Yield Loss in Hydroponically Grown Lettuce Attributed to Subclinical Infection of Feeder Rootlets by *Pythium dissotocum*

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ABSTRACT

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Pythium dissotocum, reported for the first time as a root pathogen of hydroponically grown lettuce, was responsible for significant yield reductions (35–54 and 12–17% reductions at 18 and 28 C, respectively) in the absence of visible root or foliar symptoms. The fungus was isolated from 92% of the rootlets assayed and occupied about 75% of the total root length assayed. Microscopic examination of infected roots revealed haustorialike fungal structures within healthy-appearing epidermal cells. *P. dissotocum*, in addition to *P. uncinulatum*, *P. irregulare*, *P. sylvaticum*, *P. violae*, *P. catenulatum*, and *P. rostratum*, was also consistently isolated from healthy-appearing feeder rootlets collected from field-grown head lettuce plants.

Significant yield reductions in numerous commercial crops have been attributed to fungal root pathogens that cause distinct root rot symptoms and plant death. Crop yields have been increased dramatically after control of these major root pathogens. Yet, maximum yield potential is seldom achieved even where major root diseases are absent. Soil sterilization or fumigation (2,12,13,18) commonly results in substantial increases in plant growth and yield. Such increases have been attributed, in part, to the elimination of root-invading fungi, called "minor root pathogens," which are commonly isolated from feeder rootlets showing no distinct root disease symptoms (13). Yield losses caused by these subclinical diseases often go undetected because all plants in a field, though healthy or normal in appearance, may be similarly affected.

Various species of the genus *Pythium*, in addition to their documented role as seedling pathogens and their reported role in decline diseases characterized by extensive necrosis and a reduction in the feeder rootlet system (2,6,7,9,12,14), have been circumstantially implicated as a cause of these subclinical diseases (5,13,18). However, little or no quantitative data has been reported regarding the extent of rootlet infection, the location of the fungus within asymptomatic infected rootlet tissue, or the economic losses in specific crops incurred as a result of subclinical rootlet diseases.

As previously mentioned, various *Pythium* spp. are commonly isolated from necrotic rootlets (2,6,7,9,12,14). Whether *Pythium* is responsible for the necrosis or merely occupying naturally senescent root tissue has been questioned. For example, peach tree decline in Georgia was attributed to feeder root necrosis caused by *Pythium irregulare* and *P. vexans* (7). However, Mircetch (9) in California reported that there was no correlation between peach tree decline and the ubiquitous occurrence of numerous *Pythium* spp. in necrotic feeder rootlets, and he suggested that the association was saprophytic. The latter conclusion was recently supported by Nyczepir and Lewis (10) and Funck-Jensen and Hockenhull (4). Their studies showed that various species of *Pythium* were consistently isolated from necrotic as well as "apparently healthy" feeder rootlets. Thus, the association between *Pythium* spp. and feeder rootlets remains unclear.

We became interested in subclinical diseases caused by *Pythium* spp. in 1981, when commercial production of hydroponically grown spinach and lettuce was undertaken in a 0.5-ha greenhouse in Tucson, AZ. Within 3 mo of the initial planting, commercial production of spinach was abandoned because of severe root rot caused by *P. aphanidermatum* (Edson) Fitzp. and *P. dissotocum* Dreschs. (1). Lettuce plants growing in the same raceways with diseased spinach plants did not appear affected. However, we consistently isolated *P. dissotocum* from lettuce feeder rootlets, and although all rootlets were infected, they showed no recognizable shoot or root disease symptoms. Additionally, *P. dissotocum* has been consistently isolated from roots of hydroponically grown lettuce plants obtained from commercial greenhouses in California, Illinois, and Canada (M. E. Stanghellini, *unpublished*).

