/m)	Soy
Orange oil, Nitrogen, Boron and Molybdenum	Orobor	Oro Agri	Astorga – PR, Brazil	0.35–0.75 liter/ha	Orange oil + 1.5% Nitrogen + 0.25% Boron + 0.04 Molybdenum	Annual Crops

[a]Volume of spray considered from 200 liters/ha.

evaluated in three groupings, 1) orange oil fertilizer, Azadirachtin A / B and its own control, 2) *M. anisopliae*, *B. bassiana* and its own control, and 3) copper oxychloride and own control.

Bioassay 1: Preference of *T. podisi* Parasitism Between Treated and Non-treated *E. heros* Eggs—Free-Choice Test

For each treatment, 40 cards containing 25 non-parasitized *E. heros* eggs were prepared. Half (n = 20) of the cards (replicates) were immersed in the natural product solutions (treatments) and the remaining 20 were immersed in the control Tween 80 (0.01%). After immersion, the cards were dried in a laminar flow chamber for approximately 2 h, and then two cards, one treated and one control, were placed in a flat-bottomed glass tube (10 cm height × 2.5 cm diameter), together with a 72-h-old *T. podisi* female. The tubes were sealed with polyvinyl chloride (PVC) film and kept in a chamber under controlled conditions (26 ± 2°C, 75% RH, photoperiod of 12:12 [L:D] h), as described previously by Potrich et al. (2009). After 24 h of exposure to the host, the females were removed, whereas the eggs remained in the tubes in the same chamber and conditions. Parasitism was defined as the number of parasitized eggs in each card after 5 d. Parasitized eggs were identified by their blackish coloration, due to the deposition of urate salts from the larvae excretion as described by Cônsoli (1999).

Bioassay 2: Effect of Products on *T. podisi* Parasitism—No-choice Test

Treatments applied to ***E. heros*** eggs before *T. podisi* parasitism (Pre-parasitism test): For each treatment, 20 cards containing 25 non-parasitized *E. heros* eggs were prepared and were immersed in the natural product solutions (treatments), and in the control Tween 80 (0.01%). After immersion, the cards were dried in a laminar flow chamber for approximately 2 h, and then were placed in a flat-bottomed glass tube (10 cm height · 2.5 cm diameter), together with a 72-h-old *T. podisi* female. The procedures of storage conditions and removal of females post-parasitism were the same as described for Bioassay 1. From 5 d onward, assessments were made of number of black *E. heros* eggs; percentage of emergence; offspring sex ratio; egg- adult period and longevity of emerged adults, biological parameters described by Cônsoli (1999).

Bioassay 3: Selectivity of Products to *T. podisi* Larval Stage

Treatments applied to *E. heros* eggs after *T. podisi* parasitism (Post-parasitism test): For each treatment and the control, 20 cards with *E. heros* eggs were prepared. These were individually placed in a flat-bottomed glass tube (10 × 2.5 cm), together with a 72-h-old *T. podisi* female (and conditioned in a chamber under controlled conditions, as described in Bioassay 1). After 24 h, the females were removed, and the cards were immersed in the solutions of commercial natural products, as well as the control. The cards were again placed in individualized tubes and kept in the climate chamber under the same conditions already described. The biological parameters evaluated were the same as described in Bioassay 2.

Mathematical and Statistical Analyses

For all bioassays, the percentage of parasitism for each treatment and the control were calculated, considering the number of eggs per card to be 100%.

For Bioassays 2 and 3, the percentage of emergence was calculated as $Pe = \left(\frac{Te}{To}\right) 100$, where Pe is the percentage of emergence; Te is the total emerged; and To is the total parasitized eggs.

The sex ratio was calculated as $R = \frac{Tm}{Tm + Tf}$, where R is the sex ratio; Tm is the total males; and Tf is the total females.

The developmental time (egg–adult period) was calculated using the equation: $\left[(np\ d1) + (np\ d2) + (np\ d3) \ldots + \frac{np\ dn}{To}\right]$, where np is the number of parasitoids that emerged on a given day; d is the day on which the parasitoids emerged; dn is the total days that had emergence; and To is the total number of parasitoids that emerged on all days.

Longevity was calculated as: $\left[(npm\ d1) + (npm\ d2) + (npm\ d3) \ldots + \frac{npm\ dn}{TMo}\right]$, where npm is the number of dead parasitoids on the day; d is the day on which the parasitoids died; dn is the total number of days with deaths; and TMo is the total number of parasitoids that died on all days.

