Spatial Pattern and Sequential Sampling Plan for *Meloidogyne hapla* in Muck-Grown Carrots

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ABSTRACT

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Iwao's patchiness regression technique was used to study the spatial dispersion of *Meloidogyne hapla* in muck-grown carrots. A gall index ranging from 0 to 5 was used to estimate the population densities of the northern root-knot nematode in the fields. This gall index was positively correlated with carrot yield losses in microplots and commercial fields. With current control strategies and production return, a 40% yield loss was established to be the economic threshold for *M. hapla*. This yield loss corresponds to a gall index of 1.6 for the current season and 0.65 for the preceding season. The basic components of the population were aggregates randomly distributed in carrot fields. This information was used to calculate a sequential sampling plan that requires a minimum of 10 samples and a maximum of 72 samples to determine if the mean gall index of a field is significantly different from 0.65 and if control measures are necessary for that field the following year.

The northern root-knot nematode, *Meloidogyne hapla* Chitwood, is a major nematode pest of muck-grown carrots in northeastern North America (4,15,16). In southwestern Québec, nearly 50% of the cultivated fields are infested (16), and it is estimated that yield losses and treatment costs may exceed more than CAN$1 million annually. Decision to manage the northern root-knot nematode often follows heavy damage and major crop losses. Laboratory assays of soil samples can be used to detect the presence of root-knot nematodes but are time consuming and, because of the high susceptibility of carrots, are rather inaccurate (17). A simpler technique is needed if nematode monitoring is to be added to an integrated pest management (IPM) program already in place in Québec (1).

M. hapla is known to disrupt the normal formation of the carrot taproot (4,14), and the extent of forking, stunting, and nodulations on the root is highly correlated with nematode population densities in the soil (17). The use of bioassays for detection of root-knot nematodes has been reported on other crops (10,11). Bioassays using damage indices are particularly well adapted to IPM programs because they are easy to use and require little if any special equipment. Although an economic threshold for *M. hapla* in muck-grown carrots has been established based on nematode counts (17), an economic threshold based on a bioassay method is not available.

Various approaches can be taken to optimize the sampling effort necessary to obtain a reliable estimate of a nematode infestation. Monitoring costs can be reduced without sacrificing precision by designing a sampling plan that takes into account the spatial distribution of a pest in a field (5,12). Several studies have dealt with the spatial distribution of plant-parasitic nematodes in cultivated fields, and these studies recently were reviewed (3). Based on population counts from quadrat samples, root-knot nematodes were reported to have an aggregated distribution, and the cluster size appeared to be field specific (3,13). This information is not currently available for *M. hapla* in carrot fields. Once the dispersion statistic of an organism is known, a sequential sampling plan can be calculated that allows the observer to estimate the population density of a pathogen or disease after each sample, instead of taking a fixed number of samples (2). Such a procedure enables a rapid classification of population levels relative to a predetermined threshold and can reduce by 47–63% the number of samples necessary to achieve a given level of precision (18).

In this study, we sought to describe the spatial dispersion of the northern root-knot nematode in carrot fields and to develop a sequential sampling plan for this species.

MATERIALS AND METHODS

This study was carried out in the muck soil region of Ste-Clotilde in southwestern Québec, Canada. Throughout these experiments, a root-knot nematode gall index from 0 to 5 was used to estimate nematode populations: 0 = no galling; 1 = 1–10 galls on secondary roots, taproots not affected, marketable; 2 = 10–50 galls, none coalesced, taproots with light forking, marketable; 3 = 50–100 galls on secondary roots, some coalesced, light forking, unmarketable; 4 = more than 100 galls, many coalesced, severe forking and stunting, unmarketable; 5 = more than 100 galls, mostly coalesced, severe stunting, unmarketable (Fig. 1). This index was applied to each sample unit.

