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Infection of *Narcissus* Roots by *Aphelenchoides subtenuis*[1]

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Abstract: The widespread destruction of commercially grown bulbs of *Narcissus tazetta papyraceus* (Paper White) has been reported in Israel. This phenomenon is usually characterized by a premature yellowing of the foliage, accompanied by root rot and dark, sunken basal plates. This study confirmed that *Aphelenchoides subtenuis* is the main cause of the basal plate disease of *Narcissus*. In contrast to other *Aphelenchoides* species, which feed on stems or leaves, *A. subtenuis* penetrates *Narcissus* roots. In our experiments, in winter (6 to 8 weeks after penetration), nematodes laid their eggs in the root parenchymal cells without inducing obvious symptoms on foliage or roots. Toward spring, juveniles became numerous throughout the parenchymal cells of the root cortex. Consequently, the root system collapsed rapidly, at the usual peak of bulb and foliage production. Bulbs of infected plants were small and weighed less than those of uninfected plants, and foliage became necrotic prematurely. At that time, in field conditions, secondary elements like *Fusarium* penetrate the bulb and cause it to rot, given this syndrome the common name of basal plate disease. To our knowledge, this is the first report of an *Aphelenchoides* species as a root pathogen.

Key words: Aphelenchoides subtenuis, Fusarium, Narcissus, nematode, pathogenicity.

During the past decade, considerable destruction of commercially grown bulbs of *Narcissus tazetta papyraceus* (Paper White) cv. Ziva has been reported in Israel (6,7). Symptoms usually consist of a premature yellowing of foliage of the affected plants, accompanied by root rot and dark sunken basal plates. Root and plant development are inversely correlated with the degree of damage to the basal plate of the planted bulbs (6). Plants produced by infected bulbs had a reduction of ca. 45 and 55% in height and number of flowers, respectively, when compared with plants grown from healthy bulbs (6).

Although several saprophytic fungi, mainly *Fusarium* spp., were isolated from roots or basal plates, fungal or bacterial pathogens were not (6). Moreover, when healthy bulbs were inoculated with the saprophytic fungi, no symptoms were observed (6). The nematode *Aphelenchoides subtenuis* (Cobb, 1926), however, was isolated in this study from affected bulbs (6). Experimental control treatments with several fungicide dips, hot water treatment, methyl bromide, metham sodium, ethylene dibromide, or fenamiphos (4,6,7) led to the conclusion that *A. subtenuis* was involved in the disease syndrome, possibly in combination with fungi (4). Nevertheless, nematode counts from affected bulbs revealed only several dozen per basal plate during the growth period and at bulb harvest (1,4). These low numbers did not provide a satisfactory explanation for the damage caused to the plant and the role of *A. subtenuis* in this damage. The purpose of our investigation was to evaluate the contribution of *A. subtenuis* to the basal plate disease of *Narcissus*.

Materials and Methods

Nematodes: Nematodes were extracted from bulbs and roots that had been collected from fields of diseased *Narcissus* toward the end of the growing season. Roots were cut into pieces (2 to 3 mm long) and placed on 30-μm-pore sieves covered with a thin layer of water in 14-cm-d petri dishes. The plates were incubated at room temperature (RT, ca. 22 to 26 C) for 24 hours. The nematode suspension was collected and centrifuged, and the population (mainly juveniles) were sterilized by incubation in 0.2% NaOCl for 1 minute. The nematodes were then washed three times with sterilized water.

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Pathogenicity: Sixty 750-ml plastic pots were filled in May with autoclaved sandy loam soil (6% clay, 6% silt, 88% sand; pH 7.9–8.1) and were divided into three equally sized groups that were then infested with 10-ml suspensions of 0, 5,000, or 10,000 nematodes/pot. The pots were kept unwatered for 6 months in the screenhouse to mimic field conditions; in November, apparently healthy, uninfected, uniformly small bulbs (i.e., symptom-free bulbs as determined by visual inspection) were planted, one bulb per pot, and maintained in the screenhouse. Because of the possibility that nematodes were damaged by the NaOCl and (or) the 6-month storage treatments, 20 pots were filled with an autoclaved mixture of sandy loam soil and peat soil (3:1), and half of the pots were each infested with 14,000 nematodes previously extracted from infected bulbs as described but were not surface-sterilized with NaOCl solution. Each pot was then planted with one bulb and maintained in the screenhouse; the pots containing uninfested soil were controls.

In order to investigate the possible involvement of other root pathogens in the disease syndrome, *Narcissus* roots from which nematodes had been previously extracted were dried on filter paper at RT and then mixed with autoclaved soil and transferred to 20 750-ml pots (ca. 1.5 g dry weight per pot). Bulbs were planted in November, as described before.

