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Bioefficacy of *Euphorbia peplus* latex as an antifeedant and insecticide against *Gonipterus platensis* larvae on *Eucalyptus globulus*

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Abstract

Background: *Gonipterus platensis* (Marelli) is part of the *Gonipterus scutellatus* species complex which consists of three species that have spread beyond their natural ranges. Due to its high reproductive potential and a capacity for intense defoliation by both larvae and adults, *G. platensis* causes tree growth loss and stem deformities. The antifeeding effect and insecticide efficacy of latex from petty spurge, *Euphorbia peplus* L. (Euphorbiaceae), on larvae of *G. platensis*, were evaluated through bioassays, with a view to its integrated management.

Methods: *Eucalyptus globulus* leaves treated by immersion in *Euphorbia peplus* latex solutions were infested with five third-instar larvae, and the area consumed was determined at 24 h. The antifeeding effect of five latex solutions applied with a brush on *Eucalyptus globulus* leaves, using a random experimental design of six treatments and five replications, was also evaluated. Larval mortality was recorded daily and analysed by an ANOVA and Tukey's test. The LC_{50} (lethal concentration to kill 50% of the individuals) was calculated by Probit analysis and Chi^2 tests were performed.

Results: The ethanolic solutions of the latex caused strong antifeeding effect, with total inhibition of larval feeding at all solutions. Larval mortality increased significantly over time up to 76%, due to the effect of the ethanol latex solutions, in all solutions by day 6. The lower LC_{50} values were 0.049 and 0.012% w/v on days 5 and 6, respectively.
Conclusions: These results indicate that *Euphorbia peplus* latex is a promising bioinsecticide and a possible alternative for integrated pest management. However, further tests should be carried out.

Keywords: Botanical insecticide; *Eucalyptus* snout beetle; *Eucalyptus globulus*; *Gonipterus scutellatus*; petty spurge; pichoga.

Introduction

The *Eucalyptus* snout beetle, *Gonipterus platensis* (Marelli) (Coleoptera: Curculionidae), is part of the *Gonipterus scutellatus* Gyllenhal species complex which consists of three species that have spread beyond their natural ranges. As a group, the species complex is a global pest of *Eucalyptus,* and *G. platensis* has the largest distribution outside of the three species in the complex (Mapondera et al. 2012; Schröder et al. 2020). Due to its high reproductive potential and a capacity for intense defoliation by both larvae and adults, *G. platensis* causes tree growth loss and stem deformities (Dos Santos Bobadilha et al. 2019), which are strongly related to climatic conditions (Adame et al. 2022). In Chile, *G. platensis* was detected in 1998 and since then it has affected eucalypt plantations economically (Servicio Agrícola & Ganadero 2010). In Chile, eucalypts are highly valued for their rapid growth and the quality of the wood for pulp (Rua et al. 2020), and eucalypt plantations currently cover ~860 thousand ha (Instituto Forestal 2020).

In South Africa, control trials with pyrethroids were carried out, which were quickly abandoned due to the high cost of the treatments and because of the successful introduction of a biological control agent (Romanyk and Cadahía 2002). In Chile, two biological control agents have been introduced, the egg endoparasitoids *Anaphes nitens* Huber and *A. tasmaniae* Huber & Prinsloo (Hymenoptera: Mymaridae), which are native to Australia. These species have been released in several areas, and although they have reduced the *G. platensis* populations, the problem persists probably due to the diversity of habitats and climate (Corporación Nacional Forestal 2012, 2017). In Portugal, *A. nitens* was found to be ineffective at altitudes above 400 m (Ceia et al. 2021). In Brazil, entomopathogenic fungi (*Beauveria* spp. and *Metarhizium anisoplliae*) have been evaluated against *G. platensis* adults, identifying fungal strains with superior lethality than existing commercialized strains (Jordan et al. 2021). Pest management could be improved further by controlling other life stages (larvae, pupae, and adults), using other techniques such as increasing the diversity of biological control agents, exploring environmentally friendly biopesticides, selecting and/or developing *Eucalyptus* genotypes with tolerance to infestation, and silvicultural control (Schöroder et al. 2020).

