A Method of Evaluating Postemergence Damping-Off Pathogens of Bedding Plants

Christine Taylor Stephens, C. C. Powell, and A. F. Schmitthenner

Research associate, associate professor, and professor, respectively, Department of Plant Pathology, The Ohio State University, Columbus 43210, and the Ohio Agricultural Research and Development Center, Wooster 44691. Present address of senior author: Department of Botany and Plant Pathology, Michigan State University, East Lansing 48824.

Portion of the M.S. thesis by the senior author.
Approved for publication as Journal Article 188-79 of the Ohio Agricultural Research and Development Center, Wooster.
Accepted for publication 13 March 1981.

ABSTRACT

Stephens, C. T., Powell, C. C., and Schmitthenner, A. F. 1981. A method of evaluating postemergence damping-off pathogens of bedding plants. Phytopathology 71:1225-1228.

An inoculation method was developed to allow rapid assay examination of the pathogenicity of numerous fungal isolates inducing damping-off on many plant types. Seeds of host plants were thickly sown in rows in pots containing a moistened peat-vermiculite mix. Inoculum as a 12 × 12-mm agar disk removed from a petri plate culture of the fungus, was buried at the end of the seedling row at seedling emergence. The pots were placed in plastic bags and incubated on a shaded greenhouse bench at 26 ± 2 C. After 9 days, the length of seedling row in which damping-off occurred was measured. This method maximized the amount of damping-off, minimized variability among replicates, and adequately revealed pathogenic isolates and susceptible hosts. Furthermore, the test conditions closely approximate seedling germination and early growth conditions prevalent in Ohio greenhouses.

Additional key words: soil microbiology, *Pythium, Rhizoctonia.*

Damping-off of seedlings is a major disease problem for the bedding plant industry (2,7). Control or prevention is complicated because there may be several pathogens involved either separately or in combination on as many as 80 plant types being produced in any one greenhouse. Precise host-pathogen relationships of this disease complex have not been investigated because such a study requires evaluation of many fungal isolates on many plant types under conditions approximating those found in the commercial greenhouse.

Most studies of damping-off were concerned with methods to improve seedling emergence and survival (1,3,6,8,10,11,14,15,18). These studies usually employed soil infested with damping-off pathogens. In many cases, the pathogens present in the test soil were not identified (1,4). In all these studies, seed was sown in the soils, control measures applied and seedling emergence and survival noted sometime later.

Methods for measuring magnitude of preemergence damping-off are not useful for the purpose of examining many different pathogens and host specificity to them because inoculum density and time of infection are difficult to control. The necessity of having separate soil lots infested with each fungal pathogen makes it practically impossible to deal with a large number of hosts and pathogens. Germination variability among plant types is often a problem as well.

Postemergence inoculation of individual older seedlings has been carried out with specific pathogens (10,13,17). These studies demonstrated pathogenicity; however, the methods were not useful for examining plant-to-plant spread of the pathogens on very young seedlings in soilless media such as that used by bedding plant growers. In general, bedding plant growers sow seed in thick rows in flats of soilless media (7). Seed is watered in and flats are covered, shaded, and incubated at approximately 26 C until the seedlings emerge. Seedling flats are then uncovered and moved to a cooler, less humid greenhouse for growing prior to transplanting. Most damping-off occurs when growers fail to uncover and move the seedling flats immediately after emergence.

Methods devised for studying suppression of damping-off in natural soils deviate widely from these conditions (9). Bedding plant growers rarely use natural soils for this operation and always sow seed very thickly in rows. The purpose of our study was to develop a method of evaluating fungal pathogens for ability to cause postemergence damping-off and of assessing susceptibility of bedding plant seedlings under conditions similar to those found in greenhouses in Ohio.

