MORPHOLOGICAL AND ECOLOGICAL INVESTIGATIONS OF LONGIDORUS ELONGATUS (DE MAN, 1876) THORNE AND SWANGER, 1936 IN ARIZONA

by

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ABSTRACT

Longidorus elongatus, an ecto-parasitic nematode, has received limited investigation. This study was conducted to investigate its ecology in Arizona. Morphological comparisons were also made between specimens obtained from varied ecological areas.

Soil temperatures studies illustrated that the nematode does not survive temperatures above 35°C. The most favorable temperature found in this study was 15°C.

Soil moisture of 27% yielded the highest population of the nematode while the saturated soil was the least desirable for reproduction. Moisture at 18% by weight was considered optimum for plant growth but was less satisfactory than the 27% treatment for nematode growth. At the lower moisture rate (11%), the plants were stressed for water and the nematode population was also reduced.

Loamy sand (80% sand, 12% silt, and 8% clay) was found to be the most suitable of the various soil types used in this investigation. This was followed by loamy soil and sandy loam.

Plants of economic importance to Arizona's agriculture were investigated. Tomatoes, sugarbeets, grapefruit, alfalfa and peppermint were tested as hosts of Longidorus. Sugarbeets, grapefruit, tomatoes

and peppermint were hosts to this nematode, whereas alfalfa was a doubtful host.

In vitro storage of L. elongatus indicated that the highest survival rate was at 25°C in distilled water. Temperatures of 3°C and 9°C were not favorable for more than two weeks of storage.

The conspicuous morphological difference between the specimens of L. elongatus from Arizona with those from the other areas of the United States and Europe was body length. However, other morphological characters of the Arizona specimens, such as vulva and body width, had striking similarities, typical of the species. They did not differ appreciably from those of other ecological areas of the world.

INTRODUCTION

The vinegar eelworm (Turbatrix acetae), familiar to us today, was the first free-living nematode described by Borellus in 1656. Needham's discovery of the first plant parasitic nematode in 1743 set the pace for further work in nematology. However, accent on the applied aspects of nematology dates back to 1855 when Berkeley described the root galls in cucumber as caused by Meloidogyne spp. The widespread destruction of sugarbeets in Germany during the period 1870-1910 by the sugarbeet nematode (Heterodera schachtii) accelerated investigations on its control and made the European world nematode conscious (17).

In spite of these pioneering works the progress of nematology has been very slow and only recently has it occupied a place among other sciences. Still, much research is needed to establish the fundamentals of many aspects of plant parasitic nematodes. Nematode ecology is one such aspect on which there is a dearth of information.

This study was an investigation of the ecological behavior of the ecto-parasitic nematode Longidorus elongatus as it occurs in Arizona. An added facet of the investigation was the comparative morphology of Arizona specimens with those from different geographical regions of the

United States and Western Europe. Such morphological studies are important when one considers the fact that differences in ecological habitat may have profound influence in modifying the morphology of the species (3, 17).

Until recently, Longidorus elongatus was considered to be of little economic importance. However, the extensive damage to the mint crop in Oregon caused by Longidorus sp. demonstrated its destructive potential (4, 5, 6). In Arizona, this nematode has been encountered from soil surrounding the roots of grape and citrus. Its feeding habits upon these important crops which are grown in this state have not been investigated.. The fact that Longidorus elongatus is a vector of various plant viruses, including the beet-ringspot strain of tomato black ring virus (TBRV-BRV) and raspberry ringspot virus (RRV), also enhances the importance of this species and illustrates the need for further study of this nematode in Arizona (1, 15). Longidorus elongatus, being a migratory ecto-parasite with a very long and hollow stylet, is well adapted as a good virus vector. The species may as well be involved in the transmission of more plant virus diseases (9).

Discrepancies in taxonomic literature exist since the classification of Dorylaimidae is not yet resolved. In the past, species in the genus Longidorus have been separated by use of minute morphological characters which frequently are not enough criteria for species differentiation. The following taxonomic classification is tentatively maintained

according to Thorne:

Phylum	Nematoda
Class	Adenophorea
Order	Dorylaimida
Super-family	Dorylaimoidea
Family	Longidoridae
Sub-family	Longidorinae (Tylencholaminae)
Genus	Longidorus
Species	Longidorus elongatus
Synonyms	Longidorus menthasolanus ? Longidorus sylphus ? Longidorus caespiticola ?

The analysis of the problem for this investigation has been made as follows:

1. The study of morphological comparisons of Longidorus elongatus obtained in Arizona with those specimens received from other geographical regions.

