Isolation and Differentiation of Five Strains of Curly Top Virus

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ABSTRACT

Differences in host range, mortality rate, degree of stunting, and severity of foliage distortion on selected hosts were used to differentiate five distinct strains (12A, 13B, 16C, 31A, and 35B) of curly top virus. The procedure used to isolate the individual strains from natural mixtures of strains is described. Strain 31A was distinguished from the other strains on *Nicotiana glutinosa,* V. R. Moscow tomato, and highly susceptible, moderately resistant, and highly resistant sugarbeet varieties. Strain 12A was distinguished on *N. glutinosa,* highly susceptible, and moderately resistant sugarbeets. Strain 13B was distinguished on *N. glutinosa* and moderately resistant sugarbeets. Distinction between strains 16C and 35B occurred only on *N. glutinosa,* but the distinction was clear. There was no correlation between the relative severity of symptoms caused by the strains on different hosts. Phytopathology 60:844-848.

Giddings (3) demonstrated that curly top virus (CTV) consists of a complex of strains that may be separated into recognizable entities. The leafhopper vector of CTV, *Circulifer tenellus* (Baker), can carry at least three strains simultaneously and transmit all of them to the same sugarbeet plant during a 24-hr exposure period (5). Approximately 50% of the young sugarbeet plants infected during such exposures, however, were infected by but a single strain. With rare exceptions, in the plants infected by two or three strains, one strain of the mixture became temporarily predominant. The initial symptoms were those of the predominant strain. This temporary initial advantage fell to each of the strains in mixtures approximately an equal number of times.

Giddings (3, 4, 6) described 12 strains of CTV and predicted that more eventually would be recognized. Bennett (1, 2) subsequently described three additional North American strains. Unfortunately, only a few of the original strains have been maintained in culture.

Strains of CTV were needed to evaluate resistance to CTV in tomato breeding lines. A number of *Lycopersicon* species and tomato breeding lines may collectively possess specific resistances to most, if not all, the strains of CTV (3, 8). Attempts to combine these resistances require the ability to identify sources of resistances to each strain.

MATERIALS AND METHODS.—Many plant species are susceptible to some strains of CTV and immune from others (2, 3). A wide range of species was inoculated using leafhoppers reared uncaged in an insectary on naturally infected, field-grown sugarbeets collected near Prosser, Washington. The virus strain(s) from a single plant of each species that became infected was transferred to a susceptible sugarbeet using previously nonviruliferous leafhoppers. Nonviruliferous leafhoppers were then caged on the infected sugarbeet. After a feeding period of 7 days, individual leafhoppers were transferred to small clip cages fastened on leaves, and each of 100 seedling sugarbeets was exposed to one leafhopper for 15 min. Only about 10% of the plants were infected during this short feeding period, but almost all those infected apparently contained but one strain of virus. Symptoms of the seedlings that became infected fell into distinct categories.

Finally, virus from one representative sugarbeet plant in each symptom category was transmitted successively through three additional seedling sugarbeets. Each transfer was made just as symptoms were beginning to appear. The resulting isolates were tentatively regarded as pure strains. Selected hosts were inoculated with each of the isolates, and the reactions were compared to differentiate between the isolates.

The sugarbeet cultivar NB4 was used in the isolation program because it is a highly susceptible, self-fertile inbred (7). It was hypothesized that mild strains of CTV, which might be eliminated by a more resistant beet, would be retained in NB4. Because NB4 is an inbred line, differences in symptoms could, with greater confidence, be attributed to differences in virus strains rather than to genetic differences between plants.

Test plants were grown from seed, transplanted into composted, methyl bromide-sterilized soil in 20-cm clay pots, and inoculated at an early stage of vigorous growth. All tests were conducted in a greenhouse maintained at approximately 27 C.

