

*Some Effects of Host Nutrition
on Symptoms of Exocortis*

VIRUS SYNTHESIS in plants results from a divergence of the cellular metabolism of the host, since viruses do not have the necessary means for their own replication. Virus nucleic-acid diverts the metabolic scheme of the cell from the synthesis of normal cellular constituents to that of virus nucleic-acid and virus protein which are subsequently assembled into the virus nucleoprotein. Nitrogen constitutes a considerable portion of the mass of the plant viruses and phosphorus is an important component of nucleotides. It is supposed, therefore, that deficiencies and excesses of these elements can affect virus directly by altering the supply of required building materials, and indirectly by upsetting vital functions of the cell related to protein and nucleic-acid synthesis.

This paper reports effects of graded amounts of nitrogen and phosphorus on development of exocortis virus symptoms in certain citrus plants. A brief statement of preliminary results has already appeared (9).

Materials and Methods

Experiments were conducted in a greenhouse maintained at approximately 24°C. Plants were grown in white silica sand in 12-liter earthenware crocks equipped with a siphon similar to that described by Pryor (8). The siphons automatically drained any excess nutrient solution so that each sand culture had the same moisture level. At biweekly intervals each crock received a prescribed amount of nutrient solution under study.

Nutrient solutions formulated by Hoagland and Snyder (5) and modified by Cheo *et al.* (2) were used in these investigations. All nutrients except nitrogen and phosphorus were applied in amounts adequate for

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normal plant growth. Solutions were adjusted to a pH range of 6.0-7.0.

After the plants had shown a differential growth response to the treatments, they were bud-inoculated with exocortis virus, care being taken that all plants were comparably inoculated. Inoculations were made by inserting exocortis-infected buds into T-slits in the bark of test plants. There were usually six crocks of one plant each for each treatment. Two crocks of each treatment were left uninoculated to measure effects on growth.

Results

NITROGEN.—In two experiments with Eureka lemon [*Citrus limon* (L.) Burm.] on *Poncirus trifoliata* (L.) Raf. rootstocks, plants were grown at 50, 210, 630, and 1050 ppm nitrogen in otherwise identical nutrient solutions. Fresh weights of plants, excluding roots, were recorded 21 months after inoculation (Table 1). In these experiments, maximum growth was at 210 ppm. Plants at 630 and 1050 ppm showed the effects of excess nitrogen: leaves were dark green and plants were stunted. Plants at 50 ppm showed symptoms of nitrogen deficiency. Although mean total weights of infected plants were lower than uninfected ones at each nitrogen level, the relative growth response of infected trees was similar to that of non-infected plants at the differential nitrogen treatments.

Development of exocortis symptoms and ultimate symptom severity was directly correlated with increase of nitrogen (Table 1). Bark symptoms characteristic of exocortis developed in *P. trifoliata* rootstocks at 1050 ppm nitrogen, approximately 10 to 11 months after inoculation. At 630 ppm, symptoms developed 14 months after inoculation. Bark scaling symptoms did not appear in infected plants at the 210 and 50 ppm levels during the course of the experiment (21 months). However, even in the absence of scaling, plants at these two lower levels were noticeably more stunted than control plants, indicating that the virus likely was present but not inducing scaling.

In one experiment, when nitrogen levels were reversed 21 months after inoculation, i.e., plants at 50, 210, 630, and 1050 ppm were changed to 1050, 630, 210, and 50 ppm, respectively, the plants at the two lower levels developed exocortis symptoms within a few weeks.

Bark samples were removed from the *P. trifoliata* rootstocks at 12 and 18 months after inoculation, sectioned, and treated with phloroglucinol-HCl (3). Plants showing bark symptoms of exocortis all gave positive color reactions; those without symptoms all reacted negatively.

In further studies of nitrogen effects on exocortis, Palestine sweet

TABLE 1.—THE EFFECT OF INCREASING AMOUNTS OF NITROGEN ON HOST GROWTH AND DEVELOPMENT OF EXOCORTIS IN *P. TRI-FOLIATA* ROOTSTOCKS WITH EUREKA LEMON TOPS

Nitrogen Levels (ppm)	Nitrogen as Per Cent D.M. ^a		Mean Fresh Weight of Plants (g)		Mean Incubation Period (months) ^c	Reaction to Phloroglucinol - HCl ^d	
	C ^b	I	C	I		12 mo.	18 mo.
Experiment 1							
50	2.35	2.55	226	215	—	—	—
210	3.16	2.86	297	282	—	—	—
630	5.32	5.52	217	185	14	—	+
1050	5.90	6.37	205	153	10	+	+
Experiment 2							
50	4.90	4.90	351	339	—	—	—
210	5.80	6.00	490	473	—	—	—
630	9.20	8.80	340	267	14	—	+
1050	10.30	10.70	295	245	11	+	+

^aDetermined by micro-Kjeldahl, D.M.—Dry Matter.

^bC=Control, I=Infected.

^cPeriod between inoculation and appearance of symptoms.

^d+ = positive exocortis color reaction.

