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RESEARCH PAPER

Influence of dry leaves of common tree and shrub species from southern Chile on the growth of lettuce infected with *Meloidogyne hapla*

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Abstract

N. Arismendi, and L. Böhm. 2011. Influence of dry leaves of common tree and shrub species from southern Chile on the growth of lettuce infected with *Meloidogyne hapla*. Cien. Inv. Agr. 38 (1): 75-82. Extracts of many plant species have shown nematicide activity attributable to phytochemicals present in their tissues or formed during a degradation process. Nevertheless, before the application of plant compounds in the field, it is necessary to know their effect on plant hosts. Three concentrations (1, 2.5 and 5% w/w) of dry leaves of *Buddleja globosa*, *Drymis winteri*, *Eucalyptus globulus*, *Gevuina avellana*, *Laurelia sempervirens* and *Ugni molinae* were evaluated on growth parameters (plant height, root length and leaf dry matter) in plants of *Lactuca sativa* infested with *Meloidogyne hapla*. Lettuce growth parameters were significantly affected by the interaction between the species and dry leaf tissues concentrations. *Drymis winteri* showed the biggest reductive effect on the evaluated parameters, but not significantly different as the effects from *E. globulus*. Independently of nematode infestation in lettuce plants, the incorporation of greater than 5% of dry leaves of *D. winteri*, *E. globulus*, *G. avellana*, *L. sempervirens* and *U. molinae* into the substrate had an important effect in the reduction of aerial biomass, estimating that the increase of dry leaf concentration explained about 60% of the reduction of this parameter. This suggests that the plant species evaluated have common active components with phytotoxic activity on lettuce plants, even though no complete information are available for concentrations, interactions and synthesis of derivatives during the degradation process of these tissues.

Key words: Forestry species, *Lactuca sativa, Meloidogyne hapla*, organic amendment, root-knot nematodes.

Introduction

Different plant species have shown nematicide properties over one or more species of phytoparasite nematodes (Walia and Gupta, 1995; Mareggiani *et al*., 1998; Insunza and Aballay, 1995; Insunza *et al*., 2001; Tsay *et al*., 2004; Böhm *et al*., 2009). This effect may be attributable to the presence of phytochemical compounds in their tissues, or their formation during the degradation process (Walker and Melin, 1996; Chitwood, 2002). However, their application on crops must take into account their effects on the development of the host plant where the nematode is being controlled. The allelopathic effect among plants is well known

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(Willis, 2007), which is related to the presence of root exudates but mainly to the production of secondary metabolites or allelochemicals, including phytotoxins, during the process of tissue degradation (Chung and Miller, 1995; Bais *et al*., 2006). Phytotoxins vary considerably among plant species, in their chemical structure, mechanism of action and their effects on plants and other organ isms (Bais *et al*., 2006). Therefore, metabolites from different extracts may alter germination, root development, stem elongation, cell mortality, and other biological processes (Gatti *et al*., 2004; Bais *et al*., 2006; Laynez-Garsaball and Méndez-Natera, 2006, 2007; Coelho de Oliveira *et al*., 2008), which, affect physiology, growth and survival of the plants affected, according to the concentrations and environmental conditions where these extracts are incorporated (Ismail y Chong, 2002; Caldas *et al*., 2004; Periotto *et al*., 2004; Blanco *et al*., 2007).

The effect of the incorporation of dry leaves of *Buddleja globosa*, *Drymis winteri* ("canelo"), *Eucalyptus globulus* ("eucalipto"), *Gevuina avellana* ("avellano"), *Laurelia sempervirens* ("laurel"), *Luma apiculata* ("arrayán"), *Maytenus boaria* ("maiten") and *Ugni molinae* ("murta") to the substrate with lettuce infested by *Meloidogyne hapla* was recently evaluated (Böhm *et al*., 2009). From them, *U. molinae*, *D. winteri*, *L. sempervirens* and, to a lesser extent, *E. globulus* and *G. avellana* presented the best inhibitory effects on this nematode. However, it was proposed whether these dry-milled leaves also affected the host plant. Based on the previous results, the aim of this research was to determine the effect of the five species previously mentioned on some growth parameters of lettuce plants infected with *M. hapla*.