The objectives of our investigations were 1) to determine if yield reductions occurred as a consequence of asymptomatic infection of lettuce feeder rootlets by *P. dissotocum* and, if so, the effects of rootlet temperature on the magnitude of such yield reductions; 2) to provide quantitative data regarding the extent of rootlet colonization and the location of the fungus within asymptomatic feeder rootlets; and 3) to determine if various *Pythium* spp. were associated with asymptomatic rootlets of field-grown lettuce. A preliminary report has been published (16).

MATERIALS AND METHODS

Stock cultures of *P. dissotocum* originally isolated from asymptomatic lettuce feeder rootlets were maintained at 24 C on 10% V-8 juice agar (VJA) medium. All temperatures reported in this study, unless otherwise specified, refer to temperatures of the nutrient solution. Ambient temperatures in greenhouse studies ranged daily from 23 to 36 C (mean 28 C).

Pathogenicity tests at various temperatures. Pathogenicity tests were conducted in a greenhouse under hydroponic conditions. Four 2-wk-old leaf lettuce seedlings (*Lactuca sativa* cv. Salina), started in a nursery in peat pellets, were transferred into holes cut into each of 16 Styrofoam flotation boards (32 × 27.5 × 2 cm). Boards were then placed in 13.5-L plastic tubs (37.5 × 33 × 13.5 cm) containing a continuously aerated (300 cm^3/min/tub) nutrient solution. Tubs were placed in a temperature-controlled box (3), and the nutrient solution was equilibrated to desired temperatures before transplanting (Fig. 1). After transplanting, an intact 48-hr-old VJA petri dish (9 cm diameter) culture of *P. dissotocum* was added to each tub, and within 3 hr, zoospores were detected in the nutrient solution. The petri dish cultures were removed from the tubs after 24 hr. Plants in noninfested tubs served as controls. After 3 wk of growth in the tubs, fresh weights of shoots and roots were recorded for each treatment. All treatments were replicated four times, and the experiment was repeated four times at 18 C and twice at 28 C.

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Rootlet colonization. The extent of rootlet colonization and the location of the fungus within asymptomatic rootlets were determined as follows: The rootlet systems of an inoculated and uninoculated plant from each replicate were collected after 3 wk of growth and washed in running tap water for 3 min. A portion of each root system (six to seven root segments, each 4–6 cm long) was then blotted dry, placed on water agar, and incubated at 24 C for 24–36 hr. Roots were not surface-sterilized before isolation attempts because preliminary studies showed that treating infected roots for 1 min in either 70% ethyl alcohol or 0.5% sodium hypochlorite prevented the isolation of *P. dissotocum* (17). A second portion of each washed root system was stained in acid fuschsin and examined microscopically for the location of the fungus within asymptomatic rootlet tissue.

Field studies. The occurrence and prevalence of various *Pythium* spp. associated with asymptomatic feeder rootlets of field-grown head lettuce were determined as follows: A volume of soil (about 12×8×8 cm) containing the root systems of each of 12 healthy lettuce plants 1–2 mo old were collected from each of four commercial fields in Marana, AZ. Rootlets were carefully separated from soil by agitation in water and washed in running tap water for 10 min. Five 4-cm-long segments of healthy-appearing rootlets from each plant were plated on water agar and incubated as described. *Pythium* spp. growing from the rootlets were transferred to VJA, identified, and data collected on the percentage of rootlets infested with specific *Pythium* spp.

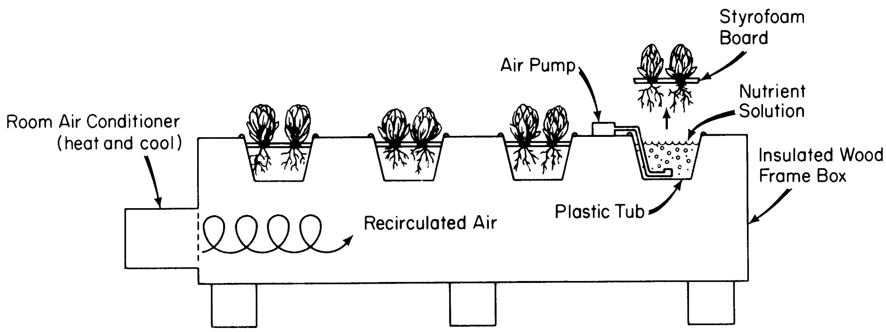

Fig. 1. Diagrammatic representation of a hydroponic cultural system employing air as a heat-exchange medium to regulate water temperature.

