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

Amanda Huerta¹, Ítalo Chiffelle², Carolina Arias¹, Tomislav Curkovic³ and Jaime E. Araya³

¹ University of Chile, Faculty of Forestry and Nature Conservation Sciences, Department of Silviculture and Nature Conservation, P.O. Box 9206, Santiago, Chile.
² University of Chile, Faculty of Agronomic Sciences, Department of Agroindustry and Enology, P.O. Box 1004, Santiago, Chile.
³ University of Chile, Faculty of Agronomic Sciences, Department of Crop Protection, P.O. Box 1004, Santiago, Chile.

*Corresponding author: ahuerta@uchile.cl*

(Received for publication 25 October 2021; accepted in revised form 22 October 2022)

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 LC₅₀ (lethal concentration to kill 50% of the individuals) was calculated by Probit analysis and Chi² 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 LC₅₀ 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).

© The Author(s). 2023 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.
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 Cadahia 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 anisopliae) have been evaluated against G. platensis adults, identifying fungal strains with insecticide efficacy of (Jordan et al. 2013). 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; Docampo 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, anti-inflammatory (Ali et al. 2013), antifeeding (Hua et al. 2017) and insecticide activities (Graham 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 Image program (Schneider et al. 2012). The percentage of antifeeding effect was calculated as $(1- [T / C]) \times 100$, 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 ≤ 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).

| Latex (% w/v) | Foliar area consumed (control) | Antifeeding effect (%)$^1$ |
|---------------|--------------------------------|--------------------------|
| 10            | 2.48                           | 98.9 ± 1 a               |
| 30            | 2.55                           | 99.3 ± 1 a               |
| 50            | 2.38                           | 99.8 ± 1 a               |
| 70            | 2.91                           | 100.0 ± 0 a              |
| 100           | 1.73                           | 100.0 ± 0 a              |

$^1$Means sharing a letter do not differ significantly according to Tukey’s test (p < 0.05%).

$^2$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$_{2,5}$ (0.05) = 16.7] and among the number of days after the initial exposure [F$_{2,5}$ (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$_{50}$ 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 *Eucalyptus 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. (Solanaceae) leaves, a South American shrub (Huerta et al. 2021).

Larval mortality
The ethanol 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 et al. 2021).
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*. 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 LC₅₀ 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

Funding
This research was supported by Laboratory of Forest Entomology (Department of Silviculture and Nature Conservation) and Laboratory Chemistry (Department of Agroindustry and Enology), both of the Universidad of Chile.

References
Adame, P., Alberdi, I., Cañellas, I., Hernández, L., Aguirre, A., Ruano, A. Moreno-Fernández, D., González, A.L., Torres, M.B., & Montes, F. (2022). Drivers and spread of non-native pests in forests: The case of Gonipterus platensis in Spanish Eucalyptus plantations. Forest Ecology and Management, 510, 120104. https://doi.org/10.1016/j.foreco.2022.120104

Ali, A.A., Sayed, H.M., Ibrahim, S.R., & Zaher, A.M. (2013). Chemical constituents, antimicrobial, analgesic, antipyretic, and anti-inflammatory activities of Euphorbia peplus L. Phyto Pharmacology, 4, 69-80.

Amri, I., Hamrouni, L., Hanana, M., & Jamoussi, B. (2013). Reviews on phytotoxic effects of essential oils and their individual components: new approach for weeds management. International Journal Applied Biology and Pharmaceutical Technology, 4, 96-114.

