Damage Threshold of Meloidogyne hapla to Lettuce in Organic Soil

N. M. Viaene and G. S. Abawi

Abstract: Lettuce was seeded in pots in the greenhouse and in field microplots in 1991 and 1992. Pots and microplots were filled with untreated or fumigated organic soil infested with Meloidogyne hapla at seven initial population densities (Pi) (0 to 32 eggs/cm³ soil). Lettuce weight, severity of root galling, and number of eggs per root system (Pf) were determined after 8 weeks. At the highest Pi, M. hapla caused yield losses up to 64% in the microplots and plant death in the greenhouse tests. The Seinhorst equation was used to describe the relation between lettuce weight and Pi (r² = 0.73 – 0.98) and to calculate the damage threshold density (T). Values of T were 7 and 8 eggs/cm³ soil in the greenhouse tests of 1991 and 1992, respectively. In the microplot tests, T was 1 egg/cm³ soil in 1991 and 2 eggs/cm³ soil in 1992. The damage threshold was the same in untreated and fumigated soils. At low Pi, root galling was more severe in the pots than in the microplots. Pf increased with increasing Pi of M. hapla in both tests, but declined at Pi > T in the greenhouse tests. The reproduction rate (Pf/Pi) of M. hapla was highest at the lowest Pi.

Key words: damage threshold, Lactuca sativa, lettuce, Meloidogyne hapla, nematode, northern root-knot nematode, organic soil, population dynamics, yield loss.

The northern root-knot nematode, Meloidogyne hapla Chitwood, is a major problem in the production of lettuce (Lactuca sativa L.) on organic soils in New York. Severely infected plants often fail to produce heads of marketable size and consequently are not harvested. Currently, soil fumigation with nematicides is the only practical control measure for M. hapla on lettuce. However, the use of soil fumigation has greatly declined in recent years because of high costs, variable effectiveness of the nematicide treatments, increasing concern about environmental contamination and human health risks, as well as increasing governmental regulation (20,23). Thus, there is a need to develop alternative control measures that are effective and environmentally sound (9,14).

In order to implement efficient management strategies against M. hapla, it is necessary to determine above which nematode density yield loss occurs. Any control method should be evaluated based on its ability to reduce the nematode population in the soil below the minimum density that inhibits plant growth (damage threshold density or tolerance level) (4). Various models have been used to describe damage functions of plant-parasitic nematodes, including linear and quadratic regressions (5,16,22), the exponential model of Seinhorst (3,18,22), a combination of geometric and exponential functions (6), and an inverse-logistic function (13).

Results of microplot experiments with the lettuce cultivars Pennlake and Great Lakes 6238 indicated that preplant densities of 666 juveniles of M. hapla/kg loam soil caused economic yield loss (loss in marketable yield exceeding 5% of the yield of the control treatment) (15,17). The main objective of the present study was to relate yield of lettuce grown in organic soil to initial soil population densities (Pi) of M. hapla, and to determine the damage threshold under greenhouse and field microplot conditions. Additional objectives were to relate root-galling severity to preplant nematode densities for use in bioassays, and to investigate the reproduction of M. hapla at different initial nematode infestation levels.

Materials and Methods

Greenhouse tests: In June 1991, seeds of lettuce cv. Montello were planted in 10-cm-diam. clay pots (500 cm³) filled with
untreated or methyl bromide-treated (24 g a.i./m², 20 cm deep, between plastic sheets) organic soil (soil containing at least 20% organic matter). 'Montello' is the most widely grown cultivar of iceberg lettuce on organic soils in New York, where direct-seeding is a common commercial practice (1). The soil had been infested with M. hapla at densities (Pi) of 0, 1, 2, 4, 8, 16, or 32 eggs/cm³ soil before seeding. Eggs of M. hapla were extracted from infected roots of tomato cv. Rutgers using the sodium-hypochlorite method (12) and thoroughly mixed with the soil before filling the pots. The organic soil was obtained from a commercial lettuce field near Oswego, New York. Pots were kept in a greenhouse at a temperature varying between 21 and 24°C. Lettuce plants were thinned from two to one plant per pot after 18 days. Plants were watered twice a day and fertilized monthly with a complete fertilizer (NPK 16-32-16). Foliar insects were controlled as needed with acephate sprayed at label rate.

