Induced Systemic Protection in Cucumber: Time of Production and Movement of the Signal

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ABSTRACT

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An inducing inoculation of the first true leaf (leaf 1) of cucumber with *Colletotrichum lagenarium* systemically protected the plants against disease caused by subsequent challenge inoculation with the fungus. Several experiments involving detachment of the inducer leaf were designed to determine the dynamics of signal production and movement. Excising leaf 1 at intervals after inoculation revealed that protection of the second true leaf (leaf 2) of two cucumber cultivars was first evident by 72 hr. Protection increased gradually and reached a maximum by 144 hr. After excision of the inducer leaf, the time of challenge (either immediately or several days later) did not affect the level of protection. Scions were previously shown to become protected when grafted onto rootstocks infected on leaf 1 with *C. lagenarium*. We found that if the inducer leaf was excised before translocation was established through the graft union, the scion never became protected. Protection was first detected when the inducer leaf was excised shortly after onset of translocation through the graft union; maximum protection required another 48–96 hr. The level of protection of leaf 2 did not differ whether the tip of leaf 1 or the whole leaf was excised at intervals after inoculation of the tip. These data suggest that the limiting step in development of systemic protection is signal production. Once produced, the signal moves swiftly through the plant and results in rapid sensitization of the plant's defense mechanisms.

Additional key words: anthracnose, immunization, induced resistance.

Systemic protection in cucumber (*Cucumis sativus* L.) can be induced by a number of infectious agents causing local lesions, including *Colletotrichum lagenarium* (Pass.) Ell. & Halst. (1–3,8,10,12,16). Mechanical injury alone or injury caused by dry ice and a large number of chemicals has not been effective. After inoculation with *C. lagenarium*, protection is first evident by about 72 hr and can remain effective through fruiting if the plant is given a booster inoculation (15).

Because protection is systemic, it has been hypothesized that an alarm signal produced and released from the host-pathogen interaction site moves systemically and sensitizes the plant's defense mechanisms (13,14,18). Isolating and characterizing the signal are paramount to a full understanding of the mechanisms involved in plant immunization against disease. Previous attempts have been only partially successful (7), although Matsumoto and Asada (17) recently reported that a lignin-inducing factor from infected cucumber leaves conferred protection against *C. lagenarium*.

We recently demonstrated that only the infected leaf is the source of the signal and that the signal is not released or remobilized from uninfected, systemically protected tissues (6). After inoculation with *C. lagenarium,* systemic protection is not detected for about 72 hr. We do not know if this lag period is due to the lack of signal production and/or its movement and processing, how rapidly or by what means the signal is distributed throughout the plant, or how rapidly the signal sensitizes the plant's defense mechanisms.

Jenns and Kuć (11) demonstrated that the signal is graft-transmissible. At what stage during the grafting process protection is transmitted from the rootstock to the scion has not been determined, however. Also not known is whether vascular translocation across the union is necessary or, if it is, how soon after protection develops.

We report here on experiments designed to determine whether the lag period is due to lack of signal production or its slow movement and sensitizing of the plant's defense mechanisms, whether the amount of signal in the tissues at any one moment can be biologically detected, and whether functional translocation is necessary for graft transmission of protection. These data provide insight into the isolation and nature of the signal.

MATERIALS AND METHODS

Pathogen and hosts. *C. lagenarium* (race 1) was maintained on green bean juice agar at 24 C in the dark. Spore suspensions were prepared from cultures 6–10 days old (10).

Cucumber cultivars Marketer and SMR-58 were grown in plastic pots (10 cm diam.) 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 of nitrogen. Plants were grown in a greenhouse at 23–31 C supplemented during the winter months with 14 hr of light (350 $\mu E/m^2/sec$ at leaf surface) from high-pressure sodium lamps. Greenhouse air was filtered through activated charcoal.