Microplot and field data were used to establish a relationship between root-knot nematode gall indices and carrot yield losses in organic soil and to calculate an economic threshold. Ten microplots (1 × 2 m) lined with galvanized steel sheets were buried in a fibrosol (pH 4.8–5.5 and >80% organic matter). After fumigation with methyl bromide, plots were infested with a local isolate of *M. hapla* reared on tomato, *Lycopersicon esculentum*

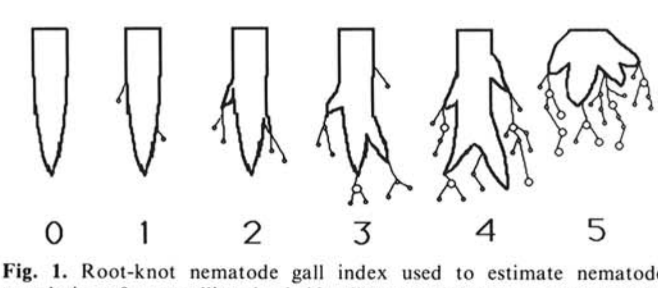

Fig. 1. Root-knot nematode gall index used to estimate nematode populations: 0 = no galling; 1 = 1–10 galls on secondary roots, taproots not affected, marketable; 2 = 10–50 galls, none coalesced, taproots with light forking, marketable; 3 = 50–100 galls on secondary roots, some coalesced, light forking, unmarketable; 4 = more than 100 galls, many coalesced, severe forking and stunting, unmarketable; 5 = more than 100 galls, mostly coalesced, severe stunting, unmarketable.

Mill. 'Rutgers,' in the greenhouse. Carrots were grown in these microplots for several years before the trial to increase root-knot nematode populations. In 1983, carrots were sown in two 2-m-long rows 45 cm apart at the rate of 150 seeds/m. At harvest, 100 carrots were sampled and indexed from 0 to 5 for galling by *M. hapla*. These carrots also were classified for marketability, and the percentage of yield loss was calculated.

The field data used in the economic threshold determination originated from six commercial fields ranging from 0.5 to 1.6 ha. In each field, 1,000 samples were taken following a regular pattern of 100 grids. Ten sampling units each consisting of a handful of carrot tops were taken at random per grid. From the five to eight carrot roots recovered, a gall index by *M. hapla* was recorded per sample. Carrots from each sample were then classified for marketability, and a percentage of yield loss was calculated for each field. These yield losses were then plotted with the average gall index for the corresponding field. Simple linear regressions were used to determine the relation between the gall indices and yield losses in both microplot and field experiments.

Because fields infested with root-knot nematodes are evaluated at or near harvesting time, thus too late for nematode control, it was necessary to establish the relationship between gall index records from a given year to those of the previous year to adjust the economic threshold for the next season of production. To gather this information, five microplots (1×2 m), identical and adjacent to those described earlier, were sown in carrots every year from 1982 through 1985. Sowing and harvesting were done as mentioned previously, and gall indices were recorded every year. Fifteen points were generated from the pairing of the gall index of the current year to the gall index of the previous year. A regression was used to determine the relation between the current and the following year's gall indices.

The spatial distribution of the northern root-knot nematode was determined from samples taken from four (1983) and six (1984) commercial fields sampled once in September of each year. Sampling areas ranged from 2.0 to 2.2 ha in 1983 and were as described earlier for 1984. For each field, 100 samples were taken at random following a zigzag pattern across the total area. The sampling unit was the same as in the economic threshold determination.

For each field, the mean index for that field was determined and used to calculate the mean crowding of that sample, $\overset{*}{X}$ (9):

$$\overset{*}{X} = \bar{X} + (s^2/\bar{X}) - 1 \tag{1}$$

where $\overset{*}{X}$ = the mean crowding of the sample, $\bar{X}$ = the mean field index, and s^2 = the variance of the mean field index.

