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RESEARCH PAPER

The effects of the essential oil and hydrolate of canelo (*Drimys winteri*) on adults of *Aegorhinus superciliosus* in the laboratory

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Abstract

R. Rebolledo, J. Abarzúa, A. Zavala, A. Quiroz, M. Alvear, and A. Aguilera. 2012. The effects of the essential oil and hydrolate of canelo (*Drimys winteri*) on adults of *Aegorhinus superciliosus* in the laboratory. Cien. Inv. Agr. 39(3): 481-488. *Drimys winteri* (Magnoliids: Winteraceae), a native Chilean species, has potential for use as a botanical insecticide. This species contains chemicals with biological activity, such as isoprenoids and polyphenols. In this study, the effects of the essential oil and hydrolate of *D. winteri* on adults of *Aegorhinus superciliosus* were determined in the laboratory. This species is one of the most important pests associated with the cultivation of blueberries in Chile. The insecticidal effect of both distillates was determined for five concentrations of the essential oil and hydrolate of *D. winteri*, with ten replicates, in a completely random bioassay. The effects of these preparations on the egg-laying, hatching and feeding activity of *A. superciliosus* were also evaluated. The essential oil produced 100% mortality at the highest concentration (40% v/v), whereas the greatest effect of the hydrolate was 12% at 100% v/v. Ovicidal and anti-feeding effects were found for both distillates. In addition, a preliminary analysis was performed with gas chromatography coupled with mass spectrometry. This analysis detected the presence of polyphenolic secondary metabolites.

Key words: Essential oil, hydrolate, secondary metabolites.

Introduction

The effects of insecticides on insect physiology are based on a complex series of physical-chemical reactions that affect a particular species (Romanyk and Cadahía, 2002). Due to their ease of use and availability in the market, synthetic insecticides have been the principal tool used by farmers to control pests. Their indiscriminate use has resulted in adverse environmental effects, including the appearance of resistance to these chemical agents and the destruction of natural control mechanisms, in addition to their high toxicity to humans (Silva *et al.,* 2006; Devine *et al.,* 2008; López *et al.,* 2008;

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Siriwong *et al*., 2009). As a result, the search for alternative and/or complementary pest control methods has increased in recent years. One alternative is the use of plant species that have developed defense mechanisms through their interaction with herbivorous insects as a result of coevolution over thousands of years (Wink, 2003). Certain plants synthesize a wide range of chemicals called secondary metabolites (Yazaki, 2006; Hartmann, 2007, Howe and Jander, 2008), many of which may provide new natural biocides (Deidre *et al*., 2005). Furthermore, these natural compounds have lower toxicity and environmental impact (Harborne, 2001).

Drimys winteri, or "canelo", a Chilean native tree, is distributed between the Río Limari in the north (30ºS) and Cabo de Hornos in the south (56ºS) (Hoffmann *et al.,* 1992).

The presence of biologically active compounds has been described in the leaves, stems and bark of this tree species. These compounds primarily include secondary isoprenoids (*e.g*., polygodial, drimenol and isodrimenol) and polyphenoids (Christine *et al*., 1982; Malherios *et al*., 2001; Muñoz *et al*., 2004; Muñoz *et al*., 2007; Limberger *et al.,* 2007, Zapata *et al*., 2009). When ingested by certain insects, polygodial inhibits their capacity to feed, either temporarily or permanently, depending on the concentration (Muñoz *et al.,* 2007, Zapata *et al*., 2009).

Recently, Monsálvez *et al*. (2010) reported a number of compounds in the essential oil obtained from canelo that displayed fungicidal activity on the fungus *Gaeumannomyces graminis* (Sacc.) Arx & D.L. Olivier. The growth of fungi was 50% inhibited by doses of 932 and 30.37 mg L^{-1} of hydrolate and essential oil, respectively.

The objective of this study was to evaluate the use of the essential oil and hydrolate of *D. winteri* as agents for the control of *A. superciliosus* (Guérin) (Curculionidae), the principal pest of blueberries. The study determined the insecticidal, ovicidal and antifeeding effects of these preparations. This curculionid is the principal pest associated with the cultivation of small fruits. Small fruit cultivation has grown exponentially in south central Chile over the past two decades, primarily for off-season exports to the northern hemisphere. The permanent establishment of these fruit plantations has created new agro-ecoystems offering favorable conditions for such native insect species as *A. superciliosus*. Because they feed on the roots, the larvae of this pest can cause the death of large numbers of plants in a plantation, and the adults cause severe defoliation (Guerrero and Aguilera, 1989; Aguilera 1994, 1995; Parra *et al.,* 2009).

