Vector Preference, a Factor of Resistance to Curly Top Virus in Certain Tomato Cultivars

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ABSTRACT

A portion of the resistance of certain tomato cultivars to infection by curly top virus appears to be the result of nonpreference by the vector, *Circulifer tenellus*. Leafhoppers released on plants of six susceptible and six resistant cultivars spent less time on certain of the resistant than on the remaining resistant and susceptible cultivars. We developed a method to remove any resistance attributable to nonpreference from results of susceptibility assays. Those cultivars upon which leafhoppers spent the least time were 21 to 23% more susceptible to virus infection when resistance attributable to nonpreference by the vector was removed; but the susceptibility of the other cultivars was not affected. The major portion of resistance could not be attributed to vector preference and, therefore, must be the result of mechanisms operative after introduction of virus into the plant by the vector. Phytopathology 61:1257-1260.

Additional key words: Lycopersicon, incidence of infection, relative resistance, susceptibility tests.

Several cultivars and breeding lines of tomatoes with resistance to curly top virus (CTV) are now available (5, 6, 7, 9, 10). All these tomatoes derived their resistance from wild species of *Lycopersicon* through interspecific crosses. They were all selected on the basis of mass inoculations (6), either through field elimination or a seedling elimination technique (4) in the greenhouse. Plants selected as resistant were those failing to develop symptoms following exposure to viruliferous beet leafhoppers, *Circulifer tenellus* (Baker).

Plants of the resistant lines appear to possess characters which reduce the chances of infection (13). Thus, a smaller percentage of plants among resistant lines than among susceptible lines develops symptoms following mass exposures to viruliferous leafhoppers in seedling tests in the greenhouse and in field tests. However, the few resistant plants which do develop symptoms express approximately the same susceptibility to injury as plants of cultivars which are easily infected. Resistance could not be associated with a capacity to recover (12).

Schwartze & Huber (8) described an escape type of resistance to virus infection in red raspberries which was attributed to low preference by the vector rather than to resistance to the virus itself. We felt that a similar mechanism might account for resistance in the resistant tomato cultivars because (i) these cultivars apparently possess an escape type of resistance (13); (ii) the beet leafhopper shows strong preferences for some hosts over others and tends to accumulate on preferred hosts (11); and (iii) the resistant tomato cultivars were selected on the basis of mass inoculations in which leafhoppers were free to choose between plants (6). These studies were undertaken to determine whether vector preference contributes toward resistance of any of the tomato cultivars and, if so, to what extent.

MATERIALS AND METHODS.—*General.*—Six tomato cultivars resistant to CTV were included in our tests. Four of these cultivars, C5 (7), CVF4 (5), C193 (1), and C27 (not yet released) were developed in a USDA breeding program initiated by H. L. Blood and now conducted by the junior author. Two of the resistant cultivars, Owyhee (9) and Payette (10), were developed at Parma, Idaho. Resistance in all of these cultivars is apparently polygenic and complex in inheritance (6).

Six susceptible cultivars were used as checks in this study. The cultivar, VR Moscow (2), has been used as a standard check in the USDA breeding program for a number of years (3). Allen's Triumph was recently developed by C. L. Allen at Vancouver, Wash. Seed of Bonny Best, Manalucie, Stone, and VF145 were obtained from commercial sources. All these commercial cultivars are very susceptible to curly top virus.

We felt justified in restricting these tests to seedling plants, as the resistance of the tomato cultivars is expressed in both seedling and adult stages (13). A great deal of the selection in developing all of the resistant cultivars except Owyhee and Payette was based on greenhouse seedling tests (6). Seedling plants were also easily adapted to our methods of measuring leafhopper preference.

Tomato seed was germinated in vermiculite in a growth chamber adjusted to 27 C, 16-hr day-length, and 3,000 ft-c. The seedlings were transplanted 7 days after seeding as their cotyledons were reaching full expansion. They were transplanted 2 cm apart in beds or flats of a prefertilized mixture of peat moss and vermiculite, and inoculated 3 days later. Susceptibility tests were conducted during the summer months in a shaded greenhouse in which midday light intensity averaged about 5,000 ft-c, and temperature varied from 24 to 30 C daily.

