Further Characterization of Mycolaminaran-Induced Resistance: Temperature Sensitivity Against Tobacco Mosaic Virus and Function Against Cauliflower Mosaic Virus and Tomato Spotted Wilt Virus

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ABSTRACT

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Mycolaminaran, a β-1,3-glucan from *Phytophthora megasperma*, inhibits initial tobacco mosaic virus (TMV) infection in greenhouse-grown *Nicotiana tabacum* and *N. glutinosa* when mixed at 100–500 μg/ml with inoculum. TMV inoculum amended with 250 μg/ml mycolaminaran produced fewer than 10% as many lesions as unamended control inoculum when applied to *Datura stramonium*, another member of the Solanaceae. Mycolaminaran similarly inhibited the infection of *D. stramonium* by four strains of cauliflower mosaic virus (Cabb-B, D4, CM1841, W260). Tomato spotted wilt virus inoculum amended with 250 μg/ml mycolaminaran also produced fewer than 10% as many lesions as unamended inoculum when applied to *N. glutinosa*. When plants (*N. tabacum* 'Xanthinc' or *N. glutinosa*) were kept at 32 C for 24–48 h before inoculation, TMV inoculum amended with mycolaminaran produced 80–100% as many lesions as unamended controls. At 30 C, the induced resistance was observed on developing leaves but not on fully expanded leaves of Xanthinc tobacco. The resistance induced by mycolaminaran is general, temperature sensitive, and not restricted to RNA plant viruses.

Certain plants, especially *Nicotiana tabacum* L. and *N. glutinosa* L., respond to applications of fungal glucans by becoming resistant to viral infection (1,8). Examples of glucans that induce resistance include mycolaminaran from cell walls (17,18) and cytoplasm (21) of *Phytophthora* spp., and a cell-wall glucan from *P. megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin (10). The resistance induced is immediate, general, localized, and appears to block an early step of infection (10,17,21). The pattern of inheritance and of the induced resistance (and the range of viruses against which it functions) are unknown.

The actual mechanism of resistance is also unknown. The resistance functions against viral RNA as well as virions (10,21). This disproves the hypothesis that mycolaminaran functions by blocking the uncoating of the virion. It is unclear, however, whether mycolaminaran blocks virus replication in the initially

infected cell, stops cell-to-cell spread, or merely inhibits lesion formation.

Singh et al (17) presented serological evidence of viral replication in the absence of lesion formation on mycolaminaran-treated leaves. However, Kopp et al (10) performed similar tests and found no evidence of viral replication in tobacco leaves inoculated with tobacco mosaic virus (TMV) amended with their glucan preparation.

Zinnen et al (21) showed that amending TMV inoculum with mycolaminaran reduced the number of starch lesions (compared to unamended inoculum) in Xanthi tobacco lacking genes for necrotic reaction to TMV. This is consistent with the hypothesis that TMV did not spread extensively from the initially infected cell.

Bawden and Freeman (1) proposed that glucans inhibit viral infection by sharply reducing the physiologic activity of treated cells. This hypothesis predicts that resistance would function against all viruses regardless of replication strategy or type of genome. All tests we are aware of, however, have used single-stranded, plus-sense RNA viruses. The hypothesis can be further evaluated by testing whether mycolaminaran induces resistance against cauliflower mosaic virus (CaMV), a DNA-containing retroid virus (13).

One objective of this work was to test mycolaminaran on different types of virus. Another objective was to find conditions in which mycolaminaran did not induce resistance in plants that normally respond under greenhouse conditions.

We report here that the viral resistance induced by mycolaminaran is temperature sensitive in the case of TMV and effective against CaMV in *Datura stramonium* L. and against tomato spotted wilt virus (TSWV) in *N. glutinosa*. A preliminary report has appeared (7).

MATERIALS AND METHODS

Viruses. TMV was obtained from R. W. Fulton (University of Wisconsin-Madison), TSWV was provided by T. German (University of Wisconsin-Madison), and four strains of CaMV (D4, W260, CM1841, Cabb-B) were obtained from J. Schoelz (University of Missouri, Columbia). TMV was purified as described by Sherwood and Fulton (15) and used at a final concentration of 0.3 μg/ml in 30 mM sodium phosphate buffer, pH 8, unless otherwise stated. Inocula of CaMV were prepared by grinding a 5-mm disk of systemically infected turnip (*Brassica rapa* L. cv. Just Right) leaf in 1 ml of the phosphate buffer. TSWV inoculum was made by grinding a 5-mm disk of systemically infected *Emilia sonchifolia* (L.) DC. in 1 ml of freshly prepared 10 mM sodium sulfite. Inoculations were made on the upper epidermis of corundum-dusted, fully expanded leaves using cheesecloth pads dipped in inoculum.

