ECOLOGY OF LONGIDORUS MENTHASOLANUS, KONICEK AND JENSEN 1961, A PARASITE OF PEPPERMINT IN OREGON

by

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Date thesis is presented May 17, 1961

Typed by Ruth Chadwick
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ECOLOGY OF LONGIDORUS MENTHASOLANUS, KONICEK
AND JENSEN 1961, A PARASITE OF PEPPERMINT IN OREGON

INTRODUCTION

Although free-living nematodes probably were on Earth before the existence of human beings, they were not reported until 1656, by Borellus (1, p. 4), who found nematodes in vinegar. The first free-living nematode was later named Turbatrix acetae, Muller - 1783. Needham, 1745, described in wheat the first plant disease caused by nematodes which he referred to as numerous minute fibers that came to life when placed in water (8, p. 5). The so-called "fibers" are now known to have been many larvae of the wheat nematode Anguina tritici (Steinbuch, 1799) Filipjew, 1936 (2, p. 105-107). Many people today are not familiar with the term nematode; however, if the pork worm, Trichinella spiralis (Owen, 1835) Railliet, 1896, or the disease trichinosis is mentioned, they are keenly aware of the importance of small nematodes. Another example of a well-known disease that is caused by a nematode is Elephantiasis.

Since Needham described the first plant disease caused by a nematode in 1745, 100 to 140 plant parasitic nematodes have been described. A convenient classification, based on
location of nematode pests in relation to the plant parts attacked, is to group the nematodes as endoparasites and ectoparasites. The endoparasitic nematodes penetrate the host by means of a short spear (10-20μ) and generally remain within that tissue as long as there is a suitable supply of food. The ectoparasitic nematodes remain outside the host plant and generally feed by means of a long spear (50-100μ). *Longidorus menthasolanus* Konicek and Jensen, 1961, belongs to the latter group and has the latter mentioned spear.

In 1954 the mint nematode was reported to cause a decline of peppermint stands in certain areas of Oregon. Since then this pest has been found throughout the peppermint culture area, and 7 per cent of the entire acreage is known to be infested. Obviously, more information is necessary before its habits can be understood and a realistic control program developed. Prior to this study very little was known about the ecology of this nematode or its destructive potential to Oregon's peppermint industry. The objectives of the investigation were to obtain fundamental information about this pest, such as life cycle, host range, seasonal population fluctuations and environmental requirements.
LITERATURE REVIEW

The genus *Dorylaimus* subgenus *Longidorus* was described in 1922 by Micoletzky (10, vol. 6, pt. 4, p. 141); however, in 1936 Thorne and Swanger raised the subgenus to generic rank, *Longidorus* Micoletzky, 1922. In 1954 Horner and Jensen (3) reported that a *Longidorus* sp. was associated with extensive stunting and dying of peppermint (*Mentha piperita* L.) in many of the older mint-growing areas of Western Oregon. In experimental tests, the nematode caused stunting and a root die-back condition. In 1956 they identified the nematode as the ectoparasite *Longidorus sylphus* Thorne (5), and in 1957 they reported that the nematode could be controlled by soil fumigation (6). Jensen, in 1958, estimated that approximately 1,000 of the 14,000 acres used for peppermint culture were infested (4, vol. 10, p. 55).
DESCRIPTION

The nematode associated with peppermint decline was first reported to be *Longidorus sylphus* Thorne, 1939 (6), but recent studies indicate that several morphological differences exist. One of the methods used to discover basic differences between two closely related nematodes is the use of the de Man formula, which compares body proportions as follows:

\[
L = \text{total body length in millimeters}
\]

\[
a = \frac{\text{total body length}}{\text{greatest width}}
\]

\[
b = \frac{\text{total body length}}{\text{length of esophagus}}
\]

\[
c = \frac{\text{total body length}}{\text{length of tail, measured from anus to tip of tail}}
\]

\[
V = \frac{\text{distance from head to vulva}}{\text{total body length}} = \%
\]

\[
T = \frac{\text{length of testes}}{\text{total body length}} = \%
\]

spear = length of spear in microns

spicule = length of spicule in microns

gubernaculum = length of gubernaculum in microns

Lengths of ovary or ovaries and post-uterine sac are expressed as a percentage of the total body length and are given as exponents of \( V \), e.g., \( V = 850^8 \). This would mean
that the vulva was midway between the head and tip of tail with each ovary extended down the body a distance equal to 8 per cent of the total body length.

**Longidorus menthasolanus, New Species** (Figure 1)

Measurements: 30 females: Length = 5.1 mm. (3.9-6.1); a = 78 (61-101); b = 13 (10-16); c = 105 (77-134); V = 7.6 [7.7](3-134.4-50.4-14); spear = 89µ (70-110); extensions = 60µ (30-68).

Male: 30 males: Length = 4.6 mm. (3.6-5.1); a = 97 (66-121); b = 21 (12-33); c = 98 (72-136); [10 males T = 48% (33-70)]; spear = 89µ (65-98); extensions = 60µ (41-62).

Female (Holotype): Length = 4.2 mm.; a = 81.5; b = 10.4; c = 111.3; V = 5.4; spear = 70.5µ; extensions = 45µ.

Male (Allotype): Length = 4.5 mm.; a = 106.7; b = 17.2; c = 100.8; t = 52.9%; spear = 72; extensions = 46µ.

