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Response of parsley to different population densities of *Meloidogyne arenaria*

Respuesta del perejil crespo a diferentes densidades de población de *Meloidogyne arenaria*

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Abstract

The effect of initial population density (*Pi*) of the peanut root-knot nematode, *Melodoigyne arenaria,* on curly leaf parsley growth was assessed in this study. The population densities of *M. arenaria* ranged from 0 to 64 eggs + second-stage juveniles (J2)/cm³ soil in sterile sandbags. The root gall index (RGI), reproduction factor (RF), fresh leaf weight (FLW), dry leaf weight (DLW), root fresh weight (RFW), root length (RL), leaf height (LH), and chlorophyll index (SPAD) were determined at 90 days after inoculation. FLW, DLW, RFW, LH, and SPAD data were fitted to the Seinhorst equation, $y = m + (1 - m) z^{Pi-T}$, to determine the tolerance limit T = 0.25 eggs +J2/cm³ soil for FLW, DLW, RFW, and LH, with relative means (*m*) of 0.52, 0.24, 0.22, and 0.4 respectively; conversely, the *T* value for SPAD was 0.125 eggs + J2/cm³ soil and with a *m* of 0.26. All biometric variables decreased with an increase in the initial population density (Pi). Nevertheless, the highest RF of *M. arenaria* in parsley was 78.92 for a *Pi* = 8 eggs + J2/cm³ soil, with an RGI value of 5 from *Pi* = 1 eggs + J2/cm³. Curly leaf parsley growth decreased with an increase in Pi of *M. arenaria*.

Key words: Tolerance limit; peanut root-knot nematode; *Petroselinum crispum*, growth reduction.

Resumen

Se realizó él estudió del efecto de poblaciones crecientes (*Pi*) del nematodo de la agalla *Melodoigyne arenaria* en el cultivo de perejil crespo, que oscilaron entre 0 y 64 huevos + juveniles del segundo estadio (J2) /cm³ de suelo en bolsas com suelo estéril. A los 90 días después de la inoculación, se determinó, índice de agallamiento (IA), factor de reproducción (FR), peso fresco follaje (PFF), peso seco follaje (PSF), peso fresco raíz (PFR), longitud de raíz (LR) altura foliar (AF) e índice de clorofila (SPAD). Los datos de PFF, PSF, PFR, AF y SPAD se sometieron a la ecuación de Seinhorst, $y = m+(1-m) z^{Pi-T}$, que permitió determinar el límite de tolerancia *T* = 0.25 huevos +J2/cm³ de suelo para PFF, PSF, PFR y AF, con promedios relativos (*m*) de 0.52; 0.24; 0.22 y 0.4 respectivamente; por otro lado, el valor de *T* para SPAD fue de 0.125 huevos+J2/cm³ de suelo y con un *m* de 0.26. Todas las variables biométricas decrecen al incremento de las poblaciones iniciales (Pi). Asimismo, el mayor FR de *M. arenaria* en perejil es 78.92 para la Pi = 8 huevos +J2/cm³ de suelo, con un valor de IA de 5 a partir de *Pi* = 1 huevos+J2/cm³. Conforme se incrementa las Pi de *M. arenaria* el cultivo de perejil crespo reduce su crecimiento.

Palabras clave: Limite de tolerancia, nematodo de la agalla, *Petroselinum crispum*, reducción de crecimiento.

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Introduction

In Peru, curly leaf parsley (*Petroselinum crispum* (Mill.)) growth has gained socioeconomic and environmental relevance for its ability to generate jobs and value added. For these reasons, the factors that can significantly reduce curly leaf parsley growth must be investigated. One of the main problems of this crop is the occurrence of diseases caused by viruses, fungi, bacteria, and nematodes (Hallmann y Meressa, 2018; Lana y Moita, 2018).

The main nematode species associated with curly leaf parsley are: *Ditylenchus dipsaci* (Kuhn) Filipjev, *Belonolaimus gracilis* Steiner, *Dolichodorus heterocephalus* Cobb, *Paratylenchus hamatus* Thorne y Allen, and *Pratylenchus penetrans* (Cobb) Filipjev y Schuurmans Stekhoven (Hallmann y Meressa 2018). In addition, economically important species of the genus *Meloidogyne* spp. include *M. arenaria* (Neal) Chitwood*,*

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M. enterolobii Uang y Eisenback*, M. floridensis* Chitwood, Hannon y Esser*, M. incognita* (Kofoid y White) Chitwood*, M. hapla* Chitwood*, M. hispanica* Hirschmann, and *M. javanica* (Treub) Chitwood (Mennan *et al*., 2011; Maleita *et al*., 2012; Barros *et al*., 2018; Hallmann y Meressa, 2018). Plants infected by these *Meloidogyne* species show symptoms related to generalized decay, leaf yellowing, reduced shoot growth, reduction of the root system, and presence of root galls (Mennan *et al*., 2011; Sangronis *et al*., 2014; Sasanelli *et al*., 2015; Hallmann y Meressa, 2018; Ntalli *et al*., 2019), and *M. arenaria* has also been reported to cause economic damages in *P. crispum* 'Bezirci', in Turkey (Mennan *et al.,* 2011).

