Strains of the Greening Pathogen

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IN A SURVEY of the geographical distribution of greening (3), differences in the abundance of symptoms were attributed mainly to the influence of climate. As it was realized, however, that the greening pathogen may exist in different strains, isolates from various areas were compared under identical conditions.

Experiments

EXPERIMENT 1.—Twig sections from 2 Valencia sister trees, both showing severe greening symptoms, were taken from Nelspruit, White River, Pretoria, Rustenburg, and Tzaneen, and each was grafted into 5 nucellar Valencia trees on rough lemon. Five noninoculated trees were kept as controls. The nucellar trees were obtained from a greening-free area (Eastern Cape). Each inoculated tree was grafted with 3 grafts and budded with 3 buds. If a graft or bud died, it was replaced with a new graft or bud from the same source. The inoculations were repeated at various times of the year. The experimental trees were grown in 200-gallon asbestos cement containers, the water supply in each drum being controlled with the aid of 3 tensiometers (6 in., 12 in., and 24 in.). The trees were protected from insects by gauze and, in addition, were sprayed regularly with an insecticide. The tree circumference was measured annually. Observations were made on the development of visible leaf symptoms. In addition, fruit and bark samples were tested with the albedo fluorescence test (AFT) and the bark fluorescence test (BFT), respectively (2, 4). The results (Table 1) indicate a distinct difference in virulence among various isolates. All 5 trees infected with the Pretoria and the Rustenburg isolates and 3 trees infected with the Tzaneen isolate showed a marked reduction in vigor as indicated by the reduced trunk circumference and strong leaf symptoms—small upright leaves, zinc-deficiency symptoms, and vein yellowing. One of the 2 trees that were infected with the White River isolate showed medium symptoms; the other had mild symptoms. Only 1 of the trees inoculated with the Nelspruit isolate was infected, and only the BFT and the AFT indicated the infection. One reason for this difference is that a much higher percentage of the Rustenburg, Pretoria, and Tzaneen grafts, after growing out, showed greening symptoms when compared with those of the Nelspruit and White River grafts. In addition, the Pretoria, Rustenburg, and Tzaneen (PRT) isolates appear to have spread much faster in the tree than did the Nelspruit and White River (NW) isolates. All the trees infected with the PR isolates and 3 infected with the T isolate showed severe symptoms 1 year after inoculation. Two years after inoculation there were very few branches that did not show signs of infection. In the two cases in which the White River isolate spread in the inoculated trees, the pathogen spread only in the second and third years, respectively, and the single infection with the Nelspruit isolate only took place in the fourth year and was identified only with the aid of the AFT and BFT.

TABLE 1. COMPARISON OF THE GREENING PATHOGEN ISOLATES FROM DIFFERENT AREAS

Isolate	Trunk circumference (mm) (mean of 5 trees)		No. trees with leaf symptoms: severe/medium /mild/absent	No. fruit in groups: A/B/C[a]	Test for bark fluorescence: pos./weak pos./neg.	No. grafts: pos./neg. for visible symptoms[b]
	Before inoculation	4 years later	Four years after inoculation			
Pretoria	38	99	4/1/0/0	11/0/24	12/1/13	8/1
Rustenburg	44	95	5/0/0/0	32/0/0	15/0/0	11/2
Tzaneen	42	134	2/1/2/0	8/1/29	4/3/8	7/1
White River	41	166	0/1/1/3	33/3/131	3/0/12	1/9
Nelspruit	39	177	0/0/0/5	37/1/87	1/2/15	1/14
Control	34	176	0/0/0/5	0/0/94	0/0/15	

a. Group A, strong violet albedo fluorescence; group B, partial violet albedo fluorescence; group C, no violet albedo fluorescence.
b. Number of grafts on 5 trees showing visible greening symptoms.

The number of severely greened fruits (group A) hanging on the trees at harvest time (Table 1) does not reflect the actual extent of infection, as a very high percentage of fruit from the trees infected with the PRT isolate dropped prematurely whereas most of the greened fruits on the NW trees that showed infection remained.

The fact that the severe strain was found in the Pretoria, Rustenburg, and Tzaneen areas and the mild one in the Nelspruit and White River areas does not necessarily mean that all or even the majority of the trees infected in Pretoria, Rustenburg, and Tzaneen contain the severe strain and those in Nelspruit and White River the mild strain. It is quite possible that the mild and severe strains exist in all areas, although they may be present in different ratios.

