# Uptake and Translocation of Phosphorus in Healthy and Curly Top-Diseased Tomatoes

G. Faccioli, N. J. Panopoulos, and A. H. Gold

Professor at the Istituto di Patologia Vegetale, Universitá di Bologna, 40126 Bologna, Italy; Assistant Research Plant Pathologist, and Professor, Department of Plant Pathology, University of California, Berkeley 94720, respectively.

Supported in part by USDA Cooperative Agreement No. 12-12-100-8456 (34). The senior author was supported by Grant No. 115.0509.05154 of Consiglio Nazionale delle Ricerche, Roma, Italy.

The authors thank James Duffus, USDA Experiment Station, Salinas, Calif., for leafhopper colonies and virus inoculum, and A. R. Weinhold for helpful suggestions during the preparation of the manuscript.

Accepted for publication 7 December 1971.

## ABSTRACT

The effect of curly top virus (CTV) infection on the uptake and translocation of phosphate by tomato plants was studied using $^{32}P$ as a tracer. In both excised roots and intact plants, the linear rates of uptake during the first 2 hr were significantly lower in CTV-infected than in healthy material. There was a positive correlation between increase in symptom severity and decrease in the linear rate. The distribution of radioactivity along the root axis after a 45-min uptake differed between the two types of roots, and was closely related to their over-all morphology and to the longitudinal distribution of root hairs. Uptake of $^{32}P$ exhibited a higher temperature coefficient ($Q_{10}$) in diseased than in healthy roots.

In intact plants, the root radioactivity increased monoexponentially, and approached a saturation level in about 6 hr. The calculated turnover rate of the "mobile" phosphate pool in the root was higher in healthy plants (0.56 $hr^{-1}$) than in diseased (0.34 $hr^{-1}$).

Phytopathology 62:524-529.

*Additional key words: Lycopersicum esculentum.*

Analysis of virus-diseased plant material often shows drastic changes in mineral composition accompanied by an over-all lessening of the ash content of the aboveground plant parts (8, 14, 16, 17). It has been concluded from such findings that the differential permeability of the cell membrane must be drastically altered by virus infection, and upward transport of the various nutrients in the diseased plant must be defective (17). Thus far, however, no kinetic investigations aimed at a more direct verification of the above conclusions have been reported. To our knowledge, only one direct investigation of uptake and translocation of an anionic inorganic nutrient for virus-diseased plants has been undertaken (4). We have already reported (11) a kinetic analysis of the impact of curly top (CTV) infection on the uptake and translocation of a cation ($^{45}Ca$). In that case, we were able to demonstrate that diseased tomato plants had a significantly higher uptake rate and a lower translocation rate than did healthy plants. The present study has been undertaken to investigate the effect of CTV infection on the uptake and translocation of an anionic nutrient, phosphate.

MATERIALS AND METHODS.—*Plant material.*—Experiments were performed with tomato seedlings (*Lycopersicum esculentum* Mill. 'VF-145') grown in 12.5-cm pots containing U.C. mix (1). Plants kept in a smog-filtered greenhouse were inoculated with curly top virus [CTV, Gidding's strain 11 (5)] 20 to 25 days after seeding, with the use of viruliferous leafhoppers (*Circulifer tenellus* [Baker]). Seedlings were removed from the pots the day after the inoculation, and the roots thoroughly washed. The seedlings were then transferred to fog-cultures and grown in Hoagland's solution (7) modified as described previously (11). Typical root symptoms appeared in about 1 week. Since the experiments were conducted over a period of several months, the plants exhibited a wide range of symptom severity and growth rates. Within each experiment, however, plants of comparable size, vigor, and symptom severity were compared.

One day prior to uptake, the plants were transferred from the fog-cultures to 250-ml Erlenmeyer flasks containing the above-mentioned nutrient solution. Plants were then placed in growth chambers under constant temperature (25 ± 1 C) and

light (400 to 500 ft-c for 14 hr) conditions. Glass wool-filtered air was constantly bubbled into the medium.

