Heat Requirements for Symptom Suppression and Inactivation of the Greening Pathogen

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IN A SURVEY in South Africa (3), it was noticed that greening symptoms are less severe in the hot, low-lying areas than in the cool, high-lying areas. There seemed to be a direct effect of heat on symptoms as well as on the build-up of psylla populations.

In this paper more information is given on the correlation between temperature and symptoms. In addition, experiments initiated to inactivate the greening pathogen by heat treatment of infected material are reported.

Experiments

SYMPTOMS OF GREENING IN RELATION TO ALTITUDE.—An attempt was made to assess greening in infected orchard trees for the Malelane, Karino, Nelspruit, and White River areas in the eastern Transvaal. Orchards were inspected in June and July, when the symptoms show up most clearly. Symptoms were classified qualitatively and related to altitude and to 2 different heat indices based on temperature records. The first index is the annual total Centigrade degree-hours above 30°C (DH/30). It was determined for the 3 years ending in June of 1966, 1967, and 1968. The second index is the number of days per annum having a maximum temperature less than 25°C (25/Tm). Values of 25/Tm were computed for 10–15 years. Fruit and leaf symptoms in orchards of the eastern Transvaal at different altitudes were as follows.

Malelane (366 m).—No symptoms were seen in in-season fruit; out-of-season fruit set in late summer had distinct symptoms. Only a few leaves of single twigs had zinc-deficiency leaf symptoms.

Karino (549 m).—A small percentage of in-season fruit had relatively indistinct symptoms. Only single branches of affected trees had typical zinc-deficiency patterns.

Nelspruit (671 m).—In general, a larger percentage of fruit had symptoms, but they were commonly limited to sectors of trees. Inside fruit had the clearest symptoms. Leaves of affected sectors usually had clear zinc-deficiency and yellow-vein patterns.

White River (914 m).—Up to 100 per cent of the fruit displayed symptoms. Both fruit and leaf symptoms were severe, often on all tree parts.

These observations imply that the altitude range more or less along latitude 25.5°S in the eastern Transvaal can be divided into zones of potential symptom development (Fig. 1). Below 450 m, for example, in-season fruit are not visibly affected by infection, and consequently there is no potential for symptom development.

The DH/30 decreases logarithmically with increasing altitude along latitude 25.5°S (Fig. 1). If heat is the primary factor in the relationship between altitude and potential symptom development, then it may be deduced that a mean annual DH/30 in excess of 1200 will prevent development of symptoms, whereas a DH/30 less than 300 will allow them to develop freely.

Table 1 indicates mean annual DH/30 values for 3 additional citrus areas. The value for Tzaneen suggests that symptom development there is similar to that of Nelspruit, a result borne out by orchard inspection. Although lack of vector activity makes Addo a greening-free area, infected experimental trees taken into the area develop distinctive symptoms as would be expected from the relatively low value of DH/30. The high value of DH/30 for Rustenburg suggests total inhibition of symptoms. Orchard inspections, however, contradict this, and indicate that the potential for symptom development is in fact slightly higher than for Nelspruit.

There is a distinct possibility that greening symptoms at Rustenburg are caused by a strain of the agent different from that in the eastern