For all bioassays the data were subjected to the normality test (Lilliefors test) and homogeneity of variance test (Bartlett's test). Data that did not present a normal distribution were transformed into an $arcsin\ \sqrt{X/100}$ using Microsoft Excel software and then submitted to the same tests. For Bioassay 1, the t-test was used. For Bioassays 2 and 3, the data were analyzed by the nonparametric Kruskal–Wallis test. The pre- and post-parasitism tests were compared by the nonparametric Mann–Whitney U test. BioEstat 5.3 (Ayres et al. 2007) was used for all statistical analyses.

Results

Bioassay 1: Preference of *T. podisi* Parasitism Between Treated and Non-treated Eggs

The products azadirachtin A/B, *B. bassiana*, orange oil fertilizer, and copper oxychloride reduced the preference *T. podisi* parasitism compared with respectively controls. Only *M. anisopliae* did not affect the preference *T. podisi* parasitism (Table 2).

Bioassays 2 and 3: Effect of Products on *T. podisi* Parasitism With No-Choice Test and Selectivity of Products to *T. podisi* Larval Stage

When *T. podisi* females were confined with *E. heros* eggs both in the pre- and post-parasitism tests, only azadirachtin A/B negatively affected the parasitism (36.00 and 33.20%) compared to the control groups (73.60 and 73.00%), respectively. For the other evaluated products, both in the pre- and post-parasitism tests, no reduction in the percentage of *T. podidi* parasitism was observed. *M. anisopliae* was the only treatment that in pre-parasitism test caused reduction in *T. podisi* parasitism comparing with the pos-parasitism test (Table 3).

Regarding the sex ratio and adults of *T. podisi* that emerged from *E. heros* eggs, in both the pre- and post-parasitism tests, the evaluated products neither affected the sex ratio nor reduced the percentage of parasitoid emergence in comparison to the respective controls. Likewise, there was no significant difference between pre- and post-parasitism tests (Table 4).

The developmental time of *T. podisi* females emerged from *E. heros* eggs, was increased by azadirachtin A/B in both the pre- and post-parasitism tests compared with the control groups. A comparative analysis between pre- and post-parasitism tests revealed a reduction in the mean developmental time when azadirachtin A/B and *M. anisopliae* were applied pos-parasitism. In contrast, fungicide copper oxychloride caused reduction in *T. podisi* mean developmental time in pre-parasitism test (Table 5).

For males, in the pre-parasitism test, the orange oil fertilizer reduced the mean developmental time. Comparing the pre- and post-parasitism tests, *M. anisopliae* was found to reduce the mean developmental time. The other products tested did not affect this

parameter when compared with the control and between pre- and post-parasitism test (Table 5).

On evaluating the longevity of *T. podisi* adults (males and females) that emerged from *E. heros* eggs immersed in solutions of commercial natural products, most of the products did not affect (prolong or reduce) this biological parameter. For females, the mean longevity was increased by the azadirachtin A/B treatment in the pre-parasitism test comparing with the control group. For males, the longevity was reduced by the orange oil fertilizer. The other products tested did not affect this parameter (Table 6).

Metarhizium anisopliae applied in pre-parasitism test reduced the mean longevity for *T. podisi* females and males, while only female longevity was decreased by copper oxychloride. Other treatments were not significantly different from the control for this parameter (Table 6).

Table 2. Preference of parasitism (±SE) of *Telenomus podisi* on eggs of *Euschistus heros* immersed in commercial natural products at the concentration recommended by the manufacturer and control

Treatment	% parasitized eggs
Control	43.80 ± 7.60a
Azadirachtin A/B	23.00 ± 3.80b
P	0.04
Control	68.40 ± 6.69a
Orange oil fertilizer	27.00 ± 6.05b
P	0.02
Control	54.00 ± 7.97a
Metarhizium anisopliae	30.00 ± 6.99a
P	0.10
Control	58.20 ± 7.24a
Beauveria bassiana	24.20 ± 5.58b
P	0.04
Control	76.40 ± 7.26a
Copper oxychloride	12.20 ± 4.85b
P	<0.01

Means followed by the same lowercase letter within the column for each treatment do not differ significantly from one another by the *t*-test ($P < 0.05$).

Discussion

In the free-choice bioassay, all treatments, except *M. anisopliae*, reduced *T. podisi* parasitism. It is important to emphasize that during oviposition, the parasitoid uses visual and olfactory stimuli to locate and recognize the host, whereas it uses gustatory and visual stimuli to accept it (Vinson 1997). This observed behavior suggests that the presence of certain substances could interfere with the recognition and acceptance of the host egg, triggering the rejection of the parasitoid, as evidenced in the free-choice bioassay.