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* (1×10^6 spores/ml) when the third leaf (leaf 3) was one-third expanded, unless otherwise indicated. Inoculated and uninoculated plants were placed in moistened humidity chambers at 22–25 C for 24 hr, then the chambers were partially opened. After a total of 48 hr, the plants were returned to the greenhouse bench and challenged with 30 5-μl drops of inoculum.

A complete randomized block design was used for all experiments, with at least six plants per treatment. Each experiment was performed at least three times.

Excision of inducer leaf at intervals after inoculation. Two series of experiments were conducted. The first series was to determine the time required after inoculation of leaf 1 (inducer leaf) for protection of leaf 2 to become evident and the time required for maximum protection: Leaf 1 on SMR-58 and Marketer plants was inoculated when leaf 2 was one-third expanded, then excised 2, 3, 4, 5, and 6 days later; leaf 2 was challenged 7 days after inoculation of leaf 1. The second series was to determine the effect of immediate and delayed challenge of leaf 2 after excision of the inducer leaf at

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various intervals after inoculation: Leaf 1 on SMR-58 and Marketer plants was inoculated when leaf 2 was one-third expanded, then excised 2, 3, 4, 5, and, in some experiments, 6 days later; leaf 2 was challenged either immediately after excision or 5 or 6 days after inoculation of leaf 1. In both series, plants were challenged with a conidial suspension (10^5/ml).

Excision of inducer leaf (leaf 1) from rootstock at intervals after grafting. Scions (leaf 4 and above) from SMR-58 plants were grafted onto SMR-58 rootstocks bearing leaves 1 and 2 (6); leaf 1 on the rootstock had been inoculated 7 days before grafting. Leaf 1 on the rootstock was excised 2, 4, 6, 8, and 10 days after grafting. Leaf 2 on the rootstock and the first leaf above the graft site on the scion were challenged 10 days after grafting with a conidial suspension (10^4/ml). At the time the inducer leaf was excised, 2 5-μl (50 nCi) drops of [^{14}C(U)]-sucrose (specific activity 673 μCi/mmol, New England Nuclear, Boston, MA) were applied to the inducer leaf on a separate plant. The leaf was punctured through the drops with a pin and enclosed in a clear plastic bag (20 × 40 cm), and the plant was incubated under fluorescent lights for 6 hr. Two leaf disks (13 mm diam.) were punched from both leaf 2 of the rootstock and the first leaf above the graft site on the scion. The disks were homogenized in a 7-ml Ten Broeck homogenizer with liquid scintillation cocktail (3a7OB) (Research Products International, Mt. Prospect, IL), and the homogenate was transferred to a cocktail vial (total volume = 20 ml).

Movement of signal across inducer leaf. Two series of experiments were conducted to follow the movement of the signal across the inducer leaf. In the first, a large number of plants were used at a single time period after inoculation of the inducer leaf. In the second, fewer plants were used at several time intervals after inoculation.

In both series, the distal tip of leaf 1 of SMR-58 plants was inoculated with 10 5-μl drops of a conidial suspension of *C. lagenarium* (10^6/ml) when leaf 2 was one-third expanded. In the first series, the inoculated leaf tip or the whole leaf was excised 110 hr after inoculation. In the second series, the leaf tip or the whole leaf was excised 64, 72, 80, 88, 96, 104, 112, 120, and 128 hr after inoculation. The tip of leaf 1 or the whole leaf was excised from unprotected plants at 64 and 128 hr to determine if the time of excision had any effect on susceptibility of leaf 2 to *C. lagenarium*. The length of leaf 2 was measured at the time of challenge to determine if the time and amount of leaf excision had any effect on plant growth. In both series, leaf 2 was challenged 6 days after inoculation with 10^4 conidia/ml. The total number of lesions was recorded daily after challenge. Diameters of necrotic lesions were measured to the nearest millimeter across the widest point; a diameter of 0 mm was recorded if the lesion was chlorotic but without necrosis. The average necrotic lesion diameter per leaf was calculated by dividing the sum of lesion diameters by the total number of lesions.

