Changes in Amounts of Auxinlike Growth Promoter, Gibberellin, and Inhibitor in Citrus Infected with Exocortis Virus

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STUNTING OF TREES on rootstocks intolerant of exocortis virus was not attributed to girdling (7), and so it appeared that the virus has an adverse effect on production of growth regulators, on their normal function, or on both. Thus, an investigation of the influence of exocortis virus on growth regulators in citrus should provide a better understanding of citrus growth and especially of host response to the virus.

In this report, which is part of a series concerned with the biochemical response of citrus to exocortis virus infection (5, 7, 8, 10), we are concerned with 1. the changes in amounts of auxinlike growth promoter, gibberellin (GA), and inhibitor; and 2. how these substances might account for the observed growth patterns in exocortis-virus-infected citrus. (Promoter, gibberellin, and inhibitor are used in the singular to simplify expression in reference to the growth-regulating materials concerned here, though each may consist of more than a single substance.)

Materials and Methods

SOURCE TREES.—The source of tissues was 4 healthy and 5 exocortis-infected trees of Hamlin sweet

orange on trifoliate orange rootstock. They were part of a group of trees budded between 1955 and 1961. They were selected on the basis of the absence or presence of typical exocortis symptoms in both trifoliate orange and citron, the phloroglucinol-HCl color test (2), and the coumarin fluorescence test (10). The source of the budwood used for propagating them had been indexed and found to be free of tristeza, xyloporosis, and psorosis viruses.

PROMOTER AND INHIBITOR.—Debladed, nonflowering terminals approximately 25–35 mm long were collected for analysis of auxinlike growth promoter and of inhibitor. The terminals from each group of trees were pooled separately and frozen immediately. Samples were collected every 2 months for 1 year (April 1968 through April 1969).

Extracts representing 1 or 2 g of terminals, prepared and processed as previously described (9), were chromatogramed on Whatman No. 1 paper with isopropanol:NH_4OH:H_2O (10:1:1, v:v:v) in the ascending direction in the dark for 23 hours at 18°C.

Chromatograms used for bioassay were cut either transversely into 10 equal sections per Rf or into areas delineated on the basis of major areas of fluorescent colors observed under UV light at 254 nm. Each chromatogram section was bioassayed by the straight-growth test with 5 wheat (*Triticum aestivum* L.) coleoptile sections per bioassay vial (9), replicated at least twice.

Other chromatograms, developed in the same manner, were scanned with an UV densitometer (Photovolt) equipped with a recorder, to determine the relative amounts of materials present in the extracts from terminals of healthy and affected trees.

GIBBERELLIN (GA).—For GA determinations, entire terminals 10–40 mm long were collected during the early stages of new flushes of growth—spring, summer, fall, and spring 1968–69—from the same trees used for determinations of promoter and inhibitor. Feeder roots (rootlets) were collected at the same time from soil depths of 15–40 cm. Terminals were frozen at the site of collection, but feeder roots required washing and were taken to the laboratory, without freezing, for immediate extraction.

Tissue samples of 100 g each were comminuted in a blender with approximately 8 volumes of ethanol to yield a final concentration of approximately 80 per cent ethanol. The extracts were stored overnight at 4°C, filtered, and the bulk of the ethanol was removed under reduced pressure at 40°C. The concentrated extracts were then processed into acidic, basic, and neutral fractions (13) for recovery and concentration of GA.

The GA fractions, equivalent to 25 g tissue, were chromatogramed in the ascending direction on Whatman No. 3 paper in isopropanol:NH_4OH:H_2O (10:1:1, v:v:v) at 23 ± 1°C for a distance of approximately 30 cm. Chromatograms were cut

transversely into 10 equal sections and each eluted in menthanol: chloroform (80:20). The eluates were evaporated to dryness, taken up in H_2O, and then stored at 4°C until bioassayed.

The aqueous eluates of the chromatogram sections were tested for growth-regulating materials; one, containing growth promoter, was usually at Rf 0.20–0.30 and another, containing growth inhibitor, was generally at Rf 0.70–0.80.

