Sources of *Rhizoctonia solani* and *Pythium* spp. in a Bedding Plant Greenhouse

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ABSTRACT

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A greenhouse operation in Ohio was studied to determine the sources of damping-off pathogens and their mode of dissemination to bedding plants. *Pythium* spp. were isolated from steam-treated soil, but neither *Pythium* spp. nor *Rhizoctonia solani* were recovered from the other components of the potting soil mix. Both *Pythium* spp. and *R. solani* were isolated from dust and soil mix particle samples collected from walkways, floors, and beds within the greenhouse and also from used seedling flats. When infested dust and soil mix particle samples were sprinkled on flats of seedlings, damping-off occurred. Sources of inoculum were present in this bedding plant greenhouse despite the use of soil steaming and routine sanitation procedures.

Rhizoctonia solani Kühn and *Pythium* spp. were determined to be major causes of the severe damping-off of plants occurring widely in the Ohio bedding plant industry (24; C. T. Stephens *unpublished*). Despite efforts to restrict these pathogens by soil pasteurization and sanitation programs, damping-off pathogens continue to cause severe bedding plant losses in greenhouses. Pathogens apparently are inadvertently introduced along with the constant flow of people, equipment, water, seed, plant material, and soil in and out of the greenhouses. Few detailed studies of the epidemiology of these fungi within greenhouses have been made.

Several studies have shown that *R. solani* can be carried on seed of various bedding plants (1,16; C. T. Stephens *unpublished*). Although seed transmission of *Pythium* is rare, it has been reported (4). Greenhouse water supply sources and irrigation ponds may also be sources of *R. solani* and *Pythium* spp. (6,8). Both *R. solani* and *Pythium* spp. are often found in many field soils (5,9,10,13,20), and field soil is frequently brought into greenhouses for use in potting mixes. Dust and larger particles of this unsterile soil may be transported by workers and equipment to other greenhouse areas.

R. solani can survive as sclerotia in soil for 5–7 mo (18) or as hyphae in dust particles for up to 9 mo (19). *Pythium* spp. survive in the soil mainly as resistant resting structures (10). *P. ultimum* has been known to survive in frozen soil for 4.5 yr (15) and in air-dry soil for as long as 12 yr (10). Sporangia of *P. ultimum* were found to survive in soil for 11 mo (22). Both *Pythium* spp. and *R. solani* can be disseminated by windblown contaminated soil and dust (2,19,25).

Evidently, damping-off pathogens can be readily introduced and can survive long enough to cause serious problems in greenhouse crops. It is difficult to totally eliminate pathogens from greenhouses. Therefore, control measures may be more feasible if based on better knowledge of potential sources of pathogens within the greenhouse and information on how pathogens are disseminated to the bedding plants. To this end, we sought to determine if pathogens are introduced with components of the growing medium, to assay dust and debris within the greenhouse for presence of damping-off pathogens and to evaluate the role of such pathogen reservoirs in soil reinfestation and bedding plant damping-off.

MATERIALS AND METHODS

A northern Ohio 7.3-ha greenhouse used for bedding plant production was selected for this 2-yr study. A brief description of relevant greenhouse operations follows. Field soil was stockpiled in a large concrete pit at one end of the headhouse area and when needed was transported to a Lindig aerated steam apparatus (Lindig Manufacturing Co., St. Paul, MN 55113) located in the same large room. The soil was steam-treated and then automatically mixed with peat, vermiculite, and perlite. The potting mix was moved by conveyor belt to a potting area separated by large sliding doors from the soil storage and steaming area. Plastic flats (36 × 50 × 6 cm) were filled, seeded, and either placed on benches or on porous floor beds of a separate seedling range. (The porous floor beds were made of concrete by a special process that provided good water drainage.) At transplant, the seedling flats were returned to the potting area; the seedlings were transplanted into plastic plant boxes (12 × 16.5 × 6 cm) and placed in large plastic flats. The transplant flats were moved on carts pulled by small tractors along the greenhouse walkways to the main greenhouse ranges where they were placed on porous beds.