2. Studies to determine the influence of soil temperature on the population of Longidorus elongatus.

3. Soil moisture and its effects on Longidorus elongatus.

4. Influence of soil texture on the growth and development of Longidorus elongatus.

5. Host range study of Longidorus elongatus.

6. Viability of Longidorus elongatus as affected by time and temperature when stored in water.

The ecological relations of Arizona Longidorus elongatus populations were investigated in the greenhouse. Specimens from other regions of the United States and Europe were studied for morphological comparisons with those from Arizona. These studies were confined to the laboratory and greenhouse.

LITERATURE REVIEW

Longidorus elongatus was collected from The Netherlands and described as Dorylaimus elongatus by de Man in 1876. Micoletzky in 1922 differentiated this species from other Dorylaimidae and named it Longidorus elongatus. Thorne and Swanger in 1936 upgraded this sub-genus to a genus. Thorne in 1939 described four species in the genus Longidorus and established the sub-family Longidorinae, Thorne, 1935. Chitwood in 1957 observed similarities between the genera Longidorinae and Tylencholaminae Filipjev, 1934, thereby synonymising Longidorinae with Tylencholaminae (10).

The first report of economic damage by Longidorus spp. was made by Horner and Jensen (4), involving peppermint decline. The same authors identified this species as the ecto-parasite Longidorus sylphus Thorne (5). After investigation they concluded that soil fumigation was an effective control of the nematode (6). Konicek and Jensen (7), however, contradicted the earlier identification and described the mint nematode as a new species and named it Longidorus menthasolanus. Morphological characters were used as the basis of separation of L. menthasolanus from the similar species L. sylphus Thorne, 1939 and L. elongatus Thorne and Swanger, 1936. The bluntly convex-conoid tail, slightly

set off lip region and presence of males differentiated L. menthasolanus from L. sylphus. L. menthasolanus again differs from L. elongatus as the lip region is not set off as much, the body width is greater in proportion to body length, the female tail is generally shorter and the male tail is bluntly convex-conoid and not tapered as much. Siddiqui (10, 11) studied specimens of Longidorus menthasolanus from Oregon soil and compared them with specimens of L. elongatus from Rhode Island and from The Netherlands. He did not find sufficient morphological differences to separate L. menthasolanus and L. elongatus from one another and hence considers them synonymous. Hooper (3) redescribed Longidorus spp., and compared morphology of L. elongatus specimens from ten different counties of Great Britain with the type specimen from The Netherlands. The lip region in all British specimens was slightly rounder than those from The Netherlands and there was also a slight difference in female tail shape. The general appearance and measurements, however, agreed closely with the type specimen. He distinguished L. sylphus Thorne, 1939 from L. elongatus Thorne and Swanger, 1936 by its continuous lip region and absence of males. Five new specimens of Longidorus from Great Britain were also described in this work.

Sturhan (13, 14) observed morphological differences in the population of L. elongatus from southern Germany and Britain and states that older descriptions of "L. elongatus" refer to other species. The

form of the female gonad in L. monohystera is regarded as an abnormality and the species is synonymised with L. elongatus. According to the same author, L. sylphus which is poorly known was hardly distinguishable from closely related species and the absence of males, used by other taxonomists in their descriptions, was not considered to be of diagnostic value.

Harrison et al. (1) reported L. elongatus to be a vector of the beet ringspot strain of tomato black ring virus (TBRV-BRV), and that the larvae but not adults transmitted this virus. Taylor (15) found that although both adults and larvae of Longidorus elongatus transmit raspberry ringspot virus (RRV), larvae may transmit more often than adults. Murant and Taylor (2) report that most L. elongatus in field soil lose their infectivity when land is fallowed through the winter. Raski et al. (9) reviewed plant parasitic nematodes as vectors of plant viruses and termed L. elongatus, along with Xiphinema spp., as an efficient vector of plant viruses. Harrison and Hooper (2) suggest that long periods of fallowing are unlikely to eradicate L. elongatus and that surviving nematodes, although probably free of tomato blackring virus, would presumbably be able to feed and transmit viruses from infected to healthy plants grown on the fallowed land. Further, the same authors investigated the survival of L. elongatus when stored in moist, light sandy loam soil samples kept in polyethylene bags at room temperature. They observed that most of

the nematodes survived over a period of 29 months in the absence of any live roots.

