Curly Top Viruliferous and Nonviruliferous Leafhopper Feeding Effects upon Tomato Seedlings

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ABSTRACT

Seedlings of *Lycopersicon esculentum* and *L. peruvianum* var. *dentatum* exhibited sudden wilting and death after attack by curly top viruliferous or nonviruliferous leafhoppers. Although reactions between treatments appeared identical qualitatively, significantly greater numbers of seedlings were affected by viruliferous than by nonviruliferous leafhopper feeding. Microscopic examination revealed death and degeneration of cells of both emerged and nonemerged secondary root tips and of primary root tips. This reaction was evident in plants of both leafhopper-fed treatments. In addition, some degree of cortical collapse was apparent in mature regions of primary roots, although this abnormality appeared distinct from the effects on young root tip cells.

Wilting and death were attributed primarily to the effects of feeding, which possibly involved a leafhopper-secreted toxin. The virus may have intensified this reaction through predisposition of seedlings to feeding effects, or through stimulation of increased toxin secretion by the insects.

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Additional key words: curly top resistance, systemic phytotoxemia.

A high degree of resistance to curly top disease exists in the wild tomato variety *Lycopersicon peruvianum* var. *dentatum* Dun. (1). Resistance, however, may vary depending upon such variables as virulence of the virus strain used for inoculation, numbers of viruliferous leafhoppers to which plants are exposed, and age of plants at the time of feeding (2, 4, 5, H. L. Blood, *unpublished data*).

In our studies of curly top, an additional factor associated with breakdown of resistance in *L. peruvianum* var. *dentatum* has been observed. This is the apparent toxic effect of leafhopper feeding upon the plants. Carter (3) listed general chloroses, veinbanding, chlorotic streaking, and wilting among known symptoms of insect-caused systemic phytotoxemias. Several genera of leafhoppers were noted as incitants of such effects. Carter stated that such feeding effects of virus vectors are sometimes easily separated from symptoms of the disease itself on the basis of different effects that each would have produced separately, but in many cases this separation is difficult. In addition, much difficulty is encountered in extraction of such toxins and in the reproduction of toxin-induced symptoms experimentally. Randall (5) reported that a number of tomato varieties exhibited leafhopper feeding injury that could not be separated from curly top symptoms.

This study was undertaken to further evaluate the early feeding effects of viruliferous and nonviruliferous leafhoppers on seedlings of *L. peruvianum* var. *dentatum* and *L. esculentum* Mill.

MATERIALS AND METHODS.—*Inoculation*.—Seeds of *L. peruvianum* var. *dentatum* and curly top-susceptible *L. esculentum* (unnamed breeding line VF7) were germinated in sand. Two to 3 days after emergence, seedlings were transplanted to soil or sand in flats, or were placed between sheets of moist absorbent paper and clamped between two small boards. With the latter arrangement, the pairs of boards, with seedlings between, were placed in shallow pans containing a weak aqueous fertilizer solution. This provided a continuous source of moisture and nutrients to the roots. A similar solution was also used to water seedlings transplanted to sand.

The transplanted seedlings were placed in an inoculation cage comprised of cheesecloth and polyethylene plastic. A 500-w incandescent light in the cage top provided supplementary illumination. Five to seven leafhoppers/plant (depending upon the particular test) carrying a virulent isolate of the virus were introduced into the cage, and were allowed to feed for specific lengths of time. Depending upon the treatment, inoculation was begun 4 to 9 days after transplanting to determine the relationship of plant age to leafhopper-feeding effects.

Identical inoculation procedures were followed using nonviruliferous insects which were placed into a separate cage containing seedlings. Control treatments were established under the same environmental conditions without leafhoppers.

External and anatomical studies.—Seedlings to be examined were carefully removed from the growth medium at specific intervals, usually 12 hr, beginning with feeding initiation. They were observed with a dissecting microscope. The seedlings grown between boards were examined in a similar manner. In the latter case, the entire set of seedlings was removed from the inoculation cage at 12-hr intervals, the roots and stems were observed, and the seedlings were returned to the cage for further leafhopper exposure.

