Unusual Symptomatology of Curly Top in Susceptible-Resistant Grafted Tomato Plants

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ABSTRACT

Grafted tomato plants comprised of *Lycopersicon esculentum* (curly top-susceptible) tops and *L. peruvianum* var. *dentatum* or *L. peruvianum* var. *humifusum* (curly top-resistant) lower stems and roots were inoculated with virulent isolates of curly top virus. Vein clearing, leaf contortion and curling, leaf stiffening, vein thickening, stunting, and phloem abnormalities of severity comparable to that in diseased nongrafted susceptible plants occurred in the scions. Yellowing typical of that in diseased-susceptible controls did not immediately accompany these symptoms in grafted plants, however, and photosynthetic tissue of these plants accumulated abnormally high chlorophyll concentrations. Longevity in grafted plant scions was greatly extended in comparison with that of controls. All of the diseased grafted plants eventually died, however.

Inoculated reciprocal grafts (*L. peruvianum* var. *dentatum* scions and *L. esculentum* roots) exhibited the opposite reaction. The resistant scions developed yellowing, but this was not accompanied by other curly top symptoms typical of susceptible tomatoes. A separation of symptoms into at least two groups was therefore evident: those involving chlorophyll degeneration and those associated with internal and external morphological abnormalities (including vein clearing).

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Additional key words: transportable chlorophyll-affecting factor.

The symptomatology of curly top has been studied anatomically by Bennett & Esau (3), Esau (6, 7, 8), and Rasa & Esau (13). These workers reported hyperplasia, hypertrophy, and necrosis in phloem tissue of infected plants. Abnormal accumulation of carbohydrates in foliar tissue is known to occur, possibly as a direct result of phloem degeneration (5, 8, 11), but it probably occurs more or less independently of this phenomenon (16, 17). A. H. Gold (*personal communication*) has shown that excessive ethylene concentration in diseased tomato plants may be responsible for many typical curly top symptoms, including leaf curling and contortion and root abnormalities. Little information concerning the yellowing symptom of curly top is available. Bawden & Pirie (1), however, stated that in plants with yellows type diseases, the C/N ratio is increased and possibly influences photosynthesis.

Bennett (2) made and inoculated reciprocal grafts between tops and roots of resistant and susceptible sugar beets. He reported no apparent interchange of material between roots and tops that affected expression in susceptible portions. Gold (9) made similar reciprocal grafts between portions of susceptible and resistant bean plants. After inoculation, the resistant-susceptible grafts developed symptoms in the roots but not in the tops. The susceptible-resistant grafts showed symptoms in both tops and roots.

This paper reports information concerning the nature of symptoms and resistance to curly top in tomato plants gained through grafting procedures.

MATERIALS AND METHODS.—*Grafting and inoculation.*—Curly top-susceptible tomato plants (*Lycopersicon esculentum* Mill. 'Loran Blood' and unnamed breeding line VF7) were grown in soil in the greenhouse. Wild relatives of *L. esculentum*: *L. peruvianum* var. *dentatum* Dun. and *L. peruvianum* var. *humifusum* C. H. Mul., which possess considerable resistance to curly top (4), were also grown in individual containers of soil.

When vigorously growing *L. esculentum* plants had attained a height of 12-15 cm, the stems were completely severed about 9 cm below the apices. These were grafted to lower, rooted stems of *L. peruvianum* var. *dentatum* or *L. peruvianum* var. *humifusum* from which the top portions had been removed 4-5 cm above the soil level. The surviving grafts were placed in the greenhouse under high temperatures (reaching 40 C). After regrowth had started, the plants were inoculated in a cage made of cheesecloth and polyethylene plastic and provided with a 500-w incandescent light for supplementary illumination. The cage was sufficiently large to contain 30 plants.

Viruliferous leafhoppers which had been reared on sugar beets infected with virulent isolates of the virus were placed in the cage with an aspiration tube. An average of 15 insects/plant was used.