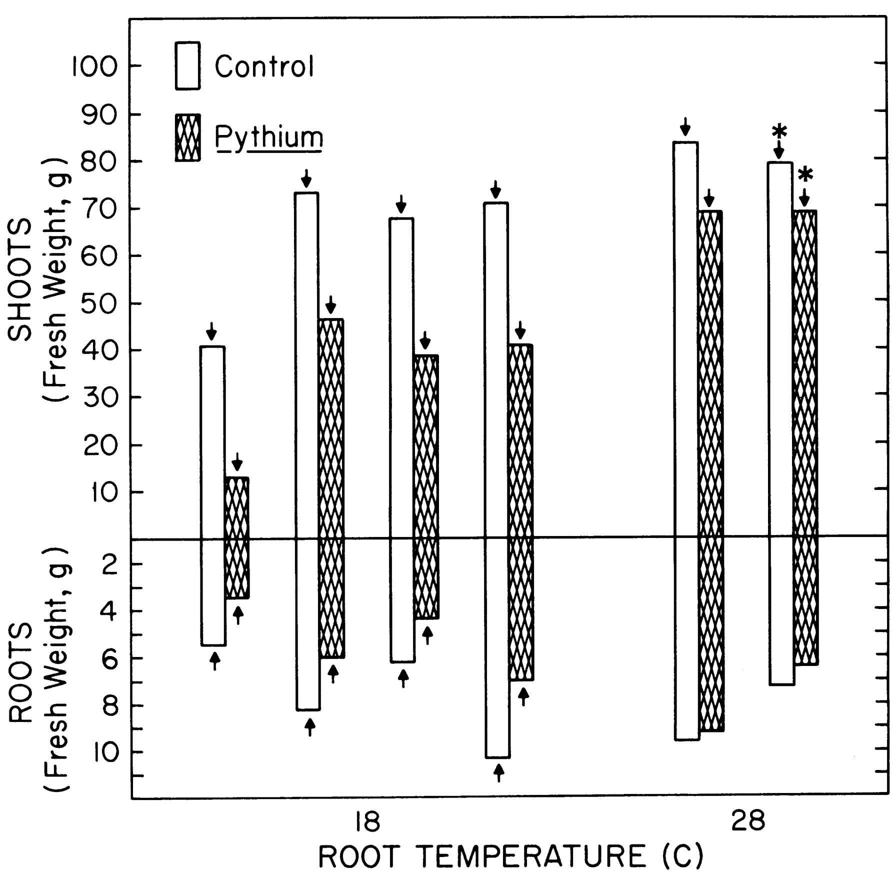

Fig. 2. Effect of root temperature and *Pythium dissotocum* on shoot and root weights of lettuce cultured hydroponically. There were four experiments at 18 C and two experiments at 28 C. Each experiment consisted of four plants per treatment, and each treatment was replicated four times. Data were analyzed by analysis of variance. Arrows indicate signficantly different mean weights between treatments at $P = 0.0001$, except * = $P = 0.05$.

RESULTS

Pathogenicity tests. Results of pathogenicity tests are presented in Figure 2. *P. dissotocum* caused significant reductions in the fresh weights of both roots and shoots at 18 C. Significant reductions in fresh weights of shoots but not roots also occurred at 28 C, but the extent of the reductions was less than at 18 C. Reductions in shoot and/or root weights occurred in the absence of visible root rot symptoms.