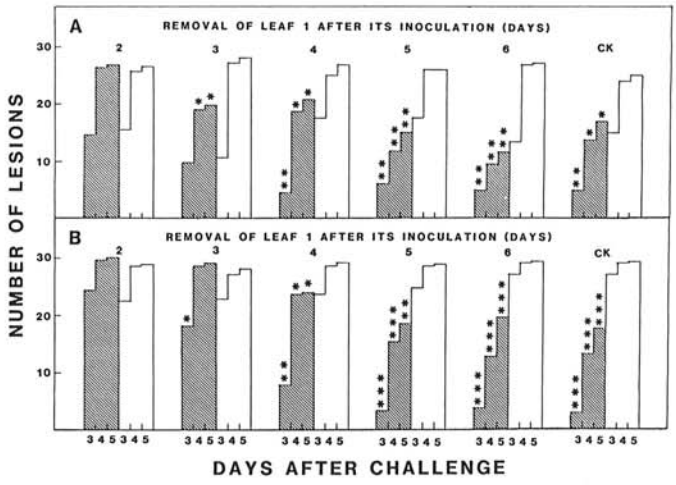

Fig. 1. Effect of the time of excision of the inducer leaf (leaf 1) from cucumber cultivars **A,** SMR-58 and **B,** Marketer inoculated with *Colletotrichum lagenarium* on the number of lesions on leaf 2 inoculated with the fungus 7 days later. On protected plants (shaded bars), leaf 1 was inoculated with *C. lagenarium*; on control plants (open bars), leaf 1 was not treated. Results shown are from one experiment, six plants per treatment; the experiment was performed five times. CK = leaf 1 not excised. Asterisks represent significant differences from corresponding control values by Student's *t* test: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