Bittner, M., Alarcón, J., Aaqueveque, P., Becerra, J., Hernández, V., Hoenisen, M., & Silva, M. (2001). Estudio químico de especies de la familia Euphorbiaceae en Chile. Boletín de la Sociedad Chilena de Química, 46(4), 1-16. https://doi.org/10.4067/S0366-1642001000400006

Ceia, R.S., Faria, N., Lopes, P.B., Alves, J., da Silva A.A., Valente, C., Gonçalves, C.I., Mata, VA., Santos S.A.P., Azevedo-Pereira H.M.V.S., Sousa J.P., & da Silva, L.P. (2021). Local and landscape effects on the occurrence and abundance of the Eucalyptus weevil Gonipterus platensis (Coleoptera: Curculionidae). Forest Ecology and Management, 500: 119618. https://doi.org/10.1016/j.foreco.2021.119618

Chaieb, I., Ben Halima, M., & Ben Hamouda, M. (2001). The effect of food containing Cestrum paquii (Solanaceae) extract on various damaging Lepidoptera. Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen), 66, 479-490.

Chiffelle, I., Huerta, A., Sandoval, C.A., & Araya, J.E. (2017). Efecto insecticida de extractos de hojas de Schinus molle en larvas de Gonipterus platensis. Revista de la Facultad Nacional de Agronomía, Medellín, Colombia, 70(3), 8263-8270. https://doi.org/10.15446/ rfna.v70n3.58272

Corporación Nacional Forestal (2012). El manejo sanitario de las plantaciones forestal. Chile Forestal, 360, 35-37.

Corporación Nacional Forestal (2017). Finaliza liberación de controladores biológicos en Región Metropolitana. Santiago, Chile: CONAF. https://www.conaf.cl/ finaliza-ultima-etapa-de-liberacion-de-controladores-biologicos-en-la-rm/ Accessed 6 May 2022.

Defagó, M., Valladares, G., Banchio, E., Carpinella, C., & Palacios, S. (2006). Insecticide and antifeedant activity of different plant parts of Melia azedarach on Xanthogaleruca luteola. Fitoterapia, 77, 500-505. https://doi.org/10.1016/j.fitote.2006.05.027

Docampo, P., Cabreriza, S., Paladino, M., Parrañob, M., Ruffolob, V., & Mutitia, O. (2010). Eritrodermia secundaria a planta productora de látex (Synadenium grantii). Archivos Argentinos de Pediatría, 108(6), 126-129.

Dos Santos Bobadilla, G., Baptista Vidaurre, G., Cajana, A.P., Fantuzzi Neto, H., Da Silva Oliviera, J.T., Salim, E., Verly Lopes, D.J., & Zanuncio, J.C. (2019). Effect of defoliator insect on growth and wood properties of eucalypt trees. European Journal of Wood Products, 77, 861-868. https://doi.org/10.1007/s10017-019-01435-6

Ghramh, H.A., Khan, K.A., & Ibrahim, E.H. (2019). Biological activities of Euphorbia peplus leaves ethanolic extract and the extract fabricated gold nanoparticles (AuNPs). Molecules, 24, 1431. https://doi.org/10.3390/molecules24071431

Hua, J., Liu, Y., Xiao, C.J., Jing, S.X., Luo, S.H., & Li, S.H. (2017). Chemical profile and defensive function of the latex of Euphorbia peplus. Phytochemistry, 136, 56-64. https://doi.org/10.1016/j.phytochem.2016.12.021
Huerta, A., Chiffelle, I., Passalaqua, C., Curkovic, T., & Araya, J.E. (2021). Antifeeding and insecticide effects of leaf extracts from Cestrum parqui (Solanaceae) on larvae of Gonipterus platensis (Coleoptera: Curculionidae). *Agriculture & Food - Journal of International Scientific Publications*, 9, 100-107.

InfoStat (2009). InfoStat versión 2009, Grupo InfoStat, FCA. Córdoba, Argentina: Universidad Nacional de Córdoba.

Instituto Forestal (2020). *Anuario Forestal 2020, Chilean Statistical Yearbook of Forestry*. Boletín Estadístico N°174. Santiago, Chile: Ministerio de Agricultura, Gobierno de Chile.