Description: Body long and slender, tapering toward anterior extremity, curving into gentle arch when relaxed with heat. Lip region expanded slightly, forming a semi-knob-like head. Six lips having two circlets of six and ten papillae. Amphids abnormally large, almost encircling head. Amphid apertures slit-like, usually obscure.
Guiding ring near apex of spear. Spear length variable (65-110\textmu\text{m}) with slight bow in posterior one-third. Spear extensions (30-68\textmu\text{m}) ranging from one-half to two-thirds as long as spear. Esophagus beginning as a slender tube which expands rather abruptly into an elongated basal bulb. Bulb length equal to or slightly greater than that of spear. Cardia bluntly conoid. Intestine six cells in circumference.

Female: Vulva a transverse slit located near middle of body. Ovaries short and reflexed one-half or more their length. Eggs, when present, approximately two and one-half times body width and three times as long as wide. Prerectum from ten to 11 times anal body width. Rectum length about equal to anal body diameter. Two pairs of caudal papillae present, and one preanal pair directly opposite rectum in a median location. Tail with conspicuous radial striae, bluntly convex-conoid, slightly longer than anal body diameter.

Male: Testes often lacking or only partially developed with granular bodies resembling sperm. Supplements consisting of an adanal pair and six to ten in a ventro-median series, beginning within range of the spicula and spaced uniformly. Prerectum seven to eight times anal body width. Spicula blunt, arcuate, and with small prong
branching from each side of proximal extremity to three-fourths of its length. Small lateral guiding pieces present. Oblique copulatory muscles prominent. Six caudal papillae present, two sub-ventral, two sub-dorsal, and two located in a median or lateral position; tail with conspicuous radial striae, bluntly convex-conoid, length slightly longer than anal body diameter.

Diagnosis: *Longidorus menthasolanus* can be separated from two other closely related species, *L. sylphus* Thorne, 1939, and *L. elongatus* Thorne and Swanger, 1936, by the morphological features given below.

In *L. menthasolanus* the lip region is set off slightly, the tail is bluntly convex-conoid (as compared to dorsally convex-conoid to blunt terminus) and males are present. It may be separated from *L. sylphus* on these bases.

*Longidorus menthasolanus* differs from *L. elongatus* as follows: The lip region or head is not set off as much; body width is greater in proportion to body length; female tail is generally shorter; male tail is bluntly convex-conoid and is not tapered as much.

Holotype: Female collected on September 3, 1958; slide deposited with Department of Botany, Oregon State University, Corvallis, Oregon.
Allotype: Male collected on September 3, 1958; slide deposited with Department of Botany, Oregon State University, Corvallis, Oregon.

Paratypes: Twenty-nine females and 29 males collected from soil about roots of mint; other data same as for holotype and allotype.

Type host: Collected from soil around roots of peppermint, *Mentha piperita* L.

Type locality: Talbot, Oregon, United States of America.
Figure 1. *Longidorus menthasolanus* n. sp.
A. Female anterior portion.  B. Female posterior portion.  C. Female face view. 
D. Male anterior portion.  E. Male posterior portion.
LIFE CYCLE

Free-living nematodes generally go through a series of four moults before they reach maturity. The first moult commonly occurs either before or after the developing nematode hatches from its egg case. The larva which emerges is known as the second-stage larva. The first, second, and third stages are devoted primarily to body growth with little or no development of genital organs. The genital organs develop in the fourth-stage larva and finish development after the fourth and final moult.

Procedure

Three attempts were made to study the life cycle in terms of time elapsed from egg to mature nematode. The first attempt was made by taking infested soil, air drying it to kill the nematodes and leave only the more resistant eggs, then planting with a known host plant. A second attempt also involved eggs; however, the procedure used to obtain eggs included the use of a sugar solution, and apparently the developing larvae were killed. The third and final attempt was to begin with first-stage larvae.

To obtain first-stage larvae, soil containing \textit{L. menthasolanus} was mixed with a large volume of water,
allowed to settle for a short time, then the liquid fraction poured through a 200-mesh screen. The nematodes retained on the screen were then washed into a pint jar by backflushing with water. The first-stage larvae were then picked out of the water with a bamboo splinter while being observed through a binocular microscope. On October 1, 1960, 20 larvae were placed in each of 15 one-gallon (120 mm.) wide-mouth jars that contained *Longidorus*-free soil and one tomato plant as a source of food. After inoculation, the jars were transferred to a constant-temperature tank which was maintained at 25° C. ± 1°. No attempt was made to control the moisture other than maintaining moisture suitable for growth of the tomato plant.

Each following week five 200-gram samples of soil were removed and examined to determine the stage of larval development.

**Results and Conclusions**

The general sequence of development was as follows: After one week only first-stage larvae were found; second week, second-stage larvae; third week, third-stage larvae; fourth week, third-stage larvae; fifth week, both third- and fourth-stage larvae; sixth week, fourth-stage larvae and adults; seventh week, adults. Soil samples were
examined for two additional weeks, and no larvae were detected. This is not too surprising, since few eggs are seen in females throughout the year. In addition to this, less than 10 per cent of the original 20 nematodes placed in each jar were recovered.

