Aspects of the Protection of Cucumber Against Colletotrichum lagenarium by Colletotrichum lagenarium

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The research reported in this paper has been supported in part by a grant from the Herman Frasch Foundation and Grant No. 316-15-51 under P. L. 89-106 of the Cooperative States Research Service of the United States Department of Agriculture.
Accepted for publication 6 October 1976.

ABSTRACT

KUĆ, J., and S. RICHMOND. 1977. Aspects of the protection of cucumber against Colletotrichum lagenarium by Colletotrichum lagenarium. Phytopathology 67:533-536.

Inoculation of a cotyledon or first true leaf (leaf 1) of cucumber with *Colletotrichum lagenarium* systemically protected cucumber plants against disease caused by subsequent challenge inoculations by this pathogen. Protection was evident as a reduction in the number and size of lesions. Inoculation of leaf 1 when the second true leaf (leaf 2) was one-fourth to one-third expanded, systemically protected plants for 4-5 wk. A second or booster inoculation, 3 wk after the first inoculation, extended the time of protection into the fruiting period. Protection was elicited by and effective against two races of the fungus and was evident, not only with susceptible cultivars, but also with two cultivars which have resistance to anthracnose. A direct relationship existed between the number of spores used for protection and the extent and duration of protection. A single lesion produced significant protection. Protection of leaf 2 was evident 96 hr after leaf 1 was inoculated. Excising leaf 1 96 hr after inoculation did not reduce protection of leaf 2. Leaf 2 was protected if excised 120 hr after leaf 1 was inoculated.

Additional key words: induced resistance, anthracnose.

Reports of acquired physiological immunity in plants are not new (2). Induced resistance to virus in virus-infected plants has been verified in numerous laboratories (6). Few reports are available describing the protection of plants against a fungal pathogen by the same pathogen (1, 3, 5, 7). In an earlier paper, we reported that cucumber was systemically protected against *Colletotrichum lagenarium* (Pass.) Ell. & Halst. race 1, by prior inoculation with *C. lagenarium* (5). This report describes the duration and characteristics of the protection.

MATERIALS AND METHODS

Plants, fungus, and inoculation procedures.—Cultures of *Colletotrichum lagenarium* (Pass.) Ell. & Halst. race 1, 2, 3 (4) were maintained on green bean agar at 24 C in the dark. Spore suspensions were prepared from 7- to 14-day-old cultures. Except as noted, the cucumber cultivar SMR-58 was used in all experiments. To determine the duration of protection, plants were grown in 20.3-cm diameter plastic pots containing a mixture of loam, peat moss, and sand (1:1:1, v/v). Plants were watered with nutrient solution (Ra-Pid-Gro, Dansville, NY 14437) every 2 wk after emerging. Plants for all other experiments were grown in 10.2-cm diameter plastic pots containing a synthetic soil mixture (Redi-Earth, Grace Products, Cambridge, MA 02140). All plants were grown in a greenhouse at 23-31 C with 14 hr of light. Except as noted, the first true leaf (leaf 1) was inoculated with 40 5-μliter drops of a conidial suspension of *C. lagenarium* (5×10^5 spores/ml) when the second (leaf 2) was one-fourth to one-third expanded. Control plants had 40 5-μliter drops of water applied to leaf 1. After inoculum or water had been applied, plants were incubated in closed moist chambers for 24 hr and then in partially opened moist chambers for an additional 24 hr at 22-28 C. Except as noted, 7 days after inoculation of leaf 1, leaf 2 was inoculated with 40 5-μliter drops of a conidial suspension of *C. lagenarium* (10^5 spores/ml). Plants were again incubated as described and symptoms recorded 4, 5, and 6 days after the inoculation of leaf 2.

Cotyledon protection.—Either one or both cotyledons, approximately two-thirds expanded, were inoculated with 20 5-μliter drops of a spore suspension of *C. lagenarium*. Control plants had 20 drops of water added to one or both cotyledons. Seven days after inoculation, either one cotyledon or leaf 1 (approximately two-thirds expanded) was inoculated with *C. lagenarium*. Each treatment contained five plants per experiment and the experiment was repeated twice.

Duration.—One week after inoculation or application of water to leaf 1, all leaves (one-third or more expanded) above leaf 1 were inoculated. The procedure was repeated weekly using three protected and three unprotected plants per wk. The experiment was repeated once.

Different races of fungus.—Leaf 1 was inoculated with a spore suspension of race 1, 2, or 3 of *C. lagenarium* and after 7 days leaf 2 was inoculated with race 1, 2, or 3 of the fungus. Nine plants were used per treatment in a single experiment.