In all experiments, plants were arranged in a randomized block design.

During the growing season, three plants were periodically harvested and roots were carefully washed, stained with 0.005% acid fuchsin in lactic acid:glycerin:distilled water (1/3/3), and then further incubated for a week at 50 C. This procedure clarified the root system and enabled observation of the early stages of juvenile penetration and the different stages of nematode development within the root tissues. Toward the end of the growing season, 20 1-cm-long root sections were chosen randomly from 10 plants, and nematode life stages (adults, eggs, and juveniles) were counted. At the end of the growing season (May), 10 plants from each treatment were harvested and the bulbs were weighed.

In the following winter, infected and uninfected bulbs from the first growing season were planted in 20 750-ml pots containing nematode-infested and uninfested autoclaved sandy loam soil, as described in the other experiments.

Results and Discussion

Our histopathological investigation revealed that *A. subtenuis* penetrated *Narcissus* roots, in contrast to other *Aphelenchoides* species, which are known as foliar feeders. To our knowledge, this is the first report of an *Aphelenchoides* species as a migratory endoparasitic root pathogen. *Aphelenchoides parietinus* has been observed feeding, to some extent, only on the surface of cotton roots (3). In winter, 6 to 8 weeks after penetration, nematodes laid their eggs in the parenchymal cells of the root tissue (Fig. 1A), but symptoms were not present on foliage or roots (Fig. 1B). Toward spring, in March, numerous juveniles were found throughout the parenchymal cells of the root tissue (Fig. 1C). Subsequently, the root system collapsed rapidly, while the plant was at its peak of bulb and foliage production. Premature yellowing of the foliage appeared in the 10,000- and 14,000-nematode-infected plants. Uninfected plants, as well as those treated with 5,000 nematodes or with the dried extracted roots, did not express the symptoms described above. Later, the prematurely yellow foliage dried (Fig. 1D), followed by shrinkage of the bulbs (Fig. 1E). At that time, neither the penetration of bulbs by secondary organisms or the resulting bulb rot typical of field situations occurred in our experiments.

At the final harvest, the average fresh weight of bulbs grown in the higher infestation levels (10,000 and 14,000 nematodes) was half that of bulbs grown in the 0- or 5,000-nematode-infested soil, or in the soil treated with the dried extracted roots (Fig. 2). The numbers of adult fe-

