Effects of Local Infection of Cucumber by *Colletotrichum lagenarium*, *Pseudomonas lachrymans*, or Tobacco Necrosis Virus on Systemic Resistance to Cucumber Mosaic Virus

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ABSTRACT

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Inoculation of leaves 1 and 2 of cucumber cultivar Marketer with *Colletotrichum lagenarium, Pseudomonas lachrymans*, or tobacco necrosis virus (TNV) induced systemic resistance to challenge inoculation with cucumber mosaic virus (CMV) rubbed onto leaf 3. Induced resistance was expressed as a decrease in the number of chlorotic, primary lesions on CMV-inoculated leaves and as a delay in the time of appearance of systemic mosaic symptoms in plants with induced resistance compared to that of control plants. Differences between TNV-induced and control plants were most pronounced with dilute CMV inocula. Local lesions of TNV enhanced the moderate resistance of cultivar Wisconsin SMR-58 cucumber to CMV. Resistance also was induced in plants challenged with CMV transmitted by melon aphids, the natural vectors of CMV. Induced resistance to CMV resembled klendusity, the tendency to escape infection. To our knowledge, this study provides the first report that localized infections by fungi, viruses, and bacteria nonspecifically induce systemic resistance in a plant against a systemic virus.

Additional key words: angular leaf spot, anthracnose, gummy stem blight, induced susceptibility, *Mycosphaerella melonis*.

Viruses are known to induce resistance, in hypersensitive host plants, to reinfection by the same or different local lesion-producing viruses and the induced resistance is often systemic (1,3,5,7,9,14,17,19–21,25,27,28,30). Gilpatrick and Weintraub (5) reported that local lesions of carnation mosaic virus on lower leaves of *Dianthus barbatus* induced resistance to the virus in upper leaves. Ross (20,21) reported that the formation of local virus lesions induced systemic resistance in tobacco and bean to challenge inoculation with the same or other viruses. Loebenstein (14) provided other examples. Localized infections with fungi, bacteria, or their metabolites also induced systemic resistance in plants to local lesion-producing viruses (7–9,15,29). Local necrotic lesions produced by fungi, viruses, and bacteria have been shown to induce resistance in whole cucumber plants to challenge with tobacco necrosis virus (TNV), a local lesion-producing virus (9).

A specialized type of acquired resistance develops when plants systemically infected with one virus are "cross-protected" against systemic infection by a second serologically closely related virus, but the protecting virus must be continually present to inhibit the second virus (4). Systemic viruses may also protect against local lesion-producing viruses (17).

This study was undertaken to determine whether the general resistance induced in cucumber by fungi, bacteria, or local lesion-producing viruses (13) extended to protection against challenge inoculation with a systemic virus, cucumber mosaic virus (CMV).

MATERIALS AND METHODS

Culture of pathogens, hosts, and vectors. Race 1 of *Colletotrichum lagenarium* (Pass.) Ell. and Halst. and a Kentucky isolate of *Mycosphaerella melonis* (Pass.) Chiu and Walker obtained from muskmelon leaves showing symptoms of gummy stem blight were each maintained on bean pod agar at 24 C in the dark. Conidial suspensions were prepared by scraping spores from the surface of 5- to 9-day-old cultures, suspending them in distilled water, and filtering them through four layers of cheesecloth to remove mycelial fragments. Spore concentrations were determined with a hemacytometer and inoculum densities were adjusted with water to give suspensions containing 10^6 conidia per milliliter. Inocula of *M. melonis*, which requires a nutritional stimulus to induce disease in uninjured cucumber foliage (23), were amended to give final concentrations of 0.1% sucrose and 0.05% hydrolyzed casein (Sigma Chemical Co., St. Louis, MO 63178).

Pseudomonas lachrymans (Sm. and Bryan) Carsner, isolate 408, was maintained on King's B agar (12) slants at 24 C in the dark. Inocula were prepared by suspending bacteria from 1-day-old cultures in distilled water and adjusting the concentration with distilled water to give suspensions of approximately 10^8 viable cells per ml according to a spectrophotometric standard curve of absorbance at 620 nm.

TNV was obtained from R. W. Fulton (University of Wisconsin, Madison 53706), and was maintained in cucumber plants. Inocula were prepared by grinding leaves with abundant necrotic lesions in a precooled mortar with 0.05 M phosphate buffer pH 7 at an approximately 1:10, w/v ratio.

