Revista Colombiana de Entomología 43 (2): 233-244 (Julio - Diciembre 2017) DOI: 10.25100/socolen.v43i2.5948

Effects of extracts from *Maytenus* on *Aegorhinus superciliosus* (Coleoptera: Curculionidae) and *Hippodamia convergens* (Coleoptera: Coccinellidae)

Efecto de extractos de *Maytenus* sobre *Aegorhinus superciliosus* (Coleoptera: Curculionidae) e *Hippodamia convergens* (Coleoptera: Coccinellidae)

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Abstract: The insecticidal effects of five ethanolic extracts produced from three species of the genus *Maytenus*: *M. boaria* leaf (MBL), *M. boaria* bark (MBB), *M. boaria* seed (MBS), *M. disticha* leaf (MDL) and *M. magellanica* leaf (MML) were evaluated on the lady beetle *Hippodamia convergens* (Coleoptera: Coccinellidae) and on the pest of berry *Aegorhinus superciliosus* (Coleoptera: Curculionidae). The anti-feeding effects of the extracts on the latter were also evaluated. Residual application was used, with five concentrations for each species of insect and ten replications of each assay. To evaluate anti-feeding action in adult *A. superciliosus*, choice (C) and no-choice (NC) experiments were established to determine the percentage of leaf area consumed. The assays lasted for 120 hours, with the mortality and anti-feeding effect monitored every 24 hours. The highest percentage of mortality in the two insect species was recorded with the MBS ethanolic extract: *H. convergens* presented 82 %, with LC_{50}: 32 mg/ml; while *A. superciliosus* presented 85 % in the choice assay and 86 % in the no-choice assay, with LC_{50}: 23 mg/ml. In both assays, the mortality increased with exposure time, reaching its highest at 120 hours. The lowest mortality was obtained with MBB extract in choice (C) and with MML extract in no-choice (NC) assays. In the choice assay, the highest percentage of leaf area consumed was recorded with MBB extract, while in the no-choice assay the highest percentage of consumed was with MML extract, which presented higher values even than the control with no application. The lowest leaf percentage consumed, in both assays, was recorded with MBS extract at the highest concentrations (20 and 30 % w/v).

Key words: Berry pest, Celastraceae, biological controller.

Resumen: Se evaluó el efecto insecticida de cinco extractos etanólicos producidos a partir de tres especies del género *Maytenus*: hojas (HMB), corteza de (CMB) y semillas (SMB) de *M. boaria*, hojas de *M. disticha* (HMD) y hojas de *M. magellanica* sobre el escarabajo *Hippodamia convergens* (Coleoptera: Coccinellidae) y en la plaga de la zarzamora *Aegorhinus superciliosus* (Coleoptera: Curculionidae). También se evaluó el efecto anti-alimentario de los extractos sobre este último. Se utilizó aplicación residual, con cinco concentraciones para cada especie de insecto y diez repeticiones en cada ensayo. Para evaluar la acción anti-alimentaria sobre adultos de *A. superciliosus*, se establecieron experimentos de elección (E) y sin elección (NE) para determinar el porcentaje de área foliar consumida. Los ensayos duraron 120 horas, con la mortalidad y el efecto anti-alimentario monitoreados cada 24 horas. El mayor porcentaje de mortalidad en las dos especies de insectos se registró con el extracto etanólico de SMB: *H. convergens* presentó 82 % con una LC_{50}: 32 mg/ml, mientras que *A. superciliosus* presentó 85 % en el ensayo de elección y 86 % en el ensayo de no elección, con una LC_{50}: 23 mg/ml. En ambos ensayos, la mortalidad aumentó con el tiempo de exposición, alcanzando su máximo a las 120 horas. La menor mortalidad se obtuvo con el extracto de CMB en el ensayo de elección (C) y con el extracto de HMM en el ensayo de no elección (NE). En el ensayo de elección, el mayor porcentaje de área foliar consumida se registró con extracto de CMB, mientras que en el ensayo de no elección el mayor porcentaje de consumo fue con el extracto de HMM, que presentó valores más altos incluso que el control sin aplicación. El menor porcentaje de hojas consumido, en ambos ensayos, se registró con el extracto de SMB en las concentraciones más altas (20 y 30 % p/v).

Palabras clave: Plaga de frutos rojos, Celastraceae, controlador biológico.

Introduction

Botanical insecticides have been used in agriculture for at least two thousand years in Asia and the Middle East (Thacker 2002). The toxicity of a large number of extracts, essential oils and components has been evaluated against insects which attack crops and stored products. They also have a limited effect on beneficial insects and are rarely toxic to mammals and humans, while their different action mechanisms mean that the insects generally develop little resistance to them (Huerta *et al.* 2010). The majority of plant species used in protection by plant extracts present an insect gowth regulators rather than an insecticidal effect, as they inhibit the insects' normal development (Quintana *et al.* 2011). In general, the protective effect of natural compounds is due mainly to the fact that they repel insects, regulate their growth or discourage them from feeding or laying their eggs (Isman 2000; Silva *et al.* 2002; Cetin *et al.* 2004; Huerta *et al.* 2010).

Plants of the genus *Maytenus* Molina have long been used in popular medicine (Alvarenga *et al.* 2001). *Maytenus magellanica* (Lam.) Hook. F., is a small evergreen tree up to 5 meters in height which in Chile grows in both the Andes and the Coastal Range from the Bío Bio to the

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Magallanes Regions; it is also present in Argentina. Dihydro-β-agarofurane sesquiterpenes, which present anti-feeding action against *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) (González *et al.* 1992; Deepa and Narmatha 2010) have been isolated from its roots, as have triterpene dimers (González *et al*. 1993; Kennedy *et al*. 2011). *Maytenus disticha* (Hook. F.) Urban, is an evergreen shrub that grows in both the Andes and the Coastal Range from the Maule to the Magallanes Regions; it also grows in Argentina. Furthermore, compounds have been isolated from this species and *Maytenus boaria* Mol. which present insecticidal action against fall armyworm *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae). *Maytenus boaria* is distributed in Chile, Argentina, Brazil and Peru. In Chile it is found from the Atacama to the Magallanes Regions and in Antarctica. Zapata *et al.* (2006) showed that *M. boaria* has a stronger anti-feeding effect than *Peumus boldus* Mol. (Monimiaceae) and *Quillaja saponaria* Mol. (Rosaceae) on the third larval stage of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae).

Aguilera (1988) cites *A. superciliosus* (Guérin) as the most economically damaging insect pest in blueberry plantations (*Vaccinium corymbosum* L.). The adults feed on leaves and twig tissue which they scarify longitudinally, leaving a characteristic scar of suberized tissue (Aguilera 1988; Parra *et al.* 2009). Chemical control has proved inefficient, since the larvae live underground while application on adults produces a residual effect that is harmful to pollinator insects (Aguilera 1995; Cisternas *et al.* 2000; Aguilera 2005).

Hippodamia convergens (Guérin-Meneville) is one of the best-known American coccinellids. It is the most widely distributed species of the genus and probably the most abundant of the family (Aguilera *et al.* 1981). In Chile it has been identified by Prado (1991) and Artigas (1994) as a predator on various species of greenfly.

The objective of this study was to evaluate the effects of ethanolic extracts of *Maytenus magellanica*, *Maytenus disticha* and *Maytenus boaria* on mortality of *H. convergens* in order to evaluate its incidence on natural enemies of pests such as aphids, and on *A. superciliosus*, and also on this latter a deterrent effect.

