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Australian Citrus Dieback

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A disease of citrus, with similarities to greening, Indian dieback, leaf mottle and likubin, is increasing in citrus growing areas of eastern Australia. Surveys have been carried out to determine distribution and extent of the disease, symptom development has been examined in major citrus varieties, a search for insect vectors commenced, observations made on its rate of spread in trees of various ages, and

effect on yield and tree deterioration examined. The similarity of many of the symptoms to those of greening prompted a search for mycoplasma-like bodies in phloem cells by electron microscopy. The effect of tetracycline treatment of infected seedlings and the status of gentioglucoside in bark and albedo have been examined.

DISEASE OCCURRENCE PRIOR TO 1968

Australian citrus dieback was first observed as a distinct condition in the Lower Murray settlement of Coomealla, New South Wales in 1942, affecting a smooth sour orange tree. Between 1942 and 1968, it was observed occasionally in grapefruit, affecting one to two per cent of the trees in some orchards in the Murrumbidgee Irrigation Area and Murray River areas, with no evidence of tree-to-tree spread. In these areas also, some deterioration of low numbers of orange trees occurred, which was not at that time diagnosed, but is now attributed to dieback.

During this period a disease affecting a few mature sweet oranges and mandarin trees in some coastal orchards caused deterioration in vigor and productivity,

without very distinctive symptoms. Sour orange trees were more severely damaged and their productive life was limited to about 15 years.

The problem was investigated but the cause was not determined. Trees failed to respond to any treatment, no pathogen could be detected, indexing for a transmissible factor by budding onto seedlings of a wide range of citrus varieties in the glass house gave no positive results. Inoculation of nursery trees in early autumn and direct propagation of affected trees by a variety of techniques produced only healthy progeny. Reexamination, in 1975, of some declining trees in this coastal area confirmed the similarity of the condition to dieback.

DISEASE OCCURRENCE—RECENT

The first indication of increase in incidence of dieback was observed in a planting of 116 Marsh grapefruit on Rough lemon and trifoliolate orange stock at the Dareton Horticultural Research Station. Two of these trees were affected before 1966 and deteriorated to a level of low productivity. Two very stunted, unthrifty trees of Washington Navel on trifoliolate orange root stock were present in an adjacent block. All these trees had, judging from records of growth and crop-

ping, contracted the disease prior to 1964.

By 1970 some increase in numbers of diseased trees of grapefruit was apparent and between 1973 and 1975 this has greatly accelerated in grapefruit and in adjacent blocks of both Washington Navel and Valencia oranges (table 1).

Surveys in 1974-75 of major citrus areas revealed that a substantial increase in numbers of trees affected with dieback had occurred in the last 3 to 5 years in

TABLE 1
INCREASE IN DIEBACK INCIDENCE, HORTICULTURAL RESEARCH STATION, DARETON,
1973-1975

Year	Marsh grapefruit on Rough lemon and <i>Poncirus trifoliata</i>			Washington Navel on <i>P. trifoliata</i>			Keenan Valencia on <i>P. trifoliata</i>		
	Posi- tive	Sus- pect	Healthy	Posi- tive	Sus- pect	Healthy	Posi- tive	Sus- pect	Healthy
1967	3	0	113	2	0	118	0	0	125
1973	14	4	98	0	10	110	0	0	125
1974	29	15	72	13	50	57	4	27	94
1975	70	27	19	41	61	18	62	32	31

inland citrus growing districts. The incidence of the disease is quite variable, large areas being free or having only isolated cases, while in other areas the numbers of affected trees is above 80 per cent. Some association between high rates of infection and proximity to areas of native shrubs appears to exist. Grapefruit

and sour orange generally seem most subject to infection but, in a few areas, orange orchards had infection rates of up to 80 per cent. The disease has been very rarely seen in trees younger than 15 years.

No recent upsurge of infection is evident in coastal orchards.

SYMPTOMATOLOGY

Symptoms of dieback have a degree of resemblance to the deterioration caused by root rot, severe stem pitting in grapefruit, deficiencies of zinc, manganese and iron, and the unthriftiness caused by heavy nematode infestation. The range and severity of symptoms vary with season, variety, age and nutritional status and can be less distinct where interaction with other disease or deficiencies occurs. For these reasons, recognition of the range of symptoms of dieback was slow, except in the case of grapefruit and sour orange, where this disease has its most distinctive and debilitating effect.

