

ANTIFEEDING AND INSECTICIDE EFFECTS OF LEAF EXTRACTS FROM *Cestrum parqui* (Solanaceae) ON LARVAE OF *Gonipterus platensis* (COLEOPTERA: CURCULIONIDAE)

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Abstract

The eucalypt weevil, *Gonipterus platensis* Martelli (Coleoptera: Curculionidae), is a main defoliator beetle of these trees worldwide. In Chile, it occurs widely in eucalypt plantations, and there are areas where parasitoids have not adapted. The antifeeding and insecticide effect of *Cestrum parqui* L'Hérit (Solanaceae) aqueous leaf extracts on third instar larvae was evaluated at various concentrations (0.0, 0.6, 1.2, 2.4, and 4.8% w/v), that were applied in laboratory bioassays to fresh eucalypt leaves, with five replicates. The antifeeding effect was estimated by the foliar area consumed at 24 h. Mortality was determined daily for 11 d. For each concentration, mortality relative to the control and LC₅₀ were determined. This extract had a high antifeeding effect at all concentrations, reaching 100% at 2.4 and 4.8% w/v. Mortality was directly related to concentration. The extract was effective as bioinsecticide, reaching 92% mortality at 4.8% w/v on day 11. The lowest LC₅₀ was 0.87% w/v on day 7. These results indicate that this extract would constitute an efficient alternative in integrated management programs of the pest in eucalypt plantations.

Key words: Antifeeding, bioinsecticide, eucalyptus snout beetle, eucalyptus weevil, *Cestrum parqui*

1. INTRODUCTION

The Australian eucalyptus weevil, *Gonipterus platensis* Martelli (Coleoptera: Curculionidae), is currently the main eucalyptus defoliator beetle worldwide (Mapondera et al. 2012). It is distributed in four continents and 25 countries (EFSA Panel on Plant Health (PLH) et al. 2018). In Chile it was detected in 1998 and since then it has affected economically eucalypt plantations from Coquimbo through Los Lagos (Servicio Agrícola y Ganadero 2010, 2017), that exploit ~860 thousand ha of this trees (Instituto Forestal 2020), that are susceptible to damage because of its capacity for intense defoliation by larvae and adults of this pest, which has a high reproductive potential and causes growth loss and stem deformity (Elliott & De Little 1984, Santolamazza & Cordero 1998).

The biology of this pest, which presents 2-4 cycles a year (Elgueta, 2010), has been studied extensively (e.g. Tooke 1953, Arzone & Meotto 1978, Mansilla 1992, Santolamazza & Cordero 1998, Estay et al. 2002, Ciesla 2011, among others).

In Chile, the egg parasitoids *Anaphes nitens* Huber and *A. tasmaniae* Huber & Prinsloo (Hymenoptera: Mymaridae) have been released, and although they have reduced the *G. platensis* populations (Corporación Nacional Forestal 2012, 2017), the problem persists given the diversity of habitats and weather. Pest management could improve by controlling other stages (larvae, pupae, and adults), and environmentally friendly botanic insecticides would be compatible with biological control.

The appearance of the high effectiveness and low cost synthetic insecticides in the 1940s, replaced those of plant origin (Silva et al. 2002). Despite of growing evidence of environmental damage and human health concerns, the global use of synthetic insecticides has continued to grow explosively over the past 50 years (Isman 2020). To reduce their negative effects, new natural chemicals, e.g., 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 natural enemies, among other properties (Amri et al. 2013).

The South American shrub, green cestrum or willow-leaved Jessamine, *Cestrum parqui* L'Heritier (Solanaceae), grows in Chile from Coquimbo through Osorno (Hoffmann 1982). Its defense saponins have anti-feeding, repellent or insecticide effects, and cause deformities on species of various insect orders, including Orthoptera (*Schistocerca gregaria* Forsskål, Acrididae) (Barbouche et al. 2001), Lepidoptera [*Spodoptera littoralis* (Boisduval), *Helicoverpa armigera* (Hübner), Noctuidae, and *Pieris brassicae* L., Pieridae] (Chaieb et al. 2001, 2007, 2009a), and also Diptera (*Ceratitis capitata* Wiedemann, Tephritidae) (Zapata et al. 2006), and *Culex pipiens* L., Culicidae (Chaieb et al. 2009b)]. Its effectiveness on Coleoptera has been proven only on four species [*Tribolium castaneum* (Herbst) (Rafael et al. 2000), and *T. confusum* Herbst), Tenebrionidae (Chaieb et al. 2006, 2009a), *Hylurgus ligniperda* F., Curculionidae (Huanquilef et al. 2020), and *Xanthogaleruca luteola* Muller, Chrysomelidae (Huerta et al. 2021)], and the report herein is the second one on a curculionid.

