Induced Systemic Protection in Cucumber: Effects of Inoculum Density on Symptom Development Caused by *Colletotrichum lagenarium* in Previously Infected and Uninfected Plants

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ABSTRACT

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Systemic protection induced by infection of leaf 1 with *Colletotrichum lagenarium* was not overcome by high levels of challenge inoculation of leaf 2 with *C. lagenarium* (10^7 conidia per milliliter). The cucumber cultivar Marketer was more susceptible than SMR-58 to *C. lagenarium*; similarly, the level of resistance induced by systemic protection was less in Marketer than in SMR-58. Lesions were more numerous, larger, and became necrotic earlier in Marketer than in SMR-58 and on unprotected leaf 2 compared to protected leaf 2. In general, the lower the concentration of challenge inoculum, the lower the total number and the more slowly chlorotic lesions became necrotic. However, the amount of added resistance induced by systemic protection, as expressed as the difference in inoculum density to obtain the same degree of symptoms in protected plants as in unprotected plants, was slightly greater in Marketer (100- to >1,000-fold) than in SMR-58 (10- to 1,000-fold). The rate of necrotic lesion expansion in leaf 2 on unprotected plants was similar for Marketer and SMR-58 and was independent of the concentration of challenge inoculum. Necrotic lesion size was dependent on the inoculum concentration. The apparent rate of necrotic lesion expansion was lower in protected leaf 2 while lesions were less than 1 mm in diameter. When lesion size exceeded this, however, expansion rates were similar. The interpretation and significance of the latter are discussed.

Additional key words: anthracnose, immunization, induced resistance.

Plant breeders are constantly trying to improve the agronomic and/or horticultural qualities of plants, and disease resistance is an important consideration. Kuć and co-workers have demonstrated that susceptible cucumber, muskmelon, and watermelon plants were made highly resistant to a wide range of fungal, bacterial, and viral pathogens (2–4,6,9,11,12,15) without a change in the plant's genome. A limited prior infection results in induced systemic protection against disease caused by members from all three pathogen classes. Protection can be maintained through fruiting if the plant receives a booster inoculation (13). Protection has been demonstrated in the field (1,5) as well as in the greenhouse. The potential economic value of disease control by induced systemic protection, in addition to its persistence, is its nonspecificity including protection against viral diseases. However, no studies have been reported on its effectiveness against high disease pressure. The dynamics of disease expression have also not been reported. Induced systemic protection has been extensively studied in tobacco (7,14,16,20,21) and bean (8,17,19,22) and may well be present in many other species.

This paper provides evidence on the effectiveness of induced systemic protection of two susceptible cultivars of cucumber against high levels of inoculum of *Colletotrichum lagenarium*. Symptom development in leaf 2 of plants previously infected or uninfected on leaf 1 was compared. These data were analyzed to help determine at which stages during pathogen penetration and establishment the mechanisms of resistance and induced systemic protection appear to function.

MATERIALS AND METHODS

Pathogen and hosts. *C. lagenarium* (Pass.) Ell. & Halst. (race 1) was maintained on green bean juice agar at 24 C in the dark. Spore suspensions were prepared from 6- to 10-day-old cultures (11).

Cucumbers (*Cucumis sativum* L. 'Wisconsin SMR-58' and 'Marketer') were grown in 10-cm-diameter plastic pots containing Canadian sphagnum peat moss and vermiculite (1:1, v/v) supplemented with a solid nutrient mix. Plants received a daily nutrient solution of 14-0-14 containing approximately 110 ppm N. Plants were grown in the greenhouse at 23–31 C supplemented during the winter months with 14 hr of light (350 μE/m^2/sec at the leaf surface) from high-pressure sodium lamps. Greenhouse air was filtered through activated charcoal (Barnebey-Cheney, Columbus, OH).

Inoculations. Systemic protection was induced by inoculating the first true leaf (leaf 1) with 30 5-μl drops of a conidial suspension of *C. lagenarium* (10^6 spores per milliliter) when the second leaf (leaf 2) was one-third expanded. Inoculated and uninoculated control plants of both SMR-58 and Marketer cultivars were placed in moistened humidity chambers at 22–25 C for 24 hr. At the end of 24 hr the chambers were partially opened; the plants were returned to the greenhouse bench after a total of 48 hr. Seven days after inoculation of leaf 1 (immunization), leaf 2 was inoculated (challenge) with 30 5-μl drops of a conidial suspension of *C. lagenarium* containing either 10^4, 10^5, 10^6, or 10^7 spores per milliliter. The plants were reincubated in eight replicate humidity chambers of the factorial experiment arranged in a completely randomized block design. Eight plants per treatment were used. Symptoms on leaf 2 were recorded 4, 5, 6, 7, and 10 days after the challenge.

