Characterization of a Factor of Resistance in Curly Top Virus-Resistant Tomatoes

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ABSTRACT

Studies on that portion of the resistance to curly top virus in certain tomato cultivars operative after inoculation suggest that resistance is not the result of: (i) selective immunities to specific virus strains; (ii) tolerance or masking of symptoms; (iii) recovery from symptoms; (iv) restriction of virus to the roots; (v) resistance to systemic translocation of virus; (vi) localization of virus at sites of insect inoculation; or (vii) slow movement of virus from sites of inoculation to sites of infection. The resistance apparently results because virus delivered by the vector fails to establish infection as often in resistant as in susceptible plants.

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Our previous research (23) suggests that resistance to curly top virus (CTV) in certain tomato cultivars is the result of mechanisms which reduce the chances of infection. All the plants of the resistant cultivars can become infected. However, under the same exposure to viruliferous vectors (*Circulifer tenellus* Baker) of CTV, fewer plants of the resistant than of susceptible cultivars develop symptoms. All of the cultivars involved are in advanced generations, and are homogenous for resistance to CTV. Progeny of plants which apparently escape infection in severe tests, whether of genetically resistant or susceptible cultivars, are no more resistant than progeny of plants which become infected (12).

Twenty to 25% of the resistance of two cultivars, C5 and CVF4, and 9% of the resistance of a third cultivar, C193, is attributable to nonpreference of the vector (24). However, the major portion of resistance in all the cultivars apparently results from mechanisms operative after introduction of virus into the plant by the vector. It is this resistance with which we are concerned here.

Several possible types of mechanisms operative after introduction of virus into the plant could produce the lower incidence of symptom development observed among plants of the resistant cultivars. The purpose of these studies was to test the various possibilities and to determine the kind of resistance possessed by the resistant cultivars.

MATERIALS AND METHODS.—The four most CTV-resistant tomato cultivars available (23), C5 (13), C193 (4), C27 (not yet released), and CVF4 (11) were used in all tests. The resistant cultivars Payette (16) and Owyhee (17), developed by W. R. Simpson at Parma, Idaho, were also used in some tests. The resistance of all these cultivars was derived from wild *Lycopersicon* species through interspecific crosses (12). The commercial cultivars, VR Moscow and VF145, were used as susceptible controls. All these cultivars are in at least the F_7 generation, and progeny tests indicate that they are essentially homogenous with regard to CTV resistance.

The curly top virus strains 12A, 13B, 16C, 31A, and 35B used in these tests were isolated at Prosser, Wash. (21). Strains 3 (6), 11, 22 (8), Los Banos (2), and Pasa Robles (3) were obtained from C. W. Bennett in Salinas, Calif.

Leafhoppers carrying specific strains of CTV were reared on infected sugar beets in cages. Leafhoppers carrying a mixture of "wild" strains were reared uncaged in an insectary maintained for the past 3 years with infected beets dug from fields near Prosser, Wash.

We inoculated plants by placing leafhoppers on leaves in small clip cages similar to those described by Giddings (7). For implant graft inoculations, we cut lengthwise the stem of the plant to be inoculated. We cut a section of stem tissue ca. 2 cm long and 0.2 mm thick from the stem of a diseased plant and inserted it into the cut. The wound was wrapped with Parafilm.

The tests were conducted in a shaded greenhouse in which midday light intensity averaged about 5,000 ft-c. The temperature varied between 24 and 30 C.

RESULTS.—*Strain-specific resistance.*—Studies conducted by Randall (15) and Martin (10) suggest that the ability of the resistant cultivars to escape infection might be the result of immunities to specific virus strains. Thus, cultivars immune to a greater number of strains or to the more prevalent strains in an area would have a better chance than others of escaping infection. To test this possibility, we inoculated each of the six resistant cultivars with each of 10 strains of CTV and with a wild mixture of strains using viruliferous leafhoppers. There were no specific immunities to any of the strains among the tomato cultivars. Each cultivar was infected by each strain (Table 1). Furthermore, the data indicate that the relative ease with which the various cultivars escape infection could be ranked by any one strain or the wild mixture of strains with a statistical confidence over 99%.

