Competition for Infection Sites and Multiplication of the Competing Strain in Plant Viral Interference

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ABSTRACT

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Interference between common tobacco mosaic virus (TMV-C), which causes mosaic in leaves of *Nicotiana sylvestris*, and a strain of the virus from petunia (TMV-P, which causes necrotic lesions in leaves of *N. sylvestris*) was investigated in leaves of infected plants showing mosaic (mosaic leaves) and in leaves on uninoculated plants (healthy leaves). TMV-C inoculum added to TMV-P inoculum reduced numbers of lesions in healthy leaves in proportion to the amount added. In mosaic leaves, however, in which TMV-P causes lesions in the dark green tissue, lesion numbers were reduced only slightly as the ratio of TMV-C to TMV-P was increased. Ultraviolet-inactivated TMV-C, TMV coat protein, or bovine serum albumin added to TMV-P reduced lesions in both healthy and mosaic leaves as did TMV-C added to TMV-P in mosaic leaves. This suggested that infection sites were being blocked nonspecifically. TMV-C RNA added to TMV-P virions reduced lesion numbers more in healthy than in mosaic leaves, but the RNA did not interfere with TMV-P to the extent that the virions did. Unlike the nonspecific interference by various proteins, yeast RNA did not interfere with lesion production by TMV-P in healthy or mosaic leaves. This suggested that the specificity of interference lies at the virus-replication stage. We conclude that both competition for infection sites and multiplication of the interfering strain are involved in the interference phenomenon.

Additional key words: competition, cross protection, TMV.

Interference, the reduction of infection by one virus when two related viruses are used as inoculum together, has been extensively investigated since it was described by Sadasivan (8). Siegel (10) proposed that an "exclusion mechanism may be operating such that when an infection is initiated with a particle of one strain of virus, a particle of a second strain cannot participate in the same infection." Wu and Rappaport (11) concluded, after studying interference by noninfectious and infectious agents, that "interference by infectious agents occurs after attachment to host cells." Helms (3) proposed that metabolic changes initiated by the interfering strain were the basis of the phenomenon. Loebenstein (4) concluded that proof of competitive exclusion at an infection site requires showing that the interfering strain does not multiply, yet reduces numbers of lesions produced by another strain. On the other hand, if the interfering strain does infect and multiply, then interference may involve interaction during multiplication.

The system using TMV and *Nicotiana sylvestris* is very suitable for testing Loebenstein's proposal. The dark green areas of the mosaic of common TMV (TMV-C) infected leaves of *N. sylvestris* contain only small amounts of virus and are susceptible to necrotizing strains of TMV. Atkinson and Matthews (1) and Fulton (2) have shown with intact virus that inoculation of mosaic affected leaves with TMV-C does not result in any increase in virus in the dark green areas, nor is there any increase in resistance to superinfection by necrotizing strains as there would be with healthy tissue. Recently, Sherwood and Fulton (9) have demonstrated these phenomena with common TMV-C RNA. Thus, upon inoculation, the interfering strain (TMV-C) multiplies in leaves of healthy plants, but not in leaves of TMV-C infected plants with mosaic; the necrotizing strain (TMV-P) multiplies in both. Since TMV-C does not multiply when inoculated onto leaves with mosaic, the effect of competition for infection sites between the two strains separate from the interaction of the two strains during multiplication can be examined.

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MATERIALS AND METHODS

Cultures of virus used were common TMV (TMV-C), which causes mosaic in *N. sylvestris* Speg & Comes, and TMV-P, which produces localized necrotic lesions. TMV-P was isolated from petunia by the second author. TMV was purified from systemically infected leaves (*N. sylvestris* for TMV-C and *N. tabacum* L. cv. Havana 307 or 38 for TMV-P) (9). Frozen leaves were ground in an equal amount (w/v) of 0.003M EDTA, pH 7.0, containing 0.02M 2-mercaptoethanol and Al_2O_3 equal to 10% of the tissue weight.

The mixture was heated to 55 C for 10 min, then centrifuged at low speed (10,000 rpm) in a Spinco No. 30 rotor for 30 min before pelleting the virus at high speed (30,000 rpm, or 40,000 rpm in a No. 40 rotor) for 2 or 1 hr. This cycle of low- and high-speed centrifugation was repeated. Virus was resuspended in 0.003M EDTA, pH 7.0. Measured amounts of virus were prepared by appropriately diluting purified preparations of known absorbance at 260 nm in 0.03M phosphate buffer, pH 8.0. We have previously reported (9) that a higher concentration of TMV-P was required to produce similar numbers of lesions on leaves with mosaic as healthy leaves. TMV-P was inoculated to leaves with mosaic at 123.5 μg/ml and to healthy leaves at 3.1 μg/ml to compensate for this difference in susceptibility. All concentrations of additive were made in proportion to the amount of TMV-P in each inoculum.

Plants were grown in 10.2-cm-diameter pots in a greenhouse kept at ~25 C. Interference tests were done with *N. sylvestris* that had developed leaves with mosaic 5–6 wk after the first 6-cm leaves were inoculated with TMV-C. Controls were healthy *N. sylvestris* of similar age.

Inoculations were made with small four-ply gauze pads saturated with inoculum by wiping leaves previously dusted with 225 μm corundum. Inoculated leaves were immediately covered for 6–12 hr with damp paper. Analysis of variance was conducted according to Little and Hills (5).

