Effects of Localized Infections of *Nicotiana tabacum* by Tobacco Mosaic Virus on Systemic Resistance Against Diverse Pathogens and an Insect

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ABSTRACT

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In experiments that were performed simultaneously, tobacco mosaic virus (TMV) inoculation of a hypersensitive tobacco cultivar induced systemic and long-lived resistance against TMV, *Phytophthora parasitica* var. *nicotianae* (Ppn), and *Pseudomonas tabaci.* In separate experiments, TMV also induced resistance against *Peronospora tabacina.* Reproduction of the aphid, *Myzus persicae* was also reduced on TMV-infected plants. Resistance to Ppn, *Ps. tabaci,* and TMV was also induced by localized infections of tobacco necrosis virus. Systemic infections by cucumber mosaic virus, however, reduced only the number of lesions caused by TMV. Levels of protection and the time resistance developed in a given leaf were not the same for the different challengers. These results show that a single viral agent may induce resistance in tobacco against diverse challengers.

Additional key words: host-parasite interaction, cucumber mosaic virus, tobacco necrosis virus.

In separate investigations, tobacco mosaic virus (TMV) has been shown to induce localized resistance of tobacco against *Thielaviopsis basicola* (4), *Peronospora tabacina* (9), and *Pseudomonas tabaci* (8), and localized as well as systemic resistance against TMV (13,14) and *Phytophthora parasitica* var. *nicotianae* (11). For the use of induced resistance in practical disease control, it is of obvious interest that one agent, such as TMV, may induce resistance of a plant against diverse challengers. Independent studies in which TMV was used to induce resistance and similar studies involving other host-parasite combinations, however, led to equivocal conclusions due to differences in experimental design. Therefore, we studied induced resistance of tobacco against diverse challengers under similar conditions and, when possible, experiments using different challengers were performed simultaneously. This approach not only permits comparisons in the development of local and systemic resistance against these challengers, but also enables conclusions to be drawn concerning the general nature of induced resistance in tobacco.

The inducing agents were TMV, tobacco necrosis virus, and cucumber mosaic virus. The challengers included: the fungi, *Phytophthora parasitica* var. *nicotianae* and *Peronospora tabacina;* a bacterium, *Pseudomonas tabaci*; and a virus, tobacco mosaic virus (TMV). These were selected because they had been studied previously, but only local resistance against *P. tabacina* (9) and *Ps. tabaci* (8) has been reported. If induced resistance is effective against diverse challengers, we hypothesized that it also may be effective against insects. Thus, we also selected the green peach aphid, *Myzus persicae,* as a challenger.

MATERIALS AND METHODS

Plants. The host plant used was *Nicotiana tabacum* 'Windsor Shade 117' (WS 117), a cultivar hypersensitive to tobacco mosaic virus (TMV) and susceptible to *Pseudomonas tabaci* (Wolf and Foster), *Peronospora tabacina* Adam., and *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker (= *Phytophthora nicotianae* van Breda de Haan var. *nicotianae*). Tobacco is also a herbaceous secondary host of the green peach aphid, *Myzus persicae* (Sulz.) (Homoptera: Aphididae).

Tobacco seed was germinated and plants were grown as previously described (11). At least 7 days before experimental use, however, plants were moved from the greenhouse to growth room A (12-hr photoperiod under high-pressure sodium vapor lamps, 649 ± 27 $\mu Em^{-2}sec^{-1}$ PAR at plant height; day and night temperatures = 25 ± 1 C and 20 ± 1 C, respectively). For some tests, pelleted tobacco seeds were germinated singly in growth room A in 350-ml styrofoam cups containing Pro-Mix (Premier Peat Moss Marketing Corporation, New York, NY 10036, USA). Plants were maintained in carts containing a fertilizer solution (12-fold dilution of 20-20-20 fertilizer; Robert B. Peters Co., Inc., Allentown, PA 18104). No differences in resistance induced against individual challengers were observed between plants transferred to or reared in the propagation room.