Materials and methods

The distillates were obtained from leaves and twigs of *D. winteri* collected in mid-October 2008 on a farm located at 38°58'S, 72°48'W, near Freire, in the central plain of the Araucanía Region. The age of the trees was approximately 10 years, and their height was 6 m. Beginning in November 2008, 500 adult specimens of *A. superciliosus* were collected on a commercial blueberry farm near Freire, Araucanía Region (38°57'S, 72°35'W). They were kept at 5° C until the beginning of the bioassays. The insects were fed on leaves of *Rubus ulmifolius* Schott (Bramble).

The study was conducted from October 2008 through March 2009 in the Entomology Laboratory of the Facultad de Ciencias Agropecuarias y Forestales of Universidad de La Frontera. The essential oil and hydrolate were obtained by steam distillation in a 90 L Chilean-made stainless steel alembic, Villarrica city, Chile.

Insect mortality and secondary and anti-feeding effects were evaluated through independent assays for each distillate. The analyses of both distillates included five treatments (concentrations) (Table 1).

The insects were fed on 10 cm twigs of *Rubus ulmifolius* sprayed with the treatments at the

Table 1. Treatments with essential oil and hydrolate of *Drimys winteri* used to determine the effects of these preparations on *Aegorhinus superciliosus*.

Treatments	Essential oil/ acetone	Hydrolate / distilled water
T0	Control with no application	Control with no application
T1	5% (v/v)	10% (v/v)
T2	10% (v/v)	20% (v/v)
T3	20% (v/v)	40% (v/v)
T4	40% (v/v)	100% hydrolate

beginning of the assay (hour 0); new treated leaves were given to the insects at 48 and 96 h. The application was performed with a manual applicator at a rate of 950 L ha^{-1}.

Insect mortality was determined after 24, 48, 76, 96 and 120 h on experimental units of five specimens in a 1 L plastic container, with ten replicates per treatment.

The effects of the distillates on egg-laying were evaluated by recording the number of eggs laid in 90 mm Petri dishes and the percentage hatched after 30 days for each treatment. The dishes were lined with absorbent paper moistened with distilled water. The eggs were obtained from the insects subjected to the different tests. In all, 78 eggs were collected for each treatment, as well as 78 eggs for the control.

To determine the antifeeding effect, the surface area of ten leaves of *Vaccinium corymbosum* L. (Blueberry) cv. Legacy was measured after spraying with the experimental treatments (Table 1). A sprayed leaf was introduced into a container with an adult *A. superciliosus* that had been held without food for the previous 24 h. The area consumed was measured after 48 h. Ten replicate measurements were performed. The differences in leaf area were determined with Arc View 3.2 Software.

A completely random experimental design was used in the assays, with five treatment concentrations and ten replicates. The efficacy percentages for control of *A. superciliosus* were calculated with Abbott's formula every 24 h up to 120 h for the degree of efficacy and ovicidal effect (Abbott, 1925). The percentages were transformed to angular values with the formula $Y = \arcsin\sqrt{x}$. Adult mortality and ovicidal and antifeeding effects were determined. The data were analyzed with an analysis of variance (ANOVA) and a Tukey multiple range test at 5% probability using SPSS 11 statistics software. The temperature and relative humidity were recorded daily using a Veto maximum and minimum thermometer and a Sundo hair hygrometer.

A gas chromatography-mass spectrometry (GC-MS) analysis was performed with a 100 ppm solution of essential oil in hexane, 1 μL of which was injected into a gas chromatograph (Focus GC; Thermo Electron Composition, Waltham, USA) coupled to a mass detector (Finnigan FOCUS DSQ). The equipment consists of a 30 m, 0.22 mm DBP-1 capillary column using helium as the carrier gas at a 1.5 mL min^{-1} flow rate and a 70 eV electron beam with injection at 250º.

The initial temperature of the oven was programmed at 40 °C and then increased by 5 °C min^{-1} to 280 °C. This temperature was maintained for 10 min. The mass spectra for each signal obtained from the chromatograms by GC-MS were compared with the NIST (V 2.0) internal library supplied by the instrument. This comparison defined the possible compounds detected at a defined percentage of certainty.

Results and discussion

The results presented below represent the cumulative effect of the bioassays. In general, measurements were taken up to 120 h with the exception of the assay that evaluated both distillates as antifeeding products, in which measurements were taken up to 48 h. After 72 h, the essential oil showed a significant insecticidal effect on the adult *A. superciliosus*, with 100% mortality observed after 120 h at the 20 and 40% v/v concentrations (Table 1).

The results demonstrated a relationship between the concentration of the essential oil and its insecticidal activity. In terms of the criterion proposed by Silva *et al.* (2005), who defined as promising only those treatments with a mortality higher than 40%, the most effective treatments were the essential oil at 20 and 40% v/v because they produced the highest percentages of mortality and efficacy from the first application (Table 2).