Tolerance or delayed symptom development.—To test the possibility that resistant plants are as easily infected as control plants but frequently fail to

TABLE 1. Susceptibility of tomato cultivars to specific strains of curly top virus[a]

Strains[b]	Tomato cultivars						
	C5	C193	C27	CVF4	Owyhee	Payette	VR Moscow
12A	5/18[c]	5/18	7/18	4/18	10/18	12/18	15/18
13B	1/18	2/18	6/18	3/18	6/18	8/18	8/18
16C	3/18	4/18	10/18	6/18	7/18	11/18	12/18
31A	6/18	6/18	5/18	6/18	9/18	13/18	17/18
35B	2/18	3/18	5/18	6/18	7/18	10/18	11/18
3	1/12	2/12	2/12	2/12	2/12	3/12	5/12
11	2/12	1/12	5/12	3/12	4/12	6/12	6/12
22	1/6	3/6	3/6	2/6	3/6	5/6	6/6
Los Banos	3/6	2/6	3/6	3/6	4/6	4/6	5/6
Pasa Robles	1/6	2/6	1/6	2/6	1/6	2/6	4/6
Wild mixture[d]	82/384	90/384	137/384	117/384	142/384	173/384	234/384

[a] Differences between strains are not comparable, but differences between cultivars inoculated with the same strain are comparable.

[b] The coefficient of concordance in the ranking of the susceptibility of the tomato cultivars by the ten strains and the wild mixture of strains (W = .77) is statistically significant at the 1% level of significance.

[c] Ratio of number of plants which developed symptoms to number inoculated.

[d] Leafhoppers used to make inoculations were reared uncaged in an insectary maintained for 3 years with infected sugar beets dug from a field near Prosser, Wash.

develop symptoms, we dug 18 healthy-appearing and 2 obviously diseased plants each of C5, CVF4, C27, and C193 from the field and transplanted them in the greenhouse. This was done near the end of the growing season after all the control plants and over 50% of the resistant plants expressed typical CTV disease symptoms. Cuttings were taken from each of the plants and rooted in the greenhouse. At the same time, tissue was taken from each of the cuttings and implant-grafted into young, susceptible, healthy control plants in the greenhouse.

Symptoms of CTV routinely developed in the young plants into which obviously diseased tissue was grafted, but, with three exceptions, plants into which tissue from healthy-appearing plants was grafted remained symptomless. In each of the three exceptions, the cutting from which the tissue was taken died; and the original plant taken from the field soon developed symptoms of CTV. The cuttings from all diseased plants and those of the exceptions noted died, but the remaining cuttings from healthy-appearing plants rooted and the resulting plants did not develop symptoms of CTV. Evidently, all the healthy-appearing plants were virus-free except three. The latter apparently had been recently infected prior to transplanting, and had not yet produced obvious symptoms.

In a second test to determine whether systemically infected, resistant plants remain symptomless, 98 plants of each resistant line were inoculated at approximately the three-leaf stage using leafhoppers. After 60 days, 18, 23, 28, 34, 37, and 45 of the C5, C193, CVF4, C27, Owyhee, and Payette plants, respectively, were obviously diseased (systemic symptoms), and the remainder appeared healthy. Each of the healthy-appearing and one obviously diseased plant of each line was indexed to sugar beet seedlings using leafhoppers, and to young, susceptible tomato plants using implant grafts. CTV was not transmitted from any of the healthy-appearing plants, but was transmitted to both tomatoes and beets from the diseased plants. It seems evident that resistant plants do not remain symptomless when systemically infected.

