ARTÍCULO CIENTÍFICO

Antifeedant activity of alkaloid extracts from calafate (*Berberis microphylla*, G. Forst, 1789) against diamondback moth larvae (*Plutella xylostella*, Linnaeus, 1758)

Actividad antialimentaria de los extractos de alcaloides de calafate (Berberis microphylla, G. Forst, 1789) sobre larvas de la polilla de la col (Plutella xylostella, Linnaeus, 1758)

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Abstract
In laboratory by dual choice test, stem and root alkaloid extracts of *Berberis microphylla* showed antifeedant activity against third-instar *Plutella xylostella* larvae. Stem extracts showed significantly lower leaf consumptions at concentrations of 500 and 1000 mg/L, as illustrated by antifeedant index of 72 and 97% respectively, whereas, root extracts were significantly active at 100, 500 and 1000 mg/L with antifeedant index of 68, 85 and 99% respectively. In addition, the pure berberine identified as the major alkaloid in the plant extracts showed significant lower food consumption by larvae only at concentration of 1000 mg/L, but the pure palmatine not affect feeding at any concentration. The results suggest that alkaloids extract of *B. microphylla* have promising antifeedant activity and could by incorporated in an integrated pest management (IPM) programs for *P. xylostella*.

Key words:
antifeedant activity, alkaloid extracts.

Resumen
En laboratorio, mediante prueba de doble elección los extractos de alcaloides obtenidos de tallos y raíces de *Berberis microphylla* mostraron actividad antialimentaria frente a larvas de tercer estadio de *Plutella xylostella*. Los extractos de tallos mostraron consumos foliares significativamente más bajos en concentraciones de 500 y 1000 mg/L con un índice antialimentario de 72 y 97% respectivamente, mientras que los extractos de raíces fueron significativamente más activos a 100, 500 y 1000 mg/L con un índice antialimentario de 68, 85 y 99% respectivamente. Además, berberina identificada como el principal alcaloide de los extractos tuvo significativamente un menor consumo de alimento por parte de las larvas mostrando su efecto solo a la concentración de 1000 mg/L. Por otra parte, palmatina no tuvo efecto en la conducta de alimentación de las larvas a ninguna de las concentración evaluadas. Los resultados sugieren que los extractos de alcaloides obtenidos de *B. microphylla* tienen una prometedora actividad antialimentaria y podrían ser incorporados en un programa de manejo integrado de plagas (MIP) para *P. xylostella*.

Palabras clave:
actividad antialimentaria, extractos de alcaloides.

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INTRODUCTION

Insect pests are one of the biotic factors that cause significant crop losses worldwide, where the use of synthetic pesticides (*eg.* organophosphates and pyrethroids) is a control strategy used traditionally for to pest control (Birch *et al.* 2011; Zhang *et al.* 2011; Chareonviriyaphap *et al.* 2013). However, the chemical control has become less effective in the last decades because of its rapid developing resistance (Shelton *et al.* 1993). In this sense, the indiscriminate use of synthetic pesticide chemicals has given rise to many ecological problems, among which, toxic residues and harm to mammals are the most crucial (Carriger *et al.* 2006; Aktar *et al.* 2009; Damalas & Eleftherohorinos, 2011). For this reason, the use of naturally derived plant products emerges as environmentally friendly alternatives to synthetic pesticide, with applications as botanical insecticides, antifeedants and insect growth regulators (Isman, 1994, 2006; Sarkar & Kshirsagar, 2014; Kedia *et al.* 2015).

In the case of natural antifeedant, has as an advantage that it never kills target insects directly allowing them to be available to their natural enemies and thus help in the maintenance of the natural balance (Jeyasankar *et al*. 2012). Several investigators reported that alkaloids are strong natural insect antifeedants (González-Coloma *et al.* 1998; Brem *et al.* 2002; Mao & Henderson, 2007; Cornelious *et al*. 2009; Sani *et al*. 2014). In this sense, *Berberis* genus are characterized to produced alkaloids with an interesting spectrum of insecticide and antifeedant properties (Tewary *et al.* 2005; Quevedo *et al*. 2007; Rehman *et al.* 2018). *Berberis microphylla* (known also *B. buxifolia*), is a native plant growing abundantly in southern regions of Chile and Argentina (Patagonia) with medicinal values attributable to the presence of alkaloids (Pitta-Álvarez *et al.* 2008; Domínguez *et al*. 2012; Manosalva *et al.* 2016). However, studies on the biological activity of *B. microphylla* against insects have not been reported.