Different cultivars of cucumber.—The effectiveness of protection was tested with the susceptible cultivars

Straight Eight and SMR-58, and with Polaris and Poinsette, both with resistance to anthracnose. Six plants were used per treatment per experiment, and the experiment was repeated once.

Effect of inoculum concentration and number of lesions.—To determine the effect of inoculum concentration on the effectiveness of protection, leaf 1 was inoculated with 10^3, 10^4, 10^5, or 10^6 spores/ml. To determine the effect of lesion numbers on leaf 1 on the effectiveness of protection, leaf 1 was inoculated with one, two, five, 10, 20, or 30 5-μliter drops of inoculum. Control plants had 30 drops of water applied to leaf 1. Nine plants were used per treatment. The experiment was repeated once.

Interval between protection and challenge.—The effect of the time interval between the inoculation of leaf 1 and leaf 2 on the effectiveness of protection of leaf 2 was studied in three experiments. Each treatment involved six plants and each experiment was done three times. In one experiment, leaf 2 was inoculated 48, 72, 96, 120, or 144 hr after the first inoculation. In the second experiment, leaf 1 was removed 24, 48, 60, 72, 96, or 120 hr after inoculation, and leaf 2 was inoculated 9 days after inoculating leaf 1. In the third experiment, leaf 2 was excised 120 hr after leaf 1 was inoculated or treated with water. Leaf 2 was supported on a sheet of aluminum foil which was spread over a pyrex baking dish. The petiole passed through a small hole in the foil and dipped into water. Leaf 2 was inoculated immediately after excision or after being held in the pyrex baking dish for 48 hr. Baking dishes containing leaves were covered with a second baking dish, the inside of which was sprayed with water. Dishes were held together with masking tape, and the tape was removed 24 hr after the leaves were inoculated. Dishes were held at 21-23 C on laboratory benches receiving approximately 12 hr of diffuse fluorescent light.

RESULTS

Cotyledon protection.—Inoculation of a single

TABLE 1. Protection of the first true leaf (leaf 1) and cotyledons of cucumber against *Colletotrichum lagenarium* by inoculating cotyledons with *C. lagenarium*

First inoculation	Time after inoculation of leaf 1 or cotyledon (second inoculation) (days)	Average number of lesions/leaf 1 or cotyledon[a]	
		Leaf 1	Cotyledon
One cotyldeon	4	0 (0-4)	0
	5	8 (2-10)	2 (0-5)
	6	12 (4-14)	5 (0-8)
Two cotyledons	4	0	
	5	5 (0-6)	
	6	6 (2-14)	
Unprotected[b]	4	25 (14-33)	3 (0-10)
	5	38 (34-40)	15 (9-17)
	6	38 (35-40)	19 (17-20)

[a]Data are the average of 15 plants. Figures in parentheses are the range in the number of lesions.

[b]Water applied to one or both cotyledons.

TABLE 2. Duration of protection in cucumber elicited by *Colletotrichum lagenarium* against *C. lagenarium*[a]

Weeks after first inoculation	Leaves/plant[b]	Average number of lesions/leaf[c]	
		Protected	Unprotected
2	3	3 (0-10)	34 (29-38)
3	6	2 (0-23)	34 (12-40)
4	8	3 (0-23)	36 (22-40)
5	12	16 (1-34)	31 (5-40)
6	22	22 (0-40)	29 (1-40)

[a]Symptoms determined 6 days after challenge inoculation. Data are the average of six plants per treatment per interval. Figures in parentheses are the range in the number of lesions.

[b]Leaves at least one-half expanded were counted.

[c]Lesions on protected plants generally are restricted and chlorotic, whereas lesions on unprotected plants enlarge and become necrotic.

TABLE 3. Protection of cucumber by and against race 1 and 3 of *Colletotrichum lagenarium*[a]

Treatment[b]	Average number of lesions/leaf 2[c]
W-1	40 (38-40)
1-1	6 (0-10)
1-3	1 (0-3)
W-3	26 (18-30)
3-1	24 (12-29)
3-3	3 (1-6)

[a]First true leaf (leaf 1) was inoculated when the second true leaf (leaf 2) was one-fourth to one-third expanded, leaf 2 was inoculated 7 days later, and the number of lesions on leaf 2 was counted 7 days after that.