Restriction of virus in roots.—Bennett (1) and Thomas (20) reported that CTV moved into the roots of certain species after inoculation, and remained confined there until the plants were severely pruned. Our first test of the possibility that this mechanism was responsible for resistance in the tomato cultivars was performed with the symptomless plants previously described which were dug from the field late in the season and transplanted in the greenhouse. The 18 symptomless plants of C5, C193, CVF4, and C27 dug from the field were severely pruned at the time of transplanting in the greenhouse. The new growth on only three of these plants expressed symptoms of curly top virus. However, the virus had not been confined to the roots of these three plants, since virus was transmitted from stem tissue to susceptible tomatoes through graft implants removed prior to pruning, and cuttings from each removed prior to pruning failed to root. If restriction of virus to the roots were responsible for resistance, nearly all of the resistant plants should have had infected root systems, since 100% of the susceptible controls were infected in the field.

In our second test to determine whether virus is present but restricted in the roots of inoculated, resistant tomato plants, 98 plants of each resistant line were inoculated with a wild mixture of virus strains in the greenhouse at the three-leaf stage of development. After 60 days, 76, 71, 64, 53, 48, and 36 of the C5, C193, CVF4, C27, Owyhee, and Payette plants, respectively, appeared healthy, and the remainder were obviously infected. The infected plants were discarded. The symptomless plants were completely defoliated to promote movement of food

TABLE 2. Time after implant graft inoculation in the stem below the cotyledonary node required for curly top virus symptoms to develop at the growing points of defoliated and nondefoliated, resistant and susceptible, tomato plants

	No. plants developing symptoms during weekly intervals (1-4) after inoculation											
	Nondefoliated plants						Defoliated plants					
Cultivar	Plant[a] no.	1	2	3	4	DI[b]	Plant no.	1	2	3	4	DI[b]
C5	39	1	18	16	4	60	35	3	22	9	1	69
C193	40	0	13	19	8	53	38	2	21	13	2	65
C27	35	0	9	19	7	51	36	4	18	11	3	66
CVF4	37	3	11	20	3	59	40	6	26	8	0	74
Owyhee	33	2	17	14	0	66	39	4	24	8	3	69
Payette	38	2	15	13	8	57	38	3	24	9	2	68
VR Moscow	40	2	18	16	4	61	37	2	23	11	1	67
Mean[c]						58.1						68.1

[a] Number of successful grafts from 40 attempted.

[b] Disease index based on number of plants and rate at which they became infected.

$$DI = \left[\frac{4\ (\text{No. 1st wk}) + 3\ (\text{No. 2nd wk}) + 2\ (\text{No. 3rd wk}) + 1\ (\text{No. 4th wk})}{4 \times \text{total number of plants}}\right] \times (100)$$

[c] Means are significantly different at the 2% level of probability. Means compared using the "Student" t-test for paired observations, sign ignored.

reserves from roots. We prevented photosynthesis in the green stems by wrapping them with black construction paper, leaving only about 0.25 inches of stem exposed at the growing point. None of the plants subsequently developed symptoms. To test the possibility that virus had actually moved into the growing points of these plants, but that it did not produce symptoms, we indexed tissue of the growing point of each plant by making implant grafts into young, susceptible tomatoes. Symptoms did not develop in any of the young tomatoes.

Systemic translocation.—Under field conditions, a single branch of C27 and, less frequently, of C193 occasionally develops symptoms several days before symptoms are obvious in the rest of the plant (23). This suggests that there may be resistance to translocation of virus within the plants.

To test for resistance to translocation, we staked 80 healthy tomato plants of each of the resistant cultivars and a control cultivar and grew them to a height of approximately 25 inches in the greenhouse. Diseased tomato tissue was implant-grafted in the stem of each plant below the cotyledonary node, and half the plants were defoliated 3 days later. The time required for expression of CTV symptoms at the growing points was noted.

Defoliation significantly decreased the time required for symptoms to appear at the growing points. However, all plants of both susceptible and resistant cultivars developed symptoms within 4 weeks (Table 2). Although symptom development was slightly delayed in C27, C193, and CVF4 as compared with the other cultivars, the results suggest that translocation is not sufficiently hindered in any of the tomato cultivars to account for the resistance observed.

