# Pathogenicity of Meloidogyne hapla to Lettuce as Affected by Inoculum Level, Plant Age at Inoculation and Temperature[1]

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*Abstract:* Pathogenicity of *Meloidogyne hapla* to lettuce was influenced by inoculum level, age of plant at inoculation and temperature. Top weight of 'Minetto' lettuce was reduced 32% when 2-week-old lettuce plants were each inoculated with five egg masses. Higher inoculum levels did not further decrease top weight significantly. Inoculation at seeding reduced top growth more than inoculation of 1-, 2- or 3-week-old seedlings. *M. hapla* reduced growth more at the intermediate (21.1 C night and 26.7 C day), than at the low (15.5 C night and 21.1 C day) or high (26.7 C night and 32.2 C day), temperature regimes. *Key Words:* Root-knot nematode, northern root-knot nematode, *Lactuca sativa*.

Root-knot disease of lettuce (*Lactuca sativa* L.) caused by the northern root-knot nematode, *Meloidogyne hapla* Chitwood, 1949, is an important disease of head lettuce grown in organic soil in New York. Infected lettuce plants are frequently left unharvested in the field because they fail to produce heads of marketable size. Most crops which are grown in rotation with lettuce are susceptible to this nematode thereby making it difficult to reduce the nematode population by crop rotation. Growers have, therefore, depended on preplant chemical soil treatment for controlling the disease. However, the effectiveness of nematicide treatments has been highly variable and the need for these treatments is frequently unpredictable (3) mainly because little is known about the biology and pathogenicity of *M. hapla* as a pathogen of lettuce in organic soil. Wilson (2), using carrot as an indicator plant, found the degree of infestation of organic soil by *M. hapla* to vary widely within short distances in the same field. Yield of carrot was reported to be reduced by 50%, celery and onion by 20% and 23%, respectively, but potato yield was not reduced when percent infection of indicator plants increased from 5.1 to 93.2. In clay drainage tiles buried in the field Olthof and Potter (1) compared the influence of 0,666, 2000, 6000 and 18,000 juveniles of *M. hapla* on lettuce yields. The weight of marketable 'Pennlake' lettuce heads decreased progressively with increasing nematode populations up to a 46% decrease at 18,000.

The statement that the severity of plant damage by *Meloidogyne* spp. is influenced by soil temperature has been published a number of times but there are few published experimental data on this subject. No data on the influence of temperature on growth or yield reductions of lettuce by *M. hapla* were noted in the literature.

This study was made to determine the effect of inoculum level, age of plant at inoculation

Received for pulbication 30 June 1972.

[1] Portion of a Ph.D. thesis submitted to Cornell University, Ithaca, N.Y. by the senior author.

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and temperature on the pathogenicity of *M. hapla* to lettuce growing in organic soil.

## MATERIALS AND METHODS

Egg masses of *M. hapla* were obtained from a greenhouse culture established from egg masses taken from 'Minetto' lettuce growing in organic soil in Oswego County, New York, and maintained by periodic transfer to 'Rutgers' tomato (*Lycopersicon esculentum* Mill.). The number of larvae obtained from one egg mass was estimated by randomly selecting 10 egg masses, placing each in a shallow layer of water in a counting dish and counting emerged larvae after 7 and 14 days. An average of 626 ± 187 larvae were obtained from each egg mass.

*Inoculum level:* 'Minetto' lettuce seeds were placed in vermiculite in a 10-cm plastic pot to germinate and 5 days later, the seedlings were transplanted, one per cup, into steam-sterilized organic soil in 90-ml paper cups. Nine days later, 2 weeks from seeding, each plant was inoculated with either 1, 5, 10 or 20 egg masses with each treatment replicated eight times. Noninoculated plants were included as checks for comparison. Inoculation was done by placing the appropriate number of egg masses on top of steam-sterilized organic soil half filling a 10-cm clay pot; a lettuce seedling with its soil ball intact from a paper cup was placed on top and additional soil was added to fill the pot. Seedlings were maintained at approximately 24 C and provided with supplemental lighting. Approximately 100 ml of fertilizer (one part of 23% N, 19% $P_2O_5$ and 17% $K_2O$ : 192 parts of water) were added weekly to each pot. Plants were observed weekly for 4 weeks, after which the tops were cut off and the roots washed free of soil. The tops and roots were dried at 27-32 C in a hot-air oven for 2 days. The dry weight of the inoculated plants was expressed as a percentage of that of the noninoculated controls.