Materials and methods

Plant materials. Leaves of *M. disticha* and *M. magellanica* were collected in the Conguillio National Park, while leaves, bark and seed of *M. boaria* were collected at the Maquehue experimental station belonging to Universidad de La Frontera. Both sites are located in the Araucanía Region, Chile. Taxonomic identification was done in the Faculty of Farming, Livestock and Forestry Sciences, Universidad de La Frontera, Araucanía Region.

Preparation of the ethanolic extract. Extracts were prepared in the Laboratory of Ecological Chemistry, Universidad de La Frontera. The plant material was macerated in ethanol p. a. at 100 % in a proportion of 3:1, and removed after 72 hours; this process was repeated three times. It was then roto-evaporated and lyophilised.

To determine the secondary metabolites present in the extract, simple and fast tests were performed. The following tests were performed to determine the phytochemical screening of the ethanolic extracts: Alkaloids: Precipitation and coloring reactions, with the reagents of Drangerdoff, Mayer and Wagner; Tannins: Reaction with Ferric Chloride; Flavonoids: Precipitation and color reaction reactions by Shinoda test; Anthraquinones: Bornträger reaction; Coumarin: Precipitation reaction and staining with NaOH; Saponins: Foam test; Terpenos / Steroids: Lieberman-Burchard reaction and Salkowski reaction.

Insects. Adults of *H. convergens* were collected from fennel (*Foeniculum vulgare* Mill.) and thistle (*Cirsium vulgare* (Savi) Ten.) plants on farms next to Angol (37°48'S, 72°43'W). In this species only the mortality was evaluated. Following the method used by Rebolledo *et al.* (2012) to evaluate mortality, four specimens were deposited in ½ litre plastic containers, with 10 repetitions. They were fed on maize flakes sprayed with the treatments, including the control with no application, using distilled water as the solvent. The treatments correspond to: 3; 6; 9; 12 and 15 % w/v. The mortality was evaluated after 24, 48, 72, 96 and 120 hours, the food being renewed each time.

Starting in November 2013 adult of *A. superciliosus* were collected from commercial blueberry fields belonging to San José Farm, owned by Exportadora Patagonia Food S.A., located in Gorbea (39°06'S, 72°41'W) in the central valley of the Araucanía Region. A second sample of insects was collected from November 2014. Following the method used by Rebolledo *et al*. (2012) the extracts were applied on leaves of *R. ulmifolius* which were put in ½ litre plastic containers with one insect adult of *A. superciliosus,* with 10 repetitions. The treatments correspond to: 2, 5, 10, 20 and 30 % w/v. The mortality was evaluated after 24, 48, 72, 96 and 120 hours, the food being renewed each time. Additionally, the method of Zapata *et al*. (2006) was used to determine food preference through two experiments:

No-choice assay (NC): Only treated or untreated food with extracts was supplied in the container.

Choice assay (C): Treated (extract) and untreated (no application) food was supplied in the container.

The food substrate given to the insects was bramble leaves (*Rubus ulmifolius* Schott (Rosales: Rosaceae)). Plant extracts were applied uniformly with an atomizer at the start of the assay and when the food was renewed at each evaluation. A control with no application was used. Leaf area consumption was evaluated after 24, 48, 72, 96 and 120 hours. The leaf area of the leaves used in the assay, and the difference consumed, were calculated using the digital image processing programme ImageJ 1.47v developed in the National Institute of Health, which is in the public domain.

Experimental design and statistical analysis. The experimental design used for all the assays was completely randomized, with 10 repetitions. Abbot's formula was used to correct the natural mortality found in the control. The effectiveness of the treatments was evaluated by analysis of variance (ANOVA), with significance level $\alpha = 0.05$, after the data was transformed using the square root of the arcsine of the percentage ($\sqrt{x}\ \%^{-1}$) in order to ensure homogeneity of the variances. Statistical analysis of the experimental data was performed using SAS® (SAS Institute, 2009) to find out the (Log probit analysis (at 95 % confidence level), regression equations and mean percentage mortality. The means were separated using Tukey's test ($\alpha = 0.05$) (Silva *et al.* 2003).

Results and discussion

The phytochemical screening revealed the presence of cardiac glycosides, saponins, terpenoids and tannins in all extracts. Moreover, coumarins and alkaloids were also present in all extracts, except in the ethanolic extract of seeds of *M. boaria*. The results are given in table 1.

Bioassays on *H. convergens*. The highest mortalities were recorded for MBS (82 %) and MML (54 %) extracts. None of the other ethanolic extracts evaluated presented a mortality higher than 50 % (MBL: 37 %; MDL: 27 %; and MBB: 25 %) (Fig. 1).

For the MBS ethanolic extract there were significant differences between the concentrations, being the mortality dependent on the dose. The median lethal concentration for this extract was 3.2 % w/v (32 mg/ml). For all concentrations evaluated, the mortality was higher than 50 %. For other extracts no significant differences were observed between the concentrations. The mortality per extract, treatment and comparison of means are given in table 2.

For all extracts the highest mortality was recorded at 24 hours of exposure (Table 3). The mortality means by extract, treatment and evaluation time are given in table 4.

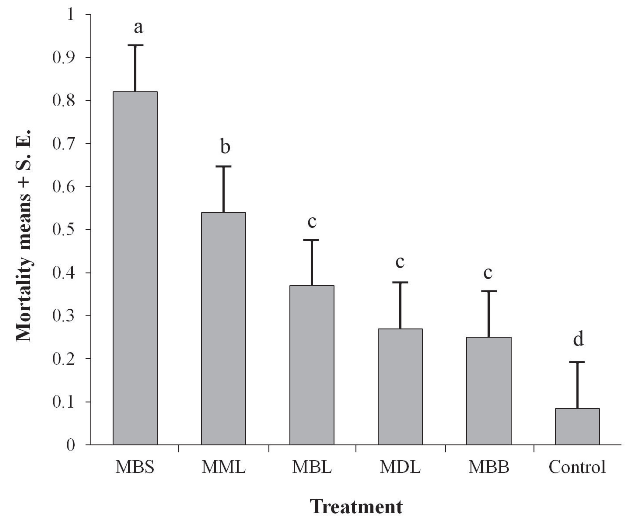

Figure 1. Mortality means ± S. E. of *Hippodamia convergens* for each extract. Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05. MBS: *M. boaria* seed; MBL: *M. boaria* leaf; MDL: *M. disticha* leaf; MML: *M. magellanica* leaf; MBB: *M. boaria* bark.

Bioassays on *A. superciliosus*. Toxicity. In the choice assay, only the MBL and MBS extracts produced mortalities higher than 50 % (Fig. 2), while this was exceeded in the no-choice assays with MBL, MDL and MBS extracts (Fig. 3). The highest mortality was obtained with MBS extract in both assays. The values obtained in the choice assays were: MBS: 85 %; MBL: 61 %; MDL: 48 %; MML: 35 % and MBB: 34 %. In the no-choice assay the values were: MBS: 84 %; MBL: 15 %; MDL: 51 %; MML: 13 % and MBB: 15 %. According to Broussalis *et al*. (1999), a mortality over 50 % is considered promising in pesticide studies with plant extracts.

The mortality percentages per extract, treatment and type of assay are given in table 5.

For the MBS extract, in the choice assay there was a significant difference between the treatments and the control, however there was no difference between the concentrations of 10-20 and 30 % w/v with LC_{50}: 23 mg/ml. In the no-choice assays there was a significant difference between the concentrations evaluated and the control, but not between the different concentrations. The mortality increased with the evaluation time, reaching its highest at 120 hours for both choice and no-choice assays.