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lime (*C. limettioides* Tanaka) plants with Lisbon lemon tops were used as test plants. These studies were made with the following levels of nitrogen: 21, 70, 210, 630, and 1050 ppm. The relative growth response of diseased plants at the various nitrogen levels was similar to that of healthy plants. That is, a gradient of growth occurred, with the maximum growth at 210 ppm. Stunting occurred at the two highest levels.

Ultimate symptom severity and incubation period of the virus varied with the treatment. No definitive symptoms of exocortis appeared in plants at 21, 70, 210 ppm levels during the course of the experiments (18 months from inoculation). Lemon tops of the infected plants grown at 1050 ppm developed yellow blotches and cracking of the bark approximately 11 months after inoculation; and at 630 ppm, symptoms appeared in 13-15 months. Bark-cracking symptoms, indicative of exocortis in Palestine sweet lime (11), did not appear in any of the sweet lime rootstocks.

When healthy Etrog citron (*C. medica* L.) buds were inserted into the trunks of the lemons of the above-mentioned experiment and forced into growth, symptoms characteristic of exocortis appeared in the citron tops of all the exocortis-inoculated trees at all nitrogen levels. Symptoms appeared at the same time in all infected plants, and over a 3-month period no differences in symptom severity could be distinguished. Citron tops showed essentially the same growth response to the nitrogen levels as did the previous lemon tops. These results show that exocortis virus was present in the Lisbon lemon Palestine sweet lime plants growing at the deficient and optimum nitrogen levels without inducing discernible symptoms during the period these experiments were in progress.

PHOSPHORUS.—*P. trifoliata* rootstocks with Eureka lemon tops were grown at 7.5, 37, 237, and 547 ppm phosphorus in otherwise identical nutrient solutions. In these experiments, the greatest growth occurred at 37 ppm phosphorus. Some stunting occurred at higher levels, but was less marked than with nitrogen. Inoculated and uninoculated plants showed similar growth responses to the various levels of phosphorus (Table 2).

The results reveal that, in general, there is a positive relationship between high phosphorus and activity of exocortis virus in *P. trifoliata*. Plants at 547 ppm phosphorus developed bark-scaling symptoms of exocortis in the *P. trifoliata* rootstocks within 14 months after inoculation. Exocortis symptoms did not appear in plants at phosphorus levels below 547 ppm within 21 months after inoculation.

Bark samples removed from the rootstocks at 12 and 18 months after inoculation were sectioned and treated with phloroglucinol-HCl. Eight-

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TABLE 2.—THE EFFECT OF INCREASING AMOUNTS OF PHOSPHORUS ON HOST GROWTH AND DEVELOPMENT OF EXOCORTIS IN *P. TRIFOLIATA* ROOTSTOCKS WITH EUREKA LEMON TOPS

Phosphorus Levels (ppm)	Mean Fresh Weight of Plants (g)		Mean Incubation Period (Months) ^b	Reaction to Phloroglucinol-HCl ^c	
	C ^a	I		12 mo.	18 mo.
7.5	380	372	—	—	—
37	417	419	—	—	—
237	358	353	—	—	—
547	340	322	14	—	+

^aC=Control, I=Infected.

^bPeriod between inoculation and appearance of symptoms.

^c+ = positive exocortis color reaction.

een months after inoculation, plants at the highest level of phosphorus reacted positively; all others reacted negatively.

Discussion

It is evident from the tests herein reported that nutrition effects on symptoms of exocortis virus in citrus plants depend upon the specific host-virus complex, and that the virus does not always follow the same pattern. The activity of exocortis virus in *P. trifoliata* and Lisbon lemon tissue, as measured by reduction in the incubation period of the virus, and symptom severity, is greater at excess nitrogen levels, even though growth of the host was greatly reduced, than at optimum and lower levels. In citron, however, symptoms developed similarly at all levels of nitrogen studied. It is quite possible that, with a larger range of nitrogen, an optimum for development of exocortis symptoms in citron would be found, but the important point here is that unmistakable symptoms are produced over a considerable range of nitrogen levels and growth responses.

In experiments with phosphorus, exocortis virus activity in *P. trifoliata*, in general, coincided with the amount of phosphorus supplied the host, and not with the differential growth response of the host.

Even though bark scaling did not develop in infected *P. trifoliata* stocks at optimum and lower nitrogen levels, these plants were stunted. This suggests that stunting without the scaling and variable incubation periods in trees infected with exocortis virus may result from variation in environmental factors. It further suggests that severe stunting in *P. trifoliata* trees infected with exocortis virus is not a secondary symptom resulting from scaling of the bark, but is a symptom resulting from other

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virus effects on the host. Other evidence in support of this view has been published (1, 12).

Some studies with certain viruses have revealed a direct relationship between host growth and virus multiplication (2, 6, 7), while other studies have shown that virus multiplication is directly related to the amount of nitrogen and phosphorus supplied to the host, even though stunting resulted from excesses of these elements (4, 6, 10). In the studies reported herein, there was no observed relation between vigor of citrus plants and exocortis virus activity as measured by development of symptoms. Conditions that favored the growth of the host did not necessarily favor the activity of the exocortis virus. Conversely, factors that disfavored the host (excess nitrogen and phosphorus) favored the virus.

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