On the other hand, in the no-choice bioassays, *Metarhizium anisopliae*, *B. bassiana*, orange oil fertilizer, and the fungicide copper oxychloride did not cause reduction of parastism of *T. podisi* in *E. heros* eggs. These results suggest that *T. podisi* parasitize host eggs, even in unfavorable in no-choice conditions. Only azadirachtin A/B reduced the parasitism in the pre and post-parasitism tests and did not affect other evaluated parameters.

In a similar study, Smaniotto et al. (2013) did not find any repellent effect when assessing six commercial natural products, including a mix of neem oil (Natuneem) on the oviposition of *T. podisi* in a free-choice test. In contrast, in a study that analyzed the repellent effect of the product composed also by neem oil - (Topneem) on *Trichogramma pretiosum* (Riley) (Hymenoptera: Trichogrammatidae) in a free-choice test, the parasitism was around 24.4%, which was significantly lower than that in the control (75.6%) (Luckmann et al. 2014). The products Natuneem and Topneem are a mixture of active ingredients, among which, azadirachtin is also present. The effect of natural products on natural enemies of pests, such as parasitoids, may vary due to several factors such as composition, formulation and concentration of the products, species of natural enemy studied, and evaluation methodology. According to Mordue and Nisbet (2000), insects can react differently when in contact with azadirachtin A/B (active principle of neem), which can inhibit feeding, affect biological development (egg, larva or nymph, pupa, and adult), affect reproduction, and destroy tissues.

The reduction in parasitism of *T. podisi* in the free-choice bioassay caused by Copper oxychloride is probably due the compound copper sulfate that has a repellent effect on some insects (Stüpp et al. 2012).

Table 3. Percentage of parasitism (±SE) of *Telenomus podisi* in eggs of *Euschistus heros* immersed in commercial natural products at the concentration recommended by the manufacturer and control

	Percentage of parasitism		
Treatment	Pre-parasitism	Post-parasitism[a]	*P*
Control	73.60 ± 4.24aA	73.00 ± 5.26aA	0.37
Orange oil fertilizer	77.40 ± 2.55aA	65.40 ± 6.83aA	0.29
Azadirachtin A/B	36.00 ± 4.24bA	33.20 ± 3.32bA	0.41
P	<0.01	<0.03	
Control	68.40 ± 5.21aA	75.60 ± 5.25aA	0.07
Metarhizium anisopliae	66.00 ± 5.21aB	81.60 ± 3.06aA	0.01
Beauveria bassiana	75.00 ± 3.19aA	73.60 ± 5.0aA	0.30
P	0.53	0.54	
Control	68.40 ± 5.21aA	75.60 ± 5.25aA	0.07
Copper oxychloride	67.80 ± 4.93aA	74.60 ± 5.49aA	0.27
P	0.87	0.93	

Means followed by the same lowercase letter within the column for each treatment group do not differ significantly from one another by the Kruskal–Wallis test ($P < 0.05$). Means followed by the same capital letter within the row do not differ from each other by the Mann–Whitney *U* test ($P < 0.05$).

[a]Pre- and post-parasitism: treatment applied respectively before and after oviposition *T. podisi*.

Table 4. Emergency (%) and sex ratio (±SE) of *Telenomus podisi* in eggs of *Euschistus heros* immersed in commercial natural products at the concentration recommended by the manufacturer and control

	Emergency percentage			Sex ratio		
Treatment	Pre-parasitism	Post-parasitism[a]	*P*	Pre-parasitism	Post-parasitism[a]	*P*
Control	72.20 ± 5.30abA	71.00 ± 6.70aA	0.89	0.62 ± 0.01 aA	0.72 ± 0.03 aA	0.22
Orange oil fertilizer	81.35 ± 3.04aA	70.55 ± 6.47aA	0.25	0.50 ± 0.01 aA	0.65 ± 0.04 aA	0.26
Azadirachtin A/B	55.61 ± 10.80bA	61.21 ± 8.60aA	0.46	0.70 ± 0.01 aA	0.70 ± 0.10 aA	0.51
P	<0.05	0.35		0.85	0.19	
Control	79.78 ± 3.97aA	65.45 ± 6.61aA	0.16	0.74 ± 0.04 aA	0.77 ± 0.05 aA	0.92
Metarhizium anisopliae	81.93 ± 6.70aA	75.29 ± 3.04 aA	0.10	0.73 ± 0.02 aA	0.73 ± 0.03 aA	0.81
Beauveria bassiana	80.15 ± 2.75aA	73.26 ± 7.54 aA	0.82	0.75 ± 0.04 aA	0.71 ± 0.02 aA	0.79
P	0.22	0.40		0.83	0.58	
Control	79.78 ± 3.97aA	65.45 ± 6.61 aA	0.16	0.74 ± 0.04 aA	0.77 ± 0.05 aA	0.92
Copper oxychloride	71.89 ± 7.73aA	70.37 ± 7.74 aA	0.78	0.73 ± 0.05 aA	0.60 ± 0.02 aA	0.64
P	0.32	0.50		0.59	0.37	