The total number of lesions and the number of lesions with visible necrosis were recorded. The necrotic lesion diameter was measured to the nearest millimeter across the widest point of the lesion. The average necrotic lesion diameter per treatment was calculated from the average lesion diameter per leaf for each treatment, since little variation was observed within a leaf. The results from a typical experiment are presented. The experiment was performed three times.

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TABLE 1. The effect of infecting leaf 1 with *Colletotrichum lagenarium* on the total number of lesions on leaf 2 resulting from different concentrations of challenge inoculum of the fungus[a]

Cultivar	Treatment of leaf 1[b]	Conc. of challenge[c] (conidia/ml)	Mean number of lesions on leaf 2[d] after challenge				
			Day 4	Day 5	Day 6	Day 7	Day 10
Marketer	Unprotected	10^7	30.0 (100)	30.0 (100)	30.0 (100)	30.0 (100)	30.0 (100)
		10^6	29.6 (100)	30.0 (100)	30.0 (100)	30.0 (100)	29.9 (100)
		10^5	29.4 (97)	29.6 (100)	29.6 (100)	29.6 (100)	29.5 (100)
		10^4	28.3 (94)	28.0 (100)	28.0 (100)	28.0 (100)	28.1 (100)
	Average		(98)	(100)	(100)	(100)	(100)
	Protected	10^7	29.6 (33)	30.0 (75)	30.0 (93)	30.0 (97)	30.0 (99)
		10^6	22.8 (6)	27.8 (46)	27.8 (74)	28.3 (87)	28.1 (98)
		10^5	12.8 (2)	18.0 (62)	18.3 (81)	18.6 (87)	18.9 (92)
		10^4	1.9 (0)	2.6 (69)	3.1 (84)	2.9 (100)	3.3 (91)
	Average		(10)	(63)	(83)	(93)	(95)
SMR-58	Unprotected	10^7	30.0 (100)	30.0 (100)	30.0 (100)	30.0 (100)	30.0 (100)
		10^6	30.0 (75)	30.0 (93)	30.0 (96)	30.0 (98)	30.0 (99)
		10^5	24.8 (50)	26.1 (94)	26.6 (98)	26.5 (100)	26.6 (99)
		10^4	13.1 (49)	13.5 (93)	14.1 (94)	14.1 (94)	14.1 (96)
	Average		(69)	(95)	(97)	(98)	(99)
	Protected	10^7	29.6 (23)	30.0 (45)	29.6 (68)	29.9 (90)	30.0 (98)
		10^6	16.0 (16)	21.3 (19)	22.5 (38)	21.9 (58)	21.9 (82)
		10^5	2.8 (4)	6.4 (28)	6.5 (46)	6.4 (55)	6.6 (67)
		10^4	0.1 (0)	0.5 (0)	0.5 (20)	0.5 (20)	1.4 (21)
	Average		(11)	(23)	(43)	(56)	(67)

[a] Results from one experiment performed three times with eight replications.
[b] Plants either inoculated with 30 5-μl drops of *C. lagenarium* (protected) spore suspension (1×10^6 conidia/ml), or were left untreated (unprotected) 7 days prior to challenge of leaf 2.
[c] Leaf 2 was challenged with 30 5-μl drops of a spore suspension of *C. lagenarium* containing either 10^4, 10^5, 10^6, or 10^7 conidia per milliliter.
[d] Data represents all lesions including chlorotic. Numbers in parentheses represent necrotic lesions as a percentage of the total number.