Although a single nematode has never been observed throughout the cycle from hatching of the egg to the development of an adult, much information may be obtained by observing several thousand nematodes and examining the moulted cuticles. Development is believed to occur as follows: the first-stage larva hatches from the egg and after a short time moults. Both the emerged second-stage and the first-stage larvae have spears and alimentary canals, but no extensive development of the genital organs is evident. After a growth period, while length increases approximately 1.5 mm. to 2.5 mm., the larva moults once more and emerges as the third-stage larva. The third-stage then moults, and the final, fourth-stage larva emerges. Fourth-stage larvae may be distinguished from the third-stage by a clear area or germinal region midway of its length. The vulva and part of the ovaries develop during the growth of fourth-stage larvae and may be seen beneath the final moulted cuticle. There appears to be very little increased growth in the length of the adult nematode after the last moult, but the genital organs finish development.
HOST RANGE STUDY

Since 1954, Oregon State University personnel have been keenly aware of the economic importance of *Longidorus* (3) (5). Approximately 1,000 of the 14,000 acres of peppermint in the state were estimated to have been infested in 1958 (4, vol. 10, p. 55). Damage in individual fields ranged from a trace to development of large barren areas (Figure 2), which may on occasion envelope an entire field.

Symptoms are most evident during late spring and early summer before the peppermint (*Mentha piperita* L.) has had a chance to cover. During this time fields have many bare areas which absorb the sun's rays, thus warming the soil to a favorable temperature for development of the nematode.

Although Oregon mint growers are now growing mint at a profit, the future does not look so bright. Because of losses from Verticillium, symphyllids, and nematodes, there is a good possibility that the mint industry may be forced to other localities. The establishment of nematode non-susceptible crops is, therefore, very important for profitable farm management as well as for the survival of the peppermint industry. Although the problem involves more than nematodes, only nematodes are dealt with here.
Figure 2. Peppermint planting with a large barren area that resulted from a heavy infestation of *Longidorus menthasolanus*. 
Procedure

Infested soil was thoroughly mixed with the aid of a shovel, passed through a 5-mesh screen to remove any plant parts and clods, again mixed in a utility cement mixer, and placed in one-gallon tin cans. One hundred ten of these cans were placed on a greenhouse bench directly, while another 110 were autoclaved at 9 pounds pressure for 4 hours, then placed in the greenhouse. The cans of soil that had been autoclaved were watered twice daily for a period of one month to remove breakdown products and salts. The other (non-autoclaved) cans of soil were watered only once daily to maintain soil moisture.

Over a period of 14 days (January 25, 1960, to February 8, 1960) 20 different plants were established in the soil thus prepared.

Bean (*Phaseolus vulgaris* L. hort. var. Royal Wonder)
Pea (*Pisum sativum* L. hort. var. Dwarf Telephone)
Corn (*Zea mays* L. hort. var. Golden Cross Bantam)
Cucumber (*Cucumis sativus* L. hort. var. Extra Long Green)
Squash (*Cucurbita maxima* Duchesne hort. var. Buttercup)
Oats (*Avena sativa* L. hort. var. Carleton Oats)
Table Beet (Beta vulgaris L. hort. var. Blood Beet)
Tomato (Lycopersicon esculentum Mill. hort. var. Bonny Best)
Cabbage (Brassica oleracea capitata L. hort. var. Extra Early Flat Dutch)
Vetch (Vicia villosa Roth. hort. var. Hairy Vetch)
Fescue (Festuca rubra L. hort. var. Penlawn Fescue)
Clover (Trifolium hybridum L. hort. var. Aliske Clover)
Carrot (Daucus carota L. hort. var. Danvers Half Long)
Clover (Trifolium repens L. hort. var. Ladino Clover)
Sugar Beet (Beta vulgaris L. Common Sugar Beet)
Turnip (Brassica rapa L. hort. var. Purple Top, White Globe)
Alfalfa (Medicago maliva hort. var. Ranger Alfalfa)
Bluegrass (Poa pratensis L. hort. var. Marion Blue-grass)
Peppermint (Mentha piperita L. Common field peppermint)
Wheat (Triticum compactum Host. hort. var. Elmar Wheat).

Two methods were used to determine if a particular plant was a host. One method was by comparing plant weights, and the other was by comparing nematode counts.
In the first method plants (roots and tops) were harvested, placed in an oven dryer at 80° C. for 48 hours, and then weighed. The roots from each plant were then detached and weighed separately. With plants such as beet and turnip, the fleshy part of the root was weighed with the foliage, and tap root and fiber roots were used for root weight. The weights of five replications were then totaled and averaged. A total weight to root weight ratio was calculated by dividing the total weight of five replications of entire plants by the total weight of the roots. The ratio of plants grown in non-infested soil was then subtracted from the ratio of plants grown in infested soil.

Example: the total dry weight of five replications of bean grown in non-infested soil was 33.2 grams. The total weight of roots was 14.1 grams. The average weight for the entire plant would be 6.6 grams (33.2 ÷ 5) and that for the roots would be 2.8 (14.1 ÷ 5). The total weight to root weight ratio would be 2.3 (33.2 ÷ 14.1). The respective averages and ratio for beans grown in infested soil were 5.4 grams, 0.9 grams and 5.9. The difference then was 3.6 (5.9 - 2.3). Averages, ratios and differences are recorded in Table 1.