Despite growing evidence of environmental damage and human health concerns, the global use of synthetic insecticides has continued to grow substantially over the past 50 years (Isman 2020). Thus, it is necessary to find alternatives replacing synthetic pesticides to control of pest insects. To reduce their negative effects, new natural botanical insecticides have been developed, based on extracts of leaves, fruits, or other plant structures, with diverse results. These compounds are biodegradable, reduce pest resistance appearance, and have a lesser impact on flora and fauna, among other properties (Amri et al. 2013).

The latex from plants in the family Euphorbiaceae, especially those in the genus *Euphorbia*, has toxic, irritant, and medicinal effects (Bittner et al. 2001; Docampoa et al. 2010), but it also has insecticide properties attributed mainly to triterpenes, flavonoids, alkaloids, coumarins, cyanogenetic glycosides and tannins and others secondary metabolites (Mendivelso et al. 2003; Ogbourne et al. 2004).

Petty spurge, *Euphorbia peplus* L., is a 15 to 40 cm tall herbaceous toxic plant, typical of gardens, sidewalks, orchards, and ruderal sites in Europe, temperate Asia, North Africa, North and South America and Oceania, in places with a temperate climate, shady, humid, and fertile soils (Mendivelso et al. 2003). This species has been tested for antimicrobial, analgesic, antipyretic, anti-inflammatory (Ali et al. 2013), antifeeding (Hua et al. 2017) and insecticide activities (Ghramh et al. 2019).

This work evaluated the antifeeding effect and insecticide efficacy of *Euphorbia peplus* latex on *G. platensis* larvae, through laboratory bioassays, with a view to integrated pest management (IPM). *Gonipterus platensis* intensely defoliates *eucalypt* plantations worldwide, without adequate management in most regions. Therefore, an antifeedant and insecticide based on *Euphorbia peplus* latex could be valuable as an alternative control method with little environmental impact.

Methods

Sampling and latex preparation

The chemical composition of *Euphorbia peplus* latex was determined by Hua et al. (2017), with 13 terpenoid compounds, including 12 diterpenoids and an acyclic triterpene alcohol. For the present study, latex was extracted from fresh stems (approx. 2.5 kg) of *Euphorbia peplus* plants collected during spring at the Antumapu Campus of the University of Chile in Santiago (33° 34'S, 70° 37'W). Approximately 5 mL of latex (density 0.958 g/mL) were extracted manually from *Euphorbia peplus* plants collected at random, to avoid effects of individual plants. Fresh stems were cut, and the latex was collected by gravity in the Chemistry Laboratory, Department of Agroindustry and Enology, Faculty of Agronomic Sciences, University of Chile, in Santiago at room temperature (19±2°C) and held in a refrigerator at -4°C until use in bioassays.

Sampling and rearing of insects

Young larvae of *G. platensis* were collected in the summer from *Eucalyptus globulus* Labill trees in San Bernardo, Metropolitan Region (33° 58'S, 70° 70'W), and were taken in cloth bags to the Forestry Entomology Laboratory, Faculty of Forestry and Nature Conservation Sciences, University of Chile, Santiago. These larvae were placed in Petri dishes (10 cm diameter) lined with Whatman No. 1 filter paper humidified with distilled water. Larvae were kept in a bioclimatic incubator (model JSPC-420C, JSR Research Inc., Chungehungnan-Do, Korea) at 20±3°C, 60±6% RH, and a photoperiod of 14:10 (day:night) until they reached the third instar. These conditions were maintained in the following bioassays.

Evaluation of the antifeeding effect

The antifeeding effect of the latex ethanolic solutions was evaluated following the method described by Defagó et al. (2006). Petri dishes (10 cm diameter) containing five third instar larvae and two *Eucalyptus globulus* leaves of the same size, one an untreated control (only ethanol at 96% v/v) and the other one treated by immersion (1 min and after air drying) in the solutions of latex (10, 30, 50, 70, and 100% w/v), with five replicates, and kept in the climatic chamber. After 24 h, the percentage of leaf area eaten (either treated or control ones) by larvae was determined by the ImageJ programme (Schneider et al. 2012). The percentage of antifeeding effect was calculated as (1 - [T / C]) x 100, where T and C were the consumed levels of the treated and control leaves, respectively. Foliar area consumed was analysed through the Wilcoxon test ($p < 0.05$), and the antifeeding effect was analysed using ANOVA followed by Tukey tests between solutions ($p < 0.05$).