Intact roots and 2-cm root segments excised 1 to 3 hr prior to uptake were blotted on paper towels and placed in radioactive nutrient solutions in which carrier-free $Na_2H^{32}PO_4$ had been added to a level of 0.01 to 0.05 $\mu$c/ml. Plants and excised roots were kept in the growth chambers throughout the period of the experiment. The uptake medium was continuously aerated as before. The pH of the uptake solution was constant within each experiment (5.3 to 5.6).

*Influx experiments.*—One healthy and one diseased plant or five to six root pieces of each type were sampled after various feeding periods, blotted on paper towels, and washed in three 200-ml changes of nonradioactive standard nutrient solution for a total of 15 min. This last step was sufficient to remove nearly all of the readily exchangeable (external) radioactivity. Increase of either phosphate concentration (up to 10-fold) or washing time did not further improve removal of the external radioactivity (Fig. 1-E). Intact plants were immediately sectioned into roots, stem, and leaves plus petioles, and dried overnight at 90 C. Excised roots were similarly treated. The temperature coefficient ($Q_{10}$) was determined by measuring the rate of uptake by excised roots at 18, 23, and 28 C. For each five-degree interval, the $Q_{10}$ was calculated from the data by means of the equation:

$$Q_{10} = Q_n^{10/n}$$

where n is the temperature interval (18).

*Uptake profile of the roots.*—Roots were taken from plants which had been allowed to feed in radioactive solutions for varying periods of time, washed in nonradioactive nutrient solution for 10 to 15 min, dried, and sectioned into 1- to 3-mm pieces under a dissecting scope. To facilitate sectioning, individual roots were placed on double-faced scotch tape, which was then placed on a millimeter scale.

*Response to different levels of phosphate.*—Plants kept in the standard nutrient solution ($10^{-4}$ M phosphate) were transferred for 24 hr to nutrient solutions containing either $10^{-5}$ or $10^{-3}$ M phosphate. The roots were then taken, sectioned in 2-cm segments, and placed in either $H_2{}^{32}PO_4^-$ or $^{35}SO_4^{--}$ solutions with a total content of $10^{-4}$ phosphate and $2 \times 10^{-4}$ sulfate, respectively. The rate of uptake was determined after 1 hr.

*Radioactivity determinations.*—Except for excised root samples and small root segments, all radioactive plant parts were ground in mortars and decolorized with 5% sodium hypochlorite to eliminate color quenching during counting (11).

Radioactivity measurements were carried out in a liquid scintillation spectrometer (Packard Tri-Carb Model 2002), using a toluene-based scintillation liquid containing 0.3 g of 1,4-bis-2(5-phenyloxazolyl)-benzene and 5.0 g of 2,5-diphenyloxazole/liter. All powdered samples were suspended in a thixotropic scintillation gel prepared by adding Cab-O-Sil (colloidal silica) to the above scintillation solution to make a 5% gel (9, 11). This procedure was adopted to avoid errors due to the sedimentation of the samples in the vials and to reduce self-absorption. Samples containing sufficiently high $^{32}P$-activity were conveniently measured by means of the Cerenkov radiation (2, 12).

*Graphical analysis.*—Long-term uptake data were plotted on semilogarithmic coordinates and analyzed for rate determination. The "curve-peeling" or "backward projection" technique (11, 13) was applied.