*Age of plants at inoculation:* Seedlings of different ages were provided by planting lettuce seeds at weekly intervals and 5 days later transplanting the seedlings into organic soil in 90-ml paper cups. Seedlings 1, 2 and 3 weeks old from date of seeding were each inoculated with 10 egg masses. In a fourth treatment, seeds were sown in soil placed over the egg masses. There were eight replications for each treatment with an equal number of noninoculated checks. Seedlings were grown and maintained as stated under "Inoculum level". Four weeks after inoculation the tops and roots were dried and weighed. The experiment was repeated and the results were combined in the statistical analysis.

*Effect of temperature:* Degree of root galling and top and root weights of infected lettuce grown at three different regimes of night and day temperatures were compared. The experiments were carried out in controlled-environment growth chambers provided with a 12-hr photoperiod at 2000 ft-c at low (15.5 C night and 21.1 C day), intermediate (21.1 C night and 26.7 C day) and high (26.7 C night and 32.2 C day) temperature regimes. Sixteen 2-week-old lettuce seedlings, eight each inoculated with 10 egg masses and eight noninoculated, were grown in each of these chambers for 4 weeks during which they were fertilized as stated under "Inoculum level". At the end of 4 weeks the roots were washed and the degree of galling was rated according to an index of 1-5 in which 1 = 0% of roots galled and 5 = 76-100% of roots galled. The tops and roots then were dried and weighed. The experiment was repeated and the results combined in the statistical analysis.

## RESULTS

*Inoculum level:* Five, 10 and 20 egg masses resulted in significantly lower ($P$ = .05) top weights than did zero or one egg mass (Fig. 1). Top weight of noninoculated plants did not differ from that of plants inoculated with one egg mass and no significant differences occurred among plants inoculated with 5, 10 or 20 egg masses. In contrast, roots of inoculated plants consistently weighed more than those of noninoculated plants. Except when the inoculum was one egg mass per plant, differences between root weights of inoculated and noninoculated plants were statistically significant ($P$ = .05).

Besides reducing top growth, root-knot nematode infection also caused considerable changes in the appearance of tops of infected plants. Plants inoculated with one egg mass were similar to noninoculated plants but those inoculated with five or more egg masses had broad and expanded leaves when the controls had begun to form small heads reaching 3 inches in diam. Leaves of the latter were dark green, whereas those of the former were light green and somewhat chlorotic. Control plants had short internodes but plants inoculated with five or more egg masses had longer internodes.

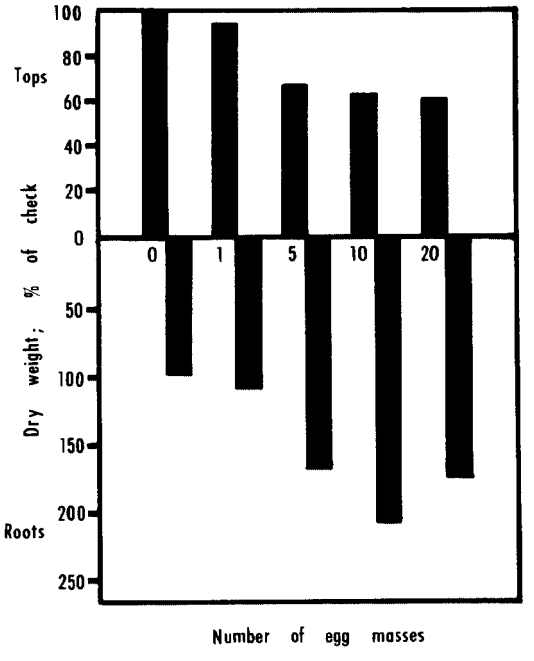


FIG. 1. Effect of inoculum levels of *Meloidogyne hapla* on dry weight of tops and roots of 'Minetto' lettuce 4 weeks after inoculation of 2-week-old plants.

Petioles were broad and stout in the control plants but spindly in inoculated plants. These symptoms also were observed in the field when root-knot nematode infected plants were examined closely.

*Age of plant at time of inoculation:* In these experiments, all inoculated plants had less top growth than did noninoculated plants. Top growth was more severely reduced when lettuce was inoculated at seeding than when inoculated 1, 2 or 3 weeks after seeding (Fig. 2). Seedlings inoculated after 2 weeks had significantly less top growth than did those inoculated at 1 week, but differences were not statistically significant between plants inoculated at 2 and 3 weeks. Roots of inoculated plants were larger and heavier than noninoculated plants except in plants inoculated at seeding; roots of these plants weighed only 54% as much as noninoculated plants. Inoculation at seeding thus caused the greatest reduction in top growth and severely restricted root growth.