Means followed by the same lowercase letter within the column for each treatment group do not differ significantly from one another by the Kruskal–Wallis test ($P < 0.05$). Means followed by the same capital letter within the row do not differ from each other by the Mann–Whitney *U* test ($P < 0.05$).

[a]Pre- and post-parasitism: treatment applied respectively before and after oviposition *T. podisi*.

Table 5. Developmental time in days, (±SE) of *Telenomus podisi* on eggs of *Euschistus heros* immersed in commercial natural products at the concentration recommended by the manufacturer and control

	Developmental time					
	Female			Male		
Treatment	Pre-parasitism	Post-parasitism	*P*	Pre-parasitism	Post-parasitism[a]	*P*
Control	10.59 ± 0.09bA	10.24 ± 0.06aB	0.01	10.60 ± 0.09aA	10.36 ± 0.08aA	0.10
Orange oil fertilizer	10.35 ± 0.06bA	10.24 ± 0.05aA	0.13	10.22 ± 0.06bA	10.36 ± 0.10aA	0.13
Azadirachtin A/B	11.02 ± 0.09aA	10.54 ± 0.16aB	0.03	10.56 ± 0.15abA	10.15 ± 0.10aA	0.08
P	<0.01	0.83		<0.01	0.20	
Control	13.62 ± 0.11aA	13.32 ± 0.10aB	0.04	13.13 ± 0.12aA	12.62 ± 0.10aB	<0.01
Metarhizium anisopliae	13.71 ± 0.10aA	13.27 ± 0.06aB	<0.01	12.84 ± 0.15aA	12.46 ± 0.10aB	0.03
Beauveria bassiana	13.63 ± 0.11aA	13.37 ± 0.10aA	0.06	12.73 ± 0.14aA	12.72 ± 0.12aA	0.48
P	0.74	0.95		0.18	0.19	
Control	13.62 ± 0.11aA	13.32 ± 0.10aB	0.04	13.13 ± 0.12aA	12.62 ± 0.10bB	<0.01
Copper oxychloride	11.00 ± 0.10aB	13.32 ± 0.06aA	<0.01	12.92 ± 0.13aA	13.25 ± 0.19aA	0.97
P	0.26	0.53		0.35	0.01	

Means followed by the same lowercase letter within the column for each treatment do not differ significantly from one another by the Kruskal–Wallis test ($P < 0.05$). Means followed by the same capital letter within the row do not differ from each other by the Mann–Whitney *U* test ($P < 0.05$).

[a]Pre- and post-parasitism: treatment applied respectively before and after oviposition *T. podisi*.

Regarding entomopathogenic fungi, we observed they did not reduce *T. podisi* parasitism. In the same way, Amaro et al. (2015) observed that *M. anisopliae* (Metarril) was not effect on the parasitism preference of *T. pretiosum* under laboratory conditions. In the other hand, two isolates of *B. bassiana* (Potrich et al. 2015) and two isolates of *M. anisopliae* (Potrich et al. 2017) caused a repellent effect against *T. pretiosum* parasitism via free-choice test. It was further stated that the parasitoid might be able to identify hosts contaminated by the fungus, thereby preventing them from being parasitized, which may explain the results obtained with *B. bassiana* in the current study.

The orange oil fertilizer (Orobor) reduced the *T. podisi* parasitism, different from that found by Luckmann et al. (2014), in similar study with *T. pretiosum*. This difference between the results for the tests with orange oil fertilizer can be attributed to the parasitoid species being different, because, as Filho et al. (2006) mentioned, various species of natural enemies of pests respond differently to natural products.