[b]Coding of treatments: W-1, water applied to leaf 1 and inoculum of race 1 applied to leaf 2; 1-1, inoculum of race 1 applied to leaf 1 and leaf 2; 1-3, inoculum of race 1 applied to leaf 1 and inoculum of race 3 applied to leaf 2.

[c]Determined 7 days after inoculating leaf 2. Data are the average of nine plants. Figures in parentheses are the range in the number of lesions.

TABLE 4. Protection against *Colletotrichum lagenarium* by *C. lagenarium* in two resistant and two susceptible cucumber cultivars[a]

Cultivar	Disease reaction	Average number of lesions/leaf 2[b]	
		Unprotected	Protected
Straight 8	Susceptible	39 (36-40)	11 (2-14)
SMR-58	Susceptible	37 (33-40)	8 (6-11)
Polaris	Resistant	29 (24-35)	7 (4-8)
Poinsette	Resistant	18 (16-22)	0

[a]First true leaf (leaf 1) was inoculated when the second true leaf (leaf 2) was one-fourth to one-third expanded, leaf 2 was inoculated 7 days later, and the number of lesions on leaf 2 was counted 5 days after that.

[b]Determined 5 days after inoculating leaf 2. Lesions on unprotected Polaris and Poinsette were chlorotic, and highly restricted with little or no necrosis. Lesions on all protected plants were similar to those on unprotected Polaris and Poinsette. The data are the average of 12 plants. Figures in parentheses are the range in the number of lesions.

cotyledon protected the other cotyledon as well as leaf 1 from disease caused by subsequent challenge inoculation (Table 1).

Duration of protection.—Inoculation of leaf 1 protected plants for 4-5 wk (Table 2). At the end of 5 wk plants were 100-120 cm long. Protection was lost over the entire plant after 5-6 wk. A second inoculation of entire plants, 3 wk after inoculating leaf 1, extended the period of protection into the fruiting period. After 8 wk, an average of 27 and 4 lesions per leaf were found on unprotected and protected plants, respectively. This enhanced protection was evident even though few lesions were apparent on plants after the second (booster) inoculation.

Different races of fungus.—Race 2 was virulent on watermelon but not cucumber and did not elicit protection against race 1 or 3. Race 1 was more virulent than race 3 and protected well against race 1 or 3 (Table 3). Race 3 protected better against race 3 than against race 1.

Different cultivars of cucumber.—When compared to cultivars Straight 8 and SMR-58, the cultivars Polaris and Poinsette had some resistance to anthracnose. Resistance was evident as a delay in lesion development (lesions visible 3-4 and 4-5 days after inoculation in susceptible and resistant cultivars, respectively) and a reduction in lesion number and extent of necrosis. Poinsette was more resistant than Polaris. Protection was evident with the four cultivars (Table 4).

Effect of inoculum concentration and number of lesions.—A spore concentration of 10^3 spores/ml applied to leaf 1 was sufficient to protect leaf 2 against disease caused by 10^5 spores/ml of inoculum (Table 5). One lesion (5×10^5 spores/ml) on leaf 1 was sufficient to protect leaf 2, and maximum protection was evident with 5-10 lesions on leaf 1 (Table 6). Though one or two lesions on leaf 1 protected leaf 2, this protection diminished with time. The average lesion diameter 7 days after inoculation of leaf 2 on protected and unprotected plants was 0.5 mm and 3.5 mm, respectively.

Interval between protection and challenge.—Protection was evident with leaf 2 96 hr after inoculating leaf 1 (leaf 2 on unprotected and protected plants had an average of 36 and 21 lesions, respectively). Excising leaf 1 96 hr after inoculation of leaf 1 did not reduce protection of leaf 2 (Table 7). Leaf 2 was protected if excised 96-120 hr after inoculation of leaf 1 (Table 7).

TABLE 5. The effect of inoculum concentration of *Colletotrichum lagenarium* applied to the first true leaf (leaf 1) on the protection of the second true leaf (leaf 2) of cucumber against the fungus

Concentration of inoculum (conidia/ml) applied to leaf 1	Lesions/leaf 2[a]
0[b]	38 (30-45)
10^3	12 (2-28)
10^4	8 (1-20)
10^5	5 (2-10)
10^6	6 (1-17)

[a]Data are the average of 18 plants. Figures in parentheses are the range in the number of lesions.
[b]Water was applied to leaf 1.

