Greening Disease, the South African Situation

J. N. Moll, S. P. Van Vuuren, and D. L. Milne

Although greening disease was first reported in South Africa in 1928 (Oberholzer, 1965), the first findings which led to its control were reported by Schwarz (1964), who trapped a "virus" in orchards where greening was common and postulated its transmission by a psyllid vector. McClean and Oberholzer (1965*a, b*) confirmed not only his findings regarding the viral incitant but also that citrus pyslla, *Trioza erytreae* (Del Guercio), is indeed the vector of greening disease in South Africa.

Since then, Laflèche and Bové (1970) showed the disease to be caused by what seemed, at first, to be a mycoplasmalike organism (MLO), which was subsequently proved to bear greater similarities to bacteria than to mycoplasmas (Moll and Martin, 1974).

The ultimate aim of these investigations was to devise a control for the disease which, especially in the cooler regions of South Africa, has reached such epidemic proportions that the citrus industry is severely threatened and, in fact, in some areas already has been abandoned. While a complete cure which could be used to eradicate the disease has not been achieved, a means has been developed to control it by chemotherapy, which now is being widely used in this country both to control light infections and to resuscitate orchards which have been all but abandoned due to heavy losses from greening.

In this paper, we sketch work done since that of Laflèche *et al.* (1970) and give a resume of current trends in the control of greening disease in South Africa.

INSECT TRANSMISSION AND VECTOR CONTROL

As early as 1965, field trials indicated citrus psylla as the vector of greening disease (McClean and Oberholzer, 1965*b*) but the mechanics of this transmission were elucidated only in 1973 (Moll and Martin, 1973), when a light and electron microscopic study was made of psyllids which had fed on infected plant tissue. Light micrographs revealed high concentrations of the organism in the hemolymph of psyllids which had fed for 30 days on infected citrus. Electron microscopy revealed organisms occuring singly in the hemolymph after 21 days feeding (fig. 1) but after 30 days (fig. 2) organisms were present in great numbers in the hemolymph and had penetrated most organs of the insect, including the salivary glands (fig. 3).

The mechanics of transmission are therefore assumed to be that the organism is ingested by the phloem-feeding adult psylla and penetrates the gut during a latent period of about 21 days after which it proliferates in the hemolymph, penetrates the salivary glands and is injected with saliva into the next plant during feeding (fig. 4).

It was found that 5 days of acquisition feeding is sufficient to initiate this transmission process and that when the insect becomes infective, after the latent period, transmission of the pathogen can occur in less than 60 minutes.

In the search for pesticides for psyllid control, the focus previously has been on foliar sprays, but now, due to the relatively universal acceptance in South Africa of biological or integrated pest control systems, a new application method had to be devised. Recently attention has been given to the possible use of soil systemics (Milne, 1976; Milne and De Villiers, 1976, 1977) applied either directly or via low-volume irrigation systems such as drip or microjet.

Dimethoate showed considerable promise in controlling not only psyllids, but thrips and aphids; also it affords long term protection. It is envisaged that this insecticide will be used as a preventive measure, being applied just

prior to flush periods and in this way protecting the trees during periods of maximum psyllid build-up and greening transmission.

IDENTIFICATION OF THE CAUSAL ORGANISM

As all attempts to culture the causal organism have failed it was decided to base the identification of the agent on the findings of a comparative electron microscopic study (Moll and Martin, 1974). The morphology of the greening organism and more specifically the structure of its cell envelope were compared with that of a plant pathogenic MLO, two vascular pathogenic bacteria, *Xanthomonas campestris* and *Pseudomonas solanacearum*, and the rickettsia-like organisms causing clover club leaf (RLO-c) and phony peach disease (RLO-p).

This study revealed that the causal organism of greening disease has a cell wall reminiscent of that of Gram-negative bacteria; the only observable difference being the total absence of an R- or mucopeptide-layer (fig. 5).

CONTROL OF GREENING DISEASE

When Lafleche and Bové (1970) showed greening to be associated with an organism, and not a virus as previously suspected, they indicated the probability that a method could be devised to control the disease by means of chemotherapy.