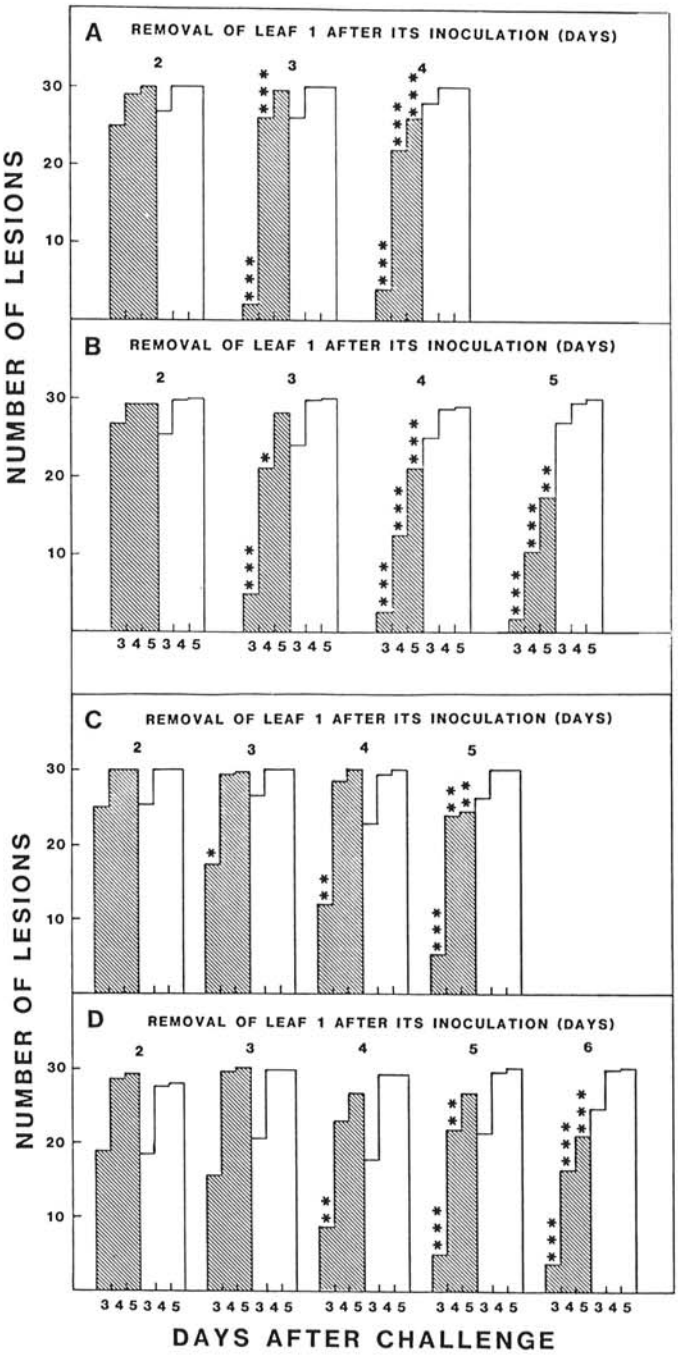

Fig. 2. Effect of the time of challenge of leaf 2 with *Colletotrichum lagenarium* after excision of the inducer leaf (leaf 1) on the number of lesions on leaf 2. **A,** Cucumber cultivar SMR-58, leaf 2 challenged immediately after excision of leaf 1. **B,** SMR-58, leaf 2 challenged 5 days after inoculation of leaf 1. **C,** Cucumber cultivar Marketer, leaf 2 challenged immediately after excision of leaf 1. **D,** Marketer, leaf 2 challenged 6 days after inoculation of leaf 1. On protected plants (shaded bars), leaf 1 was inoculated with *C. lagenarium*; on control plants (open bars), leaf 1 was not treated. Results shown are from one experiment, six plants per treatment; the experiment was performed three times. Asterisks represent significant differences from corresponding control values by Student's *t* test: *, $P = 0.05$; **, $P = 0.01$; ***, $P = 0.001$.

TABLE 1. Effect of time of excision of inducer leaf (leaf 1) from rootstock after grafting on protection of scion against disease caused by *Colletotrichum lagenarium*

Removal of inducer leaf after grafting (days)[w]	Tissue challenged[x]	Mean number of lesions[y] and days after challenge		
		4	5	7
2	Rootstock	3.3 c[z]	8.2 bc	9.7 bc
	Scion	13.8 a	16.5 a	16.7 a
4	Rootstock	3.5 c	7.7 bc	9.0 bc
	Scion	13.0 a	15.7 a	15.7 a
6	Rootstock	4.2 c	5.3 c	6.7 c
	Scion	9.5 ab	13.3 ab	14.8 ab
8	Rootstock	3.7 c	5.2 c	6.0 c
	Scion	4.7 bc	8.5 bc	8.8 bc
10	Rootstock	3.3 c	6.2 c	7.7 c
	Scion	6.3 bc	8.2 bc	8.3 bc

[w] Scions grafted 7 days after inoculation of first true leaf (leaf 1) of rootstock with *C. lagenarium*.
[x] First leaf above graft on scion and leaf 2 on rootstock challenged 10 days after grafting.
[y] Means from one experiment, six plants per treatment; experiment performed three times.
[z] Numbers followed by different letters for each day after challenge are significantly different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Movement of [^{14}C(U)]-sucrose from rootstocks to scions after grafting

Days after grafting	Tissue assayed[a]	Disintegrations per minute[b]				
		Expt. 1	Expt. 2	Expt. 3	Mean	SE
2	Rootstock	292	741	652	562	137
	Scion	1	4	123	43	40
4	Rootstock	351	1,150	629	710	234
	Scion	1,499	227	174	633	433
6	Rootstock	756	1,349	182	762	337
	Scion	751	7,697	202	2,883	2,412
8	Rootstock	515	179	216	303	106
	Scion	1,767	1,509	594	1,290	356
10	Rootstock	545	···	155	350	195
	Scion	252	···	1,300	776	524

[a] Leaf 2 on rootstock and first leaf above graft site on scion assayed for radioactivity 6 hr after application of [^{14}C(U)]-sucrose to leaf 1 on rootstock.
[b] Results from homogenizing two 13-mm disks from each leaf.

