

Nematicidal activity of leaves of common shrub and tree species from Southern Chile against *Meloidogyne hapla*

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Abstract

L. Böhm, N. Arismendi, and L. Ciampi. 2009. Nematicidal activity of leaves of common shrub and tree species from Southern Chile against *Meloidogyne hapla*. Cien. Inv. Agr. 36 (2): 249-258. The biological control of the root-knot nematode, *Meloidogyne hapla*, was evaluated through the addition of organic amendments of dry and chopped leaves of *Buddleja globosa*, *Drymis winteri*, *Eucalyptus globulus*, *Gevuina avellana*, *Laurelia sempervirens*, *Luma apiculata*, *Maytenus boaria* and *Ugni molinae* to the soil substratum. The assays were carried out in pots seeded with lettuce plants (cv. Reina de Mayo). All pots were inoculated with 2000 eggs and juveniles of *M. hapla*, and then maintained under greenhouse conditions for 45 days. Control pots without foliage additions were also seeded with lettuce. The results show that the addition of dry leaves of *L. sempervirens*, *G. avellana*, *M. boaria*, *D. winteri*, and *B. globosa* significantly reduced root-knot nematode development in soil in which lettuce plants were grown. On the other hand, while all of the treatments significantly affected the development of eggs and juveniles of *M. hapla*, the best inhibitory effect was found with dry leaves of *U. molinae*, *D. winteri* and *L. sempervirens*. For all plant species, an increase in the concentration of dry leaves incorporated into the substratum resulted in better control of nematode population.

Key words: Biological control, forestry species, *Meloidogyne hapla*, nematodes, organic-amendment; ligneous plants.

Introduction

The most important species of *Meloidogyne* in Chile are *M. hapla* and *M. incognita*. These species cause root knots in a wide range of host plants (Böhm, 1986), and are prevalent in cold to temperate climatic conditions (Hussey and Janssen, 2002). This situation favors their distribution in the Southern zone of Chile, where

Meloidogyne spp. infect, among other crops, bean, carrot, clovers, lettuce, pea, potato, sugar beet, and tomato (Böhm, 1986 and Barría, 1997; Magunacelaya and Dagnino, 1999).

Management for the control of root-knot gall nematodes is traditionally performed with cultural control methods, using resistant species or resistant cultivars, and with nematicide applications (Hussey and Janssen, 2002). The use of chemical nematicides is currently questioned due to their adverse effects in agroecosystems and because of their high cost and lack of specificity towards the different types of nematodes (Soler-Serratos et al., 1996; Chitwood, 2002).

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The current tendency is to use integrated control methods, integrating the use of antagonistic organisms into cultural practices with a minimum use of chemical nematicides. Thereby, the nematode population densities are maintained under the threshold of economic damage (Galper *et al.*, 1990; Tsay *et al.*, 2004).

Numerous plants species producing allelochemicals with antagonistic effect towards certain populations of plant parasitic nematodes have been reported worldwide (Chitwood, 2002). Many of these plants have been evaluated for their use in crop rotation, as cover crops or as green manure in arable soils. Additionally, plant extracts from allelopathic species (e.g., *Azadirachta indica* (neem), *Tagetes* spp., *Brassica* spp.) have been tested for their use as nematicides (Akhtar and Alam, 1993; Akhtar and Mahamood, 1994, 1996; Walia and Gupta, 1995; Mareggiani *et al.*, 1998; Chitwood, 2002; Insunza *et al.*, 2001; Oka and Yermiyahu, 2002; Tsay *et al.*, 2004; Walker, 2004). The nematicidal effect of these extracts can be ascribed to the presence of phytochemical compounds in their tissues or to the result of the degradation process, like some polythienyls, isothiocyanates and glucosinolates (Chitwood, 2002; Stirling and Stirling, 2003).

In Chile, the nematicidal effects of different native and cultivated plants against *Ditylenchus dipsaci* (Kuhn), *Xiphinema index* Thorne-Allen and *X. americanum sensu lato* Cobb have been evaluated (Insunza and Valenzuela, 1995; Insunza and Aballay, 1995; Insunza *et al.*, 2001; Aballay and Insunza, 2002). Their results are in accordance with the results by Halbrendt (1996) and Chitwood (2002) that suggest that the nematicidal properties of plant species vary considerably with plant species and cultivar, the plant tissue used, plant growth and development, application methods and the nematode species tested.

