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Horizontal and Vertical Distribution of *Longidorus africanus* in a Bermudagrass Field in the Imperial Valley, California[1]

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Abstract: The horizontal and vertical distribution of the needle nematode *Longidorus africanus* was studied in a bermudagrass field in the Imperial Valley in southern California. A geostatistical method involving the use of semi-variograms was used to quantify the relationship between sampling distance and variation in *L. africanus* population levels. Semi-variance between nematode numbers from different samples was very low when samples were taken close together, increased with sampling distances up to ca. 15 m, and fluctuated around a sill value at distances greater than 15 m. At very large sampling distances the semi-variance increased further. It was concluded that patches with fairly similar numbers of *L. africanus* were elongated and up to 15 m long. Seasonal fluctuations over a 2-year period, in total numbers of *L. africanus* extracted from three depths, were large and highly correlated with soil temperature. Population densities were greatest during the summer months and lowest during the winter. Averaged over the 2-year period, *L. africanus* population densities increased with increasing depth. Chances for detecting this nematode are greatest in summer at depths of 60 to 90 cm.

Key words: distribution, geostatistics, horizontal distribution, *Longidorus africanus*, nematode, sampling, temperature, vertical distribution.

The needle nematode *Longidorus africanus* initially was described from Zimbabwe (Merny, 1966). This nematode has been reported to cause damage to lettuce (Radewald et al., 1969) and carrot (Kolodge et al., 1987) in the Imperial Valley in southern California. Symptoms of damage consist of swelling of the root tips, which in carrots may result in forking of the tap root, thus rendering the crop unmarketable. *Longidorus africanus* multiplied on a wide range of different plant species in 10 families (Kolodge et al., 1987; Lamberti, 1968, 1969). Good hosts included tomato, bean, bermudagrass, sugarbeet, and lettuce, whereas cruciferous crops in general were poor hosts. The few data available on the biology of *L. africanus* suggest that nematode multiplication is greatest at relatively high soil temperatures (ca. 28 °C) and that the life cycle can be completed within 7 to 9 weeks (Kolodge et al., 1987). In southern California, damage most often is observed in fall crops on lighter soil types, although Radewald et al. (1969) reported that the nematodes were not restricted to these soil types. The horizontal and vertical distributions of related *Longidorus* and *Xiphinema* virus-vector species have been described (Brown et al., 1990; Cohn, 1969; Feil et al., 1997; Ferris and McKenry, 1974) and, from these results, sampling strategies for detection of these nematodes were developed. Such data have not been available for *L. africanus*, a species not known to transmit virus.

The objectives of this study were to determine the horizontal and vertical distribution of *L. africanus* and seasonal effects on nematode population densities. A bermudagrass field infested with *L. africanus* in the Imperial Valley was selected for this study. The results are presented here, and recommendations on sampling strategies for this nematode are made.

MATERIALS AND METHODS

L. africanus was found in six fields in a preliminary survey of 30 fields in the Imperial Valley. A field of sandy loam with bermudagrass (*Cynodon dactylon*) and a high density of *L. africanus* was selected for this study.

Horizontal distribution: In August 1997, 238 samples were collected with a Dutch auger (diam. 7 cm) from depths of 15 to 45 cm. A

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rectangular block (51.1 × 51.1 m) was set out in the field; the bottom right corner was designated as point (x,y) = (0,0) and the top left corner as point (x,y) = (5,110, 5,110). Initially, 121 samples were collected with 11 samples per row and 11 rows. Samples within each row were taken at x = 0, 5, 10, 30, 70, 150, 310, 630, 1,270, 2,550, and 5,110 cm. Correspondingly, rows were at y = 0, 5, 10, 30, 70, 150, 310, 630, 1,270, 2,550, and 5,110 cm. This sampling pattern was repeated with the top left corner (5,110, 5,110) now serving as the origin. This scheme yielded a total of 238 samples (2 × 121 = 242, less 4 samples representing the corners in the repeated sampling). The samples were stored at 15 °C in closed plastic bags, and nematodes were extracted from 200 g soil with a decanting-and-sieving method (Brown & Boag, 1988) within 10 days after sampling. Nematodes were killed with hot formalin-propionic acid (Netscher and Seinhorst, 1969) and stored until counting.