In the absence of a systemic bactericide or antibiotic, the first step was to develop a method for introducing an effective bactericide into the phloem where the causal organism occurs. Schwarz and Van Vuuren (1971) injected antibiotic solutions under pressure into the trunks of infected trees with the apparatus illustrated in fig. 6. It consists of a 2.5 liter plastic bottle (b) fitted with a bicycle valve (V) and two outlets with polythene tubes (T). The bottle is attached (fig. 7) to two holes (H), drilled to a depth of one-half the diameter of the tree trunk, by means of these polythene tubes (T) and brass connectors (C). The reason for drilling the holes to this depth is that the main movement of injected solutions is in the old nonfunctional xylem constituting approximately the central 60 per cent of the diameter of the trunk.

Using this technique, (Schwarz, Moll, and Van Vuuren (1972) determined that the most effective chemical for the control of greening disease is tetracycline hydrochloride. Tests showed (fig. 8) that a relatively high dose is required, varying from 4 g in 1 liter of water for a tree with a trunk circumference of 10 to 20 cm; to 20 g in 4 liters for a tree with a trunk circumference of 50 + cm. The concentration of the injected solution is kept below 8 g/liter as recrystallization can occur above this. The acidification of the water with about 1-2 ml of concentrated HCl per liter is often required where the water quality causes much crystallization; in these cases the antibiotic usually buffers the solution at a pH of about 3.

Despite careful examination, no trace of residues could be detected in either fruit or leaves 21 days after injection (Moll, 1974). This treatment should, therefore, present no hazards, either in itself, or in the possible disruption of intestinal flora of consumers, as fruit is picked between 90 and 180 days after injection.

The results obtained from such trunk injections are illustrated in fig. 8. It is also shown in fig. 9 that, barring reinfection by the psyllid vector, the level of infection is stable and actually tends to decrease, the reason being that the greening organism in South Africa is seemingly a relatively nonpersistent, ineffective pathogen of citrus.

Tetracycline HCl is fairly toxic to citrus, especially at the high dosage rate applied in South Africa, the symptoms being: (1) narrowing of the leaves to the shape of a peach leaf; (2) heightened photosensitivity resulting in a spotted necrosis of the leaf lamina, especially adjacent to the veins; (3) occasional defoliation and dieback of individual twigs; (4) brown staining of heartwood. None of these symptoms causes lasting