Materials and methods

Plant species and incorporation into the substrate

Five arboreal and shrubby species presenting a significant effect on the propagule reduction of *M. hapla* in previous studies were evaluated (<250 propagules per 200cc of soil) (Böhm *et al*., 2009); they corresponded to: *D. winteri*, *E. globulus*, *G. avellana*, *L. sempervirens* and *U. molinae* collected from the Arboretum of Universidad Austral de Chile, located in the north of Isla Teja, Valdivia (39°47'48" S; 73°15'45" W), during December of 2007. Shoots were cut from the middle section of the plants of each species, taking only leaves of current season, which were completely expanded and apparently healthy. The leaves were washed with tap water and left to dry on disinfected plastic trays (alcohol at 70%) at room temperature (± 20ºC) in the laboratory during 20 days. Then, they were milled (< 1 mm pieces) in an electric mill (ZM1, Retch GmbH, Haan, Germany) and incorporated to a sterile substrate (1:2, river sand: soil Series Valdivia) in pots of 200 mL expanded polysthyrene.

Three tissue doses were evaluated for each plant species: 1, 2.5 and 5% weight/weight in 200 g of substrate, in addition to a control treatment including pots without plant tissue and nematodes. The pots were irrigated periodically with tap water and maintained at room temperature (±20 ºC) during 15 days, in order to allow the substrate interaction with the dry-milled leaves. Subsequently, a bare-root lettuce plant (cv. Reina de Mayo, semillas Anasac, Chile) from seedling made in substrate sterilized in autoclave (soil: sand, 1:1) was transplanted into each pot of ±20 days from emergence, with two true leaves and 5 cm high. After 48 hours from transplanting, a pot was inoculated with a fresh suspension of 2000 eggs and juvenile of *M. hapla*. They were maintained in a greenhouse (21±5 ºC) for 45 days, applying periodical irrigation, according to the requirements of lettuce plants. After this time, the plants were withdrawn from the substrate by a careful wash of the root system, recording parameters of aerial and root development.

Design and statistical analysis

The experiment had a factorial design with five plant species for three tissue concentrations, plus a control and five repetitions per treatment. A MANOVA factorial analysis ($P \leq 0.05$) was made, because the variables of growth responses from the plants like plant height, root length and foliar biomass are significantly correlated (root length and plant height $r = 0.21$ $t_{(129)} = 2.41$

(P = 0.017); plant height and foliar biomass r = 0.47 $t_{(129)}$ = 5.98 (P ≤ 0.001) and root length and foliar biomass r = 0.23 $t_{(129)}$ = 2.72 (P = 0.007)), where the arboreal or shrubby species and their dry-milled leaves concentrations are the independent variables. Likewise, an *a posteriori* comparison of means was made by the Least Significant Difference (LSD) (P≤0.05). A one-way-ANOVA or t-student (P≤0.05) was also performed in order to compare the development of lettuce plants growing in the control pots in comparison to the plants presenting a better height, root length and dry matter production, and to those growing in presence in substrates with dry tissues from arboreal or shrubby species. In addition, the concentrations of dry tissues of the plant species, and their effect on the leaf dry matter productivity on the lettuce plants were correlated. Finally, an analysis of covariance (ANCOVA) (P≤0.05) was performed in order to determine if the effect of the dry foliage on the growth variables and development was independent from the presence of propagules. This analysis and the others in this research were performed by the program Statistica 7.0 (Statsoft Inc, USA).

Results

The aerial and root growth, as well as the foliar biomass were affected significantly by the interaction between species and the tissues concentration (MANOVA factorial Wilks λ= 0.48; $F_{(24,169)}$ = 2.03; P = 0.005) of the plants evaluated. Therefore, the incorporation of the highest concentration of *D. winteri* to the substrate reduced the lettuce height by more than 50%. *E. globulus* presented a similar reductive effect, even though its negative effects did not differ significantly from other plant species in other concentrations (Table 1). On the other hand, the incorporation of mean concentrations of milled-dry tissues of *G. avellana* and *L. sempervirens* presented the best results on the aerial growth, effects that did not differ significantly (ANOVA-1 vía $F_{(2,12)}$ = 0.118; P = 0.889) from the control treatment (mean = 26.94±2.43 cm).

In the root development, the 5% concentration of *D. winteri* leaves caused the highest reduction of root length, although this effect does not differ significantly from the results obtained with *U. molinae* at the same concentration (Table 1).

Table 1. Effect of dry leaf tissues of five common tree and shrub species on lettuce growth parameters such as plant height, root length, and leaf dry matter.