For MBL extract, there was a significant difference between treatments in the choice assay, with LC_{50}: 43 mg/ml; the highest mortality was recorded after 72 hours of evaluation. In the no-choice assay there was no significant difference between the concentrations and the control, with the highest mortality after 24 and 48 hours.

The mortality means by extract and evaluation time are given in table 6. The mortality means by type of assay, extract, treatment and evaluation time are given in table 7.

As Pamo *et al*. (2004) indicate, the mortality increases as the dose is strengthened and as the time increases (days) since botanical pesticides are slower-acting than synthetic products. The mortality percentages in the controls remained constant over the evaluation period.

Table 1. Phytochemical screening of ethanol extracts of plants of the genus *Maytenus* present in the region of La Araucanía.

	***M. boaria* seeds**	***M. boaria* leaf**	***M. boaria* bark**	***M. magellanica* leaf**	***M. disticha* leaf**
Mayer	-	-	-	-	-
Alkaloids: Wagner	-	±	±	±	±
Dragendorff	-	-	-	-	-
Tannins	±	±	±	±	±
Flavonoids	-	-	-	-	-
Anthraquinones	-	-	-	-	-
Cardiac glycosides	±	±	±	±	±
Coumarins	-	±	±	±	±
Saponins	±	±	±	±	±
Terpenoids	±	±	±	±	±

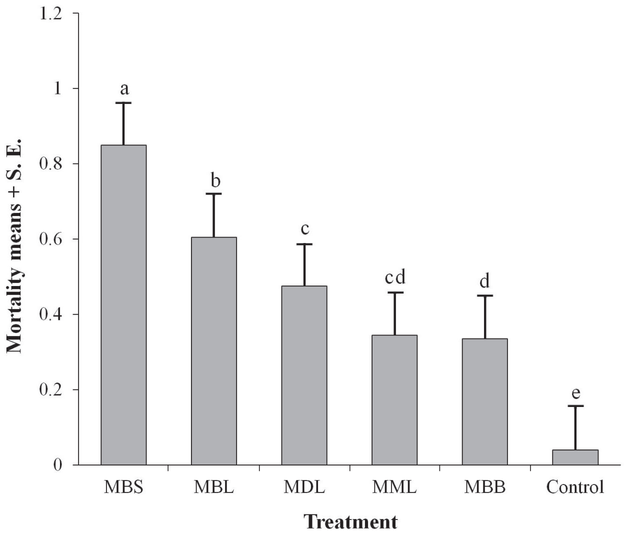

Figure 2. Mortality means ± S. E. of *Aegorhinus superciliosus* for each extract in choice assay. Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05. MBS: *M. boaria* seed; MBL: *M. boaria* leaf; MDL: *M. disticha* leaf; MML: *M. magellanica* leaf; MBB: *M. boaria* bark.

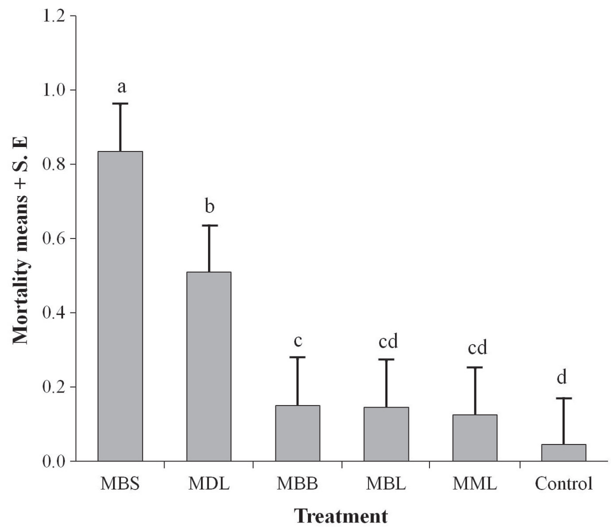

Figure 3. Mortality means ± S. E. of *Aegorhinus superciliosus* for each extract in no-choice assay. Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05. MBS: *M. boaria* seed; MBL: *M. boaria* leaf; MDL: *M. disticha* leaf; MML: *M. magellanica* leaf; MBB: *M. boaria* bark.

Alarcón *et al.* (2012), using a methanolic extract of *M. boaria* seeds, obtained the compound 9-β-furoyloxy-1α, 6β, 8α-triacetoxy-dihydro-β-agarofurane which presented strong insecticidal action (70 %) on larvae of *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae). The present study shows that ethanolic extract of *M. boaria* seed contains compounds with insecticidal action on *H. convergens* and *A. superciliosus.*

The lowest mortality was obtained with MBB extract in choice (C) and no-choice (NC) assays. There was no difference between the assessed concentrations and increased over time, in both assays. However, it has been shown that mortality with bark extracts of the genus *Maytenus* was concentration dependent. In the work of Avilla *et al*. (2000) from an unknown specie of *Maytenus*, bark of the plant roots was extracted with n-hexane: diethyl ether (1:1). The mortality of *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae) was concentration-dependent and increased over time. Of the isolated compounds, 20-R-hydroxytingenone was the most active compounds against codling moth larvae. The mortality caused by 20-R-hydroxytingenone reached 100 % five days after exposure, the LC_{50} ranging from 13.0 mg/mL (5 days later) to 8.5 mg/mL (10 days later).

Percentage leaf area consumed. In the choice assay, there was no statistically significant difference in the percentage leaf area consumed with ethanolic extracts of MBS, MBL, MML and MDL, but there was a difference between these and MBB ethanolic extract. The lowest percentage leaf area consumed was recorded with MBS extract and the highest with MBB extract (Fig. 4). A diketone isolated from the stem bark extract of *Maytenus elaeodendroides* showed feeding deterrent activity on *Sitophylus oryzae* (L.) (Coleoptera: Curculionidae), a plague that causes significant damage to rice growing (Nogueiras *et al*. 2010).

In the no-choice assay, the lowest percentages of leaf area consumed were found with MBS and MBL extracts, just as in the choice assay, with no significant differences

Table 2. Mortality means (± standard error) in *H. convergens* for each treatment and extract, for an exposure time of 120 hours.

Concentration (%w/v)	*M. boaria* seeds	*M. boaria* leaf	*M. boaria* bark	*M. magellanica* leaf	*M. disticha* leaf
Control	0.13 ± 0.05 d	0.03 ± 0.07 c	0.05 ± 0.06 a	0.18 ± 0.08 b	0.05 ± 0.07 b
3	0.53 ± 0.05 c	0.23 ± 0.07 bc	0.15 ± 0.06 a	0.48 ± 0.08 ab	0.15 ± 0.07 ab
6	0.70 ± 0.05 bc	0.30 ± 0.07 abc	0.20 ± 0.06 a	0.68 ± 0.08 a	0.20 ± 0.07 ab
9	0.90 ± 0.05 ab	0.38 ± 0.07 ab	0.30 ± 0.06 a	0.55 ± 0.08 a	0.30 ± 0.07 ab
12	0.98 ± 0.05 a	0.53 ± 0.07 a	0.30 ± 0.06 a	0.45 ± 0.08 ab	0.33 ± 0.07 a
15	1.00 ± 0.05 a	0.43 ± 0.07 ab	0.30 ± 0.06 a	0.55 ± 0.08 a	0.38 ± 0.07 a
n	240	240	240	240	240
F	38.83	6.26	2.59	4.89	3.49
P	< 0.0001	< 0.0001	0.0267	0.0003	0.0046

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05.

Table 3. Mortality means (± standard error) in *H. convergens* for evaluation time.

