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Seasonal and vertical distribution of *Meloidogyne hapla* in organic soil
Distribution saisonnière et verticale du *Meloidogyne hapla* en sol organique

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Article abstract

The seasonal population fluctuations of the northern root-knot nematode *Meloidogyne hapla* on car rot (*Daucus carota*), onion (*Allium cepa*), and weeds were observed on organic soils in southwestern Quebec. Lowest population densities of M. hapla juveniles (J_2) were recorded in July and August, followed by a peak in September and October in plots with carrot or weedy fallow. In onion, J_2 densities remained near or below the detectable level during most of the sampling period, but a small trend in population increase was also detected in the fall. The vertical distribution of *M. hapla* was similar in carrot weedy fallow, and onion plots. J_2 were regularly recovered from the four sampling depths (0-10,11-20, 21-30, and 31-40 cm). The numbers of J_2 were greater in the 0-20 cm depth than the 21-40 cm depth, with 67, 68, and 60% of the total *M. hapla* population in the 0-20 strata for carrot, weedy fallow, and onion, respectively. The tomato bioassay method was more sensitive than the Baermann pan method for detecting low *M. hapla* densities. Because of the poor correlation between J_2 densities in the soil and the number of galls on tomato roots in the bioassay, a measurement of J_2 abundance such as the Baermann pan method shoud be supported by bioassay to further assist growers in their decision process for the management of *M. hapla* in organic soil.

Seasonal and vertical distribution of *Meloidogyne hapla* in organic soil

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The seasonal population fluctuations of the northern root-knot nematode *Meloidogyne hapla* on carrot (*Daucus carota*), onion (*Allium cepa*), and weeds were observed on organic soils in southwestern Quebec. Lowest population densities of *M. hapla* juveniles (J_2) were recorded in July and August, followed by a peak in September and October in plots with carrot or weedy fallow. In onion, J_2 densities remained near or below the detectable level during most of the sampling period, but a small trend in population increase was also detected in the fall. The vertical distribution of *M. hapla* was similar in carrot, weedy fallow, and onion plots. J_2 were regularly recovered from the four sampling depths (0-10, 11-20, 21-30, and 31-40 cm). The numbers of J_2 were greater in the 0-20 cm depth than the 21-40 cm depth, with 67, 68, and 60% of the total *M. hapla* population in the 0-20 strata for carrot, weedy fallow, and onion, respectively. The tomato bioassay method was more sensitive than the Baermann pan method for detecting low *M. hapla* densities. Because of the poor correlation between J_2 densities in the soil and the number of galls on tomato roots in the bioassay, a measurement of J_2 abundance such as the Baermann pan method shoud be supported by bioassay to further assist growers in their decision process for the management of *M. hapla* in organic soil.

[Distribution saisonnière et verticale du *Meloidogyne hapla* en sol organique]

Des fluctuations saisonnières des populations du nématode des nodosités *Meloidogyne hapla* sur la carotte (*Daucus carota*), l'oignon (*Allium cepa*) et les mauvaises herbes ont été observées dans des sols organiques du sud-ouest du Québec. Les plus faibles densités de larves (J_2) ont été enregistrées en juillet et août, suivies d'un pic en septembre et octobre dans les parcelles de carotte et de mauvaises herbes. Dans l'oignon, les densités de J_2 sont demeurées faibles ou sous leur seuil de détection au cours de la période d'échantillonnage mais une légère augmentation des populations a également été enregistrée à l'automne. La distribution verticale du *M. hapla* était similaire dans les parcelles de carotte, de mauvaises herbes et d'oignon. Des J_2 ont été régulièrement retrouvées aux quatre profondeurs d'échantillonnage (0-10, 11-20, 21-30 et 31-40 cm). Les densités de J_2 étaient plus élévées dans la couche 0-20 cm que la couche 21-40 cm, avec 67, 68 et 60 % de la population totale respectivement dans la carotte, les mauvaises herbes et l'oignon. La méthode du bioessai sur la tomate s'est

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avérée plus sensible que l'assiette de Baermann pour déceler les faibles densités du *M. hapla*. À cause de la corrélation faible entre le nombre de J_2 dans le sol et le nombre de nodules sur les racines de tomate, une mesure d'abondance des J_2 comme par exemple l'assiette de Baermann devra être supportée par la méthode du bioessai pour éclairer davantage les producteurs dans leur processus de décision dans leur lutte contre le *M. hapla* en sol organique.

