SPATIAL PATTERN OF *RADOPHOLUS SIMILIS* IN THE ROOTS AND SHOOTS OF *ANTHURIUM ANDRAEANUM*[†]

B. S. Sipes

Department of Plant and Environmental Protection Sciences, University of Hawaii, Honolulu, HI, 96822, U.S.A.

RESUMEN

Sipes, B. S. 2001. Patron espacial de *Radopholus similis* en raíces y brotes de *Anthurium andraeanum.* Nematrópica 31:113-118.

El patron espacial de *Radopholus similis,* endoparásito migratorio, se caracterizó en *Anthurium andraeanum.* Los nematodos se extrajeron de raíces individuales y de secciones de tallo de 2 cm de longitud. La varianza de la de densidad poblacional siempre excedió la media en la raíces y tallos, lo cual sugiere una distribución binomial negativa con nematodos agrupados en la planta. El índice de dispersión k, fue 0.15. la ley de la fuerza de Taylor se ajustó a los datos para determinar la relación entre la varianza y la media de la densidad poblacional del nematodo en las raíces. En un segundo experimento, los nematodos se extrajeron de 1, 2, 3, 4, and 5 g de raíz y tallo por planta para determinar la precisión del muestreo. La diferencia entre la media de la muestra y la media de la planta completa correspondientes a la densidad poblacional, así como la varianza asociada con cada muestra, disminuyó con el incremento del tamaño de la muestra. Un muestra compuesta de 4 g de tallo y raíz proporcionó la mejor estimación de la densidad poblacional del nematodo en la planta como un todo. La confiabilidad en las determinaciones de las densidades poblacionales de este nematodo endoparásito migratorio depende de un método apropiado de muestreo tal como para los nematodos de suelo.

Understanding the spatial patterns of plant-parasitic nematodes in the soil has enabled scientists to develop sampling schemes that provide an acceptable estimate of the population mean. These data can be used for making management recommendations or decisions (Barker, 1985). Such data are needed for accurate mathematical and computer models of plant-parasitic nematode damage (Ferris, 1984). Precision agriculture may come to rely upon pest distribution for targeting application of control measures (Gavassoni *et al.,* 1996). Much information is available on the spatial patterns of plant-parasitic nematodes in the soil (McSorley, 1998), yet scant information is available on the spatial patterns of migratory endoparasitic nematodes in plant tissue.

Radopholus similis Cobb, a migratory endoparasite of many tropical plants, occurs in root and shoot tissue of *Anthurium andraeanum* Linden ex André (Wang *et al.,* 1999). Nematode infection causes a chronic disease called anthurium decline which can result in a 50% yield loss (Aragaki *et al.,* 1984). Alternative and better controls may be achieved or developed with accurate estimations of nematode populations in the anthurium plant. The objective of these experiments was to determine the spatial pattern of *R. similis* in the roots and shoots of *A. andreanum* and to determine appropriate sampling schemes.

Five 20-cm-tall *A. andraeanum* cv. Midori growing in 15-cm-d clay pots filled with volcanic cinder (9.5-19-mm-d) were inoculated with 2 500 mixed life-stages of *R. similis* in

5-ml aliquots. Nematodes were pipetted directly onto the media in a circle 1 cm from the base of the plant. The inoculum, originally isolated from anthurium, was extracted from alfalfa callus cultures placed on Baermann funnels (Ko *et al.*, 1995). The plants were maintained in a greenhouse under 50% shade. The plants were harvested 36 days after inoculation, sufficient time for the nematodes to infect, reproduce, and move within the plant but not cause roots to rot. Roots and leaves were separated from the stem and the leaves discarded. Starting from the bottom, each root was sequentially removed from the stem and numbered consecutively. All plants had 4 roots, 60% had 5 roots, 56% had 6 roots, 28% had 7 roots, and 12% had as many as 8 roots. The stem was divided into 2-cm-long sections and sequentially numbered beginning with the root end. Root and stem sections were assayed individually by placing them in funnels in a mist chamber. The nematodes recovered after 3 days were counted. The experiment was conducted five times. The data from each harvest date and run were tested for homogeneity of variance and pooled. The mean and standard deviation of the number of nematodes per section was calculated and the type of distribution determined (Elliot, 1983). Taylor's power law was calculated for sections across and for sections within plants (Elliot, 1983). Sample sizes necessary to achieve estimates within 50% of the mean were calculated from the power law equation.