The leafhoppers were allowed to feed on the plants for 6 to 7 days, after which the plants were removed from the cage and sprayed with insecticide. All plants were transplanted to 1-gal cans and were placed in the greenhouse under strong sunlight and temperatures of 30 to 40 C. Developing shoots from the lower resistant stem portions were removed from some grafted plants, and were allowed to develop on others. Thirty-nine susceptible-resistant grafts were inoculated. An equal number of nongrafted *L. esculentum* control plants was inoculated. Previous tests have shown that inoculated *L. esculentum-L. esculentum* grafted plants developed curly top in a manner typical of nongrafted *L. esculentum* plants. Ten noninoculated grafted plants were left as healthy controls.

Reciprocal grafts in which *L. peruvianum* var. *dentatum* scions were grafted to *L. esculentum* lower stems and roots also were made and inoculated as described above. Twenty-six resistant-susceptible plants were inoculated. Again, an equal number of nongrafted *L. peruvianum* var. *dentatum* control plants was inoculated, and six noninoculated grafts were utilized as healthy controls.

Anatomical studies.—After disease symptoms developed, stem and petiole sections were taken from near the apices of several inoculated plants of each reciprocal graft. Samples also were taken from inoculated and noninoculated nongrafted plants of *L. esculentum* and *L. peruvianum* var. *dentatum*. The tissue samples were fixed in Formalin-alcohol-acetic acid solution, 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 were mounted on microscope slides, deparaffinized, and stained with safranin-fast green (14).

Quantitative chlorophyll assay.—Typical dark-green leaves from diseased scions of susceptible-resistant grafted plants were collected, as was a sample of normal green leaves from healthy control plants. Chlorophyll was extracted in 50 ml acetone from 2 g of tissue of each treatment utilizing a method similar to that of Goodwin (10). Each chlorophyll extract was transferred from acetone to 50 ml of diethyl ether in a separatory funnel, washed repeatedly with distilled water, and dried over anhydrous sodium sulfate. Quantitative readings were made in a Beckman spectrophotometer, using pure ether as a blank. Following the method of Smith & Benitez (15), absorbency at 662 and 644 nm was determined, from which concentrations of chlorophylls *a* and *b* were calculated. These values were combined to give total chlorophyll concentration for each treatment.

RESULTS.—*Macroscopic symptoms.*—The susceptible-resistant grafted healthy controls exhibited normal development typical of nongrafted healthy *L. esculentum* plants throughout the experiment. Symptoms described in this study in diseased susceptible-resistant grafted plants occurred in the *L. esculentum* scions which comprised the dominant aboveground portions. Symptom expression in the developing shoots of the lower resistant portions varied between the two resistant varieties utilized, being nonexistent in *L. peruvianum* var. *dentatum* and occasionally present in *L. peruvianum* var. *humifusum*.

During the 10- to 14-day incubation period, both inoculated susceptible-resistant and inoculated control plants grew normally. After the incubation period, early symptoms of vein clearing, a yellowing tendency in young leaves, and contortion of young petioles became evident in both treatments simultaneously. Subsequent symptoms of stunting, stiffening, contortion and epinasty of older leaves, and thickening of veins were also similar between the grafted and control treatments.

Typical dulling, general lightening of color, and yellowing accompanied the above-mentioned symptoms among the controls. Death, or extremely