RESULTS.—The strains of CTV described here were recovered as components of the strain complexes, which infected *Nicotiana nesophila* Johnston, *Plantago virginica* L., *Salpiglossis sinuata* Ruiz & Pav., and *Solanum nigrum* L. when these species were exposed to wild sources of virus. The 15-min exposure of NB4 beet seedlings to single leafhoppers used to vector virus from infected individuals of these species resolved four components (12A, B, C, D) from *N. nesophila,* four (13A, B, C, D) from *P. virginica,* four (16A, B, C, D) from *N. glutinosa,* one (31A) from *S. sinuata,* and two (35A, B) from *S. nigrum.* Reactions of selected hosts (described below) indicated that at least five strains were represented among the 15 components. The symptoms produced by each component, except 31A, were duplicated by at least two components that were iso-

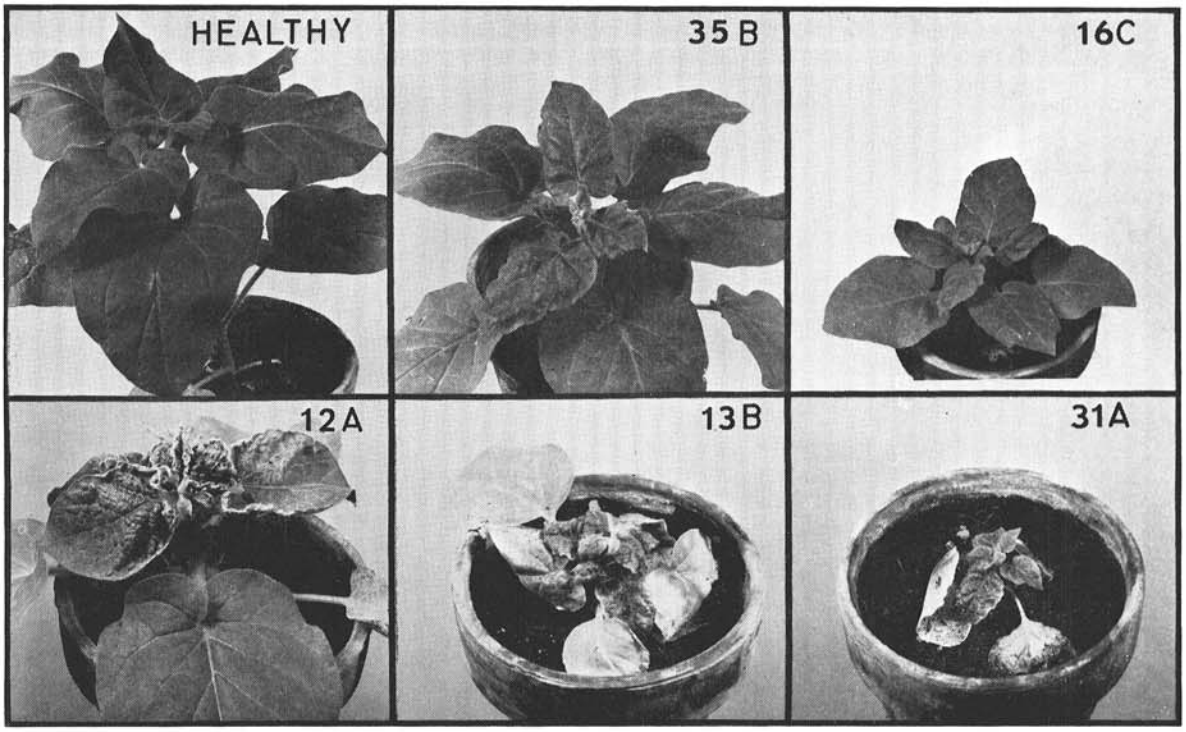

Fig. 1. Symptoms produced by five strains of curly top virus on *Nicotiana glutinosa* plants 4 weeks after inoculation.

lated from other species. Groups that caused the same symptoms were (i) 12A, 13D, and 16B; (ii) 13B, 12C, 16D, and 35A; (iii) 16C, 13C, and 12D; (iv) 35B, 12B, 13A, and 16A; and (v) 31A. A single component was chosen from each group as a strain. Those chosen were 12A, 13B, 16C, 31A, and 35B.