Stem and root sections for microscopic examination were prepared by killing and fixing these parts in Formalin-alcohol-acetic acid solution. These were dehydrated in a tertiary butyl alcohol series; passed through paraffin oil; and infiltrated with, and embedded in, paraffin. Longitudinal sections 10 μ thick were cut with a rotary microtome. Sections

TABLE 1. Effects of plant age and viruliferous and nonviruliferous leafhopper feeding (six leafhoppers/plant) on seedlings of *Lycopersicon peruvianum* var. *dentatum*

Leafhopper type	6 Days after feeding initiation			12 Days after feeding initiation		
	Total plants	No. wilted	% Wilted	Total plants	No. dead	% Dead
Feeding initiated 4 days after transplanting						
Viruliferous	70	43	61	70	51	73
Nonviruliferous	70	18	26	70	22	31
Feeding initiated 9 days after transplanting						
Viruliferous	68	15	22	68	32	47
Nonviruliferous	70	1	1.4	70	2	2.8

were mounted on microscope slides, deparaffinized, and stained with safranin-fast green (6).

RESULTS.—Viruliferous and nonviruliferous leafhopper feeding caused rapid wilting and subsequent death of seedlings of *L. peruvianum* var. *dentatum* and *L. esculentum* exposed 4 days after transplanting. Commonly recognized curly top symptoms, including vein clearing and leaf yellowing, did not occur in the plants that died rapidly. The viruliferous and nonviruliferous treatments produced qualitatively indistinguishable wilting symptoms. However, wilting became evident consistently sooner among seedlings of the viruliferous leafhopper treatment, and considerably greater numbers of plants were affected than with the nonviruliferous leafhopper treatment (Table 1).

Feeding effects upon seedlings of *L. peruvianum* var. *dentatum* were equal to or more severe than those exhibited by *L. esculentum* seedlings. Both tomato types, however, were definitely affected by each leafhopper treatment. With the viruliferous leafhopper treatment, wilting became evident in each plant type within 24 hr of feeding initiation, and occasionally within 12 hr. In no case did wilting begin as soon or affect as many seedlings in the nonviruliferous as in the viruliferous leafhopper treatment. The majority of seedlings that had not wilted and died 7 days after exposure to viruliferous leafhoppers exhibited definite stunting and dulling of color in contrast to surviving nonviruliferous-exposed seedlings. The stunted *L. peruvianum* var. *dentatum* seedlings not dead within 7 days after leafhopper feeding often recovered and assumed normal growth, whereas seedlings of *L. esculentum* so affected always eventually died. Any stunting resulting from nonviruliferous feeding was usually overcome by seedlings of both plant types. No wilting or other abnormalities were noted in control seedlings.

A substantial reduction in rapid wilting was exhibited by *L. peruvianum* var. *dentatum* seedlings exposed to leafhoppers 9 days after transplanting as compared with those exposed 4 days after transplanting (Table 1). Although the older seedlings surviving viruliferous leafhopper attack sometimes exhibited stunting, this effect was overcome soon after feeding ceased. A comparable increase in resistance to rapid wilting was also exhibited by older *L. esculentum* seedlings, although those seedlings exposed to viruliferous leafhoppers eventually developed typical curly top symptoms and succumbed to the disease.

Macroscopic and anatomical examination.—Inhibition of branch root initiation and growth, and stunting of primary root growth, occurred in seedlings of both leafhopper treatments. This effect became readily evident in seedlings collected 60 hr after start of feeding, and it was more severe in seedlings of each plant type exposed to viruliferous leafhoppers (Fig. 1). Examination under the dissecting microscope revealed that developing secondary root tips and primary root tips turned brown. In contrast, the root tips of control seedlings were white. No abnormalities were observed in stems of plants of either leafhopper-exposed treatment.