For this reason, the aim of this study was to evaluate the antifeedant activity of stem and root alkaloid extracts from *B. microphylla* against larvae of *Plutella xylostella*. In addition, two synthetic compounds, berberine and palmatine previously identified in *B. microphylla* (Manosalva *et al.* 2014) were evaluated.

MATERIALS AND METHODS

Plant material

Stems and roots samples of *B. microphylla* were collected during the flowering season (December of 2011) near the shores of Deseado Lake, Province of Tierra del Fuego (54°22'12.4"S; 68°45'45.0"W). A voucher specimen was deposited in the herbarium at the Universidad de Concepción (Voucher N° CONC 178057). The plant material was vacuum-packed and stored at -20°C for further study.

Alkaloids extraction

In this procedure, oven dried and powdered leaves (100 g), stems (300 g) and roots (300 g) of *B. microphylla* were sequentially extracted (24, 48 and 72 h) with methanol at room temperature. Methanolic extracts were evaporated in vacuo at 40°C, and the residue reconstituted with 200 mL 10% HCl for 1 h under agitation (orbital shaker, MS-NOR, Taiwan), and allowed to stand for 12 h at 10°C prior to filtering. The filtrate was washed with $CHCl_3$ (5 x 100 mL). The aqueous phase was adjusted to pH 10 with NH_4OH and extracted with $CHCl_3$ (5 x 100 mL). Finally, the solvent was evaporated under reduce pressure for obtaining dried extract containing alkaloids. The solvent was evaporated for obtaining crude extract containing alkaloids.

Alkaloid standards

Berberine chloride (purity, >98%), palmatine chloride (purity, >97%) and all analytical grade solvents used for extractions, were purchased from Sigma Aldrich (St. Louis, USA).

Insects

The larvae of *P. xylostella* were obtained from the laboratory of the Biological Chemistry and Crop Protection Department, Rothamsted Research Station (Harpenden, UK). Culture of larvae were maintained on cabbage plants at 25

± 2°C, under a 16:8 light: dark cycle. One-day-old third instar larvae were selected for antifeedant assay.

Antifeedant assay

The antifeedant activity of stem and root alkaloid extracts of *B. microphylla* and pure compounds (berberine and palmatine) identified in the plant extracts were assessed via dual-choice bioassays using third-instar larvae of *P. xylostella*. In the dual-choice test, each insect was given a choice between a treated and an untreated cabbage leaf dics. If the substance acted as an antifeedant, insects would then eat a greater amount of the untreated leaves. Concentrations of 100, 500 and 1000 mg/L of each plant extracts and 10, 100 and 1000 mg/L of pure compounds were prepared with ethanol to avoid damaging the leaves, and this solvent used as control. Each treatment was separately applied on half of a fully opened leaf by dipping both sides of the leaf blade up to the midrib in the appropriate solution. The leaf disc (5 cm diameter) was then placed on a filter paper into a tray and kept in the fume cupboard for ca. 20 min to dry. Thereafter, the leaf was placed in a Petri dish padded with a moist filter paper marked on one side to distinguish the treated half of the leaf. Five third-instar *P. xylostella* larvae previously starved for 24 h were then introduced and the dishes kept in the laboratory for 12 h. Each treatment and control were replicated ten times. The eaten leaf area of the treated leaf portion and that of control were recorded separately, and expressed in square centimeters (cm^2) using coordinate paper (Ling *et al.* 2008).

Furthemore, the percentage antifeedant (AFI) of compounds was calculated using an antifeedant index (AFI) (Krishnakumari *et al.* 2001):

$$AFI (\%) = (1 - T / C) \times 100$$

where C is the leaf area consumed in control and T is the leaf area consumed in treatments.

Statistical analysis

Insecticide and antifeedant activities of alkaloid extracts from *B. microphylla* and pure compounds were statistically analyzed by ANOVA ($P < 0.05$) with Sigma Plot test.