Virus. The U-1 strain of TMV was maintained in and purified from *N. tabacum* 'White Burley' as previously described (11). Tobacco necrosis virus (TNV, from J. Kuć, University of Kentucky, Lexington 40506) was maintained in *N. tabacum* cultivar WS 117 by frequent transfer from leaves with many lesions. Cucumber mosaic virus (CMV, a local isolate from zucchini) was maintained in systemically infected *Vigna sinensis* 'California.'

Fungi and bacterium. Race 3 of *P. parasitica* var. *nicotianae* (Ppn) (isolate M-15T) was maintained and zoospores (10^5/ml) were prepared as previously described (11). Conidia of *P. tabacina* were collected from freshly sporulating lesions on field-collected tobacco leaves by washing the leaves with distilled water. The spore suspension was adjusted to 10^5 conidia per milliliter for inoculation.

Pseudomonas tabaci (ATCC 11528, from R. N. Goodman, University of Missouri, Columbia 65201) was streaked onto King's B medium (6) from single colonies on the same medium 18–24 hr before use. Bacteria were suspended in 0.01 M potassium phosphate buffer, pH 7.2, and adjusted to 10^6 colony forming units (cfu) per milliliter for use as inoculum.

Insects. A colony of *M. persicae* was established from about 50 apterous parthenogenetic females collected from field-grown potato; their offspring were reared on tobacco cultivar WS 117 for at least 10 generations in the greenhouse (day and night minimum temperature = 24 C and 13 C, respectively, with supplemental fluorescent lighting to provide a 16-hr photoperiod). The population density of the colony was reduced periodically to maintain the production of apterous forms.

Parthenogenetic adult females (founders) for experimental use were produced by removing mature females from the main colony, allowing them to reproduce on a fresh plant for 24 hr, and then removing them. The resulting nymphs then developed on the same

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plant until they reached reproductive age, at which time they were utilized. These procedures insured that all founders were of similar age and physiological condition at the beginning of each experiment.

Inoculation with virus to induce resistance. Test plants were inoculated with TMV (purified virus) or TNV (extracts from leaves with many lesions) in 0.01 M potassium phosphate, pH 7.2, containing 1% w/v Celite as an abrasive; CMV (extracts from systemically infected leaves) inocula were prepared in the same buffer containing sodium thioglycollate (0.1%, w/v) and sodium diethyldithiocarbamate (0.3%, w/v). Leaf extracts were made at a ratio of 1 g of tissue to either 1 ml (TNV) or 5 ml (CMV) of buffer. Inoculations were made with a gauze pad.

The adaxial surfaces of entire or single lateral halves of tobacco leaves (the younget expanded leaf and the next oldest leaf) were inoculated with 1.0 μg/ml TMV. The youngest expanded leaf and the three next oldest leaves were inoculated with TNV. More old leaves were inoculated with TNV than with TMV because numbers of TNV lesions were greatest on older leaves, whereas TMV produced the most lesions on the youngest expanded leaf.

Only the youngest expanded leaf was inoculated with CMV. Leaves inoculated with buffered sap were washed with tap water immediately after inoculation. Uninoculated control plants were abraded with buffer and celite in some experiments, but this was found not to affect the results and was discontinued. Inoculated plants were maintained in growth room B (12-hr photoperiod under wide-spectrum fluorescent lights, $686 \pm 26\ \mu Em^{-2} sec^{-1}$ PAR at plant height; day and night temperatures were 25 ± 1 and 17 ± 1 C, respectively).

Challenge inoculations, fungi, and bacterium. Plants were spray-inoculated on Celite-abraded leaves as described previously (11) with Ppn zoospores or with *Ps. tabaci.* Plants were inoculated likewise with conidia of *P. tabacina* except that leaves were not abraded prior to inoculation. Plants inoculated with Ppn or *Ps. tabaci* were kept in growth room B and maintained in closed plastic bags containing a moistened towel. Plants were removed from the bags 48 hr after inoculation and lesions caused by Ppn were counted. Lesions caused by *Ps. tabaci* were counted 48–72 hr after the plants were removed from the bags. Plants inoculated with *P. tabacina* were placed in plastic bags overnight and then maintained in the greenhouse. Lesions were counted 7 days after inoculation.