Anthelmintic properties have been ascribed to a diversity of native plant species in Chile, especially the tree species. Therefore, the objective of this study was to evaluate the nematicidal effect of dry foliage obtained from eight common tree species and bush-like species in Southern Chile against *M. hapla*.

Materials and methods

Plant species

Leaves obtained from *Buddleja globosa* Hoppe ("matico"), *Drymis winteri* J.R. Foster ("canelo"), *Eucalyptus globulus* Labill (eucalyptus), *Gevuina avellana* Mol. (Chilean hazelnut), *Laurelia sempervirens* Ruiz et Pav. Tul. ("laurel"), *Luma apiculata* Burret (Chilean myrtle), *Maytenus boaria* Mol. ("maitén") and *Ugni molinae* Turz. ("murta") were used. Leaves were collected from the Universidad Austral de Chile Arbo-retum, located in the North sector of Isla Teja, Valdivia, during December of 2007. Shoots were cut from the middle sections of plants of each species. Only shoots with leaves from the current season, completely expanded and apparently healthy, were taken.

The leaves were washed and left to dry at room temperature (about 20 °C) for 20 days on plastic trays that had been disinfected with 70% ethanol. Then, the leaves were ground (< 1 mm pieces) in an electric mill (ZM1, Retch GmbH, Haan, Germany), incorporated into 200 mL pots containing a sandy soil substrate (1:2, sand:soil), watered and maintained at room temperature for 15 days in order to allow the interaction of the soil components with the plant tissue.

For each plant species, with the exception of *B. globosa*, 1.0, 2.5, and 5.0% (w/w) of plant tissue were mixed with 200 g of soil (Santa Rosa Serie). Because of its spongy texture, *B. globosa* was mixed in proportions of 0.5, 1.0, and 2.5% to avoid exceeding 200 mL. Control treatments consisted of the same soil substrate without the incorporation of plant tissue, but with equal inoculation with *M. hapla*.

Inoculum of *Meloidogyne hapla*

A pure population of *M. hapla* originally obtained from *Paeonia lactiflora* Pall. roots were used as the source of inoculum. The population was multiplied successively in tomato plants and cultivated in autoclaved sandy soil substrate

(1:1, sand:soil). The roots were processed following the methodology by Hussey and Barker (1973), and a final suspension of 1000 eggs and juveniles per mL of water was obtained for inoculum.

Pot preparation

A total of 130 pots, each containing 200 mL of soil, were used. A lettuce plant (*Lactuca sativa* L. var. Reina de Mayo) obtained from seedbed prepared on sterilized soil was transplanted into each pot about 20 days after emergence, when the lettuce were 5 cm high. After 48 h from transplanting, each pot was inoculated with a fresh suspension of 2000 eggs and juveniles of *M. hapla*. The inoculum was distributed with a micropipette into two 2-cm-deep holes around the roots, and subsequently they were covered with soil and sealed with a light irrigation.

After inoculation, the pots were arranged on plastic dishes to avoid cross contamination through the drainage, and they were maintained in a greenhouse (21 ± 5 °C) for 45 days, irrigating periodically according to the plants' requirements.

Evaluations

Evaluations were performed 45 days after inoculation. For this purpose, the roots were analyzed visually to determine the galling index on a scale from 0 to 4, where 0 = no galls (healthy root system, no infestation); 1 = 1-5 small galls (1-25% of root system galled); 2 = 2-15 small galls (26-50% of root system galled); 3 = 16-25 galls, majority of roots still functioning (56-75% of root system galled); 4 = >26 galls (>76% of root system galled) (Hussey and Janssen, 2002).

The reproduction level reached by the nematode population was estimated from the number of propagules extracted from the egg masses that developed in the roots. The whole root system from each plant was processed by the method

for the extraction of eggs and juveniles proposed by Hussey and Barker (1973). Two 1-mL aliquots were counted for each replicate, and the total number of juveniles in 100 mL of soil was calculated by multiplying the average of the two aliquots by 10. The rate of reproduction (RR) was calculated as $RR = (Pf/Pi^{-1}) \times 100$, where Pf = total of final propagules in the roots and Pi = 2000 eggs and juveniles inoculated per pot.

Design and statistical analysis

Treatments were distributed according to an 8 x 3 factorial design (eight plant species x three plant tissue concentrations), replicated five times. The experimental unit consisted of one pot per replicate. The results were analyzed for variance and means were tested for significant differences by the least significant difference (LSD), using the program JMP6 (SAS Inc., USA).