Evaluation of the insecticide efficacy

The insecticide efficacy bioassay on *G. platensis* was conducted using third instar larvae placed on fresh eucalypt leaves. Five third instar larvae were placed on fresh eucalypt leaves in 10 cm diameter Petri dishes lined with slightly moistened filter paper at the bottom and fresh washed eucalypt leaves, with the petiole wrapped in wet cotton. Six treatments (five latex solutions [10, 30, 50, 70, and 100% w/v] plus a control) were compared; the control was treated only with ethanol at 96% v/v, using a simple random design with five replicates. The treatments were applied with a hairbrush on both sides of washed eucalypt leaves. Dead and live larvae were counted daily in six times during the test. Daily and total mortality (% ± standard error) were determined. Mortality results were normalized using Bliss degrees to stabilize the variance error. After checking the assumptions of normality and homoscedasticity, the data were subjected to ANOVA (6 x 6). When significant differences occurred between treatments or days, Tukey tests were run ($p \leq 0.05$). For the statistical analysis, the first factor was set to be the treatment and the second the days after the application. Data were adjusted mathematically to obtain the best-fitting function to obtain the LC_{50} (lethal concentration to kill 50% of the individuals) using a Probit model described previously (Robertson et al. 1984). Data fit to the Probit model was confirmed with a Chi^2 test. All statistical analysis were done using InfoStat (2009) software.

Results

Antifeeding effect

The ethanol latex extracts had a strong antifeeding effect, with almost total inhibition of feeding by *G. platensis* in all treatments (Table 1).

TABLE 1: Mean antifeeding effect (%) of ethanolic solutions of *Euphorbia peplus* latex on feeding by *Gonipterus platensis* third instar larvae on *Eucalyptus globulus* leaves in a free choice test, at several latex solutions (% w/v) after 24 h.

Latex (% w/v)	Foliar area consumed		Antifeeding effect (%)[1]
	Untreated (control)	Treated	
10	2.48	0.03*	98.9 ± 1 a
30	2.55	0.01*	99.3 ± 1 a
50	2.38	0.00*	99.8 ± 1 a
70	2.91	0.02*	100.0 ± 0 a
100	1.73	0.00*	100.0 ± 0 a

[1] Means sharing a letter do not differ significantly according to Tukey's test (p <0.05%).
* Significant differences between the consumption of treated and untreated leaves in a Wilcoxon's test (p <0.05%).

Insecticide efficacy

During the test, mortality of *G. platensis* larvae increased due to the effect of the ethanol *Euphorbia peplus* latex solutions. Statistical analyses indicated significant differences between the cumulative mean mortality (%) of *G. platensis* larvae resulting from the treatments and the control [$F_{5,25}$ (0.05) = 16.7] and among the number of days after the initial exposure [$F_{5,25}$ (0.05) = 12.4]). On day 6, with the lowest solution (10% w/v) the minimum mortality was 76%. The greatest mortality occurred with the two highest solutions at 96% and 100%, which indicates the substantial insecticide potential of *Euphorbia peplus* latex on these larvae (Figure 1).

LC_{50}

The results from the Probit analysis indicate an LC_{50} decreasing over time. The lowest and most promising LC_{50s} were 0.012 and 0.049% w/v on day 6 and 5, respectively (Table 2). Thus, the latex from *Euphorbia peplus* can be considered an effective insecticide for *G. platensis* since more than 50% mortality was obtained after 4 days exposure even to a low-concentration solution of the extract (<0.15% w/v).

