Evaluating Cucurbit Seedling Resistance to *Phytophthora drechsleri*

B. MANSOORI, Former Graduate Student, and Z. BANIHASHEMI, Professor, Department of Plant Protection, College of Agriculture, University of Shiraz, Shiraz, Iran

ABSTRACT

Mansoori, B., and Banihashemi, Z. 1982. Evaluating cucurbit seedling resistance to *Phytophthora drechsleri*. Plant Disease 66:373-376.

Large numbers of similar-aged zoospores were obtained from *Phytophthora drechsleri*. A total of 116 cultivars of *Cucumis melo* (cantaloupe and long melon), *C. sativus* (cucumber), *Cucurbita pepo* (squash), and *Citrullus lanatus* (watermelon) were inoculated with zoospores at 30 C. Cultivars differed in susceptibility, with *Cucumis melo* cultivars the most susceptible and *Cucurbita pepo* cultivars the most resistant. Cucumber cultivar Ohio MR 17 and a local cultivar from Iran, watermelon cultivar Klondike No. 7, and cantaloupe cultivar Gold & Silver were highly resistant to *P. drechsleri*.

Additional key words: cucurbit root rot and damping-off, soil extract

Phytophthora drechsleri Tucker is a serious soilborne plant pathogen in Iran that causes disease in cucurbits (6,10), sugar beets (6,8), and sunflower (2). This pathogen also causes dieback in pine plantations in Australia (9) and root rot in safflower (7) and stone fruits (13) in the United States. Cother (4) recently compiled a list of 26 naturally and three artificially infected host species. There are no reports on reactions of cucurbit cultivars to *P. drechsleri*.

Zoospores probably function as the primary inoculum for infecting cucurbit plants under field conditions. A major problem in screening studies is production of the large quantities of zoospores of *P. drechsleri* required for inoculation. Because existing methods (3,5,11) are not satisfactory for obtaining large quantities of similar-aged, motile zoospores of *P. drechsleri*, the present study was undertaken to investigate various factors affecting production of zoospores in vitro. The use of zoospores in evaluating the reaction of different cucurbit cultivars to *P. drechsleri* under controlled conditions was also studied.

MATERIALS AND METHODS

Soil extract was prepared by suspending 1 kg of sandy loam field soil, pH 8.2, in 3 L of tap water containing 10 g of calcium carbonate. The suspension was allowed to settle overnight at room temperature. The supernatant was decanted, passed through cheesecloth, and cleared by centrifugation at 3,000 rpm for 10 min. The solution was sterilized either by autoclaving for 10 min at 121 C or by filtering through a 0.22-μm filter (Millipore Filter Inc., Bedford, MA 01730).

P. drechsleri isolate P-40 (Table 1) was grown on Difco lima bean agar (LBA), Difco cornmeal agar, cleared V-8 agar (CV8A), and potato-dextrose agar in petri plates at 30 C. When cultures were 6–8 days old, they were flooded with 30 ml of diluted soil extract (1 part soil extract:2 parts distilled water) for 10–12 hr at 15 C in the dark or under 1,200 lux of white fluorescent illumination to induce sporangial formation. Sporangia were counted under ×100 magnification. Ten counts were made for each of four replicates per treatment. To induce synchronous release of zoospores, cultures were transferred from 15 C to room temperature (20–22 C). Zoospores were immobilized by vigorous shaking on a vortex mixer and counted using a hemacytometer. Four counts were made in each of four replicates per treatment. Isolate P-40 was compared on CV8A with other isolates of *P. drechsleri* (Table 1).