To obtain dispersion parameters, a linear regression of $\overset{*}{X}$ on $\bar{X}$ was fitted for the 10 field surveys (6,8). In the regression equation $\overset{*}{X} = b_0 + b_1 \bar{X}$, the intercept ($b_0$) is the index of basic contagion and the slope (b_1) is the density-contagiousness coefficient. These values are used as dispersion parameters. When $b_0 = 0$, a single individual is the basic component of the distribution, and when $b_0 > 0$ or < 0, there is a positive or negative association between individuals. The density-contagiousness coefficient (b_1) indicates how the basic components are distributed in space. When $b_1 < 1$, the distribution is regular in space; when $b_1 = 1$, the basic components are randomly distributed in space; and when $b_1 > 1$, the basic components are distributed contagiously in space. In the latter case, the value of b_1 indicates the degree of aggregation of the basic components. Iwao discusses the significance and ecological implications of these distributions (8).

These parameters were used to calculate the upper and lower limits of a sequential sampling plan from the following equations (7):

$$T_{upper} = n(\text{ET}) + t[n\,(b_0 + 1)\,\text{ET} + n\,(b_1 - 1)\,\text{ET}^2]^{1/2} \tag{2}$$

and

$$T_{lower} = n(\text{ET}) - t[n\,(b_0 + 1)\,\text{ET} + n\,(b_1 - 1)\,\text{ET}^2]^{1/2} \tag{3}$$

where T = cumulative index count, n = no. samples taken, ET = economic threshold, t = value of Student's t at α = 0.025 (two-sided test, df = ∞).

The maximum number of samples to be taken was determined by

$$n_{max} = t^2[(b_0 + 1)\,\text{ET} + (b_1 - 1)\,\text{ET}^2]/d^2 \tag{4}$$

where d^2 = the confidence interval allowable for the estimation of density when the index (X) exactly equals the ET. When this maximum number of samples is reached before a sample stop line is crossed, the index is at $X = \text{ET} \pm d$.

RESULTS AND DISCUSSION

Economic threshold. A significant positive relationship was found between yield losses in marketable roots and gall indices for the current year under microplot conditions (Fig. 2A) indicating that the index could be used to estimate carrot yield loss from *M. hapla*. However, because microplots offer conditions that can be different from field situations, the same relationship was examined for field data. Again a significant positive relationship was found (Fig. 2B). Because there were no significant differences between the two regression lines ($P > 0.05$), the data were pooled and the regression $Y = 3.8953 + 21.7078\,X$ ($r = 0.88$, $P < 0.01$) was established.

Based on an average production return of 40t/ha at CAN\$60/t (CAN\$2400/ha) and standard pest control costs of CAN\$960/ha (broadcast application of 1,3-dichloropropene nematicide at 300 L/ha, 1986 prices), the loss in crop value representing that control cost was estimated to be 40%. The economic threshold for the current year for the northern root-knot nematode in muck-grown