*Effect of temperature:* Very severe galling coresponding to the highest root-knot index (Table 1) was observed at the intermediate temperature regime. At the high temperature regime, the disease was only slightly less severe than at the intermediate temperature regime but the plants grew poorly in the former. At the low temperature regime moderate disease occurred. Top weight of the infected plants at each temperature regime was less than that of the noninoculated plants. Top growth was retarded most by infection at the intermediate temperature regime, whereas increase in root weight due to infection was least at this temperature regime. The difference between the top weight at the low and high temperature regimes was not statistically significant. Although the root-knot index was 4.1 at the high temperature regime, top growth was not retarded as much as it was at the intermediate temperature regime. Observations of the noninoculated plants indicated the high temperature regime to be unfavorable for growth of this variety of lettuce and that this temperature regime may have been more limiting to plant growth than the disease. Each temperature regime was favorable for the development of the disease.

## DISCUSSION

Although there are very few experimental data to support this belief, considerable observational evidence by nematologists indicates that, in general, young plants are more

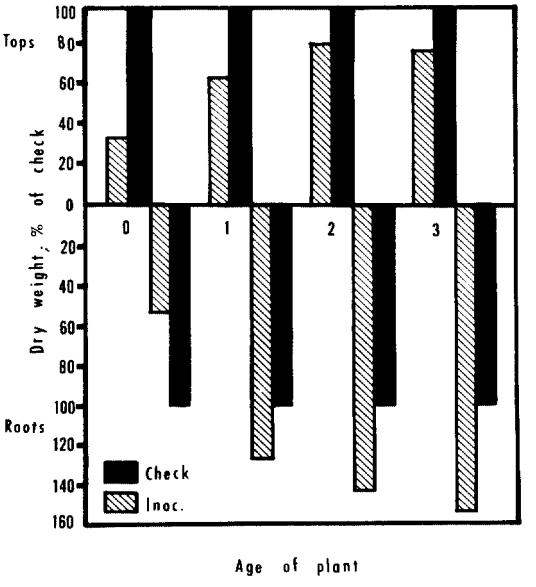


FIG. 2. Effect of plant age at inoculation on dry weight of tops and roots of 'Minetto' lettuce inoculated with *Meloidogyne hapla* and grown for 4 weeks.

TABLE 1. Relation of top and root weights and disease severity of 'Minetto' lettuce plants inoculated with *Meloidogyne hapla* and grown 4 weeks in organic soil at different temperature regimes.

| Temperature (C) | | Dry Weight (% of check) | | Root-knot |
|---|---|---|---|---|
| Night | Day | Tops | Roots | Index |
| 15.5 | 21.1 (low) | 84.3 a[a] | 147.1 a | 2.3 c |
| 21.1 | 26.7 (intermediate) | 48.8 b | 111.4 b | 5.0 a |
| 26.7 | 32.2 (high) | 76.0 a | 176.5 a | 4.1 b |

[a]Means within a column with a letter in common do not differ $P = .05$.

severely damaged by nematodes than are older plants. Results of this study suggest that, when temperature is favorable, high populations of *M. hapla* retard the growth of young seedlings more than that of older plants. This emphasizes the importance of the level of soil nematode populations at time of seeding and the need to reduce high populations during the beginning stages of plant growth.

If temperature, moisture and other soil environmental conditions are favorable for nematode activities such as hatch, movement and invasion at the time of sowing lettuce seeds, seedlings will become infected soon after germination. In heavily infested fields, such early infection may result in severe reduction of top growth and failure to form marketable heads. On the other hand, conditions unfavorable for nematode activities at planting may result in late infection after the plant has made substantial growth. Plants so infected may still be able to produce heads of marketable size. Thus, information is needed on the effects of environment on hatch, movement and root invasion of *M. hapla* in organic soil.

Rate of reproduction on lettuce or another host during the previous growing season, and overwinter survival of *M. hapla* influence population levels in the soil at planting time. Thus, experimental data are needed on the effects of environment on growth, development and reproduction of *M. hapla* in lettuce, and on overwinter survival of *M. hapla* in organic soil.

## LITERATURE CITED

1. OLTHOF, TH. H. A. and J. W. POTTER. 1971. The relation between preplant populations of *Meloidogyne hapla* and crop losses in field grown vegetables in Ontario. J. Nematol. 3:322 (Abstr.).
2. WILSON, J. D. 1957. A distribution pattern of root-knot nematode infestation on muck grown carrot. Down to Earth. 13:4-7.
3. WONG, T. K., F. C. HARPER and W. F. MAI. 1970. Soil fumigation for controlling root knot of lettuce on organic soil. Plant Dis. Rep. 54:368-370.