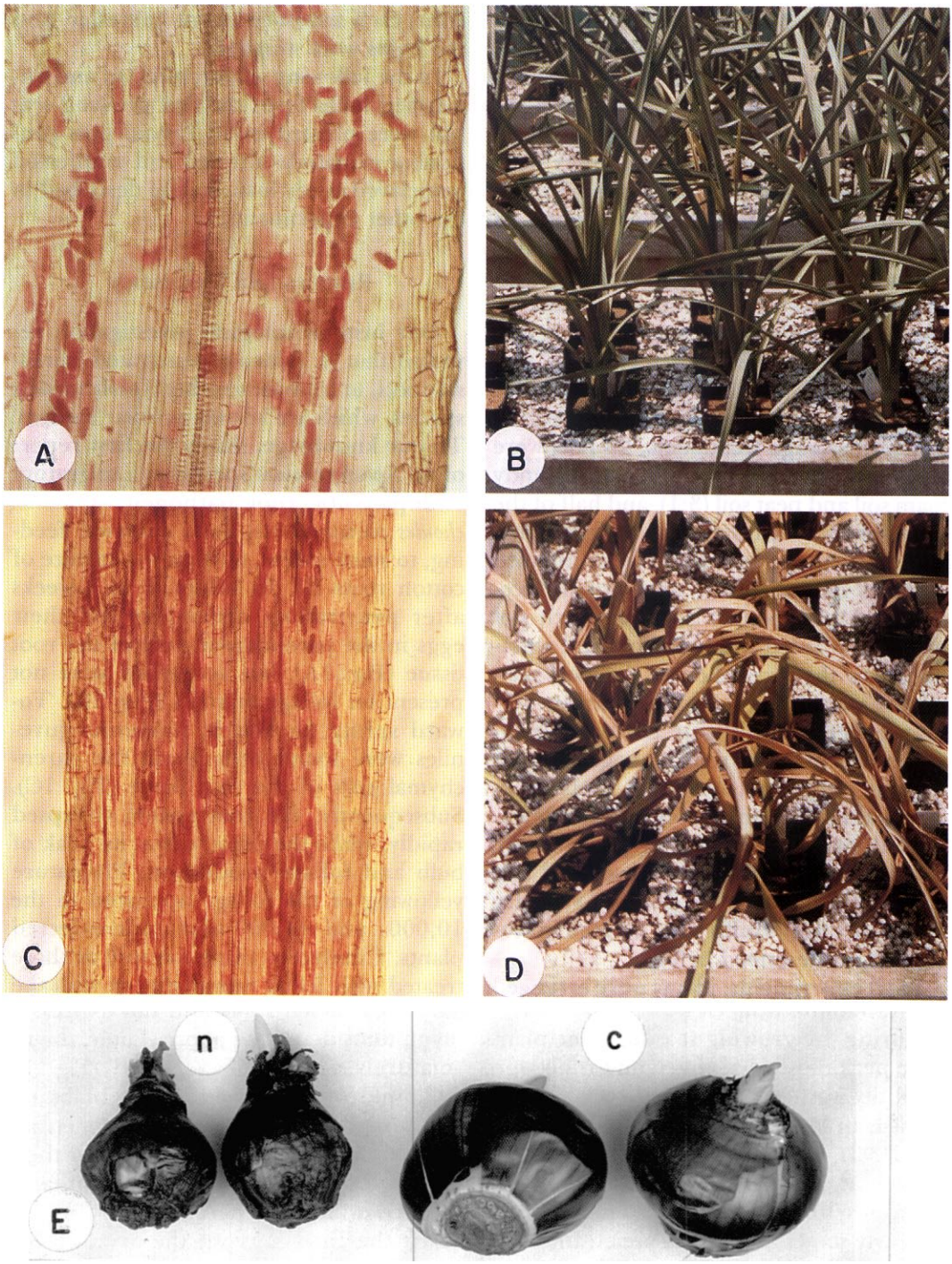

FIG. 1. Development of the basal plate disease syndrome of *Narcissus* caused by the phytoparasitic nematode *Aphelenchoides subtenuis*. A,B) 6–8 weeks after penetration, nematode eggs are widespread in the parenchymal cells of the root tissue, but symptoms are not present on foliage or roots. C) 4 months after penetration, the hatching rate increases and juveniles begin feeding on root parenchymal cells. D,E) 5 months after penetration in spring, foliage becomes dry and necrotic, followed by shrinkage of the bulbs (c = uninfected control bulbs; n = nematode-infected bulbs).

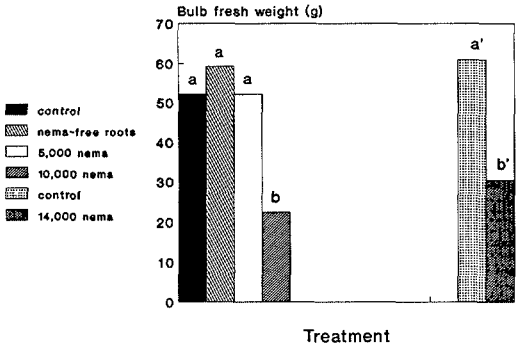

FIG. 2. Bulb fresh weight of *Narcissus tazetta papyraceus* (Paper White) var. Ziva grown in pots containing autoclaved soil infested with 0, dried roots from which nematodes had been previously extracted, 5,000, 10,000, or 14,000 nematodes of *Aphelenchoides subtenuis*. Data are from two experiments with 10 bulbs per infestation level. Bars with a common letter are not significantly different ($p = 0.05$) according to Duncan's multiple-range test.

males, juveniles, and eggs recovered per cm of root were 39 ± 5, 628 ± 21, and 574 ± 17, respectively, from the 10,000-nematode experiment; roots from either control experiment (i.e., inoculum was 0 nematodes or dried, washed roots) contained no adults, juveniles, or eggs.

In the following season, 100% of the nematode-infected bulbs subsequently transferred to uninfested soil did not flower, and their foliage dried out as harvest approached. Infected bulbs subsequently planted in nematode-infested soil barely developed foliage, daughter bulbs, or a root system. Uninfected bulbs, however, showed the symptoms detailed previously: namely, toward spring, in March, while the plant was at its peak of bulb and foliage production, premature yellowing of the foliage appeared; later, this prematurely yellow foliage dried and was followed by shrinkage of the bulbs.

Aphelenchoides subtenuis has not been regarded as an economically important parasite of narcissi (1,5). In general, *Ditylenchus dipsaci* is considered the most important nematode pest on bulbs, especially narcissi (1,2). Our work confirms the hypothesis that *A. subtenuis* is the main cause of the basal plate disease of *Narcissus* observed in field conditions in Israel. Although *Aphelenchoides* is known as the "bud and leaf" nematode, our results prove that *A. subtenuis* is primarily a root pathogen of *Narcissus*. Low numbers of *A. subtenuis* infect only the outer scale of the bulb and the basal plate roots.

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