Challenge inoculation, virus. Leaves were challenged with TMV by inoculating them in a manner similar to that used to induce resistance and the plants were maintained in growth room B. Virus lesions were counted 4–7 days after challenge inoculation. Average lesion size (millimeters diameter) was determined by measuring 30 lesions per leaf 7 days after the challenge inoculation.

Challenge inoculation, insects. A total of three (January experiment) or five (April experiment) founders of even age were placed on the lower surface of two expanding leaves. The number of surviving founders and the number of nymphs each produced was recorded daily for 14 days. Nymphs were removed after each counting.

Resistance studies. Resistance was determined by reductions in lesion numbers (Ppn, *P. tabacina, Ps. tabaci,* TMV), lesion size (TMV), or reductions in reproduction (*M. persicae*) on induced as compared to control plants. To permit comparisons of the development of resistance against Ppn, *Ps. tabaci,* and TMV, uniformly reared plants were induction-inoculated (induced) simultaneously. Sets of these plants subsequently were challenged at the same time with either Ppn, *Ps. tabaci,* or TMV. Plants to be challenged up to 7 days after induction were induced at the five-leaf stage, while plants challenged 14 or 21 days after induction were induced at the four-leaf stage. Resistance against *P. tabacina* and *M. persicae* was studied separately in plants induced up to the seven-leaf stage.

Resistance induced by TMV inoculation against Ppn, *P. tabacina, Ps. tabaci* and TMV was studied in inoculated leaves (localized protection) and in leaves above the inoculated ones (systemic protection). Only systemic resistance was studied against *M. persicae* because the insect is mobile and prefers to feed on young expanding leaves above those inoculated with inducer.

When TNV was used as the inducer, local and systemic resistance against Ppn and *Ps. tabaci* and systemic resistance against TMV was studied as described above. Since CMV infects WS 117 systemically, all leaves were challenged with Ppn, *Ps. tabaci,* or TMV.

All experiments with Ppn, *Ps. tabaci,* or TMV used as challengers had a minimum of 10 replicate plants and were performed at least twice. The experiment with *P. tabacina* had 30 replicate plants and was performed once. Data for these tests are presented as averages for all observations and were analyzed by using Student's standardized *t*-test.

The experiment with *M. persicae* had five replicate plants and was performed twice. Differences in nymph production was shown by a two-factor analysis of variance on the number of nymphs produced per founder per day. The transformation $\sqrt{x + 0.5}$ was employed to make the data conform more closely to the assumptions of the analysis of variance (15). Data from replicate plants were pooled since the variation in daily nymph production per founder among plants was negligible. The values are presented as the average percent difference in the daily reproduction rate.

RESULTS

Tobacco cultivar WS 117, which exhibited the hypersensitive response to TMV (50–100 lesions per induced leaf), was protected against Ppn, *P. tabacina, Ps. tabaci,* TMV, and *M. persicae.* Plants were typically induced when they were 10–15 cm in height and, depending upon the time of challenge, were 20 to 60 cm tall by the completion of the experiment. At the time of challenge inoculations, there were no apparent differences in stature or appearance between untreated and TMV-induced plants apart from the lesions on the induced leaves.

Whole-plant resistance against Ppn occurred 4 days after TMV inoculation (average number of lesions per leaf [$\pm SE_{\bar{x}}$] was 6.8± 1.5 and 0.6 ± 0.2 on control and induced plants, respectively, $P \leq 0.01$). Resistance lasted at least 21 days (Table 1), and from the onset of resistance through 21 days, all local systemic leaves were protected (Table 2).

Whole-plant resistance against *Ps. tabaci* had developed 3 days after inoculation with TMV (Table 1). Local resistance was evident on day 3, while systemic resistance developed by day 7 and lasted through day 21 (Table 3). The level of resistance decreased on progressively younger leaves.