TABLE 1. Severity of symptoms produced by five strains of curly top virus on four hosts

		Symptoms		
Host	Strain	Leaf distortion	Stunting	Mortality
Nicotiana glutinosa	12A	Severe	Moderate	Seldom
	16C	Mild	Severe	Never
	13B	Severe	Severe	Often
	31A	Moderate	Ex. Severe	Usually
	35B	Mild	Mild	Never
Sugarbeet var. NB4, Klien E, R&G Old Type, SL 742	12A	Severe	Mild	Never
	16C	Severe	Severe	Usually
	13B	Severe	Severe	Usually
	31A	Ex. Mild	None	Never
	35B	Severe	Severe	Usually
Sugarbeet var. US 33	12A	Moderate	None	Never
	16C	Moderate	Severe	Seldom
	13B	Severe	Severe	Usually
	31A	No infection		
	35B	Moderate	Severe	Seldom
Tomato var. V. R. Moscow	12A	Severe	Severe	Always
	16C	Moderate	Moderate	Always
	13B	Severe	Severe	Always
	31A	Severe	Severe	Always
	35B	Moderate	Moderate	Always

Symptoms produced on *N. glutinosa* L. differentiated all five CTV strains (Table 1, Fig. 1). Strains 35B and 16C produced very mild foliage distortion, but 16C caused severe stunting and 35B caused little stunting. The other three strains, 12A, 13B, and 31A, all caused pronounced foliage distortion on *N. glutinosa,* but differed in the amount of stunting produced. Plants infected by strain 12A were moderately stunted but produced at least twice as much growth as those infected by 13B, and four to six times more than those infected by 31A. Strains 12A, 13B, and 31A also could be differentiated by the types of foliage distortion produced on *N. glutinosa* (Fig. 1). Foliage distortion was most severe among plants infected by 13B. The greatly thickened leaves rolled backwards from the tip perpendicular to the mid-vein. Because the internodes were also shortened, plants infected by 13B produced tight knots of foliage. Prominent vein swelling and clearing characterized plants infected by 12A. The growing points tended to coil, which gave the appearance of more leaf distortion than was actually present. Foliage distortion produced by 31A was relatively mild, perhaps because little or no growth occurred after symptoms appeared. Strain 31A was almost always lethal to *N. glutinosa* plants, and many plants infected by 13B died. Plants infected by the other strains tended to recover from symptoms partially or completely.

Strain 31A was the most severe on V. R. Moscow tomato plants, and was clearly distinguished from the other strains (Fig. 2). Infected plants died soon after symptoms were expressed. Differences produced by the other strains were more subtle and, used alone, were

