Phytophthora Foot Rot of Black Pepper in Brazil and Puerto Rico

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ABSTRACT

Foot rot epidemics of black pepper (*Piper nigrum*) caused by *Phytophthora palmivora* are usually observed when plants are of bearing age (2 or more years old). Younger plants also are susceptible, and may collapse rapidly if inoculum concentrations are high. In areas new to black pepper cultivation, the amount of inoculum in the soil probably is low. Consequently, development of root infections into severe foot rots at the soil line occurs slowly. An increase in number and mobility of infective zoospores results in increased infection, rapid deterioration of roots, and collapse of the infected plants. Several commercial varieties are equally susceptible. Grafts on disease-resistant *Piper colubrinum* deteriorate after the 4th year.

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Foot rot (collar rot, root rot) in commercial plantings of black pepper may reach epiphytotic proportions (1, 3, 9). It is the most important disease of black pepper, and has resulted in the discontinuation of the crop on a commercial basis, both in Puerto Rico and in other countries (2, 4, 5, 6, 7, 8, 9, 10, 11). Wilting and rapid defoliation generally are the first symptoms of the disease. Death of the plant soon follows. Infection usually occurs through the root system, but direct infection of the leaves, flowers, and fruits also occurs. A distinct black rot of the collar region is usually clearly delimited from the healthy tissues by an advancing margin of infection. Infection of leaves results in circular, uniformly dark-brown fimbriate lesions which then spread and cause collapse of the affected branch.

A striking feature of the disease is its apparent absence in young plants. Plants of fruit-bearing age, 2 years or older, however, may decline rapidly, and within 1 year the entire planting may be destroyed. This rapid spread may be aided by clean-weeding where the cultivator may spread the pathogen, and where the bare earth allows rapid movement of contaminated debris and water (3).

Holliday & Mowat (3) have reported a detailed account of their investigations of the disease in Sarawak. They, as well as most other investigators, have implicated *Phytophthora palmivora* (Butler) Butler, or an undetermined species of *Phytophthora* as the pathogen. In Brazil, *Fusarium solani* f. *piperi* Albuquerque has been consistently isolated from diseased tissues (1). Isolates of this fungus have caused typical foot rot symptoms of disease in experimental black pepper plants.

We report here some observations on the development of *Phytophthora palmivora* in diseased black pepper (*Piper nigrum* L.) plants, and the use of *Piper colubrinum* L. as a rootstock for the control of the disease in field plantings. Field and laboratory observations were made at the Instituto de Pesquisas e Experimentacao Agropequarias do Norte in Belém, Brazil, and at the Federal Experiment Station, Mayaguez, Puerto Rico.

MATERIALS AND METHODS.—*Root infection.*—One-node cuttings of black pepper introductions Kudravali (P.I. 212965), Kalluvali (P.I. 212641), Kotanandan (P.I. 212962), and Uthirancotta (P.I. 213297) were rooted in vermiculite, then transplanted 4 months later to 4-inch clay pots filled with steamed soil that was infested the same day with *Phytophthora palmivora* isolate P7 or P20. The isolates were obtained from diseased black pepper plants in Puerto Rico, and proved pathogenic in previous trials. The inoculum was mixed into the soil at 2 and 4% by weight as a 4-week-old cornmeal-sand culture of the fungus. Each introduction was represented by 10 plants in soil infested with each isolate, and by 10 plants in noninfested soil. The pots each contained a single plant, and were placed randomly on a greenhouse bench in each of two trials.

The shoots were observed for disease symptoms. At 4 months, roots were washed and rated for disease. Isolations were made from diseased tissues and from soil near roots in infested soils. Cornmeal agar, consisting of 100 ppm mycostatin, 50 ppm penicillin sulfate, and 50 ppm polymixin, was used for isolation.

Rooted cuttings of the introductions also were planted in a garden of the Federal Experiment Station, where black pepper had been severely attacked by the pathogen, *P. palmivora,* in previous years. Ten plants of each introduction were planted in rows 4 ft apart. Six months later, the roots were rated for disease, and isolations of the pathogen were attempted.

One-node cuttings of black pepper introductions Kalluvali (P.I. 212641), Kudravali (P.I. 212965), Kal-balamcotta (P.I. 214301), Bangha (P.I. 240828), Accession No. 14198, and *P. colubrinum* were rooted in tap water in 125-ml flasks, then placed in 20-ml beakers (two plants/beaker) filled with a water suspension of zoospores (ca. 260,000 zoospores/ml). Four plants of each introduction which were handled similarly but not exposed to zoospores served as controls. Zoospores were produced by flooding 1-week-old cultures of the P7 isolate with sterile

distilled water at 10 C. The fungus had been grown at 26 C in cholesterol medium (5.4 g glucose, 1.5 g $NaNO_3$, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 2.0 mg thiamine-HCl, 5.0 mg cholesterol, 17.0 g agar, and 1,000 ml distilled water) under light.