The test was repeated in June 1992, but in methyl bromide-treated soil only. The infestation level of 1 egg/cm³ soil was omitted, and a new treatment of 6 eggs/cm³ soil was added. In this test, eggs were mixed with the upper 6 cm of soil using a spatula and the plants were thinned after 16 days. Pots were arranged in a randomized complete block design with six replications in 1991 and seven replications in 1992.

Field microplot tests: These tests were conducted in June 1991 and June 1992, in concert with the greenhouse tests. Microplots consisted of unglazed clay drainage tiles (25 cm in diameter and 30 cm long) inserted into the field, leaving a border of about 5 cm above the soil surface. The tiles were arranged 1 m apart in rows, with 1.5 m between the rows. The lower halves of the tiles were filled with mineral soil from the field, whereas the upper halves (7,500 cm³) were filled with untreated or methyl bromide-treated organic soil. Nematode eggs were mixed thoroughly with the organic soil using a trowel, to obtain the same number of eggs of M. hapla per cubic centimeter of organic soil as in the greenhouse tests. The untreated soil was included only in 1991. Lettuce cv. Montello was planted at a rate of six seeds per microplot in 1991 and eight seeds per microplot in 1992. Plant stands were thinned 3 weeks later to two plants per plot. A drench of chlorpyrifos (1.1 liter a.i./ha) and metalaxyl (1.2 liter a.i./ha) was applied immediately after seeding to control seed corn maggots (Hylemya cilicrura Rond.) and damping-off caused by Pythium spp., respectively. One week after seeding, each microplot received 5 g of fertilizer (NPK, 13-13-13). Watering, weeding, and insect control were performed as needed and according to commercial lettuce production recommendations (1). Treatments were repeated 10 times in a randomized complete block design.

Harvest and evaluation: Eight weeks after planting, lettuce heads were cut and weighed (yield), and roots were carefully dug, washed, and scored for root galling using a scale from 1 to 9. A rating of 1 indicated no visible galling, and consecutive ratings from 2 to 9 indicated that 1-3%; 4-10%; 11-25%; 26-35%; 36-50%; 51-65%; 66-80%; and > 80% of the root system was galled, respectively. The final population density of M. hapla (Pf) was calculated as the number of eggs extracted from the lettuce roots in each pot or microplot divided by the volume of the container. A modification of the sodium-hypochlorite method (12) was used to extract the eggs: roots were shaken for 15 minutes with a wrist-action shaker, instead of 4 minutes by hand.

A computer program (SeinFit), based on the algorithm developed by Ferris (11), was used to calculate the best-fitting Seinhorst equation for each experiment and to determine the damage threshold density. The Seinhorst model is of the form $y = y_m$ for $Pi \leq T$, and $y = y_{min} + y_{m} (1-m) z (Pi-T)$ for $Pi > T$; where $y$ = actual yield; $y_m$ = yield obtained without nematode damage; $m$ = a constant so that $y_{min}$ equals the minimum yield (yield at the highest possible nematode density); $z$, which has a value slightly less than 1, is the
slope-determining parameter of the curve; \( P_i \) is the preplant nematode density; and \( T \) is the damage threshold density or tolerance limit (nematode density below which no considerable yield reduction occurs) (18).

Initial egg densities of \( M. \) hapla (\( P_i \)) and final egg densities (\( P_f \)) were transformed to \( \ln (P + 1) \) for data analysis (Minitab, Minitab Inc., State College, PA). Regression analysis was used to relate root-galling severity and egg densities at harvest time (\( P_f \)) to initial nematode densities (\( P_i \)). A two-way analysis of variance was used to determine if there was an effect of soil treatment or year. Two-sample \( t \)-tests were applied to compare means of data obtained at the same infestation level in the different soil treatments or the different years.