The results of the azadirachtin A/B in the pre-parasitism test can be explained by the fact that the egg parasitoids use visual and olfactory stimuli for the recognition of the host. Vinson (1997) argued that the parasitoid could identify toxic or repellent substances on the eggs of the host and may not parasitize them. For the post-parasitism test, it can be inferred that the product penetrated the protective layers of the host egg, causing death of the parasitoids under development, as suggested by Smaniotto et al. (2013), who obtained similar results with our work. The death of parasitoids probably occurred due to the negative effects that azadirachtin may have on insect physiology, including an imbalance in the production of ecdysis hormones, alteration in histogenesis, cuticular melanization, interference during mitosis, and mitochondrial rupture (Moreira et al. 2010).

Table 6. Average longevity, in days, (±SE) of adult females and males of *Telenomus podisi* on *Euschistus heros* eggs immersed in commercial natural products at the concentration recommended by the manufacturer and control

	Longevity					
	Female			Male		
Treatment	Pre-parasitism	Post-parasitism[a]	*P*	Pre-parasitism	Post-parasitism[a]	*P*
Control	2.98 ± 0.05bA	3.14 ± 0.14aA	0.13	3.55 ± 0.09aA	3.24 ± 0.10aA	0.50
Orange oil fertilizer	3.29 ± 0.05bA	3.14 ± 0.09aA	0.38	3.10 ± 0.07bA	3.21 ± 0.15aA	0.87
Azadirachtin A/B	3.76 ± 0.05aA	3.45 ± 0.16aA	0.20	3.40 ± 0.13abA	3.54 ± 0.27aA	0.49
P	<0.01	0.33		0.01	0.22	
Control	4.81 ± 0.11aA	4.59 ± 0.12aA	0.11	4.39 ± 0.09abA	3.73 ± 0.05aA	0.10
Metarhizium anisopliae	5.02 ± 0.12aA	4.29 ± 0.23aB	0.002	4.93 ± 0.20aA	3.88 ± 0.20aB	<0.01
Beauveria bassiana	4.79 ± 0.10aA	4.51 ± 0.20aA	0.26	4.23 ± 0.10bA	4.13 ± 0.09aA	0.34
P	0.36	0.87		<0.01	0.69	
Control	4.81 ± 0.11aA	4.59 ± 0.12aA	0.11	4.39 ± 0.09aA	3.73 ± 0.05aA	0.10
Copper oxychloride	5.10 ± 0.13aA	4.65 ± 0.09aB	0.01	4.44 ± 0.015aA	4.19 ± 0.07aA	0.11
P	0.05	0.55		0.09	0.87	

Means followed by the same lowercase letter within the column for each treatment do not differ significantly from one another by the Kruskal–Wallis test ($P < 0.05$). Means followed by the same capital letter within the row do not differ from each other by the Mann–Whitney *U* test ($P < 0.05$).

[a]Pre- and post-parasitism: treatment applied respectively before and after oviposition *T. podisi*.

Oliveira et al. (2003) observed that neem oil reduced *T. pretiosum* parasitism via pre-parasitism test (no choice test). In contrast, Smaniotto et al. (2013) found that Natuneem did not cause a repellent effect on *T. podisi* parasitism and also did not affect the offspring percentage of emergence in the pre- and post-parasitism tests. In the same way, Luckmann et al. (2014) did not observe reduction in the parasitism of *T. pretiosum* when Topneem was applied in pre-parasitism tests. It also did not cause mortality of the larval stage parasitoid during the post-parasitism test. This difference observed for azadirachtin-based products, as already mentioned, might be related to the parasitoid species, composition and formulation of the product, and concentration of the active principle of the product used (Hohmann et al. 2010).

Analogous studies found Natuneem (Smaniotto et al. 2013), and orange oil fertilizer (Orobor) and azadirachtin A/B (Topneem) (Luckmann et al. 2014) did not interfere with the emergence of *T. podisi* (Smaniotto et al. 2013) and *T. pretiosum* (Luckmann et al. 2014) in pre- and post-parasitism tests. In a no-choice test, *M. anisopliae* (Metarril) and *B. bassiana* (Boveril) did not reduce the emergence of *T. pretiosum* (Amaro et al. 2015), which is corroborated by findings in this study. This outcome can be attributed to the embryonic phase being protected by two chorion, and the pre-pupal and pupal phase being protected by the host egg chorion, which may have prevented germination and/or penetration of the fungi, as studied by Potrich et al. (2015, 2017), who observed no interference by *B. bassiana* and *M. anisopliae* on the percentage emergence of *T. pretiosum*.

