



Reproductive and toxicological impacts of herbicides used in *Eucalyptus* culture in Brazil on the parasitoid *Palmistichus elaeisis* (Hymenoptera: Eulophidae)

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Summary

The expansion of eucalyptus tree plantations in Brazil has raised concerns that the use of herbicides may reach non-target organisms and compromise the environment where parasitoids are used to control Lepidoptera defoliators. So, the effect of herbicides used in eucalyptus crops on the parasitoid *Palmistichus elaeisis* Delvare and LaSalle, 1993 (Hymenoptera: Eulophidae) was evaluated in terms of the impact on reproduction and survival. Treatments consisted of commercial doses of the herbicides sulfentrazone, oxyfluorfen, glyphosate and isoxaflutole with a water-only control. The herbicides were sprayed on the pupae of the alternative host

Tenebrio molitor Linnaeus (Coleoptera: Tenebrionidae), which were exposed to parasitism by six females of *P. elaeisis* per pupa. Glufosinate and oxyfluorfen reduced parasitism and emergence of this parasitoid and were considered more harmful to the *P. elaeisis* females. Glyphosate and isoxaflutole resulted in higher numbers of individuals and females produced per female; thus these herbicides were less harmful to *P. elaeisis* and maybe used in IPM programmes in eucalyptus plantations.

Keywords: biological control, chemical control, IPM, non-target organisms, reproductive side effects, survival, parasitoids.

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Introduction

Eucalyptus L'Her. spp. are planted in Brazil to obtain essential oils, wood, cellulose and for other industrial uses (Moreira *et al.*, 2009; Zanuncio *et al.*, 2010). Weeds can reduce the quantity and quality of wood (Tuffi Santos *et al.*, 2005), so herbicides are frequently used because of their high efficiency, low cost and facility of application. However, the risks of contamination and

impact on non-target organisms by these products have been reported in aquatic ecosystems and for soil microorganisms and insects (Santos *et al.*, 2004; Reis *et al.*, 2008; Botelho *et al.*, 2009; Soares *et al.*, 2009a). Defoliating insect pests are important phytosanitary problems in eucalyptus plantations, because of competition, outbreaks and damage (Zanuncio *et al.*, 2009).

Palmistichus elaeisis (Delvare & LaSalle, 1993) (Hymenoptera: Eulophidae) is a generalist and gre-

gamous endoparasitoid of Lepidoptera and Coleoptera pupae. This parasitoid has been reported to parasitise pests such as *Eupseudosoma involuta* (Lepidoptera: Arctiidae), *Euselasia eucerus* (Lepidoptera: Riodinidae), *Sabulodes* sp. (Lepidoptera: Geometridae), *Thyrinteina arnobia*, *Thyrinteina leucoceraea* (Lepidoptera: Geometridae), *Hylesia* sp. (Lepidoptera: Saturniidae) found in eucalyptus plantations. This natural enemy can be reared in the laboratory on alternative prey insects, such as *Zophobas confusa* (Coleoptera: Tenebrionidae) and *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) with similar results for its biology as with the natural host (Delvare & LaSalle, 1993; Bittencourt & Berti Filho, 1999; Pereira *et al.*, 2008; Zanuncio *et al.*, 2008; Soares *et al.*, 2009b). Herbicides applied to the crop reach the adults of *P. elaeisis* by direct or indirect contact, either during spraying or through absorption post-application. In addition, active or inert ingredients of commercial formulations of herbicides may demonstrate differential effects on the beneficial organisms (Giolo *et al.*, 2005).

Eucalyptus plantations host endemic populations of natural enemies, which contribute to biological control (Steinbauer *et al.*, 2006). Therefore, sustainable practices should be studied in integrated pest management, including the evaluation of the impact of herbicides on non-target organisms. The objective of this research was to evaluate the biological and toxicological impact of herbicides used in eucalyptus plantations in Brazil on the reproduction, development and survival of the parasitoid *P. elaeisis*.

Materials and methods

This study was conducted in a constant environment room [25 ± 2°C, 70 ± 10% relative humidity and 12 h of photoperiod – 500 lux (2000 lumen at 4 m²)] in the Laboratory of Entomology of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) in Diamantina, Minas Gerais, Brazil.

One thousand larvae of *T. molitor* were placed in a polystyrene tray with wheat bran and sugarcane pieces to obtain pupae of the alternative host. *Palmistichus elaeisis* was reared in 14 × 2.2 cm glass test tubes, along with a drop of honey and capped with a cotton ball (Zanuncio *et al.*, 2008). Pupae of *T. molitor* at 24–72 h of age were each exposed to six parasitoid females for 48 h in a constant environment room. The emerging parasitoids were collected and used in the experiment.