In a second series of experiments, five *A. andraeanum* cv. Midori were inoculated with 2 500 mixed life-stages of *R. similis* collected from alfalfa callus cultures (Ko *et al.*, 1995) and grown in the greenhouse. Plants were harvested 86 days after inoculation. Leaves were removed and discarded. The remaining shoot and root tissue was cut into 1-cm long pieces and composited for each plant. Since the plants had relatively small biomass, all roots were used in the composite samples. Samples consisting of 1, 2, 3, 4, or 5 g of tissue were placed in individual funnels in a mist chamber for 3 days to collect nematodes. Multiple samples were assayed from each plant up to the total weight of the plant. The experiment was repeated five times. Data were tested for homogeneity of variance, pooled, and the mean population density/ g tissue was calculated for each plant based upon samples from that plant. The difference between the sample population density and the plant mean was derived and subjected to analysis of variance.

Radopholus similis clustered in both the roots and stems of anthurium (Table 1). One root or stem section may have contained no nematodes, whereas a nearby root or stem section may have had many individuals. The average population density for all plant sections was 19 nematodes (s^2 = 1 927). The calculated k value, an index of dispersion from the negative binomial distribution, equaled 0.15. An estimated sample size would be 328, 82, or 36 sections for a 25, 50, or 75% confidence interval half length ($P = 0.05$) about the mean, respectively (Steele and Torrie, 1960).

The average nematode population densities differed in each plant section over all of the plants (Table 1). Nematode population densities were greater in the roots than in the stems (24 and 7 nematodes/ section, respectively). The youngest (lowest) and oldest roots had fewer nematodes than those roots of intermediate age in the center of the stem. Fewer nematodes occurred in the upper portion of the stem compared to the lower section. This distribution probably reflects the inoculation procedure and the early stage of infection by the nematodes. The power law equation for sections across plants was $\sigma^2 = 0.91\mu^{2.26}$, $r^2 = 0.93$. The slope of the log variance to log mean is greater than 2.0, indicating

Table 1. Variance to mean ratios for *Radopholus similis* in different sections of *Anthurium andraeanum*.

	Section[z]	Nematode population density (_)	Variance (s²)	CV
Root	1	7	107	15
	2	12	296	24
	3	25	2276	92
	4	44	6440	146
	5	16	456	28
	6	52	6729	128
	7	28	667	24
	8	7	49	7
Stem	1	9	345	38
	2	5	336	63

[z]Increasing numbers indicate higher levels on the plant.

that relatively greater aggregation is associated with higher mean population density. Only 23 root samples would be required to estimate mean density if the lowest roots (section 1) were sampled, whereas 37 root samples would be required to determine density if roots of intermediate age (section 4) were selected (Duncan *et al.*, 1994). This result could be due to the termination of the experiment 36 days after inoculation. Nematodes may have only begun to colonize the lower roots and stem yet have laid many eggs in clusters in the roots nearest the inoculation point. Additional time for reproduction and migration may have given other results.

Nematode population per root system in a plant also differed (Table 2). Population densities per root system ranged from 1-125 nematodes. Taylor's power law was $\sigma^2 = 1.5\mu^{1.68}$, $r^2 = 0.95$ for the variance to mean relationship in the plants. As the number of nematodes per root system increased, the number of sampled roots required to predict the mean density per plant decreased. This relationship was used to provide an approximation of the numbers of root samples that would be needed to estimate the mean density per root system in a population of plants. With a mean density of 1 nematode per root system, 25 root samples would be needed to predict the mean (with a confidence interval half-length of 50% of the mean), whereas a population of 56 *R. similis* per root system would only require a sample size of 9 roots (Duncan *et al.*, 1994).