Konicek and Jensen (7) described the mint nematode from Oregon as a new species Longidorus menthasolanus. Konicek (8) investigated details of its life history, morphology and ecology. Approximately 7 weeks were required for the first larval stage of this nematode to reach maturity. A host range study indicated eight suitable hosts. Seasonal fluctuation in population was observed and the greatest peaks reported in Oregon were in April and August. The soil moisture and soil temperature studies elucidated the most favorable soil temperature to be between 25 and 35 degrees Centigrade at 13 percent and 27 percent soil moisture. Observations on the survival of the nematode under extremes of desiccation and freezing were also made. Konicek's investigations of the ecology of Longidorus menthasolanus is of further interest since the present author conducted parallel investigations on certain ecological aspects of specimens and of Longidorus elongatus from Arizona, and found the results to compare favorably with those of Konicek.

MORPHOLOGICAL COMPARISONS

Techniques and Procedure

The object of this study was a preliminary investigation of differences in morphology which exist among specimens of Longidorus elongatus recovered from Arizona and those of other geographical regions of the United States and Europe.

The Arizona specimens of L. elongatus were obtained from the soil around the roots of grape growing at the University of Arizona, Yuma-Mesa Experiment Station, Yuma, Arizona. Large samples of soil from various fields were collected to obtain a representative population of the region. This infested soil formed the original stock for isolation of the specimens, which were extracted by Cobb's gravity sieving method and by use of Baermann funnels. Adult female nematodes were hand-picked by use of the dissecting binocular microscope. Males are rare in this species and only two were encountered from among a population of approximately 1500 observed. Unfortunately both specimens were lost in the culture before a thorough morphological study was possible.

The nematodes were relaxed by gentle heat from an alcohol lamp, then transferred to FAA (formaldehyde 40%, 50 ml; glacial acetic acid, 50 ml; 70% alcohol, 900 ml) for a 24-hour fixation period. A

series of transfers through glycerine followed, viz., 1 percent glycerine; a mixture of 5 percent glycerine and 30 percent alcohol; 50 percent glycerine and finally ending in pure dehydrated glycerine. The process of grading through glycerine required a period of 10 days. The specimens were then mounted in pure glycerine on a permanent mount and ringed using zut ringing compound. An alternate technique for processing through glycerine was also adapted and found to be an improvement over the previous ones. The fixed specimens in this technique were transferred in a rather large amount of 1 percent glycerine in a vial and allowed to desiccate over $CaCl_2$ in a desiccator. The specimens were dehydrated for a period of 8 to 10 days until the pure glycerine stage was reached. The advantage of this technique over the previous method is that the dehydration process is gradual and the nematodes are spared the stress of osmotic changes from one concentration of glycerine to the other.

Specimens of Longidorus elongatus used for morphological comparisons were obtained from Wisconsin, Rhode Island, Florida and Oregon in the United States and The Netherlands, Germany and Scotland from Western Europe. Some specimens were fixed in vials in FAA while others were received as permanent mounts. All specimens chosen for the study were processed and mounted in the same manner as described above for the Arizona specimens. Camera lucida drawings were made of the body outline, the shape of the head, tail, the position of vulva

and the anus. Other distinguishing features were recorded. All drawings were projected under the low power of the compound microscope. The microscope was calibrated and the drawings were measured to scale applying a stage micrometer which had been previously calibrated. Then the measurements were completed, the different body ratios were determined according to de Man's system which is as follows:

$$a = \frac{\text{total length}}{\text{greatest width}}$$

$$b = \frac{\text{total length}}{\text{length of esophagus}}$$

$$c = \frac{\text{total length}}{\text{tail length}}$$

V = distance from head to vulva (expressed as percentage)

Results and Discussion

The morphological comparisons of the specimens were restricted to gross characters since investigations of the finer details were not necessary for the purpose of this study. Low power magnification was found sufficient to resolve most of the required details and project them for drawing by use of the camera lucida.

The measurements disclosed much variability between the specimens. However, the variations among all specimens observed

were mainly confined to general body dimensions of total length and body width. The other morphological characters showed striking similarities among all specimens from different regions regardless of size. The following morphological features were commonly observed among female specimens of this species.

Mature Female. --Body long and attenuated, gradually tapering towards head, about half the body width near the guiding ring. Relaxation by heat imparts the characteristic open "C" shape to the body and curls more ventrally in the posterior region. The guiding ring is well forward in the head region. The esophagus is characteristically the bipart, Dorylaimoid type and starts as a coiled thin tube ending with the large elongated basal bulb. The vulva is a transverse slit located approximately at the middle of the body. It is located ventrally and is typical of diadelphic species. The anus is also a short transverse slit. Tails were found conoid to bluntly conoid with the body curvature making it dorsally convex and ventrally concave. The tail is longer than the anal body width and is almost equal to the greatest body width.