RESULTS

Effect of time of inducer leaf excision and time of challenge. Protection of leaf 2 in SMR-58 and Marketer plants was first observed when leaf 1 was removed 3 days after inoculation with *C. lagenarium*. Maximum protection in both cultivars was observed when the inoculated leaf had been left on for 5–6 days. In these experiments, all plants were challenged together (on leaf 2) 7 days from the time of inoculation of leaf 1 (Fig. 1).

In other leaf detachment experiments, leaf 2 was challenged immediately after excision of leaf 1 on one-half of the plants and 5–6 days after inoculation of leaf 1 on the other half. When the inducer leaf was excised before systemic protection was evident, delaying the challenge until 5–6 days after inoculation of leaf 1 did not result in protection of leaf 2. Once protection was developing, a delayed challenge after excision of leaf 1 did not affect the level of protection of leaf 2 (Fig. 2).

Time course of protection of scion after grafting. Unprotected scions were grafted onto protected rootstocks, and the inoculated leaf (leaf 1) on the rootstocks was removed at intervals after grafting. Protection of scions was first evident when leaf 1 was excised at 6 days. Scions were maximally protected 8 days after grafting (Table 1). Leaf 2 on the rootstocks remained well protected throughout the experiments. Movement of [^{14}C(U)]-sucrose from the rootstock to the scion was clearly detected 4 days after grafting (Table 2); at this time the scion had regained full turgidity.

Detection of signal movement through inoculated leaf 1. The movement of the signal through the inducer leaf could not be detected at any time after inoculation. Analysis of variance indicated that the number, diameter, and area of lesions on challenge leaf 2 did not differ significantly whether the inoculated leaf tip or the whole inoculated leaf was removed. Leaf 2 became more protected with time, but at no time after inoculation did disease on leaf 2 differ significantly between the two excision treatments (Fig. 3). No effect was observed when a larger number of plants were used at 110 hr after inoculation (Table 3). The partial protection of leaf 2 was probably due to the reduced number of drops of inoculum applied to leaf 1, i.e., 10 instead of 30, and maximum protection was not attained by this time. The time of excision and the amount of leaf tissue removed from leaf 1 of unprotected plants had no significant effect on growth as determined by the length of leaf 2 at the time of challenge or the susceptibility of leaf 2 to *C. lagenarium*.