Data were analyzed with a geostatistical semi-variogram model, which quantifies the relationship that nematode numbers from samples taken close together tend to be more similar than nematode numbers from samples taken at greater distances from each other (Wallace and Hawkins, 1994). An (x, y) grid coordinate (in centimeters) was assigned to each sampling point based on its location in the field. A computer file was generated with three columns of numbers: the x-coordinate, the y-coordinate, and the number of *L. africanus*. Semi-variance values were calculated with a computer program written in Excel Visual Basic. Distances in the x-direction and in the y-direction were calculated separately for all possible pairs of samples. The semi-variance was calculated as $\gamma(d) = 0.5 \times \{[Z(p + d) - Z(p)]^2/n\}$, where $Z(p)$ was the number of *L. africanus* at point p, $Z(p + d)$ was the number of *L. africanus* at point $p + d$, and n was the total number of sample pairs at distance d from each other. The semi-variance was plotted against the distance d (cm) between sampling points. A curve was then fitted through the data according to the spherical model $\gamma(d) = 0.5 \times C \times d/a \times (3 - d^2/a^2)$ for $d \leq a$, and $\gamma(d) = C$ for $d \geq a$ where C is the "sill" value and a is the "range of influence" (Clark, 1979).

To obtain information on the shape and direction of patches with similar numbers of *L. africanus,* the semi-variance also was plotted against the angle between two sampling points. Angles between all pairs of sampling points were obtained from calculation of the tangent from two sampling points. For example, the tangent between sample 24 with (x,y) coordinates = (5, 10) and sample 84 with (x,y) coordinates = (310, 630) is (10-630)/(5-310) = 2.03. Thus, the angle between these points is 64°. As angles between sampling points should range between 0° and 180°, negative angles were converted to positives by adding 180 to the angle value (i.e., an angle of −24° becomes 180 − 24 = 156°). In this way, angles between sampling points that differed only in x-coordinates were 0°, and angles between points that differed only in y-coordinates were 90°.

The possible influence of soil moisture on population levels of *L. africanus* was studied by determining the soil moisture of the 20 samples with the highest number of *L. africanus* and the 20 samples with the lowest number of *L. africanus*. Soil moisture levels were determined by the loss of water from 50-g soil samples after drying for 2 weeks at room temperature.

Vertical distribution: From the grass verge of the same field, 30 soil samples were collected once every 2 months for a 2-year period, during one of the last 4 days of each month, starting in May 1996. Samples were collected at 10 sampling points (distance between sampling points 1 m) with a Dutch auger from depths of 0–30 cm, 30–60 cm, and 60–90 cm. *L. africanus* were extracted from 200-g subsamples as described above. Soil temperatures at a 15-cm depth under grass were recorded daily at a nearby (3-km distance) weather station. Analysis of variance (ANOVA) and calculation of correlation coefficients was done with SAS (SAS Institute, Cary, NC). Means were separated with Duncan's multiple-range test.

Results

Horizontal distribution: The number of *L. africanus* extracted from the field ranged from 0 to 284 (mean 116.2)/200 g soil. The *x*-direction semi-variogram (Fig. 1a) demonstrated that the semi-variance initially slowly increased with increasing distance. At distances of ca. 1,200 to 4,000 cm between sampling points, the semi-variance fluctuated around 8,500. With distances between sampling points increasing over 4,000 cm, the corresponding semi-variance sharply increased. A spherical model was fitted to describe the data for sampling points up to 4,000 cm apart. The "range of influence" a and the "sill" C were visually estimated at 1,800 cm and 8,500 *L. africanus*^{2}, respectively. Similarly, in the *y*-direction semi-variogram (Fig. 1b), the semi-variance slowly increased with sampling point distances increasing up to ca. 1,300 cm. At distances of ca. 1,300 to 4,000 cm between sampling points, the semi-variance fluctuated around 7,500 and sharply increased with further increasing distances. A spherical model was fitted as described above with range of influence a = 1,500 cm and sill C = 7,000 *L. africanus*^{2}.