The sex ratio can be determined by the quality of the host, which occurs during oviposition because the development of females requires an egg host with more nutritional resources (Vinson 1997). It is noteworthy that other factors may also interfere with sex determination, for example, environmental and ecological factors, such as temperature, parasitism time, and female density (Vinson 1997, Pacheco and Corrêa-Ferreira 1998), but these factors were not involved in the current experiment.

Results similar to those obtained in this study were reported by Potrich et al. (2009, 2017), who observed no changes in the sex ratio of *T. pretiosum* (0.68 and 0.74) when sprayed with isolates of *M. anisopliae* and *B. bassiana*, both in pre- and post-parasitism tests. Likewise, Smaniotto et al. (2013) found that some commercial natural products, including Natuneem, did not change the sex ratio of *T. podisi*.

Sex ratio is extremely important in the field for the efficiency of egg parasitoids (Potrich et al. 2009), and so low or high values for this parameter can be detrimental for the control of any given population in agroecosystems (Corrêa-Ferreira 1993).

Regarding the development of the egg parasitoid, it can be slow or fast, depending on the nutritional quality of the host, because fewer nutrients could force the emergence of adults ahead of time (Vinson 1997). However, this may not have occurred in the present study. Although significant differences were observed, the results showed variations of only a few hours in the egg–adult period, which is not expected to affect the maintenance of the population in the field. In similar work, Smaniotto et al. (2013) showed that Natuneem did not alter the egg–adult period of males and females of *T. podisi*.

Among the products evaluated, copper oxychloride, orange oil fertilizer, *B. bassiana*, and *M. anisopliae* exhibited potential for use in combination with the egg parasitoid, *T. podisi*, under the conditions of this study because they did not present any sublethal effects on this insect, in practically all evaluated parameters, particularly, in the percentage of parasitism (pre- and post-parasitism tests). Except for orange oil fertilizer and *M. anisopliae*, which altered the mean adult longevity of the parasitoid and the egg–adult period, respectively, there were no changes in other biological parameters in relation to the control. In contrast, the insecticide azadirachtin A/B, besides repelling parasitism during the free-choice test, reduced the percentage of parasitism in both the pre- and post-parasitism tests, thereby showing a non-selective effect on *T. podisi*. Complementary studies are recommended to understand the possible action of the product in the post-parasitism application, such as the possible interactions of the product with the integument of the host egg and its effect on the larva of the parasitoid.

Copper oxychloride, orange oil fertilizer, and *B. bassiana* reduced *T. podisi* parasitism in free-choice bioassay. In contrast, in the no-choice test, both pre- and post-parasitism tests, they did not reduce the percentage of *T. podisi* parasitism. Therefore, it can

be inferred that all three products have the potential to be used in combination with this egg parasitoid because the insect will prefer the host eggs that were not in contact with the products (common situation in the field) when the products do not reach all host eggs during the application. Furthermore, if the products reach all host eggs, they will not interfere with the biological parameters of *T. podisi*.

Copper oxychloride, orange oil fertilizer, *B. bassiana*, and *M. anisopliae* do not cause any sublethal effects on the egg parasitoid *T. podisi* under laboratory conditions, unlike azadirachtin A/B.

Acknowledgments

We thank the CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for funding (Agreement: 424078/2016-3); and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for granting the post-graduate scholarship to the first author. We also thank Universidade Tecnológica Federal do Paraná (UTFPR) and the company GEBANA BRASIL - Cataratas do Iguaçu Produtos Orgânicos Ltda, for all support during the development of the research.

References Cited

Agüero, M. A. F., and P. M. O. J. Neves. 2014. Seletividade de *Metarhizium anisopliae* a *Telenomus podisi* (Hymenoptera: Scelionidae). Investigação Agraria 16: 21–28.

Amaro, J. T., A. F. Bueno, A. F. Pomari-Fernandes, and P. M. Neves. 2015. Selectivity of organic products to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Neotrop. Entomol. 44: 489–497.

Ayres, M., M. J. R. Ayres, D. L. Ayres, and A. S. S. Santos. 2007. Bioestat: aplicações estatísticas nas áreas das ciências biológicas e médicas. Sociedade Civil Mamirauá, MCT-CNPq, Belém, Brazil.

Cavigelli, M. A., S. B. Mirsky, J. R. Teasdale, J. T. Spargo, and J. Doran. 2013. Organic grain cropping systems to enhance ecosystem services. Renew. Agric. Food Syst. 28: 145–159.