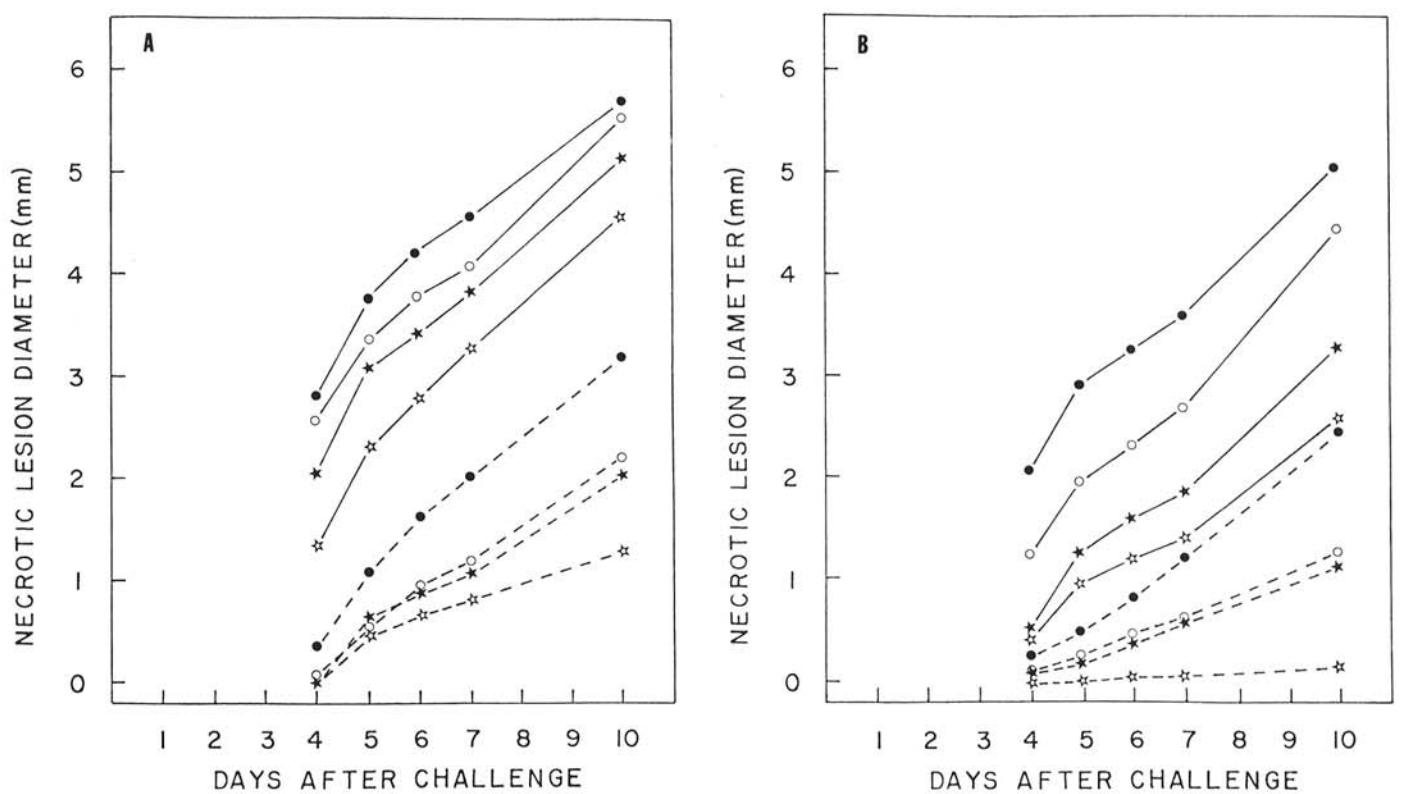

Fig. 1. The effect of inoculating leaf 1 and the inoculum concentration of *Colletotrichum lagenarium* used to challenge leaf 2 on the diameter of necrotic lesions on leaf 2. **A,** Mean necrotic lesion diameter on cultivar Marketer. **B,** Mean necrotic lesion diameter on cultivar SMR-58. Leaf 1 was either inoculated (------) with *Colletotrichum lagenarium* or untreated (——) 7 days prior to challenge of leaf 2 with 10^4 (☆), 10^5 (★), 10^6 (o), or 10^7 (•) conidia per milliliter. Results from one experiment with eight replications. The experiment was performed three times.

RESULTS

Analysis of variance was performed separately for each experiment. For each day after challenge, the analysis indicated a significant ($P < 0.0001$) effect of cultivar, immunization, and challenge inoculum concentration on total number of lesions and necrotic lesion diameter. Increasing the concentration of challenge inoculum resulted in an increase in lesion number (up to maximum of 30), and necrotic lesion diameter on leaf 2 of both unprotected and protected plants (Table 1, Fig. 1). In the overall analysis, lesions were fewer and had a smaller necrotic diameter on leaf 2 of protected as compared to unprotected plants. The cultivar Marketer was more susceptible to *C. lagenarium* than SMR-58. On unprotected plants, leaf 2 of Marketer had a greater total number of lesions ($P < 0.0001$), the lesions become necrotic faster, i.e., >90% necrotic in 4 days compared to 5 days in SMR-58 (Table 1), and had a larger necrotic lesion diameter ($P < 0.0001$).