Cultivars of *Cucurbita pepo* (squash), *Citrullus lanatus* (watermelon), *Cucumis melo* var. *inodorus* (long melon), *C. melo* var. *reticulatus* (cantaloupe), and *C. sativus* (cucumber) were used in this study (Table 2). Cucurbit seeds were sown (five seeds per pot) in a mixture of virgin soil, sand, and sphagnum peat (3:1:1, v/v) contained in 9-cm plastic pots with drain holes and grown in the greenhouse (23–35 C). The soil was determined to be free from any

Table 1. Zoospore production by isolates of *Phytophthora drechsleri*[a]

Isolate number	Source	Zoospores (no. × 10^5/ml)
P-24	*Cucumis melo* var. *flexuosus* (snake melon)	0.5
S-42	Cucumber field soil	0.6
P-42	Cucumber	1.0
P-45	Long melon	1.9
P-61	Cucumber	2.2
P-40	Cucumber	2.5
P-48	Cantaloupe	2.5
P-49	Long melon	3.0
P-62	*Spinacea oleracea* (spinach)	3.0
P-63	Cantaloupe	3.5
P-64	Cantaloupe	4.0
P-45	Long melon	4.1

[a] Isolates were grown on cleared V-8 agar for 6 days at 30 C and flooded with 30 ml of filtered, sterile soil extract (diluted 1:2) and incubated at 15 C for 12 hr. Zoospores were obtained within 30 min upon subsequent incubation at room temperature (22–24 C). All isolates were obtained from various locations in the Fars province of Iran.

Table 2. Incidence of damping-off of cultivars of *Cucurbita pepo, Cucumis sativus, Citrullus lanatus,* and *Cucumis melo* inoculated with zoospores of *Phytophthora drechsleri*[a]

Number	Cultivar	Origin	Disease incidence (%) Days after inoculation 2	5	7
		***Cucurbita pepo* (squash)**			
1	Ebony Acorn	United States	0	0	0
2	Casertra	United States	0	0	0
3	Kababi	Iran	0	0	0
4	Local cultivar (Yazd)	Iran	17	35	100

(*continued on next page*)

Portion of a thesis submitted by the senior author in partial fulfillment of the requirements for the M.S. degree, University of Shiraz, Iran.

Accepted for publication 15 June 1981.

0191-2917/82/05037304/$03.00/0

pythiaceous fungi by means of baiting with cucumber fruits (1). After 9 days, seedlings with soil blocks were transferred to pots without drain holes and placed in a water bath at 30 C under 1,000–1,400 lux of white fluorescent and incandescent illumination. The ambient air temperature was 20–22 C. After 1 day of incubation, 1 ml of a suspension containing 5×10^3 zoospores of *P. drechsleri* isolate P-40 was added around the hypocotyl of each seedling. The pots remained flooded for about 2 hr. Thereafter, the pots were irrigated to saturation twice daily. Four pots were used for each cultivar. Uninoculated pots served as controls. Seedlings were checked daily for symptom development, and the number of dead seedlings was recorded 2, 5, and 7 days after inoculation.

RESULTS

Sporangial formation and zoospore production. Sporangia were produced when *P. drechsleri* cultures on LBA or CV8A plates were flooded with either sterile or unsterile soil extract at 15 C (Table 3). Few or no sporangia were formed in cultures grown on cornmeal or potato-dextrose agar. The highest number of sporangia was obtained when cultures on LBA were flooded with filter-sterilized soil extract. Sporangia in cultures on LBA matured after 12 hr of incubation at 15 C and began to discharge zoospores within 10 min after transfer to 20–22 C. Zoospores were not released during 12 hr of incubation of cultures on LBA or CV8A; however, longer incubation resulted in zoospore discharge. Light did not affect either sporangial production or the release of zoospores. Twelve different isolates of *P. drechsleri* from cucurbits and other plants formed from 0.5×10^5 to 4.12×10^5 zoospores per milliliter on CV8A (Table 1).

Reactions of cucurbit cultivars to *P. drechsleri*. The reactions of 116 cucurbit cultivars to *P. drechsleri* are shown in Table 2. Three squash cultivars were highly resistant and a cultivar from Yazd, Iran (no. 4) was highly susceptible. Cucumber cultivars ranged from resistant to moderately susceptible and were generally less suceptible than cultivars of watermelon and melon. Cucumber cultivar Ohio MR 17 and two local cultivars from Isfahan, Iran (nos. 5 and 6) had high resistance to *P. drechsleri*.

Most cantaloupe cultivars were susceptible and succumbed rapidly to *P. drechsleri*; however, cultivar Gold & Silver (no. 95) was highly resistant. Long melon was generally more resistant than cantaloupe.