Cock, M. J. W., J. C. van Lenteren, J. Brodeur, B. I. P. Barratt, F. Bigler, K. Bolckmans, F. L. Cônsoli, F. Haas, P. G. Mason, and J. R. P. Parra 2010. Do new access and benefit sharing procedures under the convention on biological diversity threaten the future of biological control? BioControl 55: 199–218.

Cônsoli F. L., M. M. Rossi, and J. R. P. Parra. 1999. Developmental time and characteristics of the immature stages of *Trichogramma galloi* and *T. pretiosum* (Hymenoptera, Trichogrammatidae). Rev. Bras. Entomol. 3: 271–275.

Cornelius, M. L., C. Dieckhoff, K. A. Hoelmer, R. T. Olsen, D. C. Weber, M. V. Herlihy, E. J. Talamas, B. T. Vinyard, and M. H. Greenstone. 2016. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control 103: 11–20.

Corrêa-Ferreira, B. S. 1993. Utilização do parasitoide de ovos *Trissolcus basalis* (Wollaston) no controle de percevejos da soja. Circular Técnica 11, Embrapa, Londrina, Brazil.

Corrêa-Ferreira, B. S., and J. De Azevedo. 2002. Soybean seed damage by different species of stink bugs. Agric. Forest Entomol. 4: 145–150.

Desneux, N., A. Decourtye, and J. M. Delpuech. 2007. The sublethal effects of pesticides on beneficial arthropods. Annu. Rev. Entomol. 52: 81–106.

FiBL and IFOAM – Organics International. 2017. The World of Organic Agriculture. Statistics and Emerging Trends 2017. H. Willer and J. Lernoud (eds.). FiBL, Frick, and IFOAM, Bonn, Germany. https://shop.fibl.org/CHen/mwdownloads/download/link/id/785/?ref=1 (last visited 26 June 2017).

FiBL-IFOAM. 2009. The World of Organic Agriculture. Statistics and Emerging Trends 2009. FiBL-IFOAM Report. H. Willer and L. Kilcher (eds.). IFOAM, Bonn; FiBL, Frick; and ITC, Geneva, Switzerland. http://orgprints.org/18380/16/willer-kilcher-2009.pdf (last visited 10 February 2013).

Filho, W. J. M., M. Botton, A. D. Grützmacher, F. P. Giolo, and C. G. Manzoni. 2006. Ação de produtos naturais sobre a sobrevivência de *Argyrotaenia sphaleropa* (Meyrick) (Lepidoptera: Tortricidae) e seletividade de inseticidas utilizados na produção orgânica de videira sobre *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Ciência Rural 36: 1072–1078.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. 2016. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One 11: e0150275.

Hohmann, C. L., F. A. Silva, and T. G. Novaes. 2010. Selectivity of neem to *Trichogramma pretiosum* Riley and *Trichogrammatoidea annulata* De Santis (Hymenoptera: Trichogrammatidae). Neotrop. Entomol. 39: 985–990.

Koppel, A. L., D. A. Herbert, Jr, T. P. Kuhar, and K. Kamminga. 2009. Survey of stink bug (Hemiptera: Pentatomidae) egg parasitoids in wheat, soybean, and vegetable crops in southeast Virginia. Environ. Entomol. 38: 375–379.

Lairon, D. 2010. Nutritional quality and safety of organic food. A review. Agron. Sustain. Dev. 30: 33–41.

van Lenteren, J. C., K. Bolckmans, J. Köhl, W. J. Ravensberg, and A. Urbaneja. 2018. Biological control using invertebrates and microorganisms: plenty of new opportunities. BioControl 63: 39–59.

Luckmann, D., A. de Gouvea, M. Potrich, E. R. L. da Silva, B. Puretz, S. Dallacort, and T. E. Gonçalves. 2014. Seletividade de produtos naturais comerciais a *Trichogramma pretiosum* (Riley, 1879) (Hymenoptera: Trichogrammatidae). Rev. Ceres 61: 924–931.

Marangoni, C., N. F. de Moura, and F. R. M. Garcia. 2012. Utilização de óleos essenciais e extratos de plantas no controle de insetos. Revista de Ciências Ambientais 6: 95–112.

Mazzoleni, E. M., and J. M. Nogueira. 2006. Agricultura orgânica: características básicas do seu produtor. Revista de Economia e Sociologia Rural 44: 263–293.

Mordue, A. J., and A. J. Nisbet. 2000. Azadirachtin from the neem tree *Azadirachta indica*: its action against insects. Anais da Sociedade Entomológica do Brasil 29: 615–632.