In spite of the morphological similarities discussed above, the measurements of the specimens from different regions had conspicuous differences. The Arizona specimens were found to be shortest with the total length from 3.3 to 3.7 mm. The greatest lengths were attained by the specimens of L. elongatus from Holland which were measured at

6.2 to 7.5 mm. With the exclusion of these two extremes in the range of dimensions, the specimens from the other regions compared favorably with the type specimen described by de Man from The Netherlands. Dimensional fluctuations common among the specimens from the same population were observed but were of a smaller magnitude when compared with the differences between specimens from different regions.

The measurements of the ratio "b" according to de Man's system could not be recorded since many of the mounted specimens had cleared and the esophagus could not be resolved distinctly.

That differences in ecology of the nematode may bring about morphological changes, especially total length, was demonstrated during this investigation.

Table 1

Measurements of specimens of Longidorus elongatus from different geographical regions (de Man's system)

Region	Number observed	Length in mm	a	c	V%
Arizona	4	3.5 (3.3-3.7)[a]	80 (71-90)	80 (71-90)	48 (44-49)
Oregon	4	5.8 (5.0-6.7)	104 (101-112)	111 (85-134)	48 (48-49)
Rhode Island	2	5.0 (4.8-5.1)	103 (88-120)	124 (120-129)	47 (46-47)
Wisconsin	2	5.2 (5.0-5.3)	104 (101-106)	104 (101-106)	43 (43-44)
Holland	4	7.5 (6.2-7.8)	123 (97-141)	173 (145-194)	54 (53-54)
Germany	3	5.6 (4.8-6.2)	103 (78-117)	122 (96-146)	51 (50-52)
Scotland	4	6.2 (5.4-6.8)	105 (96-113)	125 (109-134)	48 (46-50)
Florida	2	5.0 (5.0-5.1)	100 (99-102)	91 (90-92)	♂

[a]Figures in brackets give the minimum and maximum measurements or percentages.

SOIL TEMPERATURE STUDIES

Techniques and Procedure

A sandy loam was well mixed with peat moss in the ratio of 3 parts sand to 1 part peat moss, and potted in 25 wide-mouthed half-gallon glass jars. The soil was watered to allow it to settle and autoclaved at 250 degrees Fahrenheit at 15 lbs pressure for 8 hours. Three-week-old tomato seedlings (Lycopersicon esculentum L. var. Bonny Best) were transplanted into the sterilized soil. The jars containing one seedling each were then transferred to constant temperature tanks and five jars were placed in each tank. For this study five temperatures were maintained in the tanks (±2°F) and were as follows:

15 degrees centigrade

20 degrees centigrade

25 degrees centigrade

30 degrees centigrade

35 degrees centigrade

A culture of Longidorus elongatus was obtained after sieving the infested soil. The adult female nematodes were hand-picked and stored at room temperature (25°C) in water, in glass vials with 20

nematodes in each vial. The nematodes were then inoculated in the soil by placing the contents of the vials around the roots of the tomato seedlings resulting in a rate of 20 nematodes per jar. Moisture was not controlled but maintained for normal optimum growth of the host plants. The water level in the temperature tanks was maintained one-half inch below the rim of the jars. Thermometers were used to detect temperature fluctuation during the study.

The experiment was determined after 14 weeks. All the soil from the five replications was sieved separately by Cobb's sieving method. The screens were backflushed and the screenings were placed on a single layer of paper tissue in Baermann funnels to eliminate organic matter. The nematodes were collected from each funnel and counts were made by the use of the dissecting microscope.

The techniques of sieving by Cobb's method and the Baermann funnels were constant for all the trials. Earlier observations had indicated that an ultimate recovery of 33 percent of the total nematodes could be expected when these techniques are employed. Hence, the nematode count from each replication was multiplied by a factor of 3 to derive the approximate number of nematodes recovered.

Results and Discussion

This investigation was established to observe the population densities at different soil temperatures. No effort was made to determine the precise optimum temperature for the nematode.

Live nematodes at different stages of growth were recovered from the soil in the temperature range of 15°C to 30°C. The treatment maintained at 35°C failed to yield any nematodes. The 15°C treatment resulted in the highest nematode population, whereas the other three treatments were found to have lower populations (Table 2). There was little difference between the populations produced in tanks maintained at 20°C, 25°C and 30°C.

No reason was attributed for the higher population at 15°C; however, it demonstrated the necessity that temperature trials should be more extensive, i.e., temperatures lower than 15°C should also be included in the trials. Such a study was beyond the scope of this investigation due to the limitations of equipment. The absence of nematodes in the 35°C treatment indicate the hypothesis that this is an inhibitory level of temperature for L. elongatus.

