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SCIENTIFIC NOTE

Palmistichus elaeisis (Hymenoptera: Eulophidae) as an indicator of toxicity of herbicides registered for corn in Brazil

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The diversity of plants in agricultural systems benefits natural enemies. Herbicides are used in weed management in corn (*Zea mays* L.) to reduce competition and productivity losses, but they can impact natural enemies and contaminate the environment. The objective was to evaluate toxicity of herbicides on pupae parasitoid *Palmistichus elaeisis* Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae). The treatments were represented by the host pupae *Tenebrio molitor* L., 1785 (Coleoptera: Tenebrionidae) and herbicides atrazine, nicosulfuron, paraquat, and tembotrione in commercial doses compared to a control treatment with water. Pupae of *T. molitor* were immersed in the solution of herbicides and exposed to parasitism by six females of *P. elaeisis* each. The herbicides atrazine and paraquat were highly toxic and, therefore, not selective to *P. elaeisis*. Nicosulfuron reduced the sex ratio of *P. elaeisis* (0.20 ± 0.03), which may affect subsequent generations. Moreover, the herbicide tembotrione was selective to *P. elaeisis*, showing results comparable to the control. Floristic diversity of weeds can increase food source, habitat, shelter, breeding places and microclimates for insect parasitoids but herbicides formulations can be toxic and these products can affect *P. elaeisis* or its hosts by direct or indirect contact, showing the importance of selectivity studies for this natural enemy. However, the herbicide tembotrione was selective to *P. elaeisis* and it can be recommended for programs of sustainable management of weeds in corn crop with this parasitoid.

Key words: Ecotoxicology, lepidopteran defoliators, parasitoids, pesticides, weed control, *Zea mays*.

INTRODUCTION

The corn (*Zea mays* L.) is cultivated in an area of approximately 13 million hectares in Brazil with a production of 55 million tons (CONAB, 2011). Increasing areas with corn plantations is followed by problems with insect pests and weeds that can reduce production (Sertkaya et al., 2004; Figueiredo et al., 2006). The adoption of sustainable management of weeds in this crop is important for a balance between pests and natural enemies.

Weeds in corn cultivation areas may benefit arthropods and natural enemies. Floristic diversity can increase food source, habitat, shelter, breeding places, and microclimates for insect predators and parasitoids (Steinbauer et al., 2006; Clough et al., 2007; Jonsson et al., 2008; Silva et al., 2010). The use of herbicides to manage weeds is a common practice with low cost and labor and high efficiency (James, 2007; Albajes et al., 2009) to reduce competition with the commercial crop (Constantin et al., 2007). However, herbicides may have direct or indirect adverse effects on natural enemies (Menezes et al., 2012a; 2012b).

Palmistichus elaeisis Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) is a generalist and gregarious endoparasitoid of Lepidoptera and Coleoptera pupae. This insect oviposits inside the pupae, where the immature feed on host body tissue until the adult stage (Pereira et al., 2008; Zanuncio et al., 2008; Soares et al., 2009). *Palmistichus elaeisis* parasitized pupae of *Spodoptera frugiperda* J.E. Smith, 1797 (Lepidoptera: Noctuidae) (Bittencourt and Berti Filho, 2004a; 2004b), demonstrating its importance in integrated pest management (IPM) in corn. Moreover, this parasitoid developed in *Tenebrio molitor* L., 1785 (Coleoptera: Tenebrionidae), a pest of stored grains. This Coleoptera is reared in the laboratory and available as egg, larva, pupae, and adult stages (Zanuncio et al., 2008).

Herbicides formulations can be toxic to parasitoids (Giolo et al., 2005; Carmo et al., 2009) and these products can affect *P. elaeisis* or its hosts by direct or indirect contact, showing the importance of selectivity studies for this natural enemy. Moreover, inert compounds of herbicide formulations can facilitate penetration of active ingredients through the insect cuticle increasing toxic effect (Malkones, 2000).

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The objective of this study was to evaluate the toxicity of four herbicides, used and registered for corn crop in Brazil, on reproduction and development of the pupae parasitoid, *P. elaeisis*.

MATERIALS AND METHODS

The study was conducted in a room at 25 ± 2 °C, 70 ± 10% RH and a photoperiod of 12:12 h (500 lumens). One thousand larvae of *T. molitor* were placed in a polystyrene tray with wheat bran buds and sugar cane until pupation (Zanuncio et al., 2008). The polyphagous habit of *P. elaeisis* favors the use of *T. molitor* as host in toxicity studies (Soltani et al., 1996; Kostaropoulos et al., 2001).