An isolate of CMV obtained from B. G. Raccah (Volcani Institute of Agricultural Research, Bet Dagan, Israel) was maintained in susceptible tobacco plants as well as in cultivar Marketer cucumber. Inocula were prepared by grinding 10 g of systemically infected tobacco leaves in 20 ml of 0.05 M phosphate buffer pH 7 in an ice-cooled mortar.

Cucumber (*Cucumis sativum* L.) cultivars Marketer (susceptible to CMV) and Wisconsin SMR-58 (moderately resistant to CMV) were grown in 10-cm-diameter plastic pots containing Pro-Mix Bx (Premier Peat Moss Co., Marketing, NY 10036) in a greenhouse at 23–31 C under daylight supplemented with fluorescent light. Plants were fertilized twice weekly with Ra-Pid-Gro (Dansville, NY 14437).

A colony of melon aphids (*Aphis gossypii*) was obtained from George Kennedy (North Carolina State University, Raleigh, 27607) and was maintained on cucumber plants.

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Inducing inoculations with various pathogens. Inducing inoculations were made on leaves 1 and 2 of cultivar Marketer plants when leaf 3 was one-fourth to one-third expanded. Plants were inoculated with *C. lagenarium* or *M. melonis* by placing 20 (5 μl) droplets of conidial suspension on each leaf. Plants were incubated in a closed humidity chamber for 24 hr followed by 24 hr in a partially opened chamber, which permitted gradual atmospheric equilibration. Plants were returned to the greenhouse after 48 hr.

Plants were inoculated with *P. lachrymans* by dipping cheesecloth pads in bacterial suspensions and then gently swabbing the abaxial surface of entire leaves; plants were rinsed with water and were kept on the greenhouse bench for the duration of the experiment.

Plants were inoculated with TNV by gently rubbing the Carborundum-dusted, adaxial leaf surfaces with cheesecloth pads dipped in sap preparations from infected plants, rinsed with water, and kept on the greenhouse bench for the duration of the experiment. Control plants were either untreated or were dusted with Carborundum and rubbed with water-soaked cheesecloth pads. In a separate experiment, rubbing was shown to have no effect on development of CMV symptoms following challenge inoculation.

Challenge inoculations with sap containing different CMV concentrations. Unless otherwise stated, plants were challenge-inoculated with a 1:10 dilution of CMV-infected tobacco sap. In certain experiments (Fig. 2A–C), plants were also challenged with the original sap preparation or with a 1:50 dilution of the original CMV sap in phosphate buffer. Each plant was challenged 7 days after the inducing inoculation by rubbing the adaxial surface of Carborundum-dusted leaf 3 with cheesecloth pads dipped in inoculum. Leaves were rinsed and the plants were kept on the greenhouse bench.

Challenge with aphid-transmitted CMV. Melon aphids, which transmit CMV in a nonpersistent manner, were allowed to acquire virus by probing systemically infected cultivar Marketer cucumber leaves for between 30 and 90 sec after a starvation period of 1–3 hr in glass vials. Probing activity was judged under a dissecting microscope by the retraction of the antennae against the thorax while the stylets were extended. Five viruliferous aphids were transferred to leaf 3 of each plant with a camel's hair brush, being careful not to injure the host. Twelve hours later, the plants were sprayed with Orthene (Chevron Chemical Co., San Francisco, CA 94119) to kill the aphids and were returned to the greenhouse.

Evaluation of symptom development. Plants were observed at ~1000 hours each day following challenge with CMV. Each plant was categorized as to whether it showed systemic mosaic symptoms or not, and the percentage of plants showing systemic symptoms was recorded for each treatment group. Faint chlorotic spots, the primary lesions of CMV, developed in leaf 3 approximately 2–3 days after inoculation. Similarly, localized vein-clearing and chlorosis appeared in 3–4 days in the third leaf of plants on which aphids had been placed. The numbers of primary CMV lesions were recorded.

Histochemical examination of CMV-challenged leaves. Leaf disks 10 mm in diameter were cut from leaf 3 of control and TNV-induced plants at 2, 3, 4, 5, and 6 days after CMV inoculation. They were cleared in boiling 70% ethanol and were examined histochemically after treatment with phloroglucinol-HCl reagent to detect lignin (11) and with the lacmoid reagent to detect callose deposition (22).

