

# Sulfate Uptake and Translocation in Curly Top Infected Tomatoes

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## ABSTRACT

Sulfate uptake by curly top virus-infected whole tomato plants or their excised roots was decreased 32-61% compared to healthy plants or roots. Translocation to the aboveground parts was also decreased. The possibility that the differences in uptake were due to different root microfloras was determined to be unlikely. Decreased uptake was found over the concentration range from  $10^{-6}$  M to  $8 \times 10^{-3}$  M. The

uptake isotherms were typically multiphasic in both healthy and diseased plant roots, and the number of phases was similar in both. Leakage of sulfate from the roots was also unaffected by infection. These and earlier results support the conclusion that curly top virus infection altered differential membrane permeability.

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*Additional key words:* *Lycopersicon esculentum*, differential permeability.

As discussed in a number of reports, curly top virus (CTV) causes on tomato a variety of growth disturbances: upward leafrolling and epinasty; shortening, thickening, and plagionastic bending of the roots; root hair proliferation; and general retardation of growth (6, 8, 14). Previous studies have suggested that these morphological derangements are probably the visible signs of physiological disturbances occurring in CTV-infected plants. It has been determined, for example, that the level of auxin and the rate of its transport in the stem are reduced in diseased plants (18, 19). The kinetics of carbohydrate turnover in the leaves and carbohydrate transport in petioles and stems of CTV-infected plants are reduced as a result of infection (16, 17).

Many CTV symptoms, both on the roots and on the above-ground part, appear on healthy plants after treatment with appropriate levels of ethylene (8), leading to the conclusion that increased production of ethylene by the infected plant is one of the causes of morphological aberrations.

In view of the morphological changes taking place in the roots, and considering the general retardation of growth, we had previously studied the impact of CTV infection on the root uptake mechanisms. We have already demonstrated that the uptake of phosphate by tomato plants or excised roots from solutions containing 0.1 mM phosphate was decreased as a result of infection (6). By contrast, calcium uptake was increased (15). Transport to the stem and leaves was decreased in both cases. Other differences between healthy and diseased plants included the formation of a new (or enlargement of a small pre-existing) pool which received that part of the absorbed calcium that was diverted away from upward transport (15), an altered uptake profile along the root (6), and some quantitative alterations in the adaptive response of the root system to experimental deprivation or oversupply of phosphorus (6).

To gain additional information on the impact of CTV infection upon disease physiology and inorganic nutrition, the uptake and translocation of sulfate was examined. Attention was paid also to the uptake isotherm

and to the leakage of sulfate from the roots to learn about the general condition of the root-cell membrane(s), and to the microflora of healthy and diseased plant roots since it can influence uptake (2), and since it had not been examined in our previous studies.

**MATERIALS AND METHODS.**—Tomato seedlings (cultivar VF145), grown in pots containing U.C. mix (1) were inoculated with CTV strain II (7) when 20 to 27 days old by means of the leafhopper vector [*Circulifer tenellus* (Baker)]. The original source of inoculum and the methods of rearing the insects and inoculating the seedlings have been described in detail previously (6, 15). The day following the inoculations the seedlings were removed from the pots, cleaned, and placed in fog-buckets in the greenhouse (8). The nutrient solution used to grow the plants was a modified Hoagland's solution (9) containing 0.1 of the nominal concentrations of its constituents with the exception of  $\text{KNO}_3$  and Fe-chelate which were added at 0.2 and 0.075, respectively, of the prescribed amounts (8, 15).

Periodic determination of root microbial population was made by grinding approximately 0.4 to 1.0 g of fresh roots in a sterile mortar and plating on King B medium (10). The population in the nutrient solution was also determined similarly. Diseased plant roots were used for the experiments 10 to 15 days from inoculation; i.e., approximately one week after symptoms appeared on the roots and on the foliage. Although showing typical curly top symptoms, they were comparable in color to healthy roots and had no visible sign of senescence or degeneration at the time of use, since such changes began usually after the third week from inoculation.

For experiments involving intact plants, and unless otherwise indicated, one day prior to the uptake trials the plants were transferred from the buckets to Erlenmeyer flasks containing nutrient solution and kept in a growth chamber at  $25 \pm 1$  C. Light was provided by fluorescent lamps 4,304-5,380 lx (400-500 ft-c) for 14 hours per day. Air filtered through glass wool was continuously bubbled through the nutrient solution. In all other experiments, plants were taken directly from the fog-buckets and the

TABLE 1. Rate of sulfate uptake by roots of intact plants or by excised roots

Exp. no.	Material used	Uptake (external concentration 0.2 mM SO <sub>4</sub> ) (nmoles/mg dry wt·hr)	
		Healthy	Diseased
1	Excised roots	0.64	0.50
2	Excised roots	1.10	0.55
3	Excised roots	1.80	1.30
4	Intact plants	2.20	1.10
5	Intact plants	3.0	1.80
6	Intact plants	0.88	0.34