Evaluation time (hours)	*M. boaria* seeds	*M. boaria* leaf	*M. magellanica* leaf	*M. disticha* leaf	*M. boaria* bark
24	0.64 ± 0.03 a	0.20 ± 0.02 a	0.27 ± 0.03 a	0.13 ± 0.02 a	0.10 ± 0.02 a
n	200	200	200	200	200
48	0.35 ± 0.05 b	0.06 ± 0.02 a	0.18 ± 0.03 ab	0.04 ± 0.02 b	0.05 ± 0.02 ab
n	72	160	146	175	181
72	0.09 ± 0.06 c	0.06 ± 0.02 a	0.09 ± 0.03 bc	0.05 ± 0.02 b	0.06 ± 0.02 ab
n	47	151	120	168	172
96	0.16 ± 0.07 bc	0.06 ± 0.02 a	0.04 ± 0.03 c	0.04 ± 0.02 b	0.05 ± 0.02 ab
n	43	142	109	159	162
120	0.00 ± 0.07 c	0.04 ± 0.02 a	0.10 ± 0.03 bc	0.03 ± 0.02 b	0.02 ± 0.02 b
n	36	133	105	152	154
F	33.47	10.06	9.98	4.67	2.48
P	< 0.0001	< 0.0001	< 0.0001	0.001	0.0427

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05.

between them. The highest percentage consumption was recorded with MML extract, higher than that obtained with MBB extract in the choice assay; there was no significant differences between these extracts and their respective controls (Fig. 5). On the other hand, González *et al.* (1993) reported that compounds isolated from leaves of *M. magellanica* showed antifeedant activity on *Spodoptera littoralis* (Lepidoptera: Noctuidae) in a choice test. This study shows that the lowest percentage of leaf area consumed by *A. superciliosus* with MML extract could be related to these compounds but their activity depends strongly on concentration.

The lowest percentage of leaf area consumed was obtained with MBS extract in choice (C) and no-choice (NC) assays. González *et al*. (1997) demonstrated that ethanol extracts obtained from *M. boaria* had antifeedant activity on *S. littoralis* (Boisduval, 1833) (Lepidoptera: Noctuidae). However, they do not specify of what part of the plant were obtained the extracts. The finding in this study shows that ethanolic extracts of seed, bark and leaves of *M. boaria* do not show the same activity on A. superciliosus. In insects kept in the laboratory and fed on with *M. boaria* branches, leaves and seeds, was observed that they first consume the bark, then the leaves and finally the seeds.

Although Aguilera (1988), Arias (2000) and Zavala *et al*. (2011) consider *M. boaria* to be the principal host species for *A. superciliosus*, in this study the lowest percentage of leaf area consumed was with *M. boaria* leaf (MBL) and seed (MBS) extracts. However, Rodríguez *et al*. (2003) affirm that feed inhibitors derived from plant extracts are secondary metabolites that according to their concentration interfere insects from responding positively to olfactory

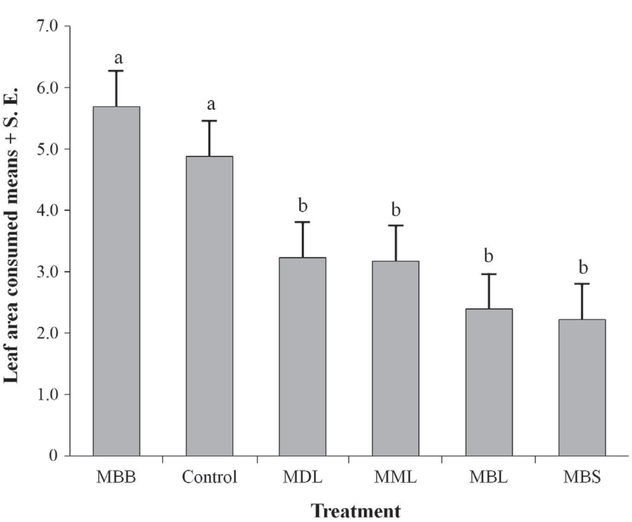

Figure 4. Percentage leaf area consumed (mean % ± standard error) of *Rubus ulmifolius* in choice assay. Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05. MBS: *M. boaria* seed; MBL: *M. boaria* leaf; MDL: *M. disticha* leaf; MML: *M. magellanica* leaf; MBB: *M. boaria* bark.

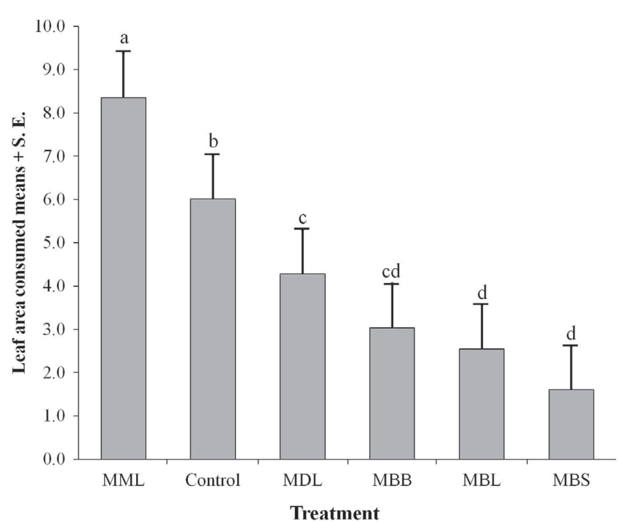

Figure 5. Percentage leaf area consumed (mean % ± standard error) of *Rubus ulmifolius in* no-choice assay. Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05. MBS: *M. boaria* seed; MBL: *M. boaria* leaf; MDL: *M. disticha* leaf; MML: *M. magellanica* leaf; MBB: *M. boaria* bark.

Table 4. Mortality percentage (means ± standard error) in *H. convergens* for extract, treatment and evaluation time.