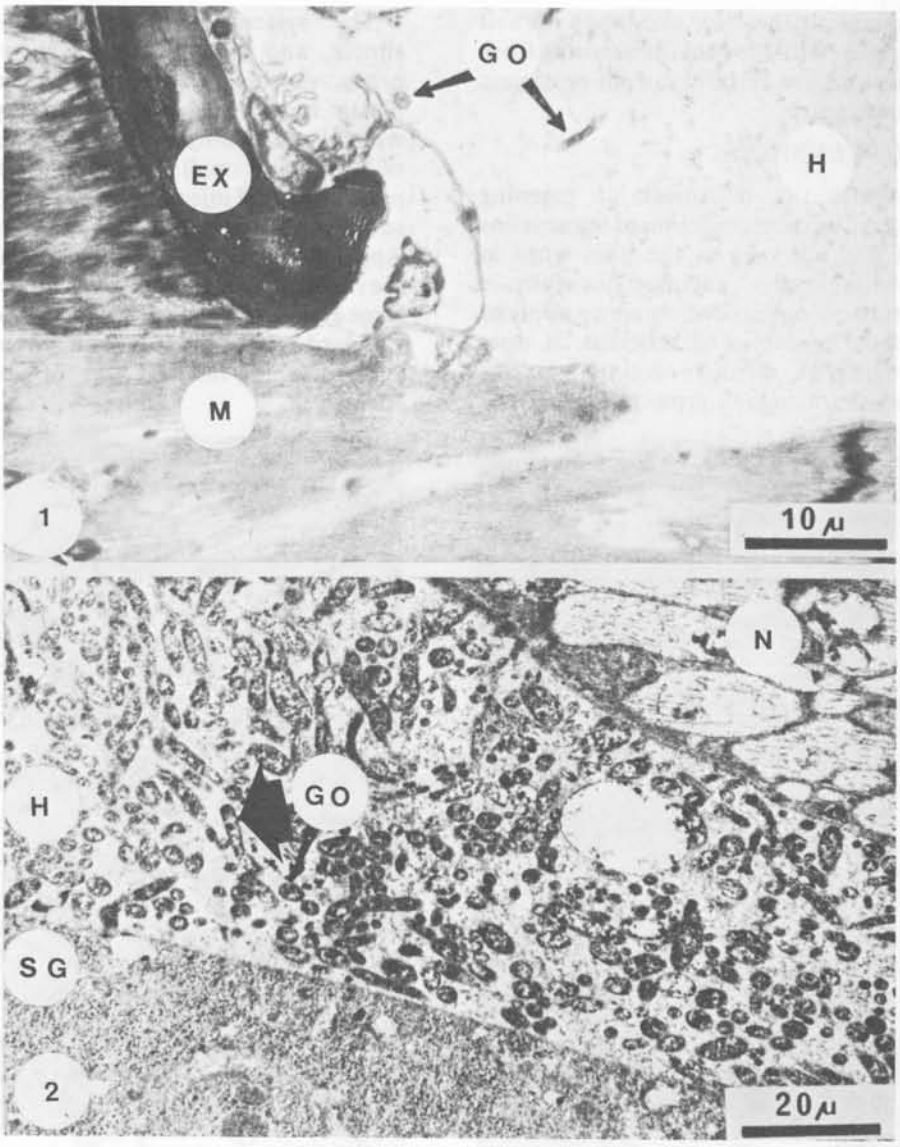

Fig. 1. Electron micrograph of greening organisms (GO) in the hemolymph (H) of an adult psyllid fed 21 days on infected citrus. EX = exoskeletal material; M = muscle tissue.

Fig. 2. Greening organisms (GO) in the hemolymph (H) of an adult psyllid fed 30 days on infected citrus. SG = salivary gland; N = nerve tissue.

damage to the tree; and when viewed together with the benefits accruing from injection are certainly of no economic importance.

CONCLUSIONS

While the treatment of greening disease by trunk injection of tetracycline HCl is not seen as the final word in greening control, and may, hopefully, in future be superseded by spray application of systemic bactericides, it does provide an effective counter to this disease for today's growers.

The system is relatively cheap, simple, and adaptable to a grower's needs, varying from the low pressure plastic bottle illustrated in this paper, which is admirably suited to the needs of relatively small growers, to high pressure, rapid injection bottles used by several large estates in South Africa. Application of this technique has led to the resuscitation of the citrus industry in large areas of South Africa and is now a standard orchard practice in virtually every greening-infested area of South Africa.

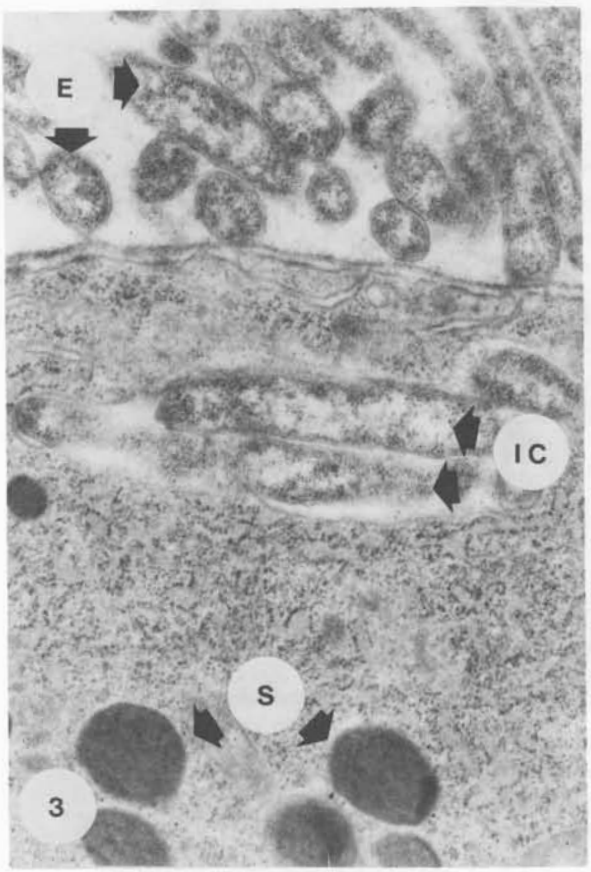

Fig. 3. Extracellular (E) greening organisms in the hemolymph and intracellularly (IC) in a salivary gland cell of an infective psyllid. S = saliva droplets.