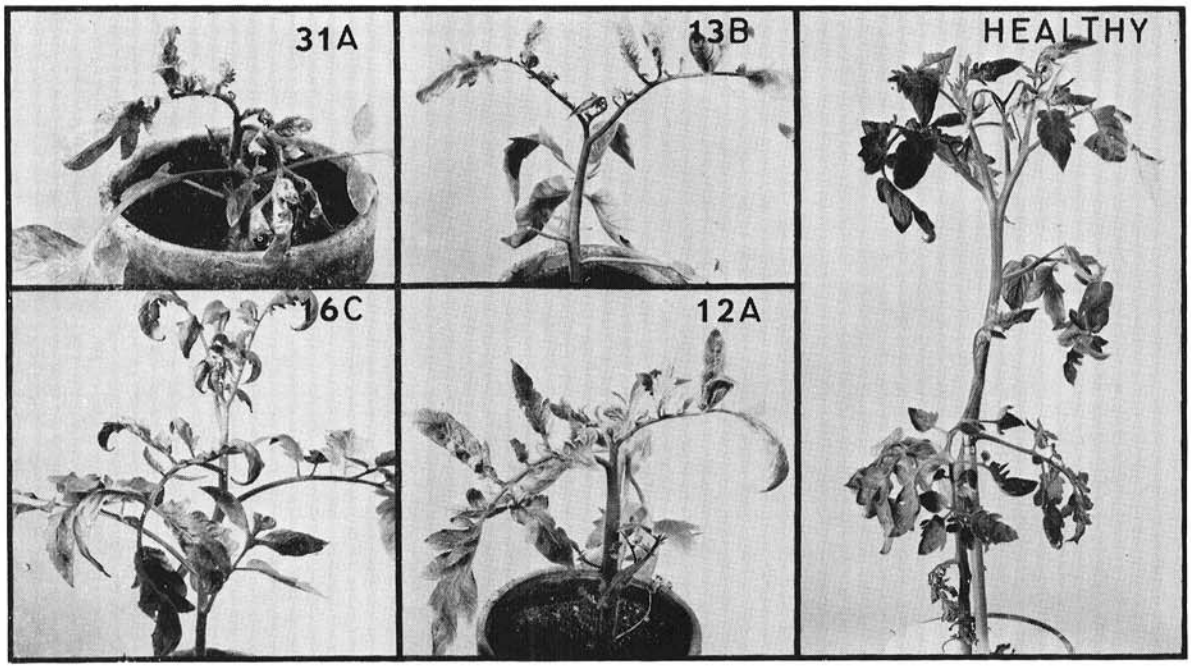

Fig. 2. Symptoms produced by four strains of curly top virus on the highly susceptible tomato cultivar, V. R. Moscow, 4 weeks after inoculation.

not sufficient to consistently distinguish between the strains. Young opposite leaflets on plants infected by 13B tended to cross over one another, wrapping themselves around the petiole, and plants took on a purplish hue. Plants infected by 12A became yellow in color, petioles drooped, and the leaflets were flat and rigid. Symptoms caused by 16C and 35B were the same on V. R. Moscow plants. The plants appeared rigid, leaflets cupped backwards, and the plants declined more slowly than those infected by other strains.

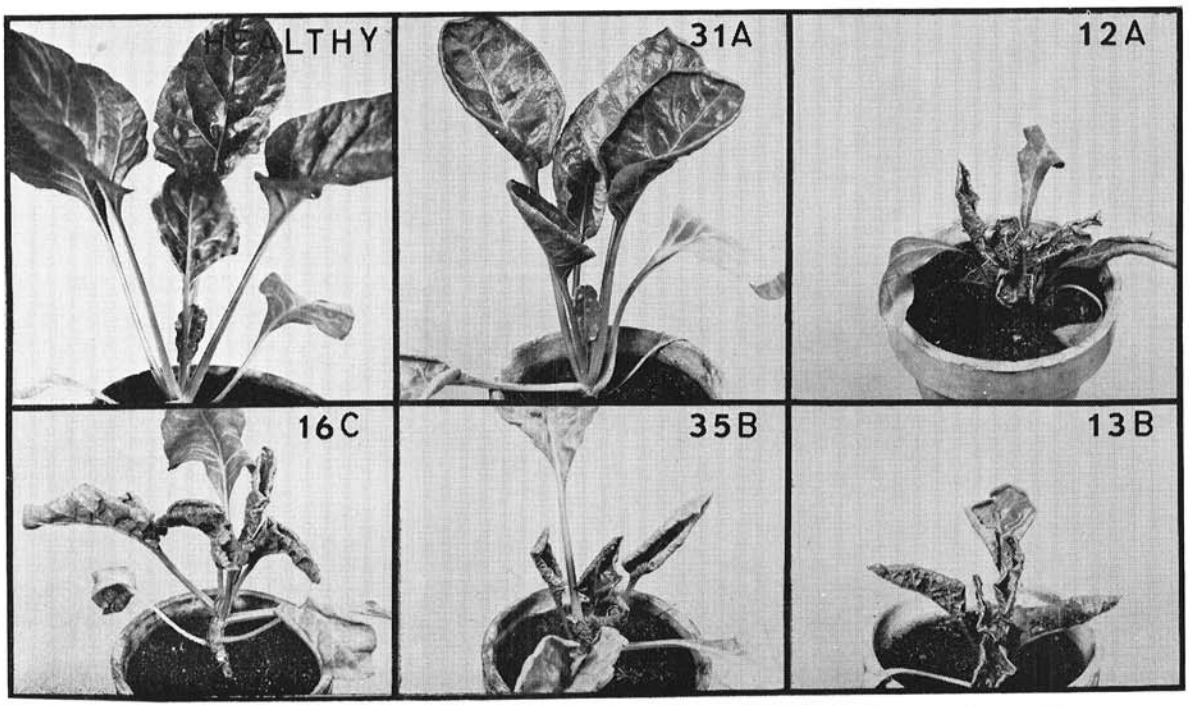

Fig. 3. Symptoms produced by five strains of curly top virus on the highly susceptible sugarbeet cultivar, NB4, 4 weeks after inoculation.