Symptoms are most conspicuous in grapefruit and sour orange in autumn, and appear first in one branch, which stands out conspicuously because of blotchy chlorotic leaf patterns. The disease spreads rather slowly in mature trees, but faster in younger trees. Trees are not killed, but remain in a state of chronic and severe dieback.

Patterns superficially suggestive of zinc or iron deficiency develop in young growth. The chlorosis may be complete or there may be some green in the midvein and some lateral veins; irregular blotches of green occur and small round

spots of green may remain in yellow tissue. Yellowing of tissue along the main vein is a prominent symptom, usually in growth made at the base of badly affected branches or on branches which have been cut back. Leaf fall is heavy on badly affected branches. Summer growth does not develop leaf patterns.

Leaves on recently affected branches of sweet orange show blotchy, chlorotic patterns generally less intense than in grapefruit and they do not persist for long; leaf fall and some regreening occur. Growth made after infection is shorter, leaves are smaller and narrower than in healthy trees and are of dull green or yellowish color. As in grapefruit, summer growth does not show leaf patterns. Infection of mature orange trees results in slow debilitation, dieback of weak growth and gradual reduction in yield (table 2). Chronically affected mature orange trees rarely show pronounced leaf patterns, but have a recognizable growth habit, foliage is thin and dull green and new growth is weak.

Fruit of affected grapefruit is small but not distorted or bitter. Fruit size is less affected in sweet orange varieties. Seed abortion has been observed only in smooth sour orange.

TABLE 2
EFFECT OF DIEBACK ON YIELD OF WASHINGTON NAVEL TREES,
HORTICULTURAL RESEARCH STATION, DARETON

Yield/tree (kg)	1964	1965	1966	1970	1972	1973
Row II avg.						
healthy	228	242	236	189	116	174
Row II diseased						
T2	203	192	257	181	130	52
T7	242	272	208	175	45	42
T8	60	24	74	35	0	17
T9	265	141	248	209	107	75

HOST RANGE

Dieback symptoms have been seen in field trees of Marsh, Foster and Thompson grapefruits; Lymon citron; Seminole tangelo; King, Emperor, Imperial, Tankan, late Emperor, Thorny, Burgess, Muscio, Dancy, and Szinkom mandarins; Ellendale tangor; Bergamot; bitter rough, bittersweet and smooth sour oranges;

yuzu, and *Poncirus trifoliata*. Many of these records were obtained from the arboretum at the Horticultural Research Station, Dareton. No recognizable symptoms of dieback have been seen in Rough lemon, Rangpur lime, Eureka or Lisbon lemons.

GRAFT TRANSMISSION

Seedlings of sweet orange and Orlando tangelo were inoculated by budding from affected Valencia orange and Marsh grapefruit trees. Very few seedlings developed symptoms. In later transmission experiments two or three shoot tips with two or three buds each were sidegrafted into seedling Maltese and Ruby blood sweet orange and Sexton and Orlando tangelo trees. Per cent transmission was low, e.g., in one experiment successful transmissions were: to sweet orange, 9/59; to Sexton tangelo, 5/34; and to Orlando tangelo, 7/37. In later inoculations, leaf pieces from partially expanded leaves were inserted under small flaps of bark cut in the stem of seedlings 15 cm tall. Transmission rates up to 50 per cent were achieved by this method. Inoculated sweet orange seedlings became severely stunted. Interveinal chlorosis, resembling zinc and manganese deficiency patterns, commenced in the youngest leaves and subsequent leaves frequently became chlorotic, slightly cupped and small with green veins; green spots sometimes persisted in the chlorotic tissue of some leaves.

Sexton and Orlando tangelo indicator seedlings were stunted. The leaves were much reduced in size and upon maturation they showed marked mottling and yellowing often similar to magnesium deficiency patterns. Infrequently, bands of tissue along midveins became yellow. However, severe aphid transmitted tristeza virus produced somewhat similar, although less severe, symptoms on Sexton and Thornton tangelo seedlings. These varieties are no longer used as indicators.

Transient leaf mottling symptoms were occasionally shown by inoculated Rough lemon and Emperor mandarin seedlings.

Seminole tangelo is a good indicator because of early symptom production, high percentage successful transmission, and the development of good leaf patterns. Symptoms are produced in the first leaf flush after inoculation; these leaves remain small and mottled as they age.