Plant growth conditions. Plants were grown in commercial soilless mix in 10-cm pots in the greenhouse and were fertilized weekly with an aqueous 1% solution of 15-16-17 fertilizer. The greenhouse thermostat was set at 25 C during cool months and at 28 C during the summer. During temperature sensitivity tests, selected plants were placed in bacteriological incubators (VWR model 1530) without light or in a Sherer Model CEL 37-14 growth chamber under cool white fluorescent lights at a flux of 240 $\mu Em^{-2}s^{-1}$.

Plants used included *N. tabacum* cv. Xanthi, *N. tabacum* cv. Xanthi-nc, *N. rustica* L., *N. glutinosa*, and *D. stramonium*. Allopolyploid (4*n*) plants of *N. rustica* v. *brasilia* × *N. tabacum* were grown from seed provided by V. Sisson (USDA Crops Research Laboratory, Oxford, NC; accession B-57).

Description of tests. Using the phenol-extraction procedure of Faro (6) with modifications as described previously (21), mycolaminaran was isolated from cell walls of *P. megasperma* 695T obtained from J. Hancock (University of California, Berkeley) and grown as described by Shumard et al (16). The identity of the mycolaminaran was established by infrared spectra obtained using the pressed KBr technique with laminaran (from *Laminaria digitata*; Sigma, Lot 77F-3885) as standard (6).

A cell-wall glucan from *P. m. glycinea* (10) was provided by Alan Darvill (University of Georgia, Athens).

To prepare amended inoculum, the appropriate glucan powder was weighed, dissolved in buffer at twice the desired concentration, and mixed with an equal volume of 2× virus (2× = twice the desired final concentration). Control inoculum was made by mixing equal volumes of the 2× virus and buffer. For tests with TMV, amended inoculum was 0.3 μg/ml in TMV and 250 μg/ml in mycolaminaran, unless otherwise stated. Amended inoculum was applied to one half of a leaf, and control inoculum was applied to the opposite half-leaf.

Infectivity was measured by counting the number of local lesions 4–7 days after inoculation. Total lesion numbers, or the average number (and standard error) of lesions per half-leaf (or per leaf, if appropriate) are presented. In cases where the amended inoculum was applied to a half-leaf and control inoculum was applied to the opposite half-leaf, the lesion counts are paired, and may also be presented as averages and standard errors of ratios of lesions from amended inoculum divided by lesions from the opposite half-leaf. All experiments were performed at least two times, and in each experiment every treatment was replicated at least three times, unless otherwise noted.

In certain tests we used starch lesions processed as described by Lindner et al (11) to detect TMV infection on the cultivar Xanthi lacking any gene for necrosis in response to TMV infection.

RESULTS

Inhibition of lesions induced by CaMV and TSWV. Half-leaves of *D. stramonium* inoculated with CaMV amended with 250 μg/ml mycolaminaran induced from 5 to 13% as many local lesions as the opposite half-leaves (Table 1). Half-leaves inoculated with TMV (0.3 μg/ml) amended with mycolaminaran (250 μg/ml) produced 5–9% as many local lesions as the opposite half-leaf inoculated with unamended TMV.

In three different tests with TSWV, a total of 22 leaves of *N. glutinosa* produced 42 lesions on the half-leaves inoculated with TSWV inoculum amended with 250 μg/ml mycolaminaran, and the opposite half-leaves inoculated with unamended inoculum produced 1,860 lesions.

Early necrotization on *N. rustica*. Singh et al reported that glucan-amended TMV inoculum induced as many lesions as unamended inoculum when applied to *N. rustica*. To determine whether the resistance induced by mycolaminaran in tobacco is inherited as a simple dominant trait, we tested allopolyploid F1

TABLE 1. Effect of mycolaminaran (myc) on lesion numbers caused by four strains of cauliflower mosaic virus and by tobacco mosaic virus (TMV) on *Datura stramonium*

Virus[a]	Lesions[b]		Ratio (paired)[c]	Number of leaves tested
	Myc	Control		
Cabb-B	47	322	0.130 ± 0.076	2
	2	36	0.062 ± 0.002	2
	11	362	0.036 ± 0.006	8
W260	6	96	0.066 ± 0.008	2
	28	448	0.061 ± 0.016	10
CM1841	7	49	0.131 ± 0.083	2
	8	189	0.047 ± 0.008	7
D4	2	42	0.049 ± 0.007	2
	13	131	0.092 ± 0.020	6
TMV	60	726	0.097 ± 0.028	4
	27	529	0.053 ± 0.010	10

[a] Cabb-B, W260, CM1841, and D4 are strains of cauliflower mosaic virus; inoculum was prepared from grinding infected turnip leaf in buffer. "TMV" is 0.3 μg/ml TMV.