RESULTS

Table 1. Antifeedant activity of alkaloid extracts from *B. microphylla* and pure compounds against the third instar larvae of *P. xylostella*.

Plant extracts	Mean leaf consumed (cm^2)[a]		AFI (%)[b]	Mean leaf consumed (cm^2)[a]		AFI (%)[b]	Mean leaf consumed (cm^2)[a]		AFI (%)[b]
	100 mg/L	Control		500 mg/L	Control		1000 mg/L	Control	
Stem	0.91 ± 0.12	1.25 ± 0.14	27	0.57* ± 0.15	2.00 ± 0.15	72	0.02* ± 0.00	0.73 ± 0.16	97
Root	0.23* ± 0.07	0.73 ± 0.16	68	0.17* ± 0.05	1.15 ± 0.15	85	0.03* ± 0.00	2.35 ± 0.26	99
Pure compounds	Mean leaf consumed (cm^2)[a]		AFI (%)[b]	Mean leaf consumed (cm^2)[a]		AFI (%)[b]	Mean leaf consumed (cm^2)[a]		AFI (%)[b]
	10 mg/L	Control		100 mg/L	Control		1000 mg/L	Control	
Berberine	0.41 ± 0.08	0.58 ± 0.18	29	0.37 ± 0.11	0.80 ± 0.01	54	0.19* ± 0.02	1.69 ± 0.11	89
Palmatine	0.60 ± 0.12	1.22 ± 0.15	51	0.82 ± 0.21	0.85 ± 0.17	4	0.23 ± 0.04	0.32 ± 0.11	28

[a] Values are mean S.E. (n=10); * indicated significant differences with the control according to Anova test ($p < 0.05$).

[b] Antifeedant Index: AFI (%)= (1- T/ C) x100, C and T represents consumption of control and treated leaf discs, respective.

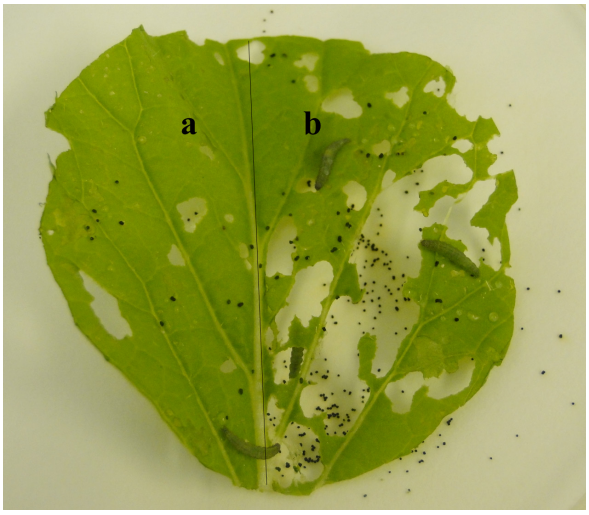

Fig. 1. Photograph of antifeedant activity of root alkaloid extract of *B. microphylla* at 1000 mg/L after 12 h treatment by via dual-choice bioassays with third-instar larvae of *P. xylostella*. (a) control, (b) treated leaf.

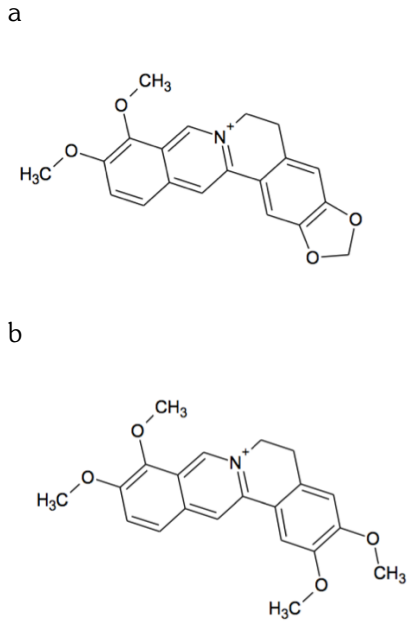

Fig. 2. Structure of berberine (a) and palmatine (b) identified in stem and root extracts of *B. microphylla*.