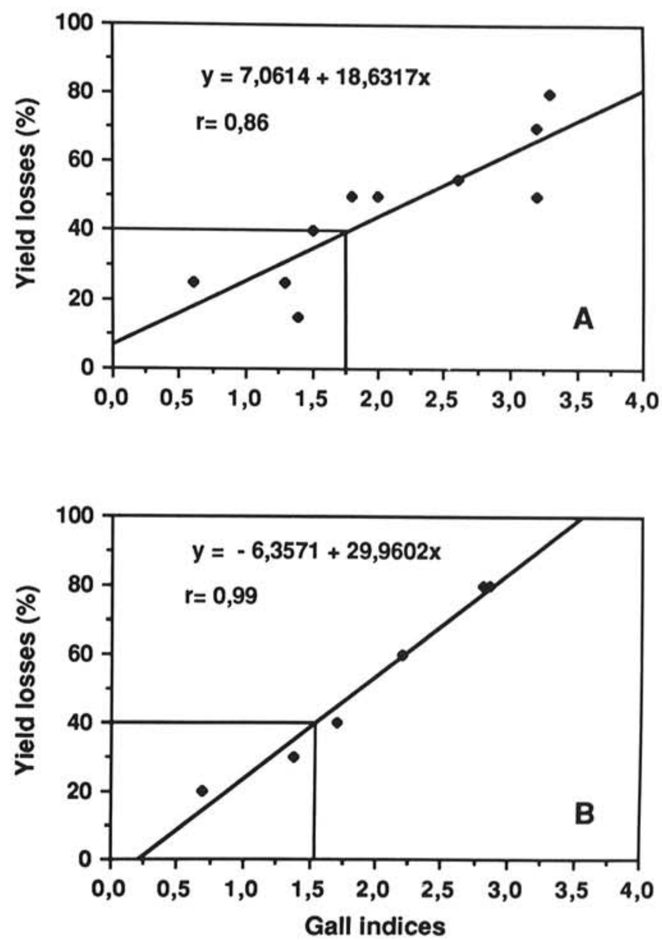

Fig. 2. Regression of carrot yield losses on root-knot nematode gall indices under **A,** microplot and **B,** field conditions.

carrots was thus estimated at a gall index of 1.6.

When the gall indices of the previous year were compared to those of the following year, a significant logarithmic relation was found (Fig. 3). Thus to reach the 1.6 mark or a 40% reduction in marketability this year, the gall index of the previous year should have been 0.65. The value of 0.65 was thus used as the economic threshold in the subsequent calculations.

Spatial dispersion. The regressions of $\overset{*}{X}$ on $\bar{X}$ were significant (1983, $r = 0.99$, $P<0.01$; 1984, $r = 0.75$, $P<0.05$) for each year of sampling, confirming the existence of a relationship between $\overset{*}{X}$ and $\bar{X}$ for the incidence of root-knot nematode on carrot roots. The data from the two years were pooled and the regression $\overset{*}{X} = 0.7286 + 0.7219\,\bar{X}$ ($r = 0.85$, $P<0.01$) was obtained (Fig. 4). The regression is highly significant and it covers the range of natural root-knot nematode infestations encountered in organic soils. The parameters of this regression were used in subsequent calculations. The b_0 value of 0.7286 was significantly different from 0 ($P<0.05$), and the b_1 value of 0.7219 was not significantly different from 1 ($P>0.05$). These results indicate that aggregates were the basic component of the root-knot nematode populations when all the fields were considered and that these aggregates were randomly distributed in carrot fields.

Sequential sampling procedures. The b_0 and b_1 values obtained were used in the sequential sampling procedure together with two other elements: an economic threshold and an acceptable error level. As determined in this study, the economic threshold used was a gall index of 0.65. An error level of 0.05 was used to calculate the

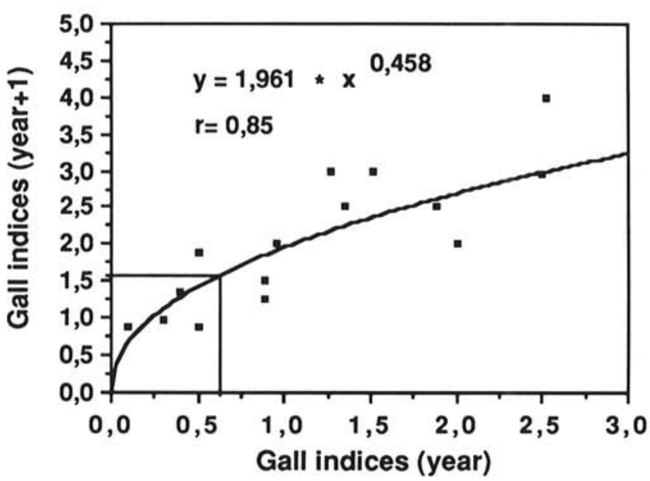

Fig. 3. Regression of root-knot nematode gall indices of the following year on gall indices of the current year.