Results

The incorporation of leaves of *L. sempervirens* ("laurel") into the soil significantly diminished ($p \leq 0.05$) the formation of galls caused by *M. hapla* in lettuce roots. Likewise, the foliar tissue of *G. avellana* (Chilean hazelnut) and *M. boaria* ("maitén") reduced the number and percentage of root galling, while only the percentage of root galling decreased in lettuce plants grown in soil substrate amended with leaves of *D. winteri* ("canelo") and *B. globosa* ("matico") (Figures 1A and 1B). On the other hand, *U. molinae* ("murta"), *E. globulus* (eucalyptus) and *L. apiculata* (Chilean myrtle) leaves had a weak, non-significant effect on gall formation, associated with a severe or high infestation in the lettuce roots (Figure 1).

Independent of the plant species, the incorporation of dry plant tissues negatively affected the reproduction of *M. hapla* in lettuce, reducing the number of eggs and the formation of second stage juveniles significantly ($p \leq 0.05$) (Figures 1C and 1D). The treatments containing dry foli-

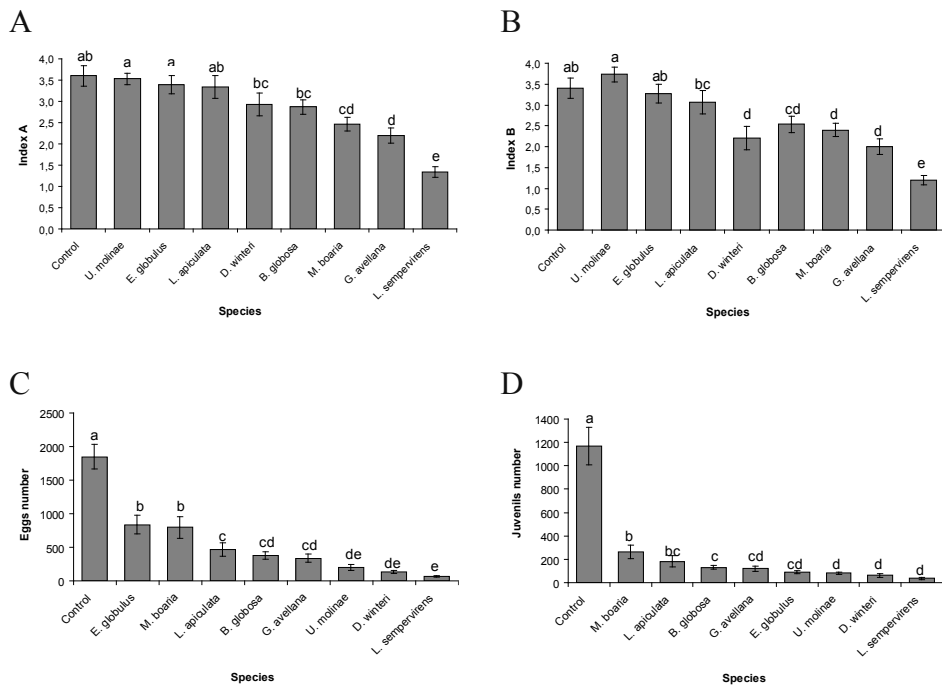


Figure 1. Effects of dry leaf tissues obtained from eight Chilean native plant species on *Meloidogyne hapla* in roots of lettuce (*Lactuca sativa*). A. Galling index by root-knot number, B. Galling index by root-knot percentage, C. Eggs, and D. Second stage juveniles. Bars followed by different letters indicate significant statistical differences according to the least significant difference (LSD) test ($p \leq 0.05$).

age of *E. globulus* (eucalyptus) and *M. boaria* (“maitén”) reduced the presence of *M. hapla* eggs by more than 50% compared to the non-amended controls. In soil amended with *L. apiculata* (Chilean myrtle), *B. globosa* (“matico”) and *G. avellana* (Chilean hazelnut), egg production was reduced by more than 30%. *Ugni molinae* (“murta”), *D. winteri* (“canelo”), and *L. sempervirens* (“laurel”) foliage had the highest impact on the reproductive activity of *M. hapla* in lettuce (Figure 1C). Likewise, the number of juveniles II was significantly reduced ($p \leq 0.05$) with respect to the control non-amended treatments. There were significant differences among the plant species, and the dry leaf tissues of *U. molinae*, *D. winteri*, and *L. sempervirens* had the highest impacts, with less than 100 juveniles II of *M. hapla* per root system (Figure 1D).

