Distribution of *Aphelenchoides fragariae* in Leaves of *Ficus elastica* and *Asplenium nidus*

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ABSTRACT

Esser, R. P., and Riherd, C. C. 1981. Distribution of *Aphelenchoides fragariae* in leaves of *Ficus elastica* and *Asplenium nidus*. Plant Disease 65:425-426.

In population distribution tests of *Aphelenchoides fragariae* in leaves of *Ficus elastica* and *Asplenium nidus*, concentrations of the nematodes were highest in the necrotic areas. A new paper punch method was used to take leaf subsamples for diagnostic purposes.

Each year, leaves exhibiting symptoms of foliar nematode damage are examined for nematodes in the Division of Plant Industry laboratory, Florida Department of Agriculture and Consumer Services. Before 1976 the leaves were teased apart in a small dish containing water and examined for foliar nematodes by Southey's method (3). Other methods that can be used to separate foliar nematodes from leaves include immersing leaves in boiling lactophenol (2) and placing finely chopped leaves in a funnel overnight (4).

In 1976 a paper punch method was implemented to demonstrate foliar nematodes in leaves. This paper describes the paper punch method and gives the results of tests designed to show where populations of foliar nematodes are highest in the leaf.

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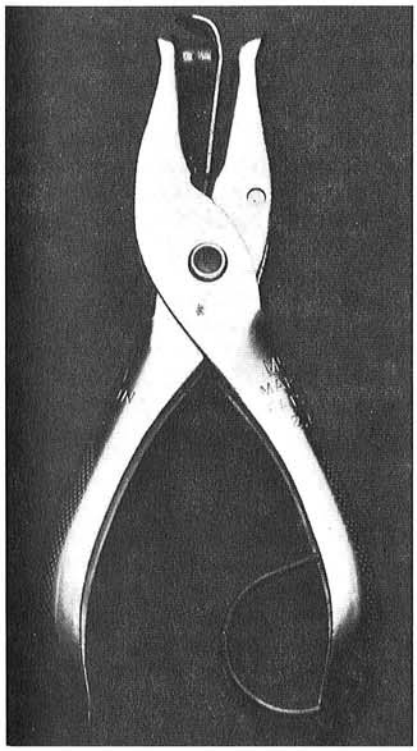

Fig. 1. Paper punch.

MATERIALS AND METHODS

Procedure. Leaves with characteristic symptoms of foliar nematode infection were held over a 65-mm Syracuse watch glass containing 2–3 mm of water. A clean paper punch (Fig. 1), ejection side over the water, was used to punch 7-mm-diameter disks from the leaves (Fig. 2). Ten to 20 foliar disks were punched into the water from the necrotic area and from the border between necrotic and healthy areas of the leaf (Fig. 3). The Syracuse watch glasses with foliar disks in water were covered, labeled, and held overnight, then examined for emerged foliar nematodes. Foliar nematodes usually emerge from heavily populated foliar disks in about 2 hr and from lightly infected foliar disks in 6–8 hr (Fig. 4). In rare cases, 16 hr pass before emergence.

After each leaf was punched, the paper punch was cleaned by using a high-pressure water spray. The punch can also be disinfected by being dipped in a 1:5 solution of sodium hypochlorite (household bleach) for 10 min (1), then rinsed in clean water.

Table 1. Numbers of *Aphelenchoides fragariae* in 7-mm-diameter foliar disks

	Ficus elastica leaf											
Character	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Total**
Healthy	0	0	0	0	0	0	0	0	0	0	0	0
Tip, healthy	0	0	0	0	0	0	0	0	0	0	A	0
Tip, necrotic	A[x]	A	A	A	A	A	A	A	A	A	0	0
Border	0	0	0	0	0	0	0	3	12	5	2	22
Midrib, healthy	0	0	0	0	A	0	A	A	0	0	A	0
Midrib, necrotic	A	A	A	A	28	A	0	10	A	A	0	38
Necrotic	19	27	24	46	17	3	1	12	50	51	500	750

	Asplenium nidus leaf															
Character	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**Total**
Healthy	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	21
Tip, healthy	40	0	0	0	0	0	0	0	A	0	0	A	0	A	0	40
Tip, necrotic	A[x]	A	A	A	A	A	A	A	500	A	A	500	A	0	A	1,000
Border	148	28	8	0	0	10	0	0	0	8	0	500	1	0	25	728
Midrib, healthy	A	A	A	A	0	A	A	0	A	A	A	A	A	A	8	8
Midrib, necrotic	500	34	35	13	A	500	0	3	500	500	0	500	3	0	A	2,588
Necrotic	48	500	500	500	500	500	500	3	500	500	500	500	7	1	500	5,559

[x]A = character absent.