RESULTS.—*Influx experiments.*—During the first 2 hr, the amount of $^{32}P$ taken up by excised roots increased linearly with time. Reduced rate of uptake was found in experiments employing excised roots (Fig. 1-F, Table 1). There was also a positive correlation between the decrease in isotope uptake and the increase in symptom severity (Fig. 1-F). In uptake experiments using intact plants, the root radioactivity increased linearly for the first 2 hr, but approached a saturation level in about 6 hr (Fig. 1-B). Radioactivity in stems and leaves, on the other hand, appeared only after a lag period, and continued to increase for more than 8 hr (Fig. 1-C, D). The amount of total radioactivity in the plant (Fig. 1-A, Table 1), as well as the $^{32}P$ content of individual plant parts, namely roots, stems, and leaves, per unit weight basis, were considerably lower in diseased plants than in healthy. In addition, the lag period for translocation to stems and leaves was longer in CTV-infected plants (ca. 40 min and 60 min, respectively) than in healthy plants (20 min and 30 min) (Fig. 1-C, D). The time-course of root radioactivity could be approximated by an equation of the form:

$$X = X_{max}(1-e^{-kt}),$$

X being the radioactivity at time t, $X_{max}$ the maximum radioactivity level reached at saturation, and k a first-order rate constant ($hr^{-1}$) (Fig. 1-G). The assumption can be made that the mobile root phosphate can be treated as a single compartment, and that the amount required for root growth during the uptake period is small compared to the amount present in the root and can, therefore, be neglected. The rate constant k will be in this case quasi-equivalent to the uptake as well as to the translocation rate. The values of k calculated according to Zilversmit et al. (19) were 0.56 $hr^{-1}$ and 0.34 $hr^{-1}$ for diseased and healthy plants, respectively. Since k has the units of inverse time, the above figures can be interpreted to mean that the mobile root phosphate pool renews itself in ca. 1.8 and 2.8 hr in healthy and diseased plants, respectively.

*Temperature coefficient ($Q_{10}$) determination.*—In the range of temperatures employed, the uptake of phosphate was more sensitive to temperature variations in diseased roots than in healthy. The $Q_{10}$ was constant between 18 and 28 C, and the values determined in four different experiments were between 2.5 and 2.8, and 2.0 and 2.4, for diseased and healthy roots, respectively.