INTRODUCTION

The northern root-knot nematode, *Meloidogyne hapla* Chitwood, is a major pest of carrot (*Daucus carota* L.) grown in organic soils of southwestern Quebec (Bélair 1989, 1992; Bélair and Benoit 1996; Vrain 1978). Symptoms of *M. hapla* infection include galling, large proliferation of secondary roots, and taproot malformation such as severe forking and stunting (Crête 1978; Vrain 1982; Wojtowicz 1989). In infested carrot fields, band application of the fumigant 1,3-dichloropropene is now the standard soil treatment used for nematode control (Bélair and Fournier 1997). Although this treatment increases marketable carrot yields and reduces nematode galling, it was shown that such a soil treatment has little or no effect on the final nematode densities in the soil. The latter often remain above the damage threshold level (Bélair and Fournier 1997). This results in poor correlation between the root damage indices and the nematode population densities in the field. Growers are no longer able to rely on the root damage indices recorded on the previous crop, extensively used as a diagnostic tool for the past 10 yr when using band injection of fumigant (Bélair and Boivin 1988; Boivin and Brodeur 1992). This new situation has promoted a renewed interest for soil analysis and nematode counts for diagnostic purposes.

Integrated management of plant-parasitic nematodes requires that population densities be monitored with sufficient precision for predictive purposes, but optimum timing of sampling is essential for obtaining meaningful estimates (Barker *et al.* 1969, 1984). In the organic soils of southwestern Quebec, information on seasonal and vertical distribution of *M. hapla* is now needed to determine the appropriate time and depth of sampling. Most plant-parasitic nematodes occur in the top 15-20 cm of soil and their vertical distribution is well correlated with the root distribution of the crop (Wallace 1963). However, physical characteristics of the soil may also influence nematode distribution. Maximum densities at lower depths are reported for some species, and vertical distribution patterns may be further complicated by the occurence of vertical migration during the season (Wallace 1963). Carrot, the major crop in this area, is highly susceptible to *M. hapla*, and so the monitoring of extremely low nematode densities is essential (Barker *et al.* 1984). The use of indicator plants has been shown to have much potential for estimating low population densities (Kinloch and Allen 1972; McSorley and Parrado 1983).

The objectives of this study were to investigate the vertical and seasonal distribution of *M. hapla* in organic soil under carrot, onion (*Allium cepa* L.), and weedy fallow. In addition, the standard Baermann extraction method was compared with a tomato bioassay for the assessment of *M. hapla* population densities.

MATERIALS AND METHODS

Trial 1: Seasonal and vertical distribution of *M. hapla* in carrot, weedy fallow, and onion

The trial was conducted at Sherrington (lat. 45°25' N, long. 73°41' W), Quebec, in a 1.2-ha commercial carrot field on organic soil with a severe root-knot nematode problem. This peaty soil had approximately 88% organic content with pH 5.0-6.0. Plots (15 m x 11 m)

were arranged in a randomized complete-block design with six replicates. From 1989 to 1991, the carrot cv. Sixpack (C), onion cv. XPH (O), and weedy fallow (F) were included in three cropping sequences of C-O-C, F-F-C, and C-C-C. In the field, each plot was surrounded by a 3-m band of barley (*Hordeum vulgare* L.) cv. Birka which was mowed regularly during the growing season. For each crop, cultural practices were conducted according to agricultural recommendations for Quebec (Conseil des productions végétales du Québec 1987). The seasonal and vertical distribution of *M. hapla* on carrot, onion, and weedy fallow was monitored by sampling each plot three times during the second year (9 August, 13 September, 16 October 1990) and three times during the third year (20 May, 18 June, 15 July 1991) of each crop sequence in order to cover one cycle of each crop. Soil samples were collected in the 0-10, 11-20, 21-30, and 31-40 cm soil layers using a 10-cm-diam bucket auger. In each plot, three cores were collected at random within rows in a stratified pattern. For each depth, the soil from the three cores was hand-mixed with a trowel and stored in a plastic bag until processing. Second-stage juveniles (J_2) of *M. hapla* in each plot were assessed by processing a 100-cm^3 soil subsample using a modified Baermann pan method (Townshend 1963).