		Mean ± (Standar error)[1]		
Species	Concentration %	Plant height cm	Root lengh cm	Leaf dry matter g
D. winteri	5.0	10.42 ± 1.12 a	8.2 ± 1.25 a	0.06 ± 0.01 a
	2.5	1 9.9 ± 0.74 bcde	10.7 ± 0.82 bc	0.71 ± 0.16 b
	1.0	24.02 ± 1.97 cdef	9.9 ± 0.51 ab	1.29 ± 0.13 cde
E. globulus	5.0	15.84 ± 2.09 ab	108 ± 0.66 bc	0.22 ± 0.07 a
	2.5	22.96 ± 1.29 cdef	10.56 ± 0.65 bc	0.97 ± 0.08 bc
	1.0	22.06 ± 1.19 cdef	11.1 ± 0.40 bcd	1.39 ± 0.10 e
G. avellana	5.0	18.5 ± 0.88 bc	12.88 ± 1.20 d	0.12 ± 0.03 a
	2.5	26.68 ± 2.81 f	11.52 ± 0.28 bcd	0.10 ± 0.04 bcd
	1.0	23.96 ± 3.06 cdef	12.1 ± 0.40 cd	0.10 ± 0.23 bcd
L. sempervirens	5.0	19.78 ± 2.65 bcd	11.06 ± 0.53 bcd	0.66 ± 0.11 b
	2.5	25.48 ± 0.54 ef	11.46 ± 0.46 bcd	1.34 ± 0.16 de
	1.0	20.48 ± 1.25 bcde	11.26 ± 0.51 bcd	1.45 ± 0.13 e
U. molinae	5.0	16.06 ± 3.14 b	10.04 ± 0.95 ab	0.14 ± 0.05 a
	2.5	22.74 ± 2.94 cdef	12.14 ± 0.40 cd	0.68 ± 0.11 b
	1.0	24.74 ± 0.88 def	11.24 ± 0.34 bcd	1.81 ± 0.22 f

[1]Mean followed by different letters in each column are stadistically different according to the least significant difference (LSD) test (P ≤ 0.05).

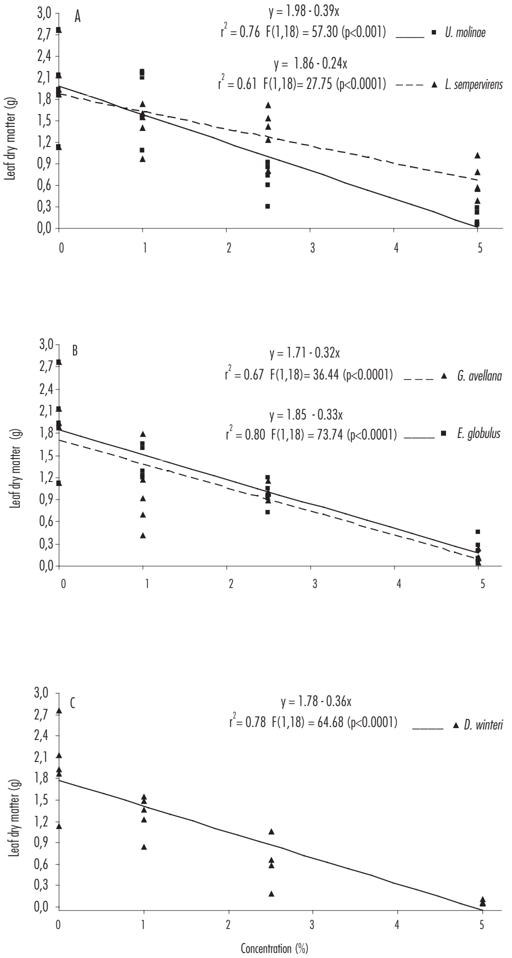

Figure 1. Linear regression analysis in absence (control) and presence of three concentrations of dry leaf tissues of five plant species on lettuce leaf biomass.

Unlike those results, the incorporation of 10 g of *G. avellana* did not induce a reduction of the root growth, and did not differ significantly (t-student $t_{(8)}$= 0.612; P= 0.557) from the control treatment (mean = 13.66±1.20 cm).

Within the most important growth variables as in the case of foliar biomass, it was observed that lettuce plants in pots with 5% dry foliage from *D. winteri*, *G. avellana*, *U. molinae* and *E. globulus* had remarkable allelopathic effects, significantly reducing the leaf dry matter productivity (Table 1). For example, *D. winteri* reduced at least 32 times this parameter in comparison to lettuce growing in absence of dry leaf tissues (mean = 1.96±0.26 g). Likewise, lower concentrations of dry tissues of these species in the substrate had a minimum effect on the foliar biomass, with *U. molinae* presenting better results at 1%, which did not differ significantly (t-student $t_{(8)}$= 0.441; P= 0.671) from lettuce plants grown in control pots. Likewise, the regression analysis shows that the effects from the incorporation of dry leaf tissues from the five plant species on the foliar biomass of lettuce are significantly dependent of the incorporated concentration. Then, the reduced biomass is explained, in most cases over 60%, by the presence of the different concentrations of dry tissue (Figures 1A, 1B and 1C). It is noteworthy that the incorporation of 2, 5 and 10 g of dry-milled leaves of *U. molinae*, *L. sempervirens*, *G. avellana*, *E. globulus* and *D. winteri* in pots with lettuce plants, infer on the productivity of foliar biomass in this lettuce specie.