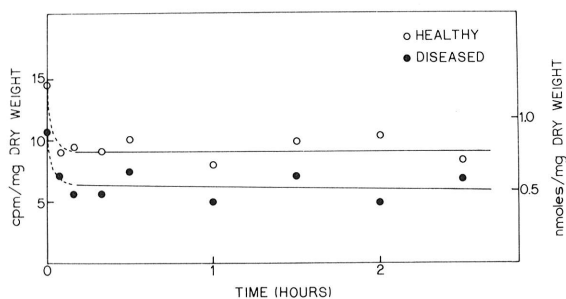


Fig. 1. Washout of "free space" <sup>35</sup>S activity from excised roots of healthy and curly top virus (CTV)-infected tomatoes. Uptake was for 0.5 hour from modified Hoagland's solution (0.2 mM sulfate, pH 5.4, 24 C). The zero-time samples were merely blotted with paper towels; all other samples were washed in modified Hoagland's solution for the time indicated.

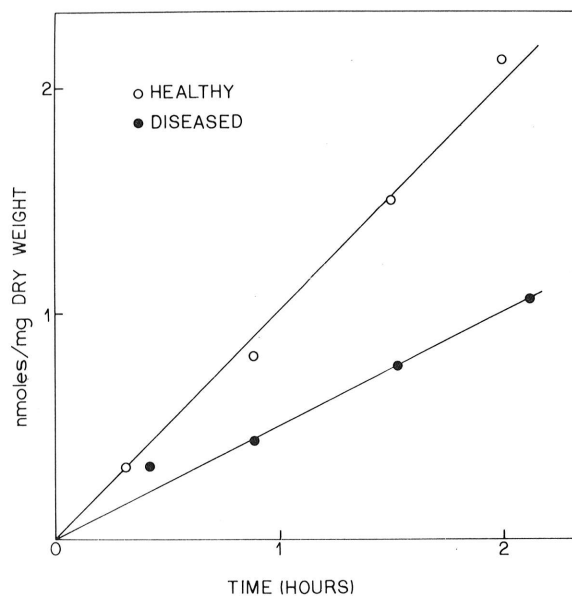


Fig. 2. Time course of sulfate uptake by excised roots of healthy and curly top virus (CTV)-infected tomatoes. Uptake conditions the same as in Fig. 1.

roots were excised shortly before use. The uptake medium consisted of nutrient solution (0.2 mM in sulfate) containing 0.02 to 0.05 mCi/ml Na<sub>2</sub><sup>35</sup>SO<sub>4</sub>. The pH of the uptake medium was 5.3 to 5.6; i.e., the same as that of the solution in which the plants were grown, and did not change during uptake. The optimum pH for uptake was not determined. Upon completion of uptake (0.3- 6 hours), individual plants were removed from the radioactive solution, the roots were blotted on absorbent paper towels, and rinsed in three changes (each approximately 200 ml) of nutrient solution, for a total of 15-20 min to remove free space radioactivity. The concentration of <sup>35</sup>S in the desorption solution relative to that in the root tissue, calculated from actual measurements made on both, never exceeded 1:600, so that external <sup>35</sup>S did not interfere with desorption. Desorption experiments (Fig. 1) showed that this treatment removed all freely exchanged <sup>35</sup>S.

In translocation experiments the plants were separated into roots and above-ground parts following this washing procedure. The different samples were dried overnight at 90 C and ground in a mortar. Green samples were decolorized with sodium hypochlorite before counting (15).

The rate of uptake by excised roots was studied at room temperature (23-25 C). For the determination of the uptake isotherm, the temperature was maintained at 25 ± 1 C by means of a water bath. The range of sulfate concentrations employed varied between experiments but was generally in the range of 1 μM to 8 mM. Individual samples consisted of 5 to 15 mg (dry weight) of excised roots. These were washed in one change of non-radioactive nutrient solution for 15 to 20 minutes upon termination of uptake, and dried as above prior to the measurement of their radioactivity.

Efflux experiments were performed by allowing excised roots to take up <sup>35</sup>S from the radioactive solution for the desired amount of time. Individual root samples were then blotted on paper towels and placed in flasks containing 200 ml of nonradioactive Hoagland's solution. Root samples were removed at various times, blotted on paper towels, and dried as previously described. The desorption solution was changed at each sampling. As already stated, the concentration of <sup>35</sup>S in the solution relative to that in the root never exceeded 1:600, so that external <sup>35</sup>S did not interfere with desorption. Radioactivity was determined in a liquid scintillation spectrometer (Packard Tri-Carb Model 2002) using a scintillation fluid consisting of 0.3 g 1,4-bis-2(5-phenyl-oxazolyl)-benzene and 5.0 g of 2,5-diphenyloxazole per liter of toluene. Cab-O-Sil was added to the scintillation fluid to make a 5.0% thixotropic gel (15) prior to determining the radioactivity of the ground samples. Uptake was expressed on mg dry weight basis, since the dry weight per fresh weight ratio was similar in healthy and diseased roots at this stage of growth (0.147 to 0.149).