Nematode counts were transformed using $\log_{10}(x + 1)$ before statistical analysis. Data were analyzed by analysis of variance using the general linear model (GLM) procedures of SAS (SAS Institute Inc. 1988), and treatment means were separated by Waller-Duncan *k*-ratio *t*-test. For analyses of population over time, data from all four depths were pooled for each sampling date.

Trial 2: Estimation of fall *M. hapla* population densities: Baermann pan vs. tomato bioassay

Soil sampling was conducted in 1995 and 1996 on an organic soil at the Agriculture and Agri-Food Canada Experimental Farm at Sainte-Clotilde (lat. 45°25' N, long. 73°41' W), Quebec, in a 0.2-ha carrot field with a severe and uniform root-knot nematode infestation. This peat soil had 80-85% organic content with pH 5.0-5.5. Carrot (cv. SixPakII) and onion (cv. Blitz) plots (7.2 m x 11 m) were arranged in a randomized complete-block design with six replicates. Soil samples were collected from the 0-10, 11-20, 21-30, and 31-40 cm soil layers in each plot on 31 October 1995 and 14 October 1996. Samples were collected, mixed, and stored as described previously. Population densities of *M. hapla* J_2 in each plot were assessed by the Baermann pan method described previously. In addition, a bioassay was performed on each sample to compare with the results from the pan method. For each soil sample, a one-month-old tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) seedling was transplanted in a 15-cm-diam pot containing approximately 800 mL of soil, and maintained in a greenhouse at 22 ± 3°C. After 60-70 d, each entire root system was washed free of soil under a stream of tap water, weighted, and chopped into 5-cm segments. The number of galls per g of root was estimated by counting the total number of galls on half of the root system. Nematode and gall counts were subjected to median χ^2 test (Dixon and Massey 1969).

RESULTS AND DISCUSSION

Seasonal variations in population densities were exhibited by *M. hapla*. The seasonal distribution of J_2 in the soil followed similar trends through the carrot and weedy fallow growing season, with a peak during late summer and early fall (Table 1). The highest average densities were recorded on 13 September with 624 and 143 J_2 per 100 cm^3 soil in carrot and weedy fallow, respectively. Numbers of J_2 dropped from May to July and went below the detectable level in July in most plots (Table 1). Because *M. hapla* is an endoparasitic species, a large proportion of the population is not in the soil but inside the root systems of host plants during the summer months. This peri-

Table 1. Number of *M. hapla* J_2 recorded under carrot, weedy fallow, and onion in an organic soil (1990-1991)

	Nematodes per 100 cm^3 soil					
	1990			1991 †		
Crop	9 Aug.	13 Sept.	16 Oct.	20 May	18 June	15 July
Carrot	3 d	624 a	479 a	211 ab	38 bc	22 c
Weed	21 b	143 a	126 a	30 b	11 b	1 b
Onion	1 a	1 a	6 a	7 a	2 a	1 a

Values in a row followed by the same letter are not significantly different ($P \leq 0.05$) according to Waller-Duncan *k*-ratio *t*-test.

† All 1991 samples collected from carrot following the 1990 crop shown.

od was followed by a significant and sharp rise in J_2 numbers ($P < 0.05$) in the months of September and October. In onion, J_2 densities remained near or below the detectable level during most of the sampling period, but a small (though not significant) rise in population was also detected in the fall. These population increases occur as newly hatched J_2 leave the egg masses deposited at the root surface of the host. Under Quebec climatological conditions, *M. hapla* require from 850 to 1000 degree-days (5°C base) to produce the first generation of newly-hatched J_2, which usually occurs by the end of July (Bélair 1989).

