Field Protection of Cucumber, Watermelon, and Muskmelon 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 316-15-51 of the Cooperative States Research Service of the United States Department of Agriculture.

Accepted for publication 18 March 1977.

ABSTRACT

CARUSO, F. L., and J. KUĆ. 1977. Field protection of cucumber, watermelon, and muskmelon against Colletotrichum lagenarium by Colletotrichum lagenarium. Phytopathology 67:1290-1292.

In three separate field trials, cucumber plants were partially protected against a challenge inoculation with *Colletotrichum lagenarium* by a prior inoculation with the pathogen. Protection of watermelon was evident in two trials and indications were that muskmelon also could be protected. Lesions produced on protected plants were reduced in number and size compared to those produced on unprotected plants. This protection phenomenon seems to be expressed in the field as well as in the greenhouse.

Additional key words: biological control, immunization.

Systemic protection by *Colletotrichum lagenarium* against *C. lagenarium* has been demonstrated in cucumber (5, 6), watermelon (2), and muskmelon (2). Since these reports concerned greenhouse experiments under controlled conditions, it was important to determine whether this phenomenon could be demonstrated in the field. This paper indicates that protection also can be elicited under field conditions. Preliminary reports of this have been published (1, 4).

MATERIALS AND METHODS

Pathogen and hosts.—*Colletotrichum lagenarium* (Pass.) Ell. & Halst. (race 1) was maintained on bean pod agar at 24 C in the dark. Spore suspensions were prepared from 6- to 14-day-old cultures.

Cucumbers (*Cucumis sativum* L. 'Wisconsin SMR 58' and 'Marketer'), watermelons (*Citrullus vulgaris* Schard. 'Sugar Baby') and muskmelons (*Cucumis melo* L. 'Iroquois') were tested. Plants were grown in 10-cm diameter plastic pots containing a mixture of soil:peat:sand (2:1:1, v/v) or Pro-Mix BX (Premier Brands, Inc., Premier Peat Moss Corp., New York, NY 10036). Plants received nutrient solution (Ra-Pid-Gro, Dansville, NY 14437) every 2 wk and were maintained in a greenhouse at 23-31 C with daylight supplemented with 14 hr of fluorescent and incandescent light.

Inoculations.—Except as noted, the first true leaf of plants (in the two-leaf stage) was inoculated as described (5). One wk after the first leaf had been inoculated, plants were transplanted to the field and shaded for 2 days. Plants were set in rows 2.5 m apart and were individually spaced 2.5 m apart in the row. Challenge inoculations were done at dusk. Inoculum was sprayed on stems and both leaf surfaces of the entire plant above ground until runoff and the plants were enclosed in plastic bags to maintain high humidity. Newspaper was placed over the plants to reduce day temperatures inside the bags. Bags were removed after 24 hr. Three separate field trials were conducted.

Trial 1 (1975).—Water or protective inoculum (10^5 spores/ml) was sprayed on the first true leaf of cucumber (SMR 58 and Marketer), watermelon, and muskmelon plants. Ten days after planting, all leaves of protected plants were sprayed with a booster protective inoculum of 10^4 spores/ml; at this stage, there were six leaves per plant. Seven days after the booster inoculation, plants were sprayed with challenge inoculum (5×10^5 spores/ml) or water. There were four treatments: (i) protected/challenged (P/B/C-inoculated on the first leaf, received booster and challenge inoculations); (ii) protected/unchallenged (P/B/UC-inoculated on the first leaf and received booster inoculation); (iii) unprotected/challenged (UP/C – received only the challenge inoculation); and (iv) unprotected/unchallenged (UP/UC – not inoculated). There were three replicates of 25 plants per treatment. The number of lesions per leaf and the number of leaves with lesions were counted 9 days after the challenge inoculation.

Trial 2 (1976).—Leaf one of cucumber (SMR 58) and muskmelon plants was sprayed with protective inoculum (10^6 spores/ml) or water. Twelve days after transplanting, plants were sprayed with challenge inoculum (10^7 spores/ml) or water. Four treatments were established: UP/C, P/C, P/UC, and UP/UC. There were 18 and 14 plants per treatment with cucumber and muskmelon, respectively. Symptoms were rated 0-5: 0 = no symptoms; 1 = trace - 20% of leaf area covered by lesions; 2 = 20-40%; 3 = 40-60%; 4 = 60-85% with growing point alive; 5 = 85-100% or complete death. Eleven days after challenge,

TABLE 1. Protection of cucumber plants against *Colletotrichum lagenarium* by *Colletotrichum lagenarium* (Trial 1)