Concentration % w/v	Evaluation time (hours)											
M. boaria seeds	24	n	48	n	72	n	96	n	120	n	F	P
Control	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.05 ± 0.02 a	40	0.08 ± 0.03 a	38	2.18	0.0728
3	0.28 ± 0.06 a	40	0.21 ± 0.07 ab	29	0.04 ± 0.07 ab	23	0.14 ± 0.08 ab	22	0.00 ± 0.08 b	19	2.76	0.0306
6	0.58 ± 0.06 a	40	0.14 ± 0.10 b	17	0.12 ± 0.9 b	15	0.14 ± 0.10 b	14	0.00 ± 0.11 b	12	9.53	< 0.0001
9	0.55 ± 0.07 ab	40	0.67 ± 0.11 a	18	0.00 ± 0.19 b	6	0.33 ± 0.19 ab	6	0.00 ± 0.23 b	4	3.68	0.0089
12	0.85 ± 0.06 a	40	0.67 ± 0.16 a	6	0.50 ± 0.28 a	2	0.00 ± 0.39 a	1	0.00 ± 0.39 a	1	2.67	0.044
15	0.95 ± 0.04 a	40	0.50 ± 0.17 b	2	1.00 ± 0.24 a	1	-----		-----		3.26	0.049
M. boaria leaf	24	n	48	n	72	n	96	n	120	n	F	P
Control	0.00 ± 0.01 a	40	0.00 ± 0.01 a	40	0.00 ± 0.01 a	40	0.00 ± 0.01 a	40	0.03 ± 0.01 a	40	1	0.4088
3	0.10 ± 0.03 a	40	0.03 ± 0.04 a	36	0.03 ± 0.04 a	35	0.06 ± 0.04 a	34	0.03 ± 0.04 a	32	0.76	0.5552
6	0.15 ± 0.04 a	40	0.03 ± 0.04 a	34	0.06 ± 0.04 a	33	0.03 ± 0.04 a	31	0.03 ± 0.04 a	30	1.63	0.1685
9	0.23 ± 0.04 a	40	0.00 ± 0.05 b	31	0.03 ± 0.05 b	31	0.07 ± 0.05 ab	30	0.04 ± 0.05 b	28	4.21	0.0029
12	0.33 ± 0.,06 a	40	0.15 ± 0.07 ab	27	0.04 ± 0.07 b	23	0.14 ± 0.08 ab	22	0.00 ± 0.08 b	19	3.81	0.0058
15	0.20 ± 0.05 a	40	0.09 ± 0.06 a	32	0.14 ± 0.06 a	29	0.04 ± 0.07 a	25	0.08 ± 0.07 a	24	1.13	0.3438
M. magellanica leaf	24	n	48	n	72	n	96	n	120	n	F	P
Control	0.03 ± 0.03 a	40	0.03 ± 0.03 a	39	0.05 ± 0.03 a	38	0.08 ± 0.03 a	36	0.00 ± 0.03 a	33	0.98	0.4211
3	0.13 ± 0.05 a	40	0.19 ± 0.05 a	36	0.07 ± 0.06 a	29	0.00 ± 0.06 a	27	0.19 ± 0.06 a	27	1.88	0.1164
6	0.30 ± 0.06 a	40	0.18 ± 0.08 a	28	0.26 ± 0.09 a	23	0.00 ± 0.10 a	17	0.24 ± 0.10 a	17	1.75	0.1433
9	0.35 ± 0.06 a	40	0.15 ± 0.07 ab	26	0.00 ± 0.08 b	22	0.18 ± 0.08 ab	22	0.00 ± 0.08 b	18	4.8	0.0012
12	0.18 ± 0.05 a	40	0.21 ± 0.06 a	33	0.12 ± 0.06 a	26	0.00 ± 0.07 a	23	0.04 ± 0.07 a	23	2.03	0.0932
15	0.40 ± 0.05 a	40	0.13 ± 0.07 b	24	0.00 ± 0.07 b	21	0.00 ± 0.07 b	21	0.05 ± 0.07 b	21	8.5	< 0.0001
M. disticha leaf	24	n	48	n	72	n	96	n	120	n	F	P
Control	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.03 ± 0.02 a	40	000 ± 0.02 a	39	0.03 ± 0.02 a	39	0.75	0.5569
3	0.10 ± 0.03 a	40	0.03 ± 0.03 a	36	0.03 ± 0.03 a	35	0.03 ± 0.03 a	34	0.00 ± 0.03 a	33	1.4	0.2368
6	0.08 ± 0.03 a	40	0.03 ± 0.03 a	37	0.06 ± 0.03 a	36	0.00 ± 0.03 a	34	0.03 ± 0.03 a	34	0.81	0.5195
9	0.15 ± 0.04 a	40	0.06 ± 0.04 a	34	0.06 ± 0.05 a	32	0.07 ± 0.05 a	30	0.00 ± 0.05 a	28	1.47	0.2123
12	0.15 ± 0.04 a	40	0.03 ± 0.05 a	34	0.09 ± 0.05 a	33	0.07 ± 0.05 a	30	0.04 ± 0.05 a	28	1.19	0.3158
15	0.18 ± 0.05 a	40	0.06 ± 0.05 a	33	0.03 ± 0.05 a	31	0.07 ± 0.05 a	30	0.11 ± 0.05 a	28	1.32	0.2632
M. boaria bark	24	n	48	n	72	n	96	n	120	n	F	P
Control	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.05 ± 0.02 a	40	0.00 ± 0.02 a	38	2.03	0.0923
3	0.08 ± 0.03 a	40	0.03 ± 0.03 a	37	0.03 ± 0.03 a	36	0.00 ± 0.03 a	35	0.00 ± 0.03 a	35	1.35	0.2533
6	0.08 ± 0.03 a	40	0.03 ± 0.03 a	37	0.06 ± 0.03 a	36	0.03 ± 0.04 a	34	0.03 ± 0.04 a	33	0.39	0.8166
9	0.10 ± 0.04 a	40	0.11 ± 0.04 a	36	0.03 ± 0.05 a	32	0.10 ± 0.05 a	31	0.00 ± 0.05 a	28	1.14	0.3417
12	0.10 ± 0.04 a	40	0.06 ± 0.04 a	36	0.09 ± 0.04 a	34	0.03 ± 0.05 a	31	0.07 ± 0.05 a	30	0.37	0.8279
15	0.13 ± 0.04 a	40	0.03 ± 0.04 a	35	0.09 ± 0.04 a	34	0.10 ± 0.05 a	31	0.00 ± 0.05 a	28	1.33	0.262

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at $P < 0.05$.

Table 5. Mortality percentage (means ± standard error) in *A. superciliosus* for type of assay, for an exposure time of 120 hours.

Concentration (%w/v)	*M. boaria* seeds	*M. boaria* leaf	*M. boaria* leaf	*M. magellanica* leaf	*M. disticha* leaf
Choice assay					
Control	0.07 ± 0.05 d	0.13 ± 0.07 c	0.08 ± 0.07 b	0.03 ± 0.07 b	0.13 ± 0.07 c
2	0.45 ± 0.05 c	0.35 ± 0.07 bc	0.23 ± 0.07 ab	0.20 ± 0.07 ab	0.20 ± 0.07 bc
5	0.80 ± 0.05 b	0.53 ± 0.07 b	0.43 ± 0.06 a	0.35 ± 0.07 a	0.38 ± 0.07 bc
10	1.00 ± 0.05 a	0.6 ± 0.07 b	0.40 ± 0.06 a	0.35 ± 0.07 a	0.45 ± 0.07 ab
20	1.00 ± 0.05 a	0.63 ± 0.07 b	0.35 ± 0.06 ab	0.45 ± 0.07 a	0.68 ± 0.07 a
30	1.00 ± 0.05 a	0.92 ± 0.07 a	0.28 ± 0.07 ab	0.38 ± 0.07 a	0.68 ± 0.07 a
n	240	240	240	240	240
F	71.69	15.17	3.43	4.94	10.55
P	< 0.0001	< 0.0001	0.0052	0.0003	< 0.0001
No-Choice assay					
Control	0.07 ± 0.05 c	0.2 ± 0.15 b	0.2 ± 0.15 ab	0.1 ± 0.15 a	0.13 ± 0.07 c
2	0.72 ± 0.05 b	0.3 ± 0.15 ab	0.5 ± 0.15 ab	0.2 ± 0.15 a	0.28 ± 0.07 c
5	0.90 ± 0.05 ab	0.9 ± 0.15 a	0.9 ± 0.15 a	0.5 ± 0.15 a	0.33 ± 0.07 bc
10	0.90 ± 0.05 ab	0.7 ± 0.15 ab	0.7 ± 0.15 ab	0.5 ± 0.15 a	0.60 ± 0.07 ab
20	0.80 ± 0.05 ab	0.5 ± 0.15 ab	0.5 ± 0.15 ab	0.6 ± 0.15 a	0.65 ± 0.07 a
30	0.95 ± 0.05 a	0.5 ± 0.15 ab	0.4 ± 0.15 ab	0.7 ± 0.15 a	0.70 ± 0.07 a
n	240	240	240	240	240
F	38.51	3.03	2.64	2.46	10.74
P	< 0.0001	0.0175	0.0331	0.0443	< 0.0001

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05.