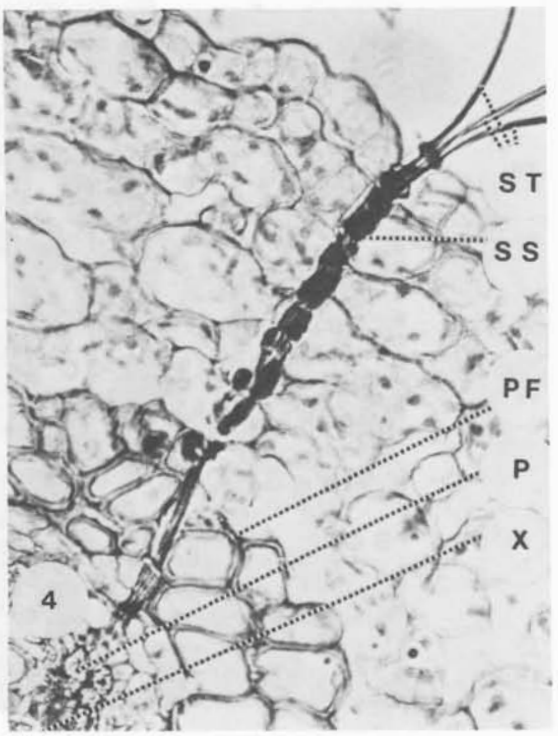

Fig. 4. Stylet of a feeding adult psyllid. The components of the stylet (ST) are coated with a salivary sheath (SS) and penetrate the phloem fibers (PF) into the phloem (P) and not the xylem (X).

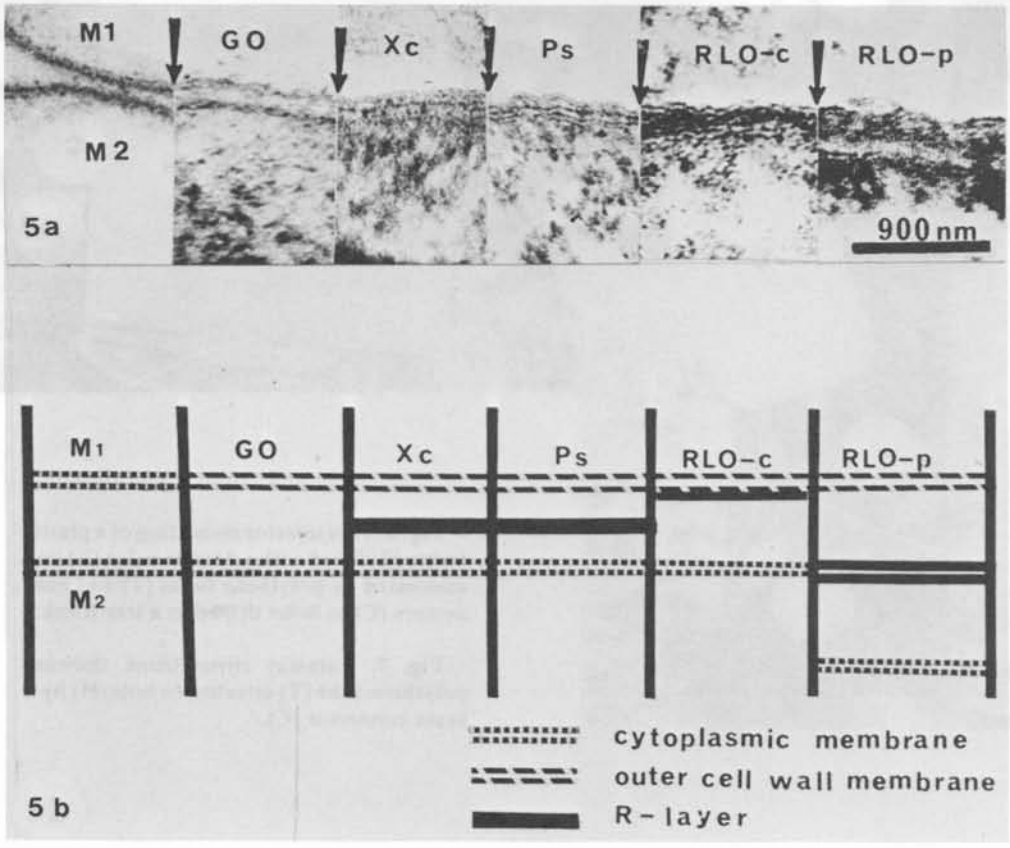

Fig. 5. Composite micrograph (a) and explanatory diagram (b) of cell envelopes of 2 adjacent MLO cells (m1 and M2), a greening organism (GO), a *X. campestris* cell (Xc), a *P. solanacearum* cell (Ps), a RLO-c cell (RLO-c) and an RLO-p cell (RLO-p).