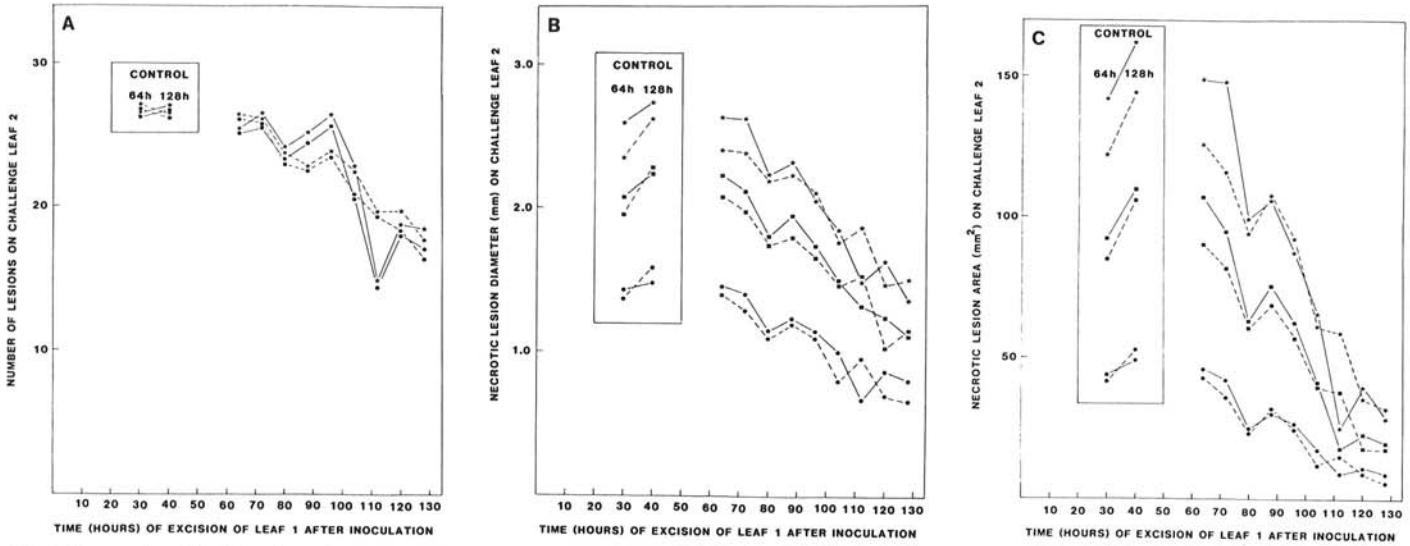

Fig. 3. Protection of leaf 2 against disease caused by *Colletotrichum lagenarium* after inoculation of leaf 1, then excision of the tip of leaf 1 or the whole leaf. Data are from 4 (•), 5 (■), and 6 (★) days after challenge of leaf 2. **A,** Number of lesions per leaf on leaf 2 (data from 5 days after challenge omitted for clarity), **B,** necrotic lesion diameter, and **C,** necrotic lesion area per leaf. The tip of leaf 1 (broken line) or the whole leaf (solid line) was excised from plants after the leaf tip was inoculated with 10 5-µl drops of a spore suspension of *C. lagenarium* (10^6 conidia/ml). On control plants (leaf 1 untreated), the tip of leaf 1 or the whole leaf was excised at 64 and 128 hr. Leaf 2 was challenged with 30 5-µl drops of spore suspension (10^4 conidia/ml). Results shown are from one experiment, six plants per treatment; the experiment was performed three times.

TABLE 3. Effect of excising tip of leaf 1 or whole leaf 1 10 hr after inoculation with *Colletotrichum lagenarium* on protection of leaf 2 against disease caused by the fungus

Treatment[x]	Mean total lesion number per leaf[y]				Mean necrotic lesion diameter (mm)[y]				Mean necrotic lesion area (mm^2) per leaf[y]			
	4	5	6	7	4	5	6	7	4	5	6	7
C/P	26.3 a[z]	26.6 a	26.6 a	26.6 a	1.26 a	1.75 a	2.45 a	3.06 a	35.4 a	67.8 a	132.1 a	201.7 a
C/T	26.2 a	26.5 a	26.6 a	26.6 a	1.20 a	1.75 a	2.41 a	2.95 a	33.8 a	73.0 a	136.8 a	202.9 a
I/P	19.6 b	20.7 b	21.2 b	21.2 b	0.77 b	1.14 b	1.67 b	2.18 b	11.1 b	24.9 b	56.7 b	98.3 b
I/T	18.9 b	21.0 b	21.3 b	21.4 b	0.75 b	1.12 b	1.62 b	2.11 b	9.8 b	24.6 b	51.3 b	88.9 b

[x] C = leaf 1 untreated, I = tip of leaf 1 inoculated with 10 5-μl drops of spore suspension of *C. lagenarium* (10^6 conidia per milliliter), P = whole leaf 1 excised, T = tip of leaf 1 excised (all infected tissue).

[y] Means from one experiment, 20 plants per treatment, 4, 5, 6, and 7 days after challenge; experiment performed three times.

[z] Numbers followed by different letters for each day after challenge are significantly different ($P = 0.05$) according to Duncan's multiple range test.