Population density of *M. hapla* is strongly influenced by the host plant. In this agricultural area, carrot is a very good host (Bélair 1992). Onion is generally referred to as an intermediate host and does maintain *M. hapla* densities at levels capable of causing severe yield losses in a susceptible crop such as carrot (Bélair 1992). Green smartweed (*Polygonum scabrum* Moench.), a major weed species colonizing the weedy plots in this experiment, was shown to be a good host of *M. hapla*, with a reproduction factor similar to that on carrot (Bélair and Benoit 1996). These results on the seasonal fluctuations of *M. hapla* populations agree with previous data on seasonal distribution (Barker *et al.* 1969; Wojtowicz 1989).

The vertical distribution of *M. hapla* was similar in carrot and weedy fallow plots (Fig. 1). Although numbers were low in onion plots, distribution followed a similar trend (data not shown). J_2 were recovered from the four sampling depths (0-40 cm). The numbers of J_2 were greater ($P < 0.05$) in the 0-20 cm depth than the 21-30 cm depth, with 67, 68, and 60% of the total *M. hapla* population located in the 0-20 cm strata in carrot, weedy fallow, and onion, respectively. The consistency in the nematode distribution in the soil profile, the synchronous rise in the number of J_2, and the short life cycle of this endoparasitic nematode suggest that the vertical distribution of *M. hapla* in these organic soils was mainly related to the root distribution of the plant. In an organic soil, carrot and onion secondary root systems were mainly located in the 0-20 cm soil layer with 74 and 90% of the total root volume, respectively (G. Bélair, unpublished data). Thus, these results agree with the general observation that root distribution is a main factor in the vertical distribution of plant-parasitic nematodes (Wallace 1963).

The two methods for *M. hapla* assessment consistently provided two different *M. hapla* population estimates (Fig. 2). From the Baermann pan extraction, J_2 population densities averaged 1740 (56-3240) and 48 (0-420) in 1995, and 604 (56-2230) and 3 (0-49) in 1996 in carrot and onion, respectively. Population densities of J_2 were always significantly greater ($P < 0.05$) in carrot that in onion at all depths, except the 21-30 cm depth in 1996 (Fig. 2 A,C). In