The nematode population density/g tissue ranged from 2-290 *R. similis* over the 25 plants tested in the second experiment. The differences between the plant and sample mean population densities did not differ (P > 0.05) but tended to decrease with increasing sample size. The 1- and 5-g samples underestimated the nematode population, whereas the 2-, 3-, and 4-g samples overestimated the population mean. The standard errors associated with each sample size generally decreased with the increasing sample size (Fig. 1). The 1-g sample probably missed nematode clusters and thus underestimated the population density. The over estimations suggest that the samples contained multiple clusters of nematodes.

Table 2. Variance to mean ratios for *Radopholus similis* in the roots of different *Anthurium andraeanum* plants.

Nematode population density (_)	Variance (s²)	CV	Nematode population density (_)	Variance (s²)	CV
0.3	0.6	2	13	150	12
0.3	0.6	2	24	729	30
0.5	0.75	15	25	153	6
2	10	5	26	306	12
3	27	9	31	468	15
3	17	6	37	455	12
4	16	4	39	1445	37
5	91	18	41	2590	63
5	17	3	56	6147	110
11	189	17	56	3667	66
12	320	27	71	3138	44
12	74	6	125	18244	146
12	46	4			

Radopholus similis is aggregated in the roots and stem of *Anthurium*. The clustering index derived for *R. similis* in anthurium tissue is similar to that reported for *Pratylenchus* in the soil (McSorley, 1987). The clustering of nematodes within the plant tissue compounds the difficulty in establishing accurate estimates of their population densities. The nematode is likely to be clustered among plants in the field as well as the within individual plants.

Based on these results, the appropriate tissues to assay to maximize detection of *R. similis* are middle roots and the lower sections of the stem. Generally in field collected plants, the oldest roots, which once probably supported high numbers of nematodes, are decomposed and support few if any nematodes. Nematodes may not have migrated to or colonized the youngest roots of a plant, therefore their population densities will be low. Nematode population densities behave similarly in the stem tissue, except that stem tissue is not likely to

decompose quickly. Some nematodes are pioneers establishing the population by migrating to new roots and apical meristem.

As nematode population densities decrease, the probability of detecting the nematodes becomes lower unless sample units are increased (McSorley and Littell, 1993). Inaccurate detection and quantification of *R. similis* is likely if a small tissue sample is taken from the wrong place on the plant because of the clustering of the nematode in anthurium. Reliable population density determinations of this migratory endoparasite depend upon collecting sufficient tissue. Samples ≥4 g tissue/plant are desirable. The principles of sampling for *R. similis* in anthurium roots and stem tissue are similar to those used for soil-borne nematodes.
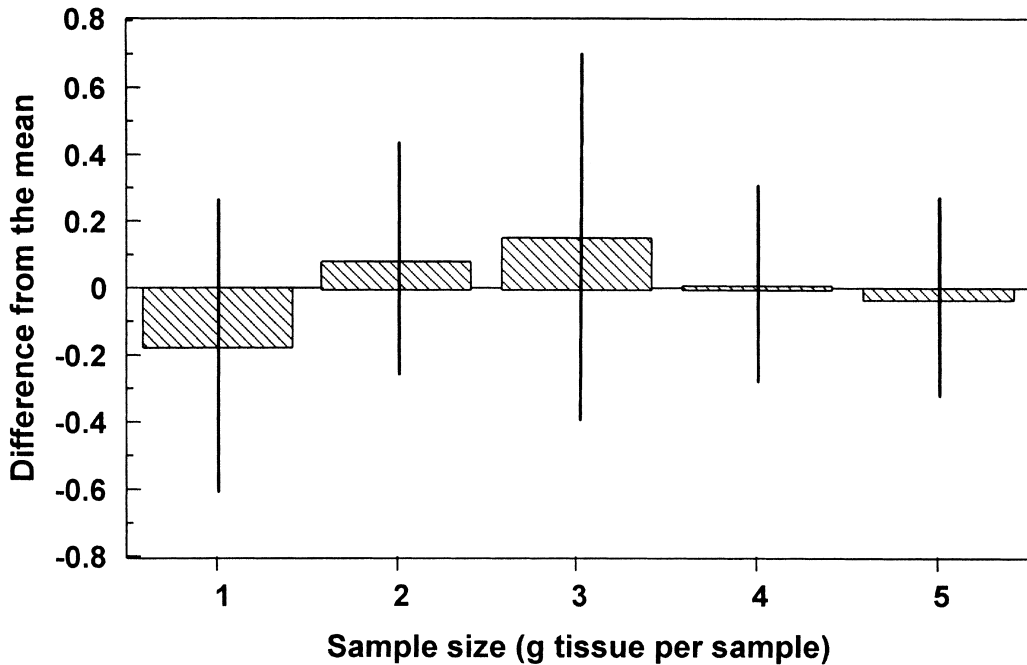
ACKNOWLEDGMENTS