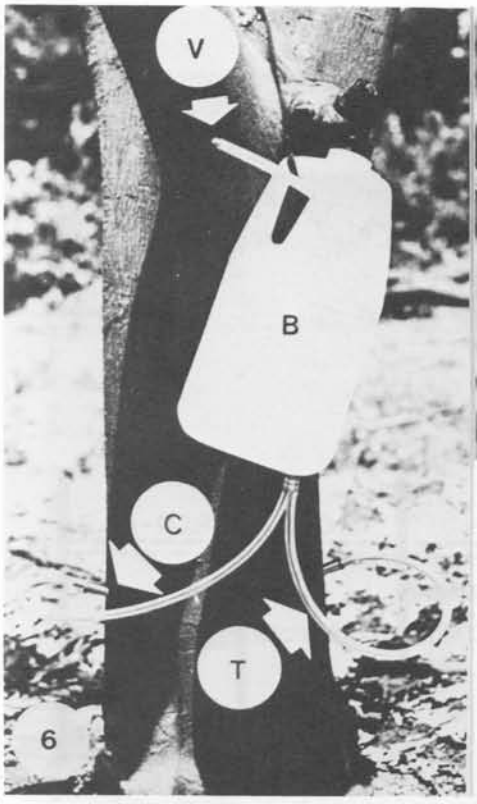

Fig. 6. Tree injector consisting of a plastic bottle (B) fitted with a bicycle valve (V) and connected by polythene tubes (T) and connectors (C) to holes drilled in a tree trunk.

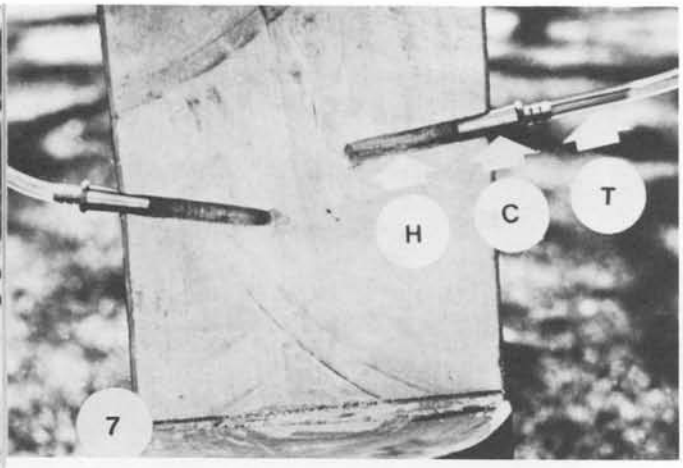

Fig. 7. Cutaway citrus trunk showing polythene tube (T) attached to hole (H) by a brass connector (C).

TABLE 1
TREES INFESTED WITH VARIOUS STAGES OF CITRUS PSYLLA, 6 WEEKS AFTER TREATMENT WITH DIMETHOATE (PERFEKTHION 40% EC AT 10 ml/m²)

	Trees infested with *psylla* per cent		
	Eggs	Nymphs	Adults
Control (18 trees)	100	78	22
Microjet treated (35 trees)	0	0	0
Dripper treated (30 trees)	3*	0	3†

* One growth point with eggs on one tree only.
† One adult on one tree only.

