Protection of Watermelon and Muskmelon Against Colletotrichum lagenarium by Colletotrichum lagenarium

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ABSTRACT

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Inoculation of the cotyledons or first true leaf (leaf one) of four cultivars of watermelon and four cultivars of muskmelon with *Colletotrichum lagenarium* systemically protected the plants from disease caused by subsequent inoculation with this pathogen. Protection was noted as a reduction in the number and size of lesions. Plants remained protected 4 wk after the protecting inoculation; at this time plants possessed 10-14 leaves. Race 1, 2, and 3 of the fungus elicited protection. A single lesion on leaf one elicited significant protection and protection increased as the concentration of spores applied to leaf one were increased from 10^3 to 10^7 spores/ml. The pattern of protection reported resembles that already observed in cucumber against this pathogen.

Additional key words: cucurbit anthracnose, immunization.

Protection of plants against a fungal pathogen by the same pathogen has been demonstrated (3, 7, 9, 10). McLean (8) noted that watermelon cultivars resistant to race 1 of *Colletotrichum orbiculare* and susceptible to race 2, were slightly protected from race 2 by prior inoculation with race 1. Protection was concluded to be localized and not systemic. This paper describes systemic protection in watermelon and muskmelon similar to that described for cucumber (6, 7). A preliminary report of this work has been published (2).

MATERIALS AND METHODS

Pathogen and host.—Races 1, 2, and 3 of *Colletotrichum lagenarium* (Pass.) Ell. & Halst. (4) were maintained on bean pod agar at 24 C in the dark. Spore suspensions were prepared from 5- to 9-day-old cultures. Watermelon (*Citrullus vulgaris* Schard.) and muskmelon (*Cucumis melo* L.) plants were grown in 10 cm diameter plastic pots containing Pro-Mix BX (Premier Brands Inc., Premier Peat Moss Corp., New York, 10036). Plants used to study the duration of protection were transplanted at the four-leaf stage to 20-cm diameter plastic pots, and were trained to grow up a plant stake. A nutrient solution (Ra-Pid-Gro, Dansville, NY 14437) was applied biweekly after emergence of seedlings. Plants were maintained in a greenhouse at 23-31 C with a 14-hr photoperiod. The watermelon cultivar Sugarbaby and the muskmelon cultivar Iroquois were used for all experiments unless indicated otherwise.

Inoculations.—Procedures were those of Kuć and Richmond (6), except that the concentration of inducer inoculum was 1×10^6 spores/ml and the concentration of challenge inoculum was 5×10^5 spores/ml. Race 1 of the pathogen was used for all experiments unless indicated otherwise. Particular care was taken upon opening moist chambers after 24 hr of incubation to insure that heat damage to watermelon leaves was minimal (5).

Different cultivars.—Watermelon cultivars Charleston Grey, New Hampshire Midget, and Crimson Sweet and muskmelon cultivars Honey Rock, Samsun Hybrid, and Delicious 51 were inoculated on the first leaf with either race 1, 2, 3, or water. Each cultivar was challenged on the second leaf with thirty drops of the race which was most pathogenic to that host. Ten plants were used per treatment in a single experiment. Symptoms were recorded 6 days after the challenge inoculation.

Duration of protection.—Plants inoculated with inducer on leaf one were allowed to grow for 4 wk, after which time they had an additional 10-14 leaves. Ten leaves above the inducer leaf were inoculated at this time with 30 drops of inoculum. There were five plants per treatment, and the experiment was performed once. Symptoms were recorded 6 days after the challenge inoculation.

Cotyledon protection.—Inoculations were as described for cucumber cotyledons (6), except that 20 drops of 1×10^6 spores/ml. were applied to the cotyledon(s). Either leaf one or the opposite cotyledon was challenged 7 days later with 30 or 20 drops of inoculum, respectively. The experiment with a single cotyledon as inducer was performed four times, with 35 test plants. The experiment with both cotyledons as inducers was done once with 10 test plants.

Inoculum concentration.—Forty drops of water or inoculum containing 10^3, 10^4, 10^5, 10^6, or 10^7 spores/ml were applied to leaf one. Seven days later, the second leaf was challenged with thirty drops of 5×10^5 spores/ml.

Data were recorded 6 days after challenge of leaf two. The experiment was done three times, using eight plants per treatment.