To insure exposure of the test plants to a wide range of CTV strains, leafhoppers used in making inoculations were reared uncaged in an insectary on naturally infected, field-grown sugarbeets collected near Prosser,

Wash. Adult leafhoppers were used. The numbers of leafhoppers used in making inoculations and the methods used were dependent upon the type of test, and are described later.

Direct observation of leafhopper preference.—We previously presented evidence (11) that accumulation of beet leafhoppers on some hosts in preference to others is oriented on the basis of feeding preference and effected through a process of trial and error. Thus, as one indication of leafhopper preference, we presented leafhoppers with a choice between plants of the 12 cultivars under identical environmental conditions, and observed the choice of the leafhoppers directly. We used a previously described (11) observation cage designed for this purpose. Tomato seedlings were transplanted ca. 2 cm apart in a row in a wooden tray 68 cm long × 7 cm wide × 7 cm deep which formed the bottom of the observation cage. Three days after transplanting, the wooden tray was placed in position in the observation cage, and the cage was placed in a room held at 24 C which had a fluorescent fixture with four 4-ft, 40-w, cool-white bulbs as its only source of light. The light fixture was laid on its side on a table, and the observation cage was placed ca. 4 cm in front of the bulbs and centralized so that each plant in the cage received the same light. On the evening of the 3rd day after transplanting, 160 leafhoppers were released in the observation cage. The leafhoppers on each seedling were counted the following day at 8 AM, 11 AM, 2 PM, and 5 PM.

Measurement of leafhopper preference in terms of resistance.—We measured the resistance attributable to leafhopper preference in each of the cultivars as the difference in susceptibility with and without the effects of leafhopper preference on susceptibility excluded. We previously presented evidence (11) that a trial feeding period of 30 to 60 min is involved in the distinction by the beet leafhopper between preferred and nonpreferred hosts. Thus, in these studies, we excluded the effects of leafhopper nonpreference on susceptibility by limiting exposure periods to 60 min or less with the leafhoppers confined on individual plants. The effects were included through long-term, mass exposures in which leafhoppers were free to choose the most desirable hosts available.

Since incidence of infection was different in the two types of susceptibility tests, results were not directly comparable. Therefore, it was necessary to express resistance in relative terms. First, relative susceptibilities were determined by expressing the incidence of infection of each cultivar as a percentage of the incidence of infection of the susceptible control, VR Moscow. Then relative resistance was calculated by subtracting relative susceptibility from 100.

Data on the incidence of infection of the six resistant cultivars based on mass exposures (vector preference included) were previously published (13). The results are considered particularly valid since they represent a variety of tests conducted at different times over the past several years and since, with the exceptions of the cultivars Owyhee and Payette, they are based on a great deal of replication. In all these tests, viruliferous leafhoppers were released at the rate of one to three leafhoppers/seedling on flats of seedlings in a greenhouse section 3 days after transplanting. The leafhoppers died within 4 to 10 days, as tomatoes will not support the sugarbeet leafhopper. Rate of infection was determined 2 weeks later.

Relative susceptibilities based on short exposures (vector preference excluded) were determined with leafhoppers confined on individual plants using exposure periods of 15, 30, and 60 min. For each exposure period, seedlings of each tomato cultivar were transplanted in rows with 32 plants/row across a bed on the greenhouse bench. The rows were arranged in 15 blocks, with one row of seedlings of each line in each block. A clip cage containing five viruliferous leafhoppers was placed on the first plant of each row. At the predetermined exposure interval, the clip cages were transferred to the second plant in each row, and on to the third and fourth until all the seedlings on the table were exposed. Infection rate was determined 2 weeks later.

RESULTS.—*Direct observation of leafhopper preference.*—Preliminary studies conducted by the junior author first suggested that leafhopper preference may be a factor in resistance to CTV. On the average, he found there were 62% more leafhoppers on flats of VR Moscow seedlings than on adjacent flats of C193 seedlings. However, extreme variation in these early experiments demonstrated that critical control of factors, affecting leafhopper behavior, particularly light, would be necessary. The average leafhopper number per flat varied from 4 to 15 among VR Moscow seedlings, and from 2 to 12 among C193 seedlings. In another instance, light coming through a small window 7 m away caused leafhoppers to congregate on the end of a cage nearest the window, even though supplementary light was placed immediately above the cage. Consequently, the previously described (11) observation cage and method of conducting these experiments were developed to reduce environmental variation as much as possible.