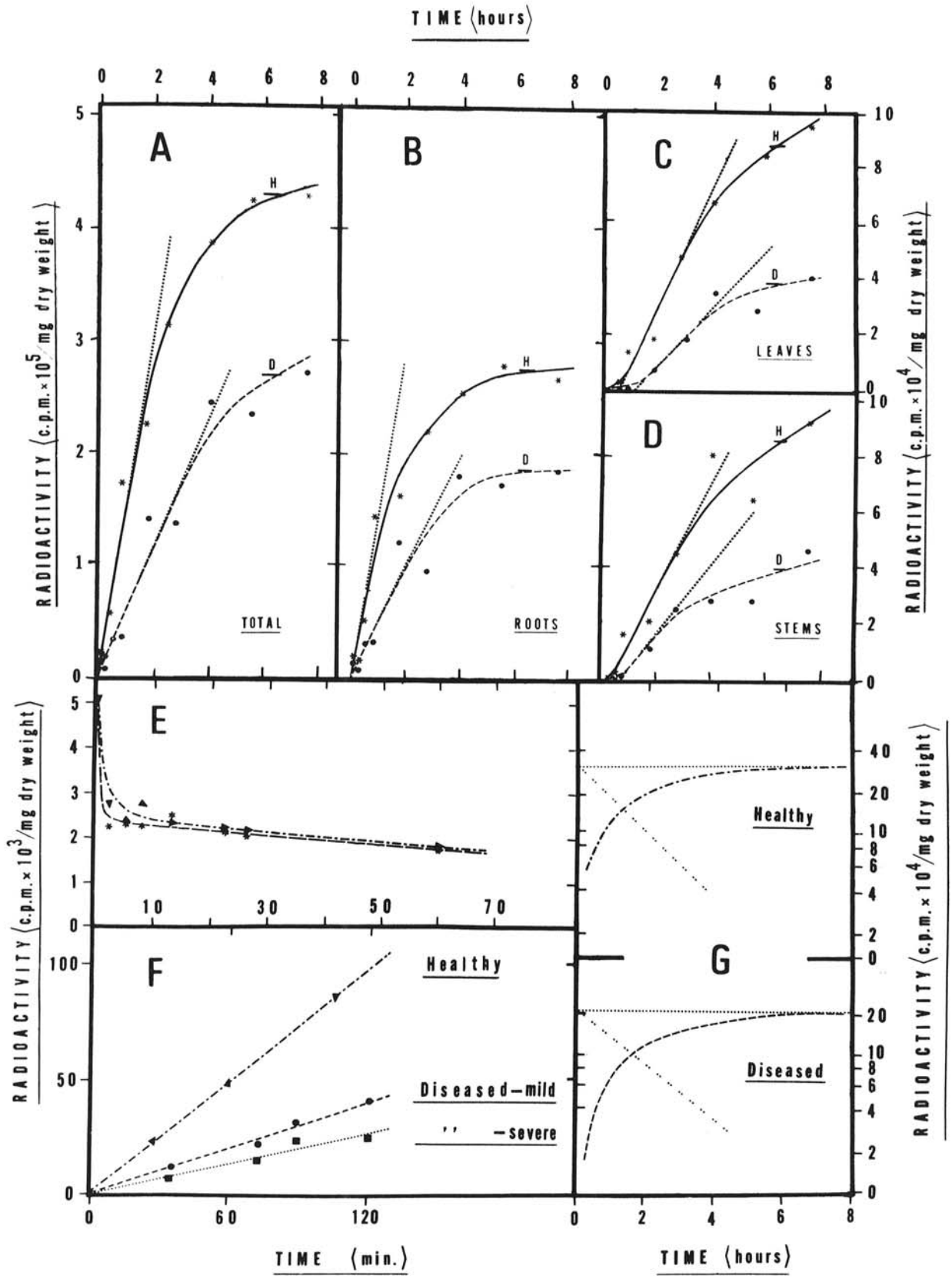
TIME (hours)
A
B
C
D
E
F
G
H
D
TOTAL
ROOTS
LEAVES
STEMS
RADIOACTIVITY (c.p.m.×10⁵/mg dry weight)
RADIOACTIVITY (c.p.m.×10⁴/mg dry weight)
RADIOACTIVITY (c.p.m.×10³/mg dry weight)
RADIOACTIVITY (c.p.m.×10⁴/mg dry weight)
Healthy
Diseased—mild
" —severe
Diseased
TIME (min.)
TIME (hours)

TABLE 1. Rate of uptake of $^{32}P$ by intact tomato plants or excised tomato roots from a solution containing $10^{-4}$ M phosphate concentration

| Exp. no. | Plant material used | Rate of uptake (cpm[a]/mg/hr) | |
|---|---|---|---|
| | | Healthy | Diseased |
| 1 | Excised roots | 1,100 | 300 |
| 2 | Excised roots | 1,600 | 460 |
| 3 | Excised roots | 1,700 | 730 |
| 4 | Intact plants | 5,000 | 1,400 |
| 5 | Intact plants | 8,300 | 1,400 |
| 6 | Intact plants | 12,000 | 5,600 |
| 7 | Intact plants | 26,000 | 10,000 |

[a]cpm = counts/min.

*Uptake profile along the root axis.*—After a 45-min uptake, profound differences were found in the longitudinal distribution of $^{32}P$ in healthy and CTV-infected roots (Fig. 2). Healthy roots showed high levels of radioactivity at the tip, followed by a characteristic region of low radioactivity at about 1 cm from the apex. Diseased roots showed a "peak" of radioactivity located at about 1 to 1.5 cm from the tip. These features appeared to be correlated to some extent with the over-all root morphology and root hair development. In healthy roots, the high surface volume ratio and the high meristematic activity at the root tip can probably account for the peak of radioactivity close to the apex. The subsequent drop can be explained by consideration of the decrease of the surface volume ratio (15) and the absence of root hairs in the first cm of root length. Diseased roots probably had a reduced meristematic activity at the tip (root elongation almost ceased after the symptoms appeared), as well as lower surface volume ratio [due to their thickening especially in the apical region (6)], as compared to the healthy. On the other hand, root hairs were already present at 2 to 4 mm from the apex, and were especially abundant in the 1- to 1.5-cm region. This can account for the location of the peak of radioactivity in this region.