[b] Total number of lesions on half-leaves inoculated with inoculum amended with 250 μg/ml myc or with unamended inoculum (control).

[c] Data are averages of the ratios of the number of lesions per half-leaf inoculated with amended inoculum divided by the number of lesions on the opposite half-leaf.

progeny of a cross between *N. rustica* and *N. tabacum*. The progeny did not display any induced viral resistance (not shown).

However, during these tests, we noticed that on greenhouse-grown, wild-type *N. rustica* and on the hybrid, TMV inoculum amended with mycolaminaran or *P. m. glycinea* induced necrotic and chlorotic lesions, whereas unamended inoculum induced chlorotic lesions that turned necrotic several days after those on the opposite half-leaf treated with mycolaminaran. This indicated that *N. rustica* and the hybrid responded to mycolaminaran and to *P. m. glycinea*, although the response was an early necrotization of lesions rather than a decrease in lesion numbers as observed with other *Nicotiana* spp.

Relative activity of mycolaminaran and the *P. m. glycinea* glucan. Kopp et al (10) reported that their *P. m. glycinea* glucan at concentrations as low as 0.1 μg/ml strongly protected some *Nicotiana* spp. against TMV infection. Because other workers had reported that other glucans required approximately 100 μg/ml for comparable protection, Kopp et al (10) calculated that their glucan might be up to 1,000-fold more active than others. However, Kopp et al placed their plants in growth chambers at 22 C for several days before and during their assays, whereas other workers (17,21) used greenhouse-grown plants. To directly compare the effectiveness of the two preparations, we tested cytoplasmic mycolaminaran and the *P. m. glycinea* glucan on greenhouse-grown Xanthi-nc plants and found that the concentration required to cause a 50% reduction in lesion numbers was 0.2 μg/ml for the *P. m. glycinea* glucan and 2 μg/ml for mycolaminaran (Table 2). In tests with other preparations of mycolaminaran, *P. m. glycinea* glucan was up to 100 times more potent than mycolaminaran (not shown).

TABLE 2. Inhibition of tobacco mosaic virus lesions on Xanthi-nc tobacco by mycolaminaran and by the *Phytophthora megasperma* f. sp. *glycinea* glucan

Glucan[a]	μg/ml[b]	Total number of lesions[c]		Ratio[d] (unpaired)
		Glucan-amended inoculum	Unamended inoculum	
Myc	15	149	819	0.18
Myc	7.5	128	450	0.28
Myc	3.7	142	360	0.39
Myc	1.9	350	668	0.52
Myc	0.93	576	627	0.91
Pmg	0.47	167	795	0.18
Pmg	0.23	287	614	0.46
Pmg	0.12	381	609	0.62
Pmg	0.058	667	674	0.99

[a] Myc = mycolaminaran; Pmg = glucan provided by Alan Darvill, isolated from *P. m. glycinea*.
[b] Concentration of glucan in amended inoculum.
[c] Lesions from four half-leaves; glucan-amended inoculum was applied to a half-leaf, and unamended inoculum was applied to the opposite half-leaf.
[d] Total number of lesions from amended inoculum divided by total number of lesions from unamended inoculum.

Temperature sensitivity of the induced resistance. Results of tests of the effect of temperature and light conditions on the mycolaminaran-induced resistance are presented in Table 3. Darkness before inoculation was not sufficient to overcome the induced resistance, but rather temperature before inoculation was a critical factor. Certain combinations of darkness and high temperature (30–32 C) before and after inoculation also reduced the resistance. None of the tested postinoculation treatments alone, however, overcame the induced resistance. For example, greenhouse-grown plants kept at 30 C for 20 h after inoculation expressed strong induced resistance, as did plants kept at 30 C for 4 days after inoculation, although the lesions formed by the latter were chlorotic rather than necrotic.

We then tested whether high temperature (30–32 C) was sufficient to overcome the induced resistance. High temperatures before inoculation, regardless of light conditions, were sufficient to block the expression of induced resistance in both *N. glutinosa* and Xanthi-nc tobacco (Table 4). *N. rustica* kept at 32 C either in the light or in the dark did not exhibit induced resistance (not shown).

