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Physiological and Biochemical Aspects

Quantitative Biochemical Changes in Healthy and Decline Sweet Orange Trees Associated with Bud-Union Crease

V. P. BHUTANI, J. C. BAKHSHI, and L. C. KNORR

IN NORTH INDIA, the cause of decline in citrus trees has been attributed mainly to greening. One type of decline, however, is distinctive in that it is restricted to Musambi and Blood Red varieties of sweet orange on rough lemon root. Decline of the tops is associated in young trees with a bud-union creasing and in older trees with an externally visible protrusion at the bud union (Fig. 1) and a separation of the cortex of the stock and scion along the line of union (1, 2). The symptoms agree closely with those described by Nour-Eldin (10) for bud-union creasing of sweet orange on rough lemon rootstock. Also present in the scion portion of the trunk is a profuse pegging of the woody cylinder (9), resembling the symptoms of fovea as described by Knorr and Price (6).

Though virus infection is suspected, the disease referred to hereafter as bud-union crease has not been reproduced by graft transmission; neither has the possibility of delayed incompatibility been disproved. To determine the actual cause, we compared biochemical differences in healthy and declining trees.

Materials and Methods

Twelve 7-year-old trees of Musambi orange on rough lemon were selected for study. Eight were obviously healthy with no externally visible bud-union crease, and the remaining 4 were in decline and showed bud-union crease. Of the 8 healthy trees, 4 were completely ringed by removing bark ⅛ in. wide at the union to determine whether ringing induces biochemical changes similar to those caused by creasing; these 4 are referred to in the text as girdled healthy trees.

In October, at the time when girdles had nearly healed, bark samples were taken above and below the union from each tree for analysis. Each tree served as a replication.

Protein nitrogen was precipitated from an extract of 5 g bark tissue

with trichloroacetic acid in accordance with the method of Slade and Birkinsaw as reported by Pirie (11) and estimated separately after digestion in micro kjeldahl (5). Bark samples were extracted with hot 80 per cent ethanol, and the extracts were filtered and cleaned by passage through glasswool and activated charcoal and dried in desiccators. A volume of 1.25 ml was made in each case with 10 per cent isopropanol in water. Ten μl samples were spotted on Whatman No. 2 chromatographic paper. The chromatograms were developed descendingly for 24 hours in one direction with the organic phase of butanol:acetic acid:water (4:1:5). The dried chromatograms were wetted with the following solution: 100 ml of 0.2 per cent ninhydrin in acetone plus 5 ml of acetic acid (7). Development was carried out at 65°C. The developed spots were eluted in test tubes containing 5 ml of 0.05 per cent copper sulfate solution in 75 per cent ethanol (4). The color intensity was read at 540 nm on a Spectronic-20 colorimeter for all amino acids except proline, which was read at 440 nm. Quantitative estimations were made from standard curves prepared earlier with analytically pure amino acids.

FIGURE 1. *Eruptive stage of bud-union crease disease in a sweet orange tree on rough lemon root. In time the swollen, scaling overgrowth separates so that only a thin connection remains between the bark of scion and stock. Creasing of the woody cylinder at the bud union is evident on removal of the bark.*

The amounts of chlorophylls *a* and *b* were determined by means of the method of Mackinney (8) and Comar and Zscheile (3), in randomly selected nonchlorotic and chlorotic leaves from normal, declined, and girdled 7-year-old Musambi sweet orange trees on rough lemon root.

Results

CHLOROPHYLL CONTENTS.—The analysis of chlorophylls *a* and *b* of nonchlorotic leaves from nongirdled healthy, girdled healthy, and declined trees showed no significant differences irrespective of tree condition. However, when chlorotic

leaves from the 3 types of trees were analyzed, chlorophyll *a* was found present to the extent of 6.1, 6.9, and 1.7 mg/100 ml and chlorophyll *b* to the extent of 6.2, 7.4, and 1.6 mg/100 ml, respectively. The chlorotic leaves from declined trees, therefore, contained significantly lower amounts of chlorophylls *a* and *b* than did the corresponding nonchlorotic leaves from the nongirdled or girdled healthy trees. The differences between chlorotic leaves of nongirdled and girdled healthy trees were not significant at the 5 per cent level.