The variance analysis shows that for each plant species there were significant differences between the levels of control of *M. hapla* by different concentrations of plant leaf incorporated into the soil, although the magnitude of this

effect differs depending on the plant species (Table 1).

For each species, an increase in the concentration of plant tissue incorporated into the soil substrate resulted in a reduction in the reproductive activity of *M. hapla*. Therefore, in most plant species, 5% concentrations (10 g) had the greatest effect on the reproductive indexes. However, significant differences ($p \leq 0.05$) among the plant species were not detected when the highest foliage concentration was incorporated into the soil substrate (Table 1). On the other hand, the responses of the reduction of eggs and juveniles varied according to the plant species when 1% of dry plant tissue was used, with *L. sempervirens* (“laurel”) having the best effect. The effect of adding 1% of dry leaf tissue of *L. sempervirens* (“laurel”) was not significantly different from the effect obtained with other plant species at 5% concentrations (Table 1).

In the special case for *B. globosa* (“matico”), the 0.5% concentration (2 g of dry leaf tissue)

Table 1. Effects of soil amended with three concentrations of dry leaf tissues obtained from Chilean native plant species against *Meloidogyne hapla* in lettuce (*Lactuca sativa*).

Species	Concentration %	Mean (\pm standard error) ¹		
		Eggs, no.	Juveniles, no.	Total, no.
<i>Buddleja globosa</i>	0.5	612 \pm 90.0 e	186 \pm 22.0 de	798 \pm 75.5 ef
	1.0	328 \pm 33.7 fg	138 \pm 14.6 def	466 \pm 40.0 g
	2.5	182 \pm 22.4 fghi	70 \pm 11.4 def	252 \pm 24.0 ghi
<i>Drymis winteri</i>	1.0	166 \pm 30.4 fghi	102 \pm 26.5 def	268 \pm 56.3 ghi
	2.5	190 \pm 34.8 fghi	68 \pm 21.0 def	258 \pm 51.8 ghi
	5.0	42 \pm 8.0 hi	16 \pm 5.1 f	58 \pm 12.4 i
<i>Eucalyptus globulus</i>	1.0	1440 \pm 69.9 b	144 \pm 20.1 def	1584 \pm 81.7 b
	2.5	898 \pm 52.0 d	72 \pm 23.5 def	970 \pm 71.8 de
	5.0	178 \pm 4.4 fghi	52 \pm 17.7 def	230 \pm 41.8 ghi
<i>Gevuina avellana</i>	1.0	608 \pm 58.6 e	196 \pm 44.8 cd	804 \pm 93.1 ef
	2.5	270 \pm 56.8 ghi	122 \pm 31.5 def	392 \pm 65.4 gh
	5.0	130 \pm 31.1 fghi	42 \pm 11.6 ef	172 \pm 37.3 ghi
<i>Laurelia sempervirens</i>	1.0	124 \pm 13.3 ghi	68 \pm 22.2 def	192 \pm 30.4 ghi
	2.5	30 \pm 15.2 i	18 \pm 8.6 f	48 \pm 23.5 i
	5.0	44 \pm 6.0 hi	28 \pm 5.8 f	72 \pm 11.1 hi
<i>Luma apiculata</i>	1.0	912 \pm 155.3 d	334 \pm 121.4 bc	1246 \pm 227.3 cd
	2.5	290 \pm 49.2 fgh	136 \pm 29.4 def	426 \pm 59.8 g
	5.0	188 \pm 44.6 fghi	78 \pm 24.2 def	266 \pm 59.4 ghi
<i>Maytenus boaria</i>	1.0	1208 \pm 284.1 bc	334 \pm 70.9 bc	1542 \pm 252.4 bc
	2.5	1040 \pm 177.7 cd	400 \pm 106.1 b	1440 \pm 271.3 bc
	5.0	140 \pm 44.4 fghi	52 \pm 19.3 def	192 \pm 46.7 ghi
<i>Ugni molinae</i>	1.0	386 \pm 94.4 ef	108 \pm 23.5 def	494 \pm 97.0 fg
	2.5	108 \pm 16.8 ghi	66 \pm 14.3 def	174 \pm 29.1 ghi
	5.0	98 \pm 23.1 ghi	70 \pm 13.8 def	168 \pm 33.7 ghi
Control	0.0	1848 \pm 180.3 a	1166 \pm 160.9 a	3014 \pm 294.7 a
Analysis of variance				
Cause of variation	d.f.	F (p)	F (p)	F (p)
Plant species (S)	7	33.13 (<0.0001)	9.25 (<0.0001)	21.80 (<0.0001)
Concentrations (C)	2	82.35 (<0.0001)	20.95 (<0.0001)	11.89 (<0.0001)
S x C	14	7.74 (<0.0001)	2.63 (<0.0028)	3.68 (<0.0001)