Quite logically one might expect a difference in root weight to total weight ratios if some organism were feeding
Table 1. Effect of *Longidorus menthasolanus* on Various Plants as Indicated by Dry Weight\(^1\)

<table>
<thead>
<tr>
<th>Plant Tested</th>
<th>Soil Without Nematodes</th>
<th>Infested Soil</th>
<th>Ratio Difference</th>
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<tr>
<td></td>
<td>Total Dry Weight (gms.)</td>
<td>Root Dry Weight (gms.)</td>
<td>Root Ratio</td>
</tr>
<tr>
<td>Peppermint</td>
<td>18.8</td>
<td>7.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Bean</td>
<td>6.6</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Tomato</td>
<td>7.4</td>
<td>2.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Table beet</td>
<td>2.4</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
<td>Vetch</td>
<td>23.4</td>
<td>15.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Ladino clover</td>
<td>8.2</td>
<td>3.2</td>
<td>2.5</td>
</tr>
<tr>
<td>Wheat</td>
<td>20.3</td>
<td>18.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Cucumber</td>
<td>5.2</td>
<td>1.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Alsike clover</td>
<td>11.6</td>
<td>6.0</td>
<td>1.9</td>
</tr>
<tr>
<td>Bluegrass</td>
<td>28.1</td>
<td>23.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Turnip</td>
<td>0.5</td>
<td>0.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Oats</td>
<td>4.6</td>
<td>1.7</td>
<td>2.6</td>
</tr>
<tr>
<td>Corn</td>
<td>16.2</td>
<td>9.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>4.7</td>
<td>3.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Penlaw fescue</td>
<td>23.1</td>
<td>19.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Pea</td>
<td>7.8</td>
<td>1.2</td>
<td>6.1</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>4.7</td>
<td>0.5</td>
<td>8.2</td>
</tr>
<tr>
<td>Cabbage</td>
<td>6.0</td>
<td>2.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Squash</td>
<td>4.8</td>
<td>0.4</td>
<td>12.1</td>
</tr>
<tr>
<td>Carrot(^2)</td>
<td>--</td>
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<td>--</td>
</tr>
</tbody>
</table>

1. All weights are averages of 5 replications. Root ratios (total plant weight \(\div\) by total root weight) have been calculated and compared to show differences between plants grown in infested and non-infested soil. Duration of experiment 1/25/60 - 6/10/60.

2. Plants apparently died from phytotoxic breakdown products.
on or damaging the roots. An arbitrary classification was therefore established. Plants that had a ratio difference from -2.9 to 1.0 were considered undamaged, while a ratio difference from 1.1 to 6.1 was considered damaged.

The other method used to determine host plant relationships was to count the nematodes in soil in which the respective host plants had been grown. An increase or a decrease in the nematode population (Longidorus) would indicate whether or not the nematodes had a suitable host plant. Prior to planting, ten of the 220 cans (five with infested soil and five with autoclaved or control soil) were taken to the laboratory. The soil from each pot was then blended well, and two samples were weighed. One 200-gram sample was mixed with water equal to ten times the volume of the soil and poured through a 200-mesh screen. This procedure was repeated two more times, then the nematodes retained on top of the screen were washed into a pint jar by backflushing with water. After the nematodes had settled to the bottom of the jar, the top portion of water was decanted. The nematodes were then transferred to plastic petri dishes where they were counted with the aid of a binocular microscope. The other sample, consisting of 100 grams, was placed in a drying oven at 110° C. for 48 hours. The number of nematodes per 100 grams of dry
soil per replication was then calculated. In order to determine whether there was an increase or decrease in the population, the average number of nematodes per five replications was determined and subtracted from the average number of nematodes found prior to planting. The difference obtained for each plant tested is recorded in Table 2. At the termination of the experiment, the remaining ten pots (out of the beginning 220), which had not been planted, were processed in a similar manner.

Results and Conclusions

Table 1 shows the effect of Longidorus on various plants, and comparative ratios indicate that bean, hairy vetch, ladino clover, peppermint, table beet and tomato were damaged. Although this table gives some criteria that might be used in assessment of the possible root damage, one should use the ratios only as a guide. Wheat, for instance, while showing a root ratio difference of only 0.7, had an average weight of 20.3 grams for the plants grown in non-infested soil and only 4.9 grams for the plants grown in infested soil. In addition, some difficulties were encountered in the establishment of some plants. One week after the vetch had germinated, slugs (Deroceras reticulatum, Muller) ate the tops off the control vetch
Table 2. Influence of Various Test Plants upon Soil Populations of *Longidorus menthasolanus*

<table>
<thead>
<tr>
<th>Plant tested</th>
<th>Nematodes per 100 grams of dry soil*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Increase</td>
</tr>
<tr>
<td>Alsike clover</td>
<td>188.2</td>
</tr>
<tr>
<td>Peppermint</td>
<td>170.8</td>
</tr>
<tr>
<td>Bluegrass</td>
<td>115.2</td>
</tr>
<tr>
<td>Tomato</td>
<td>114.6</td>
</tr>
<tr>
<td>Ladino clover</td>
<td>81.6</td>
</tr>
<tr>
<td>Table beet</td>
<td>63.4</td>
</tr>
<tr>
<td>Bean</td>
<td>45.8</td>
</tr>
<tr>
<td>Wheat</td>
<td>33.4</td>
</tr>
<tr>
<td>Turnip</td>
<td>8.0</td>
</tr>
<tr>
<td>Cabbage</td>
<td>6.8</td>
</tr>
<tr>
<td>Squash</td>
<td>0.2</td>
</tr>
<tr>
<td>Cucumber</td>
<td></td>
</tr>
<tr>
<td>Carrot</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td></td>
</tr>
<tr>
<td>Pea</td>
<td></td>
</tr>
<tr>
<td>Oats</td>
<td></td>
</tr>
<tr>
<td>Penlawn fescue</td>
<td></td>
</tr>
<tr>
<td>Sugar beet</td>
<td></td>
</tr>
<tr>
<td>Hairy vetch</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td></td>
</tr>
<tr>
<td>Control (no test plant)</td>
<td></td>
</tr>
</tbody>
</table>