Number of lesions.—Leaf one was inoculated with 0, 1, 3, 5, 10, 20, 30, or 40 drops of 1×10^6 spores/ml. The number of lesions that formed equaled the number of drops applied to leaf one. Seven days later, plants were inoculated on the second leaf with thirty drops of 5×10^5 spores/ml. Data were recorded 6 days after challenge of leaf two. The experiment was done three times, using eight plants per treatment.

RESULTS

Protection of different cultivars.—Race 1 was highly pathogenic on the three muskmelon cultivars and on New Hampshire Midget watermelon. It was chosen as the challenge for these hosts, whereas race 2 was chosen as the challenge for the other two watermelon cultivars. Protection was obtained for all cultivars (Table 1-3, Fig. 1-3). In some cultivars, protection was evident as a reduction in the number of lesions (Iroquois, Delicious 51, Samsun Hybrid, Sugar Baby, Charleston Grey, and Dixie Queen). Protection was always evident in the reduced size of the lesions, even in cultivars which expressed a hypersensitive reaction in response to the inducer race.

Duration of protection.—Watermelon and muskmelon plants were protected 4 wk after inoculation of leaf one. At this time plants had at least 10 leaves above leaf one (Table 2). Lesions were often small necrotic flecks or chlorotic areas on protected leaves as compared to expanded necrotic lesions on unprotected leaves.

Cotyledon protection.—Inoculation of one cotyledon protected the opposite cotyledon and leaf one from *C. lagenarium* (Table 3). Inoculation of two cotyledons enhanced protection of leaf one.

Inoculum concentration.—Protection of watermelon and muskmelon generally increased as the concentration of inoculum applied to leaf one increased between 10^3 to

TABLE 1. Protection in different watermelon and muskmelon cultivars against *Colletotrichum lagenarium* by *C. lagenarium*

Plant and cultivar	Treatment[a]	Host response to inducer[b]	Mean number lesions on leaf[c] two	Mean area occupied by lesions on leaf two (mm^2)
Muskmelon:				
Delicious 51	0-1	–	22.4(16-30)	171.8
	1-1	S	1.7(0-6)***[d]	6.3***
	2-1	S	2.8(1-5)***	10.4***
	3-1	mod S	2.7(1-5)***	9.4***
Samsun Hybrid	0-1	–	12.3(3-18)	68.7
	1-1	S	4.0(2-8)***	5.6***
	2-1	R	5.8(3-8)***	23.6***
	3-1	mod S	4.3(1-7)***	16.2***
Honey Rock	0-1	–	30.0(all 30)	295.8
	1-1	S	19.5(12-25)*	25.0***
	2-1	S	27.9(25-30)	114.6***
	3-1	R	29.3(28-30)	189.4***
Watermelon:				
New Hampshire Midget	0-1	–	24.2(15-30)	108.0
	1-1	S	21.2(12-28)	28.6***
	2-1	S	25.8(22-29)	54.4***
	3-1	S	26.5(23-30)	56.4***
Dixie Queen	0-2	–	14.9(8-19)*	42.8
	1-2	S	10.2(3-16)***	9.5***
	2-2	S	17.4(10-23)	7.3***
	3-2	mod S		20.7***
Charleston Grey	0-2	–	17.3(11-29)	25.6
	1-2	R	10.7(6-19)*	11.2***
	2-2	S	7.1(4-12)***	3.5***
	3-2	R	11.4(5-23)*	10.4***

[a]Treatment: 0-1, water applied to leaf one and race 1 applied to leaf two; 1-1, race one applied to both leaves; 2-1, race 2 applied to leaf one and race 1 applied to leaf two; 3-1, race 3 applied to leaf one and race one applied to leaf two.

[b]Symbols: S = susceptible, R = resistant, and mod S = moderately susceptible, lesions somewhat restricted in size but larger than the necrotic flecks characteristic of resistance.

[c]Data were recorded 6 days after challenge and are the average of ten plants. Figures in parentheses are the range in lesion numbers.

[d]Asterisks *** or * indicate that mean lesion numbers and areas were significantly different from the controls, $P = 0.001$ or 0.05, respectively.

10^7 spores/ml. (Fig. 1, 2). Lesions from the challenge inoculation were fewest on the plants inoculated with 10^7 spores/ml. A concentration of 10^3 spores/ml did not significantly protect muskmelon, but it did protect watermelon, as indicated by reduction in lesion size (Fig. 2).