Whole-plant resistance against TMV had developed by day 3 after induction and lasted at least 21 days (Table 1). Local resistance against TMV could not be assessed at the day 3 challenge because both inducer and challenger lesions were developing at the same time and could not be differentiated. Local resistance was apparent at both the 7- and 14-day challenge times (Table 4). Opposite half-leaf resistance developed 3 days after induction and lasted for at least 14 days (Table 4). Systemic resistance against TMV also was apparent by day 3 and persisted through at least 21 days (Table 5). Uppermost leaves, however, were not protected systemically at each challenge period, but they became resistant as they matured (Table 5).

Resistance against TMV also was evident as reductions in lesion size (Table 6). Resistance was again related to leaf maturity and the leaf immediately above the induced leaves had the smallest lesions while the lesions on the uppermost challenged leaf were of the same size as those on control leaves.

Resistance against *P. tabacina* was nearly absolute when plants were challenged 7 days after TMV inoculation (Table 7). All control plants had lesions on all leaves while only seven of the 30 induced plants had leaves with lesions, and only one of these plants had lesions on more than one leaf.

Reproduction of *M. persicae* was significantly ($P \leq 0.01$) reduced when aphids were placed on plants 7 days after TMV inoculation. The average reduction in the number of nymphs produced per day was 9.3% (confidence limit: 0.7–26.3) and 12.9% (confidence limit: 2.7–29.1) for the January and April experiments, respectively. Reproduction was lower on induced plants 10 (January experiment) and 12 (April experiment) days during the 14 days of

TABLE 1. Whole-plant resistance of tobacco against *Phytophthora parasitica* var. *nicotianae* (Ppn), *Pseudomonas tabaci,* and tobacco mosaic virus (TMV) induced by prior inoculation with TMV[x]

Days between inducer and challenge inoculations	Lesions per leaf (ave. no. ±SE$_{\bar{x}}$)[y]								
	Ppn			*Ps. tabaci*			TMV		
	Untreated	TMV	t Value[z]	Untreated	TMV	t Value	Untreated	TMV	t Value
3	6.2 ± 2.1	6.5 ± 1.5	0.12	31.0 ± 5.0	8.2 ± 3.1	3.89**	41.3 ± 6.7	21.8 ± 1.4	2.86*
7	6.2 ± 1.9	0	3.24**	41.3 ± 4.5	3.8 ± 1.4	7.97**	58.3 ± 3.7	41.7 ± 4.8	2.80*
14	4.8 ± 0.7	0.3 ± 0.3	6.97**	20.0 ± 3.6	2.3 ± 0.7	4.87**	55.4 ± 5.2	39.6 ± 3.4	2.52*
21	9.7 ± 3.2	0.2 ± 0.1	3.00**	40.3 ± 4.5	9.4 ± 2.8	5.77**	16.7 ± 2.3	8.0 ± 1.0	3.56**

[x] The adaxial surface of the youngest fully expanded leaf and one leaf below it on tobacco plants at the four- or five-leaf stage was inoculated by rubbing with 1 μg/ml TMV. Plants were challenged 3–21 days later by spraying leaves to runoff with suspensions containing zoospores of Ppn (10^5/ml) or cells of *Ps. tabaci* (10^6 cfu/ml) or by rubbing with 1 μg/ml TMV.
[y] Values represent the average number of Ppn, *Ps. tabaci*, or challenge TMV lesions per leaf from all leaves (locally and systemically protected).
[z] Values significant at $P \leq 0.05$ (*) or 0.01 (**).

TABLE 2. Local and systemic resistance of tobacco leaves against *Phytophthora parasitica* var. *nicotianae* (Ppn) induced by prior inoculation with tobacco mosaic virus (TMV)[x]