*Response to different phosphate levels.*—Exposure to $10^{-5}$ M phosphate in the nutrient solution for 24 hr resulted in an enhancement of $^{32}P$ uptake by the roots (Table 2). Quantitatively speaking, the response was more pronounced in healthy roots (over 200% increase) than in diseased roots (20%). Reduced supply of phosphate did not, however, increase the ability of the roots to take up sulfate (Table 2). This indicates that the effect was probably ion-specific.

TABLE 2. Effects of exposure to different levels of phosphate on the uptake of $^{32}P$ and $^{35}S$ by healthy and CTV-infected tomato roots from $10^{-4}$ M phosphate and $2 \times 10^{-4}$ M sulfate concentrations

| Exp. no. | Ratio of the uptake rates: treated/control | | | | | |
|---|---|---|---|---|---|---|
| | Uptake of phosphate | | | | Uptake of sulfate | |
| | $P^-$/Pa | | $P^+$/Pa | | $P^-$/Pa | |
| | D[b] | H | D | H | D | H |
| 1 | 0.80 | 2.17 | 0.50 | 0.95 | 0.60 | 0.72 |
| 2 | 1.60 | 2.21 | 0.42 | 0.60 | | |
| Avg | 1.20 | 2.19 | 0.46 | 0.78 | | |

[a]$P^-$ = plants transferred from $10^{-4}$ to $10^{-5}$ M phosphate for 24 hr prior to uptake determination; P = control plants kept in $10^{-4}$ M phosphate throughout; $P^+$ = plants transferred from $10^{-4}$ to $10^{-3}$ M phosphate for 24 hr prior to uptake determination.

[b]D = diseased; H = healthy.

On the other hand, diseased roots exposed to $10^{-3}$ M phosphate for 24 hr showed a decrease in $^{32}P$ uptake, as compared to healthy (Table 2). The results indicate, therefore, that CTV infection also affected physiological and/or biochemical characteristics of ion transport presumably responsible for adaptation of the roots to a relative scarcity or oversupply of inorganic macronutrients in the soil.

DISCUSSION.—In contrast to the increased uptake of calcium by CTV-infected tomato plants and excised roots (11), a profound decrease in phosphate uptake was found. Translocation to the upper parts of the plant was, however, reduced in both cases.

It is possible that the reduced rates of phosphate uptake and translocation reflect the decrease in the growth rate caused by CTV (10). The differences observed here are unexpected from the point of view of virus synthesis per se in the plant. An increased demand for this element in virus synthesis, however, could have been easily counterbalanced by reduced requirements for growth in diseased plants. The actual amount of virus synthesized is not known in this case, due to the difficulties that have been encountered in the purification and quantitative assay of CTV-particles (A. H. Gold, *unpublished data*). It is unlikely, however, that the virus concentration in the plant is high.

The effects of virus infection on ion uptake by plants have not been extensively investigated. One

**Fig. 1-A, B, C, D.** Uptake and translocation of $^{32}P$ in healthy (H) and CTV-infected tomatoes (D). Initial rates of uptake are indicated in A and B by the tangents at the origin. Relative rates of translocation to the stem and leaves are shown in C and D by the tangents to the curves at the inflection points; the intercept of the tangents with the abscissa indicates the time-lag for translocation. **E)** Removal of "free space" $^{32}P$ activity from excised healthy roots of tomato by washing with: upper curve, modified Hoagland's solution ($10^{-4}$ M in phosphate, pH 5.8); lower curve, standard Hoagland's solution ($10^{-3}$ M in phosphate, pH 4.9). **F)** Kinetics of short-term uptake of $^{32}P$ by excised tomato roots from modified Hoagland's solution; decrease in the rate of uptake is correlated with symptom severity. **G)** Semilogarithmic plot and analysis of root $^{32}P$ data (smoothed) from B; saturation of the root "mobile" phosphate pool follows a single-exponential kinetics; cpm = counts/min.