Discussion

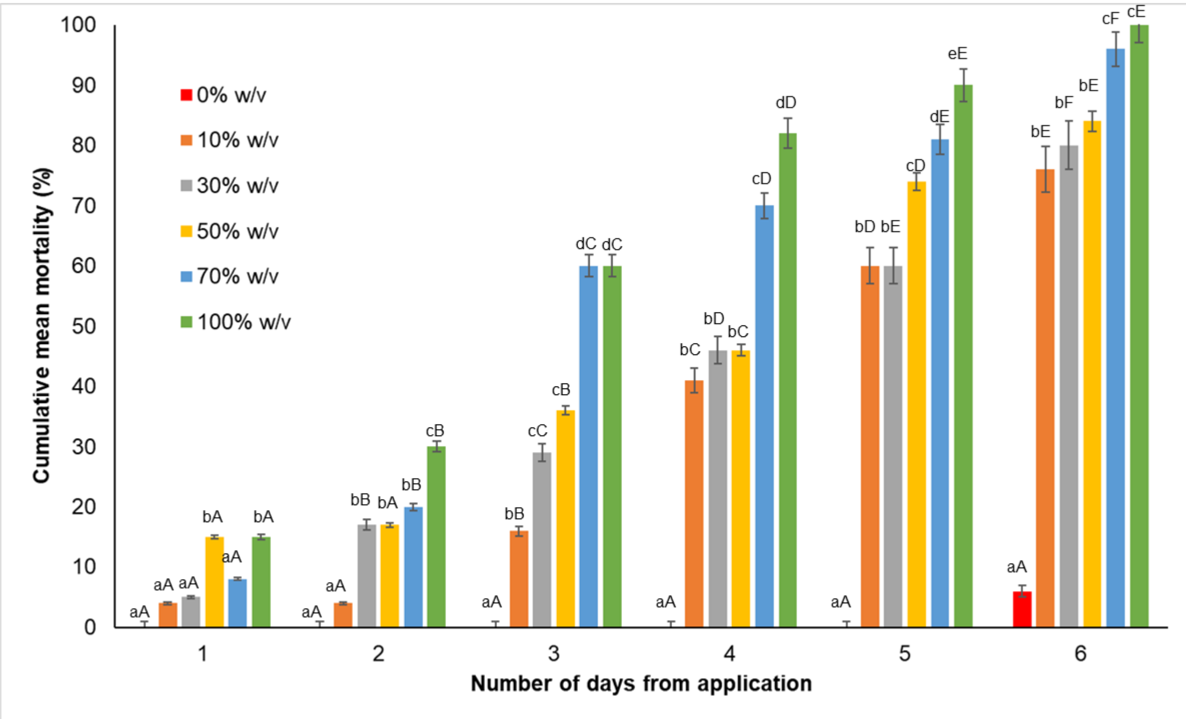
Antifeeding effects

While there are several trials with botanical insecticides in the *Euphobia* genus, there are very few with *Euphorbia peplus* or extracts tested on larvae *G. platensis*. Antifeeding results were almost 100% effective at all the solutions evaluated, thus the latex of *Euphorbia peplus* can protect against feeding by larvae of *G. platensis* on leaves of *Euphorbia globulus*. Similarly, the latex of *Euphorbia peplus* showed potent antifeedant activity against the larvae of cotton bollworm (*Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) (Hua et al. 2017). A somewhat slower feeding inhibition was observed in larvae of *Pieris brassicae* and *Spodoptera littoralis* on day 2, with 100% inhibition in *S. littoralis* and a 30% solution of *Euphorbia peplus* latex (Chaieb et al. 2001). Our results show a more potent activity of *Euphorbia peplus* latex on *G. platensis* larvae since an almost 100% antifeeding effect was obtained at 10% w/v after only 24 h. However, a 100% antifeedant effect on third-instar larvae of *G. platensis* was obtained with even lower concentrations of 2.4% w/v aqueous extracts of *Cestrum parqui* L'Heritier (Solanaceae) leaves, a South American shrub (Huerta et al. 2021).