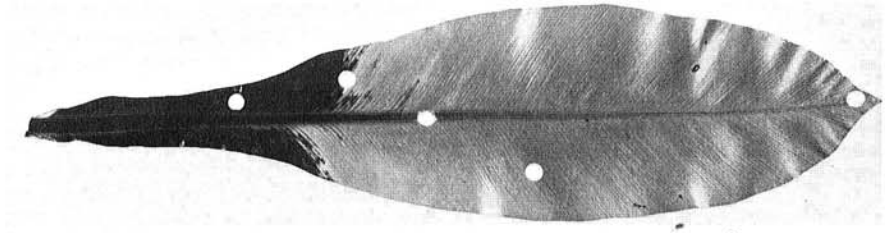

Fig. 2. Leaf with *Aphelenchoides fragariae* infestation shows punch pattern used.

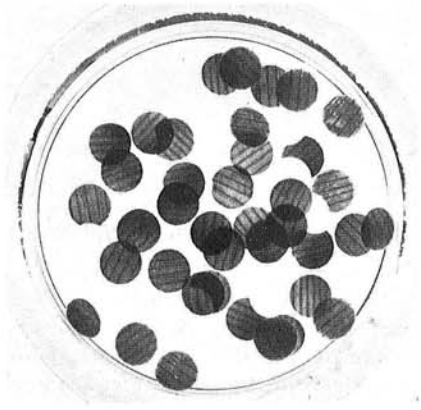

Fig. 3. Leaf disks in water.

Distribution of nematodes in leaves. One leaf of *Asplenium nidus* L. (birds-nest fern) or *Ficus elastica* Roxb. ex Hornem. (rubber-tree) was removed from a plant with symptoms of foliar nematode infestation and examined for nematodes. If the test leaf yielded large numbers of foliar nematodes, adjacent leaves with symptoms were removed for testing.

Five 28-mm USBPI watch glasses were labeled and half filled with water. Five circular disks were then punched from each leaf (Fig. 2). The first disk was punched from the healthiest tissue on the leaf and placed in one of the USBPI dishes, which had been labeled with an H for healthy. The next disk was taken from the leaf tip and placed in a dish labeled with either a TH, which indicated that the tip was healthy, or a TN, which indicated a necrotic tip. The third disk was punched from the leaf midrib and placed in another dish. This dish was labeled with either MH or MN, depending on whether the midrib was healthy or necrotic. After this, a disk was punched from the border between the necrotic and healthy tissue. This disk was then placed in a dish labeled with a B for border. The final disk was taken from the necrotic leaf tissue and placed in a dish labeled with N for necrotic.

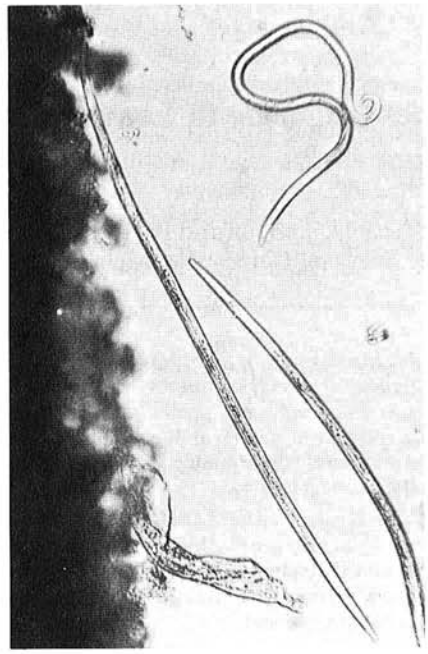

Fig. 4. Foliar nematodes emerging from cut edge of leaf disk.

After 6 hr, each dish was examined separately with the aid of a dissection microscope and the nematodes that emerged during that time were counted.

RESULTS

The data showing the distribution of foliar nematodes in 11 leaves of *F. elastica* and 15 leaves of *A. nidus* are presented in Table 1.

Where the foliar nematodes were too numerous to be counted effectively, a value of 500 was assigned to indicate their abundance.

Necrotic areas of the leaves contained the largest number of foliar nematodes. The border area between the necrotic and healthy leaf tissue contained the next largest number of nematodes. The healthy areas of *F. elastica* leaves had no foliar nematodes, and only two leaves of *A. nidus* had foliar nematodes in apparently healthy areas.

DISCUSSION

The paper punch method is easy to use and in minimal time it produces a uniform diagnostic subject that lends itself to experimental analysis. Small diseased areas or single spots on a leaf can be specifically isolated for analysis. The method has also been used in serological assays of Cymbidium mosaic and Odontoglossum ringspot viruses (5).

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