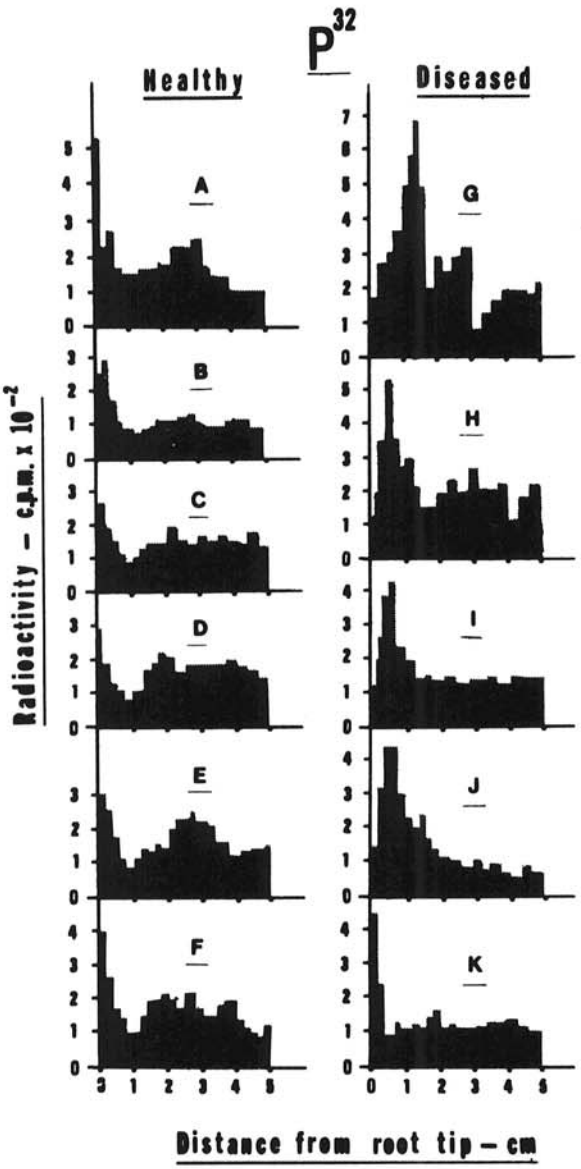


Fig. 2. Histogram representing the $^{32}$P-activity profile along individual roots of tomato following a 45 min uptake and brief wash (10 to 15 min) in modified Hoagland's solution. Note that root K has an uptake profile closely resembling those of healthy roots. This particular root was taken from an infected plant, but did not exhibit the characteristic thickening typical of the other diseased roots (G through J). Such roots are frequent in infected plants at early stages of symptom development; their occurrence is, however, rare at later times, and, whenever found, they were excluded from the samples used in uptake experiments. cpm = counts/min.

report, however, indicated that spotted wilt virus infection also decreased $^{32}$P uptake in tomatoes (4). In addition, there are reports of lower levels of phosphorus in plants for several other virus-host combinations (8, 14, 16, 17).

As to the nature of the physiological and biochemical defects underlying the depression of phosphate uptake by CTV-infected roots, some conclusions can be drawn from our data. The higher $Q_{10}$ for phosphate uptake, for instance, indicates a physical or metabolic defect in phosphate transport. Defects of this nature have been implicated for several enzymes present in virus-infected plants (3).

The results of our experiments involving low and high levels of phosphate are particularly interesting for two reasons. Firstly, they demonstrated a decreased ability of the infected root system to adjust itself to the relative availability of nutrients in the root environment. This could be significant for the growth of the infected plant under conditions in which growth is limited by a particular nutrient. Secondly, the plasticity of the ion transport capacity to external manipulation of nutrient concentration is a potentially useful tool in studying the transport system itself.

