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# Effects of *Pratylenchus penetrans* on the Infection of Strawberry Roots by *Gnomonia comari*

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*Abstract:* The fungus *Gnomonia comari,* causal agent of strawberry leaf blotch, was inoculated at the crown of young axenized strawberry plants growing in sterilized sand. Only the roots were colonized, and the infection was symptomless. When the fungus colonized the roots in the presence of the root lesion nematode *Pratylenchus penetrans,* the plants were extremely stunted and their root system was necrotic. Fungal conidiospores were found attached to the cuticle of nematodes extracted from soil inoculated with the two pathogens. These findings indicate that *P. penetrans* could transport conidiospores through soil.

*Key words:* conidiospore, *Gnomonia comari,* incitant, interaction, lesion nematode, *Pratylenchus penetrans,* strawberry leaf blotch, vector.

*Gnomonia comari* Karsten 1873 (conidial stage: *Zythia fragariae* Laiback) causes a leaf blotch of strawberry. Considered a weak parasite, the fungus generally enters through stomata and wounds. Lesions and necrosis develop on leaves and petioles, and the fungus can attack unripened fruits. *G. comari* causes heavy losses of strawberry fruits in central Europe where it has been isolated in root rot of strawberry primary roots (15). Although it can be found on the crowns in North America (6), it has not been detected in several surveys of root rot pathogens of strawberry (8,12,18). In British Columbia, infected plants develop symptoms after harvest, when necrotic areas spread on older and damaged leaves (2,3).

The root lesion nematode, *Pratylenchus penetrans* Filipjev and Sch. Stekhoven, 1941, is a widespread pathogen of strawberry in British Columbia as well as in Europe (16). It has a synergistic role in the development of certain root rot diseases of strawberry (5,7,11).

Because the fungus can be a foliar pathogen or a crown and root rot pathogen, the objectives of our study were to determine the possible role of *P. penetrans* as an incitant and as a transporter of conidiospores through soil.

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## MATERIALS AND METHODS

*Pratylenchus penetrans as an incitant:* Strawberry (*Fragaria virginianae* L. cv. UC11) plants grown in polystyrene microbeakers in 20 $cm^3$ washed sand were watered and fertilized with a dilute nutrient solution containing 60 ppm N, 26 ppm P, 50 ppm K, and micronutrients. The plants were maintained on a laboratory bench at 23 C under cool and warm fluorescent tubes with a 16-hour photoperiod and a light flow of 37 $\mu E\ m^{-2}\ s^{-1}$ at the level of the higher leaves.

When the plants reached 5–6 cm in height, they were transplanted into 200 $cm^3$ autoclaved sand in 7.5-cm-d plastic pots. Plants were inoculated with nematodes at transplanting and 16 days later with fungus singly or in combination with the nematodes inoculated previously.

Nematode inoculum consisted of all stages of *P. penetrans* reared on raspberry (*Rubus idaeus* L. cv. Willamette) and extracted from washed roots in a mist chamber. The nematodes were then axenized in an antibiotic solution containing 4 ppm of methoxy ethyl mercury chloride (17). The nematodes were rinsed in several baths of sterile water, and 400 specimens in 4 ml water were placed in a small hole 2 cm deep in the sand near the base of each plant.

Plants were grown for 16 days and then uprooted. The roots were washed free of sand, examined for signs of nematode injury, and photographed. The plants were then replanted, and half of the nematode-

free and nematode-infected plants were inoculated with the fungus. Inoculum of *Gnomonia comari* was propagated on potato dextrose agar. A 5-mm³ plug of PDA with mycelium bearing conidiophores and perithecia was mixed with sterile sand and placed at the base of plants. The fungus-free pots received equal amounts of clean agar mixed with sand. There were four treatments including the uninoculated control, each with four replications.

After 21 more days, plants were harvested and their total leaf area was measured using a portable area meter (Li-cor Inc., Lincoln, NE). The roots were washed clean of sand, observed with the aid of a stereomicroscope for symptoms of nematode and fungus infection, and photographed. A portion of the roots was stained in a solution of acid fuchsin lactophenol and destained in clear lactophenol to detect fungal structures in root tissue. Another portion of roots was rinsed several times in sterile water and plated on water agar for fungal colonization. All fungal colonies emanating from the roots were plated on PDA and grown for 14 days at 23 C for fungal isolation.