	Cultivars			
	SMR 58		Marketer	
	Lesions/leaf[a]	Leaves with lesions[b]	Lesions/leaf[a]	Leaves with lesions[b]
Test 1[c]				
UP/C	91.4(28-160)	13.3(10-9)	88.2(26-148)	18.0(14-25)
P/B/C	12.8(0-34)	6.2(0-11)	40.0(6-82)	7.5(7-8)
Test 2				
UP/C	61.2(15-128)	10.0(7-18)	67.8(16-143)	15.2(12-19)
P/B/C	10.4(2-46)	3.8(2-9)	46.7(5-151)	7.8(6-13)
Test 3				
UP/C	81.4(4-170)	12.2(7-13)		
P/B/C	14.7(2-83)	5.8(1-6)		

[a]Values are the mean numbers of lesions per leaf with ranges in parentheses.

[b]Values are the mean number of leaves with lesions per plant with ranges in parentheses. Unprotected and protected SMR 58 plants had an average of 16 and unprotected and protected Marketer plants had an average of 20 leaves per plant.

[c]Each test consisted of 25 plants per treatment. Data were recorded 9 days after challenge.

TABLE 2. Protection of Wisconsin SMR 58 cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium* (Trial 2)

Treatment	Days after challenge	Disease ratings[a]			
		UP/C	P/C	P/UC	UP/UC
1st challenge[b]	7	3.6	2.0	0.0	0.0
	8	3.6	2.1	0.0	0.0
2nd challenge[c]	4	0.4	0.3	1.3	2.4
	5	1.0	1.0	3.0	3.9
	6	1.7	2.0	3.2	3.9
	9	2.2	2.5	3.5	4.3
	12	2.5	2.6	3.5	4.1

[a]Plants were rated by at least two people each time; a 0-rating represented no symptoms, a 5-rating represented 85-100% of leaf surface covered by lesions or plant dead – see text. UP/C = unprotected and challenged, P/C = protected and challenged, P/UC = protected and unchallenged, UP/UC = unprotected and unchallenged.

[b]UP/C and P/C were inoculated until runoff with *C. lagenarium*.

[c]Plants in all treatments were inoculated with *C. lagenarium* until runoff.

plants were sending out healthy shoot growth. After allowing plants to grow for 4 additional days, plants were re-challenged (UP/C, P/C) or challenged (UP/UC, P/UC) with 10^6 spores/ml. Plants were rated with the same numerical system.

Trial 3 (1976).—Leaf one of cucumber (SMR 58) and watermelon plants was sprayed with water or protective inoculum (10^6 spores/ml). Fourteen days after the inoculation of leaf one, 40 drops (5 μliter) of challenge inoculum (5×10^5 spores/ml) were put on leaf two and 10 drops on leaf three of cucumber. Twenty drops were put on leaf two and 10 drops on leaf three of watermelon. There were 18 and 14 plants per treatment with cucumber and watermelon, respectively. Lesions were counted and their diameters determined from a random sample of 50 lesions per treatment.

RESULTS

Trial 1.—Unprotected cucumber plants (SMR 58 and Marketer) had considerably more lesions on more leaves after challenge than did protected plants (Table 1). Protection was greater for SMR 58 than Marketer. Data from one replicate with Marketer were lost.

Lesions could not be accurately counted on watermelon plants; unprotected plants were heavily covered with stem lesions and the leaves wilted and withered very rapidly. Protection was very evident 13 days after challenge when 47 of 69 unprotected plants died to the ground as compared to only 1 of the 66 protected plants. Many of the plants that died to the ground subsequently resumed growth. Muskmelon plants did not develop symptoms in any of the treatments after the challenge.

Trial 2.—Unprotected cucumber plants had a disease rating of 3.6 (Table 2); 50-60% of the leaf area was covered by lesions. Protected plants had a disease rating of 2.1; plants had 20-25% of the leaf area covered by lesions. Protection was even more striking after the second challenge. Plants not inoculated on leaf one and not receiving the first challenge (UP/UC) had the highest rating of 4.3. Plants which had been inoculated on leaf one 34 days prior to challenge (P/UC) had reduced symptoms, a 3.5 rating. Eight UP/UC plants died, whereas all of the P/UC plants survived. Symptoms also were reduced on the plants that had been inoculated with only the first challenge (UP/C), and plants which had been inoculated on leaf one and received the first challenge (P/C). Ratings for these treatments were 2.5 and 2.6, respectively.