Palmistichus elaeisis was reared in glass test tubes (14 × 2.2 cm) plugged with cotton swab. A drop of honey was placed within each tube (Zanuncio et al., 2008). One 24 h old *T. molitor* pupae was placed per tube with six *P. elaeisis* females for 48 h. Parasitoids from this rearing were used in this research.

Herbicides treatment

Fifty 48 h old *T. molitor* pupae with average weight of 0.104 g and surface area of 7.8×10^{-5} m^2 were dipped in herbicides diluted to 1 L solution, for 2 s and then removed. This methodology is efficient for toxicological tests for pupae received herbicide all over its surface and avoids death of pupae by asphyxiation (Vieira et al., 2001). After this, pupae were put in contact with *P. elaeisis* female for 2 d in the glass tube.

The herbicides used were atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine, Primoleo®) (T1), nicosulfuron (1-(4,6-dimethoxypyrimidin-2-yl)-3-(3-dimethylcarbamoyl-2-pyridylsulfonyl)urea, Sanson®) (T2), paraquat (1,1′-dimethyl-4,4′-bipyridinium, Gramoxone®) (T3), tembotrione (2-{2-chloro-4-mesyl-3-[(2,2,2-trifluoroethoxy)methyl]benzoyl}cyclohexane-1,3-dione, Soberan®) (T4), registered for the corn crop in Brazil and the control had only deionized water (T5). Solutions were prepared considering a volume equivalent to 150 L ha^{-1}, average surface area of the pupae and commercial dose of each herbicide (MAPA, 2011). Doses of active ingredient (μL) per pupa, manufacturer products, chemical groups, toxicology and formulation, and treatments are presented in Table 1.

Evaluated parameters

Each treatment had 10 replicates, with one pupae of *T. molitor* and six 72 h old *P. elaeisis* females, sexed according to the characteristics of the antennae and abdomen (Delvare and LaSalle, 1993). Pupae exposed to parasitism in the tubes were removed after 48 h and individualized in 250 mL plastic pots for 40 d and those without emergence of parasitoids discarded at the end of this period.

The longevity of *P. elaeisis* females exposed to *T. molitor* pupae treated with the herbicides was evaluated daily. The duration of the life cycle (egg to adult), percentage of parasitism, discounting the host natural mortality (Abbott, 1925), percentage of emergence of the progeny, numbers of males and females emerged, females produced per female, sex ratio, width of head capsule, and body length of parasitoids emerged per *T. molitor* pupae were obtained. The width of the head capsule and body length of *P. elaeisis* was obtained with a stereoscopic microscope (integrated digital camera and software measurement micrometer, DCM-Series, Cisco Systems, San Jose, California, USA). The sex ratio was calculated with the equation RS = number of females/total number of individuals.

Statistical analysis

Data were subjected to tests of the assumptions of the mathematical model (normality and homogeneity of variances) and ANOVA. Significant means were compared with the Tukey's test at $P = 0.05$ probability. Nonparametric data were subjected to Kruskal-Wallis test, $P = 0.05$ with the program for statistical analysis SAEG (Sistema para Análises Estatísticas, Versão 9.1, SAEG, 2007).

Table 1. Technical characteristics of herbicides registered for corn in Brazil.

Commercial formulation[1] (Manufacturer)	Active ingredient (ai) (concentration)	Dose per hectare (ai)	Dose per pupae (ai)	Chemical group	Toxicology[2] and formulation[3]
	g L^{-1}	g L^{-1}	μL		
Primoleo (Syngenta)	Atrazine (400)	2400	18.72	Triazines	CT = IV CA = II SC
Sanson 40 (Ishihara)	Nicosulfuron (4)	6	0.05	Sulfonylurea	CT = IV CA = II SC
Gramoxone 200 (Syngenta)	Paraquat (200)	600	4.68	Bipyridine	CT = II CA = II SC
Soberan (Bayer SA)	Tembotrione (420)	100	0.78	Benzoylcyclehexanedione	CT = III CA = III SC

[1]Trademark of the manufacturer.
[2]CT: toxicological class (I: extremely toxic; II: highly toxic; III: moderated toxic; IV: low toxicity), CA: environmental classification (I: highly dangerous; II: very dangerous; III: hazardous; IV: slightly hazardous).
[3]SC: suspension concentrate.