Leaf number	Time between induction and challenge inoculation (days)											
	3			7			14			21		
	Avg no. lesions (±SE$_{\bar{x}}$)			Avg no. lesions (±SE$_{\bar{x}}$)			Avg no. lesions (±SE$_{\bar{x}}$)			Avg no. lesions (±SE$_{\bar{x}}$)		
	Untreated	TMV	t Value[y]	Untreated	TMV	t Value	Untreated	TMV	t Value	Untreated	TMV	t Value
Local 1	7.2 ± 0.3	2.9 ± 1.0	1.36	13.9 ± 4.3	0	3.25**	8.9 ± 1.8	0	4.72**	-[z]	-	-
2	4.0 ± 2.2	4.6 ± 1.5	0.23	10.7 ± 4.7	0	2.26*	8.9 ± 2.6	0	3.02**	-	-	-
Systemic 3	7.7 ± 2.7	11.3 ± 3.9	0.76	4.7 ± 0.9	0.7 ± 0.3	4.07**	2.9 ± 1.0	0	3.02**	15.2 ± 4.7	0.2 ± 0.2	3.35**
4	4.9 ± 1.9	6.1 ± 3.8	0.26	1.8 ± 0.5	0.2 ± 0.2	2.84*	3.0 ± 0.9	0	3.31**	11.1 ± 4.6	0	2.73*
5				1.6 ± 0.6	0	3.24**	2.6 ± 0.7	0.1 ± 0.1	3.60**	9.2 ± 4.1	0	2.24*
6										8.1 ± 3.1	0	2.76*
7										6.5 ± 2.2	0.2 ± 0.2	2.82*

[x] The adaxial surface of the youngest fully expanded leaf and one leaf below it (leaves 1 and 2) on plants at the four- or five- leaf stage was inoculated by rubbing with 1 μg/ml TMV. Plants were challenged by spraying all leaves to runoff with zoospores of Ppn (10^5/ml).
[y] Values significant at $P \leq 0.05$ (*) or 0.01 (**).
[z] At this time, TMV-inoculated leaves were senescent and were not challenged.

TABLE 3. Local and systemic resistance of tobacco leaves against *Pseudomonas tabaci* induced by prior inoculation with tobacco mosaic virus (TMV)[x]

Leaf position	Time between induction and challenge inoculation (days)											
	3			7			14			21		
	Avg no. lesions (±SE$_{\bar{x}}$)			Avg no. lesions (±SE$_{\bar{x}}$)			Avg no. lesions (±SE$_{\bar{x}}$)			Avg no. lesions (±SE$_{\bar{x}}$)		
	Untreated	TMV	t Value[y]	Untreated	TMV	t Value	Untreated	TMV	t Value	Untreated	TMV	t Value
Local 1	40.2 ± 8.4	2.6 ± 1.6	4.42**	50.2 ± 7.8	0.6 ± 0.4	5.63**	19.7 ± 1.4	0.3 ± 0.3	2.47*	-[z]	-	-
2	32.8 ± 9.4	1.2 ± 0.5	3.35**	46.9 ± 9.4	1.2 ± 0.7	4.85**	23.5 ± 5.7	0.1 ± 0.1	3.40**	-	-	-
Systemic 3	39.2 ± 11.9	21.6 ± 10.5	1.10	46.0 ± 10.2	9.0 ± 4.5	3.42**	18.8 ± 6.1	3.9 ± 2.7	2.22*	52.7 ± 11.2	3.7 ± 0.8	4.14**
4	21.9 ± 7.3	9.9 ± 4.1	1.42	29.9 ± 7.0	2.0 ± 1.1	3.92**	18.8 ± 8.0	1.4 ± 1.2	2.15*	58.0 ± 10.3	2.5 ± 1.2	5.37**
5				21.0 ± 1.0	5.1 ± 1.7	3.88**	19.9 ± 5.2	1.9 ± 0.8	3.44**	27.9 ± 6.3	6.3 ± 2.6	3.19**
6							33.9 ± 7.2	5.0 ± 1.9	4.27**	26.2 ± 5.1	8.4 ± 3.2	2.97**
7							7.2 ± 2.4	2.4 ± 0.7	2.47*	33.3 ± 5.9	12.5 ± 4.4	2.90**

[x] The adaxial surface of the youngest fully expanded leaf and one leaf below it (leaves 1 and 2) on plants at the four- or five-leaf stage was inoculated by rubbing with 1 μg/ml TMV. Plants were challenged by spraying all leaves to runoff with cells of *Ps. tabaci* (10^6 cfu/ml).
[y] Values significant at $P \leq 0.05$ (*) or 0.01 (**).
[z] At this time, TMV-inoculated leaves were senescent and were not challenged.