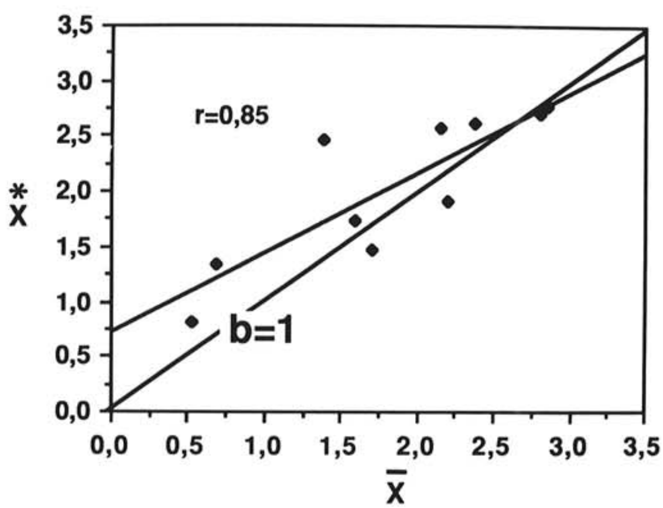

Fig. 4. Regression of mean crowding on mean root-knot nematode gall index on carrot roots.

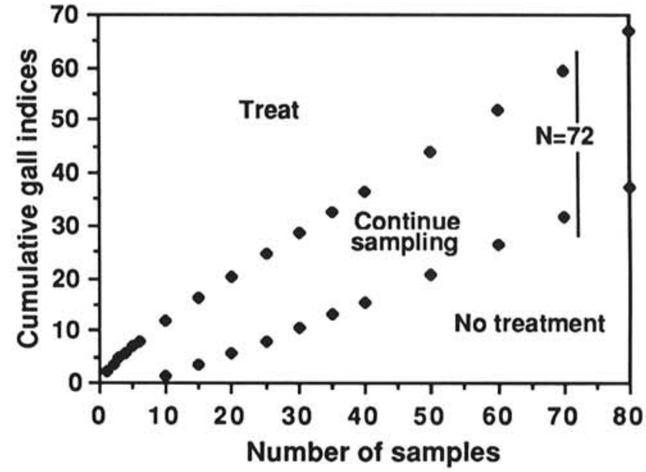

Fig. 5. Sequential sampling plan for root-knot nematode gall index on carrot roots.

stop lines of the sequential sampling plan.

Using equations 2 and 3, a sequential sampling plan is generated (Fig. 5). This sequential sampling plan calls for a maximum of 72 samples, as determined by equation 4 when d is 0.20. A minimum of 10 samples are needed and, for each root sample, if the cumulative gall index is greater than or equal to the upper limit for that number of samples, then sampling is terminated and the true mean gall index is considered to be above 0.65. A soil fumigation is then recommended. If the cumulative gall index is lower than or equal to the lower limit for that number of samples, then sampling is ended and the true mean gall index for that field is below 0.65. A soil fumigation is then not economically appropriate but other cultural practices could be recommended to maintain root-knot nematode populations below the established economic threshold. If the cumulative gall index falls between the upper and lower limits, sampling continues until a limit is reached or 72 samples are taken. At that point, the true mean gall index is considered to be at 0.65 ± 0.2 and a treatment is recommended if carrot production is scheduled in that particular field the following year.

The decision to treat a field with a fumigant nematicide implies important expenses, and growers have to be sure that such treatments are justified. It has been proposed that chemical control could be optimized by adjusting the dosage of nematicide to the nematode density (17), but lower rates of 1,3-dichloropropene reduce the fumigant's efficiency in soils with high organic content (Bélair, *unpublished*). The use of a gall index can optimize control measures by limiting treatments to fields where it is economically justified. Although it increases monitoring costs, the subdivision of large fields into 1.5 to 2.0 ha units will allow a more precise estimate of nematode population in a smaller unit and will permit treatment of these areas within a field rather than recommending an overall costly application of nematicide. Part of the extra cost of monitoring will be offset by the use of a sequential sampling plan that will reduce the number of samples to be taken before a decision can be made with a known precision level.

This method is well adapted to IPM programs that follow individual fields for insect and disease infestations. A single sampling at the end of the season can reliably determine if a chemical control of the northern root-knot nematode is needed. Based on economics (nematicide and crop values) and crop production (regional and grower level), the threshold will need to be recalculated to adjust it to the changing economic conditions.

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