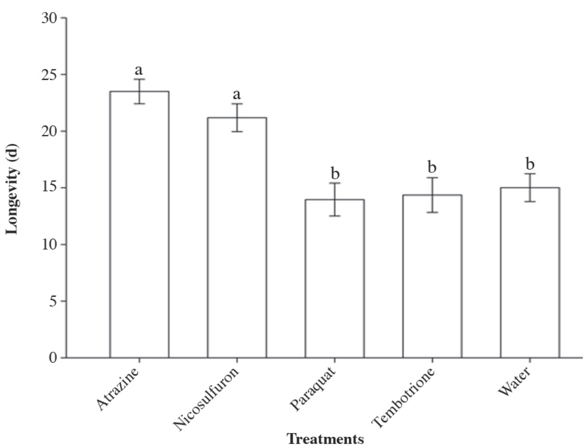
RESULTS AND DISCUSSION

Longevity of adults

Longevity of *P. elaeisis* adults exposed to *T. molitor* pupa was higher with atrazine and nicosulfuron, but lower with paraquat, tembotrione, and water ($F = 16.635$, $P = 0.000$) (Figure 1).

The herbicide atrazine and nicosulfuron may have caused the rejection of *P. elaeisis* females to *T. molitor* pupae and therefore its females had lower metabolic energy use. The largest energy reserves allowed these females to present longer longevity with these herbicide treatments compared to tembotrione and water. Atrazine was harmless (mortality < 30%) to adults of the parasitoid *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) exposed for 6 d to this product (Stefanello Junior et al., 2008). The mixture of atrazine and nicosulfuron, however, reduced the number of individuals of Collembola (Arthropoda: Ellipura), mites (Acari) and ants (Hymenoptera: Formicidae) (Vilma et al., 2007; Pereira et al., 2009).

Biological processes require energy, which is a limiting factor. Energy balances may occur in physiological processes where the energy available to a process decreases the energy to supply others (Calow and Sibly, 1990). Therefore, the lowest longevity of *P. elaeisis* in treatments with water and tembotrione, corroborates with the allocation of energy to reproduction process. Consequently, female longevity of these treatments decreased. The reduced longevity of *P. elaeisis* females after exposure to *T. molitor* pupae treated with paraquat may be due to toxicity of this herbicide (Figure 1). Paraquat, in single formulation (only the active ingredient) or in combination (paraquat + diuron), caused mortality and reduced parasitism rate of *T. pretiosum* and



Means followed by the same letter do not differ according to Tukey's test ($P = 0.05$). ($F = 16.635$, $P = 0.0000$).

Figure 1. Longevity (mean ± standard deviation) of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) females after exposure to *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae treated with herbicides registered for the corn crop in Brazil.

Anisopteromalus calandrae Howard, 1881 (Hymenoptera: Pteromalidae) (Bastos et al., 2005; Stefanello Junior et al., 2008; Lacoume et al., 2009).

Parasitism

Parasitism of *P. elaeisis* females was 30% and 40% with atrazine and paraquat without emergency of adults, and higher with tembotrione and water, 80% and 90% respectively ($F = 31.174$, $P = 0.002$) (Table 2).

The low selectivity of the herbicides atrazine and paraquat to *P. elaeisis* immature stages was similar to results with the commercial formulation of Gesaprim® GrDA and Gesaprim® 50 (atrazine) with 30%-79% mortality of adults of the parasitoid *Trichogramma cacoeciae* March, 1927 (Hymenoptera: Trichogrammatidae) (Hassan et al., 1988). However, paraquat was selective to *Telenomus remus* Nixon, 1937 (Hymenoptera: Scelionidae) parasitizing *S. frugiperda* (Carmo et al., 2009). This showed that susceptibility to the herbicides atrazine and paraquat varies with parasitoid species.

Females emerged and development of immature stages

The number of emerged *P. elaeisis* females was lower with nicosulfuron (20%) and had similar values with tembotrione and water (80% and 90%, respectively; $F = 21.474$, $P = 0.000$) (Table 2). Tembotrione did not affect the development of *P. elaeisis* immature stages.

The herbicide nicosulfuron can be toxic to *P. elaeisis* as reported for *T. pretiosum* and to non-targeted organisms such as immature of the fish *Carassius auratus* L., 1758 (Cypriniformes: Cyprinidae), inhibiting the activity of a metabolite in its brain (Bretauda et al., 2000; Stefanello Junior et al., 2008). The nicosulfuron also affected the metabolism of the algae *Chlorella vulgaris* Kessler and Huss, 1992 (Chlorococcales: Oocystaceae), *Navicula accommoda* f. *robusta* Foged, 1982 (Naviculales: Naviculaceae) and *Oscillatoria limnetica* Lemmermann, 1900 (Oscillatoriales: Oscillatoriaceae) (Leboulanger et al., 2001).