**RESULTS**

Greenhouse tests: Many seedlings died in soils with inoculum densities of 16 or 32 eggs of \( M. \) hapla/cm\(^3\) soil. Roots of plants growing in soils infested with these population densities were small and severely galled. The relationship between weight of lettuce at harvest and \( P_i \) of \( M. \) hapla was well described by the Seinhorst model \((r^2 \geq 0.90)\) (Table 1). In 1991, the mean yield in untreated soil was not different from the mean yield in the fumigated soil \((P \geq 0.05)\). Although the weight of lettuce was higher in 1992 than in 1991 \((P \leq 0.01)\), the damage thresholds were almost the same in both years: the best-fit Seinhorst equations indicated damage thresholds of 7 eggs of \( M. \) hapla/cm\(^3\) in both untreated and fumigated soils in the 1991 greenhouse test and 8 eggs/cm\(^3\) for the 1992 greenhouse test (Fig. 1A, Table 1). The minimum yield predicted by the Seinhorst model was zero for all greenhouse experiments.

Root-galling severity increased rapidly with increasing densities of \( M. \) hapla (Fig. 1C, Table 2). The galling rating at the tolerance limit varied between 7.7 (1992) and 9 (1991 fumigated soil). Galling ratings of about 7 were given to roots growing in soils infested with \( P_i \) as low as 2 eggs/cm\(^3\) soil (1992 and 1991, untreated soil). Thus, a high root-galling rating did not necessarily correlate with a loss in harvestable lettuce in the greenhouse tests. Roots were galled in the untreated soil without additional infestation with \( M. \) hapla, indicating that the soil was naturally infested with a low population density of root-knot nematode.

The densities of eggs of \( M. \) hapla at harvest time (\( P_f \)) increased with increasing preplant densities (\( P_i \)) below or equal to the damage threshold density. At higher inoculum levels, the final nematode density (\( P_f \)) decreased with increasing \( P_i \) (Fig. 1E, Table 2). In the greenhouse experi-

<table>
<thead>
<tr>
<th>Parameters(^b) and ( r^2 )</th>
<th>Greenhouse tests(^a)</th>
<th>Microplot tests(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( y_m )</td>
<td>18.3 19.8</td>
<td>27.3</td>
</tr>
<tr>
<td>( m )</td>
<td>0 0</td>
<td>0</td>
</tr>
<tr>
<td>( z )</td>
<td>0.90 0.75</td>
<td>0.83</td>
</tr>
<tr>
<td>( T )</td>
<td>7 7</td>
<td>8</td>
</tr>
<tr>
<td>( r^2 )</td>
<td>0.93 0.96</td>
<td>0.90</td>
</tr>
</tbody>
</table>

\(^a\) Tests were performed in untreated (US) or methyl bromide-fumigated organic soil (FS).

\(^b\) The Seinhorst model is of the form: \( y = y_m \) for \( P_i \leq T \), and \( y = y_m \cdot m + y_m \cdot (1 - m) \cdot z(P_i - T) \) for \( P_i > T \); \( y_m \) = yield without nematode damage, \( m \) = a constant so that \( y_m \cdot m \) equals the minimum yield, \( z \) = parameter determining the slope of the curve, \( T \) = damage threshold density.
Fig. 1. Effects of various initial densities (Pi) of *Meloidogyne hapla* on weight of lettuce (A,B), root galling (C,D), and final population densities (Pf) (E,F) in pots in the greenhouse (A,C,E) and in field microplots (B,D,F). Pi and Pf are given on a logarithmic scale. The lines in figures A and B represent the Seinhorst equation. Root galling was evaluated on a scale from 1 (no galls) to 9 (>80% of roots galled). Pf was measured as the number of eggs produced on lettuce roots divided by the volume of soil in the pot or the microplot.
Damage Threshold of *M. hapla*: Viaene, Abawi 541

TABLE 2. Relationship of root-galling severity (RGS) and final population densities of *M. hapla* (Pf) to the initial population densities of *M. hapla* (Pi) on lettuce grown in pots in the greenhouse or in field microplots. Corresponding graphs are given in Fig. 1C–F.

