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Variable Transmission of African Greening to Sweet Orange

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ABSTRACT. Bud transmissibility of three greening disease isolates from different zones of South Africa was compared. Isolates from zones of low greening incidence were transmitted more readily than an isolate from a zone of high greening incidence. The transmissibility of greening by buds and grafts was equal, but higher than with bark strips, fruit stems and fruit stem bark. Increasing the number of buds, or the length of the grafts, did not increase transmissibility. The disease may remain in transferred infectious buds, without symptom development by the host, over a 12-month period. Where infection was transmitted by confining the insect vector to the growth tip, the main reactions of sweet orange seedlings were dieback, terminal growth with severe symptoms, no terminal growth but healthy or symptomatic lateral growth. Greening symptoms developed on lateral growth 40 cm downward from the area of infection, 12 months after feeding, indicating downward movement of the organism.

Transmission of the organism of African greening was demonstrated by McClean and Oberholzer in 1965 (8, 9). Mature citrus trees usually display sectorial symptomatology indicating a restriction on the organism's movement (7). Differences in the transmissibility of greening disease from different sources were found (9, 15). Schwarz (15) and subsequently Marais and Rea (6) suggested the occurrence of different strains.

Despite present knowledge of greening disease which is being implemented to control the disease, it is still spreading (1, 2, 18). The transmissibility of the disease was therefore reassessed. Vector transmission studies has been published elsewhere (17, 20). This report is on the transmission of greening disease by plant tissue and the distribution of the organism after vector transmission in the glasshouse and in the field.

MATERIALS AND METHODS

Trial 1: Transmissibility of isolates. Greening-infected citrus budwood was collected in three citrus growing areas where the incidence of greening differs (13) and maintained in a glasshouse as three isolates (Table 1). Buds of each isolate were budded separately on disease-free glasshouse-grown Madame Vinous sweet orange seedlings. One, two or four buds were budded on each seedling for each isolate and replicated six times. After inoculation the plants were cut back to force growth and kept in a glasshouse at 20/24 C night/day temperatures. The inoculated buds were kept taped to prevent them from growing and seedling growth was kept to one shoot. The plants were inspected regularly for greening symptoms (10), and after 12 months all the plants were tested by thin layer chromatography to confirm disease infection (19).

TABLE 1
BUD TRANSMISSIBILITY OF THREE GREENING DISEASE ISOLATES

Isolate	Positive transmission (%)			
	No of buds inoculated			Mean
	1	2	4	
Rustenburg	0	17	33	17
Nelspruit	50	50	50	50
Karino	33	67	67	56
Mean	28	45	50	

Trial 2: Comparison of tissue inoculations. Greening-infected material of the Nelspruit isolate was used. Different infected tissue was used to inoculate healthy glasshouse grown Madame Vinous sweet orange seedlings. Each method was replicated six times. After inoculation the plants were treated as in the previous trial. Budding and grafting was employed according to normal practices but large tissue required additional care. The following tissues and inoculating methods were used: *i*) One bud; *ii*) Two buds; *iii*) Three buds; *iv*) Bark strip without buds, 1 cm long; *v*) Bark strip without buds, 2 cm long; *vi*) Bark strip without buds, 4 cm long; *vii*) Bark and buds of a fruitstem, 1 cm long; *viii*) Bark and buds of a fruitstem, 2 cm long; *ix*) Bark and buds of a twig/fruitstem, 4 cm long; *x*) Side grafting a 2 cm long twig; *xi*) Side grafting a 4 cm long twig; *xii*) Side grafting a 2 cm long fruitstem; *xiii*) Side grafting a 4 cm long twig/fruitstem.

Trial 3: Movement of the pathogen after infection. Tissue infection. Asymptomatic plants in Trial 2 were used after assessment of the trial. Symptomless plants in treatments *i*, *ii* and *iii* (Table 2) were cut back and the buds used for inoculation, were opened and allowed to grow. Symptomless plants in treatments *iv*, *v* and *vi*, where the infected tissue did not have buds, were used as controls. These plants

were cut back to above the inoculation site and the seedlings allowed to regrow. Plants in both groups were treated as previously.

