Mode of Entry by Pythium perniciosum into Strawberry Roots

S. Nemec

Research Plant Pathologist, Plant Science Research Division, ARS, USDA, Carbondale, Illinois 62901. Cooperative Investigations, Plant Science Research Division, ARS, USDA; and Plant Industries Department, School of Agriculture, Southern Illinois University, Carbondale, Illinois.

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ABSTRACT

Pythium perniciosum zoospores were produced in the presence of excised and intact Blakemore strawberry roots. Zoospores typically accumulated on the root in the region of elongation. Zoospore germ tubes penetrated root hairs and the root epidermal surface. Hyphae that grew into water around excised and intact roots penetrated root hairs and epidermal cells on the root surface. Phytopathology 61:711-714.

Additional key words: Fragaria chiloensis var. *ananassa*, root rots.

Middleton (9) reported that *Pythium perniciosum* Serbinow originally was described as a parasite of tobacco seedlings in Russia. Infrequent reports of *P. perniciosum* causing seedling decay and root rots have since appeared in the literature (10, 16, 17). Tompkins & Middleton (15) found it was one of several fungi associated with a root and stem disease of *Euphorbia pulcherrima*. *Pythium perniciosum* was frequently isolated from necrotic strawberry roots from plantings in southern Illinois (12). Penetration of *P. perniciosum* into main adventitious Blakemore strawberry roots and its resultant effects on root health were studied in these tests.

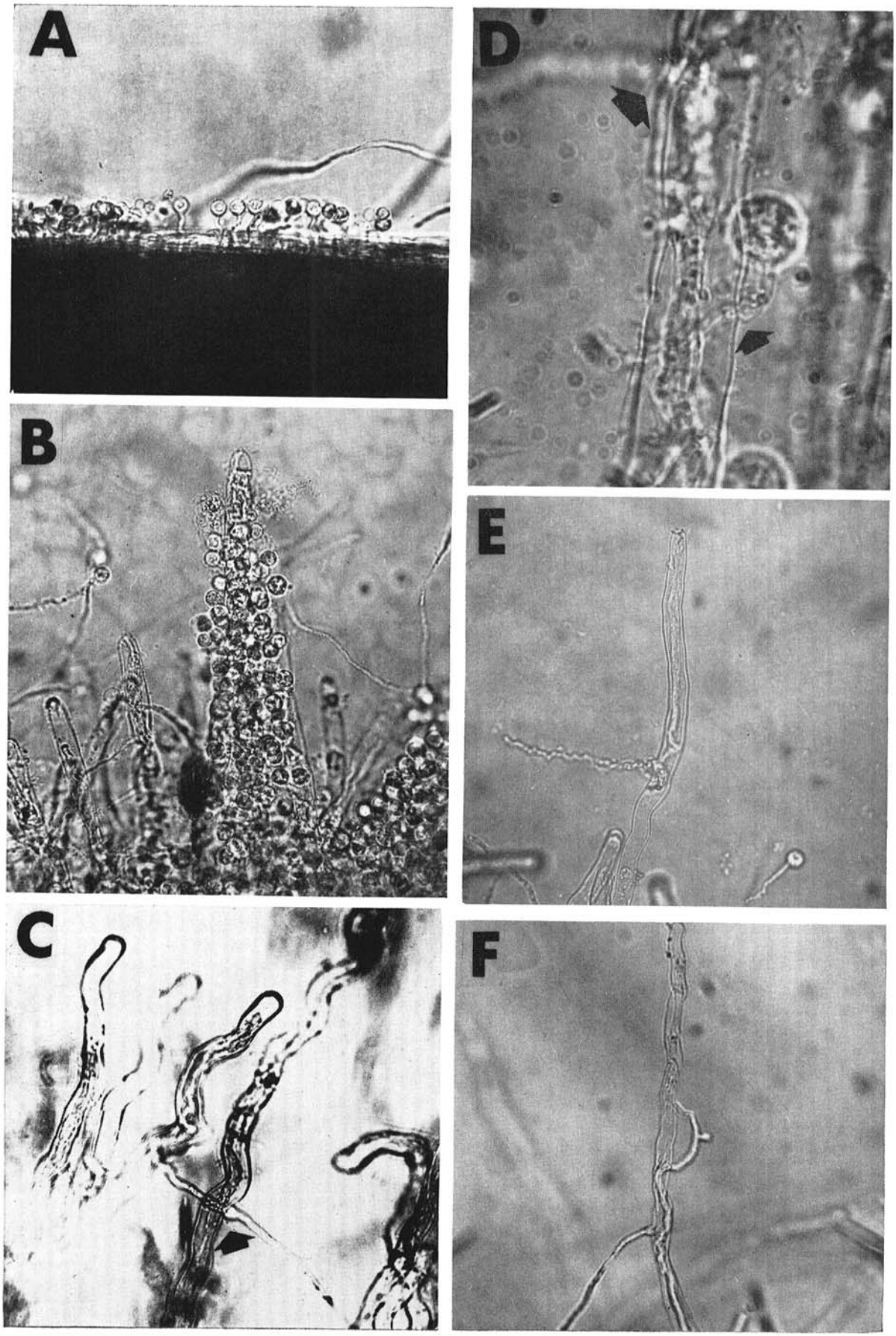
MATERIALS AND METHODS.—The isolate used originally came from diseased roots of the Surecrop cultivar. Runner nodes of the Blakemore cultivar were rooted in containers of water on a greenhouse bench. Excised and intact roots 5 to 8 cm long were placed on *P. perniciosum* colonies growing on an infusion from 20 g of hempseed in 2% agar or water agar in 100 × 15 mm polystyrene petri plates. Following the addition of sufficient sterile tap water to cover the roots, the plates were incubated at temperatures selected for each study. Initial response of zoospores to roots was determined by placing excised roots in zoospore suspensions. Other studies of fungus activity on and in the root, and root condition, were observed after 3 to 5 days. Control plates consisted of roots placed on water agar without *P. perniciosum*.