DISCUSSION

When the inducer leaf (leaf 1) was removed at any time after inoculation, the time of challenge (either immediately after excision or several days later) did not affect the extent of protection of leaf 2. The appearance of protection required about a 3-day lag period between induction and challenge. Protection developed gradually and reached a maximum by about 6 days. Removal of the inducer leaf 4 or 5 days after inoculation resulted in intermediate levels of protection in leaf 2. Protection did not increase with a delayed challenge. Apparently, within the limitations of the bioassay, the signal has a very rapid effect on sensitizing the plant's defense mechanisms.

The lag period of 72 hr coincided with the time symptoms became visible on the inducer leaf (15). Scions grafted onto rootstocks bearing leaves with well-established lesions started to show protection soon after translocation occurred, but not before. Apparently, more than direct physical contact of tissues is necessary for protection to be transmitted. The lag period of about 72 hr was probably not due to inability of the signal to move out of the inducer leaf but more likely resulted from a lack of signal production in the inoculated leaf and/or insufficient signal in leaf 2 to produce a detectable effect on disease, i.e., the threshold for protection was not attained.

Protection of the grafted scions also developed gradually, requiring 48–96 hr to reach the maximum. This indicates that even in a system where the signal is considered to be actively produced, a substantial period of time is required for attainment of full protection. The development of protection is not an all-or-nothing phenomenon. Maximum protection may be reached because the inducer leaf is no longer producing the signal or because the plant's response to the signal has been saturated. The former seems unlikely, since scions grafted onto infected rootstocks become protected even when the rootstocks were inoculated more than 144 hr before grafting (6,11).

Attempts to follow the signal movement across the inducer leaf, either with a large number of plants at a single time period or fewer plants at several time periods after inoculation, were unsuccessful. The gradual development of protection apparently is not due to slow movement of the signal out of the inducer leaf, since the data suggest the signal moves out rapidly, with no detectable concentration gradient within the inducer leaf. At any one time, the amount of signal present in the inducer leaf, then exported from the leaf to the rest of the plant is so small as to cause no detectable increase in the level of protection of leaf 2. Unpublished data also suggest that systemic protection occurs simultaneously throughout the plant.

On the basis of previous reports and the foregoing data, we propose that the signal is first produced at the time lesions in the inducer leaf begin to develop (13,14). The signal is rapidly and continuously exported to the rest of the plant, causing swift sensitization of the plant's defense mechanisms. The level of protection increases as more signal is produced and exported. Maximum protection is attained when all signal receptors have been saturated. Because the signal has not been readily extracted from infected or systemically protected tissues, it may be irreversibly bound, modified, or degraded in this interaction. The nature of the signal is unknown. It seems to move in the body of the plant and may be translocated through the vascular system (9). Matsumoto and Asada (17) recently extracted a fraction from infected cucumber leaves that induced formation of lignin and systemic protection against *C. lagenarium*. Carbohydrate and protein were associated with the active material. Whether this is the signal and whether it moves throughout the plant remain to be seen.

Sustained stimuli such as injury or disease, on the other hand, cause electrical disturbances in plants, including cucumber (19). Our data leave open the possibility that the signal is electrical. Davies and Schuster (4,5) suggested that changes in membrane potentials and ion fluxes may play a role in the rapid formation of polysomes in distal pea tissues in response to wounding. This may also explain why attempts to extract the signal have not been successful.