The data on *M. arenaria* in curly leaf parsley growth in the Arequipa region remains limited, but farmers have reported damages caused by *Meloidogyne* spp. For this reason, the present study was conducted to assess the effect of *M. arenaria* populations on plant growth and to estimate the tolerance level.

Materials and methods

The experiment was conducted, November 2019 to January 2020, in a shade house at temperatures of 25 ± 5° C and relative humidity of 40% ± 5%, and nematode analyses were performed at the Plant Pathology laboratory of the National University of San Agustín (*Universidad Nacional de San Agustín* – UNSA), Arequipa, Peru.

Curly leaf parsley 'Moss Curled' seeds were sown in propagation trays with sterile substrate (Promix®) and transplanted when the seedlings had three true leaves in 1,000 cm^3 fine sterilized sandbags. The inoculum used in this study was identified as the species *M. arenaria* (Est. A2) based on the female perineal pattern (Taylor y Netscher, 1974) and biochemical characterization by esterase isoenzyme electrophoresis (Carneiro y Almeida 2001). Subsequently, they were multiplied in tomato cultivar 'Rio Grande' seedlings grown in a plastic shade house.

The method described by Hussey and Barker (1973) was used for inoculation, applying the following inoculum levels: 0, 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, eggs + second-stage juveniles (J2)/cm^3 soil (Table 1). For inoculation, three holes were made in the ground near the base of the stem. Tomato plants (*Solanum lycopersicum* var. 'Rio Grande') were used to assess the viability of the inoculum. The plants were inoculated with 5,000 eggs + J2distributed in three holes around the plant.

At 90 days, root gall index (RGI), reproduction factor (RF=final population/initial population), fresh leaf weight (FLW), dry leaf weight (DLW), root fresh weight (RFW), root length (RL) leaf height (LH) and chlorophyll index (SPAD), determined using the Minolta SPAD 502 (Minolta, Osaka, Japan) chlorophyll meter, were evaluated. FLW, DLW, RFW, LH and SPAD were fitted to the Seinhorst equation (1965), $y = m + (1\text{-}m)\ z^{Pi\text{-}T}$. Eggs +J2 were extracted using the technique proposed by Hussey and Barker (1983). The root gall index was determined according to the methodology proposed by Taylor and Sasser (1978), where 0 = no galls, 1 = 1 to 2, 2 = 3 to 10, 3 = 11 to 30, 4 = 31 to 100 and 5 = more than 100 galls per root system. The RF was determined according to the method proposed by Oostenbrink (1996), they were considered immune (RF = 0), resistant (RF <1) and susceptible (RF> 1) species. Biometric variables were analyzed using the software SAS version 9.0.

Results

The symptoms observed in curly leaf parsley in this study, such as reduced growth, generalized decay, leaf yellowing, and reduction of the root system, are related to the presence of root galls (Table 1; Figures 1, 2 and 3).

The relationship between final population and plant growth (indicated by FLW, DLW, RFW, LH, and SPAD) was determined by fitting the data to the Seinhorst model, demonstrating good interpolation, thereby facilitating determination of the tolerance limit (*T*) of *M. arenaria* and the maximum loss of the measured variables (*m*), and establishing, in an appropriate way, the relationship between the initial populations of the nematode in the soil and the agronomic parameter considered (Figures 1, 2 and 3).

Table 1. Effect of the initial population density of *M. arenaria* on the root gall index (RGI) and reproduction factor (RF) in curly leaf parsley.

Pi[U]	D/P[V]	RGI[W]	RF[X]
0	0	0 d	0.0 e
0.065	65	3 c	50.8 a
0.125	125	3 c	45.1 a
0.25	250	4 b	37.9 b
0.5	500	4 b	33.3 b
1	1000	5 a	31.2 b
2	2000	5 a	25.2 c
4	4000	5 a	18.2 c
8	8000	5 a	17.6 c
16	16000	5 a	15.1 c
32	32000	5 a	8.4 d
64	64000	5 a	5.2 d
C.V.[Z]		27.72	25.17

[U]Pi= Initial population
[V]D/P= Population density by plant
[W]RGI= Root gall index according to the scale by Taylor and Sasser, (1983)
[X]RF= Reproduction factor (RF= final population/ initial population).
[Z]Coefficient of variation