In our first series of direct observations, a single seedling of each of the susceptible and resistant cultivars was used in each of 16 replications. The order in which they occurred in the row in each replicate was randomized. Since a slight tendency for the leafhoppers to accumulate at the two ends of the observation cage was noted, three additional seedlings were transplanted at each end of the row as a border. These border seedlings were the same as the test seedlings, and their kind and arrangement was also randomized.

In general, leafhoppers spent more time on seedlings of the cultivars susceptible to virus infection than on those of susceptible cultivars (Table 1). The correlation coefficient between susceptibility and vector preference was +0.55, which is significant only at the 10% level of probability. Despite the fact that the cultivars preferred most by leafhoppers were suscep-

TABLE 1. Vector (*Circulifer tenellus*) preference among tomato cultivars resistant and susceptible to curly top virus

Cultivar	Relative susceptibility[a]	Vector preference[b]
Bonny Best	100[c]	163
VR Moscow	100	152
Manalucie	100	152
Stone	100	150
Owyhe	49	132
C27	49	131
VF145	100	117
C193	31	115
Allen's Triumph	100	113
CVF4	29	102
C5	13	84
Payette	85	81

[a] Per cent infection relative to the susceptible control, VR Moscow, determined on the basis of mass exposures in the greenhouse.

[b] Number of leafhoppers observed on a single seedling of each cultivar in the observation cage in 16 replications, four readings/replication.

[c] Correlation coefficient = +0.55. Significant at the 10% level of probability.

tible, and that those preferred least were resistant, there was a middle ground containing both resistant and susceptible cultivars.

Further testing with greater concentration on fewer cultivars was deemed necessary. For this purpose, we selected two susceptible cultivars, one from among the most and another from among the least attractive to leafhoppers, and two resistant cultivars representing these same extremes. The cultivars selected were: (i) VR Moscow, most attractive susceptible; (ii) VF145, least attractive susceptible; (iii) Owyhee, most attractive resistant; and (iv) C5, least attractive resistant. Eight identical preference tests were conducted as previously described, with three replicates included in each test.

The results (Table 2) left little doubt that there were three levels of vector preference among the tomato cultivars: low, medium, and high. Our most highly virus-resistant cultivar, C5, was lowest in vector preference. However, the highest level of vector preference included both a resistant and a susceptible cultivar, suggesting that vector preference was not a factor of resistance to virus infection of all the resistant cultivars. A susceptible cultivar, VF145, was medium in vector preference, suggesting that it may possess a low level of resistance attributable to vector preference.

TABLE 2. Differences in the preference of the vector, *Circulifer tenellus,* for two curly top-resistant and two curly top-susceptible tomato cultivars[a]

Tomato cultivar	Mean leafhopper no.[b]
C5	6.8 a
VF145	9.5 b
VR Moscow	11.7 c
Owyhee	12.6 c

[a] C5 and Owyhee are resistant cultivars; VR Moscow and VF145 are susceptible.

[b] Mean number of leafhoppers observed on a plant of each cultivar in 24 replications. Means not followed by the same letter are significantly different at the 1% level of probability. Standard deviation = 5.1 leafhoppers.

Resistance attributable to vector preference.—In the first method used to detect vector preference, we observed that leafhoppers tend to accumulate on plants of certain cultivars in preference to others. However, this method gave little or no indication of how much resistance to CTV could be attributed to vector nonpreference. In the second method to detect vector nonpreference, we measured susceptibility of the cultivars with and without vector nonpreference expressed. This method not only indicated nonpreference, but also measured the contribution of nonpreference directly in terms of resistance to CTV.

Although the actual numbers of plants infected during the 15-, 30-, and 60-min exposure periods increased at an approximate rate of 1, 3, and 4, respectively, there were essentially no differences in the infection rate for the three exposure periods relative to that of the susceptible control, VR Moscow. Therefore, results of the three exposure periods were pooled in calculating the relative resistance, with vector preference excluded as a possible factor in resistance.