<table>
<thead>
<tr>
<th>Test*</th>
<th>Regression equation</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Greenhouse tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991 US</td>
<td>$RGS = 2.7 + 3.9\ln(Pi + 1) - 0.6[\ln(Pi + 1)]^2$</td>
<td>0.91</td>
</tr>
<tr>
<td>1991 FS</td>
<td>$RGS = 0.9 + 4.1\ln(Pi + 1) - 0.3[\ln(Pi + 1)]^2$</td>
<td>0.99</td>
</tr>
<tr>
<td>1992 FS</td>
<td>$RGS = 1.3 + 4.8\ln(Pi + 1) - 0.8[\ln(Pi + 1)]^2$</td>
<td>0.96</td>
</tr>
<tr>
<td>1991 US</td>
<td>$\ln(Pf + 1) = 0.1 + 3.3\ln(Pi + 1) - 0.8[\ln(Pi + 1)]^2$</td>
<td>0.96</td>
</tr>
<tr>
<td>1991 FS</td>
<td>$\ln(Pf + 1) = 0.7 + 3.3\ln(Pi + 1) - 0.8[\ln(Pi + 1)]^2$</td>
<td>0.99</td>
</tr>
<tr>
<td>1992 FS</td>
<td>$\ln(Pf + 1) = 0.7 + 2.3\ln(Pi + 1) - 0.6[\ln(Pi + 1)]^2$</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Microplot tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1991 US</td>
<td>$RGS = 2.3 + 1.6\ln(Pi + 1)$</td>
<td>0.95</td>
</tr>
<tr>
<td>1991 FS</td>
<td>$RGS = 0.6 + 2.2\ln(Pi + 1)$</td>
<td>0.98</td>
</tr>
<tr>
<td>1992 FS</td>
<td>$RGS = 0.7 + 2.1\ln(Pi + 1)$</td>
<td>0.97</td>
</tr>
<tr>
<td>1991 US</td>
<td>$\ln(Pf + 1) = 0.2 + 0.9\ln(Pi + 1)$</td>
<td>0.96</td>
</tr>
<tr>
<td>1991 FS</td>
<td>$\ln(Pf + 1) = -0.1 + 0.5\ln(Pi + 1)$</td>
<td>0.93</td>
</tr>
<tr>
<td>1992 FS</td>
<td>$\ln(Pf + 1) = -0.3 + 0.3\ln(Pi + 1)$</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*Tests were performed in untreated (US) or methyl bromide-fumigated soil (FS).*

In general, the reproduction factor $Rf$, which is used here to refer to the ratio of egg production on lettuce roots to the number of eggs initially added to the soil ($Rf = Pf/Pi$), decreased with increasing initial densities in both the 1991 and the 1992 tests. The reproduction factor varied between 19.3 (at $Pi = 2$ eggs of *M. hapla*/cm$^3$ soil in the 1992 test) and zero (at $Pi = 32$ eggs of *M. hapla*/cm$^3$ soil in all tests) (20).

**Microplot test:** In 1991, yields of lettuce were higher in methyl bromide-treated soil than yields obtained in untreated soil ($P \geq 0.05$) (Fig. 1B). The best-fit Seinhorst equation indicated a damage threshold of 1 egg/cm$^3$ soil for both soils (Fig. 1B, Table 1). The Seinhorst model fit better with the yields in untreated soil ($r^2 = 0.97$) than with those in fumigated soil ($r^2 = 0.73$). The predicted minimum yields were 56 and 0 g in untreated and fumigated soil, respectively (Table 1). The weight of lettuce heads obtained in the microplots was higher in 1992 than in 1991 ($P \leq 0.01$) (Fig. 1B). In 1992, the Seinhorst equation that fit the data best ($r^2 = 0.99$) suggested a damage threshold of 2 eggs of *M. hapla*/cm$^3$ soil and a minimum yield of 140 g (Fig. 1B, Table 1).

Root-galling ratings increased linearly with increasing densities of *M. hapla* (Fig. 1D, Table 2). High root-galling ratings were observed only at the highest Pi, which was in contrast to the data of the complementary greenhouse tests (Fig. 1C), where roots were already severely galled at low Pi.

The numbers of eggs of *M. hapla* at harvest time (Pf) increased as the initial nematode densities were increased (Fig. 1F, Table 2). There was an interaction ($P = 0.07$) between the inoculum densities (Pi) and the soil treatment in the microplot test of 1991. At the two lowest infestation levels (0 and 1 egg/cm$^3$ soil), Pf was higher ($P \leq 0.01$) in the untreated soil than in the fumigated soil. At the highest infestation levels (16 and 32 eggs/cm$^3$ soil), Pf was higher in the fumigated soil than in the untreated
soil ($P \leq 0.05$). Egg production ($P_f$) in 1992 was higher than in 1991, at all nematode densities ($P \leq 0.01$).