Discussion

Generally, the growth variables of lettuce plants are altered by the incorporation of dry leaf tissues in the pots, which varies considerably depending on the plant species. Therefore, *D. winteri* has the highest allelopathic effect compared to the other species evaluated, although not always significant compared to *E. globulus*, for example (Table 1).

Böhm *et al.* (2009) determined that *D. winteri* and *L. sempervirens* affected significantly the propagule formation of *M. hapla* (< 75 eggs and juveniles for each 200 cc of soil), presenting as a possible alternative for the control of this phytoparasite. However, in view of the results from this research it must be considered that the control on the *M. hapla* infestation by *D. winteri*, *E. globulus*, *G. avellana*, *U. molinae* and *L. sempervirens* is not direct or isolated, as it also involves a detrimental effect on growth parameters of host plants. Thus, aspects like plant height, root length and dry leaf matter of lettuce are reduced in presence of some of the foliar tissues added in the substrate, similar to results reported in other works (Zasada *et al.*, 2002; Meyer *et al.*, 2008).

It is also observed that there is an inverse linear relationship between the concentration of dry leaves and their dry matter production (Figures 1A, 1B and 1C); this is, increased concentrations resulted on a lower production of aerial dry matter. However, this relationship in biomass is explained between 60 and 80% by the concentration of dry-milled leaves (Figure 1), which suggests that there are other factors influencing the phytotoxic effect. It could be that the plant species incorporated, the decomposition rate of the dry leaves in the substrate, which, on the other hand, depends on environmental conditions (example: humidity), the pH and substrate characteristics, in addition to the type and quality of tissues (example: C:N relation) and the presence of microorganisms in the medium (Chung *et al*., 2003; Bonanomi *et al*., 2006; Blanco *et al*., 2007).

In the case of this work, a relevant factor in the development of lettuce plants would be the *M. hapla* infestation in the substrate (Böhm *et al*., 2009), which would combine with the phytotoxic effects of the incorporated dry leaves. It was shown in the work mentioned that the dry leaves from these plant species reduced significantly the incidence of *M. hapla*, but without eliminating the infestation completely, which could influence the development of the lettuce plants. Regardless the foregoing, the analysis of covariance considering the presence of propagules in the substrate with the dry leaves from the different species evaluated as covariable, indicated that the population present did not have a significant effect on the reduction of aerial biomass in lettuce (ANCOVA: *L. sempervirens* $F_{(1,11)} = 1.43$ $P = 0.256$; *D. winteri* $F_{(1,11)} = 0.23$ $P = 0.643$; *U. molinae* $F_{(1,11)} = 0.047$ $P = 0.832$; *G. avellana* $F_{(1,11)} = 0.086$ $P = 0.773$ and *E. globulus* $F_{(1,11)} = 0.016$ $P = 0.899$). This reveals that the effects on the reduction of one of the growth variables is more related to the presence of dry-milled leaves from the different species evaluated in the substrate than with the *M. hapla* infestation. Likewise, even though the data were not included in this analysis, it was observed that the *M. hapla* infestation did not influence significantly on the plant height and radical length reduction at 45 days from the transplant. This may be because its relatively low population (Böhm *et al*., 2009) did not influence sustantially the growth variables of the lettuce plants, although there was a presence of *M. hapla* eggs or juveniles (J2) in the substrate.

This work did not determine which are the active compounds present in the dry leaves from each species, or which were the compounds specifically acting on the lettuce plants, even though those compounds are quite likely related to the release of secondary metabolites of phytotoxins during the degradation process (Chung y Miller, 1995; Bais *et al*., 2006). In this regard, Neira *et al*. (2004) and Bittner *et al*. (2009) indicate that essential oil of *L. sempervirens* have a high content of safrol and eugenol, while Schmeda-Hirschmann *et al*. (1994) remark the presence of the laurotetanin alkaloid in its leaves. Safrol is considered the highest active ingredient in the control of some phytopathogen fungi (Bittner *et al*., 2009), but aspects on the phytotoxicity of this compound and laurotetanin have not been reported. On the other hand, eugenol has been known as a phytotoxic compound (Walker and Melin, 1996; Bowers and Locke, 2000; Meyer *et al*, 2008). Walker and Melin (1996) reported that the application of essential oils rich in eugenol for the control of *Meloigyne* spp. was phytotoxic in tomato plants when transplanted immediately after the application, but this effect did not occur when the transplant was made after seven days of applying the essential oil. Additionally, the same authors indicate that the production of tomato dry matter infected with *M. arenaria* was considerably reduced when 1,000 to 1,500 mg of eugenol oil per kilogram soil were applied.