These results confirm previous information about the essential oil of *D. winteri*, which contains chemical compounds whose biological activity is recognized. Previous studies (Muñoz *et al*., 2004, 2007 and Zapata *et al*., 2009) have cited polygodial sesquiterpene as an antimicrobial product and as a feeding inhibitor in certain insects, affecting the palatability of the food source and acting at the level of the digestive enzymes, and finally forming insoluble complexes in the digestive tract (Zapata *et al.,* 2009). In addition, certain polyphenolic compounds, including caffeic acid, ferulic acids, vanillin, quercetin and luteolin show insecticidal activity in insects of various orders in both the adult and larval phases, causing impeded movement and subsequent mortality (Regnault-Roger *et al.,* 2004). In our study, the secondary metabolites and alcohols present in high concentrations in the essential oil may have caused the preparation to act as an insecticide at the higher concentrations evaluated. In this respect, Regnault-Roger (1997) indicates that the insecticidal effects of essential oils may commonly act through inhalation, ingestion or topical absorption. For this reason, certain authors relate the insecticidal action of essential oils to the development of new alternatives for ecological pest control (Murray, 2000*,* 2005; Ricci *et al.,* 2006 and Jbilou *et al.,* 2008).

The hydrolate of *D. winteri* had no effect on the mortality of *A. superciliosus*, with no statistically significant differences between treatments (Table 3). The significant differences in biological activity between the essential oil and the hydrolate suggest that the apolar components were responsible for the mortality produced by the essential oil. This oil also showed effects on egg-laying in *A. superciliosus*. The data in Table 4 indicate that that the treatment significantly reduced egg-laying and the percentage of hatching after 120 h and 30 d, respectively. Although the cumulative number of eggs laid is directly related to mortality, the hatching percentage is independent of mortality and would be a more accurate measure of the residual effects after the consumption of shoots treated with the essential oil. These residual effects of consumption would reduce the probability of hatching. Rodriguez *et al.* (2003) state that reduced egg-laying results from the inhibition of reproduction, affecting copulation, oocyte development, the pre-laying period, and the development, number, size and hatching of the eggs. However, during the period of time from collection in the field to the beginning of the bioassays, the specimens were kept in conditions that

Table 2. Efficacy percentage of essential oil from *Drimys winteri* for the control of *A. superciliosus* (calculated according to Abbott, 1925).

	Hours after application				
Treatments	24	48	72	96	120
T0 control	0 b	0 c	0 c	0 d	0 d
T1 5% (v/v)	4 b	10 bc	18 b	20 c	22 c
T2 10% (v/v)	14 ab	28 b	36 b	42 b	54 b
T3 20% (v/v)	16 ab	58 a	86 a	90 a	100 a
T4 40% (v/v)	22 a	78 a	94 a	100 a	100 a

Different letters indicate significant differences at P≤0.05, Tukey test.

were suitable for mating; they were not separated by sex. This observation suggests that females that were already fertilized were collected in the field or that females may have been fertilized during the period prior to the start of the bioassays. In contrast, the treatments with hydrolate did not affect egg hatching (Table 3) or adult mortality. This result would indicate that the polar compounds in the essential oil are digested more easily than the nonpolar compounds in the hydrolate. Each type of compound might affect the normal development of the life cycle of *A. superciliosus*. No existing references address the use of plant hydrolate on crop pests. A hydrosoluble substance that is present in a larger proportion than the oil, that shows independent chemical properties and that contains a small fraction of essential oil and oligoelements is unknown. Urano *et al.* (2003) reported the larvicidal effect of the hydrolate from *Lippia siroides* Cham. (Verbenaceae) on *Aedes aegypti* Linn. (Diptera: Culicidae), describing the hydrolate as a product that could contribute to progress in new alternatives for ecological pest control. The effects of hydrolate on mosquito egg-laying may be explained by the low concentrations of secondary metabolites, which generally act in nanoconcentrations.

The essential oil applied on blueberry leaves showed no anti-feeding effect on *A. superciliosus* . However, adult mortality reached 78% in treatment T4 48 h after the application of essential oil (Table 2). Soon-II *et al.* (2003) indicate that these substances are highly volatile and most likely cause irreversible damage to membranes in the respiratory and digestive systems of the insects, particularly if the insects are confined in a small container. This result would indicate that the essential oil produces mortality both through consumption in low quantities and through inhalation.

Rodriguez *et al.* (2003) state that feeding inhibitors derived from plants are secondary metabolites that prevent the insects from responding positively to olfactory stimuli and therefore affect their ability to find and recognize their host plant.