Two other identical experiments using mixtures of pasteurized and unpasteurized field soil instead of autoclaved sand were conducted in a greenhouse. These experiments were added to determine any eventual suppressive effect of the pasteurized and unpasteurized soils on the expression of the symptoms caused by the two pathogens. Plants in these experiments were examined only for growth suppression and for foliar and root symptoms caused by nematode and fungus alone or in combination.

*Pratylenchus penetrans as a carrier:* Nematodes from each pot inoculated with nematodes alone or in combination with the fungus were extracted by flotation-sieving. All stages of *P. penetrans* were collected and rinsed several times in sterile water. Ten nematodes from each pot were placed on PDA in petri dishes for 14 days for isolation of the fungus carried by the nematodes. In addition, 80 nematodes from each pot were hand picked and deposited near the crown of four new, fungus-free, 21-day-old strawberry plants growing in sterilized sand. These nematode-inoculated plants were maintained for an additional 3 weeks under the growing conditions previously described. At harvest their roots were processed and examined for fungal infection as described in the incitant experiment.

Plastic tubes (BEEM embedding capsules for electron microscopy, 13 × 8 mm d), with the bottom wall replaced by a 20-μm-pore nylon membrane, were filled with wet sterile sand. Fungal mycelium from PDA cultures was placed on the surface of the sand, and 100 axenized nematodes were added to the surface of four tubes with fungus and four tubes without. The tubes were placed vertically in petri dishes for 24 hours, and the nematodes that migrated through the nylon membrane were collected in sterile water. The nematodes were placed in another BEEM capsule with both ends closed with 15-μm-pore nylon sieve. They were fixed in 4% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 6.8), dehydrated in an ethanol series starting at 20%, critical point dried with liquid $CO_2$, sputter coated with gold, and examined by SEM.

## Results

*Pratylenchus penetrans as an incitant:* Plants inoculated with nematodes and fungus were wilting during the second week after inoculation with the fungus, whereas none of the plants in the other treatments wilted. No plants from any treatments showed any symptoms of leaf blotch during the entire experiment. After 3 weeks, the foliage of plants inoculated with fungus alone appeared healthy and not different from that of uninoculated plants. The foliage of plants inoculated with nematodes alone was noticeably stunted, with smaller leaves and shorter petioles. The plants inoculated with nematodes followed by fungus were extremely stunted and necrotic. Most of their leaves died before they reached maturity, and only a few younger

