

Citrus Greening in the Indian Punjab

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ON THE BASIS of symptoms, Fraser and Singh (2) presumed the existence of greening disease in the Punjab Plain, and on the basis of an indexing of several trees in the area, Nariani, Raychaudhuri, and Bhalla (4) demonstrated the presence of the causal agent. The incidence of greening in the Punjab and the role of this disease in India's so-called citrus decline are considered here.

Geographical Distribution

The incidence of greening disease in citrus plantations of Punjab and adjacent areas of Rajasthan, Himachal Pradesh, and Uttar Pradesh was determined by chromatographic procedures that relate the presence of greening with a marker substance, gentisoyl glucose (1, 6). While it is recognized that determinations of the greening marker

substance are not equivalent to demonstrations of the causal pathogen, equating the two seems justifiable on the bases of extensive testing by Schwarz (6) and successes attending use of the chromatographic test for commercial control of greening in South Africa. Subject to this proviso, and for the sake of convenience, the following discussion assumes the relatedness of marker substance and pathogen.

Samples of bark from 2-year-old twigs—occasionally from bark from across the bud unions and from roots—were collected from April 6 through August 16, 1969. In each orchard, a random row of trees was selected, and from these trees, irrespective of their condition, samples were taken. The trees were 1–25 years in age.

Bark samples were extracted in 70 per cent ethanol, and the extractant was decanted and evaporated under reduced pressure. Residues were dissolved in ethanol and spotted on Whatman No. 1 chromatographic paper; a water-saturated n-butanol carrier was used. Chromatograms were examined under a UV lamp (Hanovia Model IV), transmitting 95 per cent of its radiation at 366 nm. Periodically, extracts were sent to Dr. R. E. Schwarz, Nelspruit, South Africa, for confirmation of results; the extent of agreement was high.

Ratios of the number of trees showing the marker substance to those tested at various locations were: Abohar (Pb.), 115/208; Attari (Pb.), 73/80; Chattoli (Himachal Pradesh), 11/15; Chanoor (H.P.), 11/11; Papahu (H.P.), 13/13; Faridkot (Pb.),

16/20; Ludhiana (Pb.), 109/147; Patiala (Pb.), 24/26; Hoshiarpur (Pb.), 21/40; Dhaulakuan (H.P.), 26/40; and Saharanpur (Uttar Pradesh), 14/26. The incidence of the marker substance for the area as a whole was 69 per cent, 85 per cent of which were clearly positive determinations and 15 per cent of which were questionable positives. The 31 per cent found negative is a combination of 77 per cent clearly negative determinations and 23 per cent questionable negatives.

Correspondence of chromatographic results with tree decline was low. Frequently, trees producing positive chromatograms showed no leaf, fruit, or dieback symptoms of greening. Failure of symptoms to appear in trees found to be positive chromatographically might result from recency of infection or from masking of symptoms by climatic factors or malnutrition. In the case of mandarin and possibly other varieties, lack of correspondence might be due to such varieties acting as symptomless carriers.

Interestingly, samples collected during May and June, when daily noontime temperatures in the Punjab range from 32 to 44°C, gave about the same percentages of positive chromatographic readings as samples taken during periods with more moderate temperatures. This result agrees with findings in Taiwan and the Philippines that the Asian greening pathogen is tolerant of high temperature. It is apparent that the marker substance under our conditions is also heat stable.

No significance is attached to variations in the percentages of positives from place to place. That there are factors vitiating correlations of findings with geographical locations is illustrated by the situation at Abohar: here the overall percentage of trees containing the marker substance was 55, but in certain blocks it was 100. Such variations may be explained by the possibility that where all plants in a block contained the marker, such plants might have been inoculated with the pathogen before leaving the nursery.

Because of the manner of spot sampling, no conclusions could be drawn regarding the rate of vectored spread at various locations; psyllids (*Diaphorina citri* Kuw.) were, however, observed in large numbers only at Abohar and Ludhiana.

Though the greening marker was found in 69 per cent of all trees sampled, the incidence of infection might well be greater because of inadequacies in sampling procedures. Twig samples were taken from 4 sides of each tree; had more positions per tree been sampled, the incidence of the marker substance would undoubtedly have been found to be greater.

In nursery plants, positives were found in 25 per cent of tested subjects. In budded nursery plants (6/14) infection could have resulted from the use of infected budwood, but in seedlings (3/22) infection can only be attributed to psyllid transmission. No positives were encountered among 17 nucellar seedlings, 1-2 years old, reared in the greenhouse.

Varietal Susceptibility

The marker substance was detected in most varieties sampled including, among sweet orange varieties: Musambi, Blood Red, Hamlin, Pineapple, Jaffa, Valencia, Tardiff, Navalencia, Golden Nugget, Vanille, and Joppa; among mandarin varieties and their hybrids: Santra, Kinnow, Orlando, Kara, Honey, Wilking, Minneola, and Pearl; among trifoliate orange varieties and their hybrids: common trifoliate orange, Carrizo, Savage, and Sacaton; among grapefruit varieties: Marsh Seedless; and among miscellaneous varieties: rough lemon, table lemon, and sweet lime. According to Dr. R. E. Schwarz, it is not yet certain whether the marker substance in trifoliate orange, its hybrids, rough lemon, and sweet lime correlates specifically with greening infection. Significance of the marker substance in these varieties remains to be determined. Those varieties for which the marker substance is known to indicate infection are given by Schwarz (7).