Root colonization. Isolations from asymptomatic roots from inoculated and uninoculated plants are presented in Table 1. *P. dissotocum* was isolated only from roots of inoculated plants. The fungus was recovered from 92% of the roots assayed and was shown to occupy about 75% of the total root length assayed. The extent of rootlet infection was similar in inoculated plants grown at 18 or 28 C. To verify the linear extent of root colonization and eliminate the possibility that secondary spread of the fungus within infected roots during the isolation period contributed to the overall extent of infestation, five 5-cm-long infected roots were cut into 2-mm-long segments and plated on water agar in sequential order. The fungus was recovered from 86% of these segments. Microscopic examination of infested roots revealed haustorialike structures in epidermal cells of inoculated roots (Fig. 3).

Field studies. *Pythium* spp. were consistently isolated from asymptomatic roots of all plants sampled from the four commercial lettuce fields at Marana, AZ. Colonization of roots from individual plants varied from 15 to 80%. *P. dissotocum* was isolated from segments of roots from all four fields and was the predominant species isolated (>50% of the isolates) from roots from two of the fields. *P. uncinulatum* Van der Plates-Niterink & Blok and *P. sylvaticum* Campbell & Hendrix were the predominant species isolated from roots in the other two lettuce fields. In addition to the above species, *P. irregulare* Buisman, *P. violae* Chesters & Hickman, *P. catenulatum* Matthews, and *P. rostratum* Butler were isolated from healthy-appearing roots.

Laboratory assessment of the potential pathogenic capabilities of the *Pythium* spp. isolated from asymptomatic roots was determined as follows: Lettuce seeds were sown on the surface of water agar in a 9-cm-diameter petri dish. A 5-mm-diameter plug of mycelium from a representative isolate of each *Pythium* sp. was placed in the center of the dish. Sown and inoculated dishes were then incubated at 25 C in illuminated (14 hr of light, 11,000 lux per day) growth chambers for 10 days. Uninoculated, seeded dishes served as controls. At the end of each experiment, plants were rated for disease severity. All experiments were repeated twice. All *Pythium* spp. tested grew across the petri dish within 4 days. Seed and/or seedling death occurred on plates inoculated with *P. irregulare* and *P. sylvaticum*, whereas only root tip necrosis and inhibition of lateral root formation occurred on plates inoculated with *P. dissotocum*, *P. uncinulatum*, and *P. violae*. *P. catenulatum* and *P. rostratum* had no obvious effect on lettuce.

DISCUSSION

Results of our studies showed that *P. dissotocum*, reported for the first time as a root pathogen on lettuce, was responsible, in the absence of any visible foliar symptoms, for significant reductions in yield (35–54 and 12–17% reductions at 18 and 28 C, respectively). The root systems of inoculated plants appeared healthy, although there was a significant reduction in fresh weights of roots from inoculated plants at 18 C but not at 28 C. A recent report by Hodges and Coleman (8), who found that *P. aristosporum* Vanterpool and *P. arrhenomanes* Dreschs. were responsible for significant reductions in shoot and root weights of *Agrostis palustris* Hud. in the absence of root rot symptoms, substantiates our findings, which demonstrate the insidious effects of subclinical root disease caused by *Pythium* and illustrate the importance of root temperature in the host-pathogen interaction. Our hydroponic system enabled us to study the host-pathogen interaction at temperature regimes more closely resembling those encountered under field conditions. Additionally, we could visually and quantitatively assess the entire rootlet system, a parameter difficult at best to measure under field conditions or in pot culture, where plants rapidly become root-bound.

Regarding the level of root infestation, isolations from inoculated plants revealed that about 92% of the rootlets were infected by the fungus and microscopic examination revealed haustorialike fungal structures within healthy-appearing epidermal cells of infected roots. The latter structures were present in almost all of the roots and were observed along the entire root length of individual roots. It should be pointed out that at the time of inoculation, transplanted seedlings had only one to three roots extending beyond the peat pellet in which they were reared. Thus, essentially all root production and colonization by *P. dissotocum* occurred after transplanting and inoculation.