Table 2

Total number of Longidorus elongatus recovered from each replication at varying temperatures following a 14-week incubation period

Temp. °C.	Replications					
	1	2	3	4	5	Avg.
15	165	0	84	177	147	115
20	0	33	69	0	48	30
25	84	36	0	0	93	43
30	0	51	78	63	105	59
35	0	0	0	0	0	0

SOIL MOISTURE AND ITS EFFECTS

Techniques and Procedure

Sterile sandy loam soil and peat moss were put into 20 earthen pots of 6" diameter. Two-week-old tomato seedlings (L. esculentum, var. Bonny Best), germinated in a peat moss seed bed, were transplanted, one seedling to each pot. Females of L. elongatus, hand-picked from the culture and stored in glass vials, were introduced at the rate of 20 nematodes to each pot.

The influence of soil moisture was studed at four levels:

	Approx. percentage moisture by wt.
Stress	13
Field capacity	27
Saturated	41
Control	18

The soil moisture was maintained by the use of tensiometers. A correlation was figured out for the tensiometer reading and soil moisture content by pre-determining the soil moisture percent by wet and dry weighings of the soil at different moisture levels. The moisture was controlled by addition of water to the pots periodically.

so as to maintain the desired stress on the tensiometer dial. A tray filled with water was placed under the pots holding the saturation treatment, thus providing a constantly saturated soil. The temperature during this trial was not controlled. Greenhouse temperature was maintained at approximately 27°C.

The test was terminated after 14 weeks thereby providing enough time for two life cycles of Longidorus to be completed. The soil was sieved, passed through Baermann funnels and the nematode population counted.

Results and Discussion

An average nematode population of 10 was recovered from the stressed soil treatment (13% moisture). At field capacity (27% moisture) an abrupt rise in population was observed. The saturated soil (41% moisture) failed to yield any nematodes. The control approximately (18% moisture) which received normal watering for the tomato host yielded an average of 31 nematodes which compared favorably with the field capacity (Table 3).

The above data indicated that the preferred soil moisture content for Longidorus elongatus development was at field capacity. The failure of the saturated soil moisture treatment to yield any nematodes points to the possibility that waterlogging of the air spaces may lead to oxygen deficiency and consequent inhibition

Table 3

Soil moisture and its influence on Longidorus elongatus development

Moisture level	Total number of nematodes per pot Replications					
	1	2	3	4	5	Avg.
Stress	6	0	0	15	27	10
Field cap.	45	54	33	27	75	47
Saturated	0	0	0	0	0	0
Control	30	39	0	63	21	31

of the nematodes. Increased moisture may also increase fungi and bacteria which are parasitic to the nematodes.

Maintaining constant soil moisture was found to be a complex procedure. Different techniques were considered but the use of tensiometers was found to be simple and practical. The treatments of soil moisture were arbitrarily taken up for investigation and represented approximate soil moisture content.

INFLUENCE OF SOIL TEXTURE

Techniques and Procedure

Four soil samples were acquired from different locations in Arizona to represent textural grades ranging from loam to sand. These soils formed the different treatments for investigations on soil texture. Soil from the location where L. elongatus was originally found was used as a control. The soil samples were mechanically analyzed and the following information was recorded.

Soil type	% sand	% silt	% clay
Sand	94	3	3
Loamy sand (control)	80	12	8
Sandy loam	64	26	10
Loam	32	49	19

Range of particles:

Sand	2 mm-.05 mm
Silt	.05 mm-.002 mm
Clay	<.002 mm

Twenty tin cans of one gallon capacity were filled with the four types of soil, each soil treatment being replicated five times.

Two-week-old tomato seedlings were transplanted into the cans which had been previously sterilized. One week after the plants were established, 20 gravid females of L. elongatus, hand-picked from a stock culture, were inoculated to the soil around each plant. Normal watering was maintained but temperature and humidity were subject to fluctuations in the greenhouse. The pots were retained in shallow trays to which water was added as needed, thereby avoiding damage to the plants or nematode during any period of high temperature. Since this was a study in soil texture, the desired texture was obtained from field sources and no humus was added. Nutrient solution (Hoagland's) was added at the rate of 500 ml per pot twice during the period of trial so as to sustain the plant normally in the absence of the organic matter.

At the end of 14 weeks of growth, the plants were removed and the soil was subjected to sieving and Baermann funnel as previously described. The nematodes recovered from each replications were counted by use of a binocular microscope.