In the case of *U. molinae,* it has been determined that the leaves have a high content of phenolic compounds, where some flavonoids like myricetin, quercetin, kaemferol and their derivates (Rubilar *et al*., 2006) are included. Kalinova and Vrchotova (2009) evaluated the allelopathic effect of myricetin and quercetin in the development of different weeds species, without any significant effects of these flavonoids. On the contrary, Bais *et al.* (2003) found a phytotoxic effect from kaempferol and dihidroquercetin and other flavonoids, affecting more than 60% the differentiation of shoots and roots, and the efficiency of germination of weeds, wheat and tomato. Eugenol, kaemferol, dihydroquercetin also have been reported in *D.*

winteri and other Wintereaceae (Williams and Harvey 1982; Montes and Wilkomirsky, 1985), which may condition *D. winteri* to be more phytotoxic than the other species evaluated in this work.

In regard to eucalyptus, 1.8-cineol (eucalyptol) is one of the main monoterpens among essential oils of this species (Lawrence, 1997). Evaluations with 1.8-cineol have reported phytotoxic alterations, like for example, a reduction of the root growth and rate of seeds germination of *Echinochloa crusgalli* (Poaceae) and *Cassia obtusifolia* (Fabaceae) (Romagni *et al*., 2000). A different situation occurs with *G. avellana*, species where the leaf components have not yet been characterized, which does not allow inferring their phytotoxic effect on lettuce plants.

According to the information presented, common active components may exist among some of the vegetal species evaluated, which may involve a similar effect in nematode control or in their phytotoxic activity towards lettuce plants. However, no information is available on the concentration, interactions and synthesis of derivatives from each of the active ingredients, being difficult to determinate the real effect from the tissues evaluated in this study. This does not mean that those species presenting a high phytotoxicity are not recommendable for use or for further research, but that it is important to define more precisely the moment and dose application in order to avoid phytotoxicity. The use of phytotoxic plants on pre-sowing or pre-transplant may be feasible, as well as a further use of non phytotoxic plants. Likewise, an important aspect to be considered is the amount of dry leaves to be applied, which might hinder a large scale use; therefore, it is necessary to evaluate alternatives based on the application of aqueous or alcholic extracts, but mainly the knowledge on which the chemical components are involved.

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Resumen

N. Arismendi y L. Böhm. 2011. Influencia de follaje seco de especies arbóreas y arbustivas comunes del sur Chile en el crecimiento de plantas de lechuga infectadas con *Meloidogyne hapla*. Cien. Inv. Agr. 38 (1): 75-82. Numerosas especies vegetales y/o sus extractos muestran propiedades nematicidas, atribuibles a los compuestos fitoquímicos presentes en sus tejidos o formados durante el proceso de degradación. Sin embargo, previo a su aplicación en el campo es necesario conocer su efecto sobre las plantas hospedantes. Se evaluó el efecto de la incorporación al sustrato de tres contracciones (1, 2,5 y 5% peso/peso) de hojas secas trituradas de *Buddleja globosa*, *Drymis winteri*, *Eucalyptus globulus*, *Gevuina avellana*, *Laurelia sempervirens* y *Ugni molinae* sobre variables de crecimiento (altura de la planta, largo radical y materia seca foliar) en plantas de *Lactuca sativa* infestadas con *Meloidogyne hapla.* Los parámetros de crecimiento de las lechugas fueron afectados significativamente por la interacción entre la especie y las concentraciones de tejido, mostrando *D. winteri* el mayor efecto reductivo, aún cuando similar a *E. globulus.* Independientemente del efecto de la infestación del nemátodo en las plantas, la incorporación al sustrato de concentraciones superiores al 5% de hojas secas de *D. winteri*, *E. globulus*, *G. avellana*, *L. sempervirens* y *U. molinae*, redujeron la biomasa aérea, estimándose que el aumento de concentración de hojas secas explica alrededor de un 60% la reducción de este parámetro. Esto sugiere, que las especies vegetales evaluadas presentarían componentes activos comunes, teniendo actividad fitotóxica hacia plantas de lechuga, aún cuando, se carece de información de la concentración, interacciones y síntesis de derivados durante su proceso de degradación de esos tejidos.

Palabras clave: Especies forestales, enmiendas orgánicas, *Lactuca sativa*, *Meloidogyne hapla*, nemátodos agalladores.

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