Table 6. Mortality percentage (means ± standard error) in *A. superciliosus* for evaluation time.

Evaluation time (hours)	*M. boaria* seeds	*M. boaria* leaf	*M. magellanica* leaf	*M. disticha* leaf	*M. boaria* bark
Choice assay					
24	0.09 ± 0.03 d	0.08 ± 0.03 b	0.01 ± 0.02 c	0.06 ± 0.02 b	0.00 ± 0.02 c
n	200	200	200	200	200
48	0.14 ± 0.03 cd	0.15 ± 0.03 ab	0.01 ± 0.02 c	0.08 ± 0.02 b	0.01 ± 0.02 c
n	182	184	199	187	200
72	0.22 ± 0.03 bc	0.23 ± 0.03 a	0.03 ± 0.02 c	0.13 ± 0.02 ab	0.09 ± 0.02 b
n	156	156	198	172	197
96	0.31 ± 0.04 b	0.18 ± 0.03 ab	0.13 ± 0.02 b	0.19 ± 0.03 a	0.09 ± 0.02 b
n	122	120	193	150	179
120	0.64 ± 0.04 a	0.22 ± 0.04 a	0.24 ± 0.02 a	0.14 ± 0.03 ab	0.19 ± 0.02 a
n	84	99	168	122	163
F	34.13	4.65	30.18	4	16.2
P	< 0.0001	0.001	< 0.0001	0.0032	< 0.0001
No-Choice assay					
24	0.01 ± 0.01 b	0.22 ± 0.06 ab	0.06 ± 0.04 b	0.06 ± 0.01 a	0.12 ± 0.04 b
n	200	50	50	200	50
48	0.02 ± 0.01 b	0.36 ± 0.06 a	0.02 ± 0.05 b	0.02 ± 0.01 ab	0.07 ± 0.04 b
n	198	39	47	188	44
72	0.03 ± 0.01 b	0.12 ± 0.08 ab	0.41 ± 0.05 a	0.04 ± 0.01 ab	0.02 ± 0.05 b
n	194	25	46	185	41
96	0.04 ± 0.02b	0.09 ± 0.08 ab	0.07 ± 0.06 b	0.01 ± 0.01 a	0.03 ± 0.05 b
n	188	22	27	177	40
120	0.14 ± 0.02 a	0.05 ± 0.09 b	0.08 ± 0.06 b	0.02 ± 0.01 ab	0.31 ± 0.05 a
n	180	20	25	175	39
F	11.61	3.03	11.77	2.92	6.25
P	< 0.0001	0.0178	< 0.0001	0.0205	0.0001

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at P < 0.05.

Table 7. Mortality percentage (means ± standard error) in *A. superciliosus* for evaluation time.

Concentration (% w/v)	Evaluation time (hours)											
	24	n	48	n	72	n	96	n	120	n	F	P
					Choice assay							
M. boaria seeds												
No aplication	0.02 ± 0.02 a	40	0.03 ± 0.02 a	39	0.00 ± 0.02 a	38	0.03 ± 0.02 a	38	0.00 ± 0.02 a	37	0.48	0.7496
2	0.05 ± 0.05 a	40	0.08 ± 0.05 a	38	0.14 ± 0.05 a	35	0.13 ± 0.06 a	30	0.15 ± 0.05 a	26	0.73	0.5703
5	0.10 ± 0.06 b	40	0.20 ± 0.07 b	35	0.18 ± 0.08 b	28	0.30 ± 0.08 ab	23	0.56 ± 0.10 a	16	4.14	0.0034
10	0.13 ± 0.06 b	40	0.14 ± 0.06 b	35	0.27 ± 0.07 b	30	0.41 ± 0.08 b	22	1.00 ± 0.,11 a	13	14.84	< 0.0001
20	0.08 ± 0.06 c	40	0.14 ± 0.06 bc	37	0.22 ± 0.06 bc	32	0.36 ± 0.07 b	25	1.00 ± 0.09 a	16	21.77	< 0.0001
30	0.10 ± 0.06 c	40	0.17 ± 0.06 bc	36	0.30 ± 0.07 bc	30	0.43 ± 0.08 b	21	1.00 ± 0.11 a	12	14.12	< 0.0001
M. boaria leaf												
No aplication	0.00 ± 0.03 a	40	0.03 ± 0.03 a	40	0.03 ± 0.03 a	39	0.03 ± 0.03 a	38	0.05 ± 0.03 a	37	0.55	0.6982
2	0.08 ± 0.05 a	40	0.08 ± 0.05 a	37	0.09 ± 0.05 a	34	0.10 ± 0.05 a	31	0.14 ± 0.06 a	28	0.25	0.9067
5	0.02 ± 0.05 a	40	0.13 ± 0.05 a	39	0.24 ± 0.06 a	34	0.12 ± 0.07 a	26	0.17 ± 0.07 a	23	1.96	0.1038
10	0.13 ± 0.06 a	40	0.20 ± 0.06 a	35	0.18 ± 0.07 a	28	0.13 ± 0.08 a	23	0.20 ± 0.08 a	20	0.29	0.8837
20	0.02 ± 0.06 b	40	0.13 ± 0.06 ab	39	0.18 ± 0.06 ab	34	0.29 ± 0.07 a	28	0.25 ± 0.08 ab	20	2.72	0.0315
30	0.15 ± 0.07 c	40	0.24 ± 0.08 bc	34	0.54 ± 0.09 ab	26	0.33 ± 0.13 abc	12	0.63 ± 0.16 a	8	4.36	0.0025
M. magellanica leaf												
No aplication	0.00 ± 0.01 a	40	0.00 ± 0.01 a	40	0.00 ± 0.01 a	40	0.00 ± 0.01 a	40	0.03 ± 0.01 a	40	1	0.4088
2	0.00 ± 0.03 a	40	0.00 ± 0.03 a	40	0.03 ± 0.03 a	40	0.08 ± 0.03 a	39	0.11 ± 0.03 a	36	2.42	0.0499
5	0.00 ± 0.04 b	40	0.00 ± 0.04 b	40	0.05 ± 0.04 b	40	0.11 ± 0.04 ab	38	0.24 ± 0.04 a	34	5.57	0.0003
10	0.00 ± 0.04 b	40	0.00 ± 0.04 b	40	0.03 ± 0.04 b	40	0.10 ± 0.04 b	39	0.26 ± 0.04 a	35	7.3	< 0.0001
20	0.00 ± 0.04 b	40	0.03 ± 0.04 b	40	0.00 ± 0.04 b	39	0.21 ± 0.04 a	39	0.32 ± 0.05 a	31	9.72	< 0.0001
30	0.02 ± 0.04 b	40	0.00 ± 0.04 b	39	0.03 ± 0.04 b	39	0.16 ± 0.04 ab	38	0.28 ± 0.05 a	32	6.77	< 0.0001
M. disticha leaf												
No aplication	0.00 ± 0.03 a	40	0.00 ± 0.03 a	40	0.00 ± 0.03 a	39	0.00 ± 0.03 a	39	0.00 ± 0.03 a	37	1.07	0.3719
2	0.00 ± 0.03 a	40	0.05 ± 0.03 a	40	0.05 ± 0.03 a	38	0.08 ± 0.03 a	36	0.03 ± 0.04 a	33	0.87	0.4844
5	0.08 ± 0.05 a	40	0.11 ± 0.05 a	37	0.06 ± 0.05 a	33	0.10 ± 0.05 a	31	0.11 ± 0.05 a	28	0.18	0.9496
10	0.08 ± 0.05 a	40	0.05 ± 0.05 a	37	0.14 ± 0.05 a	35	0.20 ± 0.06 a	30	0.08 ± 0.06 a	24	1.19	0.3156
20	0.10 ± 0.06 a	40	0.08 ± 0.06 a	36	0.21 ± 0.07 a	33	0.35 ± 0.07 a	26	0.24 ± 0.09 a	17	2.46	0.0476
30	0.08 ± 0.06 b	40	0.11 ± 0.06 ab	37	0.18 ± 0.06 ab	33	0.26 ± 0.07 ab	27	0.35 ± 0.08 a	20	2.48	0.0463
M. boaria bark												
No aplication	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.03 ± 0.02 a	40	0.05 ± 0.02 a	39	1.39	0.2388
2	0.00 ± 0.03 a	40	0.00 ± 0.03 a	40	0.10 ± 0.03 a	40	0.06 ± 0.04 a	36	0.09 ± 0.04 a	34	1.97	0.1015
5	0.00 ± 0.04 b	40	0.02 ± 0.04 b	40	0.10 ± 0.04 ab	39	0.11 ± 0.05 ab	35	0.26 ± 0.05 a	31	4.47	0.0018