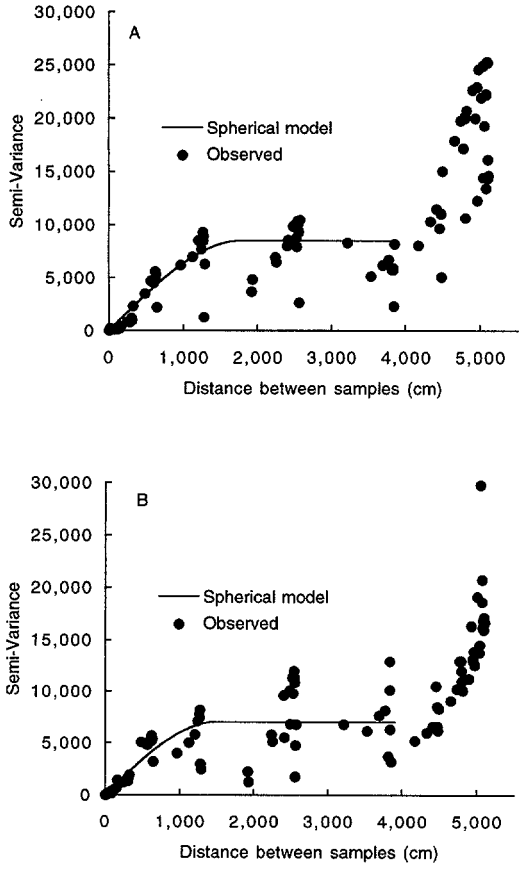

FIG. 1. Semi-variogram (spherical model) for *Longidorus africanus* in a bermudagrass field. A) *X*-direction. B) *Y*-direction.

The soil-moisture levels in the 40 samples ranged from 12.8% to 18.8%, but no significant correlation between soil moisture and number of *L. africanus* was found (r = 0.13; P = 0.67).

The angle between sampling points was plotted against the semi-variance (Fig. 2), and sampling points forming angles of 110° to 170° appeared to have small semi-variance values, whereas sampling points forming angles of close to 90° and close to 180° had higher semi-variance values.

Vertical distribution: Averaged over all sampling dates, the number of *L. africanus* increased with increasing depth ($P \leq 0.01$) (Table 1). Averaged over all sampling depths, nematode numbers found at each of the September or July samplings were significantly higher than those at the January or March samplings ($P \leq 0.01$), with intermediate numbers extracted during the May and November samplings. From May 1996 to May 1997 and from November 1997 to May 1998, significantly more *L. africanus* were found in the 60- to 90-cm layer than in the upper layers, and at none of the sampling dates were the numbers of nematodes found in the upper layer significantly higher than in the middle layer ($P \leq 0.01$).

Average monthly soil temperatures at the 15-cm depth ranged between 31.3 °C in August 1996 and 12.9 °C in January 1998. The average soil temperature during the month of sampling was correlated significantly with the total number of *L. africanus* (r = 0.85; $P \leq 0.0002$), as well as with the *L. africanus* numbers in each of the three depths (0–30 cm: r = 0.77, $P \leq 0.002$; 30–60 cm: r = 0.79, $P \leq 0.001$; 60–90 cm: r = 0.84, $P \leq 0.0003$)