Microscopic examination of longitudinal root tip sections from leafhopper-damaged seedlings revealed cell disorganization and collapse with accompanying cytoplasm degeneration. These abnormalities often involved the entire meristematic region, and possibly extended into the region of maturation. Although a great deal of deterioration was evident, neither hypertrophy nor hyperplasia appeared characteristic of this condition. Secondary root tips which had not yet emerged from primary root cortical tissue at the time of collection exhibited similar effects. In the latter case, the degeneration of embryonic root cells did not extend to immediately adjacent pericyclic and vascular tissues of the primary roots. These effects appeared qualitatively similar in root tips of both viruliferous and nonviruliferous treated seedlings. In contrast, both emerged root tips of control seedlings were comprised of organized, nucleated cells which were obviously functional at the time of collection (Fig. 2).

Some degree of cortical tissue collapse was also evident. This condition appeared more severe in viruliferous-exposed than in nonviruliferous-exposed seedlings. This abnormality occurred mainly in mature regions of primary roots, and may have disrupted vascular tissue, although primary effects were not observed in vascular tissue itself. The cortical collapse appeared distinct from the effects upon root tip cells. Stem tissue appeared normal in plants of all treatments.

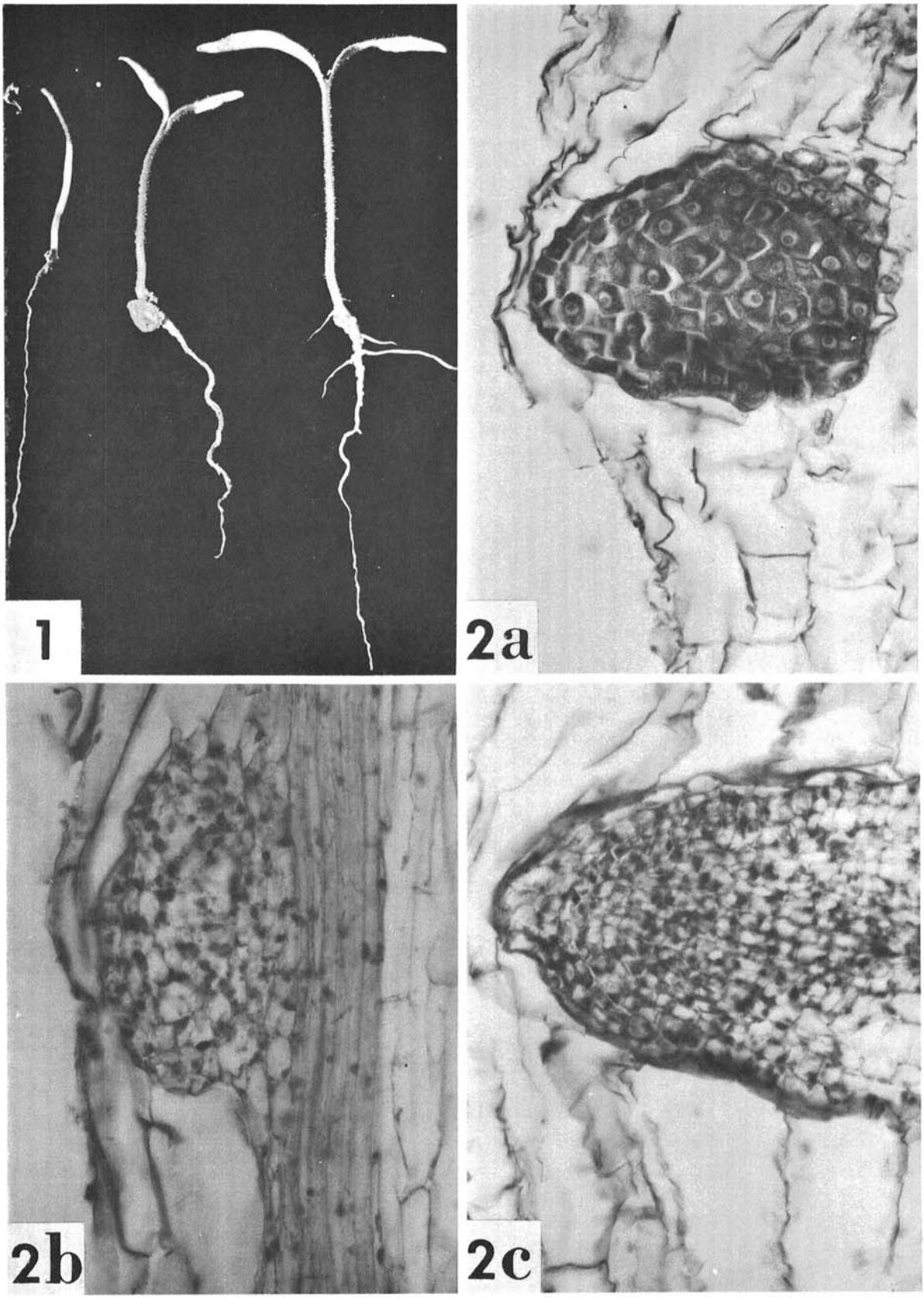

Fig. 1-2. 1) Comparison of effects of viruliferous, nonviruliferous, and no leafhopper feeding upon *Lycopersicon esculentum* seedlings 60 hr after initiation of leafhopper feeding. (Left) Exposed to viruliferous leafhoppers, exhibiting severe wilting and tissue collapse. (Center) Exposed to nonviruliferous leafhoppers, exhibiting stunting and inhibition of branch root initiation. (Right) Healthy control (not exposed to leafhoppers), exhibiting normal growth and branch root development. **2)** Comparison of effects of viruliferous, nonviruliferous, and no leafhopper feeding upon nonemerged secondary root tips of *L. esculentum* seedlings 60 hr after initiation of feeding by leafhoppers. **a)** Healthy control (not exposed to leafhoppers); consisting of normal, functional cells. **b)** Nonviruliferous; consisting of dead, degenerate cells. **c)** Viruliferous; consisting of dead, degenerate cells.