Because the necrotic reaction of Xanthi-nc is temperature sensitive (14), we tested whether the mycolaminaran-induced resistance was also temperature sensitive in tobacco lacking genes for necrosis in response to TMV infection. We previously showed that Xanthi tobacco lacking such genes exhibits mycolaminaran-induced resistance against TMV when grown at 24–28 C (21). Results shown in Table 3 show that mycolaminaran reduced the number of TMV starch lesions on greenhouse-grown plants to a greater extent than on plants kept at 32 C, indicating that temperature sensitivity is independent of necrosis genes.

We also tested whether the resistance induced by the *P. m. glycinea* glucan was temperature sensitive in *N. glutinosa*. Plants were kept in dark chambers at either 24 C or 30 C for 48 h before and 15 h after inoculation. A total of eight half-leaves in two trials were rubbed with TMV amended with 10 μg/ml *P. m. glycinea* glucan; opposite half-leaves were rubbed with unamended TMV. At 24 C, amended inoculum produced no lesions, and control inoculum produced 353. At 30 C, amended inoculum produced 266 lesions, and control inoculum produced 234.

Effect of leaf position on temperature sensitivity. During one trial with Xanthi-nc we noticed that the temperature sensitivity was more pronounced on older leaves than on younger ones. To test for a position effect, Xanthi-nc plants were placed in the dark incubators at 30 or 32 C for 48 h before and 15 h after inoculation with amended TMV inoculum on one half-leaf and unamended inoculum on the opposite half. Greenhouse-

TABLE 3. Effect of preinoculation and postinoculation temperature on the induction by mycolaminaran of resistance to tobacco mosaic virus (TMV) lesions on *Nicotiana glutinosa*

Conditions before inoculation[a]			Inoculate	Post-inoculation	Ratio[b]		Lesions[c]	
−48 h	−36 h	−24 h	0 h	20 h	Trial 1	Trail 2	Trail 1	Trail 2
Greenhouse					0.02 ± 0.002	0.087 ± 0.01	8/431 (8)	5/57 (4)
24 C dark					0.061 ± 0.012	0.066 ± 0.023	41/706 (11)	14/326 (4)
30 C dark					0.95 ± 0.036	0.93 ± 0.065	1125/1182 (11)	664/715 (7)
	30 C dark				0.77 ± 0.08	0.96 ± 0.041	624/836 (8)	814/842 (9)
		30 C dark			0.65 ± 0.064	0.84 ± 0.22	465/794 (9)	227/289 (5)
30 C dark				Greenhouse	0.60 ± 0.044	0.55 ± 0.094	485/826 (9)	278/511 (5)
	30 C dark			Greenhouse	0.23 ± 0.028	0.66 ± 0.11	173/742 (6)	360/532 (6)
		30 C dark		Greenhouse	0.37 ± 0.050	0.30 ± 0.071	254/684 (6)	89/272 (4)
Greenhouse				30 C dark	0.08 ± 0.02	0.081 ± 0.02	41/549 (4)	6/81 (4)

[a] Leaves were inoculated on one half with 0.3 μg/ml TMV amended with 250 μg/ml mycolaminaran and on the opposite half-leaf with unamended TMV. All plants were kept in the greenhouse after the first 20 h postinoculation.
[b] Ratios are the averages and standard errors of ratios calculated by dividing the number of lesions on a half-leaf inoculated with amended inoculum by the number of lesions on the opposite half-leaf inoculated with unamended inoculum.
[c] The first number is lesions from half-leaves rubbed with amended inoculum; the second number is lesions from opposite half-leaves. The total number of leaves tested per treatment is given in parentheses.

grown plants served as a control. Plants kept at 32 C showed uniformly weak resistance, but those at 30 C showed strong resistance on the youngest inoculated leaf and progressively weaker resistance on the next two or three leaves beneath the youngest (Table 5).

Leaf dip tests. Yarwood (19,20) reported that dipping leaves briefly (~1 min) in water at 45–50 C increased the susceptibility of certain plants to viruses. We tested whether such brief treatments would block expression of the mycolaminaran-induced resistance in *N. glutinosa* and in Xanthi-nc tobacco. Mycolaminaran-amended TMV inoculum induced 22–72% as many lesions as unamended inoculum on leaves dipped in hot water, but on leaves dipped in 27 C water the amended inoculum produced fewer than 15% as many lesions as control inoculum (Table 6).