0031-949X/81/11122504/$03.00/0

MATERIALS AND METHODS

Seeds of vegetable and flower bedding plants were obtained from the George J. Ball Company, West Chicago, IL 60185. Test plants included tomato (*Lycopersicon esculentum* 'Early Girl'), pepper (*Capsicum frutescens* 'California Wonder'), cabbage (*Brassica oleracea* var. *capitata* 'Golden Acre'), celosia (*Celosia argenta* 'Red Fox'), ageratum (*Ageratum houstonianum* 'Blue Blazer'), impatiens (*Impatiens wallerana* 'Dwarf Blaze'), salvia (*Salvia splendens* 'Fireball'), eggplant (*Solanum melongena* var. *esculentum* 'Black Beauty'), and vinca (*Vinca major* 'Little Blanche'). Seeds were sown in moistened peat-vermiculite medium (Jiffy Mix, Jiffy Products, West Chicago, IL 60185) in 9-cm-diameter plastic pots. The seeds were placed in rows so that they were just touching. Three rows, radiating from a common point at one side of the pot were sown in each pot. Each row contained a different species of plant. Plant types in the same pot all had similar emergence times. To determine the best method to insure rapid but even and predictable emergence times of the seedlings, germination conditions were varied, but kept strictly in the range of conditions naturally found in commercial greenhouses. Placing seeded pots under a mist system, covering seeded pots with shaded glass, and enclosing seeded pots in plastic bags were all evaluated in separate experiments. For all host-pathogen studies, seeded pots were enclosed in 25 × 17.5 × 43-cm plastic bags (three pots per bag). Stakes in the pots kept bags away from seedlings.

Numerous isolates of *Pythium* species and *Rhizoctonia solani* were obtained from the collection of A. F. Schmitthenner. The *Pythium* species included in the test were *Pythium aphanidermatum* (Edson) Fitzpatrick, *P. irregulare* Buisman, *P. mammillatum* Meurs, Wortelrot, *P. pulchrum* von Mindon, *P. ultimum* Trow, *P. vexans* deBary, *P. deBaryanum* Hesse, *P. graminicolum* Subramaniam, *P. paroecandrum* Dreschler, *P. spinosum* Sawada, *P. splendens* Braun, and *P. sylvaticum* Campbell and Hendrix. Most cultures were isolated from greenhouse-grown ornamentals within the last 5 yr. Several isolates of *P. ultimum* and *R. solani*

were obtained from bedding plants with symptoms of damping-off in the previous season. *Pythium* spp. were cultured on cornmeal agar (Difco Laboratories, Detroit, MI 48232) in petri plates for 3 days at 24 C. The isolates of *R. solani* were grown on potato-dextrose agar (Difco) for 4 days at 24 C. Disks of mycelium (12 mm in diameter), taken from the edge of the colonies, were used as inoculum in all experiments. Inoculations were done on the day the seedlings emerged by placing inoculum at the converging end of the seedling rows. At least three replications were made of each fungus-plant-environment variable in each experiment.

After inoculation, further experimentation was carried out to determine an environment that resulted in a consistant expression of damping-off. Three or more replications were made of a number of combinations of inoculum placement and incubation temperatures.

The progress of damping-off was observed at various times after inoculation to determine a suitable incubation period. A seedling was considered to be damped-off if the lower stem of the plant appeared water-soaked and the seedling had tipped over, or if there was a brown lesion at the soil line and the seedlings were wilted or stunted. The length of seedling row in which symptoms were apparent was recorded. Seedlings exhibiting damping-off were plated on Ko's medium (12) or sucrose-asparagine-pentachloro-nitrobenzene medium (16) to reisolate the causal agent.

To determine whether older plants could remain susceptible if inoculation was delayed, impatiens and pepper seedlings were inoculated with an isolate of *Pythium* sp. or *R. solani* at emergence and at nine 3-day intervals after emergence. For the later inoculations, the seedlings were kept in the bags until 3 days after emergence, removed from the bags until inoculation, and then mist irrigated and returned to the bags for incubation.

To determine if the amount of damping-off was simply a result of differences in growth rate among fungal isolates, pots of planting media without plants were infested by burying inoculum disks at the edge of the pot. Four 12-mm-diameter agar disks of sterile selective medium were buried as baits in each pot, two at 4.5 cm and two at 9 cm from the inoculum disk. Disks of Ko's medium were used to bait for *R. solani* and SA-PCNB to bait for *Pythium* sp. The pots were incubated for 4 and 8 days, after which the baits were examined. Baits were examined for hyphae of *Pythium* sp. or *R. solani* microscopically and colonies were identified by plating the baits on a suitable medium. In vitro growth rate of the isolates was determined by placing disks of inoculum at the edge of cornmeal agar plates. Four replications of each isolate were incubated at 22 C. Fungal growth was measured every 24 hr.