RESULTS.—*Microbial population of roots.*—The population of bacteria in the nutrient solution of the fog-bucket was 1 × 10<sup>5</sup> to 2.4 × 10<sup>5</sup> cells/ml. On the roots the number of bacteria varied from 3.0 × 10<sup>6</sup> to 1.1 × 10<sup>8</sup>/g of fresh roots, but no attempts were made to characterize these bacteria other than to notice the presence among them of approximately 5-20% of fluorescent

Pseudomonads. No appreciable differences between healthy and diseased roots were observed. In the experiment showing the highest bacterial count, healthy roots had  $1.1 \times 10^8$  cells/g whereas diseased roots had  $0.95 \times 10^8$  cells/g. In another experiment healthy and diseased roots had  $2.0 \times 10^7$  and  $2.1 \times 10^7$  cells/g roots, respectively. These results differ, therefore, from those of other workers (4) who found increased microbial population in the rhizosphere of virus-infected peas. The lack of any significant difference in our case is not surprising since both healthy and diseased plants were grown in the same fog-bucket and bacteria were continuously washed away from the roots, and brought back on them with the mist. Only occasional fungal colonies were observed on the plates, indicating that the number of fungal propagules on the roots was 250- to 600-fold lower than the bacterial population.

**Sulfate uptake and translocation.**—The linearity of sulfate uptake by roots of intact plants as well as by excised roots during the first few hours is established by the results shown in Fig. 2 and 3. These data also show that CTV-infected plants and excised roots absorbed less sulfate compared to healthy plants and roots. The decrease in uptake varied from 22-61% (Table 1). The assertion that these results measure true uptake rather than passive diffusional influx is based on the lack of any measurable leakage of absorbed tracer from the roots (Fig. 3), excluding of course the initial exchange of "free space" material, as discussed later. If there was free diffusion across a damaged plasmalemma and/or tonoplast, and since the ratio of  $^{35}\text{S}$  in the root to that in the desorption solution was kept very high ( $>600$ ), we would expect extensive loss of radioactivity in the pulse-chase experiment. The accumulation ratio (internal to external  $^{35}\text{S}$ ) in these experiments was 3.2 to 11.6, but no attempt was made to determine the distribution of radioactivity into various compounds.

Some authors[see review by Barber (2)] have reported that positive or negative effects of the bacterial flora of aseptically grown roots to the measured uptake can be significant. The differences found here, however, could not have been caused by a direct participation or by indirect effects of the root microbial population in the absorption, since there were no differences, either quantitative or qualitative, in the microflora of healthy and CTV-infected tomato roots.

Translocation of radioactivity to the above-ground parts was also decreased by CTV infection (Fig. 3). In diseased plants proportionally less radioactivity was translocated to the leaves than in healthy plants (only 21% compared to 39% in 6 hours). Although our data did not permit determinations of translocation rates, they show that decreased uptake by the diseased plant roots accounts only in part for the lower radioactivity level in the diseased plant leaves.

**Uptake isotherm.**—The effect of disease on uptake at concentrations above and below  $2 \times 10^{-4}$  M was studied in a series of experiments using overlapping concentration ranges from  $10^{-6}$  M to  $8 \times 10^{-3}$  M. Uptake was reduced through the entire range (Fig. 4). The isotherms were clearly multiphasic; i.e., they exhibited sudden increases in uptake for relatively small increases in concentration at certain sulfate concentrations. Double reciprocal plotting

(not shown here) confirmed the existence of the phases. Multiphasic isotherms are characteristic of sulfate uptake by plant roots (11, 13). Broadly speaking, the different transition points separating consecutive phases were within a relatively narrow range of concentrations in both healthy and diseased plants and in different experiments. Furthermore, the number of phases was the same in both cases. Thus, CTV infection did not alter the basic features of the sulfate uptake mechanism but decreased its efficiency throughout the range of concentrations tested by approximately the same proportion.