The greatest amounts of growth promoter were found in healthy terminals collected in June, Decem-

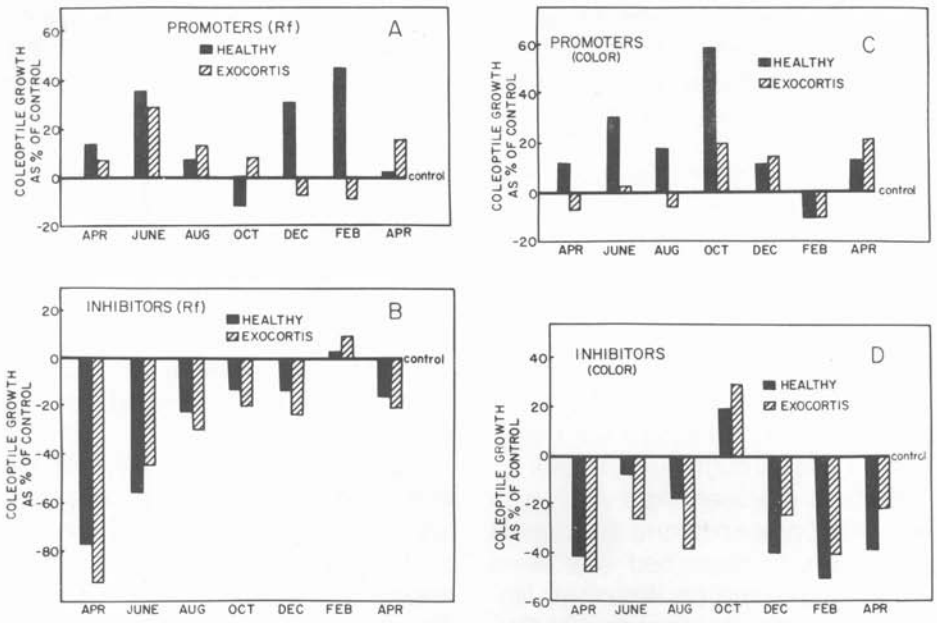

FIGURE 1. *A. Amounts of growth promoter (Rf 0.20–0.30). B. Amounts of growth inhibitor (Rf 0.70–0.80). As determined by wheat coleoptile bioassay of chromatograms of terminals from healthy and infected trees. C. Amounts of promoter. D. Amounts of inhibitor. As found in color zones 3 and 7, respectively, of bioassay of similar chromatograms. For delineation of color zones, see text and Fig. 2.*

concentration of GA by use of the barley (*Hordeum distichum* L.) endosperm bioassay (3). Replicated trials were made with 3 "half seeds" per replicate.

Results

PROMOTER AND INHIBITOR: ASSAY BY Rf.—Wheat bioassay of chromatograms indicated 2 areas of ber, and February. Infected terminals collected in June contained more growth promoter than infected terminals collected in any of the other periods (Fig. 1,A).

Growth inhibitor was present in greater amounts in both healthy and exocortis terminals collected in April, June, and August than at any other time (Fig. 1,B). It was always

present in a greater amount in infected terminals than in healthy terminals except in June.

ASSAY BY UV COLOR.—Chromatograms of the same type as those mentioned above, but bioassayed by sections delineated on the basis of UV fluorescent color, produced a

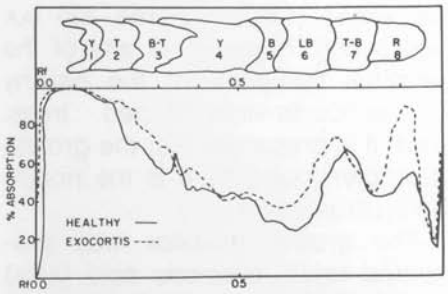

FIGURE 2. *Differences in ultraviolet densitometer absorption (254 nm) between extracts of healthy and exocortis-virus-infected terminals on chromatograms showing the fluorescent color zones 1 to 8 (across top of figure). (Y, yellow; T, tan; B, blue; LB, light blue; R, red.) The corresponding Rf for each color zone is also indicated.*

different pattern of distribution of promoter and inhibitor in relation to the time of tissue collection (Fig. 1, C,D). The fluorescent color zones of these chromatograms are indicated in the upper portion of Figure 2. Color zone 3 contained growth promotive materials and zone 7 contained growth-inhibitory materials. These color zones corresponded generally with the Rf zones 0.20–0.30 and 0.70–0.80, respectively.

Healthy terminals collected in October contained the greatest amount of promoter, followed by samples collected in June and August. The materials removed from the inhibitor zones for October terminals (healthy and infected) were promotive in the bioassay (Fig. 1,C,D) rather than inhibitory.

Exocortis terminals collected in April, June, and August contained more growth inhibitor than did their corresponding healthy terminals, but healthy terminals collected in December, February, and April contained more inhibitor than did exocortis terminals collected in these same 3 months (Fig. 1,D).

Ultraviolet light densitometry of chromatograms indicated 4 zones of higher concentration of materials in exocortis tissue than in healthy tissue. These zones were primarily nos. 3, 6, 7, and 8 (Fig. 2).

GIBBERELLIN.—The GA content in healthy terminals was more than 10 times, and in healthy roots about 3 times, the amount of GA in the corresponding exocortis tissues collected in the spring of 1968 (Fig. 3). The amounts of GA in healthy and exocortis-infected tissues collected in the 3 following seasons, including spring 1969, were essentially the same low amounts found in exocortis tissues collected in spring 1968.