Number of lesions.—A single lesion on leaf one elicited significant protection in both watermelon and muskmelon (Fig. 3). The area of necrosis on leaf two of protected muskmelon leaves was greatly reduced in size by even a single lesion on leaf one (Fig. 3). An increased number of lesions on leaf one did not significantly increase protection in muskmelon. Protection of leaf two of watermelon appeared to reach a maximum with 10-20 lesions on leaf one.

DISCUSSION

Infection by *C. lagenarium* in four cultivars of watermelon and muskmelon provided systemic protection against subsequent infection by *C. lagenarium*. This protection was analogous to that

TABLE 2. Duration of protection of watermelon and muskmelon by *Colletotrichum lagenarium* against *C. lagenarium* race 1[a]

Host	Leaf above inducer leaf[b]	Mean number of lesions per leaf	
		Protected	Unprotected
Watermelon	1	5.0(3-10)	6.2(3-8)
	2	2.0(0-3)	10.2(1-18)
	3	1.2(0-3)	6.4(3-11)
	4	3.0(0-7)	16.2(0-24)
	5	3.4(2-5)	17.6(3-26)
	6	3.6(1-6)	18.4(0-25)
	7	6.2(2-17)	17.4(5-27)
	8	8.8(2-21)	20.6(9-28)
	9	8.6(3-19)	19.8(3-29)
	10	10.0(1-25)	13.3(6-22)
Total lesions		51.8	146.1
Muskmelon	1	1.7(1-2)	7.6(4-14)
	2	1.7(1-3)	7.8(5-11)
	3	1.5(0-2)	7.0(6-10)
	4	2.0(1-3)	6.6(3-10)
	5	2.5(0-5)	8.0(6-11)
	6	1.5(0-4)	8.6(6-13)
	7	3.5(1-9)	9.0(6-15)
	8	3.5(0-10)	7.0(5-9)
	9	2.2(1-4)	7.0(4-11)
	10	2.3(0-6)	7.0(3-10)
Total lesions		22.4	75.6

[a]Symptoms determined 6 days after challenge. There were five plants per treatment. Figures in parentheses are the range in the number of lesions. The experiment was performed once.
[b]Plants were challenged 4 wk after the inducer inoculation.

TABLE 3. Protection of leaves and cotyledons of watermelon and muskmelon against *Colletotrichum lagenarium* race 1 by inoculating the cotyledon(s) with *C. lagenarium* race 1

First inoculation	Host	Mean number of lesions on either leaf one or cotyledon[a]		Mean area occupied by lesions on either leaf one or the cotyledon (mm^2)	
		Leaf one	Cotyledon	Leaf one	Cotyledon
One cotyledon	WM	9.0(8-30)***[b]	5.6(1-15)***	9.7***	5.6***
	MM	2.5(1-26)***	6.7(1-16)***	3.2***	14.8***
Two cotyledons	WM	5.0(3-10)***	...	4.2***	...
	MM	1.4(0-5)***	...	1.4***	...
Unprotected	WM	18.2(12-30)	9.8(1-20)	44.9	19.5
	MM	12.6(12-30)	12.3(4-18)	42.8	57.4

[a]Data were recorded 6 days after challenge. Figures in parentheses are the range in the number of lesions.
[b]Asterisks *** indicate that mean lesion numbers and areas were very highly significantly different from the controls, $P = 0.001$.

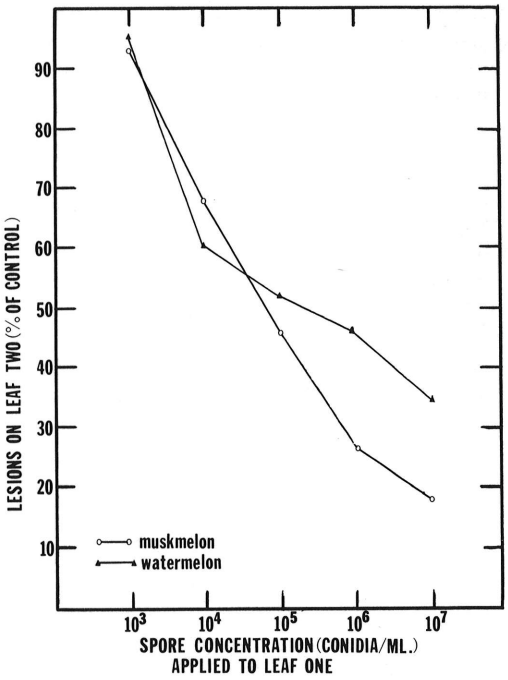

Fig. 1. Effect of the inoculum concentration of *Colletotrichum lagenarium* race 1 applied to leaf one of watermelon and muskmelon on the number of lesions on leaf two inoculated with *C. lagenarium*.