¹Means followed by different letters in each column are statistically different according to the least significant difference (LSD) test ($p \leq 0.05$).

reduced the number of eggs by almost 30% and the presence of juveniles II of *M. hapla* by six times, respectively, compared to the non-amended control. The decrease in the formation of eggs was more than double the impact accomplished with the 1% dry leaf tissue of *E. globulus* (eucalyptus) and *M. boaria* ("maitén"). These differences were significant ($p \leq 0.05$) (Table 1).

Discussion

Considering that there was a significant reduction in the galling and reproductive activity of *M. hapla*, the detrimental effect of the foliar dry tissue from the different plant species evaluated against *M. hapla* was clearly demonstrated.

This is one of the first studies to evaluate the effect of dry leaf tissues from some native Chilean species against *M. hapla*. Previous works have shown that certain native species (e.g., *Aristotelia chilensis*, *Cestrum parqui*, *Quillaja saponaria*, *Chenopodium ambrosioides*, *Oxalis rosea*, and *Ovidia pillopillo*) have nematostatic or nematocidal effects against *Ditylenchus dipsaci*, *M. incognita*, and *Xiphinema americanum sensu lato* (Insunza and Valenzuela, 1995; Insunza *et al.*, 2001).

It is noticeable that native species like *L. sempervirens* ("laurel") cause the highest reduction in the gall indexes of lettuce roots. In addition, laurel showed a significant effect on altering the reproductive activity of *M. hapla*. These effects are in concordance with previous findings indicating that the essential oils of *L. sempervirens* have growth regulatory, insecticidal and acaricidal activity (Neira *et al.*, 2004; Torres *et al.*, 2004). Although the identities of the compounds that reduce the presence of eggs and juveniles of *M. hapla* in the present test are unknown, Schmeda-Hirschmann *et al.* (1994, 1996) have reported the presence of alkaloids (e.g., laurotetanine and seco-isotetrandrine) in *L. sempervirens* leaves. In addition, saffrole and eugenol derivatives have been isolated (Neira *et al.*, 2004); insecticidal properties have been reported for both compounds (Huang *et al.*, 1999; Huang *et al.*, 2002). Likewise, the effects asserted on the presence of *M. hapla* by *U. molinae*

("murta") and *D. winteri* ("canelo") were not significantly different from the effects of laurel, and even these effects were similar in presence of juveniles II (Figure 1).

On the other hand, extracts obtained from "murta" have been widely used in the pharmaceutical industry because of their medicinal activity, mainly associated with the polyphenolic compounds present in the leaves (Rubilar *et al.*, 2006). Some polyphenols are secondary metabolites that defend the plant against infections caused by microorganisms (Heath, 2002), which suggests that some compounds of this nature assert negative effects on *M. hapla*. In addition, other extracts from Myrtaceae leaves as well as *E. globulus* leaves (eucalyptus) have been used as antibacterial components, where the main active ingredient is eucalyptol (1,8-cineole) (Salari *et al.*, 2006). The 1,8-cineole has been reported to have a high insecticidal activity against fruit flies (Clemente *et al.*, 2007). In addition, α -pinene extracted from eucalyptus leaves has shown some effect on reducing egg enclosure in *M. incognita* (Ibrahim *et al.*, 2006). The compound α -pinene has also been detected in Chilean myrtle (*L. apiculata*), a species that also contains flavonoids (e.g., quercetin, kaempferol, mirecetin), cineole and myrtol (Montes and Wilkomirsky, 1985; Hoffmann *et al.*, 1992). Some flavonoids (e.g., kaempferol) considerably inhibit the development of lepidopteran larvae (Onyilagha *et al.*, 2004).