Individual soil samples of at least 150 g were collected, air-dried, sieved through a 0.25-cm sieve, and stored at 4 C until used. Adaptations of the beet seed colonization technique (17) were used to assay each sample for *R. solani* and *Pythium* spp. Soils were remoistened for assay by placing 75 g of an air-dried soil sample on filter paper in a 10-cm-diameter Buchner funnel and immersing the filled funnel in water for 3 hr. The funnel was then attached to a filter flask and subjected to suction at 254 torr for 15 min, after which it was considered to be approximately at the moisture equivalent (14), ie, roughly minus one-third atmosphere tension (14). Use of this comparatively rapid, approximate method of moisture adjustment was necessitated by the variable particle composition (both nature and size of particles) of the dust and soil particle samples assayed. The sample was then placed in a plastic bag with 120 autoclaved garden beet seeds (*Beta vulgaris* L. 'Detroit Dark Red'). After 2 days of incubation, the seeds were separated from the soil by sieving and plated on Ko's medium (12), selective for *R. solani,* and on sucrose-asparagine-pentachloronitrobenzene-benomyl-neomycin sulfate-chloramphenicol medium (SAPBNC) (21), selective for *Pythium* spp. Sixteen plates of Ko's medium and eight plates of SAPBNC medium, each containing five seeds, were used per sample. After 48 hr, the plates were examined for *R. solani* and *Pythium* spp.

To determine if components of the bedding plant medium (steamed field soil, vermiculite, Canadian peat, and perlite) contained *R. solani* or *Pythium* spp., 10 samples of each component were

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assayed, using the beet seed technique. Additionally, in the first year, 10 samples of different steamed soil batches were collected over a 2-wk period and assayed. In the second year, three samples from separate steamed soil batches were collected every 2 wk for 4 mo (24 samples) and assayed.

Twenty used and 10 new seedling flats were assayed for the presence of *R. solani* and *Pythium* spp. by placing 75 g of moistened autoclaved soil into each. The flats were placed in plastic bags (36 × 72 cm) and incubated at room temperature (24 C) for 2 wk. Each soil sample was then assayed for the presence of *Pythium* spp. and *R. solani* by the beet seed technique.

To determine if *R. solani* and *Pythium* spp. were present in greenhouse dust and soil mix particles, 16 samples were collected with a manual revolving brush-type walk sweeper from various floor and walkway areas in the greenhouse every 2 wk over a 4-mo period and were assayed as described previously.

Soil mix particles and dust tended to collect in the porous beds on which the seedling flats were placed. Several techniques were used to assay these beds for *R. solani* and *Pythium* spp. In the first year of sampling, autoclaved garden beet seeds were placed under bedding plant flats directly in and on the porous bed and incubated for 3 days. A plastic sheet was placed over the seeds to isolate them from the flats above. Assays were made of both new bed areas lacking appreciable soil accumulation and old bed areas with evident soil accumulation. After incubation, seeds were plated on *R. solani*- and *Pythium*-selective media as described earlier. Different locations were assayed monthly for 4 mo.

In the second year of sampling, a J 105 Hydrovac vacuum cleaner (Pullman/Holt Products, Tampa, FL 33617) was used to extract soil mix deposits from the porous beds. Five different locations on old soil-filled beds and five locations on new relatively clean beds were assayed twice a month for 4 mo.

To determine if pathogen-infested greenhouse dust can reinfest soil in seedling flats and cause damping-off, the following experiment was performed. Celosia seeds (*Celosia argentea* L. 'Red Fox') were thickly sown in a moistened peat-vermiculite mix (Jiffy Mix, Jiffy Products, West Chicago, IL 60185). After seeding, the flats were lightly covered with additional Jiffy Mix, then misted, put in plastic bags (61 × 30 cm) and placed in a growth chamber with a 14-hr day, 25,000-lux light intensity at 24 C and 10-hr nights at 21 C. At seedling emergence, 75-g portions of soil samples from greenhouse dust and soil mix particles collected from greenhouse floors and walkways and known to be infested with *Pythium* spp. and *R. solani* were sprinkled over each of 10 flats. Two control flats were sprinkled with autoclaved soil. After the soil was added, each flat was lightly misted, bagged, and placed in the growth chamber for 6 days. Damped-off seedlings were removed from the flats and placed directly on agar plates of selective media for isolation of pathogens.

RESULTS

Neither *R. solani* nor *Pythium* spp. were recovered from the nonsoil components of the bedding plant medium or from the 10 steam-treated soil batches tested in the first year of this study, but three of the 24 steamed soil batches collected in the second year contained *Pythium* spp. In the three infested lots, 10, 55, and 90% of beet seeds plated on the *Pythium*-selective medium were colonized.