* Figures represented are the differences between average number of nematodes obtained from five replications prior to and after plants were grown in infested soil, which contained at the beginning of the experiment 39.6 nematodes/100 grams of dry soil.
plantings. What appeared to be phytotoxicity from breakdown products and salting-out was encountered with plantings of sugar beet and carrot. Other variable factors to be considered are the type of plant and root system. Such plants as clover and grass have well-developed root systems and could, no doubt, in this short-time experiment outgrow or maintain roots in spite of the nematodes. The best criteria for host plant relationships appear to be the increase or decrease in the number of nematodes and a comparison of root systems.

From Table 2, where the increase and decrease in the number of nematodes per replication is calculated, one can conclude that suitable host plants are alsike clover, bean, bluegrass, ladino clover, peppermint, table beet, tomato and wheat. Apparently these nematodes can survive on such plants as cabbage, squash and turnip; however, such plants as corn and hairy vetch are seemingly unsuitable. One can conclude that different plants affect the nematode population and that Longidorus menthasolanus has adverse effects upon certain plants. In some genera (alsike and ladino clover) there was a trend for susceptibility, but in other genera (table beet and sugar beet) no consistent trend was noted. Similar results were encountered within plants of the same family in that some were susceptible while others were not.
Since symptoms on most crops are somewhat similar, only those of peppermint will be discussed here. On September 22, 1960, some of the plants used in the host range study were sown in non-infested soil and some in infested soil. These plants were established for photographic purposes only. Plants were washed free of soil after five months and are shown in Figures 3 through 7.

Peppermint plants grown in heavily infested soil are generally shorter than those grown in non-infested soil. Attacked plants often have a reddish appearance; however, this is probably a common reaction of peppermint to any factor that retards its growth. Roots of plants from infested areas are often malformed, stubby, or lacking (Figures 7 and 8). Feeder roots are entirely absent. During the summer months, July and August, if the plant is dug and the soil removed the nematode may be seen coiled around rudimentary rhizomes (Figure 8). Field and temperature tank studies indicate that warm soil (20° to 25° C.) favors development of *Longidorus menthasolanus*. 
Figure 3. Alsike clover grown in non-infested soil (top) and in infested soil (bottom). Note differences in root systems.
Figure 4. Elmar wheat grown in non-infested soil (top) and in infested soil (bottom). Note striking differences in tops and root systems.
Figure 5. Marion bluegrass grown in non-infested soil (top) and in infested soil (bottom). Notice top growth apparently shows little effect; however, differences in root growth are obvious.
Figure 6. Bonny Best tomato (top) and table beet (bottom) grown in infested soil. Note absence of feeder roots.
Figure 7. Peppermint grown in non-infested soil (top) and infested soil (bottom). Note striking differences in root systems.
Figure 8. Peppermint rhizome with coiled *Longidorus menthasolanus*. 
SEASONAL FLUCTUATION OF POPULATION

Common questions essential to the development of control programs are: How deep is the nematode found in the soil? Does the pest move up to the top layers of soil then move back to lower depths under favorable and unfavorable conditions? At first one might conclude that such factors are unimportant, but a closer evaluation will show a need for this type of information. A farmer would indeed be foolish to fumigate the soil at depths where there were no nematodes or while the pests were concentrated in another area. Likewise, county agents and experiment station personnel might reach a false conclusion if they were to take soil samples for nematological evaluation at depths where there were no nematodes or during periods when there were none.

Procedure

To answer these questions and others, a field survey was conducted. The field selected for study was located approximately one mile south of Talbot, Oregon. This field was chosen for the following reasons: (1) it contained areas that were sometimes flooded during the winter as well as areas that were not; (2) the soil was Willamette silt loam, which is a soil where peppermint and *Longidorus* are commonly associated; (3) Oregon State University personnel
had established fumigation plots, and the control plot could be utilized; and (4) the area around the experimental plots represented a normal peppermint culture. Tentatively the experimental control plot was labeled plot A, and another area nearby, but outside of the fumigation region, was labeled plot B. Soil samples were collected once each month for a period of one year with a soil sampling tube (Figure 9) which removed soil cores two centimeters in diameter and up to 23 centimeters long (Figure 9). Soil cores were taken at each of the following depths: 0-6", 6-12" and 12-24". The upper one inch of each soil core taken below 6" was removed to prevent possible contamination from soil that fell down the hole. Fifteen to 20 soil cores from each depth per plot were combined in a plastic bag and brought to the laboratory for analysis. To insure that collection of soil cores was unbiased, they were taken every three paces and in a serpentine path. Temperature readings were taken at each site (at 3, 9 and 15-inch depths) after digging a hole two feet by two feet and inserting a mercury thermometer approximately two inches into the soil profile at the selected depths.

The number of female nematodes and larvae per 100 grams of dry soil per sample was then calculated in the same manner as in the host range study. The combined total of the number of nematodes found per 100 grams of dry soil
Figure 9. Soil sampling tube and soil core.
at three depths and the temperature at the 3-inch level are shown in Figures 10 and 11.