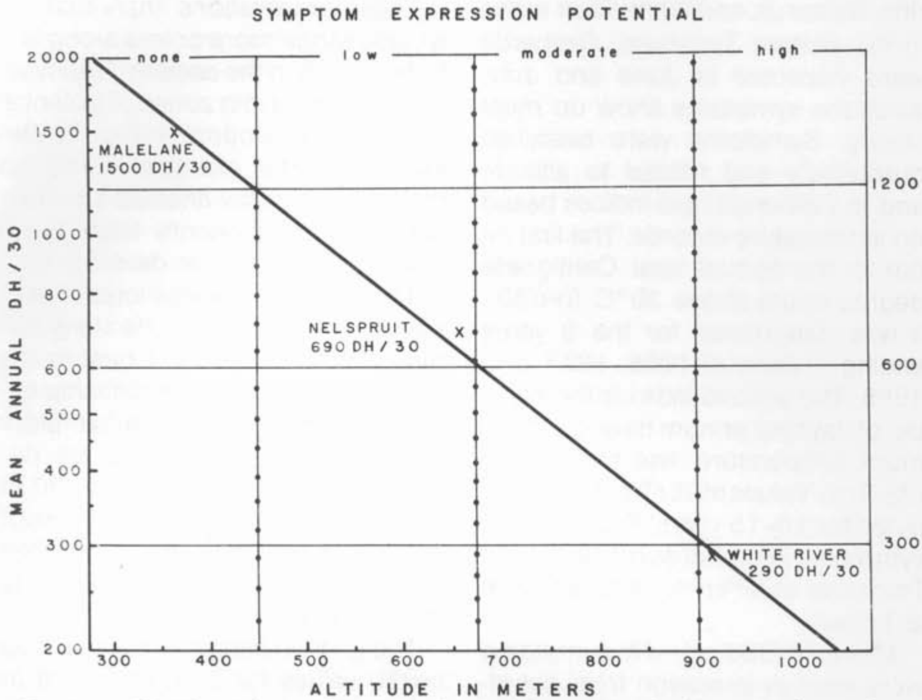

FIGURE 1. *The dependence of classes of potential symptom development of greening on altitude and heat along latitude 25.5°C near the escarpment of the eastern Transvaal.*

Transvaal (6), which may react differently towards heat. However, it must also be stressed that the high DH/30 value for Rustenburg is primarily the result of a short but extremely hot summer period, the remainder of the year being considerably cooler than, for instance, Malelane, which also has a high DH/30 value. The temperature range between seasons at Rustenburg is in fact considerably greater than for all areas along the eastern Transvaal Escarpment including Tzaneen, Nelspruit, White River, and Malelane. The high proportion of cool days at Rustenburg in relation to similarly "hot" (± 1200 DH/30) escarpment areas may therefore counteract the effect of the high DH/30 level.

Consideration of the value of 25/Tm in relation to symptoms at Malelane, Nelspruit, and White River shows that the higher the value, the more extensive the symptoms (Table 1). The importance of cool days under Rustenburg conditions appears to be confirmed by the fact that 25/Tm, in contrast to DH/30, suggests a slightly higher prevalence of symptoms at Rustenburg than at Nelspruit. This index, furthermore, implies a high potential for symptom development at Addo, a potential at Tzaneen similar to that at Nelspruit, and high and very low potentials for Malkerns and Big Bend (Swaziland), respectively.

HEAT INACTIVATION EXPERIMENTS: ARTIFICIAL HEAT TREATMENT.—In a preliminary study, budwood from greening-infested Washington Navel, Pineapple orange, and Olinda Valencia orchards in the Nelspruit area was heated for 1 hour at 49°C. The budwood was taken from twigs immediately behind an infected fruit. It was placed on a grid suspended over the water surface in a thermostatically controlled hot water bath. The thermostat was so regulated that the water-saturated air above the grid had the desired temperature. Ten rough lemon seedlings were each inoculated with 2 treated buds, and another 10 were similarly inoculated with untreated buds. One year after the buds had grown out, the shoots were checked for visible leaf symptoms of greening and the bark tested with the thin layer chromatography (TLC) fluorescence method (5). When the latter test was positive the material was recorded as infected, irrespective of the presence or absence of definitive external symptoms.

The results (Table 2) show that a heat treatment of 1 hour at 49°C is not sufficient for inactivation. On the other hand, because of the low percentage of transmission even in the control, the greening-infected "Nelspruit" material proved to be unsuitable for the heat treatment experiment (4, 6). It appears possible from the data that there is a higher bud transmissibility of the greening pathogen in Navel trees than in the Valencia and Pineapple cultivars.

In a second experiment, budwood from infected Valencia trees from the university orchard at Pretoria was used because it gave a high percentage of transmission. The procedures were otherwise the same as in the preliminary experiment. After budding, the inoculated rough lemon seedlings were transferred to a glasshouse with a constant temperature of 21–23°C. The results are based both

TABLE 1. MEAN ANNUAL VALUES OF TWO HEAT INDICES IN CERTAIN CITRUS-PRODUCING AREAS, DH/30 BEING THE TOTAL CENTIGRADE DEGREE HOURS ABOVE A THRESHOLD OF 30° C, AND 25/TM THE NUMBER OF DAYS WITH THE MAXIMUM TEMPERATURE LOWER THAN 25° C

Area	DH/30	25/Tm	Severity of symptoms[a]
White River, eastern Transvaal	290	176	5
Nelspruit, eastern Transvaal	692	122	3
Malelane, eastern Transvaal	1498	105	1
Tzaneen, northeastern Transvaal	727	131	3
Rustenburg, western Transvaal	1244	149	4
Addo, Eastern Cape	572	186	4
Malkerns, Swaziland		171	5
Big Bend, Swaziland		67	1

a. The class numbers being from 1 (very mild) to 5 (very severe).

on the greening symptoms visible 1 year after grafting and the TLC fluorescence test, but the fluorescence test was taken as the main criterion.