RESULTS.—As a preliminary experiment to the study of zoospore behavior, a method to produce zoospores was devised. Zoospores did not develop on hempseed and water agar plates when they were flooded with 10 ml sterile tap water and incubated for 1 week at 2, 7, 13, 21, and 31 C. Numerous zoospores appeared on colonies of cultures within 48 hr after intact or excised roots were added and covered with water. The number of zoospores produced at each of these five temperatures in the presence of roots was determined on three plates of water agar, each containing three excised roots of uniform length incubated at each temperature for 48 hr. Zoospores developing in each of the three plates were pooled in 80 ml of water for this determination. This test was repeated 3 times, and an average number of zoospores per ml determined for each temperature. Counts were made with a hemacytometer. At 2, 7, 13, 21, and 31 C, average zoospore counts/ml (80-ml sample) were 1,675, 2,911, 8,273, and 18,450, respectively.

At 21 C, zoospores were attracted to the region of elongation of freshly excised roots within the first min of exposure. Movement of zoospores in this area was random, with frequent root contact occurring before the encystment process was initiated. After a 1-hr exposure, some zoospores had encysted along the root epidermis. Zoospore germ tube growth occurred often toward the root at the region of elongation (Fig. 1-A). Zoospores did not appear to be attracted to other regions of the root; however, zoospores moved among the root hairs and along the epidermis. In one test (13 C), large numbers of zoospores accumulated on the root in the region of elongation and maturation by the end of 48 hr. Masses of zoospores encysted on the surface of root hairs (Fig. 1-B).

Zoospores were also attracted to cut areas of the root. When 12-μ-thick cross and longitudinal sections were excised near the crown and placed in zoospore suspensions, zoospores swam to the sections. Zoospores in this case appeared to be attracted more to the stele and inner cortex than to the outer cortex or epidermis.