RESULTS

Systemic effects of various inducers against CMV. Induced resistance to disease caused by CMV was observed as a tendency toward delayed symptom development in populations of induced plants as compared to uninduced plants; this trend was consistently observed in greenhouse experiments conducted at different times of year. Faint, chlorotic spots developed on CMV-inoculated leaf 3 in 2–3 days regardless of whether the plants were induced or uninduced. The typical mosaic symptoms were first evident on the new foliage of uninduced plants 4–5 days after challenge of leaf 3 depending on the experiment (Fig. 1). The average time of appearance of systemic symptoms was delayed in TNV-induced

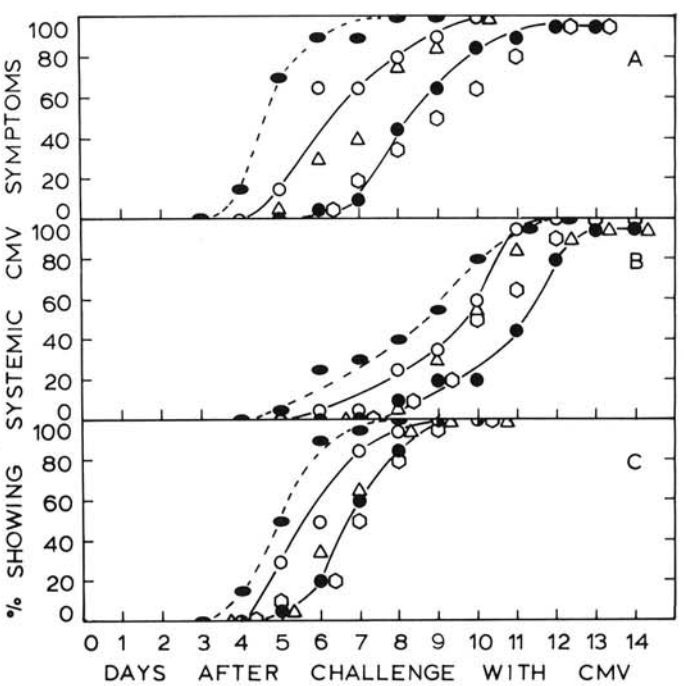

Fig. 1. Effects of various inducers on the time of appearance of systemic mosaic symptoms in populations of cultivar Marketer cucumber plants after challenge with cucumber mosaic virus (CMV). Leaves 1 and 2 were either uninoculated o, or were inoculated with *Mycosphaerella melonis* ●, *Colletotrichum lagenarium* Δ, *Pseudomonas lachrymans* ⬡ 1, or tobacco necrosis virus • 7 days prior to challenge of leaf 3 with a 1:10 dilution of CMV inoculum. Graphs A, B, and C are the results of three separate experiments. Each data point is the percentage of 20 plants per treatment with systemic symptoms.

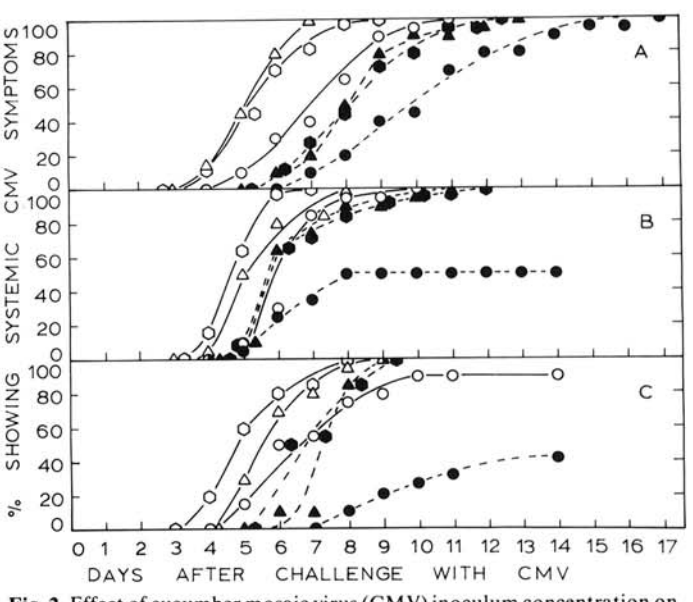

Fig. 2. Effect of cucumber mosaic virus (CMV) inoculum concentration on the time of appearance of systemic cucumber mosaic symptoms in populations of uninduced and tobacco necrosis virus (TNV)-induced cultivar Marketer cucumber plants after challenge with CMV. Leaves 1 and 2 were inoculated with TNV (solid symbols and dotted lines) or uninoculated (open symbols and solid lines) 7 days prior to challenge to leaf 3 with the original CMV sap preparation (Δ, Δ), or with 1:10 (⬡ , ⬢) or 1:50 (O, ●) dilutions of CMV inoculum. Graphs A, B, and C are the results of three separate experiments. Each data point is the percentage of 20 plants per treatment with systemic symptoms.