Symptoms appeared on indicator seedlings in the glasshouse 3 to 5 months after inoculation and were more severe in winter than in summer. Controlled environment studies confirmed that symptom

expression of dieback in indicator seedlings was better in plants held under a 22°C, 6-hour night and 25°C, 18-hour day, than under the warmer conditions of 28°C, 6-hour night and 32°C, 18-hour day.

Higher transmission rates were ob-

INSECT TRANSMISSION

Although numerous native psyllid species occur in Australia, none are recorded as being pests of citrus. *Trioza erytraea* and *Diaphorina citri* do not occur.

Attempted transmissions from affected sweet orange indicator seedlings to healthy sweet orange seedlings using the black citrus aphid (*Toxoptera citricidus* Kirk.) were unsuccessful.

Insect trapping was commenced by N.

FLUORESCENT MARKER SUBSTANCES

Work was carried out at Rydalmere and subsequently by Dr. A.W. Feldman, University of Florida, to determine the occurrence of gentistic glucoside in the bark and albedo of dieback-affected trees and of Marsh grapefruit with severe stem pitting (Feldman and Hanks, 1969). Samples of albedo collected in winter and bark collected during the spring, summer, and autumn were examined. The accumulation of gentistic glucoside in the bark and albedo of trees with dieback or stem pitting was found to be highest in the autumn or early winter with little or none

tained from material collected in early spring or late autumn than in summer or winter. Percentage of successful transmission from chronically affected trees was nil or very low.

Grylls, Division of Entomology, CSIRO, Canberra, in November 1974. Traps were set up in a block of Marsh grapefruit with a high incidence of dieback, and in adjacent native vegetation, at the Horticultural Research Station, Dareton. Several species of flattedids, cicadellids, and psyllids have been trapped and are being used in transmission trials (N. Grylls personal communication).

present in spring or summer. Trace amounts were also found in winter in the albedo of healthy nucellar Valencia and navel oranges on trifoliolate orange stock. Trees severely affected either by chronic dieback or by stem pitting showed very small amounts of the substance or lacked it altogether. It would, therefore, appear that stem-pitting disease and environmental stress, as well as dieback may induce formation of gentistic glucosides (Feldman and Hanks, 1969) so estimation of these cannot be used as an indexing method for Australian dieback.

TETRACYCLINE APPLICATIONS

Sweet orange seedlings infected with dieback by graft transmission from field trees, and showing leaf patterns, were divided into two groups of ten plants each. Seedlings were grown in U.C. mix. One group was treated by drenching the soil twice weekly with a 55 ppm (active ingredient) solution of aureomycin (chlorotetracycline-HCl). After four months no difference between control and treated plants was apparent. The

experiment was repeated with similar results.

This negative result does not exclude the possibility that a mycoplasma-like organism sensitive to tetracycline antibiotics is involved in Australian dieback. Igwegbe and Calavan (1973) obtained suppression in stubborn symptom development by tetracycline compounds applied to roots as a dip, or in hydroponic solution but not as a sand drench.

ELECTRON MICROSCOPY

Vascular tissue of midveins and petioles of young partially expanded leaves were prepared for examination by the electron microscope, using the method of Saglio

et al., (1971), but with a variety of resins including Spurr's low viscosity resin, Epon 812 and Araldite.

Young leaves of Marsh grapefruit,

Joppa orange, Sampson tangelo, Tankan mandarin, smooth sour orange, and Washington Navel orange, all showing symptoms of dieback in the field, and indicator seedlings of Orlando tangelo, Ruby blood sweet orange, and Red Blush grapefruit showing dieback symptoms following leaf grafting with the above varieties

were examined for the presence of mycoplasma-like organisms. Mycoplasma-like organisms (fig. 1) were observed in one sample only, a field collection of smooth sour orange. There were few organisms per sieve tube and usually only one sieve tube per section was infected.