The results (Fig. 2) give an indication of the lethal, the inactivating, and the noninactivating temperature-time combinations. The area between the solid and the broken line is more or less the area in which inactivation of the greening pathogen occurs. The best results were achieved with the following treatments: 51 °C at 60 min, 49°C at 120 min, and 47°C at 240 min. A problem in assessing the results was the low percentage of transmissions in the control (4 pos., 16 neg.) despite the fact that the budwood was taken at a time of maximum transmissibility and that the "Pretoria" strain (4, 6) was used. Thus, the apparent inactivation in some less severe heat treatments may have been due to the low rate of transmission. Lin (1) obtained inactivation of the yellow shoot pathogen—possibly related to the greening pathogen—with similar temperature-time combinations. His citrus material, however, appears to have been more tolerant to heat since he obtained survival of a certain number of buds after subjecting them to treatments found to be lethal in the present study. Olson and Rogers (2) obtained heat inactivation of the stubborn pathogen at treatments of 51 °C for 90 min and 120 min. The citrus material appears again to have been more tolerant to heat than ours since a 45 per cent survival was obtained at temperature-time combinations that proved fatal for the budwood in the present experiment. Olson and Rogers did not obtain inactivation at 51 °C for less than 60 min and at 49 °C or 50 °C for less than 2 hours, treatments that are sufficient for inactivation of the greening pathogen. The stubborn pathogen thus appears less sensitive to heat than the greening pathogen. This finding is in accordance with the observations that greening symp-

TABLE 2. INCIDENCE OF GREENING SYMPTOMS IN THE SHOOTS FROM UNTREATED AND HEAT-TREATED BUDS FROM NAVAL, VALENCIA, AND PINEAPPLE SWEET ORANGE TREES; HEAT-TREATED BUDWOOD WAS EXPOSED FOR 1 HOUR TO WATER-SATURATED HOT AIR AT 49 °C

	Palmer	Navel	Olinda	Valencia	Pineapple	
	Untreated	Heat-treated	Untreated	Heat-treated	Untreated	Heat-treated
No. buds	40	40	40	40	40	40
No. buds surviving	27	21	29	35	38	53
No. buds with shoots showing symptoms (8 months after budding)	4	2	1	1	1	2

toms show up best under cool conditions (4), and that those of stubborn show up best under hot conditions (2).

In a third experiment, 5-year-old Valencia trees with severe greening symptoms were removed from an orchard at Nelspruit in July; the soil was washed carefully from their roots. Six trees per treatment were subjected to the various temperature-time treatments in the water-saturated atmosphere of a tobacco drying oven. One year after treatment 3 bark samples from each tree were subjected to the fluorescence test.

The results (Table 3) show that whole trees can be treated at higher temperature-time combinations than budwood. A treatment for 30 min at 54–56°C is fatal for budwood, but when whole trees are treated in the same way some survive. Possibly the meristem is more protected under the bark of the trunk and thick branches than under the bark of the thin branches from which buds were obtained. The new growth of the heat-treated trees did not show