TABLE 4. Local and opposite half-leaf resistance of tobacco leaves against tobacco mosaic virus (TMV) induced by prior inoculation with TMV[x]

Days after induction that challenge was applied	Avg no. of challenge lesions/half-leaf (±SE$_{\bar{x}}$)					
	Local half-leaf			Opposite half-leaf		
	Untreated	TMV	t Value[y]	Untreated	TMV	t Value
3	-[z]	-	-	9.4 ± 1.3	3.3 ± 0.5	4.46**
7	13.1 ± 2.2	6.2 ± 1.2	2.77*	17.7 ± 2.5	5.9 ± 1.7	3.93**
14	27.4 ± 4.1	9.0 ± 1.8	5.20**	27.3 ± 4.1	16.2 ± 6.1	3.42**

[x] One-half of the adaxial surface of the youngest fully expanded leaf on plants at the four- or five-leaf stage was inoculated by rubbing with 0.3 μg/ml TMV. Plants were challenged by rubbing the entire adaxial surface with 0.1 μg/ml TMV.
[y] Values significant at $P \leq 0.05$ (*) or 0.01 (**).
[z] The number of lesions for the challenge inoculation on the induced half-leaf was determined by the difference between the total number of lesions and the number of lesions caused by the induction inoculation. Induction and challenge lesions on the local half-leaf could not be differentiated for the day 3 challenge inoculation.

observation. Reproduction declined with founder age on both induced and untreated plants, and reproduction was only 7–23% of the maximum during the last 3 days of observation. It was during this period that there was little if any effect on reproduction on induced plants.

When TMV was replaced by TNV as the inducer, whole plant resistance, as evidenced by reduced lesions numbers, also occurred against Ppn, *Ps. tabaci,* and TMV 7 days after induction (Table 8). Resistance against TMV also was apparent as a reduction in sizes of lesions (Table 6). The inducing lesions caused by TNV could be discerned visually from TMV lesions; only occasional plants had a TNV lesion that resembled a TMV lesion.

No protection against Ppn or *Ps. tabaci* developed when CMV was used as the inducer. Whole-plant protection against TMV had developed when plants were challenged 14 days after CMV inoculation (Table 8).

DISCUSSION

We demonstrated, in experiments performed simultaneously, that both TMV and TNV induce resistance in tobacco against Ppn, *Ps. tabaci,* and TMV. We also show that TMV induces resistance against *P. tabacina* and *M. persicae.* This provides unequivocal evidence that a single agent may induce systemic and persistent resistance in tobacco against diverse challengers. This further suggests the potential usefulness of induced resistance as a means of pathogen and pest control in the field.

Reduced aphid reproduction on TMV-induced plants is the first demonstration that a localized infection by a plant pathogen can induce systemic antibiosis against a phytophagous insect. Biotic or abiotic stresses on an entire plant may make it less suitable for insects, usually by increasing the concentration of plant chemicals that are detrimental to insects (1), or by reducing the nutritional quality of hosts for insects (10). Such whole-plant stresses, however, are in marked contrast to our report of a localized plant infection inducing systemic resistance against an insect.

Our results using TMV and TNV to induce systemic resistance in hypersensitive hosts agree with those of others (5,11,12,14). In contrast, CMV, a virus that systemically infects tobacco, caused resistance only against TMV, and only after systemic CMV symptoms appeared. Thus, the CMV-TMV response may not be the same as the general induction of resistance caused by TMV or TNV. Rather it may be an interaction between viruses in which one

TABLE 5. Systemic resistance of tobacco leaves against tobacco mosaic virus (TMV) induced by prior inoculation with TMV[y]