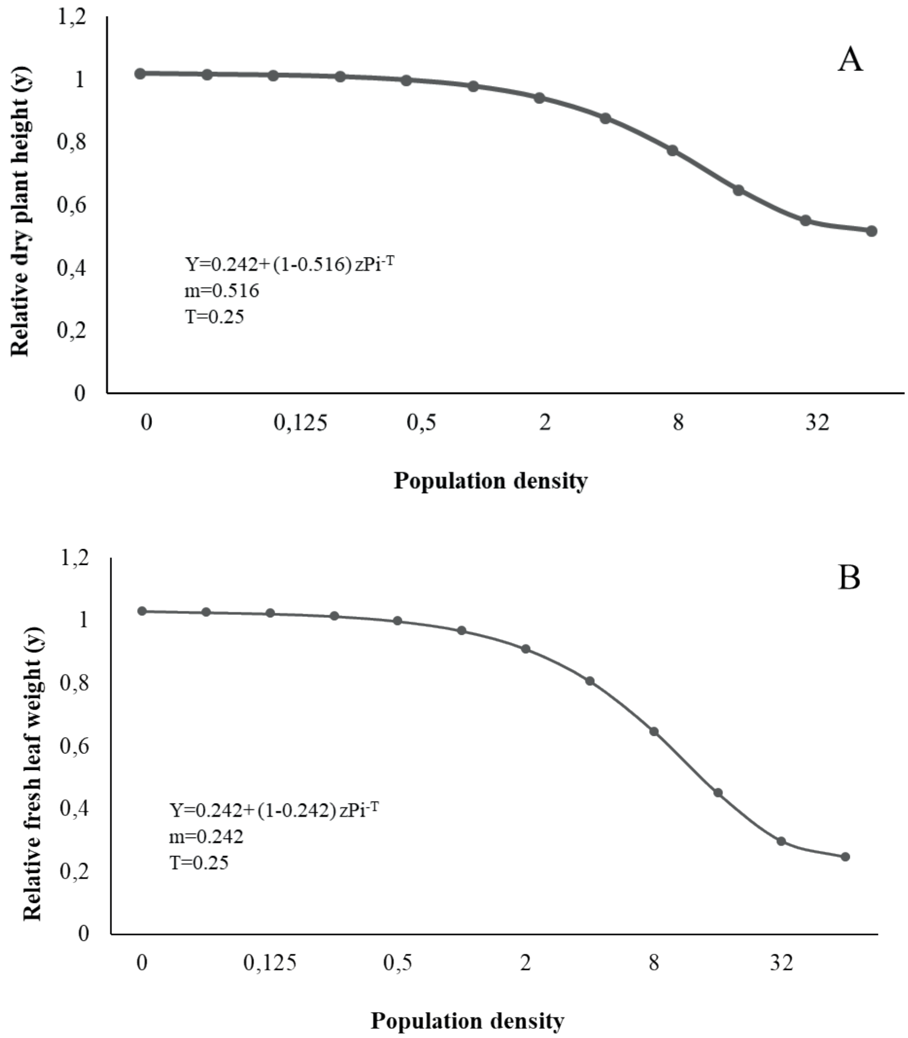
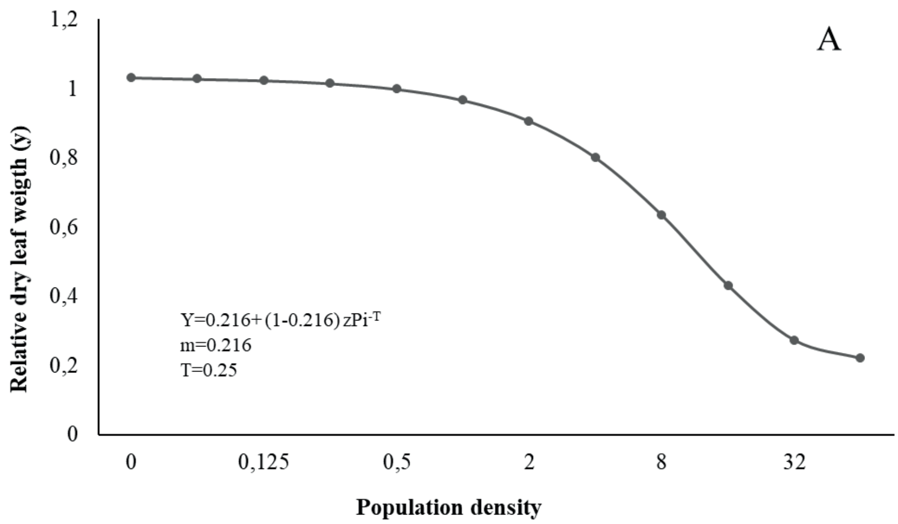


Figure 1. Relationship between plant height (A), fresh leaf weight (B) and initial population (Pi) of *Meloidogyne arenaria* in curly leaf parsley



