S. P. MONSELISE and R. GOREN

Some Physiological Properties of Leaves and Bark of Psorosis-Infected Valencia Orange Trees

METHODS ALLOWING the early and rapid diagnosis of citrus virus diseases are still lacking, with very few exceptions. Changes in activity of different enzymatic systems or of relative amounts of different metabolites may prove to be useful in diagnosis if they can be correlated with presence of a certain virus in a host plant. The present study has been carried out to explore these possibilities.

An orchard of Valencia orange [*Citrus sinensis* (L.) Osbeck] trees on sour orange (*C. aurantium* L.) stock provided leaves and bark for chemical and enzymatic determinations. In this orchard, near Rehovot, 3 different categories of trees were found: (a) Young replants about 4 years old in 1961 propagated from certified psorosis-free sources; (b) replants about 5-6 years old that had been budded with buds from trees infected with psorosis virus before regulations on budding with such material were enforced; (c) trees about 27 years old showing various degrees of damage from psorosis, trees which had provided buds for category (b). Trees were graded into 5 classes according to frequency of scales on twigs and to general degree of decline. Young certified trees, which were always symptomless, were considered as disease-free controls. Replants budded with diseased wood but not yet showing bark symptoms of psorosis will be considered as class 1; older trees are divided into damage classes 2, 3, and 4 according to increasing intensity of symptoms.

The trees chosen grew mixed over an area of about half an acre, and were selected in November, 1961. Three separate samplings were carried out as follows: In December, 1961, mature healthy leaves about 4-5 months old were collected at 4 weekly intervals from 5 positions in each of 20 trees (in all 100 samples). In December, 1962, 1 sample only of comparable leaves was collected from each of the same 20 trees. In May, 1964, 1 sample of bark was collected from each of the above-mentioned trees from normal portions of branches near areas heavily scaled.

The following determinations were performed: fresh and dry weight; total and alcohol-insoluble (protein) nitrogen; hesperidin content (of leaves only) by a U.V. spectrophotometric method (2); total content of phenols by the diazo reagent (4); peroxidase activity, using both guaiacol and pyrogallol as H-donors (3, 6, 7); catalase activity (1); ascorbic acid (8). Results were tested by standard statistical methods.

Trees of class 1 evinced a lower content of hesperidin and of total phenols in leaves and a higher content of total phenols in bark than certified controls (Fig. 1). With respect to these factors, leaves of old psorosis-affected trees evinced trends of progressive revergence toward values of virus-free controls with increasing damage and decline.

The largest divergence from controls with respect to the remaining factors occurs in classes 2 and 3 (low and medium damage classes of old trees, respectively). In class 2 we find the minimum for peroxidase (pyrogallol = PeP) and catalase activities of leaves and for ascorbic acid content of leaves and bark and the maximum for peroxidase (guaiacol = PeG) activity of leaves. In class 3, we find the minimum for PeP and the maximum for PeG in bark. The ratio of PeG to PeP attains a very high peak for both leaves and bark in classes 2 and 3, respectively (141 per cent and 203 per cent of the control).

Total nitrogen and protein nitrogen contents have been found to be essentially identical for all classes, including the controls.

The above-mentioned trends have been confirmed when calculated on the basis of fresh or dry weight or on the basis of leaf area (leaves only), or of total nitrogen (bark only). With leaves, however, the most significant results have been found when calculated on the basis of leaf area, since all classes evince a lower dry weight per unit area than certified controls. Results for leaves have been confirmed at both successive sampling dates.

The extensive data collected for leaves in 1961 allow the calculation of coefficients of variability for different classes of trees separately. In certain cases (especially PeG and hesperidin), a very pronounced peak (55 per cent and 30 per cent, respectively) has been found for class 2 whereas the values for class 4 was again near those for the certified controls (about 21 per cent for both factors). These results indicate increased variability when tissue reaction to disease is highest.