The results obtained by McClean and Oberholzer (1) in graft-transmissions of greening also indicate a higher percentage of transmission of greening with some isolates than with others. In inoculations with 4 grafts from greening-infected trees from White River to indicator seedlings, the authors failed to obtain transmissions, whereas 34 inoculations with material from Tzaneen, Rustenburg, and Pretoria yielded 6 transmissions. When 17 twig tips of greening-infected material from White River and Nelspruit were top-grafted onto stock seedlings, none, after growing out, showed greening symptoms, whereas 44 of 153 twig tops from Tzaneen, Rustenburg, and Pretoria grafted in the same way showed greening symptoms.

In the Rustenburg area, it has been reported that greening generally shows a progressive development in affected trees. Even if only part of the tree is infected, the disease spreads to other parts in the following years, leading to a total invasion of the tree. In the White River area, on the other hand, the disease is reported to have "disappeared" in 1946, after severe crop losses from greening from 1939 to 1945. Although the areas are not directly comparable because of the differences in climatic conditions, the observations may indicate the prevalence of a highly systemic strain in Rustenburg and the prevalence of a strain with a slow and uneven translocation in White River.

Although the symptoms of the PRT strain are more severe than those of the NW strain, there is no basic difference in the symptoms produced by them, as both induce zinc-deficiency and yellow-vein symptoms. In the field the difference in the severity of the strains may be canceled out in cases of severe infestation by the citrus psylla.

EXPERIMENT 2.—In this experiment, originally laid out to study the effect of soil and various water levels on the symptoms of greening, 48 trees in 200-gallon containers were each inoculated with 3 buds and 3 grafts of material from the same 2 trees at White River from which the material for the first experiment was taken. The inoculations were carried out in June and July.

Eight of the trees developed symptoms of greening 2 years after inoculation. Of the 112 grafts that had taken, 23.2 per cent showed symptoms of greening and 76.8 per cent showed no symptoms 1 year after inoculation.

EXPERIMENT 3.—The percentage of transmissions in the controls of a heat inactivation experiment (6) also gave information on the transmissibility of the Nelspruit isolate. The budwood used, however, was obtained from an orchard different from that used in experiment 1. Two buds, taken from twigs immediately behind a greened fruit, were each budded to 10 rough lemon seedlings in September. This experiment was carried out with 3 varieties: Washington Navel, Pineapple, and Olinda Valencia.

The shoots from 4 of 27 surviving Navel buds, 1 of 29 Olinda Valencia buds, and 1 of 38 Pineapple buds showed greening symptoms 1 year after inoculation.

Experiments 2 and 3 confirm the relatively low transmissibility of the White River and Nelspruit isolates. The better transmission in experiment 2 might be due to differences in the isolates, but also might be due to the fact that the transmission was carried out in midwinter, whereas the transmission in experiment 3 was carried out in spring.

EXPERIMENT 4.—During a period of 25 months, 10 seedlings each month were side-grafted with graft-wood from greening-infected nucellar Valencia trees from two areas: Pretoria and Nelspruit. The trees from which the material was taken were sister trees of those from which the material for experiment 1 was obtained. After inoculation, the seedlings were kept in a temperature-controlled glasshouse at 21–23°C. Although the main aim of this experiment was to study the seasonal fluctuation in the transmissibility of the greening pathogen (5), it also gave information on the differences between the Pretoria and Nelspruit isolates. The seedlings were regularly inspected for visible greening symptoms, and bark samples were tested with the bark fluorescence test 6 and 12 months after inoculation.

Of the 250 seedlings inoculated with Pretoria material, 24 per cent showed external greening symptoms, and of the same number inoculated with Nelspruit material, only 2.5 per cent showed symptoms under cool conditions. In the Pretoria group, there was a distinct maximum of transmission in midwinter.

When the BFT was applied, 67.6 per cent were positive in the Pretoria group and 59.2 per cent in the Nelspruit group. Thus, when the BFT is taken as a criterion for infection, there is a relatively high percentage of transmissions throughout the year for both isolates.

It is suggested that the following strains of the greening pathogen exist: strains with rapid and slow systemic spread and a latent strain that does not induce symptoms under any conditions. If such a latent strain exists and is fully systemic, it might be used for preimmunization of sweet orange against infection with the greening pathogen.

Observations made during the large-scale indexing with the BFT at Letaba and Zebediela Estates also indicate the presence of strains that are latent under the conditions prevailing in these areas. Although there was a good correlation between the results of the BFT and the incidence of greening in the mother trees from which the budwood had been obtained, the majority of the BFT positives did not show visible greening symptoms within 3 years after testing.

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