Discussion

In these exocortis-infected Hamlin sweet orange trees on trifoliate orange rootstock, the general decrease in promoter, along with an increase in inhibitor content, is in agreement with the reports of similar changes in amounts of promoter, inhibitor, or both in other kinds of virus-infected plants (4).

The general decrease in growth

promoter and the consistently low ratio of promoter to inhibitor seem to be sufficient to account, in part, for the stunting observed in exocortis-virus-infected citrus. The increases in growth inhibitor, though general, appear to be insufficient to account for much, if any, of the growth reduction noted in the virus-infected trees.

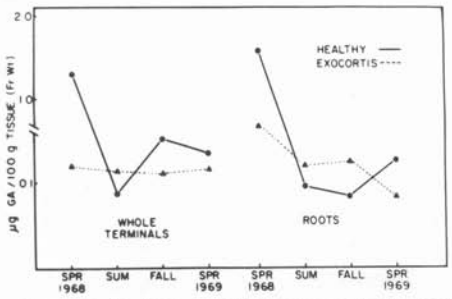

FIGURE 3. *GA content of terminals and feeder roots collected in early stages of flush collected from healthy and exocortis-virus-infected trees for four successive seasons.*

There is, however, a correlation between the higher amounts of inhibitor in the infected terminals in April, June, and August and the accumulation of free and bound coumarins in the exocortis-virus-infected rootstock (bark of trifoliate orange) during the same period (5). Although the same coumarins have been implicated in indole-3-acetic acid oxidase inhibition (5), it is, at present, difficult to account for any of the growth mediating effects from their accumulation in the roots, especially since indole-3-acetic acid (IAA) is not a normal constituent of citrus terminals (9). Perhaps it should also be mentioned that we could find no increase in the amounts of coumarins (10) in the infected terminals.

Indole-3-acetic acid has been reported to be present in Valencia orange blossoms (14) and was found in young terminals collected in August from trees suffering from spreading decline (9) caused by the burrowing nematode *Radopholus similis* (Cobb) Thorne. No IAA was found, however, in any of the terminal tissues from the healthy or exocortis-virus-infected trees; thus, it is presumed that the growth promoter found here is the nonindole citrus auxin (12).

The growth inhibitor was presumed to be abscissic acid (ABA) (1) since it was located in the well-known β-inhibition zone of chromatograms and possessed the characteristic Rf and fluorescent color of ABA. In citrus, there is a phenolic compound in the β-inhibitor zone that may also be involved, but it is not one of those phenolics previously identified for citrus (6, 8).

Infected young terminals collected in the spring of 1968 contained about one-tenth the amount of GA found in healthy flush, a difference great enough to account for some of the stunting in growth of the infected trees. Subsequent collections of seasonal flushes, including spring 1969, failed to show as great a difference between the amount of GA in healthy and infected terminals. The GA present is probably GA_1, the only GA reported to date for citrus (15).

Debladed terminals collected in

April 1969 contained much less inhibitor than did similar terminals collected in spring 1968 (Fig. 1,B). There was also less GA in entire terminals collected in spring 1969 than in similar terminals collected in spring 1968 (Fig. 3). It is possible that this dissimilarity in content of inhibitor and of GA was due to delayed growth and development of these terminals collected in 1969. Tree growth in the spring of 1969 began nearly a month later than in the spring of 1968.

The long-range influence of temperature and time on the functions of promoter, GA, or inhibitor, or any combination of these growth materials in citrus, is unknown. Studies should be made in environmental control chambers to determine whatever relationships may exist. Such studies are necessary to determine the time lag, if any, between the peak production of growth regulators and the visible evidence of their activity.

A reduction by as much as 50 per cent in amino acid and amide content of exocortis-virus-infected citrus has been reported (5). Thus, the changes in the amounts of growth promoter, GA, and inhibitor reported here for infected trees is possibly an indirect effect of the virus, exerted through nitrogen metabolism and its attendant consequences, rather than through any direct effect on growth substances per se.

Though stunting or reduced growth of citrus may be somewhat similar for exocortis virus infection and spreading decline disease, the biochemical responses of trees with these diseases are different. Trees infected with the burrowing nematode contained as much as 4 times the total amino acid content of healthy trees (11) and indicated an increase in growth promoter in some seasons, but still exhibited reduced growth (7). Thus, in spreading decline, the interference with, or the blocking of, growth regulators probably occurs in an entirely different biochemical system of the tree than it does in exocortis-virus-infected trees. If stunting of citrus is the result of an indirect effect of exocortis virus on growth-regulating substances, then other biochemical aspects should be investigated to determine the system or systems that are directly affected by the virus.

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