**Efflux of  $^{35}\text{S}$  from the roots.**—Some virus diseases cause increased leakage of various substances from the roots (4). Accordingly, one could argue that reduced

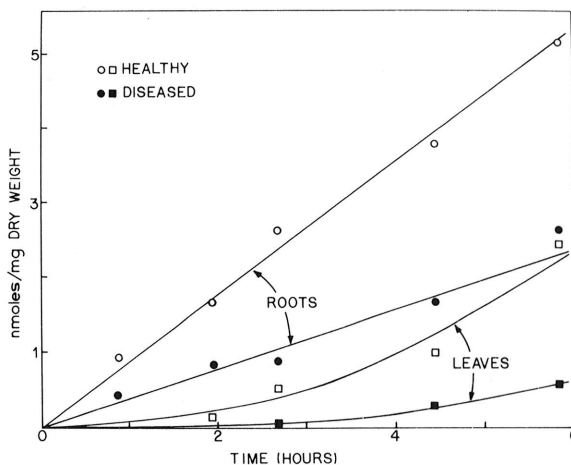


Fig. 3. Time course of sulfate uptake and translocation in intact healthy and curly top virus (CTV)-infected tomatoes. Uptake conditions as in Fig. 1 except that pH was 5.6.

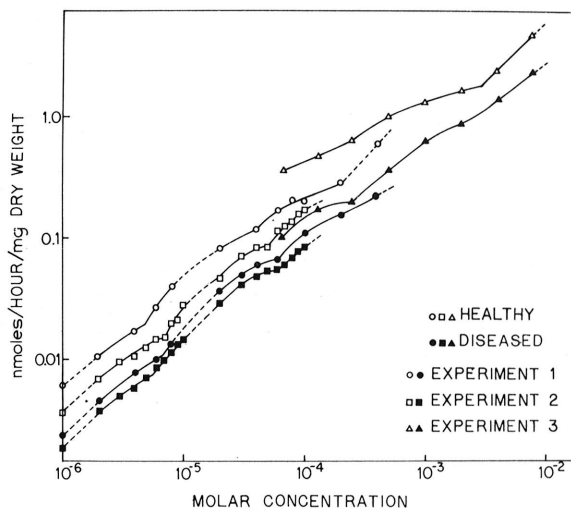


Fig. 4. Sulfate uptake isotherms in excised roots of healthy and curly top virus (CTV)-infected tomatoes. Uptake conditions as in Fig. 3. Three different experiments are shown covering the concentration range from 1.0  $\mu\text{M}$  to 8.0 mM. Five different phases are present in the isotherms of both types of roots.

uptake rates for sulfate could reflect increased leakiness of the ion from the diseased roots, since these were washed for 15 minute following uptake. This was examined by following the loss of  $^{35}\text{S}$  from healthy and diseased roots (Fig. 1). After a 30 minute pulse  $^{35}\text{S}$  activity was chased from the roots in a manner suggesting a rapid initial "free space" exchange [apparent  $t_{1/2}$  approximately 2-3 minutes (Fig. 1)] and no further tracer leakage from the roots into the medium for at least 2.5 hours. Differential leakage, therefore, could not have caused the differences in uptake found in our studies.

**DISCUSSION.**—Curly top-affected tomatoes absorbed significantly less sulfate than did healthy tomatoes. This was true both for whole plants and for excised roots indicating that decreased uptake was not caused by decreased transpiration which characterizes curly top infected tomatoes (Panopoulos and Gold, *unpublished*). Since decreased uptake could not be attributed to increased leakage of sulfate from the roots or to effects of the unaffected root microbial population, it must be attributed to other causes. Decreased translocation to the leaves could have been caused to some extent by the lower transpiration rates (3).

Leakage of sulfate from the roots was not affected by CTV infection. Similarly unaffected was reported to be the leakage of phosphate (14) which, like sulfate, exchanged very little in the healthy roots, and the leakage of calcium which showed extensive exchange in the healthy roots (15). These results differ from those of other investigators who reported increased leakage of organic substances and electrolytes from other virus-infected plant roots (4). Regardless of the reason for these different results, the lack of effect of curly top disease on root leakiness for these three ions and the opposite changes found in sulfate and phosphate uptake on one hand and calcium on the other hand suggest that diseased roots display an altered differential permeability but show no signs of gross membrane damage. The latter conclusion is further supported by the multiphasic nature of the sulfate uptake isotherm if we accept the most current views about the origin of the phases in sulfate uptake (5, 11, 12, 13).

Assuming that the curly top agent is a virus, our present and previous studies (6, 15) provide direct demonstration of alterations in the differential permeability of plant roots to inorganic nutrients caused by a virus disease. This opinion was expressed earlier by Wynd (20) for virus diseases in general based on compositional data obtained by various workers for virus-infected plants.

Translocation of sulfate, phosphate, and calcium to the above-ground parts was decreased (6, 15, and present study). These findings also confirm the conclusion (20) that upward transport of inorganic substances in general in virus-infected plants is impaired.

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