Vector infection. Healthy glasshouse-grown Madame Vinous sweet orange seedlings with an approximate height of 50 cm were used as receptor plants. Adult citrus psylla (*Trioza erytreae* Del G.), the insect vector of greening disease (8), were collected from diseased plants and transferred to the active growth points of the healthy receptor plants. Small transparent plastic cages were used to keep 20 insects confined to each growth point. After 7 days of feeding, the plants were sprayed with a suitable insecticide to kill all insects and eggs. The plants were kept in a glasshouse at 20/24 C night/day temperatures. Side shoots which developed after insect feeding were not removed. The development of greening symptoms were monitored for 12 months.

Trial 4: Distribution of greening in field trees. Trees with sectorial single branch infections were selected during winter in a 25-yr-old Valencia sweet orange orchard. The infected branches were removed by cutting them at different thicknesses towards the main trunk (Table 3). Wounds were treated with a tree-sealing compound. Suckers which developed at the cut during the following season were not removed. The number of branches

TABLE 2
GREENING TRANSMISSION WITH DIFFERENT INFECTED PLANT TISSUE

Infected tissue	Survival (%)	Positive transmission (%)
One bud	100	50
Two buds	100	50
Four buds	100	50
Bark strip, 10 mm	100	0
Bark strip, 20 mm	100	0
Bark strip, 40 mm	100	17
Fruit stem bark and buds, 10 mm	67	0
Fruit stem bark and buds, 20 mm	83	0
Twig and fruit stem bark and buds, 40 mm	100	17
Side graft, 20 mm (twig)	100	67
Side graft, 40 mm (twig)	83	50
Side graft, 20 mm (fruit stem)	0	17
Side graft, 40 mm (twig and fruit stem)	67	50

TABLE 3
DEVELOPMENT OF SUCKERS AND THE PERCENTAGE WITH GREENING SYMPTOMS
AFTER THE REMOVAL OF INFECTED BRANCHES

Branch diameter (mm)	Number	Branches with sucker development (%)	Suckers greening positive (%)
10 to 19	30	0	0
20 to 29	30	23	86
30 to 39	20	35	43
40 to 60	20	35	29

which developed suckers and the number of suckers which developed greening symptoms were assessed 12 months later.

RESULTS

Transmissibility of isolates. The percentage transmissibility of the three isolates is given in Table 1. Transmissibility of the Karino isolate increased when the inoculated diseased buds were increased from one to two but not from two to four. No differences occurred with the Nelspruit isolate when one, two, or four buds were used for inoculation. The percentage transmissibility of the Rustenburg isolate was always low. No transmission occurred when one bud was used for inoculation but transmission increased according to the increased number of buds.

Root and top-growth of the plants with the three different isolates did not differ but leaf symptomatology did. Symptoms of the Rustenburg isolate were a blotchy mottle with normal sized leathery leaves (primary symptoms), while symptoms of the Nelspruit and Karino isolates were zinc deficiency-like, with small leaves (secondary symptoms) (5).

Comparison of tissue for transmission. The percentage survival of the inoculated material and greening transmission with each treatment are given in Table 2. Except for fruit stems, the survival of the tissue was good and negative transmissions could not be attributed to the absence of diseased tissue. The best transmission

was achieved using buds and grafts. Increasing the number of buds and the length of grafts did not contribute to a higher transmissibility. Transmission using bark strips and fruit stems was low. A combination of fruit stems and twig stems increased the survival rate and transmission of the disease was equal to that of twig stems only.

Movement of the pathogen after infection. Tissue infection. Twenty-one infected buds on nine plants grew and all developed greening symptoms. Two of 17 plants with bark strips without buds (control) developed greening symptoms.