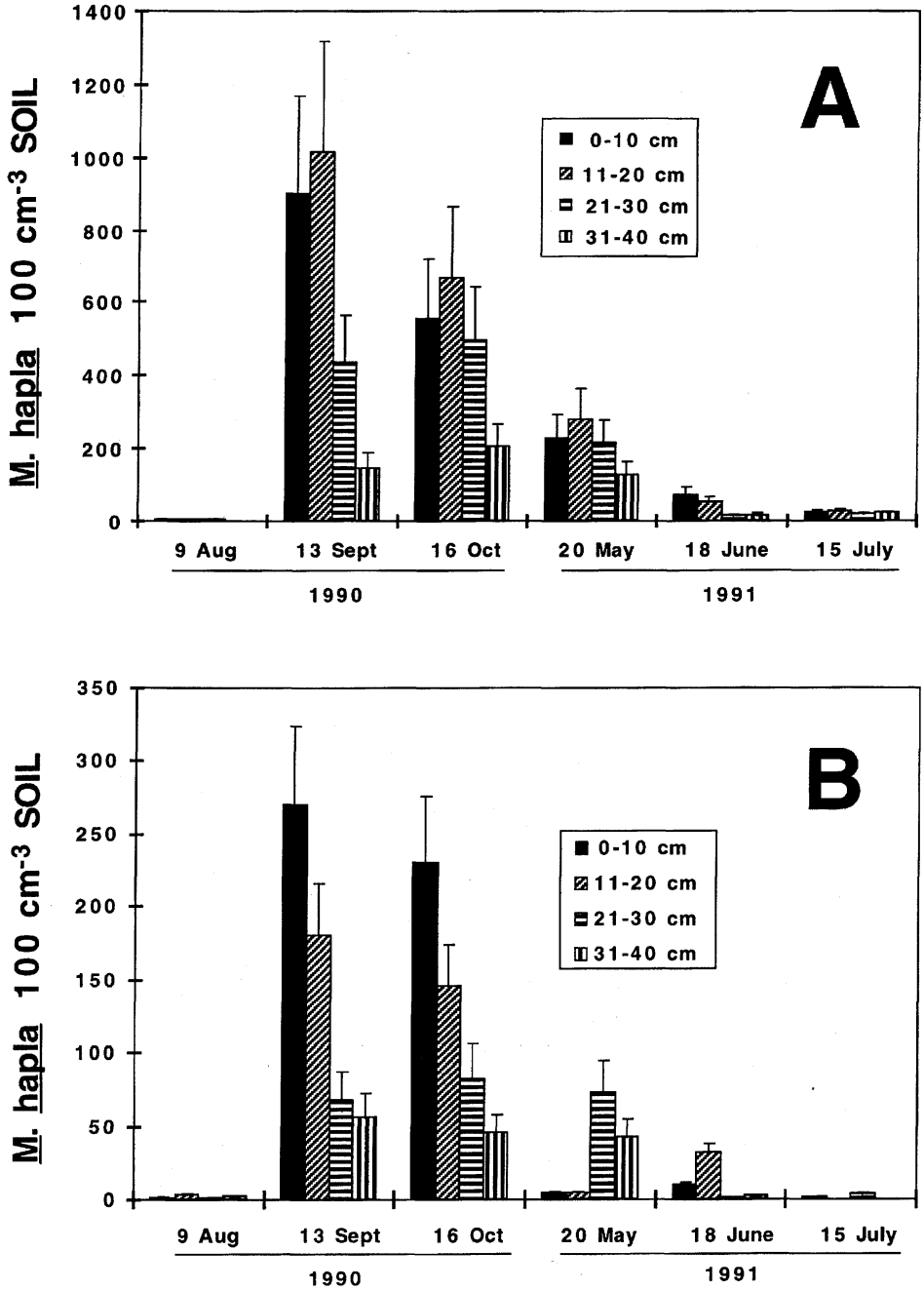

Figure 1. Mean numbers (± SE) of *M. hapla* J_2 extracted from four soil depths in carrot (A) and weedy fallow (B) during Fall 1990 and Spring 1991.