DISCUSSION

Uniform inoculum production is important in the development of an effective disease-screening method. Using the methods we described, large numbers of *P. drechsleri* sporangia and zoospores

Table 2. (*continued from preceding page*)

Number	Cultivar	Origin	Disease incidence (%) Days after inoculation 2	5	7
		***Cucumis sativus* (cucumber)**			
5	Ohio MR 17	United States	0	0	0
6	Local cultivar (Isfahan)	Iran	0	0	0
7	Local cultivar (Isfahan)	Iran	0	5	5
8	Local cultivar (Fars)	Iran	0	0	10
9	Basmench	Iran	0	10	10
10	Wisconsin SMR	United States	9	9	13
11	Cucumber-3440	Iran	0	0	20
12	Local cultivar (Isfahan)	Iran	0	10	20
13	Cucumber-3436	Iran	5	12	20
14	Local cultivar (Isfahan)	Iran	0	15	27
15	Long Marketer	United States	7	28	39
16	Beith Alpha	Holland	0	8	42
17	Local cultivar (Gorgan)	Iran	4	42	42
18	Darofarman	Iran	0	10	57
19	Local cultivar (Rudbar)	Iran	10	35	57
20	Local cultivar (Fars)	Iran	0	17	58
21	Local cultivar (Fars)	Iran	10	35	62
		***Citrullus lanatus* (watermelon)**			
22	Klondike No. 7	United States	0	0	0
23	Local cultivar (Yazd)	Iran	0	0	3
24	Charleston Gray	United States	0	5	5
25	Peacock	United States	0	8	13
26	Jubilee	United States	5	10	14
27	Local cultivar (Tehran)	Iran	0	27	27
28	New Hampshire Midget	United States	21	21	30
29	Dixie Queen	United States	0	8	33
30	Golchin	Iran	25	25	33
31	Isin	Iran	16	16	37
32	Black-seeded Klondike	United States	5	15	40
33	Watermelon-10063	Iran	0	35	42
34	Hosein-Abadi	Iran	10	15	47
35	Koorpal	Iran	14	21	50
36	Local cultivar (Kermanshah)	Iran	16	25	50
37	Sharif-Abadi	Iran	42	50	50
38	Irani	Iran	20	40	35
39	Sugar Baby	United States	15	55	55
40	Afrapooli-Zoodras	Iran	33	55	55
41	Neishapoori	Iran	0	14	57
42	Congo	United States	50	50	58
43	Watermelon-10064	Iran	0	20	60
44	Irani	Iran	0	53	64
45	Family Fun	United States	35	58	64
46	Watermelon-10068	Iran	5	25	65
47	Watermelon-10065	Iran	30	40	65
48	Kleckley's Sweet	United States	38	56	68
49	Local cultivar (Kermanshah)	Iran	32	68	68
50	Watermelon-10072	Iran	10	15	72
51	Watermelon-10073	Iran	10	50	72
52	Watermelon-10070	Iran	58	76	76
53	Watermelon-10074	Iran	15	77	77
54	Setalaghi	Iran	14	35	78
55	Crimson Sweet	United States	48	78	86
56	Dixie Queen F_1	United States	62	68	88
57	Black Diamond	United States	44	77	88
58	Northern Sweet No. 6	United States	17	91	91
59	Afrapooli	Iran	46	84	92
60	Watermelon-10066	Iran	0	41	100
61	Watermelon-10071	Iran	30	76	100
62	A-36 Shiraz	Iran	63	81	100
		***Cucumis melo* var. *inodorus* (long melon)**			
63	Local cultivar (Mashad)	Iran	0	3	7
64	Local cultivar (Guilan)	Iran	0	6	12
65	Mesri	Iran	0	9	13
66	Local cultivar (Isfahan)	Iran	14	18	18
67	Abbas-Shoori-Varamin	Iran	0	14	21
68	Local cultivar (Mashad)	Iran	8	12	23

(*continued on next page*)

Table 2. (*continued from preceding page*)