DISCUSSION

Protection of eight cucumber cultivars against *C. lagenarium,* race 1, has been reported (5). Data in this paper indicate that protection is enhanced in resistant cultivars and is not limited to a single race of the fungus. The development and nature of symptoms on the two unprotected resistant cucumber cultivars and protected susceptible cultivars appear similar. In both, appearance of symptoms was delayed, lesion number and size were reduced, and lesions were chlorotic with reduced necrosis. The efficacy and systemic nature of protection is clearly depicted by the duration of protection (4-5 wk) and the protection obtained from a single lesion. The loss of protection from the entire plant, rather than from foliage most distant from leaf 1, indicates a dilution of protectant is unlikely to be the only reason for the loss of protection.

TABLE 6. The effect of the number of lesions caused by *Colletotrichum lagenarium* on the first true leaf (leaf 1) of cucumber on the protection of the second true leaf (leaf 2) against *C. lagenarium*

No. of lesions on leaf 1 (first inoculation)	Area (mm^2) of lesions on leaf 2 after:[a]		
	4 days	5 days	6 days
0[b]	15	120	135
1	1	15	58
2	1	4	30
5	1	4	10
10	<1	2	3
20	<1	2	3
30	<1	1	2

[a]Data are the average of 18 plants.
[b]Water (40 5-μliter drops) applied to leaf 1.

TABLE 7. The effect of the time of the excising of the first true leaf (leaf 1) of cucumber or the second true leaf (leaf 2) on the protection of leaf 2 against *Colletotrichum lagenarium*[a]

Inoculation of leaf 1 followed by excision of:	Time between inoculation of leaf 1 and excision (hr)	Average number of lesions per leaf 2 5 days after inoculation of leaf 2[b]
Leaf 1	24	30 (27-33)
	48	32 (29-34)
	60	37 (35-40)
	72	19[c] (10-24)
	96	13 (11-16)
	120	12 (6-14)
Leaf 2	120[d]	3 (0-8)
	120[e]	5 (0-7)

[a]Leaf 2 was inoculated 9 days after the inoculation of leaf 1 in experiments where leaf 1 was excised. Leaf 2 was inoculated immediately or 48 hr after excision in experiments in which leaf 2 was excised.
[b]Data are the average of 18 plants per treatment. Figures in parentheses are the range in the number of lesions. Control plants with water applied to leaf 1 had 30-40 lesions per leaf 2.
[c]An average of 37 lesions per leaf were evident with this treatment 9 days after inoculation.
[d]Leaf 2 inoculated immediately after excision.
[e]Leaf 2 inoculated 48 hr after excision.

The ability to enhance protection with a booster inoculation, which itself produces few lesions, suggests injury of tissue is not the only determinant of protection. This is consistent with our report that injury with dry ice did not elicit protection (5). Pollution and chemical injury to leaf 1 also did not elicit protection in other tests in our laboratory. Though approximately 96 hr are necessary between the inoculation of leaf 1 and the onset of definite protection in leaf 2, once protection is initiated, leaf 1 is not necessary for the maintenance of protection. This is supported further by the observation that once protection is elicited in leaf 2, leaf 1 can be excised without loss of protection. The initiation, maintenance, and loss of protection appear systemic.

The sensitivity and systemic character of the response, duration of protection, effect of a booster inoculation, and limited requirement of leaf 1 for initiation of protection all outwardly resemble the immunization process in animals. At the molecular level, however, the immune response as described for animals has not been reported in plants.

LITERATURE CITED

1. BRAUN, J. W., and A. W. HELTON. 1971. Induced resistance to Cytospora in Prunus persica. Phytopathology 61:685-687.
2. CHESTER, K. 1933. The problem of acquired physiological immunity in plants. Quart. Rev. Biol. 8:129-151, 275-324.
3. CRUICKSHANK, I. A. M., and M. MANDRYK. 1960. The effect of stem infestation of tobacco with Peronospora tabacina Adam. J. Austr. Inst. Agric. Sci. 26 (4):369-372.
4. GOODE, M. J. 1958. Physiological specialization in Colletotrichum lagenarium. Phytopathology 48:79-83.
5. KUĆ, J., SHOCKLEY, G., and K. KEARNEY. 1975. Protection of cucumber against Colletotrichum lagenarium by Colletotrichum lagenarium. Physiol. Plant Pathol. 7:195-199.
6. LOEBENSTEIN, G. 1972. Localization and induced resistance in virus-infected plants. Annu. Rev. Phytopathol. 10:177-206.
7. RANDALL, H., and A. W. HELTON. 1976. Effect of inoculation date on induction of resistance to Cytospora in Italian prune trees by Cytospora cincta. Phytopathology 66:206-207.