On systemically protected plants, disease was also more severe on Marketer than on SMR-58. Leaf 2 of protected Marketer had more lesions ($P < 0.0001$), the lesions became necrotic faster, i.e., >90% necrotic in 7 days compared to greater than 10 days on leaf 2 of protected SMR-58, and were larger in diameter ($P < 0.0001$) (Table 1, Fig. 1). In general the lower the concentration of challenge the more slowly chlorotic lesions became necrotic.

Where lesions appeared, their development was delayed by approximately 6 days on leaf 2 of protected plants compared with lesions on unprotected controls. In general, it was not until 10 days after challenge that lesions on protected leaf 2 reached the necrotic diameter which their unprotected controls had reached at 4 days after challenge.

The relative amount of systemic protection can be expressed in terms of a reduction in inoculum density required to cause a similar level of disease on unprotected and protected leaves. In terms of total lesion number, this reduction for Marketer was approximately 100-fold at 10 days after challenge; i.e., the total number of lesions on leaf 2 of control plants challenged with 10^4 conidia per milliliter were similar to the total number on protected plants challenged with 10^6 conidia per milliliter. For SMR-58 this reduction was between 10- to 100-fold for lesion number. Analysis of variance, however, indicated that there was no interaction of cultivar with immunization, suggesting no difference in the amount of added resistance between the two cultivars. However, a marginally significant ($P = 0.0455$) interaction was observed for necrotic lesion diameter. The reduction of inoculum density was about 1,000-fold for SMR-58 and much greater than 1,000-fold for Marketer, 10 days after challenge. This suggested, if anything, that the amount of added resistance was slightly greater in Marketer than in SMR-58.

However, when the protection of leaf 2 was expressed as the proportional reduction of lesion number or necrotic diameter, at each concentration of challenge, then the amount of added resistance was slightly greater in SMR-58 than in Marketer.

Analysis of variance for the daily rate of necrotic lesion expansion between 4 and 10 days after challenge indicated a significant ($P < 0.0001$) effect of the concentration of challenge inoculum on protected leaf 2, but no effect on unprotected controls. The lower the concentration of challenge, the lower the apparent rate of necrotic lesion expansion on protected leaf 2.

On protected leaf 2, however, necrotic lesions were difficult to measure because of their small size, and were generally few in number. Also, over the first few days of data collection, chlorotic lesions slowly turned necrotic, especially on protected leaf 2 (Table 1). This had the effect of artificially reducing the average necrotic lesion expansion rate because both the sum of the necrotic lesion diameters and the number of necrotic lesions were increasing. To overcome these problems, only data from 7 to 10 days after challenge and treatments with necrotic lesions greater than 1 mm in diameter at 7 days were used. The data from leaf 2 of the lowest three concentrations of challenge inoculum on protected SMR-58 and the lowest on protected Marketer were omitted. The remaining data (Table 2) were subjected to analysis of variance by using balanced contrasts to compare the effects of particular treatments on the rate of necrotic lesion expansion. This analysis indicated that there was no effect of cultivar, challenge inoculation level, or immunization of SMR-58. The only effect of a treatment that was found was a marginally significant ($P = 0.044$) overall decrease in necrotic lesion expansion rate on Marketer with immunization. Further comparisons of individual challenge inoculum levels indicated that this effect was only significant ($P = 0.037$) at 10^6 conidia per milliliter.

TABLE 2. The effect of infecting leaf 1 with *Colletotrichum lagenarium* on the daily rate of necrotic lesion expansion on leaf 2 resulting from different concentrations of challenge inoculum of the fungus

Cultivar	Conc.[w] of challenge (conidia/ml)	Daily rate of lesion expansion on leaf 2 7–10 days after challenge[v]	
		Unprotected	Protected[x]
Marketer	10^7	0.39 b [y]	0.39 b
	10^6	0.48 b	0.34 b
	10^5	0.43 b	0.34 b
	10^4	0.43 b	...[z]
SMR-58	10^7	0.49 ab	0.43 b
	10^6	0.58 a	...
	10^5	0.47 b	...
	10^4	0.41 b	...