The first challenge inoculum on muskmelon (10^7 spores/ml) caused many stem lesions and mortality was high. However, the ratings of the surviving plants in the UP/C (0.7) and P/C (0.3) treatments after the second challenge were considerably lower than that in the P/UC (2.1) and UP/UC (2.5) treatments.

Trial 3.—Excellent protection was achieved in cucumber plants (Table 3). On leaf two, 90% of the drops

TABLE 3. Protection of Wisconsin SMR 58 cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium* as expressed by the number of lesions (Trial 3)

Days after challenge	Mean number of lesions per leaf[a]			
	UP/C[b]		P/C[b]	
	leaf two	leaf three	leaf two	leaf three
4	14.4	7.3	1.7***[c]	0.3***
5	26.5	9.1	5.8***	2.8***
6	30.2	9.7	7.6***	2.4***
7	31.8	9.8	5.8***	2.6***
8	36.1	10.9	7.2***	3.6***

[a]Forty drops of inoculum were put on leaf two, ten drops on leaf three.

[b]UP/C = unprotected and challenged; P/C = protected and challenged.

[c]Asterisks *** indicate that mean lesion numbers were significantly different from the controls, $P = 0.001$.

TABLE 4. Protection of Wisconsin SMR 58 cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium* as expressed by lesion diameter and area of lesions (Trial 3)

Challenged leaf	Mean diameter of lesions (mm)[a]		Area of lesions per leaf (mm^2)[a]	
	UP/C[b]	P/C[b]	UP/C	P/C
leaf two	4.1	1.4***[c]	485.9	11.6***
leaf three	3.5	0.4***	108.5	0.5***

[a]Area of necrosis recorded 8 days after challenge.

[b]UP/C = unprotected and challenged, P/C = protected and challenged.

[c]Asterisks indicate that mean diameters and areas of lesions were significantly different from the controls, $P = 0.001$.

of challenge inoculum developed into lesions on unprotected plants versus 18% for protected plants. On leaf three, 100% of the drops of inoculum developed into lesions by 8 days after the challenge on unprotected plants versus 36% for protected plants. The area of lesion necrosis on both leaves two and three was considerably less for protected plants (Table 4). The total lesion area on protected plants was 2.4% (leaf two) and 0.4% (leaf three) of the lesion area on unprotected plants.

Although symptoms after challenge were poor on watermelon plants, protection was indicated. The UP/C plants had an average of 7.2 and 2.4 lesions on leaves two and three, respectively, whereas the P/C plants had 1.7 and 0.3 lesions on their leaves.

DISCUSSION

The three field trials indicate that both cucumber and watermelon plants can be systemically protected against *C. lagenarium* by prior inoculation with the pathogen. The phenomenon probably also occurs in muskmelon although the evidence was inconclusive.

Protection was expressed in the two cucumber cultivars as a reduction in the number and size of lesions.

Although protection also was noted in watermelon as a reduction in the number and size of lesions, it was demonstrated best in the survival of these plants (65 of 66) versus the unprotected plants (22 of 69). This was chiefly due to the greater numbers of stem lesions on watermelon as compared to cucumber. The stem lesions caused rapid wilting and death of plants.

To test protection in muskmelon, a more susceptible cultivar should be employed in future trials, perhaps either Delicious 51 or Honey Rock (2).

In a separate experiment, inoculation of one cotyledon gave negligible protection with watermelon, and muskmelon. The plants were rated as in trial 2, and 10 days after challenge the following ratings were noted: cucumber UP/C = 2.6, P/C = 1.5; watermelon UP/C = 1.9, P/C = 1.3; and muskmelon UP/C = 3.1, P/C = 2.8. The inoculation of one cotyledon may not be sufficient to elicit protection in watermelon and muskmelon in the field as compared to the greenhouse.

Application of the challenge inoculum on plants by spraying in trials one and two made it difficult to count lesions accurately, determine lesion size, and differentiate chlorotic from necrotic lesions. Application of the challenge inoculum in droplets as done in trial three permitted a more accurate estimation of the extent of protection.

Induced protection may provide a means of disease control. It is observed in the greenhouse and field. Inconclusive results have been obtained from fragmentary data concerning yield from UP/C, P/C, P/UC, and UP/UC treatments. The effect of treatments on yield will be studied further in 1977.

Induced protection may permit the use of high yielding and high quality cultivars that have been discarded. The elicitor of protection, once isolated and characterized, might be as effective as the living pathogen in eliciting protection. Though the concept of acquired physiological immunity was reviewed by Chester (3) more than 40 yr ago, few investigators have tested its validity in the greenhouse and field.

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