RESULTS AND DISCUSSION

TMV-C was added in various amounts to TMV-P and the mixtures inoculated to healthy leaves and to leaves with mosaic. In healthy leaves the number of necrotic lesions was reduced significantly ($P = 0.01$) and proportionately to the amount of TMV-C added (Table 1) as had been reported by Siegel (10) and Wu and Rappaport (11). In leaves with mosaic, however, there were nonsignificant ($P = 0.05$) reductions in lesion numbers (in dark green tissue) as the ratio of TMV-C to TMV-P was increased (Table 1).

This difference in response of healthy leaves and leaves with mosaic to mixed inoculum was further investigated by comparing other mixtures. TMV-C inactivated by UV irradiation (exposed to a Raytech model SW-18 lamp [Ultraviolet Products, Inc., San Gabriel, CA 91778] at 15 cm for 4 days), or isolated TMV coat protein (6) or bovine serum albumin (BSA) (Sigma No. A 9647, St. Louis, MO 63178) added to TMV-P caused similar small significant ($P = 0.05$) reductions in lesions in both healthy leaves and leaves with mosaic (Table 1). This suggests that a nonspecific competition for infection sites is involved. UV-inactivated TMV-C, TMV coat protein, and BSA probably had slightly greater interfering capability than infectious TMV-C because there are more interfering particles per unit weight.

TABLE 1. Number of necrotic lesions per half-leaf (average of six) and percent interference (% I) produced by TMV-P in healthy (H) or mosaic (M) *Nicotiana sylvestris* when mixed in several proportions with materials that might interfere with infection

		Ratio, additive to TMV-P (w/w)						
Additive[a]	Host	0	1	(% I)	5	(% I)	25	(% I)
Infectious TMV-C[z]	H	57 ± 4	24 ± 2	(58)	10 ± 2	(82)	4 ± 2	(93)
do[x]	M	28 ± 11	25 ± 8	(11)	25 ± 9	(11)	24 ± 8	(14)
UV-irradiated TMV-C[y]	H	36 ± 2	33 ± 3	(8)	30 ± 2	(17)	24 ± 2	(33)
do[y]	M	32 ± 4	29 ± 3	(9)	30 ± 3	(6)	24 ± 3	(25)

		Ratio, additive to TMV-P (w/w)						
		0	50	(% I)	100	(% I)	200	(% I)
TMV coat protein[y]	H	37 ± 4	37 ± 3	(0)	34 ± 3	(8)	28 ± 1	(24)
do[y]	M	31 ± 3	27 ± 3	(13)	30 ± 4	(3)	24 ± 3	(23)
Bovine serum albumin[y]	H	71 ± 3	71 ± 5	(0)	60 ± 7	(15)	62 ± 9	(13)
do[y]	M	41 ± 8	33 ± 4	(20)	38 ± 6	(7)	30 ± 4	(27)

		Ratio, additive RNA to RNA in TMV-P (w/w)						
		0	100	(% I)	500	(% I)	1,000	(% I)
TMV-C RNA[z]	H	41 ± 5	27 ± 3	(34)	21 ± 2	(49)	12 ± 1	(68)
do[y]	M	25 ± 1	20 ± 1	(20)	16 ± 1	(36)	15 ± 1	(40)
Yeast RNA[x]	H	42 ± 2	42 ± 3	(0)	42 ± 1	(0)	46 ± 2	(−10)
do[x]	M	36 ± 3	40 ± 2	(−11)	35 ± 3	(3)	39 ± 3	(−8)

[a]Superscript denotes treatment had no significant effect (x = >0.05) or significant effect at y = <0.05, z = <0.01 level as indicated by an analysis of variance.

We previously demonstrated (9) that TMV-C RNA does multiply in healthy tissue, but does not multiply detectably in dark or light green mosaic tissue. Therefore, the effect of TMV-C RNA in mixtures was tested on the assumption that it might bypass the competition at the infection site. This would permit determination of the effect of multiplication of the interfering strain on interference. RNA was prepared by the phenol method of Ralph and Berquist (6) except that residual phenol was removed by three extractions with chloroform rather than ether. Preparations had 260/280 nm absorbance ratios of 2 or more. The ratio of TMV-C RNA to the RNA in TMV-P inoculum was based on intact TMV at 20 μg/ml being equivalent to its RNA at 1 μg/ml. TMV-C RNA was about 5% as infectious as intact virions (5 ± 1 lesions compared to 94 ± 7 lesions based on an average of eight half-leaves of *N. tabacum* cv. Xanthi-nc). To compensate for the lower infectivity, the ratio of RNA was increased to 500 to 1 or higher.

The addition of TMV-C RNA to TMV-P reduced lesion numbers more in healthy leaves ($P = 0.01$) than in leaves with mosaic ($P = 0.05$) (Table 1). The difference in interference in healthy leaves and in leaves with mosaic is probably due to inability of the interfering strain to multiply in the dark green tissue of leaves with mosaic. In contrast to the interference by noninfectious proteinaceous agents, yeast RNA (Calbiochem. No. 55712, La Jolla, CA 92037) had no significant interfering capability ($P = 0.05$) (Table 1), suggesting that specificity involved RNA replication.

These data indicate that both competition for infection sites and multiplication of the RNA of the interfering strain are involved in interference. The competition for infection sites seems to be nonspecific because both noninfectious and infectious addenda affect healthy leaves and leaves with mosaic similarly. The specificity evidently lies in the usurpation of replicative locations or functions of the challenge RNA by the RNA of the interfering virus. As Ross (7) hypothesized, the addition of interfering viral RNA may bind ribosomes and prevent the binding of challenge RNA of a necrotizing strain and its subsequent replication. The failure of RNA to interfere with a necrotizing strain in dark green tissue of *N. sylvestris* leaves with mosaic is probably due to its inability to replicate there.

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