Symptoms produced by strains 31A and 12A were distinctive on the highly susceptible sugarbeet cultivars NB4, Klien E, R & G Old Type, and SL742, but those caused by 16C, 35B, and 13B were not consistently distinguishable (Fig. 3, Table 1). Strain 31A caused only faint vein clearing in the youngest leaves and mild vein swelling in an occasional leaf on the susceptible beets. At an early stage of development, symptoms produced by 12A were severe and similar to those produced by 16C, 35B, and 13B (Fig. 3). Subsequently, the latter three strains were usually lethal. However, NB4 plants infected by 12A responded much like plants of the sugarbeet cultivar US33 infected by the same strain (Fig. 4). They continued to grow, and eventually produced as much foliage as healthy plants, although they continued to express vein clearing and swelling.

The reactions of the moderately resistant sugarbeet cultivar, US33, to strains 31A, 12A, and 13B were distinctive, but 16C and 35B could not consistently be distinguished from each other on this host (Fig. 4, Table 1). Strain 31A did not infect US33; 12A produced moderate symptoms and appeared to actually stimulate foliage growth; 16C and 35B produced moderately severe symptoms and stunting, but were seldom lethal; and 13B usually killed infected plants.

The more resistant sugarbeet cultivar, US75, was less effective in differentiating between strains than US33. Strain 31A did not infect this cultivar. The other four strains were not readily distinguishable on US75. All caused moderately severe symptoms and stunting, but none was routinely lethal.

The distinguishing symptoms reported here were remarkably clear and reliably reproduced on three occasions when six plants of each host were simultaneously inoculated with each of the strains. In addition to the major differences in symptomatology noted, less consistent and more subtle differences were exhibited that would be recognized by an observant investigator.

Some of the strains, notably 31A, 16C, and 35B, produced relatively severe symptoms on certain host species, and relatively mild symptoms on others. In view of these striking differences in relative severity, it seemed possible that some of the presumed single strains might actually consist of more than one strain, and that certain host species were infected by one component of the mixture and others were infected by another component. Consequently, transfers were made from each test host back to NB4 beet. The symptoms produced on NB4 by virus from the test hosts were always the same as those produced by transfers from the original isolate. If strains were eliminated by any of the test hosts, this elimination did not change the symptoms produced on NB4.

DISCUSSION.—Evidence is strong that the strains of CTV reported here consistently produce different symptom patterns. Strain 31A was distinguished from the other strains on five hosts, 12A was differentiated on three hosts, and 13B on two hosts. Although distinction between 16C and 35B occurred only on *N. glutinosa*, the distinction was clear.

There is not necessarily a relationship between the relative severity of a strain of CTV on one host and on another. Although 31A was the most severe of the