According to Williams and Harvey (1982), Montes and Wikomirsky (1985) and Hoffmann *et al.* (1992), *D. winteri* ("canelo") leaves contain different essential oils such as ascaridole, pinene, limonene and eugenol. In addition, they have tannins, terpenoids (e.g., drimenol, drimenine, cryptomeridiol, winterine) and flavonoids (e.g., cirsimaritin, taxifoline, quercetin, kaempferol). *Drymis winteri* ("canelo") extracts have been used for insect control (Torres *et al.*, 2004), and ascaridole derivatives have shown nematocidal activity against *Aphelenchoides besseyi* and *M. incognita*, with better results against this last species (Yen *et al.*, 2007).

In *G. avellana* (Chilean hazelnut), the identification of active compounds has been focused on the fruit (nut), mainly to study the content of

fatty acids as a source of antioxidants (Bertoli *et al.*, 1998; Moure *et al.*, 2000). The possible active components present in the leaves have not been identified yet. However, the presence of phenolic and tannin compounds, which may have a direct effect on *M. hapla*, has been characterized (Chacón and Armesto, 2006).

The concentrations of flavonoids and tannins in *B. globosa* ("matico") are remarkable (Vogel *et al.*, 2004). The presence of terpene compounds (e.g., buddlejone, deoxybuddlejone, buddledin-A, buddledin-B and maytenone) with high antifungal activities against dermatophytes has also been reported (Mensah *et al.*, 2000), where buddledin-A is the main compound responsible (Houghton *et al.*, 2003). Likewise, daucosterine, dulcitol, lupenone, β -amyryne, oleanoic acid, β -sitosterol and α -spinasterol have been isolated from *M. boaria* ("maitén") leaves and roots (Hoffmann *et al.*, 1992), but there are not data on the effects of these compounds on nematodes. However, β -dihydroagarofuran obtained from *B. globosa* ("matico") seeds has an inhibitory effect on *Spodoptera frugiperda* (Lepidoptera) and causes 100% larval mortality (Céspedes *et al.*, 2001).

The nematicidal activity on eggs and juveniles II of *M. hapla* obtained from the dry plant tissues in this study may serve for the eventual development of biocides. The nematicidal activity of these plant extracts may be due to either a single, or more than one compound and an interaction among compounds is also possible. Further research is needed to clarify this point. However, the data show that all of the dry plant tissues evaluated may be used for the integrative control of *M. hapla*.

In conclusion, all of the dry leaf extracts of the plant species used in this study exerted control on *M. hapla*, reducing gall formation and the presence of eggs and juveniles, with the best results shown by the native Chilean species like *L. sempervirens* ("laurel"), *D. winteri* ("canelo"), and *U. molinae* ("murta").

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Resumen

L. Böhm, N. Arismendi y L. Ciampi. 2009. Actividad nematocida del follaje de especies arbóreas y arbustivas comunes del sur de Chile sobre *Meloidogyne hapla*. Cien. Inv. Agr. 36 (2): 249-258. Buscando alternativas biológicas para el control del nemátodo de las agallas radicales, *Meloidogyne hapla*, se evaluó la incorporación al sustrato de follaje seco y molido de *Buddleja globosa*, *Drymis winteri*, *Eucalyptus globulus*, *Gevuina avellana*, *Laurelia sempervirens*, *Luma apiculata*, *Maytenus boaria* y *Ugni molinae*. Se utilizó como control un tratamiento sin follaje. El ensayo se realizó en macetas en las que se trasplantaron plantas de lechuga (cv. Reina de Mayo). Todas las macetas se inocularon con 2000 huevos y juveniles de *M. hapla* y se mantuvieron por 45 días en invernadero. Los resultados indicaron que las plantas de lechuga desarrolladas en sustratos con hojas secas de *L. sempervirens*, *G. avellana*, *M. boaria*, *D. winteri* y *B. globosa* redujeron significativamente el agallamiento radical. Mientras que todos los tratamientos afectaron significativamente la formación de huevos y juveniles de *M. hapla*, destacando el efecto inhibitorio sobre este parámetro el follaje seco de *U. molinae*, *D. winteri* y *L. sempervirens*. En todas las especies, el incremento de la concentración de follaje incorporado al sustrato resultó en un mayor control en la multiplicación de *M. hapla*.

Palabras clave: Árboles nativos, control biológico, especies forestales, *Meloidogyne hapla*, nematodos.

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