In the microplot test with untreated soil, the reproduction factor of *M. hapla* ($R_f = P_f/P_i$) decreased with increasing $P_i$. In fumigated soils, $R_f$ increased with increasing $P_i$ until a maximum was reached (at 2 eggs/cm$^3$ soil in 1991 and at 6 eggs/cm$^3$ soil in 1992), after which $R_f$ declined. The reproduction factor was higher in 1992 than in 1991, as a result of the higher final nematode densities obtained in 1992 compared to 1991. The values of $R_f$ in the microplot tests varied between 1.1 and 0.7 (20).

**DISCUSSION**

Results of this research suggest that the damage threshold density of *M. hapla* to lettuce is 7 to 8 eggs/cm$^3$ organic soil in a greenhouse environment and 1 to 2 eggs/cm$^3$ organic soil in field microplot conditions. It is difficult to compare these numbers with the economic loss threshold of *M. hapla* to lettuce (666 juveniles/kg loamy soil) reported by Olthof and Potter (15) because their experiments were conducted differently. For example, they used different lettuce cultivars, a different soil type, and transplanted 2-to 6-week-old seedlings in the microplots instead of direct-seeding the lettuce. Moreover, an economic loss threshold was not calculated in the research presented here because economic loss depends on many variable factors, such as the nematode control practices used. However, the number of 666 juveniles/kg soil suggests that the damage threshold density of *M. hapla* to lettuce is quite low, as was observed in this study. Very low damage threshold densities of *M. hapla* (<10 juveniles/100 cm$^3$) have been reported for tomato, strawberry, and pea (4). The tolerance level to *M. hapla* on carrot grown in organic soil in a field microplot test was 2.7 nematodes (juveniles + eggs)/100 cm$^3$ (22). However, the reported damage thresholds of *M. hapla* calculated for most vegetables are higher than that of lettuce: the number of juveniles of *M. hapla* per kg of loam soil that caused economic losses was 2,000 for onion and spinach, 6,000 for cauliflower, and 18,000 for cabbage and red beet (15,17).

In addition to field microplots, tests also were performed in pots in the greenhouse to have an idea of the population dynamics of *M. hapla* in this environment, and to base future evaluation of control methods in pot experiments on the damage threshold density in the greenhouse. Environmental conditions are known to influence plant-growth responses to nematode infection (3,4,9,16). This effect was clearly demonstrated by the higher damage threshold densities of *M. hapla* on lettuce found in the greenhouse tests, as compared to those in the field microplots. Since the root systems of lettuce plants grown in the greenhouse were confined to the pots, weight of greenhouse-grown lettuce was much lower than that of lettuce grown in the field microplots. However, growth conditions in the greenhouse were less stressful than in the field microplots because plants were watered regularly and there were fewer extremes in temperature. Therefore, higher initial soil densities of *M. hapla* were required in the greenhouse than in the field to obtain suppression in lettuce yield. The higher lettuce yields obtained in 1992 in field microplots, as compared to those of 1991, were probably due to the better growing conditions that prevailed that season. The mean air temperatures during June and July were 20.9 and 17.8°C in 1991 and 1992, respectively, while the mean monthly precipitation during these months was 3.0 cm in 1991 and 14.0 cm in 1992, respectively. The cooler temperatures and higher rainfall in 1992 were both more favorable to lettuce growth. Consequently, a higher damage threshold density of *M. hapla* was obtained in the microplot test of 1992 (2 eggs/cm$^3$ soil) than in 1991 (1 egg/cm$^3$ soil). Similar effects of weather conditions on threshold densities.
have been reported in studies with oat and tomato (5,6). Higher yields did not always result in higher damage threshold densities. In the microplot test of 1991, the damage threshold density (T) of M. hapla to lettuce was the same in untreated soil and in fumigated soil, although the yields of lettuce in the latter were higher. In most cases, Seinhorst equations with T-values that are slightly higher or lower than the T-value of the best-fitting equation still give satisfactory goodness-of-fit assessments of the damage functions. For this reason, T-values for the 1991 experiments reported previously (21) were different from the more (mathematically) accurate T-values reported here. Calculation of the best-fitting Seinhorst equation involves error due to variability in nematode infestation and yield estimation (10). This error, although stabilized by using the means of yields at each infestation level, makes it difficult to calculate the damage threshold density with great precision. Therefore, the T-values should be considered as best estimates, which can vary with prevailing weather conditions, cultural practices, geographic locations, and other factors (9).