LITERATURE CITED

1. Bergstrom, G. C., Johnson, M. C., and Kuć, J. 1982. Effects of local infection of cucumber by *Colletotrichum lagenarium, Pseudomonas lachrymans,* or tobacco necrosis virus on systemic resistance to cucumber mosaic virus. Phytopathology 72:922-926.
2. Bergstrom, G. C., and Kuć, J. 1981. Induction of systemic resistance in cucumber to bacterial wilt and gummy stem blight. (Abstr.) Phytopathology 71:203.
3. Caruso, F. L., and Kuć, J. 1979. Induced resistance of cucumber to anthracnose and angular leaf spot by *Pseudomonas lachrymans* and *Colletotrichum lagenarium*. Physiol. Plant Pathol. 14:191-201.
4. Davies, E., and Schuster, A. 1985. Intercellular communication in plants: Evidence for a rapidly generated, bidirectionally transmitted wound signal. Proc. Nat. Acad. Sci. USA 78:2422-2426.
5. Davies, E., and Schuster, A. 1981. Polyribosome formation in response to wounding. (Abstr.) Plant Physiol. 65:33.
6. Dean, R. A., and Kuć, J. 1986. Induced systemic protection in cucumbers: The source of the "signal." Physiol. Mol. Plant Pathol. 28:227-233.
7. Garas, N. A., and Kuć, J. 1984. The extraction, assay and general properties of an inducing resistance factor from cucumber leaves induced with *Pseudomonas lachrymans*. (Abstr.) Phytopathology 74:873.
8. Gessler, C., and Kuć, J. 1982. Induction of resistance to Fusarium wilt in cucumber by root and foliar pathogens. Phytopathology 72:1439-1441.
9. Guedes, M. E. M., Richmond, S., and Kuć, J. 1980. Induced systemic resistance to anthracnose in cucumbers as influenced by the location of the inducer inoculation with *Colletotrichum lagenarium* and the onset of flowering and fruiting. Physiol. Plant Pathol. 17:229-233.
10. Hammerschmidt, R., Acres, S., and Kuć, J. 1976. Protection of cucumber against *Colletotrichum lagenarium* and *Cladosporium cucumerinum*. Phytopathology 66:790-793.
11. Jenns, A. E., and Kuć, J. 1979. Graft transmission of systemic resistance of cucumbers to anthracnose induced by *Colletotrichum lagenarium* and tobacco necrosis virus. Phytopathology 69:753-756.
12. Jenns, A. E., Caruso, F. L., and Kuć, J. 1979. Non-specific resistance to pathogens induced systemically by local infection of cucumber with tobacco necrosis virus, *Colletotrichum lagenarium* or *Pseudomonas lachrymans*. Phytopathol. Mediterr. 18:129-134.
13. Kuć, J. 1982. Plant immunization—mechanisms and practical implications. Pages 157-178 in: Active Defense Mechanisms in Plants. R. K. S. Wood and E. Tjamos, eds. Plenum Press, New York.
14. Kuć, J. 1983. Induced systemic resistance in plants to diseases caused by fungi and bacteria. Pages 191-221 in: The Dynamics of Host

Defense. J. A. Bailey and B. J. Deverall, eds. Academic Press, New York.
15. Kuć, J., and Richmond, S. 1977. Aspects of the protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. Phytopathology 67:533-536.
16. Kuć, J., Shockley, G., and Kearney, F. 1975. Protection of cucumber against *Colletotrichum lagenarium* by *Colletotrichum lagenarium*. Physiol. Plant Pathol. 7:195-199.
17. Matsumoto, I., and Asada, Y. 1984. A role of lignification-inducing factor in resistance induction demonstrated in Japanese radish downy mildew and cucumber anthracnose. Ann. Phytopathol. Soc. Jpn. 50:483-490. (English summary)
18. McIntyre, J. L., Dodds, J. A., and Hare, J. D. 1981. Effects of infections of *Nicotiana tabacum* by tobacco mosaic virus on systemic resistance against diverse pathogens and an insect. Phytopathology 71:297-301.
19. Van Sambeck, J. W., and Pickard, B. G. 1976. Mediation of rapid electrical, metabolic, transcriptional, and photosynthetic changes by factors released from wounds. I. Variation potentials and putative action potentials in intact plants. II. Mediation of the variation potential by Ricca's factor. III. Measurements of CO_2 and H_2O fluxes. Can. J. Bot. 54:2642-2671.