Number	Cultivar	Origin	Disease incidence (%) Days after inoculation 2	5	7
69	Ivankay	Iran	0	10	30
70	Local cultivar (Orumieh)	Iran	0	16	33
71	Local cultivar (Tehran)	Iran	8	10	36
72	Khaghani	Iran	8	11	41
73	Isin	Iran	8	11	43
74	Local cultivar (Kermanshah)	Iran	25	25	44
75	Mosagholi-e-Marand	Iran	0	37	47
76	Ebrahimi	Iran	4	28	50
77	Dr. Kopaie (Guilan)	Iran	0	16	53
78	Shadekan	Iran	0	36	54
79	Local cultivar (Khorramshahr)	Iran	0	11	61
80	Local cultivar (Yazd)	Iran	5	45	70
81	Local cultivar (Isfahan)	Iran	10	73	73
82	Local cultivar (Isfahan)	Iran	73	73	79
83	Ivankay-Danesh Field	Iran	16	34	87
84	Local cultivar (Isfahan)	Iran	7	60	93
85	Zarandi	Iran	19	93	93
86	Molabashi	Iran	9	9	97
87	Local cultivar (Kashan)	Iran	3	22	97
88	Local cultivar (Khash)	Iran	50	64	100
89	Seied-Mirza	Iran	34	75	100
90	Makhaloote	Iran	45	82	100
91	Afrapooli	Iran	16	92	100
92	Local cultivar (Varamin)	Iran	61	95	100
93	Lakie	Iran	69	95	100
94	Local cultivar (Isfahan)	Iran	50	100	100
	***Cucumis melo* var. *reticulatus* (cantaloupe)**				
95	Gold & Silver	Japan	0	0	0
96	Local cultivar (Mirnab)	Iran	0	0	3
97	Local cultivar (Isfahan)	Iran	0	12	17
98	PMR-45	United States	4	12	25
99	Semsoori	Iran	0	31	38
100	Persian Small Type	United States	8	38	38
101	Local cultivar (Isfahan)	Iran	4	23	41
102	Local cultivar (Mashad)	Iran	3	56	63
103	Local cultivar (Isfahan)	Iran	4	28	72
104	Semsoori-6003	Iran	8	67	74
105	Local cultivar (Kerman)	Iran	26	61	83
106	Cici	Iran	16	84	84
107	Harvest Queen	United States	36	91	91
108	Local cultivar (Mashad)	Iran	2	70	97
109	Local cultivar (Kermanshah)	Iran	13	40	100
110	Afrapooli	Iran	6	67	100
111	Doar-e-Shahri	Iran	9	81	100
112	Shahd-e-Shiraz	Iran	13	81	100
113	Local cultivar (Varamin)	Iran	6	82	100
114	Local cultivar (Mashad)	Iran	40	86	100
115	Semsoori-3642	Iran	6	88	100
116	Israeli-6014	Israel	39	91	100

[a] Seedlings 15–20 days old were inoculated with 1 ml of a suspension containing zoospores (5×10^3/ml) of isolate P-40 around the hypocotyl of each plant and kept at 30 C under 1,400 lux of white fluorescent and incandescent illumination.

were obtained within a short period of time. In contrast to other reports (12), light did not affect sporangial formation. It is not known whether light intensity would affect development of sporangia.

In our seedling screening tests for resistance to *P. drechsleri,* cantaloupes were the most susceptible of the various cucurbits evaluated. Their susceptibility likely accounts for the widespread occurrence of root rot in cantaloupe fields throughout the Fars province of Iran. Cultivars of *Cucurbita pepo* resistant to *P. drechsleri* in our tests also have resistance to *Pythium aphanidermatum* (Edson) Fitz. (14). Although we have never observed root rot caused by *Pythium aphanidermatum* or *P. drechsleri* in squash or pumpkin under field conditions, we found root rot due to *Phytophthora capsici* Leonian in a field of squash in the Fars province. Cucumber Ohio MR 17 and watermelon cultivar Klondike No. 7 were susceptible to *Pythium aphanidermatum* (14) but were highly resistant to *P. drechsleri,* whereas watermelon cultivar Charleston Gray and cantaloupe cultivar Gold & Silver were resistant to both pathogens.