TABLE 4. Effect of temperature and light on the induction by mycolaminaran (myc) of resistance to lesions caused by tobacco mosaic virus (TMV) on *Nicotiana glutinosa* and Xanthi-nc tobacco[a]

Species	Conditions[b]	Ratio (paired)[c]	Lesions[d] Myc	Lesions[d] Control
N. glutinosa	greenhouse	0.023 ± 0.004	0.3	51
	24 C, dark	0.022 ± 0.004	3	136
	30 C, dark	0.94 ± 0.059	87	96
	30 C, dark	0.75 ± 0.22	18	25
N. tabacum cv. Xanthi-nc	greenhouse	0.028 ± 0.006	4	138
		0.11 ± 0.030	18	139
	24 C, dark	0.022 ± 0.007	4	173
		0.074 ± 0.022	7	81
	32 C, dark	0.86 ± 0.036	112	128
		1.04 ± 0.038	92	89
	32 C, light	0.53 ± 0.082	46	94
		0.62 ± 0.063	55	86
N. tabacum cv. Xanthi	greenhouse	0.14 ± 0.058	2	20
	32 C, light	0.80 ± 0.087	28	35

[a] Half-leaves were inoculated with 0.3 μg/ml TMV, and opposite half-leaves were inoculated with 0.3 μg/ml TMV amended with 250 μg/ml mycolaminaran.
[b] Plants were kept at the described conditions for 48 h before and 15–20 h after inoculation; afterward, all were placed in the greenhouse.
[c] Data are averages of the ratios of the number of lesions per half-leaf inoculated with amended inoculum divided by the numbers of lesions on the opposite half-leaf.
[d] Data are the average number of lesions per half-leaf; Myc denotes inoculum amended with mycolaminaran. Lesions are necrosis for *N. glutinosa* and Xanthi-nc, and starch lesions for Xanthi.

During the temperature sensitivity tests, lesion size and color often varied with temperature (10,12), but the mycolaminaran-treated half-leaves produced lesions indistinguishable from those on the opposite half-leaf inoculated with unamended inoculum.

DISCUSSION

These results show that the response to mycolaminaran is not restricted to *Nicotiana* spp. inoculated with single-stranded RNA viruses, but is also detectable in *D. stramonium* against both TMV and CaMV. Furthermore, in *N. glutinosa*, the inducible resistance functions against TSWV, an enveloped virus containing ambisense RNA (4,5). The practical application of mycolaminaran to prevent infection of crops by TSWV is being evaluated.

Induced resistance against CaMV excludes the hypothesis that the induced resistance functions solely against single-stranded RNA viruses. The results are consistent with the hypothesis that mycolaminaran causes a general resistance, perhaps by reducing the rate of physiologic activity in treated cells (1). For example, mycolaminaran may generally block translation or transcription.

Since temperature manipulations are useful ways to investigate viral infection of plants (2,3,9,14), it is significant that the mycolaminaran-induced resistance was temperature sensitive and that the sensitivity occurred in both Xanthi and Xanthi-nc tobacco. The key sensitive period was before inoculation: greenhouse-grown plants placed at 30 C after inoculation did not show temperature sensitivity. Manipulating the postinoculation conditions of plants kept at 30 C before inoculation, however, had some effect. In particular, darkness before or after inoculation was a secondary factor in amplifying the temperature sensitivity. In Xanthi-nc kept at 30 C, the temperature sensitivity also exhibited positional effects: resistance was more strongly induced in younger, physiologically more active leaves than in the older leaves. Moreover, the induced resistance was markedly reduced in leaves dipped for 1 min in water at 45–50 C.

In a direct comparison, *P. m. glycinea* was 10–100 times more inhibitory than mycolaminaran. It is not known whether our mycolaminaran preparations are active because they contain a small amount of *P. m. glycinea* glucan or because mycolaminaran itself is active but less potent than *P. m. glycinea* glucan. Both *P. m. glycinea* glucan and mycolaminaran induce a temperature sensitive resistance, and both induce early necrotization in *N. rustica*.

The temperature sensitivity of the induced resistance may be key to resolving two opposing observations concerning glucan-induced resistance. In similar work, Singh et al (17) detected symptomless local infection on greenhouse-grown plants treated with mycolaminaran, whereas Kopp et al (10) did not detect symptomless local infection on plants treated with the *P. m. glycinea* glucan.