[v] Results from one experiment performed three times with eight replications.

[w] Leaf 2 was challenged with 30 5-μl drops of a spore suspension of *C. lagenarium* containing either 10^4, 10^5, 10^6, or 10^7 conidia per milliliter.

[x] Leaf 1 was inoculated with 30 5-μl drops of a spore suspension of *C. lagenarium* (10^6 conidia per milliliter) 7 days prior to challenge.

[y] Numbers followed by different letters are significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Data omitted; necrotic lesion diameter <1 mm in diameter 7 days after challenge.

DISCUSSION

Cucumber cultivars SMR-58 and Marketer are both susceptible to *C. lagenarium*. However, susceptibility is not absolute but relative, since, as reported here, SMR-58 is more resistant to *C. lagenarium* than is Marketer. Even "susceptible" plants have differing capacities to resist disease. Plant reaction to pathogens generally represents a continuum as do many aspects of nature. Plant reaction to pathogens is often classified as "resistant" or "susceptible" to disease. Once made, however, the assignment may be mistakenly considered to be absolute. Even the classification itself may depend on the investigator's definition of plant disease. Wheeler (24) defines plant disease as all malfunctions which result in unsatisfactory plant performance or which reduce a plant's ability to survive and maintain its ecological niche. Plant disease as defined has two components: one economic and the other ecological. Plant pathologists have generally emphasized the economic aspects of plant disease of major crops over the ecological impacts. There is also a third academic aspect of disease at the physiological or biochemical level. Infected cells in a few small lesions are diseased, but they are unlikely to have an economic or ecological impact.

Local lesion development may be restricted. However, restriction of disease may be influenced by the inoculum concentration and the environmental conditions. In the experiments reported here, it appeared that the lesions of *C. lagenarium* on cucumber leaves did not become restricted, at least within the experimental time frame. In fact, placing infected leaves in humidity chambers generally results in the rapid collapse and death of the whole leaf and petiole.

The data presented demonstrate that systemic protection against *C. lagenarium* is not broken down or overwhelmed by high levels of disease pressure under greenhouse conditions. Protection was observed of both Marketer and SMR-58 even when plants were challenged with 10^7 conidia per milliliter. Even by 10 days after challenge the total necrotic lesion area on protected leaves

challenged with 10^7 conidia per milliliter was approximately one third of that on unprotected leaves. Where lesions appeared on protected leaves their development was delayed by approximately 6 days. This natural enhancement of resistance may be of considerable economic value and result in a reduction of the use of expensive and potentially environmentally damaging chemicals to control plant disease.

Disease resistance may be expressed at many levels; for example, reduced penetration and establishment, lesion expansion, and sporulation. On unprotected leaf 2 of both Marketer and SMR-58, the daily rate of necrotic lesion expansion was independent of the concentration of challenge inoculum. Lesion size was determined by the initial size, which was dependent on the number of conidia in the drops of inoculum and the cultivar. This may suggest that at least part of the difference in susceptibility between the two cultivars is in the germination, penetration, and early establishment of the fungus in the tissues. Richmond et al (18) reported that 44% of the appressoria penetrated into unprotected Marketer leaves compared to 20% into SMR-58 leaves.

On systemically protected leaves, in addition to being smaller and fewer, necrotic lesions between 4 and 10 days after challenge appeared to expand less rapidly than those in unprotected leaves. This may suggest that other mechanisms, as well as reduced penetration (18) and rapid lignification (10,23) are operating to retard the growth of fungus through the leaf tissue, assuming symptom development parallels fungal development equally in both unprotected and protected leaves. However, this reduced rate of expansion may reflect precision errors in measuring small necrotic lesions less than 1 mm in diameter coupled with the fact that new lesions turn necrotic over a period of several days which reduced the average lesion diameter. The rate of lesion expansion in protected leaves for both cultivars between 7 and 10 days after challenge was generally the same as the rate in unprotected tissues when the lesion diameter exceeded 1 mm (Fig. 1). This may indicate that the mechanism(s) of induced systemic protection is (are) also only effective during the early stages of pathogen invasion. Richmond et al (18) reported that immunization reduced appressorial penetration of *C. lagenarium* by a similar proportion in Marketer and SMR-58 leaves, from 44 to 7% and from 20 to 3%, respectively.

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