Fig. 1. The difference in *Radopholus similis* population estimations between the plant mean and means from various sized samples of *Anthurium andreanum* tissue. Standard error bars are given for each difference.

LITERATURE CITED

ARAGAKI, M., W. J. APT, R. K. KUNIMOTO, W. H. KO, and J. Y. UCHIDA. 1984. Nature and control of anthurium decline. Plant Disease 68:509-511.

BARKER, K. R. 1985. Sampling nematode communities. Pp. 3-17 *in* K. R. Barker, C. C. Carter, and J. N. Sasser, eds. An Advanced Treatise on *Meloidogyne* Vol. II. North Carolina State University Graphics, Raleigh, NC, U.S.A.

DUNCAN, L. W., M. M. EL-MORSHEDY, and R. Mc-SORELY. 1994. Sampling citrus fibrous roots and *Tylenchulus semipenetrans*. Journal of Nematology 26:442-451.

ELLIOT, J. M. 1983. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biological Association, Ambleside, Cumbria, U.K.

FERRIS, H. 1984. Nematode damage functions: The problems of experimental and sampling error. Journal of Nematology 16:1-9.

GAVASSONI, W. L., G. P. MUNKVOLD, and G. L. TYLKA. 1996. Spatial dependence of soybean cyst nematode and egg populations. Phytopathology 86:S86.

KO, M. P., D. P. SCHMITT, and B. S. SIPES. 1995. Axenizing and culturing endomigatory plant-parasitic nematodes using Pluronic® F127, including its effects on population dynamics of *Pratylenchus penetrans*. Journal of Nematology 8:115-123.

McSORLEY, R. 1987. Extraction of nematodes and sampling methods. Pp. 13-48 *in* R. H. Brown, and B. R. Kerry, eds. Principles and Practice of Nematode Control in Crops. Academic Press Sydney, Australia.

McSORLEY, R. 1998. Population dynamics. Pp. 109-133 *in* K. R. Barker, G. A. Pederson, and G. L. Windham, eds. Plant and Nematode Interactions. American Society of Agronomy, Crop Science Society of America, Soil Science of America, Madison, WI, U.S.A.

McSORLEY, R., and R. C. LITTELL. 1993. Probability of detecting nematode infestations in quarantine samples. Nematropica 23:177-181.

STEELE, R. G. D., and J. H. TORRIE. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Company, NY, New York, U.S.A.

WANG, KOON-HUI, B. S. SIPES, and A. R. KUEHNLE. 1999. *Radopholus similis* in *Anthurium* shoot tissue. HortScience 34:296-297.

BLANK PAGE USED IN PAGE COUNT

BLANK PAGE USED IN PAGE COUNT

BLANK PAGE USED IN PAGE COUNT

BLANK PAGE USED IN PAGE COUNT