Larval mortality

The ethanolic solutions of the latex obtained from *Euphorbia peplus* caused >76% larval mortality on *G. platensis* in all treatments (starting at 10% w/v) six days after exposure. A consistent dose-dependent insecticide effect of the *Euphorbia peplus* latex was observed with >90% larval mortality at >70% w/v. By comparison, the insecticide effect of extracts from *Azadirachta indica* A.Juss. (Meliaceae) neem oil on *G. platensis* larvae, reached only a maximum of 40% mortality at 3% w/v concentration on day 8 after exposure (Pérez Otero

FIGURE 1: Cumulative mean mortality (% ± standard error) of *G. platensis* third instar larvae resulting from ethanolic solutions of latex from *Euphorbia peplus* at one to six days from initial exposure.



[1] Means with different small letters, and capital letters, indicate significant differences between solutions and days after initial exposure, respectively, according to Tukey tests (p≤0.05).

et al. 2003). In another study, ethanol extracts from new and mature leaves of the Peruvian pepper tree *Schinus molle* L. (Anacardiaceae) were evaluated at 3.4% and 4.8% w/v, respectively, leading to 100% and 94.7% cumulative mortality 10 days after application (Chiffelle et al. 2017). The insecticide effect of leaf, seed, and root extracts of *Antelaea azadiracha* L. (Meliaceae) was also evaluated on larvae of the eucalypt weevil, providing 72.5% mortality on day 7 (Santolamazza & Fernández 2004), a considerably lower effect than in our tests with *Euphorbia peplus* latex. Finally, 2.4% w/v aqueous extracts from *Cestrum parqui* leaves provided 52% mortality of third-instar larvae of *G. platensis* on day 6, being more effective than low concentrations of *Euphorbia peplus* latex tested here (Huerta et al. 2021).

The lower LC_{50} was 0.012 % w/v for the ethanolic latex extract on day 6 in our study. In comparison, the LC_{50} of ethanol extracts from new and mature leaves of *S. molle* against larvae of *G. platensis* on day 6 was 0.79 and 0.63% w/v, respectively (Chiffelle et al. 2017), therefore less effective than those achieved in our current study. *Euphorbia peplus* ethanol leaf extract was also lethal against fourth instar larvae of *Culex pipiens* Linnaeus (Diptera, Culicidae), with an LC_{50} of 0.14 % w/v after 24 h exposure (Ghramh et al. 2019). Although aqueous extracts of leaves of *Cestrum parqui* were shown to be effective as an antifeedant and an insecticide against *G. platensis* larvae, the LC_{50} on day 6 was 1.84% w/v (Huerta et al. 2021), higher than that obtained in the present study. This means less plant material of *Euphorbia peplus* latex is required than of *Cestrum parqui* leaves to achieve a similar control effect.

TABLE 2: Mean lethal concentration (LC_{50}) and parameters of Probit regression for effects of ethanol solutions of *Euphorbia peplus* latex on *G. platensis* larvae at different evaluation times.

Time (Days)	Slope (mean ± error standard)	LC_{50} (% w/v) (95 % CI[1])	Chi-square[2]
4	14.27 ± 6	0.125 (0.061-0.135)	8.52
5	13.34 ± 4	0.049 (0.012-0.053)	2.54
6	10.43 ± 3	0.012 (0.009-0.015)	2.52

[1] CI: confidence interval.
[2] Goodness of fit for Probit model, critical Chi-square value 9.49 (df=4; p≤0.05).

Conclusions

The latex of *Euphorbia peplus* had an almost 100% antifeeding effect on *G. platensis* larvae. In addition, the ethanolic solutions of the latex caused >76% larval mortality in all treatments, with the least LC_{50} of 0.012% w/v on day 6. These results indicate that *Euphorbia peplus* is a possible source of botanical insecticide compounds that could be used in IPM of *G. platensis*. However, further studies on adults as well as field tests are required to determine the effectiveness of *Euphorbia peplus* latex application under field conditions.

Competing interests

The authors have no competing interests to declare.

Abbreviations

ANOVA: analysis of variance
CI: confidence interval
LC50: lethal concentration to kill 50% of the individuals
% w/v: Percentage weight/volume

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