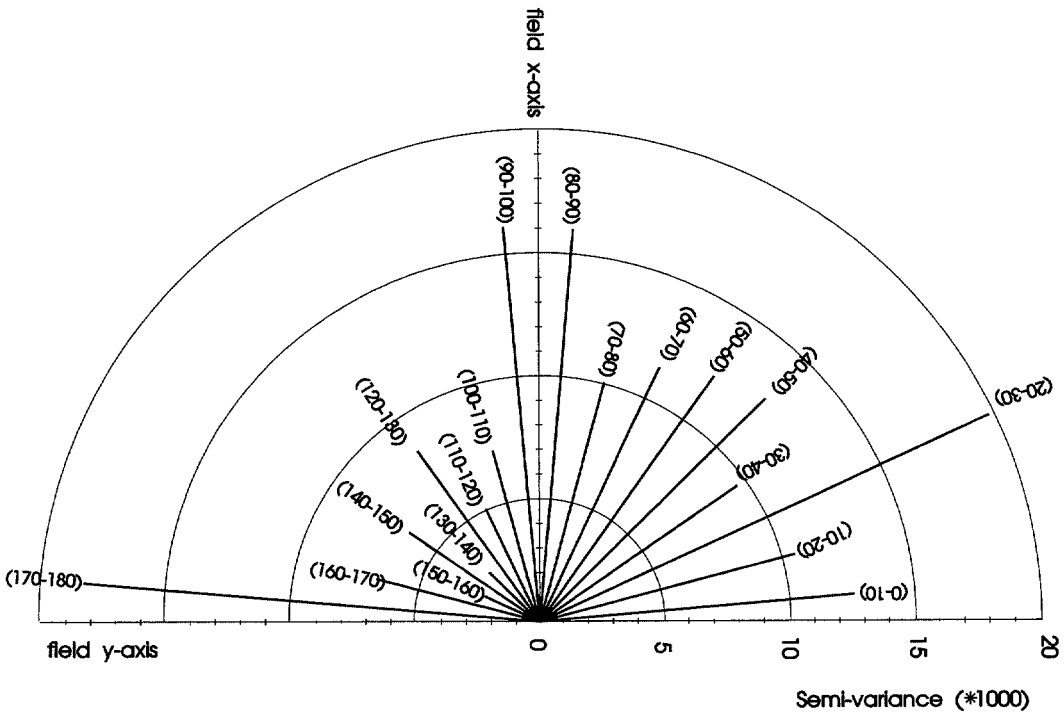

FIG. 2. Relation between the semi-variance of *Longidorus africanus* numbers found in samples (200 g) and the angle (degrees; in parentheses) between those samples.

(Fig. 3). The correlation between *L. africanus* numbers and temperatures in the month preceding sampling was significant only for the 60- to 90-cm layer ($r = 0.72$; $P \leq 0.005$).

The fluctuations between numbers of *L. africanus* counted at consecutive sampling dates were calculated as absolute numbers (difference in numbers of *L. africanus* at two consecutive sampling dates) and as the percent change. The largest fluctuation in nematode numbers, both absolute and as percent change, occurred between March 1997 and May 1997 in the total number of *L. africanus* as well as in the number of nematodes at each of the three depths (Table 1). Between these sampling dates, the total number of nematodes throughout the sampled soil increased from 32/200 g to 125/200 g (3.9× increase), with the highest increase (10.7×) at the 30- to 60-cm depth. The average fluctuations throughout the 2-year period were, however, not significantly different among the three depths ($P \leq 0.05$).

DISCUSSION

In this study, the *x*- and *y*-direction semivariograms (Fig. 1) showed similar trends. The semi-variance between samples taken very close together was almost zero. The

TABLE 1. Mean numbers of *Longidorus africanus* collected at different sampling dates and from different depths.

Sampling date	Sampling depth (cm) 0–30	30–60	60–90	All depths
May 1996	75.5[a]	83.7	124.0	94.4
July 1996	33.7	76.3	177.5	95.8
Sept. 1996	85.5	93.8	168.0	115.8
Nov. 1996	22.5	53.8	110.6	62.3
Jan. 1997	8.7	17.7	80.8	35.7
March 1997	15.3	10.4	70.0	31.9
May 1997	109.2	112.2	154.0	125.1
July 1997	84.4	187.1	183.9	151.8
Sept. 1997	105.7	148.0	210.1	154.6
Nov. 1997	20.3	56.1	149.3	75.2
Jan. 1998	31.0	40.5	100.8	57.4
March 1998	8.0	13.6	79.2	33.6
May 1998	53.8	49.2	91.1	64.7
All dates	50.3	72.5	130.7	84.5

[a] Each number is the mean of 10 replicates.