Results and Discussion

The greatest population of nematodes were encountered in the loamy sand, the soil originally infested with nematodes in Arizona (Table 4). This was followed by the loamy soil which yielded an average of 63 nematodes per replication, approximately a threefold increase over the initial population. The sandy loam treatment had a

Table 4

Influence of soil texture on the growth and development of Longidorus elongatus

Soil type	Number of nematodes recovered Replications					
	1	2	3	4	5	Avg.
Sand	0	0	0	0	0	0
Sandy loam	9	15	9	27	6	13
Loam	42	84	48	63	78	63
Loamy sand (control)	78	117	183	114	51	109

low nematode count of 13 specimens. There was no nematode recovery from the treatment consisting of the sandy soil.

The high nematode population in loamy sandy soil demonstrated that this type of soil is better suited for the development of L. elongatus when compared with other soil types used in this study. This factor probably accounts for its presence in large numbers in fields where this soil occurs. Sandy soils do not seem to favor L. elongatus development since no specimens were recovered from this treatment. Further, the soil moisture is difficult to uniformly maintain in sandy soils. Under normal greenhouse conditions of this study such soil required more frequent watering and tended to get either too wet or too dry during very short time periods. Sandy loam was the next finer in texture to that found as optimum (loamy sand). However, fewer nematodes reproduced in this soil than in loamy soil of finer texture. This observation could not be explained as, hypothetically, sandy loam should be more conducive to nematode development than a loamy soil.

Although the soils selected for the textural study were representative of field soil, some differences were expected between field soil and the assembled treatments in the greenhouse. The presence of organic matter and other environmental factors on a field scale impart a definite structure (flocculation, crumb structure, etc.), whereas

soils artificially assembled for greenhouse study are devoid of such structure. As such, the data obtained from this investigation, though valid, should be substantiated by field investigations.

HOST RANGE STUDY

Techniques and Procedure

This study was a limited investigation of the host range of Longidorus elongatus in Arizona. Only a few plants which are of economic importance and are grown in the presently known region of the state where the nematode occurs were investigated.

Host plants for this study were grown in seed beds two to six weeks prior to the commencement of the investigation. The following were the plants which comprised the host range:

Alfalfa (Medicago sativa L. hort. var. Sirsa)

Grapefruit (Citrus grandis L.)

Tomato (Lycopersicon esculentum L. hort. var. Bonny Best)

Sugarbeet (Beta vulgaris L.)

Peppermint (Mentha piperita L.)

Although peppermint is of no economic importance in Arizona, it was used to determine how closely related this species found in Arizona is to that found in Oregon.

Grapefruit, alfalfa and tomato were grown in seedbeds before transplanting to trial pots. Peppermint was developed from cuttings planted earlier in pots. Sugarbeet was grown from seed.

Fifty 10-inch earthen pots were filled with a sterilized mixture of loamy sand and peat moss. Seedlings and cuttings were transplanted in these pots in groups of ten for each of the five host plants. Adult female nematodes in batches of 20 were hand-picked from stock culture and stored in water in vials. Each of the five pots for every host plant was inoculated with 20 nematodes, the remaining five pots of each host being retained as control. Watering was maintained to keep the plants at optimum growth. The temperature and humidity were not controlled and were subject to the normal greenhouse fluctuations of 60°F to 95°F and the relative humdity 25% to 90%. The plants of alfalfa, tomato and peppermint made brisk growth and the top growth had to be cut back to keep the plants confined to the pots. The investigation began on February 24, 1964, and the harvest terminated on June 6, 1964.

The plants were harvested by carefully removing them from the pots, the soil adhering to the roots was washed off with a spray of water and this water collected in a pan. The soil from all the treatments was sieved and later the screenings were put through Baermann funnels for recovery of nematodes. The cleaned root systems were severed from their stems at the soil line. The point at which they were severed was uniform for both treatments and control. The root system was then dried in an air circulating oven at 70°C for 48 hours. The

individual root weights of all five replications of each treatment were averaged and recorded. Root weight comparisons were then made to determine the influence of the nematode population on its host.

Results and Discussion

All treatments under study failed to yield nematodes at the time of harvest. In some treatments a few free-living nematodes were observed but no Longidorus were encountered. Failure to recover the nematode was attributed to the wide environmental fluctuations which occurred during the study. Temperature and humidity in the greenhouse fluctuated over a range of 60°F to 95°F and the relative humidity 25% to 90%, respectively. During the twelfth week of this trial the plants were temporarily wilted during the spell of three hot days and it is expected that the nematodes might have succumbed during this short period of desiccation.