Excised and intact roots were incubated for 72 hr at 7, 21, and 31 C on 4-day-old water agar cultures of *P. perniciosum*. Three roots were placed in each of three plates for each temperature. At the end of this period, zoospores were present in the water, and mycelial growth had developed in the water and along the root. Growth of many hyphae near the root was directed toward the root. Observation of root hairs, up to 3 cm from the root tip, indicated that many had become infected. More root hairs appeared to be infected at 7 than at 21 and 31 C. Most root hairs were infected near the base and midway along the sides. Zoospore germ tubes grew among the root hairs and were seen penetrating them, but most root hair infections appeared to be initiated by hyphae from the surface of the agar colony. Hyphal growth and branching was extensive along the sides of the root, and hyphae penetrated epidermal cells as well as root hairs. A slight swelling developed in the hyphae at the site of penetration (Fig. 1-C, D). Hyphae which had penetrated root hairs grew toward the root, toward the root hair tip (Fig. 1-E), or sometimes branched and grew in both


A
B
C
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E
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Fig. 1. *Pythium perniciosum* on Blakemore strawberry roots. **A)** Zoospores germinating on a root in region of elongation. **B)** Zoospore accumulation on a root hair. **C)** Appressorium formed at point of root hair penetration (arrow). **D)** Swelling of zoospore germ tube at site of root hair penetration (small arrow); hair contains hypha from previous infection (large arrow). **E)** Hyphal growth in root hair towards the tip. **F)** Hyphal growth in root hair toward the base and tip.

directions (Fig. 1-F). Other hyphal branching resulted in growth of hyphae out of the root hair through the side or tip of the root hair. No oospores, sporangia, or hyphal enlargements were seen within hairs.

Roots placed in control petri plates were free of *Pythium* infection. After 3 to 5 days' incubation at 21 C, they appeared white and healthy. Inoculated roots held at this temperature for the same period of time were typically brown 1 to 2 cm behind the tip. Some necrosis was observed on the cut ends of excised roots in both control and inoculated petri plates. The fungus had penetrated into the cortex within 48 hr after inoculation.

DISCUSSION.—*Pythium perniciosum* recently was isolated from necrotic strawberry root tissue of plants growing in eight southern Illinois plantings (12). Because this species is suspected of causing damage to plants, its behavior on excised and intact roots was observed in petri plates. Results of microscopic observations showed that root penetration was direct by hyphae penetrating root hairs and epidermal cells on the root surface, and by germinating zoospores on the root surface and zoospore germ tube penetration of hairs.

Zoospores typically accumulated on the root in the region of elongation, but in one test, zoospores were attracted all along the surfaces of 3- to 6-cm-long excised roots. On these roots, encysted zoospores frequently accumulated on hairs. This zoospore accumulation pattern on strawberry roots is similar to the formation of a sheath of *Phytophthora megasperma* var. *sojae* zoospores on soybean roots (4), although the soybean root apex and hairs were usually devoid of cysts.

Zoospore penetration of root hairs, by biflagellate Phycomycetes, is reported for *Pythium aphanidermatum* (5) and *Aphanomyces euteiches* (2); but they are much less attractive to zoospores than is the zone of elongation (13). Cunningham & Hagedorn (1) reported that zoospores of *A. euteiches* did not accumulate around root hairs. *Phytophthora fragariae* zoospores encysted in the root-hair zone of strawberry, germ tube origin and growth was random, and penetration of root hairs did not occur (3). Root hair penetration by zoospore germ tubes, even though not often reported, nor even a selective phenomenon, may serve as an important avenue for entry into areas of the root which may have greater root surface resistance to pathogen invasion.

Possibly unique among the means by which the fungus penetrated the root was hyphal invasion of root hairs. These hyphae grew into the water around the root from the surface of the agar. Root epidermal surface penetration by hyphae has been reported for *Phytophthora* (6) and *Pythium* (8, 11, 14); but additional evidence for root hair penetration by hyphae of *Aphanomyces, Pythium,* and *Phytophthora* has not been found. Results with other fungus parasites, however, indicate that root hair invasion is a major means of entry. Mathre et al. (7) observed *Thielaviopsis basicola* directly penetrating root hairs of cotton seedlings. More recently, Williamson & Hadley (18) showed that individual hyphae of *Rhizoctonia* isolates penetrated root hairs of protocorms of the orchid, *Dactylorhize purpurella.*

Environmental conditions that favor sporangium and zoospore production, such as periodic water-saturation of soils, may also favor hyphal activity. Actively growing hyphae should be considered potentially infectious to the root surface and hairs. *Pythium perniciosum,* when it is present in strawberry plantings, could be expected to contribute to the development of symptoms commonly described as root rot.

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