(Continúa)

Concentration (% w/v)	Evaluation time (hours)											
	24	n	48	n	72	n	96	n	120	n	F	P
10	0.00 ± 0.04 b	40	0.00 ± 0.04 b	40	0.15 ± 0.04 ab	40	0.12 ± 0.05 ab	34	0.20 ± 0.05 a	30	3.93	0.0044
20	0.00 ± 0.04 b	40	0.03 ± 0.04 b	40	0.05 ± 0.04 b	39	0.11 ± 0.04 ab	37	0.24 ± 0.05 a	33	4.82	0.001
30	0.00 ± 0.04 b	40	0.02 ± 0.04 b	40	0.05 ± 0.04 ab	39	0.05 ± 0.04 ab	37	0.17 ± 0.04 a	35	3.01	0.0194
No-Choice assay												
M. boaria seeds												
No aplication	0.00 ± 0.02 a	40	0.00 ± 0.02 a	40	0.03 ± 0.02 a	40	0.00 ± 0.02 a	39	0.05 ± 0.02 a	39	1.38	0.2426
2	0.00 ± 0.04 b	40	0.00 ± 0.04 b	40	0.02 ± 0.04 b	40	0.03 ± 0.04 b	39	0.71 ± 0.04 a	38	73.56	< 0.0001
5	0.00 ± 0.04 b	40	0.03 ± 0.04 b	40	0.05 ± 0.04 b	39	0.08 ± 0.04 b	37	0.82 ± 0.04 a	34	75.89	< 0.0001
10	0.02 ± 0.05 b	40	0.08 ± 0.05 b	39	0.11 ± 0.05 b	36	0.16 ± 0.05 b	32	0.81 ± 0.06 a	27	33.51	< 0.0001
20	0.10 ± 0.06 c	40	0.14 ± 0.07 bc	36	0.19 ± 0.07 bc	31	0.40 ± 0.08 b	25	0.73 ± 0.10 a	15	9.02	< 0.0001
30	0.03 ± 0.06 c	40	0.10 ± 0.06 c	39	0.26 ± 0.06 bc	35	0.50 ± 0.07 b	26	0.85 ± 0.10 a	13	17.35	< 0.0001
M. boaria leaf												
No aplic/ation	0.00 ± 0.07 a	10	0.00 ± 0.07 a	10	0.10 ± 0.07 a	10	0.11 ± 0.07 a	9	0.00 ± 0.07 a	8	0.74	0.5703
2	0.10 ± 0.08 a	10	0.11 ± 0.09 a	9	0.00 ± 0.09 a	8	0.00 ± 0.09 a	8	0.13 ± 0.09 a	8	0.45	0.7701
5	0.30 ± 0.16 a	10	0.57 ± 0.19 a	7	0.67 ± 0.30 a	3	0.00 ± 0.51 a	1	0.00 ± 0.51 a	1	0.79	0.5452
10	0.40 ± 0.14 a	10	0.33 ± 0.18 a	6	0.00 ± 0.23 a	4	0.25 ± 0.23 a	4	0.00 ± 0.26 a	3	0.86	0.5026
20	0.10 ± 0.11 a	10	0.33 ± 0.12 a	9	0.00 ± 0.14 a	6	0.17 ± 0.14 a	6	0.00 ± 0.16 a	5	1.19	0.3356
30	0.20 ± 0.14 a	10	0.50 ± 0.15 a	8	0.25 ± 0.22 a	4	0.00 ± 0.25 a	3	0.00 ± 0.25 a	3	1.19	0.3418
M.magellanica leaf												
No aplication	0.00 ± 0.04 a	10	0.00 ± 0.04 a	10	0.00 ± 0.04 a	10	0.00 ± 0.04 a	10	0.10 ± 0.04 a	10	1	0.4175
2	0.10 ± 0.07 a	10	0.00 ± 0.07 a	9	0.00 ± 0.07 a	9	0.00 ± 0.07 a	9	0.11 ± 0.07 a	9	0.71	0.5889
5	0.10 ± 0.10 a	10	0.00 ± 0.10 a	9	0.00 ± 0.10 a	9	0.11 ± 0.10 a	9	0.38 ± 0.11 a	8	2.13	0.0948
10	0.20 ± 0.11 a	10	0.25 ± 0.13 a	8	0.00 ± 0.15 a	6	0.00 ± 0.15 a	6	0.17 ± 0.15 a	6	0.73	0.5762
20	0.10 ± 0.11 a	10	0.11 ± 0.12 a	9	0.13 ± 0.12 a	8	0.00 ± 0.13 a	7	0.43 ± 0.13 a	7	1.53	0.2145
30	0.10 ± 0.11 ab	10	0.22 ± 0.12 ab	9	0.00 ± 0.13 b	7	0.00 ± 0.13 b	7	0.57 ± 0.13 a	7	3.37	0.0196
M. disticha leaf												
No aplication	0.03 ± 0.03 a	40	0.03 ± 0.03 a	39	0.05 ± 0.03 a	38	0.00 ± 0.03 a	36	0.03 ± 0.03 a	36	0.49	0.7424
2	0.00 ± 0.04 a	40	0.08 ± 0.04 a	40	0.03 ± 0.04 a	37	0.06 ± 0.04 a	36	0.15 ± 0.04 a	34	2.07	0.086
5	0.02 ± 0.04 a	40	0.03 ± 0.04 a	39	0.13 ± 0.04 a	38	0.06 ± 0.04 a	33	0.13 ± 0.05 a	31	1.56	0.1881
10	0.35 ± 0.06 a	40	0.12 ± 0.07 ab	26	0.30 ± 0.08 ab	23	0.00 ± 0.09 b	16	0.00 ± 0.09 b	16	4.56	0.0018
20	0.30 ± 0.06 a	40	0.14 ± 0.08 a	28	0.33 ± 0.08 a	24	0.06 ± 0.10 a	16	0.07 ± 0.10 a	15	2.28	0.0647
30	0.45 ± 0.06 a	40	0.05 ± 0.08 b	22	0.43 ± 0.09 b	21	0.00 ± 0.11 b	12	0.00 ± 0.11 b	12	7.45	< 0.0001

(Continúa)

(Continuación Tabla 7)