Results and Conclusions

The population of larvae and females in plot A (Figure 10) appears to reach a peak in June, decreases from July to September, and then increases from September to January. The high population recorded in the latter part of the year may be, however, a false population. The peppermint crop is normally harvested in the summer, thus leaving plants that are less actively growing and developing new roots. Logically then, one might assume that nematodes will wander in search for food. Since soil cores were taken at random, this type of sampling would tend to increase the number of nematodes per sample because the nematodes would be uniformly distributed throughout the soil. A second factor which could influence the apparent rise in population is that the fields are plowed in the fall to control rust (Puccinia menthae Pers.) and powdery mildew (Erysiphe cichoracearum DC.), thus blending the large numbers of nematodes found around the roots with the rest of the soil.

Population trends in plot B (Figure 11) throughout the year do not show much fluctuation until November; however, this is understandable because the peppermint plants
were sparse, and results may be explained by the previously made assumptions. There is a noticeable difference in both plot A and plot B between the number of nematodes found in January and those found in February of the previous year. Two factors are believed to be responsible. First, the total population under favorable conditions increases until the host plants die, then decreases because there is a lack of food. Second, and probably most important, is that in December 1958 the upper two inches of soil were frozen. This factor, plus cool soil (3°C) the following month, probably preserved the nematodes even though many were dead. When the nematodes were counted, no attempt was made to determine if they were alive or dead, with the exception of partially decomposed nematodes. Additional samples taken since this survey indicate that many nematodes die in fall and winter months and are decomposed by bacteria.

If the previous assumptions are correct, there appears (Figures 10 and 11) to be a direct correlation between reproduction and temperature. However, from the data presented one can not be sure but must speculate.

Most of the nematodes (81.2%) were found in the upper 0-6" soil profile with a few (14.4%) from 6-12" and only a trace (4.4%) were found from 12-24". There was no detectable migration of nematodes from one level to another throughout the year.
Figure 10. Seasonal fluctuations of nematodes and temperature in plot A. The totals of nematodes per 100 grams of dry soil at depths of 0-6", 6-12" and 12-24" and the temperature taken at the midpoint between 0-6" are plotted for each month with the exception of October, at which time no sample was taken because the field had recently been plowed.
Figure 11. Seasonal fluctuations of nematodes and temperature in plot B. The totals of nematodes per 100 grams of dry soil at depths of 0-6", 6-12", and 12-24" and the temperature taken at the midpoint between 0-6" are plotted for each month with the exception of October, at which time no sample was taken because the field had recently been plowed.
Longidorus menthasolanus is commonly associated with soils that are frequently flooded during the winter months. Consequently, one might expect that the nematode would thrive and reproduce readily in cold and water-logged conditions. Temperature studies were, therefore, designed to test this assumption.

Procedure

Soil infested with L. menthasolanus was obtained from a farm located near Jefferson, Oregon. The soil was then spread out on the floor of a greenhouse headhouse, allowed to air dry for four days (soil being mixed each day with a shovel to ensure uniform drying) and passed through a 5-mesh screen. The soil was blended in a utility cement mixer, potted (4375 grams per jar) in 95 wide-mouth one-gallon (120 mm.) jars and sealed with lids. The soil was air dried and sealed in jars to minimize moisture fluctuation while handling. Five of the 95 jars containing infested soil were selected at random and brought to the laboratory for moisture and nematological analysis. Two samples were removed and weighed from each of the five jars. One 100-gram sample was placed in an oven at 110° C.
for 48 hours then weighed to determine the moisture percentage. Another sample consisting of 200 grams was used to determine the number of nematodes present per 100 grams of dry soil. The average percentage moisture for the five jars was 3.5 (3.2 to 3.9%), and the average number of nematodes per 100 grams of dry soil was 22.5 (19.3 to 28.0).

The 90 remaining jars were divided into three equal groups of 30 jars, and the percentage moisture per group was adjusted to 41 per cent (saturation), 27 per cent (average percentage moisture found in the field during winter and spring months), and 13 per cent (a moisture content equal to one-half of 27). The amount of water needed was calculated from the 3.5 per cent average mentioned in the previous paragraph. Total weight, including weights of jar, soil, and water, was then recorded on each jar. Finally, a hole two centimeters in diameter and a length equal to one-half the depth of the soil (Figure 12) was made down the center of the soil to ensure even moisture distribution.

On July 6, 1959, each jar was planted with a peppermint cutting (Figure 12) five inches in length and then transferred to constant-temperature tanks (Figure 13) maintained at temperatures of 10, 15, 20, 25, 30 and 35° C. Each temperature tank had five replications of each
Figure 12. Top view of jar with peppermint planted prior to being placed in temperature tank.
Figure 13. One of six temperature tanks used for the temperature studies. Note jar on top in which the soil water has not yet reached an equilibrium.
moisture level (41%, 27% and 13%). One thermograph recorder (Auto-lite) was placed at each tank to record temperature fluctuation.

Moisture content was maintained ± 2% by weighing each jar twice daily and adding the amount of water equal to twice that lost by evaporation. Care was taken to ensure that an equal amount of water was added to the top and bottom (through the hole in the soil) layers of soil. After peppermint cuttings had become established and produced sufficient roots for nematode feeding, the tops were cut back to reduce the amount of water lost through transpiration and to reduce the effect of the added plant growth on the total weight of each jar.

Temperature records, with the exception of a few short time fluctuations caused by sticking thermostats, indicated that the temperatures were maintained at plus and minus one degree. Air temperatures, however, varied from 90° F. in the day to 55° F. at night.