TABLE 1. Damping-off by *Pythium* spp. and *Rhizoctonia solani* of celosia as determined by two methods of inoculation[a]

Fungal isolate	Length of row damped-off[b] (cm)	
	A	B
Pythium aphanidermatum #246[c]	2.0	6.3
P. irregulare #33	0.0	0.0
P. mammillatum #37	0.0	0.0
P. pulchrum #93	0.0	0.0
P. ultimum #166	0.0	5.5
P. ultimum #248	0.0	4.8
P. vexans #72	0.0	0.0
Rhizoctonia solani #1076	5.8	6.3
R. solani #1078	2.3	5.3
R. solani #1082	2.3	6.3
Check	0.0	0.0

[a] A = inoculum disk placed on the surface of planting media in the pot, germinated seedling rows inoculated and resealed in plastic bags without additional water. B = agar inoculum disk buried 3 mm into the planting media in the pot and germinated seedling rows inoculated and pots irrigated lightly prior to being resealed in the bag.
[b] Numbers are the centimeters of seedling row damped off in a 7.5-cm row after a 9-day incubation period. Each number is the average of three replications.
[c] Numbers in A. F. Schmitthenner's culture collection.

RESULTS

Germination of seed was poor in pots placed on a concrete bench and misted automatically for 5 sec every 6 min during the day. The poor germination resulted from temperature fluctuation caused by

TABLE 2. Damping-off of tomato and ageratum by *Pythium* species and *Rhizoctonia solani* in conditions of fluctuating and controlled temperatures after a 9-day incubation period

Fungal isolate	Length of seedling row damped-off (cm)			
	Tomato		Ageratum	
	Fluctuaring[b] temperature	Controlled[c] temperature	Fluctuating temperature	Controlled temperature
Pythium aphanidermatum #246[d]	0.0	1.3	0.0	1.3
P. deBaryanum #228	0.0	0.0	0.0	0.0
P. graminicolum #223	0.0	0.0	0.0	0.0
P. irregulare #33	0.0	0.0	0.0	0.0
P. irregulare #250	0.0	1.5	0.3	1.3
P. mammillatum #37	0.0	0.0	0.0	0.0
P. paroecandrum #94	0.0	0.0	0.0	0.0
P. pulchrum #93	0.0	0.0	0.0	0.0
P. spinosum #28	0.0	0.3	0.0	0.0
P. splendens #134	0.0	0.0	0.0	0.0
P. sylvanticum #78-79	0.0	0.0	0.0	0.0
P. ultimum #32	0.0	0.3	0.0	0.0
P. ultimum #166	0.0	3.5	0.0	4.8
P. ultimum #247	0.0	2.5	0.0	3.5
P. ultimum #248	0.0	3.8	0.0	2.0
P. ultimum #249	0.0	6.3	0.0	7.5
P. ultimum #251	0.0	0.3	0.1	4.5
P. vexans #72	0.0	0.0	0.0	0.0
P. vexans #129	0.0	0.0	0.0	0.0
Rhizoctonia solani #1076	0.4	7.5	1.9	7.5
R. solani #1078	1.8	7.5	7.5	7.5
R. solani #1082	2.2	7.5	7.5	7.5
Check	0.0	0.0	0.0	0.0

[a] Numbers are the centimeters of seedling row in which damping-off occurred in a 7.5-cm row. Each number is the mean of four replications.
[b] "Fluctuating temperatures" were those naturally occurring on a greenhouse bench and varied between 20 and 33 C.
[c] "Controlled temperatures" were achieved by growing plants on a shaded greenhouse bench held at 26 ± 2 C.
[d] Numbers in A. F. Schmitthenner's culture collection.

the cold mist. Seeds in pots placed on a bench covered with panes of glass and cheesecloth germinated evenly when temperatures were held at 26 ± 2 C. However, the plants quickly grew to the point where they were pressing up against the glass. Seed in pots placed inside plastic bags and placed on a shaded bench at 26 ± 2 C germinated rapidly and uniformly.