populations of plants as compared to control populations (Fig. 1). *P. lachrymans* consistently induced a delay in systemic mosaic development comparable to that induced by TNV (Fig. 1). *C. lagenarium* induced consistently detectable, but less effective resistance than did TNV and *P. lachrymans*. Inoculation of leaves 1 and 2 of cultivar Marketer cucumber with TNV, *P. lachrymans*, or *C. lagenarium* caused no visible alterations in plant growth. However, inoculation of leaves 1 and 2 with *M. melonis* resulted in rapid necrosis, caused stunting of the plants, and induced somewhat greater susceptibility to CMV rubbed onto leaf 3 (Fig. 1). The rate of mosaic appearance varied with greenhouse climate, but the relative trends among the treated populations remained fairly constant under the environmental conditions of different experiments (Fig. 1A–C).

TABLE 1. The effect of tobacco necrosis virus (TNV) lesions on leaves 1 and 2 of cultivar Marketer cucumber on the development of primary, chlorotic cucumber mosaic virus (CMV) lesions on leaf 3 following challenge inoculation with CMV

Experiment	Primary CMV lesions on leaf 3 (no.)	
	Control	TNV-induced
1	39.8±4.1[a]	28.6±3.9[a]
2	13.5±1.6	5.7±0.6
3	9.7±1.0	4.9±0.5

[a] Mean ± standard error based on sample size of $n = 10$ for experiment 1 and $n = 20$ for experiments 2 and 3. Leaf 3 was challenged with a 1:10 dilution of CMV inoculum 7 days after the inducing inoculation. CMV lesions were counted 3 days after challenge in experiments 1, 2, and 3 on plants of the same populations which were observed for systemic symptoms in Fig. 1 A, B, and C, respectively.

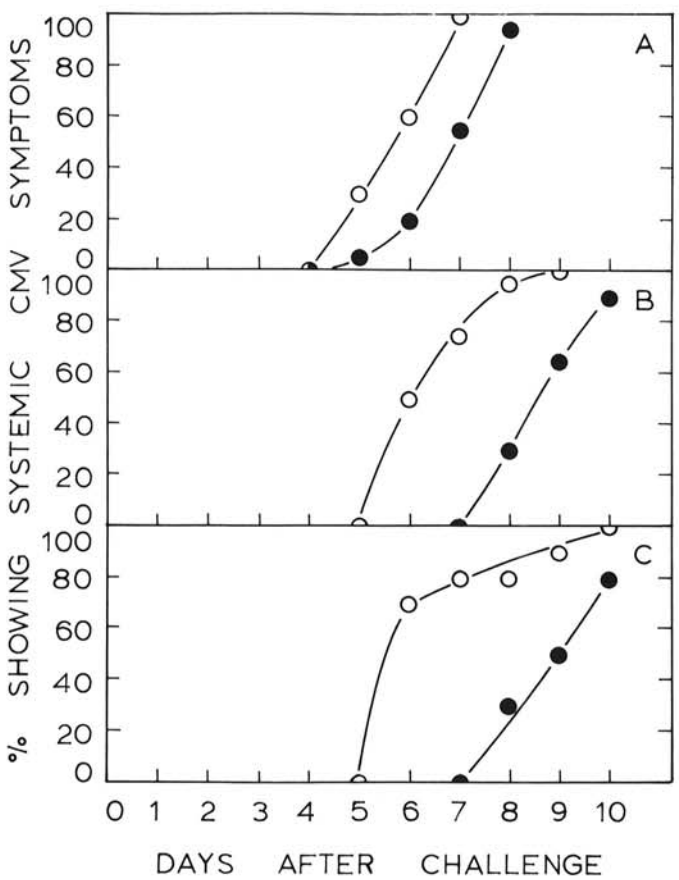

Fig. 3. Effects of tobacco necrosis virus (TNV) on the time of appearance of systemic cucumber mosaic symptoms in cultivar SMR-58 cucumber (moderately resistant to cucumber mosaic virus [CMV]) plants after challenge with CMV. Leaves 1 and 2 were inoculated with TNV (solid circles) or were uninoculated (open circles) 7 days prior to challenge of leaf 3 with 1:10 dilution of CMV inoculum. Graphs A, B, and C are the results of three separate experiments. Each data point is the percentage of 20 plants per treatment with systemic symptoms.