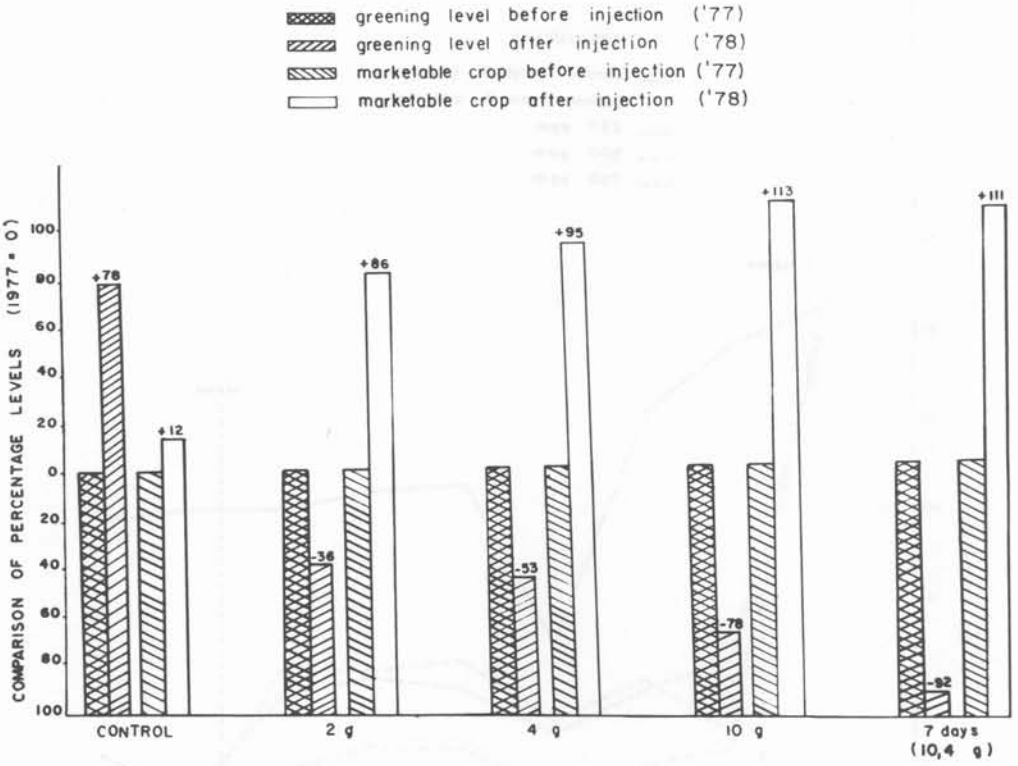

Fig. 8. Comparison of levels of greening infection and marketable crop before and after various injections; fruits individually assessed on five trees/treatment.

TABLE 2
TREES INFESTED WITH PSYLLIDS AND APHIDS 7-1/2 WEEKS AFTER TREATMENT WITH DIMETHOATE (PERFEKTHION 40% EC AT 10 ml/m^3).

	Percentage of trees infested with			
	Psyllids			Aphids
	Eggs	Nymphs	Adults	
Control (28 trees)	54	64	50	29
Microjet treated (46 trees)	26	2*	24	2
Dripper treated (23 trees)	39	4	30	8

* One nymph on one tree only.