	Days between induction and challenge inoculation											
	3			7			14			21		
	Avg no. lesions ($\pm SE_{\bar{x}}$)			Avg no. lesions ($\pm SE_{\bar{x}}$)			Avg no. lesions ($\pm SE_{\bar{x}}$)			Avg no. lesions ($\pm SE_{\bar{x}}$)		
Leaf position	Untreated	TMV	t Value[z]	Untreated	TMV	t Value	Untreated	TMV	t Value	Untreated	TMV	t Value
Systemic 3	46.0 ± 6.0	21.0 ± 3.4	3.63**	63.4 ± 9.7	30.4 ± 4.6	3.08**	37.2 ± 6.9	13.0 ± 4.4	2.95**	6.6 ± 1.6	1.4 ± 1.2	2.59**
4	36.6 ± 11.1	23.8 ± 2.9	2.89**	63.4 ± 5.7	45.0 ± 2.4	2.65*	53.1 ± 8.1	25.5 ± 3.5	3.1 **	12.8 ± 2.5	2.0 ± 0.5	4.26**
5				48.2 ± 5.0	53.2 ± 9.4	0.47	53.6 ± 12.6	36.3 ± 4.4	1.17	13.6 ± 1.4	6.0 ± 0.6	5.06**
6							50.6 ± 12.5	61.2 ± 15.7	0.53	24.2 ± 5.6	10.4 ± 1.2	2.40*
7							72.0 ± 13.2	66.8 ± 9.8	0.33	23.2 ± 4.3	13.2 ± 2.6	1.99
8										21.7 ± 7.2	17.3 ± 5.3	0.51

[y]The adaxial surface of the youngest fully expanded leaf and one leaf below it (leaves 1 and 2) on plants at the four- or five-leaf stage was inoculated by rubbing with 1 μg/ml TMV. Plants were challenged by rubbing the adaxial surface of leaves 3-8 with 1 μg/ml TMV.
[z]Values significant at $P \leq 0.05$ (*) or 0.01 (**).

TABLE 6. Size of tobacco mosaic virus (TMV) challenge lesions on leaves of TMV- or tobacco necrosis virus (TNV)-induced tobacco plants as influenced by leaf position[w]

	Lesion diameter (mm ± $SE_{\bar{x}}$)[y]					
Leaf no.[x]	Untreated	TMV	t Value[z]	Untreated	TNV	t Value
3	2.2 ± 0.1	1.3 ± 0.1	7.20**	3.1 ± 0.1	1.7 ± 0.2	7.81**
4	2.5 ± 0.2	1.6 ± 0.1	4.76**	3.1 ± 0.1	2.4 ± 0.2	3.47**
5	2.5 ± 0.2	2.5 ± 0.2	0.01	3.3 ± 0.1	2.9 ± 0.1	2.16*

[w]The adaxial surface of the youngest fully expanded leaf and one leaf below it (leaves 1 and 2) on tobacco plants at the five-leaf stage was inoculated by rubbing with 1 μg/ml TMV, or the first fully expanded leaf and up to three leaves below it were inoculated by rubbing with TNV. Plants were challenged 7 days later by rubbing younger expanding leaves with 1μg/ml TMV.
[x] Leaf 3 is the first leaf above the leaves induced with TMV or TNV.
[y]Challenge lesions were measured 7 days after inoculation; values represent averages for 30 lesions.
[z]Values significant at $P \leq 0.05$ (*) or 0.01 (**).

TABLE 7. Whole-plant resistance and local and systemic resistance of tobacco against *Peronospora tabacina* induced by prior inoculation with tobacco mosaic virus (TMV)[x]

	P. tabacina lesions per leaf (avg no. ± $SE_{\bar{x}}$)		
Leaf number	Untreated	TMV	t Value[y]
Local 1	7.5 ± 1.2	0	6.20**
2	6.7 ± 1.2	0	6.37**
Systemic 3	16.1 ± 3.2	1.0 ± 0.4	4.72**
4	15.8 ± 2.6	0.1 ± 0.1	6.03**
5	9.5 ± 3.1	0	3.05**
6	2.3 ± 0.7	0	3.92**
Avg lesions/leaf[z]	10.0 ± 0.2	0.2 ± 0.1	6.44**

[x]The adaxial surface of the youngest fully expanded leaf and one leaf below it (leaves one and two) on plants at the five- or six-leaf stage was inoculated by rubbing with 1 μg/ml TMV. Plants were challenged 7 days later by spraying all leaves to runoff with a suspension of *P. tabacina* spores (10^5/ml).
[y]Values significant at $P \leq 0.01$ (**).
[z]Value represents the average number of lesions per leaf on all leaves (locally and systemically protected).