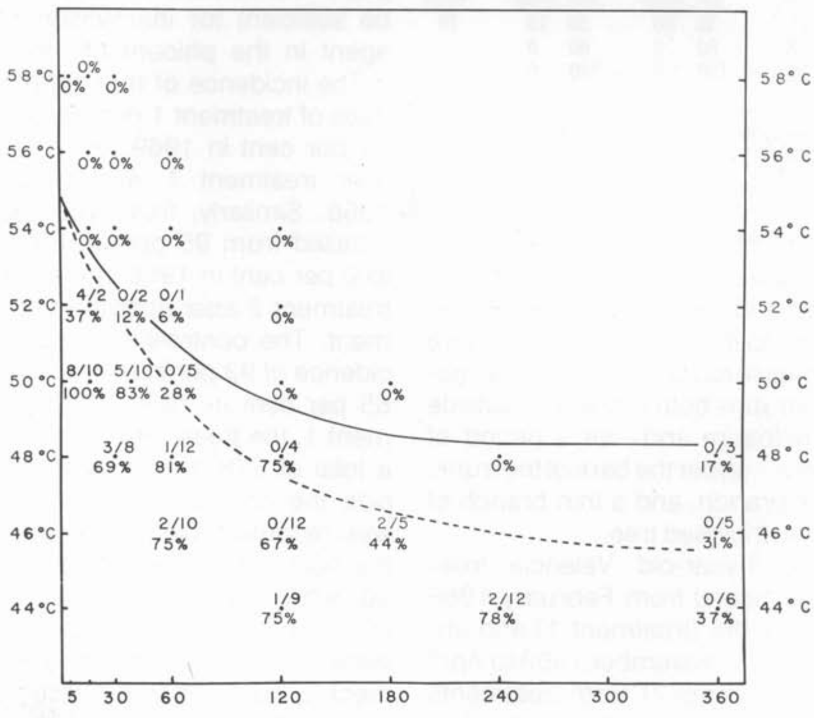

FIGURE 2. *Heat treatment of greening-infected sweet orange buds (Pretoria strain) at various combinations of time and temperature. Per cent figure is survival of treated buds. Fractions are positives/negatives surviving treatment. The results are based on the albedo fluorescence test. The curve was fitted visually. Untreated control = 4 positive, 16 negative.*

greening symptoms; otherwise the experiment has so far given no evidence of the effect of the treatments on the pathogen.

NATURAL HEAT TREATMENT.—Tree enclosures were constructed by screwing together four 3 m x 1 m fiber glass sheets to form a hollow cylinder, the top being securely covered by a sheet of polythene perforated by a small hole to allow rain water to drain freely. An enclosure was placed over each tree to be treated and secured to 2 iron poles driven into the ground. Temperature was measured by means of thermo-hygrographs both inside and outside the enclosure and—for a period of 3 weeks—under the bark of the trunk, a thick branch, and a thin branch of a single enclosed tree.

TABLE 3. EFFECT OF TREATING WHOLE TREES INFECTED WITH GREENING VIRUS AT DIFFERENT TEMPERATURE-TIME COMBINATIONS

46–48°C		50–52°C		54–56°C		Control
Min	Index[a]	Min	Index	Min	Index	Index
60	18	30	20	30	13	19
120	22	60	0	60	0	
180	14	180	0	120	0	
240	20					

a. Derived by summing class numbers for the 6 trees for each treatment; the class numbers are as follows: 0, dead; 1, tree alive but no growth; 2, weak growth; 3, medium growth; 4, vigorous growth.

Eight 3-year-old Valencia trees were enclosed from February 1968 to April 1968 (treatment 1) and another 8 from November 1968 to April 1969 (treatment 2). Both treatments were carried out in the orchard at Woodhouse (altitude 823 m), where symptoms of greening are severe and where the population density of citrus psylla is normally high. Regular spraying, however, prevented a build-up of psylla on the treated trees.

At the time of the daily maximum, the temperature in the fiber glass enclosure was 8–10°C higher than the outside ambient temperature. When compared with the temperature of the air in the fiber glass enclosure, the temperature under the bark of the trunk was about 6° lower, and under the bark of thin branches 2°C higher when sun-exposed and 1°C lower when shaded. Thus, even if the temperature in a cage is sufficient for inactivation of the pathogen in the phloem of thin branches, it might not be sufficient for inactivation of the agent in the phloem of the trunk.

The incidence of fruit greening in trees of treatment 1 decreased from 94 per cent in 1969 to 4 per cent after treatment 1 was applied in 1968. Similarly, fruit greening decreased from 95 per cent in 1968 to 0 per cent in 1969 in the trees of treatment 2 after applying the treatment. The controls showed an incidence of 93 per cent in 1968, and 85 per cent in 1969. During treatment 1, the trees were subjected to a total of 678 DH/30, whereas outside the enclosure only 13 DH/30 was recorded. During treatment 2, the heat application was 1911 DH/30, while outside the cage it was 299 DH/30. The fruit symptoms were totally suppressed by treatment 2, but treatment 1 also had a partial effect. This was in spite of the fact that the treatment was carried out late in the development of the fruit and during relatively cool months.

Conclusions

From the altitude-heat-symptom prevalence relationship, it could have been predicted that greening symptoms would be totally inhibited by a seasonal heat application in excess of the 1,500 DH/30 (Malelane) level. The fact that fiber glass enclosures raised the natural heat level at Woodhouse from 298 DH/30 (approximately the same as for White River) to 1911 DH/30 and simultanously inhibited symptoms of greening would confirm such a prediction. The fact that infection persists in Malelane, and also that the difference in DH/30 levels between Malelane-untreated and Woodhouse-treated is relatively small, implies that infection is still present in Woodhouse-treated trees and may be expressed in future years under normal heat conditions.

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