Concentration (% w/v)	Evaluation time (hours)											
	24	**n**	**48**	**n**	**72**	**n**	**96**	**n**	**120**	**n**	**F**	**P**
M. boaria bark												
No aplication	0.00 ± 0.06 a	10	0.00 ± 0.06 a	10	0.00 ± 0.06 a	10	0.10 ± 0.06 a	10	0.11 ± 0.06 a	9	0.8	0.5341
2	0.10 ± 0.10 a	10	0.00 ± 0.11 a	9	0.33 ± 0.11 a	9	0.17 ± 0.14 a	6	0.00 ± 0.11 a	5	1.42	0.247
5	0.00 ± 0.05 a	10	0.00 ± 0.05 a	10	0.10 ± 0.05 a	10	0.00 ± 0.05 a	9	0.00 ± 0.05 a	9	0.95	0.447
10	0.00 ± 0.07 a	10	0.00 ± 0.07 a	10	0.30 ± 0.07 a	10	0.00 ± 0.09 a	7	0.00 ± 0.09 a	7	3.23	0.0221
20	0.00 ± 0.11 a	10	0.10 ± 0.11 a	10	0.22 ± 0.11 a	9	0.14 ± 0.13 a	7	0.17 ± 0.14 a	6	0.58	0.6809
30	0.00 ± 0.10 b	10	0.00 ± 0.10 b	10	0.50 ± 0.10 a	10	0.00 ± 0.14 b	5	0.20 ± 0.14 ab	5	4.77	0.0035

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at $P < 0.05$.

stimulants, affecting their ability to find and recognize their host. These metabolites are probably those obtained in this case with ethanol as solvent. Zapata *et al.* (2006) showed that the anti-feeding effect of *M. boaria* on the third larval stage of *Spodoptera littoralis* was attributable to the presence of dihydro-β-agarofurane sesquiterpenes, which are very abundant in this plant.

Table 8 give the leaf area percentages consumed per extract and treatment for choice (C) and no-choice (NC) assays respectively. These tables show that percentage leaf area consumed was lowest in the C and NC assays with ethanolic extract of seeds of *M. boaria* at higher concentrations. There were no significant differences in consumption by evaluation time, decreasing to 72 hours in the no-choice assay. The mortality values and leaf area consumed obtained for this extract are consistent with a high insecticide activity recorded by Céspedes *et al*. (2001) on *S. frugiperda* (Smith) (Lepidoptera: Noctuidae), who conclude that the effect seems to be a combination of antifeedant action and post-gastrointestinal toxicity. While with MML extract, in the C assay for all concentrations the consumption was lower than in the control. However, in the NC assay at lower concentration the consumption was higher than control.

Most works that antifeedant and insecticidal activity of plants belonging to the genus *Maytenus* reported have been tested against the insect *S. littoralis* such as: *M. senegalensis* (Lam.) Exell showed a high antifeedant and insecticidal activity on third larval instar of *S. littoralis*. It has effects on behavior and post ingestive toxicity on the developmental stages of *S. littoralis* (El-Aziz and El-Din 2007). From *M. canariensis* (Loes.) G. Kunkel & Sund. five new sesquiterpenes with skeleton dihydro-β-agarofurano were isolated, which showed antifeedant activity against *S. littoralis* at concentration 10 µg/ml (González *et al*. 1993); and from leaves of *M. chiapensis* Lundell wilfordine, euonine and alatusinine compounds, that showed strong antifeedant activity on *S. littoralis*, were isolated (Nuñez *et al*. 2004).

Conclusions

Based on our results, the ethanolic extract of *Maytenus boaria* seeds shows promise due to its insecticidal action, and their possible use the anti-feeding agents, on adults of *Aegorhinus superciliosus*. For the percentage leaf area consumed, the highest consumption in the choice assay was recorded with the ethanolic extract of *M. boaria* bark with no differences of control, while in the no-choice assay the highest consumption was reached with the ethanolic extract of *Maytenus magellanica* leaf, being greater than control at the lowest concentration, and decreasing as it increases. Therefore, from these species it is possible to develop an inhibitor of feeding and a bait for *A. superciliosus*. Furthermore, *Hippodamia convergens* was less susceptible to the ethanolic extract of *Maytenus boaria* seeds than *A. superciliosus*.

Acknowledgements

The authors of the present work are grateful for the support of DIUFRO Project DI14-0111, of Universidad de La Frontera, Temuco, Chile.

Table 8. Percentage leaf area consumed (mean % ± standard error) of *Rubus ulmifolius* per assay.

Concentration (%w/v)	*M. boaria* seeds	*M. boaria* leaf	*M. boaria* bark	*M. magellanica* leaf	*M. disticha* leaf
Choice assay					
Control	5.14 ± 0.36 a	4.01 ± 0.38 a	6.34 ± 0.64 abc	6.50 ± 0.40 a	2.40 ± 0.50 ab
2	2.74 ± 0.36 b	3.72 ± 0.38 a	8.13 ± 0.64 a	4.63 ± 0.40 b	3.84 ± 0.50 ab
5	4.64 ± 0.36 a	2.75 ± 0.38 ab	3.68 ± 0.64 c	5.51 ± 0.40 ab	2.53 ± 0.50 ab
10	2.23 ± 0.36 bc	1.47 ± 0.38 b	7.00 ± 0.64 ab	4.05 ± 0.40 b	4.49 ± 0.50 a
20	0.78 ± 0.36 c	2.09 ± 0.38 b	4.66 ± 0.64 bc	0.68 ± 0.40 c	3.06 ± 0.50 ab
30	0.72 ± 0.36 c	1.93 ± 0.38 b	4.96 ± 0.64 bc	0.97 ± 0.40 c	2.20 ± 0.50 b
n	60	60	60	60	60
F	26.4	7.06	6.6	36.01	3.26
P	< 0.0001	< 0.0001	0.0001	< 0.0001	0.0121
No-choice assay					
Control	3.78 ± 0.29 a	5.60 ± 0.62 a	5.37 ± 0.68 a	9.46 ± 0.87 bc	5.84 ± 0.66 ab
2	2.88 ± 0.29 ab	3.30 ± 0.62 ab	4.16 ± 0.68 ab	15.56 ± 0.87 a	3.16 ± 0.66 b
5	1.32 ± 0.29 cd	2.14 ± 0.62 b	5.28 ± 0.68 a	10.36 ± 0.87 b	3.18 ± 0.66 b
10	2.30 ± 0.29 bc	2.18 ± 0.62 b	3.23 ± 0.68 abc	6.83 ± 0.87 bc	6.21 ± 0.66 a
20	0.92 ± 0.29 d	2.28 ± 0.62 b	1.73 ± 0.68 bc	6.17 ± 0.87 cd	3.46 ± 0.66 ab
30	0.60 ± 0.29 d	2.84 ± 0.62 b	0.79 ± 0.68 c	2.86 ± 0.87 d	5.37 ± 0.66 ab
n	60	60	60	60	60
F	17.74	4.61	7.51	24.85	4.65
P	< 0.0001	0.0014	< 0.0001	< 0.0001	0.0013

Different letters in the columns indicate significant differences after analysis by ANOVA followed by the Tukey's test at $P < 0.05$.

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Received: 15-Jan-2016 • Accepted: 22-Sep-2017

Suggested citation:

ZAVALA H., A.; HORMAZABAL U., E.; MONTENEGRO R., G.; ROSALEZ V., M.; QUIROZ C., A.; PAZ R., C.; REBOLLEDO R., R. 2017. Effects of extracts from *Maytenus* on *Aegorhinus superciliosus* (Coleoptera: Curculionidae) and *Hippodamia convergens* (Coleoptera: Coccinellidae). Revista Colombiana de Entomología 43 (2): 233-244. Julio - Diciembre 2017. ISSN 0120-0488.