Because no effective controls have been found to prevent cucurbit root rot incited by *P. drechsleri* or *Pythium aphanidermatum,* the use of resistant cultivars remains the most promising means of control. Our results and those reported by others (14) indicate that a pool of resistant genes exists among cucurbits that could be incorporated into more desirable cultivars in a breeding program. The present screening method should be useful for evaluating resistance of cultivars and lines in breeding programs.

ACKNOWLEDGMENT

The authors are grateful to Dr. Iraj Poostchi, Department of Agronomy, University of Shiraz, for supplying seeds of native cultivars of cucurbits.

LITERATURE CITED

1. Banihashemi, Z. 1970. A new technique for isolation of *Phytophthora* and *Pythium* species from soil. Plant Dis. Rep. 54:261-262.
2. Banihashemi, Z. 1975. Phytophthora black stem rot of sunflower. Plant Dis. Rep. 59:721-724.
3. Barash, I., Klisievicz, J. M., and Kosuge, T. 1965. Utilization of carbon compounds by zoospores of *Phytophthora drechsleri* and their effect on

Table 3. Production of sporangia and zoospores of *Phytophthora drechsleri* as affected by culture medium and sterility of soil extract

Medium[a]	Age of culture (days)	Unsterile soil extract Sporangia (no.)[b]	Zoospores[c] (no. × 10^3/ml)	Sterile soil extract Autoclaved[d] Sporangia (no.)	Zoospores (no. × 10^3/ml)	Filtered[e] Sporangia (no.)	Zoospores (no. × 10^3/ml)
LBA	5	5.6	5	13.1	100	42.8	450
CV8A	7	2.4	Few	14.1	100	38.5	300
CMA	7	0.0	0	0.0	0	0.0	0
PDA	8	0.0	0	0.0	0	0.0	0

[a] LBA = lima bean agar, CV8A = cleared V-8 juice agar, CMA = cornmeal agar, and PDA = potato-dextrose agar.
[b] Mean of 10 counts (×100 magnification field) in four petri plates incubated for 10 min.
[c] Mean of four counts in four petri plates incubated at room temperature for 10 min.
[d] Autoclaved for 15 min at 121 C.
[e] Cleared soil extract passed through 0.22-μm Millipore filter.

motility and germination. Phytopathology 55:1257-1261.
4. Cother, E. J. 1975. *Phytophthora drechsleri*: Pathogenic testing and determination of effective host range. Aust. J. Bot. 23:87-94.
5. Cother, E. J., and Griffin, D. M. 1973. The role of alternate hosts in survival of *Phytophthora drechsleri*. Aust. J. Biol. Sci. 26:1109-1113.
6. Ershad, D. 1971. Beitrag zur Kenntnis der Phytophthora Arten in Iran und ihrer phytopatholoischen Bedeutung. Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem 140. 84 pp.
7. Erwin, D. 1952. Phytophthora root rot of safflower. Phytopathology 42:32-35.
8. Fatemi, J. 1971. Phytophthora and Pythium root rot of sugarbeet in Iran. Phytopathol. Z. 71:25-28.
9. Heather, W. A., and Pratt, B. H. 1975. Association of *Phytophthora drechsleri* Tucker with death of *Pinus radiata* in southern New South Wales. Aust. J. Bot. 23:285-288.
10. Mansoori, B. 1980. The biology and ecology of *Phytophthora drechsleri* Tucker, the incitant of cucurbit root and crown rot in Fars Province, M.S. thesis. University of Shiraz, Shiraz, Iran. 62 pp.
11. Mehrotra, R. S. 1970. Techniques for demonstrating accumulation of zoospores of *Phytophthora* species on root in soil. Can. J. Bot. 48:879-887.
12. Merz, W. G. 1964. Effect of light on sporulation of heterothallic species of *Phytophthora*. (Abstr.) Phytopathology 54:900.
13. Mircetich, S. M., and Matheron, M. C. 1976. Phytophthora root rot and crown rot of cherry trees. Phytopathology 66:549-558.
14. Rahimian, M. K., and Banihashemi, Z. 1979. A method for obtaining zoospores of *Pythium aphanidermatum* and their use in determining cucurbit seedling resistance to damping-off. Plant Dis. Rep. 63:658-661.