The success of the method depended on several factors. The data in Table 1 illustrate the effects of inoculation by two contrasting methods. A method that was more conducive for disease expression was burying the disk 0.5 cm deep at the end of the seedling row and lightly watering the plants after inoculation (Table 1). Controlling the temperature at 26 ± 2 C (Table 2) further improved the method. Some isolates that failed to cause disease in early attempts now proved to be pathogenic.

The progress of damping-off in the seedling rows where isolates of *R. solani* were introduced was more rapid than that in the rows inoculated with isolates of *Pythium*. *R. solani* usually completely destroyed susceptible seedlings in 6 or 7 days. Steady progression of disease up to 9 days after inoculation was observed on seedlings in rows infested with *Pythium*. Little additional damping-off occurred from 9 to 23 days after inoculation among 16 isolates of *Pythium* that were tested. Nine isolates that did not induce symptoms 9 days after inoculation were still nonpathogenic 23 days after inoculation. Both pepper and impatiens remained highly susceptible to isolates of *Pythium* and *R. solani* up to 27 days after sowing when inoculated and placed back under the conditions conducive to damping-off (Table 3) that were described earlier.

In infested pots without plants, the mycelium rarely colonized the bait disks nor was mycelium of *Pythium* or *R. solani* observed to be growing across the soilless potting medium. In one instance, mycelium of *Pythium* was isolated from a bait disk that had been placed 4.5 cm from the inoculum. None of the bait disks at the 9-cm distance contained mycelium of either *Pythium* or *R. solani*. Although most of the bait disks remained sterile, hyphae of other fungi colonized small areas of several of them.

In vitro growth rates were not related to the length of row affected by damping-off. For example, *P. ultimum* #249 grew 2.8 cm per day; *R. solani* #1082, 1.6 cm; *P. de Baryanum* #228 2.3 cm; *P. ultimum* #251, 0.6 cm; and *P. vexans* #129, 2.6 cm. Damping-off occurred along the entire 7.5 cm of row 9 days after inoculation when seedlings were exposed to *P. ultimum* #249 and *R. solani* #1082. *P. ultimum* #251 damped-off 4.5 cm of the row 9 days after inoculation. *P. vexans* #129 and *P. de Baryanum* #228 did not cause damping-off.

TABLE 3. Damping-off of impatiens and pepper seedlings following inoculation at varying times after seeding

Days after seeding	Plant and plant height	Length of 7.5-cm row affected (cm)[a]	
		Pythium ultimum	*Rhizoctonia solani*
	Pepper		
9	Emergence	4.5	7.5
18	58–63 cm	3.0	7.5
27	98–100 cm	3.5	7.5
	Impatiens		
9	Emergence	7.5	7.5
18	43 cm	7.5	7.5
27	88–90 cm	7.5	7.5

[a] Damping-off observed at 9-day intervals after inoculation. Numbers are the centimeters of seedling row damped off in a 7.5-cm row. Each number is an average of three replications.

DISCUSSION

The test conditions derived in this study enabled maximization of the amount of damping-off, minimization of the variability among replications, and more adequate detection of which isolates were pathogenic. The method finally developed was highly sensitive; some isolates that failed to cause disease in earlier attempts under different conditions proved to be pathogenic. We employed a number of fungal isolates and plant types in this study to insure wide applicability of our method. This method closely approximates seedling-holding conditions that often lead to damping-off in commercial operations. Thus, results should correlate well with commercial situations.

Little additional damping-off occurred after 9 days of incubation with our method. Reasons for this were not determined, although it was demonstrated that plants up to 23 days old are highly susceptible when freshly inoculated and placed back under the warm, high humidity conditions. This suggests that failure of damping-off to progress further down the row of seedlings did not involve increasing resistance of host plants as they matured.