Moreira, M. D., M. C. Picanço, E. M. Silva, S. C. Moreno, and J. C. Martins. 2010. Uso de inseticidas botânicos no controle de pragas, pp. 89–120. *In* M. Venzon, T. J. de Paula Jr., and A. Pallini (eds.), Controle alternativo de pragas e doenças na agricultura orgânica. Epamig/CTZM, Viçosa, Brazil.

Nava, D. E. 2007. Controle biológico de insetos-praga em frutíferas de clima temperado: uma opção viável, mas desafiadora. Documentos 208, pp. 1–22. Embrapa Clima Temperado, Pelotas, Brazil.

Oliveira, R. C., D. Pratissoli, and A. F. Bueno. 2003. Efeito da *Azadirachta indica* (NIM) sobre o parasitismo de *Trichogramma pretiosium* (Hymenoptera: Trichogrammatidae) em ovos de *Anasgasta kuehniella* (Lepidoptera: Pyralidae). Revista Ecossistema 28: 75–78.

Pacheco, D. J. P., and B. S. Corrêa-Ferreira. 1998. Potencial reprodutivo e longevidade do parasitóide *Telenomus podisi* Ashmead, em ovos de diferentes espécies de percevejos. Anais da Sociedade Entomológica do Brasil 27: 585–591.

Pacheco, D. J. P., and B. S. Corrêa-Ferreira. 2000. Parasitismo de Telenomus podisi Ashmead (Hymenoptera: Scelionidae) em Populações de Percevejos Pragas da Soja. Anais da Sociedade Entomológica do Brasil. 29: 295–302.

Panizzi, A. R., and F. Slansky Jr. 1985. Review of phytophagous pentatomids (Hemiptera: Pentatomidae) associated with soybean in the Americas. Fla. Entomol. 68: 184–203.

Panizzi, A. R., and B. S. Corrêa-Ferreira. 1997. Dynamics in the insect fauna adaptation to soybean in the tropics. Trends Entomol. 1: 71–88.

Parra, J. R. P. 2014. Biological control in Brazil: an overview. Sci. Agric. 71: 345–355.

Potrich, M., L. F. Alves, J. Haas, E. R. Da Silva, A. Daros, V. Pietrowski, and P. M. Neves. 2009. [Selectivity of *Beauveria bassiana* and *Metarhizium anisopliae* to *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae)]. Neotrop. Entomol. 38: 822–826.

Potrich, M., L. F. A. Alves, E. Lozano, J. C. Roman, V. Pietrowski, and P. M. O. J. Neves. 2015. Interactions between *Beauveria bassiana* and *Trichogramma pretiosum* under laboratory conditions. Entomol. Exp. Appl. 154: 213–221.

Potrich, M., L. F. A. Alves, E. R. Lozano, A. K. Bonini, and P. M. O. J. Neves. 2017. Potential side effects of the entomopathogenic fungus *Metarhizium anisopliae* on the egg parasitoid *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) under controlled conditions. J. Econ. Entomol. 110: 2318–2324.

Siegwart, M., B. Graillot, C. Blachere Lopez, S. Besse, M. Bardin, P. C. Nicot, and M. Lopez-Ferber. 2015. Resistance to bio-insecticides or how to enhance their sustainability: a review. Front. Plant Sci. 6: 381.

Silva, D. M., and A. F. Bueno. 2014. Toxicity of organic supplies for the egg parasitoid *Telenomus podisi*. Ciência Rural 44: 11–17.

Smaniotto, L. F., A. de Gouvea, M. Potrich, E. R. L. da Silva, J. da Silva, and C. S. Pegorini. 2013. Seletividade de produtos alternativos a *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae). Semina: Ciências Agrárias 34: 3295–3306.

Stüpp, J. J., P. A. de Souza Gonçalves, and M. I. C. Boff. 2012. Efeito da calda bordalesa e extrato de adultos de *Diabrotica speciosa* no manejo fitossanitário de feijoeiro cultivado sob o sistema orgânico. Revista de Ciências Agroveterinárias 11: 222–229.

Tognon, R., J. Sant'Ana, and S. M. Jahnke. 2013. Aprendizagem e memória de *Telenomus podisi* (Hymenoptera, Platygastridae). Série Zoologia 103: 266–271.

Vinson, S. B. 1997. Comportamento de seleção hospedeira de parasitoides de ovos, com ênfase na família Trichogrammatidae, pp. 67–120. *In* J. R. P. Parra and R. A. Zucchi (eds.), *Trichogramma* e o controle biológico aplicado. FEALQ, Piracicaba, Brazil.