TABLE 5. Effect of leaf position on temperature sensitivity of mycolaminaran-induced resistance to tobacco mosaic virus (TMV) in Xanthi-nc tobacco

Leaf[a]	Greenhouse Lesions[c]	Greenhouse Ratio[d]	Inoculation temperature[b] 30° Lesions	30° Ratio	32° Lesions	32° Ratio
1	ND[e]	ND	78/464	0.17 ± 0.06	219/211	1.04 ± 0.054
2	ND	ND	170/521	0.27 ± 0.036	380/470	0.81 ± 0.055
3	ND	ND	442/530	0.94 ± 0.13	316/419	0.82 ± 0.085
1	3/116	0.038 ± 0.017	70/94	0.39 ± 0.19	271/292	0.92 ± 0.11
2	3/279	0.016 ± 0.006	218/426	0.49 ± 0.142	309/355	0.85 ± 0.09
3	5/358	0.014 ± 0.001	117/357	0.32 ± 0.07	321/310	1.08 ± 0.16
4	7/494	0.015 ± 0.025	217/241	0.98 ± 0.20	115/112	1.03 ± 0.19

[a] 1 = youngest of the inoculated leaves, not fully expanded; 4 = oldest of the inoculated leaves.
[b] Temperature for 48 h before and 15 h after inoculation.
[c] Total number of lesions on half-leaves inoculated with 0.3 μg/ml TMV amended with 250 μg/ml mycolaminaran, followed by the total number of lesions on opposite half-leafs inoculated with unamended 0.3 μg/ml TMV.
[d] Paired ratio: data are averages and standard errors of ratios generated by dividing the number of lesions on a half-leaf inoculated with amended inoculum by the number of lesions on the opposite half-leaf inoculated with unamended TMV.
[e] ND = not data.

TABLE 6. Effect of dipping leaves in 45–50 C water for 1 min before inoculation with tobacco mosaic virus (TMV) amended with 250 μg/ml mycolaminaran and with unamended TMV

Temperature	Trial	*Nicotiana tabacum* Xanthi-nc		*N. glutinosa*	
		Lesions[a]	Ratio[b]	Lesions	Ratio
27C	1	17/386	0.086 ± 0.018	36/199	0.16 ± 0.035
	2	8/186	0.043 ± 0.009	5/106	0.048 ± 0.006
	3	59/1664	0.060 ± 0.026	37/317	0.115 ± 0.027
	4	68/1092	0.062 ± 0.012	121/672	0.197 ± 0.045
45C	1	ND[c]	ND	ND	ND
	2	ND	ND	124/162	0.77 ± 0.16
	3	362/1964	0.19 ± 0.036	320/825	0.38 ± 0.034
	4	424/1573	0.25 ± 0.037	700/1369	0.51 ± 0.031
50C	1	270/431	0.66 ± 0.062	90/80	1.01 ± 0.11
	2	181/177	1.00 ± 0.003	ND	ND
	3	ND	ND	ND	ND
	4	260/573	0.52 ± 0.10	ND	ND

[a]Total number of lesions on half-leaves inoculated with 0.3 μg/ml TMV amended with 250 μg/ml mycolaminaran, followed by the total number of lesions on opposite half-leaves inoculated with unamended 0.3 μg/ml TMV.

[b]Paired ratio: data are averages and standard errors of ratios generated by dividing the number of lesions on a half-leaf inoculated with amended inoculum by the number of lesions on the opposite half leaf inocualted with unamended TMV.

[c]No data; also, *N. glutinosa* leaves were often damaged by the 50 C treatment, so 27 C and 45 C treatments were tested in trials 2, 3, and 4 of *N. glutinosa*.

Only Kopp et al, however, used greenhouse-grown plants transferred to growth chambers at 22 C for several days before inoculation.

Although plants that normally respond to mycolaminaran by becoming resistant can be treated so that they respond only weakly, so far we have not found any conditions in which *N. rustica* shows induced resistance. *N. rustica* and *N. rustica* v. *brasilia* × *N. tabacum* F1 progeny, however, responded to mycolaminaran by exhibiting early necrotization of TMV lesions. To our knowledge, this is a new kind of response. Furthermore, the failure of the F1 progeny to show induced resistance means that the resistance is not inherited as a simple dominant trait.

These results are further evidence that glucans reduce infection by inducing resistance rather than by inactivating virus. The temperature sensitivity of the induced resistance may reduce the practical usefulness of mycolaminaran as an antiviral agent. However, the necrosis resistance conditioned by the N gene in tobacco is also sensitive above 32 C, yet it is considered agronomically indispensable.

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