Localization at points of insect inoculation.—Martin (9) described a type of resistance to cucumber mosaic virus in squash similar to that observed in the CTV-resistant tomatoes. He found that infection was established as readily in resistant as in susceptible plants, but movement of virus from sites of infection was hindered.

We tested the possibility that resistance to CTV infection in tomatoes could be due to a tendency toward localization of virus at infection sites near points of leafhopper inoculation. A clip cage containing five viruliferous leafhoppers was attached to a cotyledon of each of 32 healthy seedlings of the four most resistant tomato cultivars and the susceptible cultivar, VR Moscow, for 2 hr. Inoculated cotyledons were removed from eight plants of each cultivar at 2, 4, and 8 hr after inoculation began, and were left on eight control plants of each cultivar. This experiment was replicated 12 times.

There were no significant differences between the number of plants infected at any interval of cotyledon removal and the number infected among control plants on which cotyledons were not removed (Table 3). The relative numbers of plants infected in the different cultivars reflected the approximate levels of resistance previously established (23), indicating that the resistance of these cultivars was expressed in these tests.

Rate of movement from points of inoculation.—The previous tests suggested that the CTV particles injected by the vector routinely move some distance from points of inoculation to points of initial infection. A slower rate of movement in resistant than in susceptible plants could account for fewer infections if the particles were undergoing inactivation during this period of movement.

To measure the rate of movement of CTV from points of inoculation in resistant and susceptible cultivars, we determined the extent to which virus had moved from inoculated cotyledons at short intervals after inoculation. A clip cage containing five viruliferous leafhoppers was attached to one

cotyledon of each of 32 plants of each cultivar for 15, 30, or 60 min. After the specified interval, the clip cages and the inoculated cotyledon of half the plants were removed. This was replicated 15 times at each interval. The numbers of plants infected varied according to the susceptibilities of the cultivars and the length of the exposure. Therefore, it was necessary, for comparative purposes, to express the extent to which virus had moved from inoculated cotyledons during each interval as the ratio:incidence of infection with inoculated cotyledons removed to incidence of infection with inoculated cotyledons remaining attached (Table 4). The ratio could vary from 0 to 1.

Virus did not move from inoculated cotyledons more rapidly in susceptible than in resistant cultivars (Table 4). Although it may have moved somewhat more rapidly in CVF4 than in the other cultivars, CVF4 is a resistant cultivar. There were few, if any, additional plants infected in any of the lines when inoculated cotyledons were left attached more than 60 min.

The number of plants infected, including all cultivars, was 3.6% and 14.2% of the number inoculated at the 15-min and 30-min intervals, respectively, when cotyledons were removed. The corresponding percentages with cotyledons remaining attached were 6.1 and 15.9%, respectively. Extrapolation to 0% infection from plots of these values against time suggests that the minimum time required for a leafhopper to deliver virus to a cotyledon is ca. 3 min. Only an additional 6 min are required for the virus to move out of the cotyledon.

DISCUSSION.—Randall (15) found that individuals of certain wild *Lycopersicon* species, from which the resistance in the tomato cultivars used in this study were derived, were immune to some strains of CTV and susceptible to others. Martin (10) tested two of the resistant tomato cultivars, C193 and Owyhee, in various areas of the west and found that each cultivar was more resistant than the other in the area in which it was developed. The two areas of development are in different leafhopper dispersal areas (5). This information suggested that the capacity of resistant cultivars to escape infection might be the result of immunities to specific virus strains. In our tests, however, all of the resistant cultivars were infected by each of 10 strains of CTV. A second indication that resistances of the cultivars are not strain-specific is that the relative ease with which the various cultivars escaped infection could be ranked by any one strain or the wild mixture of strains. Van der Plank (14) pointed out that, if resistance is strain-specific, resistant and control lines are equally susceptible to a strain capable of infecting both.