Root samples of Kal-balamcotta plants were taken 6, 9, 12, 24, 48, and 96 hr after their immersion in the zoospore suspension. Infection and development of the fungus in roots were studied histologically from sections made of paraffin-embedded tissues stained with safranin-fast green.

Root systems of eight 4-year-old black pepper plants were washed and observed for infection. These plants were taken from the vicinity of wilted plants, but were free of foliar symptoms. Isolations of the pathogen were attempted.

Stem infection.—Cuttings of the same black pepper introductions and *P. colubrinum* which were used for root-infection tests were rooted in vermiculite and planted in steamed soil in 4-inch clay pots. Their stems were wounded slightly with a sterile razor blade ca. 10 cm from the soil line, and a small piece of mycelium of P7 was introduced into the wound. Three inoculated, and two noninoculated, plants/introduction were tested in each of two trials. Samples of the inoculated stems were taken for histological examination. Stem inoculations also were made on cuttings rooted in water. These inoculations were made concurrently with those that involved zoospore suspensions.

Leaf infection.—Five-cm^2 sections were cut from the center of the leaf blades of mature or young leaves of black pepper introductions Kalluvali (P.I. 213292), P.I. 214301, Balamcotta (P.I. 205364), Accession No. 14198, and *P. colubrinum*. The sections were wounded slightly in the center with a sterile needle, then placed in separate petri plates. A plug of a cholesterol-agar culture of P7 was obtained with a sterile No. 1 cork-borer and placed on the wound. Leaf sections were incubated at room temperature (26-28 C). Leaves wounded but not inoculated served as controls. Measurements of lesion size were made 24, 48, 72, and 96 hr after inoculation.

Field tests for resistance and graft compatibility of Piper colubrinum.—The performance of black pepper clones Balamcotta (P.I. 205364) and Kal-balamcotta (P.I. 214301) grown on *P. columbrinum* as rootstock, and nongrafted plants of the same introduction, were determined in two experimental field plantings in Puerto Rico. The plants were kept in the greenhouse for 5 months after wedge-grafting them, and then were placed beside concrete posts or bucare (*Erythrina berteroana* Urban) under full sunlight. Seven plants of each introduction for each treatment in each of two experiments were arranged in a randomized split block design. The plants were placed in soil known to be heavily and naturally infested with the pathogen.

Piper colubrinum was tested in Brazil for its resistance to *Phytophthora palmivora* (isolate PBr) and *Fusarium solani* f. *piperi*. Young green branches were wounded, and mycelium of either fungus was introduced into a wound. Other *P. colubrinum* plants whose branches were wounded but not inoculated were used as controls. In addition, *P. colubrinum* was planted in clay pots that contained soil infested with fungus cultures (40 g/ca. 5 kg soil) of *F. solani* f. *piperi* (12-day-old culture grown on sterilized wheat stems) or *Phytophthora palmivora* (12-day-old culture grown on a sand-dextrose-sorghum meal medium). *Piper nigrum* 'Singapura' was also tested in the same manner for its resistance to both pathogens.

The graft compatibility of *P. nigrum* 'Singapura' and *P. colubrinum* was tested in Brazil. One month after the grafting of Singapura onto *P. colubrinum* root rootstock in the greenhouse, the plants were taken to a naturally infested field under full sunlight. Ten grafted plants were placed in the field, and observations were made on their growth and yield. The same number of nongrafted plants in infested soil was placed in the field after a 10-day period in clay pots.

RESULTS.—*Root infection.*—No foliar symptoms of foot rot were observed in plants growing in infested soil during the 4 to 6 months of observation. A few plants in the greenhouse and in the garden died soon after they were transplanted, but these did not show the typical symptoms of foot rot, and were discarded from the experiment. Other plants showed a slight necrosis of root tips and branches. The damage was not serious in most plants, and had not developed into the stem near the soil line. Root decay was more serious in a few plants in the garden, but in all these, insect larvae were attacking the roots.

Isolations of the pathogen were easily made from root samples from infested soil in the greenhouse. The pathogen was also isolated directly from soil close to the roots. Isolation from plants grown in the garden was not so successful (3 roots of 22). *Phytophthora palmivora* was not obtained from noninfested soil. There were no differences in degree of root infection among the black pepper introductions.

Roots of the eight 4-year-old black pepper plants had typical black lesions, but the plants were free of foliar symptoms. Infection was well developed, and large lesions could be observed in the main root branches. However, the collar (foot) region at the soil line was still healthy in all of them. A *Phytophthora* was isolated from the diseased tissues and proved pathogenic to young black pepper cuttings.