Effect of concentration of CMV challenge inoculum on induced resistance. Mosaic symptoms developed most rapidly in plants challenged with the most concentrated CMV inocula (Fig. 2). The average time of mosaic appearance was earlier in the uninduced populations compared to that of the corresponding TNV-induced populations challenged with each concentration of CMV inoculum. In two experiments (Fig. 2B and C), as many as 50% of plants in the induced populations challenged with dilute (1:50) CMV inoculum escaped mosaic development completely.

Systemic effects of TNV lesions on CMV primary lesion development. There was a greater number of primary CMV lesions on leaf 3 of uninduced plants than of TNV-induced plants 3 days after CMV inoculation (Table 1); no new primary lesions appeared after 3 days. CMV lesions were counted in experiments 1, 2, and 3 on plants of the same populations that were observed for systemic symptoms in Fig. 1 A, B, and C, respectively.

Induced resistance in a CMV-resistant cultivar. Systemic symptoms developed in cultivar SMR-58 cucumber at about the same time as in cultivar Marketer but the mosaic symptoms were much less striking. Inoculation of lower leaves with TNV enhanced the CMV resistance of cultivar SMR-58 by delaying the appearance of mild mosaic symptoms (Fig. 3) analogous to the delay in severe mosaic in induced cultivar Marketer plants (Figs. 1 and 2).

Induced resistance to aphid-transmitted CMV. There were delays in the appearance of systemic CMV symptoms in the TNV-induced compared to uninduced populations following inoculation of leaf 3 with aphid-transmitted CMV (Fig. 4). However, there were no detectable differences in the number of primary lesions (Table 2) as there were in the case of rubbed CMV challenge inoculation (Table 1).

Histochemical examination of CMV-rubbed leaves. No gross

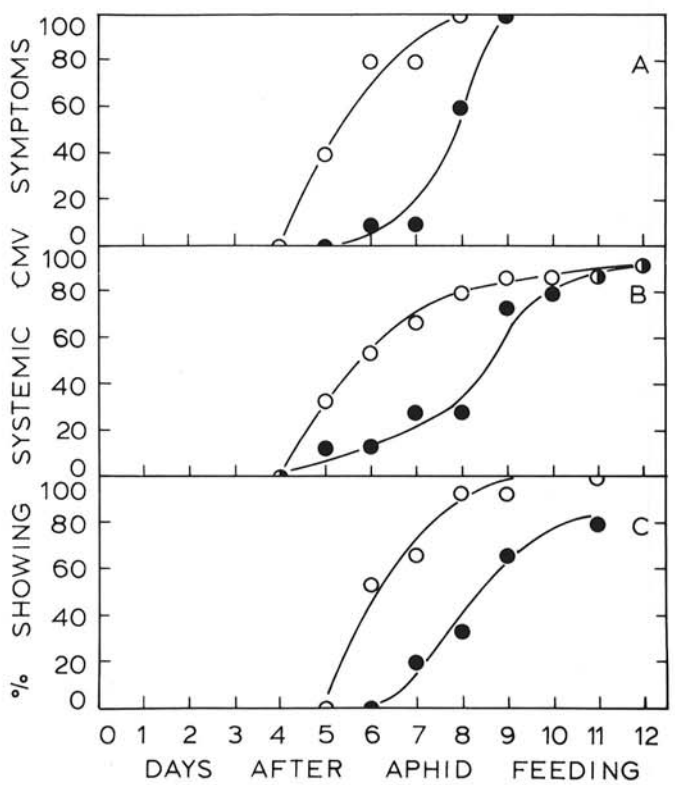

Fig. 4. Effect of tobacco necrosis virus (TNV) on the time of appearance of systemic cucumber mosaic symptoms in populations of cultivar Marketer cucumber plants after challenge with aphid-transmitted cucumber mosaic virus (CMV). Leaves 1 and 2 were inoculated with TNV (closed circles) or were uninoculated (open circles) 7 days prior to commencement of probing by viruliferous aphids on leaf 3. Data points are percentages of 10, 15, and 15 plants per treatment in experiments A, B, and C, respectively.

differences in callose were found in association with primary CMV lesions between induced and uninduced plants. Lignin was seldom detected in nonvascular cells except in wounded trichomes.

DISCUSSION

Localized lesions of *C. lagenarium, P. lachrymans*, or TNV induced systemic resistance in cultivar Marketer cucumber to systemic cucumber mosaic development. This is to our knowledge, the first report of resistance to development of systemic viral symptoms induced nonspecifically in a plant by a fungus, a bacterium, and a local lesion virus. Induced resistance was detected as a delay in the onset of systemic symptom development similar to a delay in bacterial wilt development in protected cucumber plants challenged with the systemic bacterium, *Erwinia tracheiphila* (2).