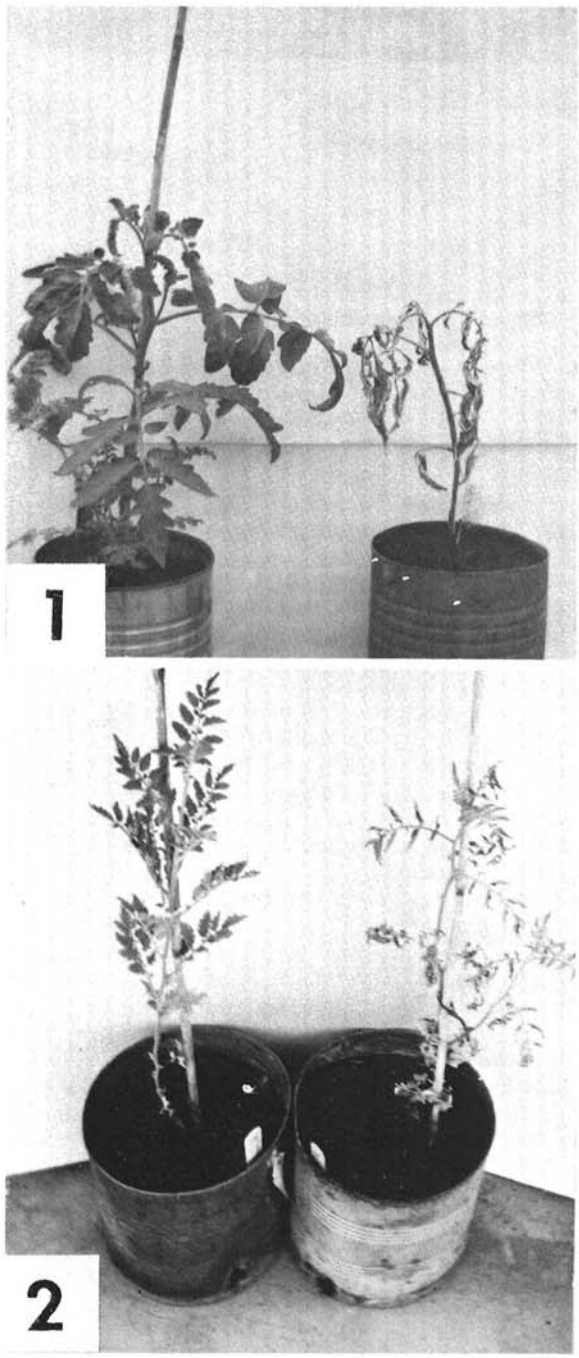

Fig. 1-2. 1) Comparison between diseased grafted *Lycopersicon esculentum-dentatum* (left) and diseased, necrotic *L. esculentum* control (right) plants about 1 month following simultaneous inoculation. **2)** Comparison between diseased grafted *dentatum-L. esculentum* (right) and inoculated *dentatum* control (left) plants about 1 month following simultaneous inoculation. Leaf curling on the grafted plant is due to necrosis.

severe symptoms typically occurred 7 to 10 days after the initial symptoms appeared. In contrast, the grafted plants resisted general yellowing except that which occasionally began in young apical leaves. They assumed a distinctly darker-green color than is normal for healthy plants. The abnormal color intensity occurred uniformly throughout the leaves and stems of each diseased susceptible-resistant grafted plant with the occasional exception of a somewhat lighter color at the plant apex. The grafted plants remained in a static, dark-green condition accompanied by leaf stiffening, contortion, epinasty, and vein thickening comparable with diseased control plants. All or most of the control plants became yellow and died while the grafted plants were still green (Fig. 1).

During an interval of 7 to 14 days after most control plants were dead, yellowing began to occur in some grafted plants. This yellowing was not typical of that which occurred in the infected control plants. Instead of yellowing occurring in the entire plant, this symptom occurred in each diseased grafted plant in distinctly delimited portions independently of the remainder of the plant. Thus, the yellow portions contrasted sharply with the dark-green portions, which showed no tendency toward yellowing. Over-all longevity of diseased grafted plants was therefore greatly extended as compared with the life span of the control plants. In some cases, portions of grafted plants remained alive, although in a static, moribund condition, for 2 months after the death of all control plants. No diseased grafted plant recovered from the disease, however.

No diseased grafted plant failed to become abnormally dark green and to express the symptoms of growth cessation, vein clearing, leaf contortion, epinasty, and eventual yellowing and death. The presence or absence of developing shoots from lower, resistant stem portions apparently had no effect upon these symptoms. In no case was the symptom development of a control plant typical of that of the diseased grafted plants. A definite contrast in symptom expression was therefore evident between the treatments, with each plant falling clearly into one of the two groups. No diseased grafted plant developed yellowing prior to, or simultaneously with, yellowing in a control plant. All of the inoculated control plants were dead of curly top before the death of any grafted plant.

Inoculated resistant-susceptible grafted plants demonstrated a reaction opposite to that of inoculated susceptible-resistant grafts. Yellowing typical of curly top in *L. esculentum* occurred in the *L. peruvianum* var. *dentatum* scions, and led to the deaths of these portions. Other external symptoms, including vein clearing, vein thickening, and leaf contortion and stiffening, did not accompany yellowing, however (Fig. 2). No symptoms were noted in either the nongrafted inoculated *L. peruvianum* var. *dentatum* controls or the noninoculated resistant-susceptible controls.