Smith et al. (18) reported that auxin content decreased equally after inoculation in certain of the resistant and susceptible cultivars used in this study, whereas only susceptible plants developed symptoms. They (19) suggested that the resistant cultivars may possess a form of tolerance which prevents damage after infection occurs. However, our tests showed that systemically infected resistant plants do not remain symptomless.

It seemed possible that CTV might move from the site of inoculation into the roots of the resistant tomatoes and remain confined there as it does in certain other species (1, 20). This now seems unlikely in the case of the resistant tomatoes. Defoliation, which caused CTV confined in the roots of other species to move into the growing points, also caused

TABLE 3. Number of plants of resistant and susceptible tomato cultivars which developed curly top symptoms when inoculated cotyledons were removed at 2, 4, or 8 hr after inoculation, or were left attached

Tomato cultivars	Intervals after inoculation at which cotyledons were removed (hr)			
	2	4	8	Not removed
C5	23[a]	19	22	18[b]
C193	25	22	24	19
CVF4	28	28	25	36
C27	30	39	36	31
VR Moscow	52	57	63	62
Total	158	165	170	166

[a] Number of plants infected from 96 inoculated (8 plants inoculated in each of 12 replications).

[b] Chi-square contingency tests indicate that the number of plants infected at the various times of cotyledon removal is in no case significantly different from the number of control plants (without cotyledons removed) infected.

TABLE 4. Extent to which curly top virus moved from inoculated cotyledons of resistant and susceptible tomato cultivars during short intervals after inoculation

Tomato cultivar	Mean ratio of incidence of infection among plants with cotyledons removed at intervals after inoculation to incidence of infection among plants with cotyledons remaining attached[a,b]		
	15 min	30 min	60 min
C5	0.45	0.81	0.93
C193	0.70	0.88	1.13
C27	0.64	0.84	1.08
CVF4	0.80	1.16	1.00
Owyhee	0.50	0.74	0.84
Payette	0.75	0.88	0.94
VF145[c]	0.65	0.90	0.87
VR Moscow	0.43	0.88	1.08

[a] One cotyledon of each of 32 plants of each cultivar was exposed to five viruliferous vectors during each interval. The exposed cotyledon was removed from half the plants at the end of the interval. Infection was determined on the basis of subsequent symptom development (replicated 15 times).

[b] An analysis of variance was conducted on the data at each interval using a completely random design. There were no significant differences between means at any interval.

[c] The cultivars VF145 and VR Moscow are susceptible controls. The remaining cultivars possess various levels of resistance.

more rapid upward movement of virus from grafts below the cotyledonary node in the resistant tomatoes. But the same treatment did not cause virus to move into the growing points of heavily inoculated, but symptomless, resistant plants.

The relative levels of resistance of the tomato cultivars were the same in tests in which inoculations were made on cotyledons as those previously established (12, 23) when vectors were not confined on cotyledons. Thus, it is probably valid to draw inferences concerning the nature of resistance from experiments in which cotyledons are inoculated.

The very short interval after inoculation required for CTV to move from an inoculated tomato cotyledon suggests that the virus particles which move from the cotyledon are those injected by the leafhoppers, not their progeny. The fact that few, if any, additional plants became infected when inoculated cotyledons remained on plants more than 60 min suggests that infection is seldom, if ever, initiated in the cotyledon. Thus, it seems probable that the virus particle must move some distance from the site of inoculation to a site receptive to infection. Since movement from cotyledons was as rapid in resistant as in susceptible cultivars, it is unlikely that the virus particles were exposed to inactivating influences during their movement to infectible sites for a longer period in resistant than in susceptible plants.

We were unable to devise a direct test to determine whether infection by CTV particles is less likely in plants of the resistant than of susceptible cultivars. However, we tested the plausible alternative possibilities which could account for the lower incidence of symptom development in resistant than in susceptible cultivars. We (22) previously showed that the resistance did not reflect an ability of resistant plants to recover. Further studies may now be undertaken with greater confidence that factors involved in the infection process account for the resistance of the tomato cultivars to CTV.

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