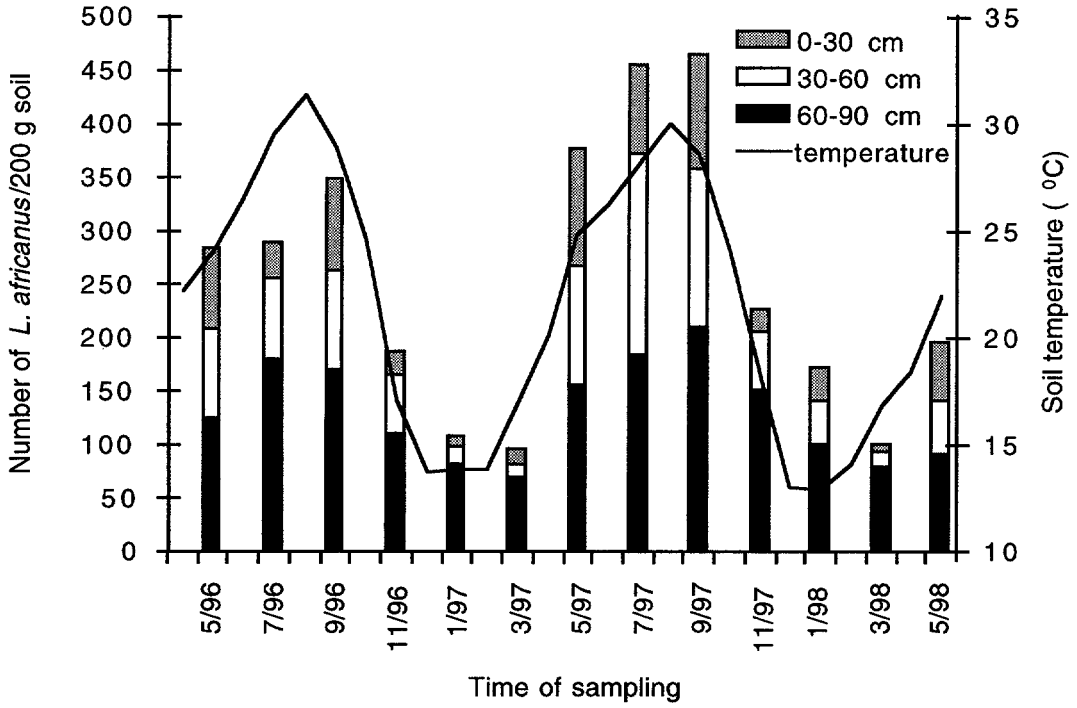

FIG. 3. Seasonal fluctuations of average monthly soil temperature and average (n = 10) numbers of *Longidorus africanus* (one sample every 2 months) at three soil depths.

semi-variance consists of two components: the inherent variance between nematode population densities at different sampling points and the variance resulting from measurement error, which may result from subsampling, extraction, or counting. Since, theoretically, the nematode numbers in samples taken at exactly the same point (distance = 0) should be equal, the semivariance at distance = 0 (the "nugget value") represents the variance due to measurement error. Thus, as the nugget value in our study is close to zero, it can be concluded that the observed semi-variance over the complete distance range is almost exclusively due to the inherent variation among *L. africanus* population densities in this field. In a study by Wallace and Hawkins (1994), nugget values for six plant-parasitic nematode species (not including *Longidorus* spp.) ranged between 40 and 25,000. As the smallest distance between sampling points in their study was 10 m, it was difficult to accurately estimate the nugget values at distances close to zero. Their suggestion to increase precision by decreasing the distance between sampling points was validated by our results (Fig. 1). An accurate estimation of nugget values from our data would have been difficult when data corresponding to sampling point distances smaller than 10 m would have been omitted. The range of influence *'a'*, estimated from Figs. 1a and 1b, was close to 15 m for both the *x* and *y* directions (15 m and 18 m, respectively). Thus, the nematode densities at any sampled point has some indicative value for the nematode density at any other sampling point less then 15 m apart. When samples are taken farther apart, this is no longer true. Wallace and Hawkins (1994) estimated ranges of influence varying between 45 m and 160 m for the six nematode species studied, which is substantially larger than our finding. Thus, patches with similar population densities were smaller for *L. africanus* than for six other plant-parasitic nematodes. Our data also show that, at the perimeter of the sampled area, patches with high or low *L. africanus* population densities occurred

since the semi-variance values dramatically increased when distances between sampling points approached the total length or width of the sampled area (51.1 m).

The data on the relation between semivariance values and angles between sampling points (Fig. 2) suggest that patches with similar *L. africanus* numbers are elongated, with the main axis making an angle of 110°–170° with the field *x*-axis. Although the main factors influencing the horizontal distribution of *L. africanus* in this field are unknown, it is unlikely that soil moisture was a major component since no correlation between soil-moisture level and *L. africanus* numbers was detected.

The seasonal fluctuation in the total numbers of *L. africanus* extracted from the three sampled depths was large and strongly correlated with soil temperature. A similar correlation recently was found for the longidorid nematode *Xiphinema index* under grape in California (Feil et al., 1997). Cohn (1969), however, did not observe major differences between numbers of *L. africanus* found in winter and summer samplings in Israel, and also failed to find a correlation between nematode population levels and seasonal factors (e.g., temperature). In our study, nematode numbers responded quickly to temperature changes, suggesting that temperature is a major factor regulating population density. Since population levels were highest during the warm months of September and July, and lowest during the winter samplings, *L. africanus* appears to prefer soil temperatures close to 30 °C. This corresponds with conclusions by Kolodge et al. (1986), who designated *L. africanus* as a "relatively high-temperature organism," and with their observation that damage is usually most severe in fall crops, which are planted in late summer when soil temperatures and population levels are highest. From our data it is not possible to separate nematode multiplication from nematode migration as the factor resulting in population fluctuations. However, *Longidorus* spp. can migrate 2 cm/day through the soil profile up to depths of 160 cm, and temperature, rather than soil moisture or root growth, is an important factor in this migration (Rössner, 1972). The preferred depths of several longidorid nematodes varied greatly among different nematode species and among studies (Boag et al., 1989). Results by Cohn (1969), who reported in a study in Israel that most *L. africanus* were present in the upper 10 cm of the soil with very few nematodes found at depths below 60 cm, are in contrast with our findings, in which most nematodes were present in the 60 to 90-cm depth. Although the reasons for these contrasting results remain unknown, differences in soil temperatures, host crops, or agricultural practices may be important factors.