We have so far demonstrated that CTV infection affected the uptake of calcium and phosphorus by the plant differently. It would be interesting to determine whether the uptake of other cations and anions is influenced in the same way. To this purpose, experiments on $^{35}$S uptake by CTV-infected plants are currently in progress.

## LITERATURE CITED

1. BAKER, K. F. [ed.]. 1957. The U.C. system for producing healthy container-grown plants. Calif. Agr. Exp. Sta. Manual 23. 332 p.
2. ELRICK, R. H., & R. P. PARKER. 1967. The use of Cerenkov radiation in the measurement of β-emitting radionuclides. Int. J. Appl. Radiat. Isotopes 18:263-271.
3. ESANU, V. 1967. Activation energy of some enzymes as influenced by plant pathogen viruses and fungi, p. 161-163. *In* T. Hirai, Z. Hidaka, & I. Uritani [ed.]. Biochemical regulation in diseased plants or injury. Kyoritsu Printing Co., Ltd., Tokyo, Japan.
4. GALDAS, A. R., O. J. CROCOMO, A. B. GUSMAN, & L. O. BASSO. 1968. Influência do virus vira cabeça na adsorçao e transloçacão de fosforo en plantas de tomate. Ôyton 25:29-34.
5. GIDDINGS, N. J. 1954. Two recently isolated strains of curly top virus. Phtyopathology 44:123-125.
6. GOLD, A. H., & G. FACCIOLI. 1968. Relationships of ethylene to curly top symptoms. Phytopathology 58:1052 (Abstr.).
7. HOAGLAND, D. R., & D. I. ARNON. 1938. The water culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ. 347. 39 p.
8. HOLDEN, M., & M. V. TRACEY. 1948. Effect of infection with tobacco mosaic virus on the levels of nitrogen, phosphorus, protease and pectase in tobacco leaves and on their response to fertilizers. Biochem. J. 43:151.
9. LERCH, P., & M. COSANDEY. 1966. The comparison of different procedures to measure simultaneously $^{32}$P and $^{45}$Ca in inorganic salts by liquid scintillation counting. New England Corp. Bull. 52:1-8.
10. PANOPOULOS, N. J. 1971. Physiological effects of curly top virus infection on tomato plants. Ph.D. Thesis. Univ. Calif., Berkeley. 167 p.
11. PANOPOULOS, N. J., G. FACCIOLI, & A. H. GOLD. 1972. Effect of curly top disease on uptake,

transport, and compartmentation of calcium. Phytopathology 62:43-49.
12. PARKER, R. P., & R. H. ELRICK. 1966. The assay of $\beta$-emitting radioisotopes using Cerenkov counting. Int. J. Appl. Radiat. Isotopes 17:361-362.
13. RIGGS, D. S. 1963. The mathematical approach to physiological problems. The Williams & Wilkins Co., Baltimore, Md. 445 p.
14. RYZHKOV, V. L., & M. W. VOROBJEVA. 1942. Phosphate distribution in the leaves of healthy and mosaic-infected tobacco. Biokhimiya 7:79.
15. SCOTT, B. I., HEATHER GULLINE, & C. K. PALLAGHY. 1968. The electrochemical state of cells of broad bean roots. I. Investigations of elongating roots of young seedlings. Australian J. Biol. Sci. 21:185-200.
16. VAYONIS, G. C. 1954. Phosphorus disturbances in mosaic-virus infected tobacco plants. Physiol. Plantarum 7:687-699.
17. WYND, L. F. 1943. Metabolic phenomena associated with virus infection in plants. Bot. Rev. 9:395-465.
18. YARWOOD, C. E. 1965. Temperature and plant disease. World Rev. Pest Control 4:53-63.
19. ZILVERSMIT, D. B., C. ENTENMAN, & M. C. FISHER. 1943. On the calculation of the turnover time and turnover rate from experiments involving the use of labelling agents. J. Gen. Physiol. 26:325-331.