A number of 4-year-old plants which appeared healthy at the time of sampling in 1967 subsequently wilted, and collapsed 1 year later. Their root systems (including the collar region) were then heavily decayed, and the pathogen was isolated from them.

Infection of roots by zoospores was first detected by superficial observations of roots in beakers 24-48 hr after immersion of the roots. Root tips darkened first, and infection appeared to develop acropetally. The roots were completely decayed, and shoots wilted within 4 days. Evidence of infection was apparent in root samples of Kal-balamcotta taken 6 hr after immersion of the roots. In some cases, the fungus was well established in the cortex within 9 hr.

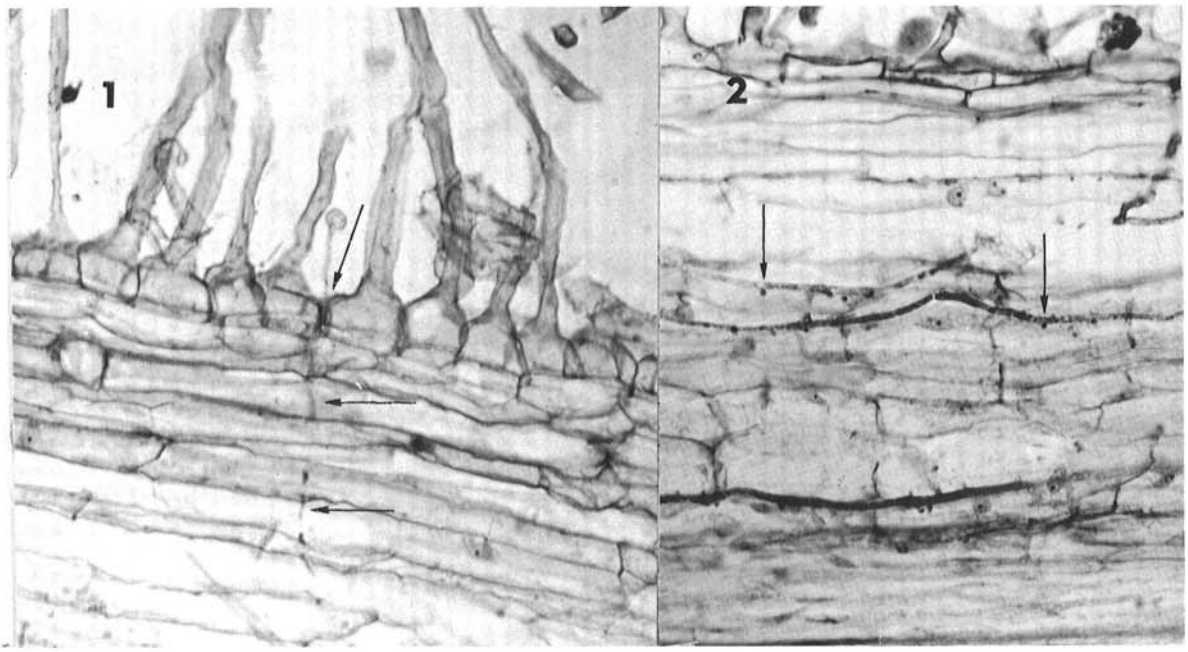

Fig. 1-2.1) Black pepper root infection after 6 hr in a water suspension of zoospores. Arrows indicate initial intercellular infection and intracellular development in the cortex. **2)** Root infection after 9 hr of immersion in a water suspension of zoospores. Note haustorialike projections into host cells (arrows).

Zoospores usually effected penetration of the epidermis between two cells (Fig. 1). Growth of the fungus was then intracellular. One zoospore infected at least 10 cells within 6 hr of root immersion, without apparent branching (Fig. 1). In other cases, the fungus initially developed intercellularly, and subsequently produced haustorialike projections into the root cells (Fig. 2, see arrows). These bulbous short projections of the intercellular hyphae were not seen in later stages of infection where the fungus had grown more deeply into the cells. At 9 hr after root immersion, the pathogen was well established in the cortex of the root within 2 cm of the root tip, but tissues at this time were not obviously macerated. After 12 hr, maceration of tissues was readily observed macroscopically. In these darkened and soft tissues the fungus was not easily seen; however, many of the hyphae observed were devoid of protoplasm.

Stem infection.—Inoculation by wounding the stems of rooted cuttings planted in soil or in water resulted in rapid decay but did not produce the wilt symptoms observed in field infections. Wounded inoculated stems darkened within 12-24 hr, and sometimes the leaves became infected before appreciable wilt was detected. Leaves often abscissed soon after the stem next to the petiole became darkened.