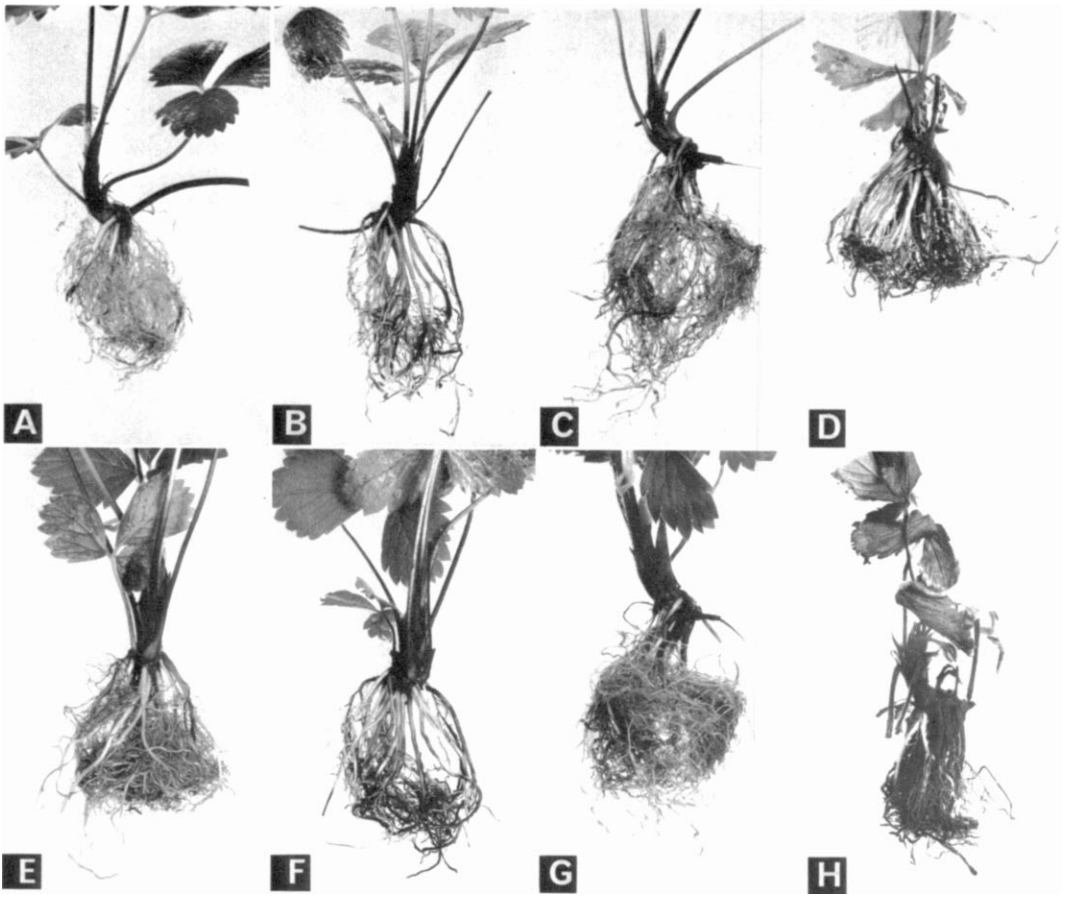


FIG. 1. Strawberry root systems before *Gnomonia comari* inoculation (Top) and 3 weeks later (bottom). A, E) Nematode-free and fungus-free control plants. B, F) *Pratylenchus penetrans*-infected roots left fungus free. C, G) Nematode-free roots infected with *G. comari*. D, H) Roots infected with *P. penetrans* and *G. comari*.

leaves appeared functional at the end of the experiment. Average leaf areas per plant at harvest were 61.4 $cm^2$ for uninoculated control; 74.6 $cm^2$, plants inoculated with *G. comari* only; 40.2 $cm^2$, plants inoculated with *P. penetrans* only; and 2.4 $cm^2$ for plants inoculated with both fungus and nematodes.

The root systems of uninoculated plants (Fig. 1A, E) showed no discoloration or lesions. Roots of plants inoculated with nematodes (Fig. 1B, D, F) showed root lesions typical of *P. penetrans* and grew less rapidly than the roots of uninoculated plants. Roots of plants inoculated with fungus alone (Fig. 1G) were somewhat darker than those of uninoculated plants, but they showed no lesions and were well developed. Roots of plants inoculated with nematodes and then fungus were extensively necrotic (Fig. 1H). Staining with acid fuschin lactophenol showed mature darkened perithecia only in the roots of plants inoculated with both nematodes and fungus; no perithecia were observed in the roots of any of the other plants. However, *G. comari* was consistently isolated on water agar from roots of plants inoculated with fungus, irrespective of the presence of nematodes.

In the two greenhouse experiments with mixtures of pasteurized and unpasteurized field soil, the plants showed no sign of fungal infection and we could not repeat the results of the nematode plus fungus interaction obtained from plants grown in ster-