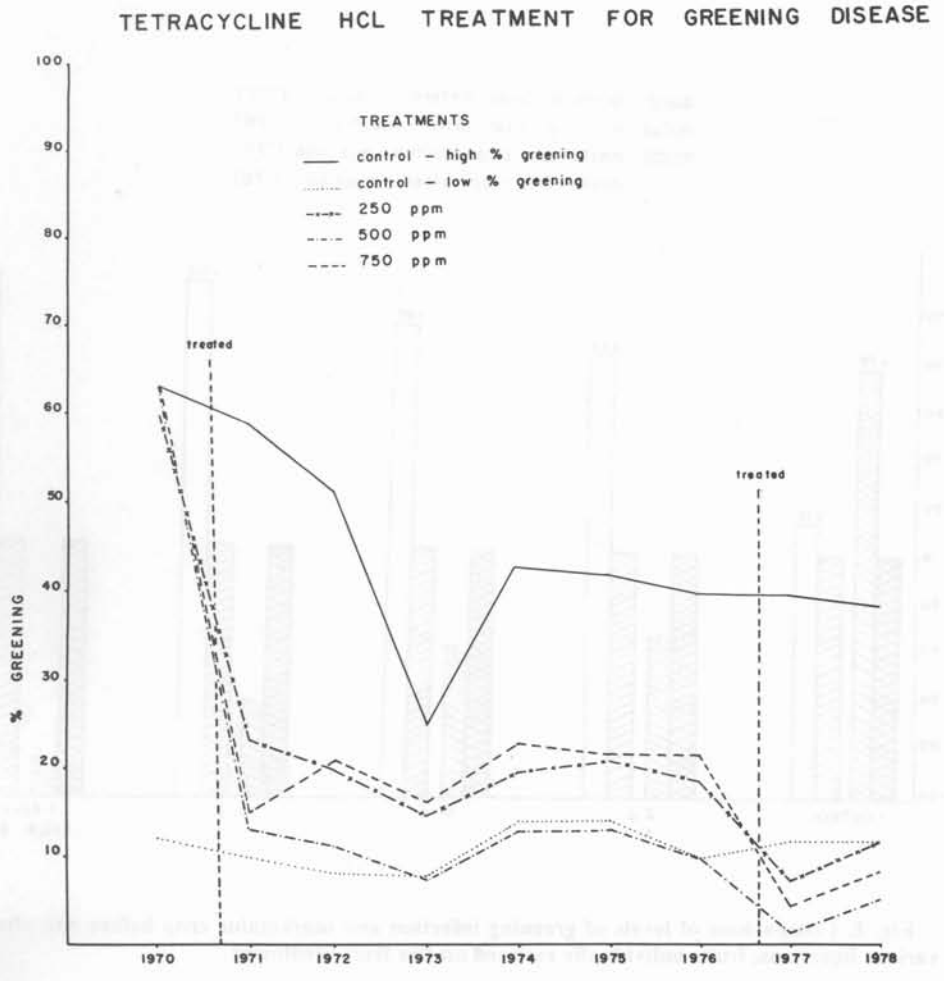

Fig. 9. Levels of greening infection as monitored for several years after tetracycline injections. Fruits were cut and examined under UV light for five trees/treatments.

LITERATURE CITED

LaFLÈCHE, D., and J. M. BOVÉ
1970. Structures de type Mycoplasme dans les feuilles d'orangers atteints de la maladie du "Greening." C. R. Acad. Sc. Paris, Ser. D., 1270: 1915-17.

McCLEAN, A. P. D., and P. C. J. OBERHOLZER
1965*a*. Greening disease of the sweet orange; evidence that it is caused by a transmissible virus. S. Afr. J. Agr. Sci. 8: 253-76.

McCLEAN, A. P. D., and P. C. J. OBERHOLZER
1965*b*. Citrus psylla, a vector of the greening disease of sweet orange. S. Afr. J. Agr. Sci. 8: 297-98.

MILNE, D. L.
1976. Experimental control of citrus thrips (*Scirtothrips aurantii*) in glasshouse trials. Citrus and Subtropical Fruit J. No. 483: 16-18.

MILNE, D. L., and E. A. DE VILLIERS
1976. Soil systemics against citrus thrips. Results of the 1974-75 field trials. Citrus and Subtropical Fruit J. No. 506: 17-20.

MILNE, D. L., and E. A. DE VILLIERS
1977. Soil application of systemic pesticides for control of thrips and nematodes on citrus. Citrus and Subtropical Fruit J. No. 518: 9-18.

MOLL, J. N.
1974. Fluorometric determination of antibiotic residues in citrus trees injected with tetracycline hydrochloride, p. 198-201. *In* Proc. 6th Conf. IOCV. Univ. California Div. Agr. Sci., Richmond.

MOLL, J. N., and M. M. MARTIN
1973. Electron microscope evidence that citrus psylla (*Trioza erytreae*) is a vector of greening disease in South Africa. Phytophylactica 5: 41-44.

MOLL, J. N., and M. M. MARTIN
1974. Comparison of the organism causing greening disease with several plant pathogenic Gram-negative bacteria, rickettsia-like organisms and mycoplasma-like organisms. INSERM 33: 89-96.

OBERHOLZER, P. C. J.
1965. Oorsig oor ondervindinge met vergroening, p. 9-20. *In* Report on Colloquium on citrus greening. Citrus and Subtropical Fruit Research Institute, Nelspruit.

SCHWARZ, R. E.
1964. An insect-transmissible virus, trapped on sweet orange seedlings in orchards where greening disease is common. S. Afr. J. Agr. Sci. 7: 885-89.

SCHWARZ, R. E., J. N. MOLL, and S. P. Van VUUREN
1972. Control of citrus greening and its psylla vector by trunk injections of tetracyclines and insecticides, p. 26-29. *In* Proc. 6th Conf. IOCV. Univ. California Div. Agr. Sci., Richmond.

SCHWARZ, R. E., and S. P. Van VUUREN
1971. Decrease in fruit greening of sweet orange by trunk injection of tetracyclines. Plant Dis. Rep. 55: 747-50.