Since nonchlorotic leaves showed no differences that could be related to tree condition, it may be helpful in future studies to use chlorotic leaves to ascertain relative magnitudes of biochemical changes in healthy and declined trees.

DRY WEIGHT, TOTAL, AND PROTEIN NITROGEN.—The percentage dry weight of scion bark collected from above the union in the case of declined and girdled healthy trees was 52.5 and 51.4, respectively, and was significantly higher than that, 44.7, of nongirdled healthy trees. An interruption in downward translocation and consequent accumulation of synthates above the union resulted from both girdling and bud-union crease. The differences in dry weight of rootstock bark from nongirdled, girdled, and declined trees were not significant.

Differences in total nitrogen or in protein nitrogen in scion bark samples were not significant. On the other hand, the rootstock bark samples from declined trees contained 0.74 per cent protein nitrogen on a dry weight basis, a significantly higher amount than the 0.50 per cent in girdled healthy trees or the 0.62 per cent in nongirdled healthy trees.

The higher level of TCA-precipitable nitrogenous material from bark samples of declined trees (but not from girdled healthy trees) and the earlier published observations of Nauriyal et al. (9), showing pigmentation to start in the rootstock bark, suggest that mere girdling of bark and interruption in downward flow of synthates may not be the sole reasons for decline. A possibility can be entertained of a rootstock reaction to scion infection that may cause formation of anomalous proteins. This point needs, however, to be confirmed by qualitative analyses of proteins through electrophoresis —particularly so when the changes in amino acids (as detailed subsequently) are of the same order in declined and girdled healthy trees.

AMINO ACIDS.—The changes in amino acid content of scion and rootstock bark from nongirdled healthy, girdled healthy, and declined trees are in Table 1. A general conclusion that can be derived from these data is that the amino acid content in rootstock bark of declined and girdled healthy trees was much less than that in the rootstock of nongirdled healthy trees. If the results are compared with those of protein nitrogen determinations mentioned earlier, it becomes difficult to suggest whether

TABLE 1. AMINO ACIDS CONTENT AS μG OF AMINO NITROGEN PER G FRESH WEIGHT OF BARK FROM NORMAL, DECLINED, AND GIRDLED 7-YEAR-OLD MUSAMBI SWEET ORANGE TREES ON ROUGH LEMON

	Healthy trees		Girdled trees		Declined trees	
Amino acid	Above union	Below union	Above union	Below union	Above union	Below union
Alanine	6.77	1.72	5.84	Trace	6.00	Trace
Gamma aminobutyric acid	9.34	2.22	6.81	Trace	8.71	0.14
Arginine and asparagine	3.07	3.03	2.50	Trace	5.62	Trace
Aspartic acid	5.27	3.91	4.66	1.97	4.33	0.78
Cystine	2.33	3.35	0.91	2.12	3.37	1.71
Glutamic acid and threonine	3.81	4.81	3.72	Trace	6.16	Trace
Leucine containing isoleucine	3.30	1.91	2.75	0.37	4.09	Trace
Lysine	0.56	1.17	0.77	Trace	1.03	Trace
Proline	115.00	45.60	120.60	Trace	110.60	Trace
Valine	1.67	0.47	1.37	0.53	1.94	0.16
Total	151.12	68.19	149.93	4.99	151.85	2.79

the causes of depletion of amino acids from the rootstock bark are similar in the case of declined and girdled healthy trees. One conclusion, however, is permissible: in both cases the downward translocation has been hampered. Bud-union crease is, therefore, an important symptom of the malady, and the biochemical changes induced as a consequence indicate that this symptom must be taken into consideration.

Conclusions

It is recognized that greening is widespread in citrus trees of north India and accounts for much of the decline problem. The greening virus may also have been present in the trees used by us, but the decline associated with creasing is distinctive in producing a much earlier and more complete collapse of trees than does the decline caused by greening in Musambi and Blood Red orange on rootstocks other than rough lemon (1, 9). It seems probable that even in the absence of greening, trees affected by bud-union crease would collapse because of interferences in downward translocations. In this light, bud-union crease disease should be regarded as another major contributor to India's so-called citrus decline problem.

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