Although the new seedling flats did not yield damping-off pathogens, the used flats were contaminated. *Pythium* spp. were found in six and *R. solani* in two of the 20 used flats tested. The percentage of beet seeds colonized per soil sample of 40 seeds for *Pythium* and 80 seeds for *R. solani* ranged from 5 to 58% for *Pythium* spp. and from 3 to 5% for *R. solani.*

Both *Pythium* spp. and *R. solani* were isolated from dust and soil mix particle samples collected from floors and walkways in the greenhouse (Table 1). *Pythium* spp. were isolated on five sampling dates from both the greenhouse walkway and the seedling transplant room floor and *R. solani* was isolated on six of the eight sampling dates.

During the first year of sampling, a high percentage (avg. 65%) of the beet

Table 1. Percentage of beet seeds colonized by *Pythium* spp. or *Rhizoctonia solani* in soil samples of dust and potting mix particles collected from greenhouse walkway and floor areas on designated assay dates

Date	Soil sample source	Beet seed colonized by *Pythium* (%)	Beet seed colonized by *Rhizoctonia* (%)
15 February	A[a]	100.0[c]	3.3[d]
	B[b]	96.7	0.0
28 February	A	11.1	0.0
	B	93.3	3.3
15 March	A	0.0	6.6
	B	0.0	3.3
30 March	A	0.0	0.0
	B	0.0	16.6
15 April	A	93.3	3.3
	B	90.0	13.3
30 April	A	0.0	0.0
	B	0.0	6.6
15 May	A	96.7	0.0
	B	80.0	0.0
30 May	A	100.0	0.0
	B	36.6	0.0

[a] A = greenhouse walkway.
[b] B = seedling transplant room floor.
[c] Percentage of beet seeds colonized of a total of 40 seeds per soil sample.
[d] Percentage of beet seeds colonized of a total of 80 seeds per soil sample.

Table 2. Percentage of beet seeds colonized by *Pythium* spp. after incubation in various locations in porous beds on four assay dates

Date	Location	Beet seed colonized[a] (%)
March	1A[b]	100
	2A	100
	3A	100
	4A	100
	5A	10
	1B[c]	0
	2B	0
	3B	0
	4B	0
	5B	0
April	1A	40
	2A	15
	3A	50
	4A	80
	5A	0
	1B	0
	2B	0
	3B	0
	4B	0
	5B	0
May	1A	100
	2A	65
	3A	100
	4A	45
	5A	30
	1B	0
	2B	0
	3B	0
	4B	0
	5B	0
June	1A	100
	2A	80
	3A	40
	4A	100
	5A	40
	1B	0
	2B	0
	3B	0
	4B	0
	5B	0

[a] Percentage of beet seeds colonized of 40 seeds tested.
[b] A = older porous beds.
[c] B = new porous beds.

seeds incubated in old porous beds were colonized by *Pythium* spp. (Table 2). Only one older bed location on one date (5A, April) failed to give a positive assay. In contrast, there was no colonization of beet seed in the newer floor bed areas. No *R. solani* was obtained from either the new or old porous beds.

In the second year of sampling, the vacuum-extracted soil mix deposits from the porous beds contained both *Pythium* spp. and *R. solani* (Table 3). *Pythium* spp. were detected on all eight sampling dates in soil samples (five samples per date) obtained from locations on older beds as opposed to detection on only four sampling dates from locations on new beds. Average percentages of beet seeds colonized per sampling date ranged from 28 to 77% for old and from 0 to 68% for new porous bed soil samples. *R. solani* was detected on fewer sampling dates from both old (six dates) and new (three dates) beds. The average percentage of beet seeds colonized per sampling date ranged from 0 to 45% for old and from 0 to 22% for new beds.

The infested dust and soil mix particle samples that were sprinkled on the flats of celosia seedlings caused damping-off in seven of 10 flats. A total of 18 spots with damping-off symptoms (each involving several seedlings) appeared in all seven flats within 6 days. *R. solani* was isolated from 16 spots, *Pythium* spp. from one spot, and both *R. solani* and *Pythium* spp. from one spot.

DISCUSSION

Assay of the greenhouse potting soil mix and of the dust and soil mix particle samples obtained from within the greenhouse area for *R. solani* and *Pythium* spp. was relatively simply accomplished by use of adaptations of the beet seed colonization (baiting) technique (17). The usefulness of the beet seed method was enhanced by use of two plating media, one selective for *R. solani* (12) and the other selective for *Pythium* spp. (21). Additionally, the Buchner funnel technique (14) provided a practical means of moisture adjustment adequate for handling the variable particle composition of the dust and soil mix particle samples.