Vector infection. Greening symptoms were always first observed on the part of the plant where the vector fed. The following reactions of the plants were observed: *i*) They grew normally. Possibly no transmission of the pathogen occurred; *ii*) The growth point where the insects fed died. Subsequent growth was normal; *iii*) Terminal growth with severe symptoms developed; *iv*) No terminal but lateral growth developed. The shoots were either normal or developed greening symptoms; *v*) Dieback of symptomized feeding area. Subsequent lateral growth was symptomless.

Distribution of greening in field trees. The development of suckers and the percentage with greening symptoms after removal of infected branches of different sizes are given in Table 3. Sucker development increased when thicker branches were removed but greening infection was lower in these suckers.

DISCUSSION

One isolate from each of the zones described by Schwarz (13) was used in this study. The Rustenburg isolate which was from an area with a high degree of infection, had the lowest transmission rate. A high level of transmission was achieved with the Nelspruit and Karino isolates. The high percentage transmission using bud tissue is in contrast with McClean and Oberholzer (9) and McClean (7) but agrees with the results of Marais and Rea (6). The high transmissibility of the Nelspruit and Karino isolates indicates a higher titre of pathogen which will also favour insect transmission (20).

Except with fruit stems, a good survival rate was achieved with the inoculated material. Negative transmissions therefore cannot be attributed to the absence of diseased tissue. The highest transmission rate was achieved by the use of infected buds and graft sticks. The number of buds and the length of the sticks did not make a difference on the transmission rate. Transmission using bark strips and fruit stems (bark and sticks) was low. Although Marais and Rea (6) also had a lower transmission rate with bark than with buds, their percentage transmission was higher than in this study.

Several researchers attempted to transmit African greening using various infected tissue with mixed success (6, 7, 9, 15). In the transmission trial with different plant tissue, only 50% of the plants developed symptoms after inoculation with infected buds. When the asymptomatic plants were cut back and the inoculated buds allowed to grow, all developed symptoms. This showed that the organism was present but did not move in the host plant over a period of 12 months. This result corresponds with the findings of McClean (7) that the pathogen does not readily pass to progeny trees propagated by buds from infected trees, possibly because of sieve tube necrosis (9). The

uneven distribution of the greening pathogen (3) and low titre because of climatic conditions (14) can contribute to a low transmission rate in which case higher transmissions can be achieved by using stem pieces.

Host plants reacted differently after vector feeding. The most severe reaction was dieback. Two types of dieback occurred. Firstly, the growth point died within two weeks after feeding. The dead area varied from a few millimeters to 4-5 cm with a definite abscission point. Second, symptoms developed at the feeding site after 3 months and subsequently died. In both cases regrowth was either healthy or diseased. The host apparently abscised tissue carrying the organism. This agrees with the statement by Moll and van Vuuren (12) that citrus is not a good host for the greening organism and that partly infected trees can recover if re-infections by the vector do not occur (16). It is possible that a high concentration of the greening organism will induce dieback (protection mechanism or hypersensitivity) since the organism usually occurs in low concentrations in citrus material (4, 11).

In this study greening symptoms developed approximately 40 cm back from the site of vector feeding after 12 months. This is in contrast to the findings by McClean and Schwarz (10) that the greening organism does not move back in a plant. The results of this study explain why a high percentage of suckers which develop after the removal of infected branches are infected even if effective vector control is applied.

No suckers developed when branches 1-2 cm thick were removed. Where branches of 2-3 cm thick were removed, 23% developed suckers of which 86% were infected with greening. Although no special control measures were applied to control the greening vector, it appears that less greening inoculum was removed in comparison with the removal of 4-6 cm branches. More suckers developed in the latter treatment but a lower percentage was infected with greening. This indicates

that when infected branches are removed, the cut should be made well into apparently healthy tissue. Sucker

development can be reduced by the correct cut (18) or by chemical treatment (1).

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