A comparison of the root weights of the treated and control plants (Table 5) demonstrated the influence of Longidorus on their host. The root weights of the control were higher than the inoculated plants and demonstrated the damage done by the nematode. The only plant which did not follow this trend was alfalfa. The average weight of the root system of the control plants was found to be lower than those plants which had been inoculated. This may indicate alfalfa is a suitable host, but that the nematode may not be sufficiently pathogenic to reduce

Table 5

Host range study of Longidorus elongatus from Arizona[a]

Host plant	Replications					
	1	2	3	4	5	Avg.
Treatments						
Tomato	11.0	10.6	17.7	21.4	14.3	15.0
Alfalfa	20.7	13.9	12.1	4.1	6.7	11.5
Peppermint	42.4	40.9	48.7	55.9	57.5	49.1
Sugarbeet	89.0	88.4	101.4	51.4	50.7	76.2
Grapefruit	1.5	1.2	3.3	2.9	1.8	2.1
Control						
Tomato	22.9	26.3	11.6	17.2	24.9	20.6
Alfalfa	14.4	15.5	5.3	4.8	6.1	9.2
Peppermint	52.4	61.5	55.1	47.2	54.4	54.1
Sugarbeet	65.2	87.5	122.7	75.0	113.2	92.7
Grapefruit	2.2	1.5	2.6	1.3	2.1	2.5

[a]Weights of root system in grams.

the root weight. On the basis of reduced root weights of the host subjected to nematode feeding as compared to the control plants which had no nematodes, it is postulated that the tomato, sugarbeet and grapefruit used in this investigation are suitable hosts for L. elongatus and the nematode is pathogenic to them.

MOTILITY AS AFFECTED BY STORAGE

Techniques and Procedure

A range of four temperatures was investigated for *in vitro* storage of *Longidorus* in distilled water, viz.: 3°C, 9°C, 15°C, and 25°C.

A culture of *Longidorus* differing in stages of growth from the second larval stage to the adult stage was obtained by sieving fresh samples of infested soil. Sixteen 10-ml glass vials were half filled with distilled water and kept ready to receive the nematodes for four replications of each of the four temperature treatments mentioned above. In each of the vials 10 nematodes were placed at approximately the same stage of growth. These were segregated and hand-picked from the culture by the aid of the dissecting microscope. Thus, the four replications under each treatment represented specimens at different stages of growth. The vials were corked and stored in darkness at the above mentioned temperatures. The freezer and the lower chamber of the refrigerator were set to register a constant temperature of 3°C and 9°C, respectively, for the two lower temperature treatments. Incubators were used for 15°C and 25°C treatments.

The nematodes in the vials were examined under the dissecting microscope for motility. The mortality counts were recorded after each

examination. The large size of the nematodes facilitated this visual examination. After examination and removal of the dead specimens, the vials were immediately restored to their respective temperatures for subsequent observations.

This test was repeated using tap water instead of distilled water following the same techniques and procedure as in the first study. Observations were made and the data recorded.

Results and Discussion

The purpose of this investigation was to observe the in vitro storage of Longidorus elongatus and their longevity at different water temperatures. These data were required as live specimens from the same culture of nematodes were often used repeatedly for subsequent tests and had to be stored for short or longer durations of time.

Storage at 3°C was not found to be favorable for this nematode. In distilled water most of the specimens died during the first four days. There was no viability after 7 days. In tap water the storage period was further reduced by two days.

At 9°C the nematodes survived over a period of 14 days in distilled water, total mortality being observed after 15 days. In tap water, the maximum period of storage declined to 10 days. Small amounts of a fungus were found clinging to the anterior and posterior

regions of the nematodes. The same fungus was not observed in the distilled water treatment.

A slight improvement in storage time was observed at 15°C as the nematode survived over a period of 16 days. However, storage in tap water resulted in a rapid decline as all the nematodes succumbed by the eighth day. A fungal mass was found clinging all over the surface of the nematodes and it was assumed that here as well as at the other temperatures studied, the tap water was contaminated with a fungus which attacked the nematode.

The maximum period of live storage of this nematode was observed at room temperature. A period of 33 days was required for total mortality in distilled water. However, in tap water all the nematodes died within a period of 12 days with a profusion of fungus found attached all over the nematodes.