Anatomical symptoms.—Extensive phloem hypertrophy, hyperplasia, and some necrosis were found in stem and petiole sections of scions of diseased susceptible-resistant grafted plants which were dark green at the time of tissue collection. The phloem abnormalities observed in diseased grafted plants were comparable in severity with those observed in nongrafted diseased *L. esculentum* controls. Phloem of healthy nongrafted *L. esculentum* controls was normal.

Phloem of diseased (yellow) *L. peruvianum* var. *dentatum* scions of resistant-susceptible grafted plants was normal as compared with that of inoculated nongrafted and noninoculated *L. peruvianum* var. *dentatum* control plants.

Quantitative chlorophyll assay.—Total chlorophyll in 2 g of healthy control leaf tissue was found to be 0.53 mg/g fresh wt as compared with 0.88 mg/g fresh wt in dark-green leaves of diseased scions of susceptible-resistant grafts. This represents an increase of ca. 65% above normal chlorophyll concentration.

DISCUSSION.—Although symptomatology in resistant or susceptible scions was definitely influenced by the resistant or susceptible lower stems and roots of grafted plants, general resistance or susceptibility was not conferred by the lower portions. Whereas chlorophyll in susceptible-resistant grafts was not only maintained, but increased considerably in concentration, other external and anatomical symptoms were inhibited little, if at all. The diseased resistant-susceptible grafts exhibited the opposite effect in the development of yellowing in *L. peruvianum* var. *dentatum* scions with an absence of other overt and anatomical symptoms. Curly top-induced chlorophyll degeneration in nongrafted *L. peruvianum* var. *dentatum* plants of size comparable with that of the scions tested in this experiment has not been reported previously.

Symptoms of curly top were therefore separated into two categories: (i) yellowing, which appeared to be influenced specifically by the lower stems and roots; and (ii) the morphological and anatomical symptoms (including vein clearing) which appeared to be influenced completely by resistance or susceptibility on a local level. Resistance to curly top in *L. peruvianum* var. *dentatum* therefore probably is caused by at least two separately operating factors that may occur quite independently of each other. Since phloem abnormalities were observed in conjunction with the vein clearing, leaf contortion, and leaf-stiffening symptoms in green diseased susceptible-resistant grafts, these external symptoms may originate directly from internal disruptions, as some workers have suggested (5, 6, 8, 11). If such a cause-and-effect relationship does not exist (12, 16, 17, 18), both external and internal symptoms probably belong to the same symptom complex, and are etiologically closely related.

The significant stimulation of chlorophyll production or active chlorophyll accumulation in diseased susceptible-resistant grafts indicates the actual presence of a factor in roots of plants of the resistant varieties tested which stimulates chlorophyll production. The alternative hypothesis, therefore, that a factor exists in diseased susceptible roots which

causes chlorophyll degeneration, does not appear likely.

The factor which influences chlorophyll maintenance appeared to be transportable, and was evidently not dependent upon resistant photosynthetic shoots for production. Susceptible scions, however, may have furnished the necessary metabolites for production of this substance. The failure of abnormal concentrations of chlorophyll to accumulate in inoculated nongrafted *L. peruvianum* var. *dentatum* plants may result from differences in virus titer between resistant plants and the susceptible portions of grafted plants. High virus concentrations in susceptible scions may have directly stimulated production of the suggested chlorophyll-affecting substance, or may have acted indirectly through possible metabolic imbalances resulting from phloem disruption.

Yellowing, which eventually did occur in isolated portions of diseased susceptible-resistant grafted plants, appeared to take place on local levels and may have resulted from disruptions in vascular tissue proximal to affected areas. The transport of the suggested chlorophyll-affecting factor, as well as other substances, may therefore have been inhibited, allowing chlorophyll degeneration to proceed.

To positively establish the existence of the indicated factor responsible for chlorophyll maintenance in inoculated plants of the *L. peruvianum* varieties tested, further investigations should be directed toward isolation and characterization of this substance.

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