Direct-seeding of lettuce resulted in severe infection and damage at the highest initial densities of M. hapla, especially in the greenhouse tests. In the 1992 greenhouse test, staining of the roots of extra seedlings revealed that an average of 77 ± 37 juveniles of M. hapla had invaded the root system of lettuce seedlings 2 weeks after seeding in the soil infested with 16 eggs/cm³ soil. However, only 19 ± 15 juveniles were observed in roots of 2-week-old seedlings in the microplots infested with the same nematode density. Slower hatching of the eggs or greater distances between juveniles and lettuce roots in the microplots might have affected the rate of root infection by M. hapla, allowing the seedlings to develop a larger and more tolerant root system.

Root galling increased with increasing nematode infestation and was more severe in the greenhouse than in the field microplots at medium nematode densities (2 to 8 eggs/cm³ soil). Greater galling under greenhouse conditions is probably the result of the earlier and simultaneous hatching of eggs and more localized presence of the nematodes around the roots (8,24). The galling ratings on lettuce growing in the greenhouse at Pi equal to the damage threshold densities of M. hapla in the field microplots (1 to 2 eggs/cm³ soil) varied between 3.5 and 6.7. This large variability in root galling makes it impossible to rely on greenhouse bioassays to evaluate field soils for nematode damage potential. Because root galling increased more steadily with increasing nematode infestation in the microplots, root galling of a previous lettuce crop in the field might be used to more accurately predict potential damage (3,7).

Galling ratings at the damage thresholds (1 to 2 eggs/cm³ soil) ranged between 2.2 and 3.4 in field microplots, indicating that 1% to 10% of the root system was galled. The final densities of nematodes (Pf) were based on the numbers of eggs extracted from the lettuce roots, as it was assumed that most of the population of M. hapla was present as eggs in egg masses on the roots at harvest time. The final densities were higher in 1992 than in 1991, which could be the result of the higher plant weights in 1992 and resulting ability to sustain higher nematode populations (19). The reproduction curves of the greenhouse tests showed a decline at high nematode infestation levels, resulting from the extensive damage caused to the lettuce by such high densities (16). This decline was not observed in the microplot tests, where Pf increased with increasing Pi. The latter suggested that the carrying capacity of the roots had not been reached at the highest infestation levels. The higher Pf of M. hapla in untreated soil at low infestation levels as compared to the Pf in fumigated soil (1991 microplot test) was due to the presence of low numbers of M. hapla in the organic soil used. The presence of a natural infestation with M. hapla also was noted in the greenhouse test, where roots of
plants growing in soil without artificial infestation exhibited some galling. It was estimated that the untreated soil contained about one juvenile of *M. hapla* per cm³ soil prior to its use in the experiments, based on extraction from soil with Baermann pans. The Pf was calculated as the number of eggs extracted from the lettuce roots divided by the volume of the container (pot or field microplot). Therefore, the low values of Pf and Rf computed for the microplots may be due to the relatively larger volume of the microplots and may be considered as an artifact of the calculation method used.

This investigation provided information about the relationship between lettuce growth and the initial population densities of the northern root-knot nematode in organic soil. Similar information has been used to design nematode advisory programs for a number of plant-parasitic nematodes, including *Meloidogyne* spp., infecting corn, peanut, and soybean (2). The data obtained in the present study could be used in the establishment of a management program, provided that the damage threshold density is verified under field conditions. Knowledge of the damage threshold in the greenhouse and in microplots will be a useful criterion for the evaluation of novel control methods tested under these experimental conditions.

**Literature Cited**

20. Viaene, N. M. 1996. Damage threshold and bi-