The objective of this study was to evaluate the anti-feeding effect and the insecticide efficacy of aqueous extracts of *C. parqui* leaves on *G. platensis* larvae through bioassays, as a contribution to the sustainable, effective, and efficient control of this pest.

2. MATERIAL AND METHODS

During the summer, 1 kg foliage from *C. parqui* (Fig. 1) was collected randomly at 1.5 m from the ground, from ten trees with a trunk at least 10 cm diam to ensure diversity, in Los Pelambres, Salamanca County, Province of Choapa (29°53'S; 71°15'W), Coquimbo Region, Chile. First instar larvae of *G. platensis* (Fig. 2) were collected on juvenile *Eucalyptus globulus* Labill trees in Peñuelas, San Pedro County, Province of Melipilla, Metropolitan Region (33°54'S, 71°29'W), Chile, and taken in cloth bags to the Laboratory of Entomology, Department of Forestry and Nature Conservation, Faculty of Forestry and Nature Conservation, Universidad de Chile, Santiago, Chile.



Fig. 1. Individuals of *Cestrum parqui* in nature conditions.



Fig. 2. Larvae of *Gonipterus platensis* feeding on eucalypt leaves.

The larvae were fed fresh, washed and air dried eucalypt leaves in plastic containers, under natural photoregime at $20\pm 2^{\circ}\text{C}$ and $35\pm 5\%$ HR in the laboratory, through the third instar.

The extracts used to evaluate the antifeedant and insecticide properties from *C. parqui* were prepared with leaves (stems were discharged) washed and dried 60 h in a forced air oven (Memmert, Schwabach, Germany) maintained at 37°C at the Chemistry Laboratory, Department of Agroindustry and Enology, Faculty of Agronomic Sciences, University of Chile, Santiago, Chile. The dried leaves were ground in a mechanical mill into 0.25 mm particles and stored in labeled glass vials. Later, the leaf powder was mixed with water until obtaining the highest concentration at 1/10. This mixed was set 18 h in a magnetic stirrer (Heidolph, MR 3001K, Schwabach, Germany) at 37°C the first hour, then filtered through a Whatman N° 1 filter paper, centrifuged (HN-S Centrifuge, International Equipment Co., Philadelphia, USA) 15 min at 1,500 rpm and re-filtered to obtain the base extract. In order to calculate maximum concentration (mc) of total solids, 15 mL of each extract were dried until constant weight, at 100°C in a forced air oven. Four concentrations were prepared by dilution (0.6, 1.2, 2.4, and 4.8% w/v) starting from the respective mc.

Fresh and washed eucalypt leaves were immersed 30 s in each extract, air dried at room temperature for 30 s. Two leaves (treated and control) were set with two third instar larvae for 24 h in an observation arena. The foliar area consumed was determined with the ImageJ program (Schneider et al. 2012). The antifeeding effect was estimated by $(1 - T/C) \times 100$, where T and C are the areas consumed in treated and control leaves, respectively. The foliar consumed area was analyzed through the Wilcoxon ranks test ($P < 0.05$) and the antifeeding effect through Anova ($P < 0.05$), followed by Tukey' test to compare concentrations ($P < 0.05$). The bioassay included a water control; all treatments had five replicates. The experimental unit was a Petri dish lined with slightly wet filter paper at the bottom and a fresh and washed eucalypt leaves, with the petiole wrapped in wet cotton. For mortality assessment, five *G. platensis* larvae were used/replicate. The leaves were immersed for 1 min in the respective treatment, let to dry at room temperature and set on the humidified filter paper lined Petri dishes along with the insects. Daily and total mortality were determined until mortality stabilized on day 11. The results were normalized by Bliss degrees. After the evaluation, data were submitted to an Anova factorial analysis (5×11), where factor A was the concentration and factor B the day from treatment.