The herbicides used were: sulfentrazone (Solara[®]) for product label recommended, oxyfluorfen (Goal[®]), glyphosate (Scout[®]), glufosinate-ammonium salt (Finale[®])

and isoxaflutole (Fordor[®]), which are registered for eucalyptus plantations in Brazil. A control containing only deionized water was also included. Sixty *T. molitor* pupae at 48 h old, with an average weight of 102.33 g and a mean surface area of 7.85 × 10⁻⁵ m² were sprayed with an electronic pressure sprayer to simulate a field application (spray nozzle Teejet Albuz API 110, spray pressure of 300 kPa and zero wind speed), spraying the equivalent of 200 L ha⁻¹ of herbicide solution. The herbicides were applied at the recommended field rate per hectare, assuming that each pupa received a dose equivalent to 50% of its body surface as calculated by its mean surface area (Table 1).

Each herbicide treatment consisted of ten replicates, with one pupa of *T. molitor* and six 72-h-old females of *P. elaeisis*. The test was only conducted once with a high number of replicates, because several preliminary tests conducted to determine dose showed minimal variation among tests, low standard errors from the replicates and the controlled environmental conditions contributed to tests with high repeatability. These females were sexed according to the morphological characteristics of their antenna and abdomen (Delvare & LaSalle, 1993). The herbicide-treated pupae subsequently exposed to parasitism were removed from the tubes after 48 h and placed in 250-mL plastic bottles until emergence of *P. elaeisis* adults. The experiment was completed after 30 days of parasitism and the pupae without emergence of the parasitoid were discarded.

The longevity of *P. elaeisis* adult females exposed to *T. molitor* pupae sprayed with herbicides was evaluated daily. The duration of the life cycle (egg to adult), the percentage of parasitism (discounting the natural mortality of the host) (Abbott, 1925), the percentage of emergent progeny, number of individuals emerged, number of males and females, sex ratio, width of the head capsule and body length of parasitoids emerging from each pupa of *T. molitor* were obtained from parasitised pupae. The width of the head capsule was obtained using a stereoscopic microscope with an integrated digital camera and micrometre measurement software (DCM-Series: Nova). The sex ratio was calculated as the proportion of females out of the total number of individuals.

The data were submitted to tests of normality, homogeneity of variances and analysis of variance (ANOVA). Significant means were compared by Tukey's test at *P* = 0.05. Non-parametric data were analysed using the Kruskal–Wallis test at *P* = 0.05 (Sistema para Análises Estatísticas (SAEG), Universidade Federal de Viçosa, <http://www.ufv.br/saeg/Saeg.htm>).

Table 1 Technical characteristics of the herbicides registered for the cultivation of eucalyptus in Brazil

Commercial formulations* (manufacturer ®)	Active ingredient (a.i.) (concentration)	Dose per hectare a.i. (g L ⁻¹ or g kg ⁻¹)	Dose per pupa a.i. (µL or µg)	Chemical Group	Toxicology and† formulation‡
Solara 500 (FMC)	Sulfentrazone (500 g L ⁻¹)	500	3.90	Triazolone	CT = IV CA = III SC
Goal BR (Dow AgroSciences)	Oxyfluorfen (240 g L ⁻¹)	960	7.48	Diphenyl ether	CT = III CA = II CE
Scout§ (Monsanto)	Glyphosate (720 g kg ⁻¹)	720	5.61	Glycine replaced	CT = IV CA = II WG
Finale (Bayer S.A)	Glufosinate-ammonium salt (200 g L ⁻¹)	800	6.24	Homoalanina replaced	CT = I CA = II SL
Fordor 750 WG (Bayer S.A)	Isoxaflutole (750 g kg ⁻¹)	150	1.17	Isoxazol	CT = I CA = II WG

*Registered trade mark ®;

†CT, Toxicological class (I: extremely toxic; II: highly toxic; III: medium toxicity; IV: low toxicity); CA, Ambiental (I: extremely dangerous; II: highly dangerous; III: danger; IV: low danger);

‡SL, soluble concentrate; CE, emulsifiable concentrate; WG, dispersible granules; SC, concentrated solution;

§Acid equivalent.

Results

The herbicide glufosinate-ammonium salt reduced the % survival of *P. elaeisis* females after 24–48 h exposure to *T. molitor* treated pupae (Table 2). None of the other herbicides reduced survival of the parasitoid over time. After 72 h, glufosinate-ammonium salt was the most toxic herbicide relative to the water control and the other herbicides. Oxyfluorfen, glyphosate and isoxaflutole were the least toxic to parasitoid survival.

