Greening and Citrus Decline in India

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CITRUS DECLINE in India refers to a particular syndrome known as "dieback." It involves the defoliation of young shoots and dying back of twigs from the tip downwards, resulting in loss of vigor, general health, and decreased fruit production. The disorder is reported to have been present as early as the eighteenth century (1), but it has assumed alarming proportions during the last few decades.

Dieback has been attributed in the past to various factors, including soil disorders, nutritional deficiencies, and parasitic agents such as fungi and viruses. Recently it has been shown that the major cause of the malady is a complex in which the greening pathogen and certain fungi play an important role (3). The greening pathogen is transmitted in India by the citrus psylla *Diaphorina citri* Kuw. (2), which is present in most of India's citrus-growing areas. The psylla reaches its maximum incidence during spring, but also has a high incidence in the autumn.

Material and Methods

Colonies of the citrus psylla were raised in an insectary. Cylindrical, tinned metal frames with muslin were used as cages for feeding insects on individual seedlings. The seedlings were sprayed with 0.1 per cent Ektatox after the insects were removed.

Determination of free amino acids was made by the descending paper chromatographic technique; standard amino acids were used as controls. About 5 g each of greening-affected and healthy leaves of sweet orange were cut into pieces and processed in a blender with 80 per cent ethanol for 2 min. The leaf extracts obtained were stored at 4°C for 7 days and were then allowed to dry in an incubator at 60°C. The residue was dissolved in 1 ml of n-butanol and spotted on Whatman No. 1 chromatographic paper by means of finely pointed pipettes. A mixture of n-butanol, acetic acid, and water (4:1:5) was used as a solvent. The chromatograms were removed after 14 hours, sprayed with 0.2 per cent ninhydrin in n-butanol, and dried in an oven for about 15 min at 110°C, when colored spots appeared.

Inoculations with the fungi were made by the cut split technique, i.e., cutting the stem longitudinally and inserting a culture of the fungus in the split. A piece of absorbent cotton soaked in sterilized water was placed over the cut end, which was then covered with a polythene film to maintain moisture.

Results

RELATION OF THE NUMBER OF PSYLLA TO TRANSMISSION OF THE PATHOGEN.—Transmission of the greening pathogen by the psylla has been demonstrated (2). Experiments were laid out to determine the relationship of the number of psylla to successful transmission of the greening agent. Citrus psylla singly, as well as in groups of 2, 5, and 10, were fed on the diseased source (sweet orange) for 3 days and then transferred to sweet orange seedlings (var. Pineapple). Six plants were inoculated under each treatment. The results (Table 1) indicate that even a single psylla is capable of transmitting the greening pathogen.

TABLE 1. RELATION OF THE NUMBER OF VECTORS TO TRANSMISSION OF THE GREENING PATHOGEN

Number of psylla employed	Time of feeding on source plant (days)	Time of feeding on test seedlings (days)	No. infected/ no. inoculated
1	3	10	4/6
2	3	17	5/6
5	3	13–16	4/6
10	3	10–17	4/6

ABILITY OF NYMPHS TO TRANSMIT.—To determine whether nymphs of psylla can pick up and transmit the greening pathogen, nymphs were allowed to feed for 2 days on a greening-affected sweet orange plant and were then released on 8 healthy sweet orange (Mosambi) seedlings. The insects were killed as soon as they were observed to become adults. None of the seedlings were infected. Thus, there was no evidence that nymphs are able to pick up and transmit the greening agent during the span of their nymphal life.

LATENT PERIOD OF THE GREENING PATHOGEN IN ITS VECTOR.—To determine whether there is a latent or incubation period of the greening agent in its vector, experiments were designed in which the insects, after moulting from nymphs, were allowed to feed on a diseased plant for a definite period and then were transferred to a series of sweet orange plants after definite intervals in succession. Three such series were maintained.

TABLE 2. LATENT PERIOD OF THE GREENING PATHOGEN IN ITS VECTOR

Feeding period on source plant (days)	Time of feeding in serial transfers					
	1st transfer (2 days)		2nd transfer (4 days)		3rd transfer (4 days)	
	No. of insects	Results	No. of insects	Results	No. of insects	Results
2	20	*	15	*	12	+
2	20	*	11	*	9	+
2	25	*	13	*	5	+

*Denotes no infection, + denotes infection with greening.

The results (Table 2) indicate that the psylla does not become infective immediately after acquisition of the pathogen and that there is a latent period of about 8–12 days in the vector.

DETERMINATION OF FREE AMINO ACIDS IN HEALTHY AND GREENING-AFFECTED LEAVES OF SWEET ORANGE.—The results of chromatographic determination of the free amino acid composition of the healthy and diseased sweet orange plants are in Table 3. Glycine, glutamic acid, phenylalanine, and leucine were absent

from the diseased leaves or present in undetectable quantities. Histidine, lysine, aspartic acid, threonine, alanine, tyrosine, and methionine were present in comparatively lower concentrations than in healthy leaves.

TABLE 3. COMPARISON OF AMINO ACIDS IN HEALTHY AND GREENING-AFFECTED LEAVES OF SWEET ORANGE

Amino acid	R value	Healthy leaves	Diseased leaves
Histidine	0.043	+++	++
Lysine	0.045	+++	++
Arginine	0.060	0	+
Aspartic acid	0.106	++	+
Glycine	0.132	+	0
Glutamic acid	0.155	++	0
Threonine	0.185	++	+
Alanine	0.244	++	+
Tyrosine	0.314	+++	+
Tryptophan	0.428	0	+
Methionine	0.430	++	+
Phenylalanine	0.532	+	0
Proline	0.561	+	+
Leucine	0.622	++	0

+++, High; ++, medium; +, low; 0, none.

Role of Fungi

It has been established (3) that citrus plants infected with the greening pathogen are especially liable to attack by several fungi including *Colletotrichum gloeosporioides, Diplodia natalensis, Curvularia tuberculata,* and *Fusarium* spp. The combination causes the dieback syndrome. Greening-affected plants of kagzi lime and sweet orange artificially inoculated with *C. tuberculata, C. gloeosporioides,* and *D. natalensis* developed a more severe dieback than did the healthy seedlings inoculated with these fungi. The fungi are, however, capable of independently causing dieback symptoms in these citrus species when trees are weakened by other causes.

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