Table 1. Extent of *Pythium dissotocum* colonization in asymptomatic rootlets of hydroponically grown plants

Treatment	Roots assayed (no.)	Roots infected (%)	Total root length assayed (mm)	Root length infected (%)
Control	98[a]	0	4,231	0
Inoculated	111	92	5,254	75.1

[a] Cumulative data based on six or seven root segments (each 40–60 mm long) from one plant per treatment, each treatment replicated four times and repeated on four separate tests.

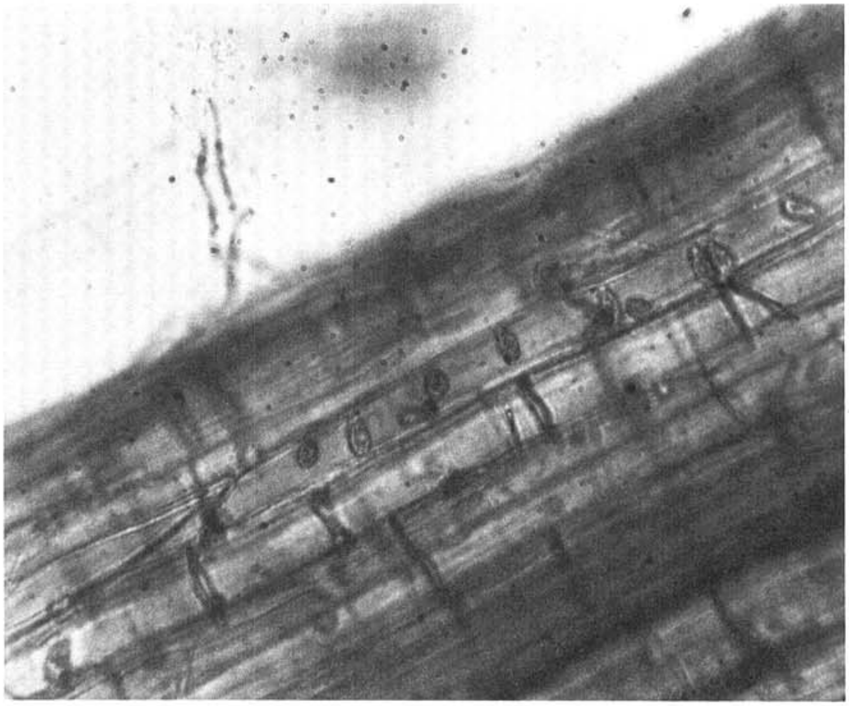

Fig. 3. Haustorialike structures of *Pythium dissotocum* within epidermal cells of healthy-appearing lettuce feeder rootlets.

The mechanism(s) involved in yield reduction resulting from asymptomatic infection of feeder roots is not known. *Pythium* may accelerate rootlet maturation and senescense. Perry (11) recently reported that asymptomatic potato root tips lapse into maturity and cease meristematic activity when infected by *Verticillium dahliae* Kleb. Also, early stages of infection of wheat rootlets by *P. arrhenomanes*, a major root rot pathogen, resulted in cessation of root elongation and lateral root formation (15). Asymptomatic feeder roots infected by *Pythium* spp. may not be functioning properly with respect to the uptake and translocation of water as suggested by Hodges and Coleman (8). Additionally, plant growth reductions may occur as a result of impairment of the uptake and translocation of minerals and the production of root-synthesized hormones. Quantitative physiological studies regarding rootlet dysfunction resulting from asymptomatic infection by *Pythium* are warranted.

The significance of lettuce feeder rootlet infection by species of *Pythium* under field conditions is not known. However, our study showed that *Pythium* spp. were consistently associated with asymptomatic rootlets of almost all plants sampled from four commercial lettuce fields. *P. dissotocum* was identified as the predominant species isolated from two of the fields, and *P. uncinulatum*, a known seedling pathogen of lettuce not previously reported in the United States, and *P. sylvaticum* predominated in rootlets from the other two fields, respectively. These results suggest that undetected but significant yield losses may be occurring in the production of lettuce under field conditions as a result of subclinical diseases caused by *Pythium* spp.

ACKNOWLEDGMENTS

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