The effect of herbicide treatments on the reproduction of the parasitoids is shown in Table 3. Herbicide treatments did not affect the duration of the parasitoid life cycle relative to the water control. However, oxyfluorfen and glufosinate-ammonium salt reduced the percentage parasitism and percentage emergence by 70–80% relative to the water control. The number of individuals per pupa were significantly lower when

sulfentrazone, oxyfluorfen and glufosinate-ammonium salt were applied (39, 20 and 14 individuals, respectively), as compared with the number with glyphosate, isoxaflutole and water (60, 91 and 95, respectively). With the exception of isoxaflutole, there were fewer females parasitoids produced after being exposed to the herbicides. There were minimal differences among the treatments in the length of the head capsule in females, but in males the head capsule length was wider after exposure to sulfentrazone, oxyfluorfen and glufosinate-ammonium salt. Isoxaflutole reduced the body length in females and males; sulfentrazone, oxyfluorfen, glufosinate-ammonium salt significantly increased body length in males, but only marginally in females relative to the water control. Exposure to any of the herbicides increased female and male longevity relative to the water control. However, oxyfluorfen altered the sex ratio, such that there were fewer females after exposure.

Table 2 Survival (%) of *Palmistichus elaeisis* adults from 0 to 96 h after exposure for 48 h to pupae treated with the herbicides registered for eucalyptus culture in Brazil

Treatments	0	24	48	72	96
Sulfentrazone	100.0Aa	94.9Aa	94.9Aa	94.9Aab	94.9Aab
Oxyfluorfen	100.0Aa	100.0Aa	100.0Aa	100.0Aa	98.3Aa
Glyphosate	100.0Aa	100.0Aa	98.0Aa	96.6Aa	96.6Aa
Glufosinate-ammonium salt	100.0Aa	93.0ABa	89.0Ba	85.0Bc	85.0Bc
Isoxaflutole	100.0Aa	96.7Aa	96.7Aa	96.7Aa	96.7Aa
Water	100.0Aa	100.0Aa	96.0Aa	95.0Aab	95.0Aab

Means followed by the same upper case, per line, or lower case letters, per column, do not differ by Tukey's test at $P = 0.05$.

Table 3 Reproductive parameters of the first generation of *Palmistichus elaeisis* (Hymenoptera: Eulophidae) from pupae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) treated with herbicides registered for the cultivation of eucalyptus and the water control

Reproductive variables	Sulfentrazone	Oxyfluorfen	Glyphosate	Glufosinate-ammonium salt	Isoxaflutole	Water
Duration of the life cycle (days)	28.5 ± 1.70A	27 ± 0.70A	27.5 ± 0.88A	27.6 ± 1.15A	27.3 ± 0.85A	27.0 ± 0.80A
Parasitism (%)*	80.0A	30.0B	90.0A	30.0B	100.0A	100.0A
Emergence (%)*	80.0A	20.0B	90.0A	30.0B	100.0A	100.0A
Number of individuals per pupa	38.6 ± 12.9B	19.5 ± 6.4B	60.3 ± 24.7AB	13.8 ± 1.56B	91.1 ± 35.1A	95.3 ± 38.1A
Females produced by female	5.3 ± 0.40B	2.3 ± 0.60B	8.2 ± 0.40AB	2.0 ± 0.50B	12.1 ± 0.60A	12.9 ± 0.55A
Female head capsule (mm)	0.63 ± 0.02A	0.60 ± 0.06AB	0.62 ± 0.02A	0.60 ± 0.01AB	0.59 ± 0.01AB	0.57 ± 0.01B
Male head capsule (mm)	0.45 ± 0.02B	0.44 ± 0.02B	0.40 ± 0.01C	0.52 ± 0.01A	0.40 ± 0.03C	0.39 ± 0.02C
Female length (mm)	2.14 ± 0.14A	2.10 ± 0.16AB	2.17 ± 0.15A	2.10 ± 0.07AB	1.92 ± 0.06C	1.96 ± 0.07B
Male length (mm)	1.62 ± 0.06A	1.58 ± 0.04AB	1.50 ± 0.10BC	1.63 ± 0.09A	1.39 ± 0.07D	1.43 ± 0.04C
Female longevity (days)	28.1 ± 2.42AB	31.8 ± 2.50AB	28.0 ± 3.64AB	32.6 ± 2.39A	30.1 ± 3.78AB	24.2 ± 2.49B
Male longevity (days)	32.1 ± 4.03A	34.4 ± 2.34A	28.8 ± 4.38AB	28.6 ± 4.69AB	27.8 ± 3.50AB	19.4 ± 3.21B
Sex ratio	0.81 ± 0.11A	0.70 ± 0.05B	0.83 ± 0.07A	0.87 ± 0.02A	0.81 ± 0.09A	0.80 ± 0.08A

Means followed by same letter per line, do not differ by Tukey's test at $P = 0.05$.