Females produced per female and sex ratio

The emergence of *P. elaeisis* and females produced per female were higher with tembotrione and in the control, but lower with nicosulfuron ($F = 21.074$, $P = 0.000$) (Table 2). The sex ratio of parasitoid was lower with nicosulfuron (0.2) and higher with tembotrione and water (0.8 and 0.9, respectively; $F = 9.439$, $P = 0.0008$) (Table 2).

The highest values of adult emergence and female produced per *P. elaeisis* female with tembotrione and water were similar to that of this parasitoid with pupae of this host without treatment (Zanuncio et al., 2008). However, the sex ratio with nicosulfuron can affect population dynamics of *P. elaeisis* due to the lower number of females compared to tembotrione and water. *Melittobia clavicornis* Cameron, 1908, *Melittobia australica* Girault,

Table 2. Reproductive variables (mean ± standard deviation) of the first generation of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) from pupae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) treated with herbicides registered for corn crop in Brazil.

Reproductive variables	Atrazine	Nicosulfuron	Paraquat	Tembotrione	Water
Duration of life cycle, d	-	21.00 ± 2.82a	-	21.30 ± 2.13a	23.00 ± 1.81a
Parasitism, %1	30.0b	50.0ab	40.0ab	80.0a	90.0a
Emergence, %1	-	20.0b	-	80.0a	90.0a
Number of individuals	-	5.70 ± 3.70b	-	56.60 ± 17.5a	80.90 ± 23.0a
Females produced per female	-	0.80 ± 0.05b	-	8.30 ± 0.30a	11.90 ± 0.40a
Head capsule of females, mm	-	0.43 ± 0.03a	-	0.45 ± 0.03a	0.59 ± 0.03a
Head capsule of males, mm	-	0.37 ± 0.03a	-	0.40 ± 0.02a	0.40 ± 0.02a
Length of females, mm	-	1.95 ± 0.04b	-	2.10 ± 0.10a	2.07 ± 0.11a
Length of males, mm	-	1.78 ± 0.04a	-	1.58 ± 0.08b	1.60 ± 0.10b
Longevity of females, d	-	29.00 ± 3.27a	-	18.00 ± 4.20a	28.00 ± 3.01a
Longevity of males, d	-	27.10 ± 3.98a	-	22.90 ± 4.00a	26.00 ± 3.46a
Sex ratio	-	0.20 ± 0.03b	-	0.80 ± 0.05a	0.90 ± 0.02a

Means followed by the same letter per row do not differ according to Tukey's test ($P = 0.05$).
[1]Means followed by same letter per row do not differ according to Kruskal-Wallis test ($P = 0.05$).

1912 and *Melittobia digitata* Dahms, 1984 (Hymenoptera: Eulophidae) showed a sex ratio of 0.97, 0.95, and 0.95, respectively (Silva-Torres and Matthews, 2003; González et al., 2004), suggesting that this rate is characteristic of this family.

Width of head capsule and body length

The width of the head capsule of *P. elaeisis* males and females was similar, but their body length varied between treatments, with higher values for females treated with tembotrione and water (2.096 and 2.071 mm, respectively), and for males with nicosulfuron (1.778 mm, $F = 27.420$, $P = 0.0000$) (Table 2). The body length of females (1.948 mm) was lower with nicosulfuron ($F = 43.986$, $P = 0.0000$) (Table 2).

The shorter length of the body of *P. elaeisis* females with nicosulfuron may be due to toxicity of this herbicide, as evidenced by fewer parasitoids emerged.

Longevity of both sexes

The longevity of *P. elaeisis* females and males was similar with the herbicides tembotrione (18.0 ± 4.20 and 22.9 ± 4.00 d), nicosulfuron (29.0 ± 3.27 and 27.1 ± 3.98 d) and water (28.0 ± 3.01 and 26.0 ± 3.46 d, respectively; $F = 3.507$, $P = 0.0543$) (Table 2).

The longevity of *P. elaeisis* with nicosulfuron, tembotrione, and water shows that the herbicides did not affect the first generation, with similar longevity numbers for males and females of this parasitoid in other study, with 22.65 ± 1.13 and 28.3 ± 2.38 d, respectively (Zanuncio et al., 2008).

CONCLUSIONS

The herbicides atrazine, paraquat, and nicosulfuron were toxic and reduced parasitism and emergence of *P. elaeisis*. Nicosulfuron reduced the sex ratio of *P. elaeisis* and therefore it should be used with caution or substituted with more selective products. The herbicide tembotrione was selective to *P. elaeisis* and may be suitable for programs of sustainable management of weeds in corn with this parasitoid. More studies are needed, especially in field and using natural hosts to confirm the results obtained in the laboratory.

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