Statistical differences between treatments were separated using the Tukey test ($p \leq 0.05$). The results were examined with mathematical functions to identify the best fit obtaining the LC_{50} (lethal

concentration to kill 50% of the individuals) through the Probit method (Robertson et al. 1984), using χ^2 to verify the model. All statistical analysis were done using the InfoStat (2009) software.

3. RESULTS

The aqueous extracts had a significant difference in leaf consumption by larvae at 2.4% w/v ($z = 2.05$, $p < 0.05$) and 4.8% ($z = 3.02$, $p < 0.05$) % w/v. At 24 h after exposure, a significant antifeeding effect was observed, reaching almost 100% at the two highest concentrations and almost 80% at 0.6% w/v (Table 1).

Table 1. Foliar area consumed and antifeeding effects of aqueous extracts from *Cestrum parqui* leaves on *Gonipterus platensis* third instar larvae, at various concentrations (% w/v) after 24 h.

Concentration (% w/v)	Foliar area consumed (%)		Antifeeding effect (%) ¹
	Control	Treated	
0.6	1.84	0.10	79.08±2a
1.2	1.35	0.01	85.16±1a
2.4	2.13	0.01*	99.52±1b
4.8	3.49	0.00*	99.86±1b

¹ Means with different letter are significantly different according to a Tukey's test ($P < 0.05$).

*Indicate significant differences between control and treated for each concentration according to Wilcoxon's test ($P < 0.05$).

There were significant differences in larval mortality due to the exposure to *C. parqui* aqueous extracts [$F_{4,40} (0.05) = 10.15$], and between days after the initial exposure [$F_{10,40} (0.05) = 18.2$]. From day 6, significant differences occurred with the lowest concentration (0.6% w/v), reaching a maximum of 92% at 4.8% w/v on day 11. This indicates that the mortalities recorded were due to the biocide action of the extract and were directly related to the concentration (Table 2).

Table 2. Cumulative mean mortality (%±SE) of 3rd stage *Gonipterus platensis* caused by water extracts from *Cestrum parqui* leaves at several days from initial exposure.

Days from application	Concentrations (% w/v)				
	0.0	0.6	1.2	2.4	4.8
1	0 ± 0 aA	0 ± 0 aA	0 ± 0 aA	0 ± 0 aA	0 ± 0 aA
2	0 ± 0 aA	0 ± 0 aA	0 ± 0 aA	0 ± 0 aA	4 ± 4 aA
3	0 ± 0 aA	0 ± 0 aA	0 ± 0 aA	4 ± 4 aA	16 ± 2 aA
4	0 ± 0 aA	4 ± 4 aA	8 ± 1 aA	20 ± 4 bA	24 ± 0 bB
5	0 ± 0 aA	20 ± 0 aA	24 ± 0 aB	32 ± 0 bB	44 ± 2 cC
6	0 ± 0 aA	32 ± 5 bB	40 ± 6 bC	52 ± 5 bcC	68 ± 5 cD
7	0 ± 0 aA	44 ± 2 bC	56 ± 2 cD	64 ± 2 dD	76 ± 1 eE
8	0 ± 0 aA	56 ± 0 bD	64 ± 2 cE	72 ± 3 dE	84 ± 0 eF
9	4 ± 4 aA	64 ± 0 bE	76 ± 2 cF	88 ± 1 dF	88 ± 1 dG

10	4 ± 4 aA	68 ± 5 bF	76 ± 2 bF	88 ± 1 cF	88 ± 1 cG
11	6 ± 2 aA	72 ± 2 bF	80 ± 2 cF	88 ± 1 dF	92 ± 1 eH

Means with different small letters in a row, and capital letters in a column, indicate significant differences between concentrations and days after initial exposure, respectively, according to Tukey tests ($p \leq 0.05$).

The results of the Probit analysis indicated a LC_{50} of 0.87% at day 7 after treatment (Table 3).

Table 3. Mean lethal concentration (LC_{50}) and parameters of Probit regression for aqueous extracts from leaf *Cestrum parqui* on *Gonipterus platensis* third instar larvae at different evaluation times.

Day	Slope ± E.S.	LC_{50} (% p/v)	Chi-square
6	0.42 ± 0.06	1.84	7.7
7	0.40 ± 0.03	0.87	4.5

*Goodness of fit for Probit model, critical Chi-square value 7.82. (df = 3; $p \leq 0.05$).