TABLE 8. Whole-plant resistance of tobacco against *Phytophthora parasitica* var. *nicotianae* (Ppn), *Pseudomonas tabaci,* or tobacco mosaic virus (TMV) induced by prior inoculation with tobacco necrosis virus (TNV) or cucumber mosaic virus (CMV)[x]

Inducer	Postinduction time before challenge was applied (days)	Lesions per leaf (avg no. $\pm SE_{\bar{x}}$)[y]								
		Ppn			*Ps. tabaci*			TMV		
		Untreated	Treated	t Value[z]	Untreated	Treated	t Value	Untreated	Treated	t Value
TNV	7	5.3 ± 0.6	0.5 ± 0.2	8.08**	14.4 ± 1.4	5.4 ± 1.6	4.25**	172.5 ± 18.2	42.4 ± 6.2	6.76**
CMV	7	4.1 ± 0.4	2.8 ± 0.7	1.71	19.5 ± 3.4	22.8 ± 3.1	0.70	23.1 ± 5.8	27.8 ± 3.4	0.70
	14	1.6 ± 0.3	1.9 ± 0.3	0.72	21.5 ± 5.3	23.7 ± 3.3	0.35	23.9 ± 3.6	12.1 ± 2.2	2.65*

[x]The adaxial surface of the youngest fully expanded leaf on tobacco plants at the four- or five-leaf stage was inoculated by rubbing with CMV, or the youngest fully expanded leaf and up to three leaves below it were inoculated by rubbing with TNV. Plants were challenged 7 or 14 days later by spraying all leaves to runoff with zoospores of Ppn (10^5/ml), cells of *Ps. tabaci* (10^6 cfu/ml), or by rubbing with 1 μg/ml of TMV.
[y]Values represent average number of Ppn, *Ps. tabaci,* or TMV lesions per leaf on all leaves (locally and systemically protected).
[z]Values significant at $P \leq 0.05$ (*) or 0.01 (**).

virus is attempting to invade a plant that has been altered markedly both physiologically and biochemically due to systemic infection by another virus.

Different models could explain induced resistance of tobacco against diverse challengers: several will be discussed. First, the induced plant could produce and transport several different antimetabolites, each active against only certain challengers. This, however, would seem energy-inefficient for the plant. Second, a single antimetabolite with activity against diverse challengers could be produced and translocated. This also seems unlikely since such an antimetabolite might exhibit autotoxicity. Third, a single metabolite, a signal with no activity against potential challengers, could be produced and translocated. The signal would condition the plant to respond, when challenged, with defense mechanisms appropriate to specific types of challengers. We suggest this model, which is similar to one proposed by Kuć (7), to be the most appealing. Although our data do not predict any of these models to be the most appropriate, the third proposal adequately explains why differences were noted in both development and level of resistance against the various challengers. That is, the resistance mechanisms activated by the signal are more effective against certain challengers than others. Since induction of the hypersensitive response in tobacco to infection by TMV results in altered levels of indoleacetic acid (17), cytokinins (2), several proteins (16) and several other compounds, their role as possible signals requires investigation.

Our results suggest that tobacco has the capacity to respond in a resistant manner to several diverse pathogens, but that this resistance is not expressed until the plant has been induced and subsequently attacked. The evolutionary advantage of this strategy is easily understood. If pathogen attack is unpredictable, and if metabolites normally used for plant defenses can otherwise be used to increase plant reproduction, then natural selection would favor genotypes with the ability to "turn off" resistance genes when they are not needed and channel the energy saved into increased reproduction. By understanding the mechanisms of induced resistance, we may be able to selectively "turn on" these mechanisms when a crop is threatened by certain pests. In this way, induced resistance could be substituted for constitutive resistance that may be lost or reduced as a consequence of breeding plants for increased vigor or productivity. The use of pathogens to induce resistance provides useful models by which this phenomenon may be studied, but which would often not be suited for a practical application. The recent report (3), however, of a synthetic agent that induces resistance of rice against *Piricularia oryzae* further indicates the potential and practical use of induced resistance in the field.

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