The hydrolate presented a significant anti-feeding effect (Table 4). This finding may be a result of the occurrence of polar secondary metabolites, such as polygodial and flavonoids, in this fraction. Although these compounds do not make the hydrolate an insecticidal product *per se*, they do provide anti-feeding qualities that affect the palatability of the food substrate for *A. superciliosus.* These results are consistent with those of Zapata *et al.* (2009) on *S. littoralis.* The effects on *S. littoralis* were attributed to the anti-feeding effects of the sesquiterpenes polygodial and drimenol contained in the bark of *D. winteri.*

Based on our results, the essential oil of *D. winteri* shows promise due to its insecticidal action on adults of *A. superciliosus*. The hydrolate of *D. winteri* had no insecticidal effect. In the investigations of egg-laying and hatching during the bioassay, the high mortality of the adult specimens made it difficult to determine whether the essential oil showed ovicidal action. In this sense, the hydrolate may be a useful alternative because it produces anti-feeding effects.

Table 3. Efficacy percentage of hydrolate from *Drimys winteri* for the control of *Aegorhinus superciliosus* (calculated according to Abbott, 1925).

	Hours after application				
Treatments	24	48	72	96	120
T0 control	0 a	0 a	0 a	0 a	0 a
T1 10% (v/v)	2 a	6 a	10 a	12 a	12.2 a
T2 20% (v/v)	0 a	0 a	6 a	8 a	10.2 a
T3 40% (v/v)	2 a	4 a	6 a	6 a	4.10 a
T4 100% Hyd.	2 a	4 a	6 a	6 a	12.2 a

Different letters indicate significant differences at P≤0.05, Tukey test.

Table 4. *Aegorhinus superciliosus* eggs laid after 120 h and larvae hatched after 30 days in the presence of different concentrations of essential oil from *Drimys winteri.*

Treatments	N° of eggs laid	N° of larvae hatched	% Hatching
T0 Control	78 a	76 a	97
T1 5 % (v/v)	32 b	17 b	53
T2 10 % (v/v)	12 c	5 c	41
T3 20 % (v/v)	6 cd	2 c	16
T4 40 % (v/v)	1 d	0 c	0

Different letters indicate significant differences at P≤0.05, Tukey test.

The essential oil of *D. winteri* had no anti-feeding effect on *A. superciliosus* but did produce high mortality and efficacy. In contrast, the hydrolate showed an anti-feeding effect. A chromatographic analysis demonstrated the presence of polyphenols (pinocmebrin and quercetin) in the essential oil and of caffeic acid in the hydrolate.

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Resumen

R. Rebolledo, J. Abarzúa, A. Zavala, A. Quiroz, M. Alvear y A. Aguilera. Efecto del aceite esencial e hidrolato de canelo (*Drimys winteri*), en adultos de A*egorhinus superciliosus*, bajo condiciones de laboratorio. Cien. Inv. Agr. 39(3): 481-488. *Drimys winteri* (Magnoliales: Winteraceae) especie nativa de Chile, tiene potenciales para ser utilizada como un insecticida de origen botánico. Trabajos previos han mostrado que esta especie presenta compuestos químicos con actividad biológica, tales como isoprenoides y polifenoles. En el presente estudio se determinó el efecto, bajo condiciones de laboratorio, de dos productos de la destilación de *D. winteri* (aceite esencial e hidrolato), sobre adultos de *Aegorhinus superciliosus*, considerado una de las plagas más importantes asociadas a cultivos de frutales menores en Chile. Los bioensayos se desarrollaron en el Laboratorio de Entomología de la Facultad de Ciencias Agropecuarias y Forestales de la Universidad de La Frontera (Temuco, Chile). Se determinó el efecto insecticida de ambos destilados sobre los insectos, utilizando cinco tratamientos incluyendo un testigo, con diez repeticiones, asignando a cada tratamiento diferentes concentraciones de aceite esencial e hidrolato de *D. winteri*. También se evaluaron sus efectos sobre oviposición, eclosión y la actividad alimentaria sobre *A. superciliosus*. El diseño experimental de los bioensayos correspondió a un diseño completamente aleatorizado. Los resultados, mostraron que el aceite esencial alcanzó un porcentaje de mortalidad del 100%, con la mayor concentración; mientras que el mayor efecto del hidrolato llegó a 12%, sobre insectos adultos. Se detectaron efectos ovicidas y antialimentarios en ambos destilados. Complementariamente se realizó un análisis preeliminar por cromatografía de gases acoplada a espectrometría de masas, encontrándose metabolitos secundarios polifenólicos.

Palabras clave: Aceite esencial, *Aegorhinus superciliosus*, *Drimys winteri,* hidrolato, metabolitos secundarios.

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