Isman, M. (2020). Botanical Insecticides in the Twenty-First Century-Fulfilling Their Promise? *Annual Review of Entomology*, 65, 233-249. https://doi.org/10.1146/annurev-ento-011019-025010

Jordan, C., dos Santos, P.L., Oliveira, L., Domingues, M.M., Gêa, B., Ribeiro, M.F., Mascarín, G.M., & Wilcken, C.F. (2021). Entomopathogenic fungi as the microbial frontline against the alien Eucalyptus pest Gonipterus platensis in Brazil. *Scientific Reports*, 11, 7233. https://doi.org/10.1038/s41598-021-86638-9

Mapondera, T.S., Burgess, T., Matsuki, M., & Oberprieler, R. (2012). Identification and molecular phylogenetics of the cryptic species of the Gonipterus scutellatus complex (Coleoptera: Curculionidae: Gonipterini). *Australian Journal of Entomology*, 51, 175–188. https://doi.org/10.1111/j.1440-6055.2011.00853.x

Mendivelso, D., Palacios, C., Pinzón, A., & Victoria, M. 2003. Estudio morfológico y anatómico de Euphorbia peplus Linneo (Euphorbiaceae). *Acta Biológica Colombiana*, 8(2), 99-103.

Ogbourne, S., Suhrbier, A., Jones, B., Cozzi, S., Boyle, G., Morris, M., McAlpine, D., Johns, J., Scott, T., Sutherland, K., Gardner, J., Le, T., Lenarczyk, A., Aylward J., & Parsons, P. (2004). Antitumor activity of 3-ingenyl angelate: Plasma membrane and mitochondrial disruption, and necrotic cell death. *Cancer Research*, 64, 2833-2839. https://doi.org/10.1158/0008-5472.CAN-03-2837

Pérez Otero, R., Mansilla Vásquez, P., & Rodríguez Iglesias, J. (2003). Eficacia y efectos en laboratorio de diferentes insecticidas en el control del defoliador del eucalipto Gonipterus scutellatus y de su parasitoide Anaphes nitens. *Boletín de Sanidad Vegetal Plagas*, 29, 649-658.

Robertson, J., Smith, K., Savin, N., & Lavigne, R. (1984). Effects of dose selection and sample size on the precision of lethal dose estimates in dose-mortality regression. *Journal of Economic Entomology*, 77(4), 833-837. https://doi.org/10.1093/jee/77.4.833

Romanyk, N., & Cadahí, D. (2002). *Plagas de insectos en las masas forestales*. Madrid, Spain: Sociedad Española de Ciencias Forestales. Ediciones Mundiprensa, 336 p.

Rua, J.C.P., Barreiro, S., Branco, M., & Tomé, M. (2020). Estimating defoliation impact of Gonipterus platensis on Eucalyptus globulus stands productivity using a forest simulator based on 3-PG. *Forest Ecology and Management*, 478: 118495. https://doi.org/10.1016/j.foreco.2020.118495

Santolamazza, S., & Fernández, F. (2004). Efectos de dos insecticidas de síntesis y de dos bio-insecticidas sobre el defoliador del eucalipto Gonipterus scutellatus Gyllenhal y su agente de control biológico Anaphes nitens Girault. *Boletín de Sanidad Vegetal Plagas*, 30, 265-277.

Schneider, C.A., Rasband, W.S., & Eliceiri, K.W. (2012). NIH Image to Image: 25 years of image analysis. *Nature Methods*, 9, 671-675. https://doi.org/10.1038/nmeth.2089

Schröder, M., Slippers B., Wingfield, M., & Hurley, B. (2020). Invasion history and management of Eucalyptus snout beetles in the Gonipterus scutellatus species complex. *Journal of Pest Science*, 93, 11-25. https://doi.org/10.1007/s10340-019-01156-y

Servicio Agrícola & Ganadero. 2010. *Informativo Fitosanitario Forestal No 4 (3). Unidad de Vigilancia y Control de Plagas Forestales y Exóticas Invasoras*. Santiago, Chile: Ministerio de Agricultura. 8 p.