The experiment was terminated after 107 days, and the number of nematodes per 100 grams of dry soil per replication was determined.

**Results and Conclusions**

The nematode is able to survive in saturated soil at
moderate soil temperatures, although their rate of reproduction and survival is low (Figure 14). At 13 per cent and 27 per cent soil moisture, the population of nematodes appears to decrease in the temperature range from 10° C. to 15° C., then increase throughout a temperature range of 15° C. to 30° C., and again decline from 30° C. to 35° C. There were, however, two replications that had an unusually low number of nematodes. One replication was at 15° C. with 13 per cent moisture, and the other was at 15° C. with 27 per cent moisture. The decline in the population at temperatures from 10° C. to 15° C. may therefore be superficial. A soil moisture content of 13 per cent appears to be somewhat more favorable for nematode reproduction; however, this may be due to cooling of the upper layer of soil by evaporation.

Thermometer readings taken from the upper inch of soil showed that the soil temperature was the same as that of the water in the temperature tanks up to 20° C.; however, at 25° C., 30° C. and 35° C. there were some discrepancies noted. The upper inch of soil at 13 per cent moisture was found to be 24.5° C. at the tank set at 25° C., 29° C. at the tank set at 30° C., and 34° C. at the tank set at 35° C. The respective soil temperatures at the 27 per cent moisture level were 24° C., 28° C. and 32.5° C. Regardless
Figure 14. Effect of various soil temperature and moisture conditions on the population of *Longidorus menthasolanus*. Averages of five replications are plotted. Experiment duration 107 days (7/6/59 to 10/21/59).
of these discrepancies, the most favorable temperature for reproduction lies somewhere between 25° C. and 35° C.
EFFECT OF FREEZING ON SURVIVAL

When a pest is disseminated to other localities, its establishment does not necessarily follow. Environmental conditions, for example, may be unsuitable for growth and reproduction of the pest. Although the soils of the Willamette Valley are seldom frozen, transporting the nematode with soil around mint rhizomes to other localities is possible. Many people prohibit the transporting of a nematode pest to their farm, but, on the other hand, if they were in a region where the soil is frozen throughout the winter, this may not be a serious problem because the nematode might perish. The upper soil crust was frozen during the month of December 1958. Even though no immediate consideration was given to the effect of freezing on nematode survival, the previous temperature studies indicated that temperature certainly plays an important role.

Procedure

Three 4000-gram samples of infested soil were adjusted to 9.5, 16.2 and 33.0 per cent moisture levels. Each sample was then divided into 20 equal sub-samples weighing 200 grams, placed in a double plastic bag, and sealed. Five bags from each moisture level were placed in a refrigerator at \(-1^\circ C. (\pm 1^\circ C.)\); five were placed in another
refrigerator set at -19° C. (± 1° C.); and another five were left in the laboratory at room temperatures (23-25° C.). The remaining five were sealed in glass jars, one sample per jar, then packed with dry ice. These were placed in an ice chest which had previously been lined with three-inch fiberglass insulation. The soil samples were sealed in jars to prevent possible buildup of CO₂ which could asphyxiate the nematodes or change the pH, thus causing death. The ice chest with its samples was then transferred to a refrigerator set at 2° C. Additional dry ice was added every three days to replenish the supply. Although no temperature readings were taken in the ice chest, the temperature was assumed to be down to -70° C.

After one week the frozen samples were removed and allowed to warm gradually to room temperatures. The nematodes were then washed out of the soil and retained in pint jars. Three days later the number of surviving nematodes was determined with the aid of a binocular microscope.

Results and Conclusions

The only visible sign of injury to those nematodes that were dead was in the esophageal region. The esophagus of dead nematodes had an opaque appearance in comparison to
the semi-transparent esophagus of surviving nematodes. Apparently, freezing temperatures have a very deleterious effect upon these nematodes. One hundred and forty-six nematodes survived out of 150 examined at room temperatures (23-25°C); two survived at -1°C; three survived at -19°C; and three at -70°C. Since insufficient data were obtained from this experiment, it is not possible to state that soil moisture plays an important role in survival of nematodes when the soil freezes. There appeared to have been a slight trend for a higher survival rate in drier soil, however.
EFFECT OF DESICCATION ON SURVIVAL

Temperature and moisture studies indicated that saturated soils were unfavorable for the development of the mint nematode. This condition is an extreme which is found sporadically throughout peppermint fields in the winter and following irrigation. Another field condition that sometimes exists is the development of dry areas throughout the field prior to irrigation. To answer the question, "What happens to the nematode population during these dry periods?" a desiccation experiment was conducted.

Procedure

Fifty 250-ml. Pyrex beakers were filled with infested soil containing 20.8 per cent moisture. Five of the 50 were utilized to establish the number of active nematodes out of the first ten nematodes observed. The remaining 45 beakers were placed upon a laboratory bench and allowed to air dry at room temperatures (23-25°C). Each week thereafter, five samples were utilized for nematological counts, and one was used to determine the soil moisture percentage. When a moisture level was found to have a lethal effect upon the nematodes, five samples were used to determine the percentage moisture. The upper three-fourths of the soil was removed, and only the bottom one-fourth was used for
determinations. This was done because the upper portion dried out more rapidly and became stratified. The bottom one-fourth was used to minimize this condition. The results are shown in Figure 15.