*Means followed by the same letter per line, do not differ by Kruskal–Wallis test at $P = 0.05$.

Discussion

The lower toxicity of oxyfluorfen on *P. elaeisis* agrees with a previous report where this herbicide was found to be only moderately toxic to *Trichogramma atopovirilia* Oatman and Platner, 1983 (Hymenoptera: Trichogrammatidae) and *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae), with a reduction in parasitism of 95.8% and 95.6%, respectively (Manzoni *et al.*, 2006; Giolo *et al.*, 2007). On the other hand, the higher reduction in parasitism of *P. elaeisis* indicated that the susceptibility of parasitoids to this product differs because of the species of the natural enemy. The herbicide oxyfluorfen probably caused the herbicide-treated alternative host to reject parasitism by *P. elaeisis* females and, therefore, these did not reproduce and spent less metabolic energy. This favoured longevity compared with the other treatments, with 80–100% parasitism, except for glufosinate-ammonium salt, which resulted in lower female longevity. Oxyfluorfen resulted in a negative risk of ecological impact on the fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae), an important biological control agent of insects (Andalo *et al.*, 2004).

The reproductive responses of *P. elaeisis* to isoxaflutole were similar to the water control, demonstrating the safety of this herbicide to the parasitoids; similar observations were noted for adults of the parasitoid

T. pretiosum and its impact on parasitism of their females (Stefanello Júnior *et al.*, 2008). The herbicide glyphosate showed intermediate harm to *P. elaeisis*, with survival and reproductive values between those of isoxaflutole (least harmful) and oxyfluorfen and glufosinate-ammonium salt (most harmful). Glyphosate was also less harmful for the natural enemies *Euseius victoriensis* (Acari: Phytoseiidae), *Microctonus hyperodae* (Hymenoptera: Braconidae) and the egg parasitoid *Telenomus remus* (Hymenoptera: Scelionidae), but some commercial formulations of this product were slightly harmful to pupa of *T. remus* and *T. pretiosum*, suggesting that the differentiation between the type of salt present and inert ingredients is a factor in toxicity to parasitoids (Addison & Barker, 2006; Noernberg *et al.*, 2008; Carmo *et al.*, 2009; Bernard *et al.*, 2010). Even with the application of herbicides (sulfentrazone, glyphosate and isoxaflutole), there was efficient parasitism of *P. elaeisis* in *T. molitor* pupae, as previously reported for this parasitoid (Zanuncio *et al.*, 2008). However, the lowered sex ratio from exposure to oxyfluorfen affected the population dynamics of *P. elaeisis*. The other sex ratio values were similar between treatments and close to that of *P. elaeisis* with pupae of *T. molitor* (Zanuncio *et al.*, 2008) and other Eulophidae parasitoids, such as *Melittobia clavicornis* Cameron (González *et al.*, 2004), suggesting that this rate is characteristic of this family.

The smallest head capsule width and body length in *P. elaeisis* in treatments with glyphosate, isoxaflutole and the water control can be explained by the greater number of individuals of this parasitoid per pupa, which increases intraspecific competition and reduces the supply of food and increases the production of smaller individuals (Pereira *et al.*, 2009). On the other hand, the greater body length and head capsule width of *P. elaeisis* in treatments of sulfentrazone, oxyfluorfen and glufosinate-ammonium salt were due to the smaller number of individuals per parasitised pupae and less competition between their larvae, which improved the acquisition of resources by individuals of *P. elaeisis*. The smaller number of parasitoids within the host may be due to the repellent effect of the herbicides, which led to the death of females during oviposition or mortality of eggs or larvae of *P. elaeisis* within these pupae. However, the body length and head capsule width (mm) in all treatments were close to that of the progeny of *P. elaeisis* in pupae of *T. molitor*, 2.00 ± 0.03 , 0.58 ± 0.01 and 1.34 ± 0.02 , 0.45 ± 0.01 for females and males, respectively (Zanuncio *et al.*, 2008). The longevity of *P. elaeisis* was similar to this parasitoid in the pupae of *T. molitor*, 22.65 and 28.3 days for females and males, respectively, showing that the herbicides did not affect this parasitoid (Zanuncio *et al.*, 2008).

Conclusions

The herbicides, glufosinate-ammonium salt and oxyfluorfen, reduced the parasitism and emergence of *P. elaeisis*, as they were more toxic to this parasitoid. Oxyfluorfen reduced the sex ratio of *P. elaeisis*, which can affect their population dynamics in consecutive generations and, therefore, must be used with caution or substituted by more selective herbicides. Sulfentrazone, glyphosate and isoxaflutole offered little risk to *P. elaeisis* and could be used in IPM programs with this parasitoid.

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