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

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RESUMEN


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.

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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. andraeanum* 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

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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 = 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
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 1. Variance to mean ratios for $Radopholus similis$ in different sections of Anthurium andraeanum.

<table>
<thead>
<tr>
<th>Section'</th>
<th>Nematode population density ($)</th>
<th>Variance (s²)</th>
<th>CV</th>
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<tr>
<td>Root</td>
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<td></td>
</tr>
<tr>
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<td>107</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>296</td>
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</tr>
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</tr>
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</tr>
<tr>
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<td>5</td>
<td>336</td>
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</table>
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.

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