Results and Conclusions

A soil moisture range from 6 to 20 per cent apparently has little effect upon survival. Soil moisture from 2.4 down to 0.7 per cent had a pronounced effect upon survival, with moisture contents at or near 0.7 per cent being lethal. Although not recorded here, nematodes from the original soil source, with soil moisture percentage at or near 20, were checked at the end of the experiment, and no dead nematodes were observed. This, of course, rules out the possibility that the nematodes died from starvation.

Nematodes obtained by dry screening the soil (from the remaining 18 beakers) and observed with a binocular microscope showed a difference between those from air dried soil and those from moist soil. There appeared to be a film of water around the nematodes in moist soil, but when the soil dried out this film broke. After this water film breaks, the nematode is believed to dry out rather rapidly and die.
Figure 15. Relation of soil moisture to survival of *Longidorus menthasolanus*. Numbers plotted represent the average of 5 replications of the first 10 counted.
Current taxonomic studies have shown that the mint nematode, previously identified by Jensen and Horner (6) as *Longidorus sylphus*, is a new species and is described herein as *Longidorus menthasolanus*. The slightly set off lip region and the bluntly convex-conoid female tail clearly distinguish this nematode from *L. sylphus*. The discovery of males, which have not been reported for *L. sylphus*, also serves as an excellent distinguishing feature for *L. menthasolanus*. This is the only known plant parasite of the genus *Longidorus*. Members of this genus are well adapted for ecto-parasitism, as they possess very long spears, attain a tremendous size, and often are associated with plant roots. Future investigations will probably disclose additional species as plant parasites.

The mint nematode is the largest plant parasitic nematode reported. The exceptionally long spear (70-110μ) makes this nematode especially adapted for an ecto-parasitic mode of life and enables the pest to feed deeply in plant roots.

Peppermint plants in heavily infested soil have few feeder roots. The plants that die in these areas are probably not killed directly by the pest, but, rather by the inability of the plant to take up sufficient nutrients and water. Damage in peppermint fields ranges from a trace to
the development of large barren areas. The amount of damage done to the crop is associated with population trends of this nematode and ecological factors which affect the pest and host plants.

Eight plants out of twenty tested were suitable host plants. This showed that care must be taken when selecting crops to be grown in infested soil. Such crops as bean, clover (alsike and ladino), table beet, tomato or wheat should not be used in the crop rotation program because they would be severely injured and would also increase the nematode population rather than decrease it. Although some plants such as clover and bluegrass may grow satisfactorily in spite of the nematode, the possibility of re-establishing peppermint following these crops is unlikely because the nematode population is maintained.

A field survey taken for a period of one year showed that *L. menthasolanus* does not migrate up and down within the soil profiles, but remains within the peppermint root zone (0-6" depth). One might suspect that the pest would migrate from one soil depth to another as the soil temperature changed; however, this was not the case.

Moisture and temperature, however, are important to the development and reproduction of this nematode. Not only does temperature affect the population directly, but
also indirectly. Temperature affects the nematode directly by favoring growth and reproduction, and indirectly by stimulating plant growth which furnishes food for the nematode. Studies on the effect of temperature and moisture indicate that conditions optimum for plant growth are also preferred by the nematode. Extreme conditions such as air dried soil (2.4 down to 0.7% moisture), saturated soil, and temperatures below freezing are unfavorable for survival of *L. menthasolanus*. The low survival rate encountered in saturated soil is probably a matter of oxygen deficiency rather than toxicity. The inability of the pest to reproduce readily in these adverse conditions gives some indication as to areas where the nematode can become established. If the pest is disseminated to areas where the soil is frozen throughout the winter months or to areas where summer fallow is practiced, the probability of the pest becoming established is very slight.

Farmers should not use unwashed rhizomes from infested fields to plant new peppermint stands, regardless of the location of the new field. The possibility of establishing this pest is a threat that can not be overlooked. An alternate crop, such as Marion bluegrass, should not be planted in infested fields because this would maintain high population levels of the destructive pest. Although the
The economical importance of this pest is difficult to estimate, the shifting of the mint industry to other areas as in the past two years must be attributed in part to injury by *Longidorus menthasolanus*. 
SUMMARY

The mint nematode was determined to be a new species and was described and named *Longidorus menthasolanus*.

The nematode requires approximately seven weeks to reach maturity, starting from the first-stage larva.

Eight plants out of twenty tested were suitable hosts: bean, bluegrass, clover (alsike and ladino), peppermint, table beet, tomato, and wheat.

The greatest population occurred between November and December; however, this was believed to be a false peak. The greatest population throughout the year probably occurs between April and August. Most (81.2%) of the nematodes were found in the upper six inches of soil, few (14.4%) were found from six to 12 inches, and only a trace (4.4%) were found from 12 to 24-inch soil depths.

The most favorable soil temperature at 13 per cent and 27 per cent soil moisture was between 25° C. and 35° C. (being close to 30° C.).

Nematodes were unable to survive in saturated soil at temperatures above 25° C., and only a few survived at 25°, 20°, 15°, and 10° C.

A few nematodes survived soil temperatures of -1°, -19°, and -70° C.; however, 98 per cent of the nematodes were killed when frozen.
Few nematodes survive in soil moistures from 2.4 per cent down to 0.7 per cent, and none at 0.7 per cent soil moisture. The cause of death is probably due to the breaking of a water film which normally surrounds the nematodes.
BIBLIOGRAPHY