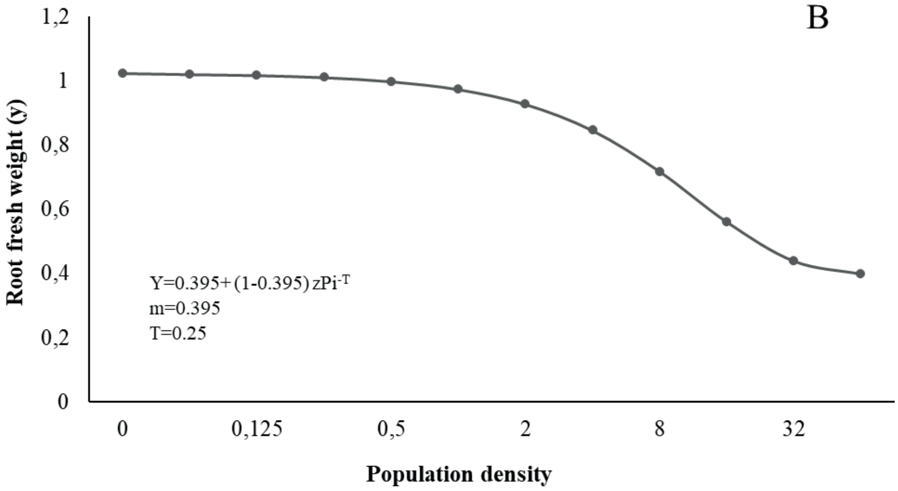


Figure 2. Relationship between dry leaf weigth (A), root fresh weight (B) and initial population (Pi) of *Meloidogyne arenaria* in curly leaf parsley

Discussion

The *T* value for LH, FLW, DLW, and RFW was estimated at 0.25 eggs + J2/cm^3 soil, while the *m* value was 0.518, 0.246, 0.220, and 0.398 respectively; therefore, from Pi ≥ 64 eggs + J2/cm^3 soil, the biometric evaluations 90 days after inoculation showed 49.14%, 76.14%, 78.68%, and 61.14% reductions, respectively (Figures 1 and 2).

The *T* value for SPAD was 0.125 eggs + J2/cm^3 soil, and the value of m was 0.265, decreasing to 74% (Figure 3); these values indicate that the damage increases with the increase in population density, and therefore the damage to crop growth increases as a result of increased reproduction (Sangronis *et al*., 2014; Mennan *et al.,* 2011; Silva *et al*., 2020).

For the RF, the *Pi* levels < 16 eggs +J2/cm^3 soil showed the highest multiplication rates with RF values = 15.1–50.8; this could be attributed to the fact that the nematodes are exposed to decreased intraspecific competition in the rhizosphere of the plant and thereby to increased food availability (Aguirre et al., 2002; Crozzoli et al., 2012; Crozzoli et al., 2013; Sangronis et al., 2014). The highest RF was measured at *Pi* = 8 eggs + J2/cm^3 soil, which caused reduced crop growth. In turn, the RF decreased from *Pi* ≥ 16 eggs +J2/cm^3 soil due to limited food and space (Perichi *et al.*, 2019).

Root damage due to gall formation induced by *M. arenaria,* determined as the RGI according to the scale by Taylor and Sasser (1983), ranged from 3 to 5; as such, RGI = 3 for 0.065 Pi and 0.125 eggs + J2/cm^3 soil, RGI = 4 for 0.25 Pi and 0.5 + J2/cm^3 soil e RGI = 5 para Pi ≥ 1 eggs + J2/cm^3 soil, corroborating the results reported by Sangronis *et al.,* (2014). Similarly, high nematode *Pi* may deteriorate infection sites and generate metabolic waste accumulation, which would affect crop root development, as indicated by Ferris (1985).

The biometric variables of parsley such as LH, FLW, DLW, RL, and SPAD (Figure 1,2,3) vary similar to the growth variables, decreasing with the increase in Piroot infection with *Meloidogyne* spp. markedly affecting plant physiology, especially the photosynthetic rate and nutrient uptake, and therefore its normal growth and development (Niño *et al.,* 2008; Moosavi 2015). Fresh leaf and root weight tended to decrease with the increase in initial population density, corroborating Niño *et al.,* (2008). Similarly, Aguirre *et al*., (2002) and Salazar et al. (2013) report height reductions at higher population densities of *Meloidogyne* spp.

The findings of this study confirm that *M. arenaria* adversely affects curly leaf parsley 'Moss curled' growth, showing a very low *T* of 0.125 eggs + J2/cm^3 soil and high DLW reductions, reaching 78.68%; therefore, owing to its high reproduction rate, *M. arenaria* should not be used in crop rotation because it would maintain and/or increase its populations.

Conclusions

With the determination of lost income that has shown the need to reduce *M. arenaria* populations by tolerance level before it seems at least to reduce poblaciones at a level that does not cause economic damage.

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