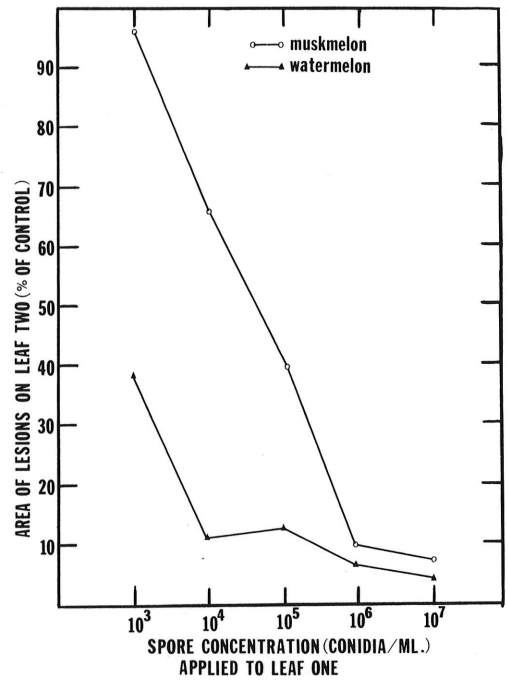

Fig. 2. Effect of the inoculum concentration of *Colletotrichum lagenarium* race 1 applied to leaf one of watermelon and muskmelon on the area of necrosis on leaf two inoculated with *C. lagenarium*.

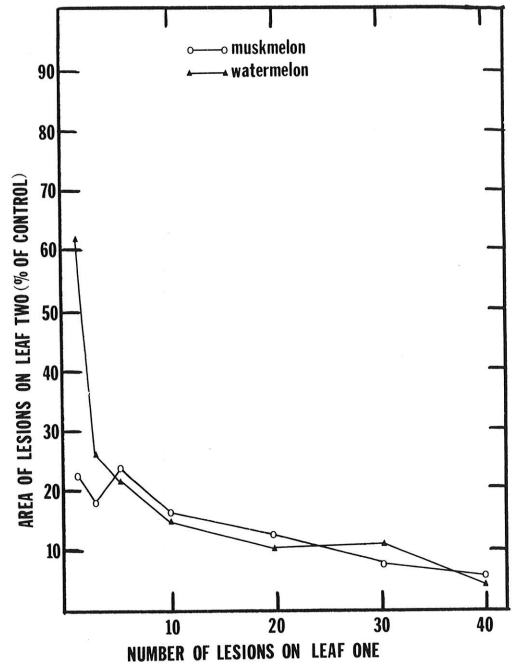

Fig. 3. Effect of the number of lesions produced by *Colletotrichum lagenarium* race 1 on leaf one on the area of necrosis of leaf two inoculated with *C. lagenarium*.

achieved in eight cucumber cultivars (7). Lesions were generally smaller, and the effectiveness of drops of inoculum to produce lesions was less on watermelon and muskmelon than on cucumber. The second leaf was protected by one lesion on leaf one. Plants remained protected 4 wk after inoculation of leaf one, and the protection was effective on the entire plant. Races 1, 2, and 3 elicited protection in muskmelon and watermelon even if leaf one developed hypersensitive flecks. The areas of lesions on protected leaves versus unprotected leaves were sometimes vastly different; i.e., 295.8 and 25.0 mm^2 on Honey Rock, 171.8 and 6.3 mm^2 on Delicious 51, 63.1 and 19.6 mm^2 on Iroquois, 66.8 and 8.6 mm^2, respectively, on Sugar Baby. Data for the cultivars Iroquois and Sugar Baby are derived from the experiment illustrated by Fig. 2. Though protection was expressed as a reduction in number of lesions and their size in three of the four watermelon and muskmelon cultivars studied, it was not true for Honey Rock and New Hampshire Midget. In these cultivars, protection was most evident as a reduction in lesion size. This suggests that two distinct mechanisms may control resistance and protection; one mechanism influences lesion number and the other lesion size. This suggestion is consistent with the report by Akai, et al. (1).

It is important to know that other members of the Cucurbitaceae in addition to cucumber can be protected. Watermelon and muskmelon also possess efficient resistance mechanisms against *C. lagenarium* which can be elicited by the pathogen. These plants also might possess latent resistance mechanisms against other pathogens. The protection phenomenon may be a commonplace occurrence in plant-parasite interactions.

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