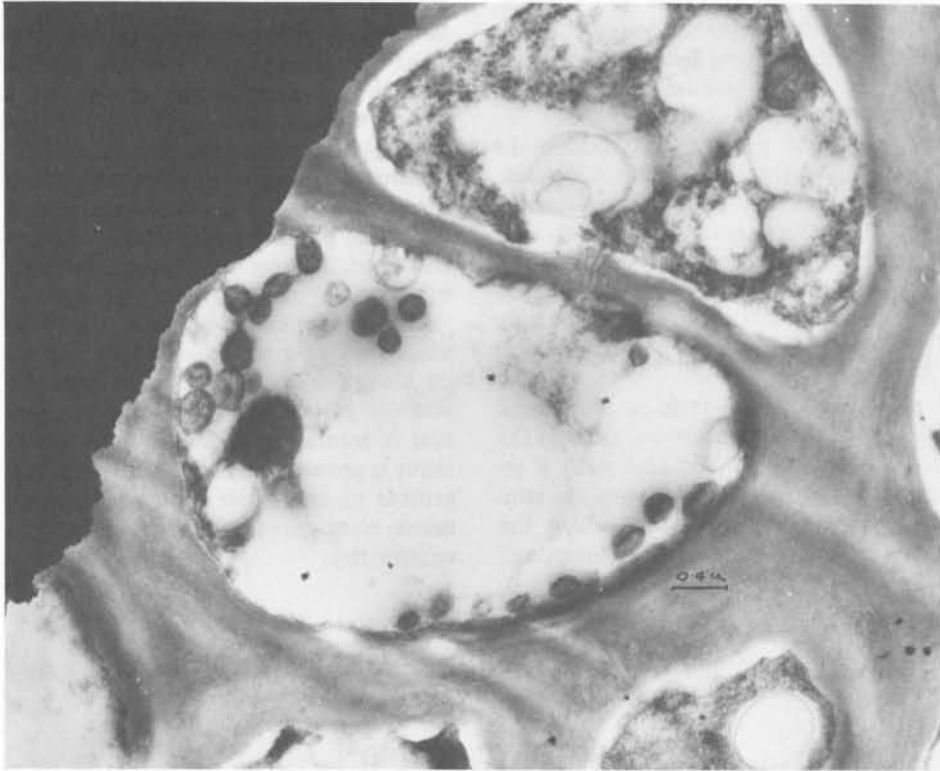


Figure 1. Section of phloem cells in young leaves of smooth sour orange showing mycoplasma-like bodies.

ATTEMPTED ISOLATIONS OF MYCOPLASMA-LIKE ORGANISM

Isolation from seeds and leaves of the infected smooth sour orange was attempted repeatedly using the method of

Fudl-Allah *et al.*, (1972). No "fried-egg" colonies developed following transfer of the liquid cultures to agar media.

DISCUSSION

The range and pattern of symptom production in the field and on indicator seedlings in the glasshouse suggest that Australian dieback is probably related to greening disease (McClellan and Oberholzer, 1965) and Indian dieback (Fraser, 1966). Symptom production is best under cool conditions as for greening

(Schwarz, 1968). Susceptibility of citrus species and varieties to Australian dieback and to Indian dieback is generally similar (Fraser, 1966); the symptom pattern is the same but intensity of reaction of susceptible species varies. Australian dieback, for example, produces milder symptoms in orange varieties and is more

severe in grapefruit than Indian dieback.

As yet no convincing correlation between Australian dieback and the presence of a mycoplasma-like organism in sieve tubes of affected trees has been obtained. The membrane of the organism seen in the one sample of affected smooth sour orange resembled in thickness that of *Spiroplasma citri* (Igwegbe and Calavan, 1970) rather than that of the organism associated with greening disease (Saglio *et al.*, 1971). Attempted culturing of the organism has been unsuccessful.

Work is in progress to establish the vector, or vectors, of Australian dieback, fluctuation of vector populations, and the seasonal factors involved. Further electron microscopy is being done to confirm the presence of a mycoplasma-like organism in dieback-affected trees and to study histological changes in the cells of dieback-affected material.

The considerable increase in dieback incidence in widely scattered areas in the last five years is coincident with a sequence of seasons of above-average rainfall in spring and autumn, which has encouraged growth of native shrubs and

weeds and a build-up in populations of native insects. There has also been a trend towards dependence on biological control of red scale and consequent decreasing use of insecticides.

The very wide, but sporadic, distribution of Australian dieback, its appearance in isolated orchards, sometimes in remote areas surrounded by native vegetation, and in trees of mature age and previous good record or known budline, suggests that this is an endemic disease with a native vector which until recently has visited citrus only rarely. Several psyllid-transmitted diseases of vegetable and field crops in this category are known in Australia, particularly in arid and semi-arid districts where crops are grown under irrigation.

The danger seems to lie in a possible adaptation of a native vector to feeding on citrus, or to the continuance of the seasonal pattern which has caused explosion of insect populations in areas where citrus is grown under irrigation and could provide an alternative food source when native plant growth becomes mature and unattractive.

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