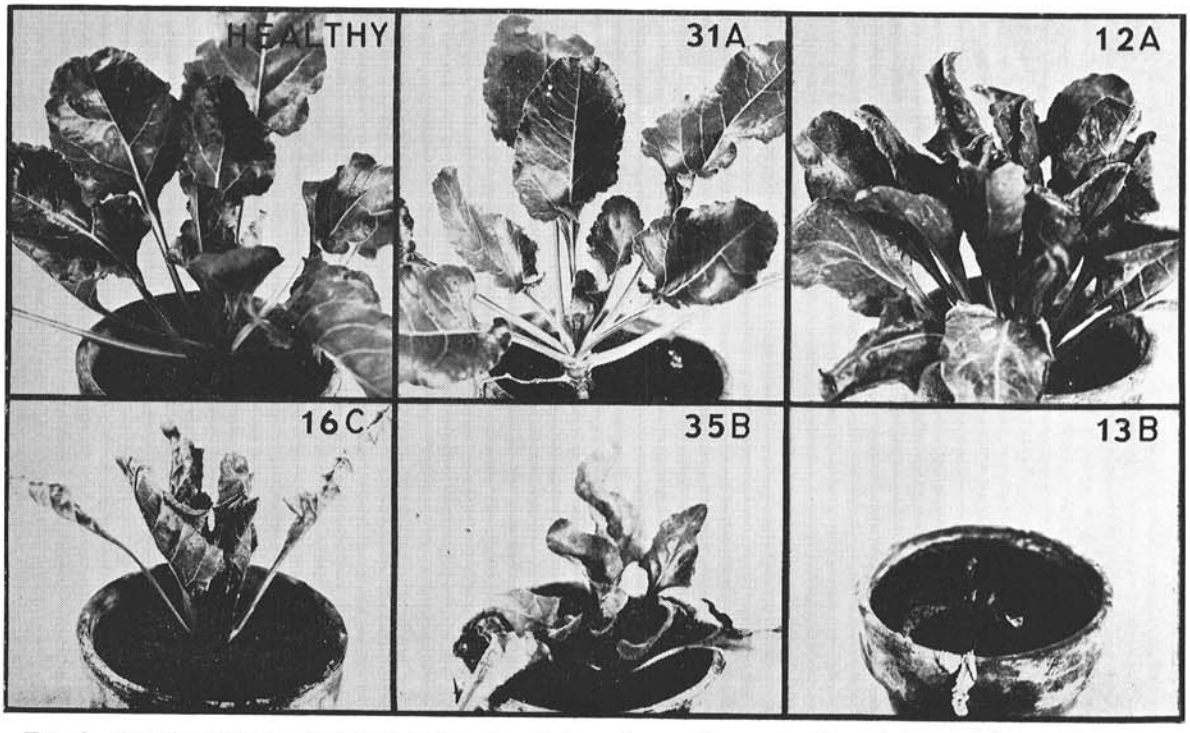

Fig. 4. Symptoms produced by five strains of curly top virus on the moderately resistant sugarbeet cultivar, US33, 6 weeks after inoculation.

five strains on *N. glutinosa* and V. R. Moscow tomato, it was extremely mild on the most susceptible sugarbeets and did not infect more resistant beets. Conversely, strains 35B and 16C were relatively mild on *N. glutinosa,* but relatively severe on sugarbeets.

Strain 31A illustrates the importance of using only the most highly susceptible sugarbeets when isolating strains of CTV. This strain, which promises to be the most useful in developing CTV-resistant tomatoes, would have been lost had even a moderately resistant sugarbeet cultivar been used.

Most strains of CTV described in this report appear to be different from previously described strains. No previously reported strain behaved like 31A, which causes extremely mild symptoms on susceptible sugarbeets and severe symptoms on *N. glutinosa.* No previously reported strain caused mild symptoms on *N. glutinosa* and severe symptoms on sugarbeets as does 35B. Strain 12A is unique in that it causes pronounced vein swelling and vein clearing in both susceptible and resistant sugarbeets, but causes little if any stunting. Strains 13B and 16C caused symptoms on susceptible and resistant sugarbeets similar to those reported by Giddings (6) for strain 11, and one of these may be a duplicate of strain 11. However, both 13B and 16C could not duplicate strain 11 since they are different from one another.

LITERATURE CITED

1. Bennett, C. W. 1962. Highly virulent strains of curly top virus in sugar beet in Western United States. J. Amer. Soc. Sugar Beet Technol. 12:515-520.
2. Bennett, C. W. 1967. Apparent absence of cross-protection between strains of curly top virus in the beet leafhopper, *Circulifer tenellus*. Phytopathology 57:207-209.
3. Giddings, N. J. 1938. Studies on selected strains of curly top virus. J. Agr. Res. 56:883-894.
4. Giddings, N. J. 1944. Additional strains of the sugar beet curly-top virus. J. Agr. Res. 69:149-157.
5. Giddings, N. J. 1950. Some interrelationships of virus strains in sugar-beet curly top. Phytopathology 40: 377-388.
6. Giddings, N. J. 1954. Two recently isolated strains of curly-top virus. Phytopathology 44:123-125.
7. McFarlane, J. S., & I. O. Skoyen. 1965. Sugar beet breeding lines combining resistance to bolting and disease. J. Amer. Soc. Sugar Beet Technol. 13:555-562.
8. Randall, T. E. 1966. The utility of the reactions of selected host plants to known isolates of the curly-top virus in developing a different approach to breeding problems in tomatoes. Washington Agr. Exp. Sta. Tech. Bull. 49. 18 p.