Among major scion varieties, the ratios of trees exhibiting the marker substance to the total number of trees examined were: Mosambi, 22/22; Blood Red, 87/119; Hamlin, 37/41; Pineapple, 26/28; Jaffa, 25/25; Valencia, 67/86; Santra mandarin, 23/29; and Kinnow, 12/42.

Unusual was the failure to obtain, either in twig or root samples, positive readings in a block of 8 16-year-old Cleopatra mandarin seedling trees free of decline or greening

symptoms. In the same block, low incidences of the marker substance were shown by many of 23 other varieties imported 16 years ago from the United States. The overall rate of positives for these trees was 37 per cent, with some varieties having few or no trees with the marker—Carrizo citrange, 1/4; Troyer citrange, 1/3; Dancy tangerine, 0/4—whereas high incidences of the marker were shown by Pearl tangelo, 3/4; Valencia sweet orange, 4/4; and Marsh

the search led to information on the distribution of the marker substance within individual trees.

In an experiment at Ludhiana involving 4 7-year-old greening-affected Blood Red sweet orange trees on rough lemon rootstock, samples were taken from twigs 6 months old, 1 year old, and 2 years old on 4 sides of each tree. Samples of bark were also taken from above and below the bud union and from roots.

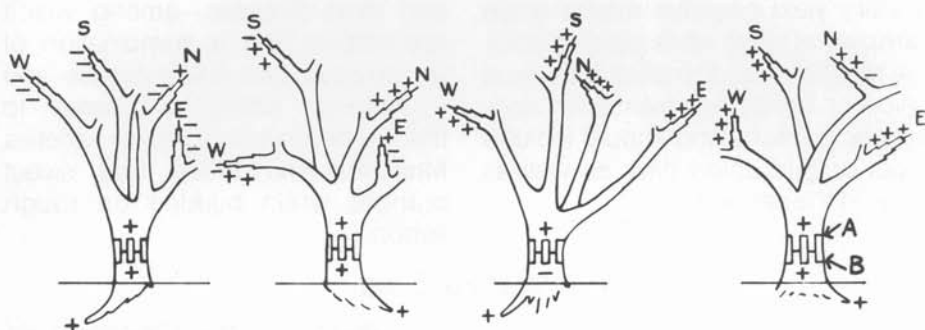


FIGURE 1. Diagram of four 7-year-old Blood Red sweet orange trees on rough lemon rootstocks at PAU Orchard, Ludhiana, showing localization of greening marker substance on July 12, 1969. Terminal portions in canopy represent 6-month-old twigs, penultimate portions 1-year-old twigs, and subpenultimate portions 2-year-old twigs. A. Bark patch above union. B. Bark patch below union.

Seedless grapefruit, 4/4. These rates closely parallel those given for tolerance by Fraser and Singh (3) and suggest that "tolerance" may actually involve resistance.

Localization of the Marker Substance within the Tree

Attempts were made to find the most reliable single site in sampling trees for the marker substance. Though this site was not determined,

The marker substance was encountered in 11 of 16 6-month-old twigs, in 11 of 16 1-year-old twigs, in 10 of 16 2-year-old twigs, in 4 of 4 bark patches above the bud union, in 3 of 4 bark patches below the bud union, and in 4 of 4 roots (Fig. 1).

The encouraging lead that the greening marker substance might be detected most consistently by sampling roots or bark above the bud

union was not borne out, however, in subsequent testing of other trees where it was found that positives were registered in 20 of 23 trees in 2-year-old twigs, in 16 of 23 trees in bark above the bud union, in 11 of 23 trees in roots, and in 6 of 23 trees in bark below the bud union. In some cases, negative root chromatograms may have resulted from an inability of the rootstock species to elaborate the marker substance rather than to an absence of infection.

As shown in Figure 1, trees occasionally yield negative results when sampled at twigs while yielding positive results when sampled at the bud union or roots. To cover such contingencies, sampling should include roots or bud-union bark as well as twigs in the canopy.

Conclusions

Since 69 per cent of trees sampled contained the marker substance of greening disease, it is evident that greening is an important factor in the "citrus decline" problem of the Punjab. It is misleading, however, to suggest that greening alone is responsible for all the recent decline in the area. There are many other factors involved in the poor growth of trees, including high soil pH values, salinity, inadequate drainage, pernicious intercropping, poor pest control, and virus diseases—among which are tristeza and a combination of an eruptive bud-union crease and a fovealike pitting destructive to the predominantly grown varieties Musambi and Blood Red sweet oranges when budded on rough lemon (5).

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