We found no correlation between in vitro growth rate of our test isolates and amount of seedling row the isolates damped-off in 9 days. Although the in vitro growth rate of the *R. solani* isolates was relatively slow, they were highly pathogenic. This is in agreement with Blair's conclusions (5). Similarly, there was no relationship between growth rate of *Pythium* spp. and pathogenicity. Further, in the absence of seedlings, selective media baits placed in the soilless media were not colonized by the inoculum. This suggests that the fungi were limited in their ability to grow through the Jiffy Mix, a low-nutrient medium. Thus, the fact that some fungal isolates caused damping-off in more of the seedling row than others within a given time period probably is related to the virulence of the isolates and to the susceptibility of the host.

Studies are currently underway using this method to survey which *Pythium* spp. are causing damping-off in Ohio greenhouses. In addition, we shall attempt to evaluate the significance of associations of particular *Pythium* spp. with certain hosts and to develop host-range hypotheses. Finally, the method should prove useful in rapidly screening the many species and cultivars of bedding plants grown in greenhouses to search for resistance to the most prevalent and important damping-off fungi. Integration of such data will provide the basis for epidemiology studies of the organisms and improved disease management strategies.

LITERATURE CITED

1. Anderson, N. W., Kadow, K. J., and Hopperstead, S. L. 1937. The evaluation of some cuprous oxides recommended as seed treatment products for the control of damping-off. Phytopathology 27:575-587.
2. Anonymous. 1973. Bedding Plant News, Nov. 1973. 6 pp.
3. Beach, W. S., and Chen, S.-Y. 1942. Experimental control of damping-off. Penn. Agric. Exp. Stn. Bull. 434.
4. Bewley, W. F. 1920. Damping-off and foot-rot of tomato seedlings. Ann. Appl. Biol. 7:156-172.
5. Blair, I. D. 1942. Studies on the growth in soil and the parasitic actions of certain *Rhizoctonia solani* isolates from wheat. Can. J. Res. 20, C:174-185.
6. Brien, R. M., and Chamberlain, E. E. 1936. Tomato seedling damping-off control by soil treatment. N. Z. J. Agric. 52:267-275.
7. Brooks, W. M., Kiplinger, D. C., Lindquist, R. K., Partyka, R. E., Powell, C. C., Staby, G. L., and Tayama, H. K. 1977. Tips on Growing Bedding Plants. Ohio Coop. Ext. Serv. (Columbus) Bull. MM-265. 29 pp.
8. Greeves, T. N., and Muskett, A. E. 1937. A temperature study of *Pythium* attack on swede seedlings. Ann. Appl. Biol. 23:264-270.
9. Henis, Y., Ghaffar, A., and Baker, R. 1978. Integrated control of *Rhizoctonia solani* damping-off of radish: effect of successive plantings, PCNB, and *Trichoderma harzianum* or pathogen and disease. Phytopathology 68:900-907.
10. Horsfall, J. G. 1938. Combating damping-off. N. Y. Agric. Exp. Stn. (Geneva) Bull. 683. 46 pp.
11. Kadow, K. J., and Anderson, H. W. 1937. Damping-off control on evaluation of seeds and seed treatment. Pages 291–348 in: Ill. Agric. Exp. Stn. Bull. 439.
12. Ko, W. H., and Hora, F. H. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 67:707-710.
13. Mircetich, S. M., and Zentmyer, G. A. 1964. Rhizoctonia seed and root rot of avocado. Phytopathology 54:211-213.
14. Person, L. H., and Chilton, S. J. P. 1940. Seed and soil treatment for the control of damping-off. La. Agric. Exp. Stn. Bull. 349:3-16.
15. Porter, R. H. 1945. Germinability of treated and untreated lots of

vegetable seeds in *Pythium* -infested soil in the field. Pages 951–982 in: Iowa Agric. Exp. Stn. Bull. 345.
16. Schmitthenner, A. F. 1973. Isolation and identification methods for *Phytophthora* and *Pythium*. Proc. Woody Ornamentals Dis. Workshop, 24–25 January 1973, University of Missouri, Columbia.
17. Shaw, F. J. F. 1912. The morphology and parasitism of *Rhizoctonia*. Mem. Dep. Agric. India (Butan Ser.) 4:115-153
18. Stanghellini, M. E., and Phillips, J. M. 1975. *Pythium aphanidermatum*; its occurrence and control with pyroxychlor in the Arabian desert at Abu Dhabi. Plant Dis. Rep. 59:559-563.