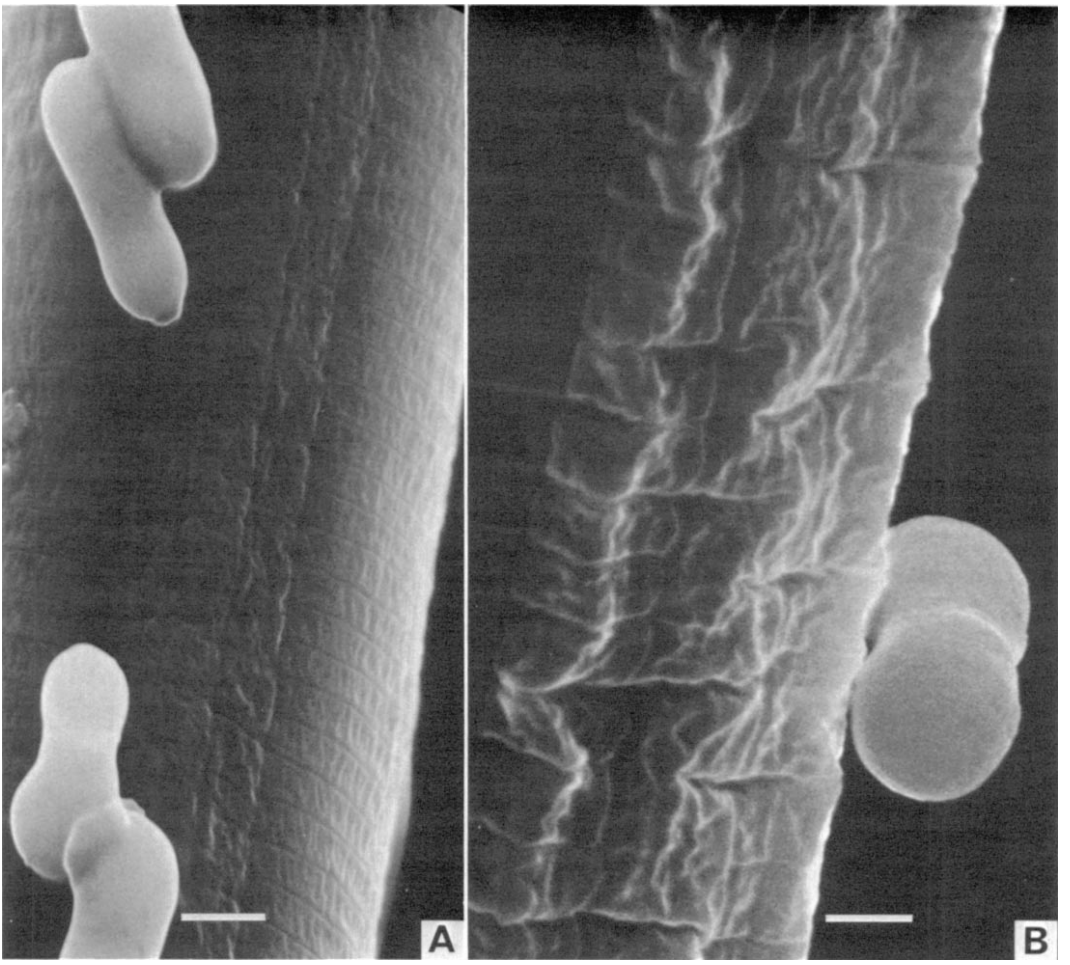


FIG. 2. *Gnomonia comari* conidiospores, in clumps (A) or singly (B), adhering to the cuticle of *Pratylenchus penetrans* near the lateral line. Bar is 2 μm in A, 1 μm in B.

ile sand. The greenhouse plants growing in nematode-infested soil were stunted, but there was no necrosis on the foliage and no root symptoms developed in plants inoculated with nematodes and fungus in combination.

*Pratylenchus penetrans as a carrier:* Of the nematodes that migrated down the BEEM tubes through the sand mixed with fungal mycelium and spores, 22% were carrying one or more conidia attached to the cuticle (Fig. 2A, B). Nematodes that migrated in tubes without fungus did not carry any conidia. When 10 nematodes extracted from pots inoculated with fungus and nematodes were rinsed extensively and plated on PDA, half of the plates showed *G. comari* colonies after 14 days incubation. All young strawberry plants inoculated with 80 *P. penetrans* extracted from pots with plants previously inoculated with fungus and nematodes became infected by the nematodes and their roots were colonized by *G. comari.*

## DISCUSSION

*Pratylenchus penetrans* is known for its role as an incitant in several fungal diseases such as verticillium wilt in potato (4) or alfalfa (20). In our experiments, when *P. penetrans* and *G. comari* were present in the roots of plants grown in sterile sand, *G. comari* developed perithecia in the root tissue and was extremely pathogenic. Factors that

could cause such a drastic change of response in the plant are not known. This response may be analogous to that observed by Powell (13) in tobacco plants, where several nonpathogenic fungal soil inhabitants could infect and damage roots previously inoculated with *Meloidogyne incognita.*

There are few reports of nematodes acting as carriers of spores of plant-parasitic fungi (1,9). There are, however, numerous examples of nematophagous fungi whose spores adhere to their hosts before infection. *G. comari* did infect strawberry roots without the presence of the nematode. Clearly, *P. penetrans* is not essential to the fungal infection process. Results suggest, however, that conidiospores of *G. comari* adhered to the cuticle of *P. penetrans* and could be transported through sand to the root surface, or into the roots, in the same manner that the nematode picked up spores in the sand of the BEEM tubes and transported them through the sand and the 20-μm-pore sieve. To our knowledge this is the first report showing *P. penetrans* carrying fungal spores. The role of endoparasitic nematodes as vectors of small fungal conidia may be more important than the current literature suggests.

*Gnomonia comari* has been isolated infrequently from the roots and crowns of healthy plants of several cultivars in field surveys (6,13). It is usually a foliar pathogen, however, and was not reported in other surveys (8,12,18). We hypothesized that *P. penetrans* or other root pathogens may be involved in the expression of *G. comari* pathogenicity to roots and crowns of strawberry. In our experiments with sterilized sand, the fungus inoculated alone near the crown of the plants developed in the roots of young strawberry plants but did not develop perithecia and was not pathogenic.