Based on our results, the chances for detection of *L. africanus* would be greatest when sampling deeper soil layers (60–90 cm), especially during the fall and winter months, when nematode numbers in the upper soil layers decline. An accurate estimation of the horizontal distribution of *L. africanus* would involve taking samples fairly close (ca. 15 m) together, as the variation between nematode numbers in samples taken at greater distances apart becomes large. This nematode has the potential of becoming a serious threat to a variety of economically important crops in southern California. Our data show that, under high soil temperatures, *L. africanus* populations can reach high levels in short periods of time. Future research on factors determining *L. africanus* distribution, the influence of cultural practices on nematode population dynamics, and on nematode density-plant damage functions is needed to allow a prediction of risks on crop damage and for the development of control strategies.

Literature Cited

Boag, B. B., D. J. F. Brown, and A. S. G. Banck. 1989. Optimizing sampling strategies for nematode-transmitted viruses and their vectors. Bulletin OEPP/EPPO Bulletin 19:491–499.

Brown, D J. F., and B. B. Boag. 1988. An examination of methods used to extract virus-vector nematodes. Nematologia Mediterranea 16:93–99.

Brown, D J. F., B. Boag, A. T. Jones, and P. B. Topham. 1990. An assessment of the soil-sampling density and spatial distribution required to detect virulifer-

ous nematodes (Nematoda: Longidoridae and Trichodoridae) in fields. Nematologia Mediterranea 18:153–160.

Clark, I. 1979. Practical geostatistics. London: Applied Science Publishers.

Cohn, E. 1969. The occurrence and distribution of species of *Longidorus* and *Xiphinema* in Israel. Nematologica 15:179–192.

Feil, H., B. B. Westerdahl, R. J. Smith, and P. Verdegaal. 1997. Effects of seasonal and site factors on *Xiphinema index* populations in two California vineyards. Journal of Nematology 29:491–500.

Ferris, H., and M. V. McKenry. 1974. Seasonal fluctuations in the spatial distribution of nematode populations in a California vineyard. Journal of Nematology 6:203–210.

Kolodge, C., J. D. Radewald, and F. Shibuya. 1986. Host range and life cycle of *L. africanus*. California Agriculture 40:13–14.

Kolodge, C., J. D. Radewald, and F. Shibuya. 1987. Revised host range and studies on the life cycle of *Longidorus africanus*. Journal of Nematology 19:77–81.

Lamberti, F. 1968. The effect of cropping on the population levels of *Longidorus africanus*. Plant Disease Reporter 52:748–750.

Lamberti, F. 1969. Pathogenicity of *Longidorus africanus* on selected field crops. Plant Disease Reporter 53: 421–424.

Merny, G. 1966. Nématodes d'Afrique tropicale: Un nouveau *Paratylenchus* (Criconematidae), deux nouveaux *Longidorus* et observations sur *Longidorus laevicapitatus* Williams, 1959 (Dorylaimidae). Nematologica 12: 385–395.

Netscher, C., and J. W. Seinhorst. 1969. Propionic acid better than acetic acid for killing nematodes. Nematologica 15:286.

Radewald, J. D., J. W. Osgood, K. S. Mayberry, A. O. Paulus, and F. Shibuya. 1969. *Longidorus africanus* a pathogen of head lettuce in the Imperial Valley of southern California. Plant Disease Reporter 53:381–384.

Rössner, J. 1972. Vertikalverteilung wandernder Wurzelnematoden im Boden in Abhängigkeit von Wassergehalt und Durchwurzlung. Nematologica 18: 360–372.

Wallace, M. K., and D. M. Hawkins. 1994. Applications of geostatistics in plant nematology. Supplement to the Journal of Nematology 26:626–634.