The only component of the bedding plant medium that contained damping-off pathogens (*Pythium* spp.) was the steamed field soil. This was rather surprising because none of the other three components of the soil mix (peat, vermiculite, and perlite) were steamed on site. Presumably, their packaging in closed bags prevented contamination by unsterile field soil dust, at least within the limits of our ability to detect pathogen presence.

The presence of *Pythium* spp. in low proportions of steamed soil represents a considerable danger because of the high recolonization potential of such soils due to their readily available nutrient content and absence of competing microorganisms (3). During mixing of the steamed soil with the other potting soil mix ingredients, small quantities of insufficiently heated soil containing *Pythium* spp. could be generally dispersed in the medium, thereby contaminating large numbers of flats. Despite the failure to demonstrate *R. solani* in steam-treated soil, it seems likely it could be a source of inoculum because *R. solani* is more tolerant of heat than are *Pythium* spp. (2).

The presence of both *R. solani* and *Pythium* spp. in samples of dust and soil mix particles collected from greenhouse walkways and the seedling-transplant room floor indicates that movement of unsterile contaminated soil takes place on machinery (tractors, carts, etc.) by workers (shoes, clothing, tools) and by windblown dust (2,18,25). Once present on floors and walkways, the contaminated soil could be blown by air currents (eg, caused by passage of tractors and carts and the ventilation system) and/or splashed during watering onto the seedling flats and porous beds. We have demonstrated that contaminated dust and soil mix particle samples obtained from floors and walkways can induce damping-off in seedling flats.

The porous beds, especially the older beds with appreciable soil accumulations in the pores, can harbor large populations of *Pythium* spp. and *R. solani* (Tables 2 and 3) and serve as inoculum reservoirs. Flats of seedlings placed on these beds can be contaminated by water-splashed soil, or in the case of transplants, their roots may grow into the porous beds and contact the pathogens. Disinfestation of these beds would probably be an important element in any successful damping-off control program.

The presence of damping-off pathogens in used flats from the previous season indicates that *Pythium* spp. and *R. solani* propagules can survive for at least 9 mo in very dry soil deposits. Earlier, Baker (2) reported that *R. solani* can survive in soil on used containers. Most flats are shipped out filled with the saleable bedding plants. The flats retained will probably include those having seedling damping-off problems and consequently will have a high probability of being contaminated by pathogens. Our results indicate that flats should not be reused without being disinfested.

Bedding plant growers commonly combat damping-off by using soil fungicides, generally soil drenches, after the disease is evident (7). Another approach is to attempt to make steamed (pasteurized) potting mixes less favorable for recolonization by pathogens. Baker (3) advocated use of low-temperature aerated steaming (60 C) to avoid creating a biological vacuum devoid of soil microorganisms. Although the greenhouse operation studied had an aerated steaming apparatus, it was consistently operated at temperatures above 80 C, negating its potential advantages for retarding pathogen recolonization.

Another method for suppressing the activity of pathogens through use of composted hardwood bark in the potting soil mix has been suggested by Hoitink (11). A study of the feasibility of using composted bark mixtures for greenhouse bedding plant production was made by Stephens et al (23). Composted bark potting soil mixtures definitely reduced damping-off, but their general use in bedding plant operations could not be recommended without additional testing.

Table 3. Percentage of beet seeds colonized by *Pythium* spp. or *Rhizoctonia solani* after incubation in soil samples vacuum-extracted from porous beds on designated assay dates

Sampling date	Soil source	*Pythium* spp. colonization (%)	*R. solani* colonization (%)
30 January	Old[a]	48[b]	1[b]
	New[c]	0[d]	0[d]
15 February	Old	39	45
	New	0	22
30 February	Old	71	1
	New	8	0
15 March	Old	76	7
	New	35	1
30 March	Old	35	0
	New	0	0
15 April	Old	48	32
	New	22	3
30 April	Old	28	0
	New	0	0
15 May	Old	77	4
	New	68	0

[a] Older porous beds.
[b] Mean percentage colonization of beet seeds in soil samples extracted from five older porous bed areas (40 seeds for *Pythium* spp. and 80 seeds for *Rhizoctonia solani* per soil sample).
[c] New porous beds.
[d] Mean percentage colonization of beet seeds in soil samples extracted from five new porous bed areas (40 seeds for *Pythium* spp. and 80 seeds for *Rhizoctonia solani* per soil sample).

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