Klinkenberg (10) also isolated many fungi from strawberry plants growing in "root-rot" soil. While several of these fungi infected sterile seedlings in tube culture, they did not infect strawberry plants grown in pasteurized or unpasteurized soil. Another report (19) mentions that small runner strawberry plants transferred to soil heavily inoculated with the fungus *Gnomonia fructicola* (a synonym of *G. comari*) grew well and remained symptomless. The authors noted that root inoculations were unsuccessful. We suggest that *G. comari* may be a weak competitor with the natural flora in soil, on the root surface, or inside of roots. It can show severe pathogenesis when associated with *P. penetrans,* but only in the absence of inhibitory or competitive micro-organisms.

## Literature Cited

1. Bird, A. F., and A. McKay. 1987. Adhesion of conidia of the fungus *Dilophospora alopecuri* to the cuticle of the nematode *Anguina agrostis,* the vector in annual ryegrass toxicity. International Journal of Parasitology 17:1239–1247.

2. Bolay, A. 1971. Contribution a la connaissance de *Gnomonia comari* Karsten (syn. *G. fructicola* [Armand] Fall). Etude taxonomique phytopathologique et recherches sur sa croissance in vitro. Bulletin de la Societe Botanique Suisse 81:398–482.

3. Bolton, A. T. 1954. *Gnomonia fructicola* on strawberry. Canadian Journal of Botany 32:172–181.

4. Burpee, L. L., and J. R. Bloom. 1978. Influence of *Pratylenchus penetrans* on the incidence and severity of Verticillium wilt of potato. Journal of Nematology 10:95–99.

5. Chen Tseh-An, and A. E. Rich. 1962. The role of *Pratylenchus penetrans* in the development of strawberry black root rot. Plant Disease Reporter 46:839–843.

6. Gourley, C. O. 1969. Microfungi of crowns and roots of apparently healthy dormant strawberry roots. Canadian Journal of Botany 47:945–949.

7. Hastings, R. J., and J. E. Bosher. 1938. A study of the pathogenicity of the meadow nematode and associated fungus *Cylindrocarpon radicicola.* Canadian Journal of Research 14:11–26.

8. Hildebrand, A. A. 1934. Recent observations on strawberry root rot in the Niagara peninsula. Canadian Journal of Research 11:18–31.

9. Jensen, H. J. 1967. Do saprozoic nematodes have a significant role in epidemiology of plant diseases? Plant Disease Reporter 51:98–102.

10. Klinkenberg, C. H. 1955. Nematode diseases of strawberries in the Netherlands. Plant Disease Reporter 39:603–606.

11. McKinley, R. T., and P. W. Talboys. 1979. Effects of *Pratylenchus penetrans* on development of strawberry wilt caused by *Verticillium dahliae.* Annals of Applied Biology 92:347–357.

12. Nemec, S. 1970. Fungi associated with strawberry root rot in Illinois. Mycopathologie et Mycologia Applicata 41:331–346.

13. Parikka, P. 1981. Strawberry root rot in Finland. Annales Agriculturae Fenniae 20:192–197.

14. Powell, N. T. 1979. Internal synergisms among

organisms inducing disease. Pp. 113–133 *in* J. G. Horsfall and E. B. Cowling, eds. Plant Disease, vol. 4. New York: Academic Press.

15. Seemuller, E. 1969. Uber das Auftreten von *Gnomonia fructicola* (Arnaud) Fall als Erreger einer Fruchtfaule der Erdbeere. Nachrichtenblatt fur den deutschen pflanzenschutzdienst. Stuttgart 21:35–37.

16. Szczygiel, A., and H. Profic-Alwasiak. 1986. Interaction of *Pratylenchus penetrans* and *Cylindrocarpon destructans* on strawberries. Zeszyty Problemowe Postepow Nauk Rolniczych 323:109–114.

17. Townshend, J. L. 1963. The pathogenicity of *Pratylenchus penetrans* to celery. Canadian Journal of Plant Science 43:70–74.

18. Truscott, J. H. L. 1934. Fungus root rots of strawberry, Canadian Journal of Research 11:1–17.

19. Van Adrichem, M. C. J., and J. E. Bosher. 1958. Leaf blotch and petiole blight of strawberry caused by *Gnomonia fructicola.* Plant Disease Reporter 42:772–775.

20. Vrain, T. C. 1987. Effect of *Ditylenchus dipsaci* and *Pratylenchus penetrans* on Verticillium wilt of alfalfa. Journal of Nematology 19:379–383.