The results of antifeedant activity of alkaloid extracts from *B. microphylla* and pure compounds are presented in Table 1. Stem and root alkaloid extracts at concentration of 500 and 1000 mg/L significantly reduced leaf consumptions by *P. xylostella* larvae. Thus, at 500 mg/L, stems and root extracts elicited a decrease the insect feeding behavior with antifeedant index of 72% and 85% respectively. At de concentration of 1000 mg/L, stem and root extracts deterrent feeding by larvae of *P. xylostella* with antifeedant index of 97 and 99% respectively (Fig. 1). The antifeeding activity depending on the concentration of the plant extracts and all the activities are dose dependent.

Furthemore, the *P. xylostella* larvae were fed with leaves treated with berberine and palmatine (Fig. 2). Berberine exhibited significant antifeedant activity only at the highest concentration of 1000 mg/L, whereas palmatine did not show such activity.

DISCUSSION

Our results showed that *B. microphylla* stem and root alkaloid extracts have antifeedant activity against *P. xylostella* larvae because reducing leaf consumptions. Some investigators have reported feeding deterrent activity of plant alkaloids against *P. xylostella*. Banaag *et al.* (1997) shown that alkaloid fractions from *Dioscorea hispida* not only inhibited the feeding of *Plutella xylostella* larvae, but had toxic activity and disrupted of adult emergence. In the same way, Guo *et al.* (2014) found that two alkaloids (7-demethoxytylophorine and 6-hydroxyl-2,3-dimethoxy phenanthroindolizidine), isolated from *Cynanchum komarovii* were active against the third-instar larvae of *P. xylostella* as insecticides, antifeedants and growth inhibitors. Other study by Paulraj *et al.* (2014) reported that leaf fraction from *Adhatoda vasica* contain alkaloids called vasicine acetate and 2-Acetyl benzylamine that present antifeedant activity against *P. xylostella* larve. Furthermore, in relation to biological activity of *Berberis* species has been report that alkaloids identified in stem and leaf extracts of *B. glauca* were showed antifeedant effects on larvae 3rd y 4th stage of the moth *Spodoptera sunia*, as well as toxic effects (Moreno-Murillo *et al.* 1995).

In previous studies, we have reported that stem and root extracts of *B. microphylla* showed a complex mixture of isoquinoline alkaloids. The roots contain of the following alkaloids: allocryptopine, berberine, calafatine, jatrorrhizine, palmatine, protopine, reticuline and thalifendine. Stems also contain of the following alkaloids: allocryptopine,

berberine, isocorydine, jatrorrhizine, protopine, scoulerine and thalifendine (Manosalva *et al.* 2014). In this sense, two synthetic alkaloids, berberine and palmatine identified in stem and root extracts were tested, where only berberine reduced larval feeding of *P. xylostella*. Shields *et al.* (2008), evaluated feeding deterrents of nine alkaloids (acridine, aristolochic acid, atropine, berberine, caffeine, nicotine, scopolamine, sparteine and strychnine) against *Lymantria dispar* larvae. Results suggested that berberine and aristolochic acid were the two most potent feeding deterrents. The results obtained for berberine are interesting because this compounds is a major constituent in stems and roots of *Berberis* plants (Ivanovska *et al.* 1996). Park *et al.* (2000) indicating that berberine and palmatine identified in roots of *Coptis japonica* had antifeeding activity against *Hyphantria cunea* larvae when treated by leaf-dipping assay. They suggested that the antifeeding activity was much more pronounced in applications of mixtures of berberine and palmatine, indicating a synergistic effect.

Considering that the alkaloid extracts from stem and root of *B. microphylla* are a complexely mixture of alkaloids (Manosalva *et al.* 2014) the antifeedant activity against *P. xylostella* it could be related to the synergistic effect among alkaloids present in the extracts. Future studies will be focused in identifying other bioactive components present in plant extracts of *B. microphylla* against *P. xylostella* larvae to allow the development of botanical insecticides with greater effectiveness than the crude plant extracts evaluated in this study.

CONCLUSION

Alkaloid extracts from stems and roots of *B. microphylla* showed antifeedant activity against larvae of *P. xylostella* suggesting extracts have compounds that could be contributing towards feeding suppression against the *P. xylostella* larvae.

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