4. DISCUSSION

Some insects have demonstrated antifeeding and insecticide effects after exposure to *C. parqui* aqueous extract. For example, Chaieb et al (2001) found feeding inhibition in larvae of both *Pieris brassicae* and *Spodoptera littoralis* at day 2, and a 100% inhibition in *S. littoralis* at a concentration of 30%, so our results shown a more potent activity from *C. parqui* extracts on *G. platensis* larvae since almost 100% antifeeding effect was reached at 2.4% w/v. Larvae of *Ceratitis capitata* and *S. littoralis* fed by Zapata (2005) with extract at 2% w/v presented antifeeding activity >50% in both species, and again our results were more promising, as twice the antifeeding activity occurred almost at the same concentration. Chaieb et al. (2009a) evaluated also the antifeeding effect of crude saponins extracted in water from *C. parqui* leaves, by measuring the weight of the consumption by juveniles of *Schistocerca gregaria* and *S. littoralis*. Whereas no significant differences with the controls occurred on *S. gregaria*, they did occur in *S. littoralis* species at day 8. Thus, our results herein were more effective as significant differences occurred at 24 h. Therefore, larvae of *G. platensis* are more sensitive to these extracts from *C. parqui* leaves.

The insecticide effect from *C. parqui* aqueous extracts (leaves) tested by Zapata et al. (2006) on larvae of *C. capitata*, at a concentration ranging from 0.2 to 2.0% w/v, caused 80 and 100% mortality on days 3 and 6 using the highest conc.; the LC_{50} were of 0.90 and 0.40% w/v on days 3 and 4, respectively. Those results are slightly greater than ours on *G. platensis*.

The insecticide effect of aqueous extracts from new and mature leaves of *C. parqui* was also evaluated by Huerta et al (2021) on adults of *X. luteola*, determining toxicity at 0.5 to 5.5% w/v, with maximum mortalities on day 8 after the initial exposure to the extracts obtained from young and mature leaves at 73 and 51% w/v, respectively. Also, for those extracts they found at the 8 day LC_{50} values of 0.63 and 0.80% w/v, respectively. Those results of mortality are lower than herein, indicating that *G. platensis* larvae are more sensitive than *X. luteola* adult to these extracts. Although the LC_{50} are lower than those obtained in our work, a higher concentration of the extract was necessary to kill 50% of *X. luteola* adults. It is important to notice that in that work there were no difference between extracts from new of mature leaves. Zapata et al. (2006) and Huerta et al. (2021) used the same methodology described in our study, and both groups found, as we herein, that mortality increased with concentration, indicating a dose response effect.

Rafael et al. (2000) determined the insecticide activity from *C. parqui*, *Solanum sisymbriifolium* Lam and *Chenopodium album* L. extracts (1000 ppm), using different (ethanol, hexane, chloroform) solvents,

on *Tribolium castaneum* adults, concluding *C. parqui* extracts were the most toxic among the three plant species.

Santolamazza & Fernández (2004) evaluated the insecticide effect of azadirachtin, a limonoid from *Melia azedarach* L, against larvae of *G. platensis*. Mortality by contact and ingestion reached 72.5% at day 7 (0.278 % w/v), more effective than our results presented herein. Furthermore, Chiffelle et al. (2017) evaluated the effect of aqueous extracts from new and mature leaves of *Schinus molle* L. on *G. platensis* third instar larvae, at 0.5 to 4.8% w/v, obtaining 92 and 88% mortality (on day 10) with the highest concentration, respectively. CL₅₀ values of 2.08 and 12.11% w/v on day 6 were estimated, respectively, with the same extracts. Those levels of mortality are similar to our results, where 92% mortality was reached on day 11, so the extracts from *C. parqui* and *S. molle* would be similarly effective against *G. platensis*. However, the LC₅₀ values indicate that the extract from *C. parqui* requires less than half the concentration, and similar effects one day later than those from *S. molle*, indicating a higher efficiency of the former against *G. platensis* larvae.

5. CONCLUSIONS

The aqueous extract from *C. parqui* leaves inhibited feeding of *G. platensis* third instar larvae and was effective as bioinsecticide, reaching 92% mortality at 4.8% w/v on day 11 after initial exposure in laboratory bioassays. Mortality was directly related to the *C. parqui* concentration in aqueous extracts. The lower LC₅₀ was 0.87% w/v on day 7. It is concluded that this extract would become an efficient alternative to be incorporated in integrated management programs in forest plantations against this pest.

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