The fungus was isolated readily from the darkened tissues, but not from the healthy green tissues next to them. A fairly distinct margin was observed histologically between the healthy and diseased tissue, and the fungus was usually best observed near this margin. No apparent differences in resistance among the black pepper introductions were determined by the inoculation methods employed. Although immature leaves were generally more susceptible to infection than were mature leaves, all the black pepper introductions tested proved highly susceptible.

Resistance of P. colubrinum to infection by Phytophthora palmivora and F. solani f. piperi, and graft compatibility with P. nigrum.—*Piper colubrinum* was highly resistant to infection by *Phytophthora palmivora* isolate P7 in Puerto Rico, and to isolate PBr in Brazil. Shoot infection was limited essentially to the wounded portion, and roots immersed in zoospore suspensions remained healthy in all tests.

In field tests made in Brazil, *P. colubrinum* remained healthy, whereas *P. nigrum* 'Singapura' showed disease symptoms 15 to 30 days after planting. *Fusarium solani* f. *piperi* infections were limited to the wounded portion of the shoot. *Piper colubrinum* became diseased in soil heavily infested with *F. solani* f. *piperi*.

Graft compatibility to Balamcotta, Kalluvali, and Singapura was good for the first 4 years. In Brazil, Singapura grafts fruited 1 year after grafting. A 1-year-old plant may produce 500 g black pepper, and a 2- or 3-year-old plant, 2 to 3 kg (Fig. 3). Most of the grafted Singapura died within 4 years of planting. The plants developed longitudinal cracks in the grafted portion 8 to 12 months before showing any wilting (Fig. 4). The grafted portion eventually rotted. In Puerto Rico, grafted Balamcotta and Kalluvali developed much better than nongrafted plants, especially when provided with concrete posts.

Fig. 3-4. 3) Black pepper (Singapura variety) grafted to *Piper colubrinum*, 3.5 years old in Brazil. **4)** Longitudinal cracks in grafted black pepper (Singapura variety) in Brazil.

Mortality in nongrafted plants was twice as high as in grafted plants during the 3 years of observation.

DISCUSSION.–Unusually high amounts of inoculum generally resulted in rapid development of disease in the black pepper introductions tested. Roots became infected within 6 hr of inoculation with zoospores, and collapsed within a few days. Stems also became decayed shortly after inoculation. The inoculation methods employed may be useful for gross tests for resistance in black pepper varieties, but they do not simulate field conditions.

In the field, root infection may occur soon after contact is made with the pathogen in soils containing low concentrations of inoculum. The rate of spread of the pathogen may then be determined mainly by the number of infections in susceptible tissues. Our experience in artificially infested soils suggests that rate of spread may be quite slow in young plantings. During the 4 months of observation, none of the plants showed foliar symptoms. The fungus, however, had attacked a number of roots, and the pathogen was detectable from soil isolations. In garden soil, where the distribution of the pathogen may not be uniform and infective propagules are low in number, infection was even less evident.

Holliday & Mowat (3) reported that fruit-bearing plants were somewhat more susceptible than were younger plants; the incubation time before the appearance of aboveground symptoms was 29 and 77 days for 1- and 5-year-old plants, respectively. They also found that pathogen penetration of the stem from roots, and shoot collapse, occurred with the greatest frequency in plants treated with large amounts of inoculum.

Plants which appear healthy may have an extensive root infection. The onset of foliar symptoms in the field seems to be associated mainly with a strangulation of the collar region, except in cases where there is a heavy infection through the leaves. The period from initial infection to foliar wilt may vary in proportion to the number of root infections, providing other factors are favorable for disease.

Very little is known about the biology of the fungus in soils where black pepper is planted. Holliday & Mowat (3) believed that the fungus was usually introduced into new plantings from outside the garden, as initial outbreaks were associated with paths and entrances. It is probable that in Puerto Rico the pathogen was already established in soils used later for commercial plantings of black pepper. The fungus which attacks black pepper in Puerto Rico can also infect other plants known to be susceptible to that species and, therefore, apparently is not specific to black pepper. The possibility that infected cuttings were introduced in Puerto Rico and used for propagation seems unlikely.

We were not able to detect any resistance to the disease in the black pepper introductions. Uthirancotta, which was reported as resistant (3), was very susceptible in our tests. The use of *P. colubrinum* as a disease-resistant rootstock cannot be recommended for commercial plantings of black pepper grown under full sunlight. Additional tests are necessary to determine if this species will perform better when grown under partial shade. Our experiments suggest, however, that *P. colubrinum* is not entirely graft-compatible with *P. nigrum*.

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