The type of resistance induced in cucumber against CMV resembled that described as klendusity, the inherent tendency to escape infection (26). This is supported by the fact that several protected cucumber plants escaped disease when challenged with dilute CMV inoculum and by the reduction in the number of primary CMV lesions in challenged, protected plants. Expression of induced resistance against TNV, a local lesion-producing virus, in cucumber was also dependent on challenge virus concentration (9). Reduced numbers of primary virus lesions appear to be analogous to reduced penetrations by fungi into the epidermis of protected cucumber leaves (6,10,18). Troutman and Fulton (26) reported that plants of tobacco cultivar T.I. 245 exhibited klendusity to CMV and other viruses. Thomas and Fulton (24) concluded that klendusity was based on reduced number of ectodesmata which served as initial infection sites. Darkening or temperature treatments of T.I. 245 tobacco increased susceptibility as well as increased the numbers of ectodesmata (24).

In addition to klendusity, which reduced the number of local lesions, Thomas and Fulton (25) reported that there was resistance in cell-to-cell virus movement in T.I. 245 tobacco, which resulted in a reduction in the size of local lesions. This type of resistance resembled systemic acquired resistance to viruses in that both were eliminated at 28 C and both were nonspecific (25). Furthermore, systemic acquired resistance was induced in susceptible tobacco, but not in T.I. 245. However, we found that cultivar resistance of cultivar SMR-58 cucumber to CMV could be enhanced by inducing systemic resistance. That two mechanisms, one against initial infection and one against systemic virus movement, may be expressed in induced systemic resistance to CMV is suggested by the differences between mechanical and vector transmission of the CMV challenge. Mechanical transmission revealed a difference in both the number of primary lesions and in the time of appearance of systemic symptoms in protected compared to unprotected plants. Even though there was no reduction in the number of primary lesions in protected plants following aphid transmission, the plants were protected in terms of reduced systemic symptom development. Perhaps the aphid stylets bypassed the klendusity barrier while resistance to virus movement was still operational. This is a potential example of the involvement of multiple mechanisms in induced resistance as suggested by Kuć (13).

Localization or resistance to virus movement has been postulated to occur either by inhibition of viral multiplication or by structural barriers (paramural bodies, callose deposition, cell collapse) that seal off plasmodesmata to uninfected cells (14,16). Lignin or toxic lignin precursors were recently implicated as part of the localization mechanism in induced resistance to fungal pathogens in cucumber (6). We found no gross differences between induced and uninduced, CMV-challenged leaves in the deposition of lignin or callose. Whether or not induced resistance to viruses affects the multiplication of the challenge virus needs to be resolved. Some investigators (9,21,28) reported that infectivity was reduced in the challenged leaves of protected plants while others (1,3) reported no effect on the infectivity or accumulation of viral RNA in the challenged tissues. Fraser (3) suggested that induced systemic resistance to viruses may not be resistance to virus multiplication or movement, but may be just a suppression of symptom development.

The reason for the apparent induction of systemic susceptibility to CMV by inoculation of the lower leaves with *M. melonis* is not understood, but could simply be a matter that challenged leaves were more susceptible owing to their physiological condition in the stunted plants.

This report extends the general resistance potential of induced systemic resistance in cucumber as reviewed by Kuć (13) to protection against systemic viral pathogens. The potential usefulness of this phenomenon as a biological control measure is enhanced in that induced resistance was expressed even when CMV was transmitted by melon aphids, natural vectors of the virus.

TABLE 2. The effect of tobacco necrosis virus (TNV) lesions on leaves 1 and 2 of cultivar Marketer cucumber on the development of primary, chlorotic cucumber mosaic virus (CMV) lesions on leaf 3 following exposure to melon aphids contaminated with CMV

Experiment	Primary CMV lesions on leaf 3 (no.)	
	Control	TNV-induced
1	4.0±0.4[a]	3.3±0.3[a]
2	2.1±0.4	2.9±0.4
3	2.5±0.5	2.4±0.3

[a] Mean ± standard error based on a sample size of $n = 10$ for experiment 1 and $n = 15$ for experiments 2 and 3. Five viruliferous aphids were placed on leaf 3 of each plant 7 days after the inducing inoculation. CMV lesions were counted 4 days after commencement of aphid probing in plants of the same populations which were observed for systemic symptoms in Fig. 4 A, B, and C, repsectively.

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