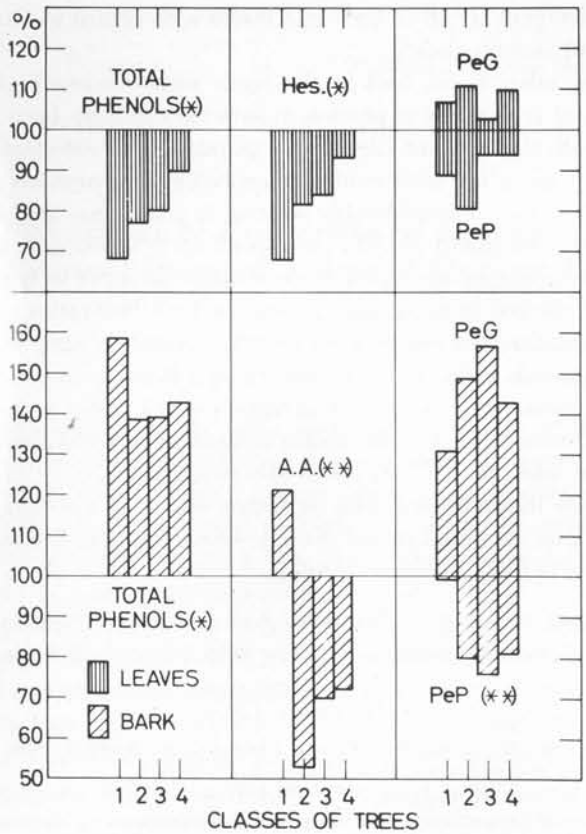

FIGURE 1. *Content of total phenols (Diazo), hesperidin (Hes), and ascorbic acid (AA) and peroxidase activities by guaiacol (PeG) and by pyrogallol (PeP) of leaves and bark of psorosis-affected Valencia orange trees (classes 1-4, see text) in percentage of certified psorosis-free controls (**,*, significant at 0.01 and 0.05 levels, respectively).*

Data for leaves and for bark show certain similarities in peroxidase and catalase activities and ascorbic acid content. The main discrepancy concerns phenolic compounds. From previous unpublished work, we know that very little hesperidin is present in bark of orange trees whereas relatively large amounts of it are in leaves (up to 2 per cent of dry matter). The total phenolic compounds tested by the diazo reaction are therefore not identical in leaves and bark; this conclusion is confirmed by differences in the absorption curves for the products of diazo reaction in bark and in leaf extracts (unpublished results). Qualitative differences in phenolic compounds found in bark and leaves could possibly account for the divergent trends of bark and leaves with regard to total phenols in different psorosis classes.

On the other hand, bark is the tissue most obviously affected by psorosis and an increase in phenols in bark has similarly been shown for cachexia-affected trees of Clementine mandarin (*C. reticulata* Blanco) (5) where again the most evident morphological symptoms are in the bark. In that case, a considerable increase in peroxidase activity of bark (PeG) was also found (5). In psorosis-affected Valencia orange trees, peroxidase activities are again more strongly affected in bark than in leaves as reflected by much higher peaks of PeG/PeP ratios.

In conclusion, our results seem to reflect relatively early reactions of tissue to psorosis, especially that concerning a decrease in phenolic compound in leaves. The quite general feature that in older trees there is a reversion toward values more similar to those of certified controls as the amount of damage increases seems also to suggest that we probably do not measure in most cases plain secondary effects of increasing decline, and that after an initial strong reaction of tissues there is some adaptation of trees to their diseased condition.

ACKNOWLEDGMENT.—This work has been supported by a grant (4, A-5) of the Ford Foundation. The help is gratefully acknowledged.

Literature Cited

1. GAGNON, M., HUNTING, W. M., and ESSELEN, W. B. 1959. New method of catalase determination. Anal. Chem. 31: 144-145.
2. GOREN, R., and MONSELISE, S. P. 1964. Determination of hesperidin in dry matter of citrus tissues by ultraviolet spectrophotometry method. J. Assoc. Offic. Agr. Chemists 47: 677-681.
3. GOREN, R., and MONSELISE, S. P. 1965. Interactions of hesperidin, some natural components and enzymatic systems in developing Shamouti orange fruits. J. Hort. Sci. (in press).
4. HENDRICKSON, R., and KESTERSON, J. W. 1957. Chemical analysis of citrus bioflavanoids. Proc. Florida State Hort. Soc. 70: 196-203.
5. JARDENY, A., MONSELISE, S. P., and CHORIN, M. 1965. Some morphological and physiological features of Clementine mandarin trees affected by cachexia, p. 291-294. *In* W. C. Price [ed.], Proc. 3d Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
6. MAELY, A. C., and CHANCE, B. 1954. The assay of catalases and peroxidases. Vol. I, p. 358-424. *In* D. Glick [ed.], Methods of Biochemical Analysis. Interscience Publ., New York.
7. MONSELISE, S. P., and HALEVY, A. H. 1962. Effects of gibberellin and Amo-1618 on growth, dry matter accumulation, chlorophyll content and peroxidase activity of citrus seedlings. Am. J. Bot. 49: 405-412.
8. ROE, J. H. 1954. Chemical determinations of ascorbic, dehydroascorbic and diketogulonic acids. Vol. I, p. 115-139. *In* D. Glick [ed.], Methods of Biochemical Analysis. Interscience Publ., New York.