Some interesting observations were elucidated through this investigation. It was seen that the larval stages perish earlier in storage than the adults. Although the survival of the nematode at different stages of growth was not observed in detail, the early mortality of the larvae and survival of only adults in older cultures substantiated the above observation. The type of water used for the storage of the nematodes also might account for their longevity. The infestation of the nematode with fungus might be attributed to the type of water. The

fungus attacks the nematode rapidly (within 2 to 4 days) in tap water, whereas there was seldom an infestation in distilled water. Further research into the nature of the fungi infesting the nematode may be revealing.

GENERAL DISCUSSION

The principal objective of this investigation was the study of some of the ecological aspects of L. elongatus that may limit its distribution in Arizona. The other objective was to determine if there are ecological factors which influence morphology of the species.

Morphological comparisons in this study were based on the work of Hooper (3), who enunciated that morphological variability occurs in specimens of the same species collected from different habitats. The results in the present investigation tend to confirm his observation. Wide variations were observed in total body length of specimens from Arizona with those from other regions. However, gross differences in morphology, such as ratio of body width to length, position of vulva and testes and stylet length, were not observed in the specimens used in this study.

Unlike the work of Konicek (8), soil temperature studies and their influence on Longidorus development were not coupled with soil moisture studies. By maintaining the moisture at a level conducive for normal growth of the tomato host, the highest L. elongatus population was encountered when the soil temperature was maintained at 15°C. Such data do not collaborate completely those results obtained by

Konicek (8). He observed a population decline at 15°C and 13% soil moisture. However, the higher temperature of 35°C confirmed Konicek's findings that the nematode does not reproduce at this temperature or any temperature exceeding it.

Data from the soil moisture studies on Longidorus in this work were found to parallel those of Konicek's. Saturated soil was found to inhibit the nematode population in the studies. This observation illustrates the possibility of controlling the nematode in the field by flooding the soil for extended periods of time. Such control measures have been used successfully for other nematode species in areas where water is plentiful and drainage is no problem.

The effect of soil texture on Longidorus elongatus development has not previously been studied. The results of this investigation showed that soil texture is an important factor limiting the distribution of Longidorus. Specimens from Arizona were found to develop favorably in loamy sand. This accounts for its occurrence in the Yuma Valley. However, various other agricultural areas in Arizona have soil types and temperature very similar to the Yuma-Mesa area and as a consequence are potential areas for infestations of this nematode species. The possibility of it becoming a pest in sandy soils or heavy clays is thus reduced.

Sugarbeets which are expected to become an important crop in Arizona in the near future were found to be satisfactory host plants of Longidorus elongatus in this investigation.

Curly-top virus of sugarbeets is transmitted by a species of leafhopper but the possibility of Longidorus elongatus as a vector of the same virus has not been explored. Grapefruit which is also susceptible to a number of viruses was found to be a host of this nematode species. In addition to direct pathogenicity to citrus, the transmission of viruses to this important crop is also a possibility.

SUMMARY

The ecology of Longidorus elongatus was investigated in this study with particular reference to the specimens available in Arizona. A further study concerned the morphological comparisons between different specimens obtained from other regions and the specimens recovered from Arizona soil.

1. The morphological comparisons showed significant differences in the body dimensions of different specimens. The specimens from Arizona were the smallest of all specimens observed attaining a total length of 3.3 to 3.7 mm. However, in spite of these differences, marked similarities in all gross morphological characters were observed in all the specimens.

2. Soil temperature studies demonstrated the survival and reproduction of L. elongatus from the treatments maintained at 15°C, 20°C, 25°C and 30°C. There was no recovery of nematodes from the treatment held at 35°C. The greatest increase in the population of the nematodes was obtained at 15°C.

3. The optimum soil moisture was found to be at the field capacity for the particular soil used in this study. The soil under stress of soil moisture yielded low nematode counts, whereas there was no recovery of nematodes in saturated soil.

4. Loamy sand was found as the most suitable medium for the reproduction of Longidorus. This was the soil found infested with this nematode in field locations in Arizona. Loamy soil and sandy loam followed in order of favorable texture. Sandy soil was found not congenial for Longidorus, as evidenced by the absence of the nematode in this treatment.

5. Host range studies indicate that tomato, peppermint, sugarbeet and grapefruit were hosts to this nematode, whereas alfalfa was a doubtful host. However, since there was no recovery of nematodes, these data were not confirmed.

6. In vitro storage of nematodes in water at different temperatures yielded the following results. Storage at room temperature (25°C) was found most favorable, as the nematodes survived for a period of 33 days. Quickest mortality was at 3°C. Adults were found to survive longer in storage than larvae. Type of water, whether distilled or tap water, also affected storage. Nematodes survived longer in distilled water since in tap water a fungus infested the nematodes, and the nematodes succumbed earlier.

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