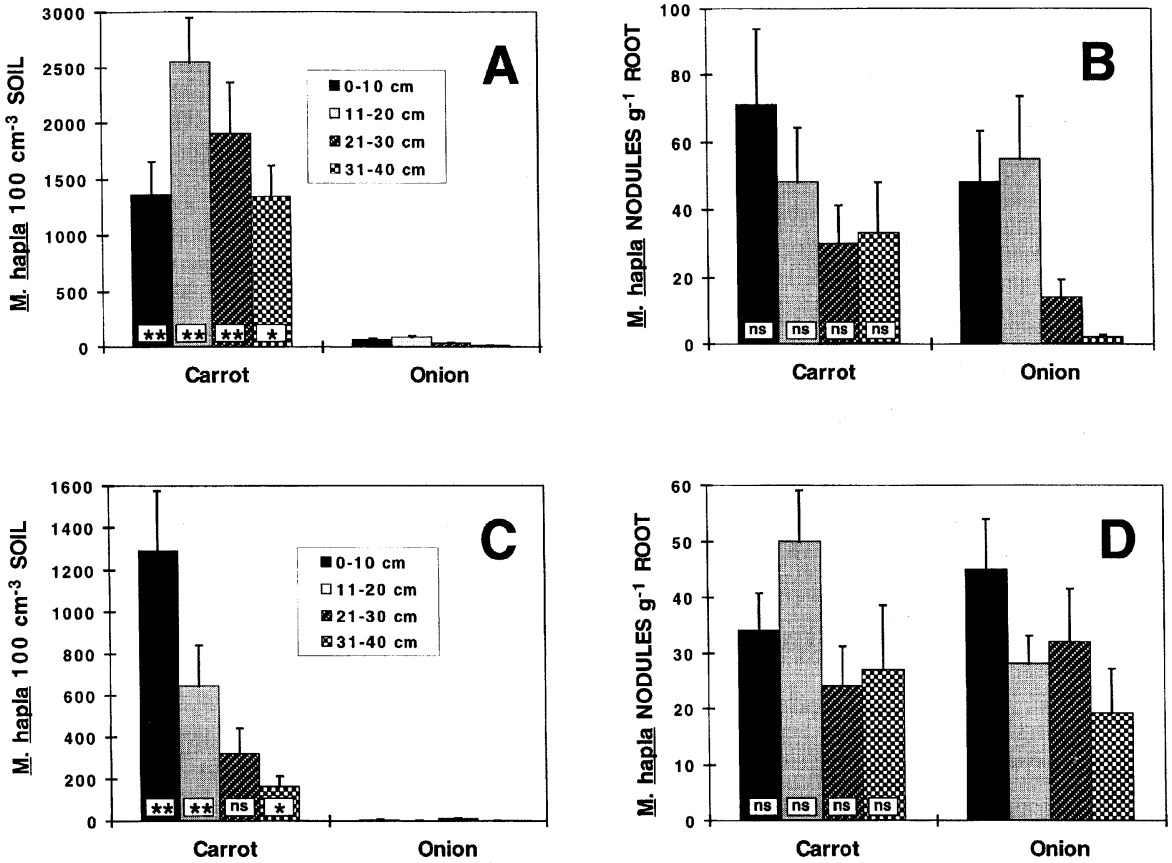

Figure 2. Mean numbers (± SE) of *M. hapla* J_2 and galls (tomato bioassay) from four soil depths in carrot and onion during Fall 1995 (A,B) and Fall 1996 (C,D). Between each crop, bars with an asterisk for the same depth are significantly different (* = 0.05 and ** = 0.01) according to median χ^2 test; ns indicates no significant difference.

the tomato bioassay, the numbers of *M. hapla* galls per g of root were numerous, with an average of 38 and 40 in 1995, and 53 and 34 in 1996 for carrot and onion, respectively. No significant differences in galling between these two crops were detected at any soil depth in both 1995 and 1996 (Fig. 2 B,D). It appears that the bioassay was more sensitive than the Baermann pan method for detecting low *M. hapla* population densities. From the same data, it also appears that a poor correlation exists between the number of *M. hapla* in the soil and the number of galls on tomato roots in the bioassay, a phenomenon that has been previously reported by Kinloch and Allen (1972). Thus, our data suggest that *M. hapla* gall counts from a bioassay should supplement other measures of abundance such as the Baermann pan.

Based on this study, it is suggested that soil sampling be performed preferably in September or October. It is also suggested that soil sampling should be done in the upper 20 cm of the soil profile for both carrot and onion in organic soil. Detection of *M. hapla* following onion could be significantly improved by performing a tomato bioassay along with a nematode soil extraction method such as the Baermann pan method. In southwestern Quebec, half of the vegetable production in organic soils is scouted by a private integrated pest management (IPM) company (Boivin and Brodeur 1992). Carrot, the major crop, is also scouted for presence of the root-knot nematode by using a damage and galling index at harvest (Bélair and Boivin 1988). Onion is the second major crop and is routinely introduced in the rotation program. Based on the present findings, the tomato bioassay would be a valuable tool for improving the scouting for the presence of *M. hapla* in infested field soils. Use of a tomato bioassay is also supported by the finding of a significant negative correlation ($r = -0.97$, $P < 0.01$) between the marketable carrot yields of the current year and the bioassay gall counts from the previous year of onion, in a microplot test (G. Bélair, unpublished data). Similar results were reported for the damage function in carrot monoculture (Bélair and Boivin 1988). A tomato bioassay performed with soil from the upper 20 cm of the soil profile collected during the previous year will be a valuable tool in the decision-making process for the grower.

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