DISCUSSION.—A degree of limitation was inherent in these tests, because it was not possible to exclusively observe the effects of the virus upon seedlings as it was to observe the effects of leafhopper feeding alone. Therefore, it was not possible to definitely determine whether the curly top virus itself was capable of causing symptoms similar to those of leafhopper feeding. The absence of qualitatively distinct symptoms between seedlings exposed to feeding by viruliferous and nonviruliferous leafhoppers indicates that subsequent wilting may have been caused primarily by leafhopper feeding. The virus definitely contributed to the severity of feeding symptoms quantitatively, however. Rapid death of young root tips, inability to absorb moisture, and arrested root development were assumed to be the major cause of the observed seedling wilting and death. The occasional collapse of cortical tissue and possible resulting disruption of vascular tissue also may have contributed secondarily to wilting.

The leafhopper-feeding effect upon developing root tips did not appear to result from general removal of photosynthates from the shoots, although this conceivably may have been the origin of cortical collapse in mature roots. A specific leafhopper-secreted toxin is therefore implicated as the cause of sudden death of root tip cells. If the virus alone is not capable of causing wilting, establishment of viral infection in plant tissue may have predisposed the seedlings to a more severe reaction to leafhopper feeding. An alternative possibility would involve the stimulation of increased toxin production by viruliferous leafhoppers as compared with nonviruliferous leafhoppers. This explanation may better account for the short interval (within 12 hr) between the start of feeding and wilting.

The older seedlings apparently acquired considerable resistance to such a phytotoxemia, possibly a result of dilution of the secretion by the larger amount of plant tissue. The distinction between resistance to leafhopper feeding and to curly top itself became discernible in older inoculated plants. As many *L. peruvianum* var. *dentatum* seedlings as *L. esculentum* seedlings wilted and died shortly after exposure to feeding by viruliferous and nonviruliferous leafhoppers. This number was substantially lower, however, among both plant types as compared with that of younger seedlings. Whereas *L. peruvianum* var. *dentatum* seedlings surviving viruliferous leafhopper feeding became stunted but were often able to resume normal growth, *L. esculentum* seedlings that survived the feeding effects later developed typical curly top disease symptoms and died.

Further work using isolation procedures is necessary to definitely establish the presence and nature of the proposed leafhopper-secreted toxin.

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