The results of preventing the expression of vector nonpreference (Table 3) agreed closely with those in which choices of leafhoppers for the various cultivars were observed directly. Relative resistance of C5,

TABLE 3. Relative resistance of tomato cultivars to curly top virus attributable to nonpreference by the vector, *Circulifer tenellus*

Tomato cv	Relative resistance[a]		
	Excluding vector preference factor[b]	Including vector preference factor[c]	Attributable to vector nonpreference[d]
C5	64	87	+23
CVF4	50	71	+21
C193	60	69	+ 9
C27	49	51	+ 2
Owyhee	61	51	−10
Payette	21	15	− 6
VR Moscow (control)	0	0	0

[a] Relative resistance = 100 − relative susceptibility. Relative susceptibility = incidence of infection among test cultivar ÷ incidence of infection among VR Moscow × 100.

[b] Mean relative resistance determined with exposure periods of 15, 30, and 60 min. Based on inoculation of 480 plants of each cultivar at each of the three exposure periods, a total of 1,440 inoculations. Influence of vector preference was excluded from results by limiting the period of exposure to leafhoppers to less time than that required by the vector to express a preference.

[c] Relative resistance based on inoculation of 3,028, 632, 1,912, 3,612, 131, 130, and 779 plants of C5, C193, C27, CVF4, Owyhee, Payette, and VR Moscow, respectively. Influence of vector preference was included in results by using mass exposures of 4-10 days in which leafhoppers were free to accumulate on plants of their choice.

[d] The difference between relative resistance with vector preference included and excluded as a possible factor in results.

CVF4, and C193 increased 23, 21, and 9%, respectively, with vector nonpreference included as compared with excluded in susceptibility tests. These cultivars were second, third, and fourth least preferred by leafhoppers according to our direct observations. Leafhoppers showed little or no nonpreference for C27 and Owyhee in our direct observations; as expected, preventing the expression of vector nonpreference did not decrease their relative resistance. Payette was the only exception. Although it was approximately equal to C5 in leafhopper nonpreference, excluding vector nonpreference in susceptibility tests did not decrease its relative resistance.

DISCUSSION.—Although high levels of resistance to CTV have been developed in tomato, the resistance is complex in inheritance and is difficult to incorporate with desirable characteristics of susceptible lines (6). Part of this difficulty undoubtedly arose from the fact that there is no knowledge of the specific factors which make up the resistance of the resistant lines. In this study we have, for the first time, identified a factor of resistance to CTV in tomato, determined its availability in specific lines, and developed a simple method to detect and measure this factor directly in terms of resistance. The factor is vector preference. This knowledge should improve our efficiency in breeding and selecting tomatoes, at least for this one factor.

Results of two different types of tests to detect differences in vector preference for various tomato cultivars suggest that vector nonpreference may be an important factor of resistance to curly top virus in two cultivars, C5 and CVF4, and a minor factor in a third, C193. The remaining three resistant cultivars apparently possess little, if any, resistance attributable to vector nonpreference.

Payette is the only cultivar for which the results of the two methods used to detect vector nonpreference were not in substantial agreement. Payette showed the same high level of vector nonpreference as C5 and CVF4, as measured by the tendency of leafhoppers to accumulate on preferred hosts. However, preventing the expression of vector preference in susceptibility tests did not similarly decrease its relative resistance. Our susceptibility tests, with vector nonpreference included, detected only a slight degree of resistance in Payette. Had we detected levels of resistance in Payette similar to those reported by Simpson (10), there would be no inconsistency. It seems possible that our measurement of susceptibility was inaccurate.

Although we previously regarded VF145 and Allen's Triumph as fully susceptible to CTV, we had noted (*unpublished data*) that fewer plants of these cultivars than of the other susceptible cultivars became infected in field tests when leafhopper populations were low. Therefore, it was not surprising to find that these two cultivars possessed a low level of leafhopper nonpreference. The leafhopper does not distinguish between preferred and nonpreferred hosts until after feeding for 25 to 50 min (11). It then departs from nonpreferred hosts, and tends to accumulate on the most preferred hosts available. Thus, a low level of nonpreference as an only source of resistance would not result in fewer infections under the severe exposures we provide in susceptibility tests.

After resistance attributable to vector preference is removed, none of the resistant lines is more than 50% as susceptible as VR Moscow. Thus, the major portion of resistance has not yet been identified. However, all of the resistance of the resistant lines has been tentatively characterized as being of the escape type; i.e., resistance to establishment of infection (13). Thus, it seems probable that the major portion of the resistance remaining after accounting for that attributable to vector preference results from mechanisms operative after introduction of virus into the plant and before infection is established. Further studies are under way to determine the nature of this resistance.

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