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THE CONTROL OF GREENING AND STUBBORN, TWO MYCOPLASMALIKE DISEASES OF CITRUS

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Greening and stubborn are two very destructive citrus diseases generally attributed to tetracycline-sensitive mycoplasma-like pathogens. Stubborn, called little leaf in Israel, is caused by *Spiroplasma citri* Saglio *et al.* (23). The causal agent of greening may be more bacteria-like than mycoplasma-like (27). Both pathogens are transmitted by insect vectors (6, 8, 21, 23, 25) and invade sieve tubes of infected plants (15, 19).

Following the report by Ishiie *et al.* (17) that treatment with tetracyclines caused suppression of symptoms of mulberry dwarf, considerable experimentation was done with antibiotics on citrus affected by greening or by stubborn.

This paper lists some of the methods available for antibiotic treatment, summarizes results obtained, and, for stubborn, reports some preferred alternative measures to tree injection.

TREE INJECTION METHODS

Methods of tree injection were described by May in 1941, primarily for control of fungus and bacterial diseases. More recently several systems, either pressurized or gravity flow, have been used for injecting antibiotics into fruit trees. Some of these systems are briefly described below.

Nyland and Moller (29, 30) applied transfusion treatments of 25-200 µg/ml oxytetracycline HCl (terramycin) in water by gravity flow for the control of pear decline. The solution was fed into the xylem from

1/4-1-gal plastic reservoirs through 1/4-inch diameter plastic tubes fitted with "quick connectors" that made water-tight seals to the reservoirs and into hollow-lag bolts in 1/4-inch diameter holes drilled into the tree trunk. The plastic reservoirs were suspended 3-4 feet above the holes by wires. The reservoirs usually emptied in 30 min to 2 hr. The treatments were highly successful, resulting in remission of symptoms and stimulation of growth and production.

Similar equipment and methods were used by Nyland and Sachs (31) for the successful terramycin treatment of cherry buckskin and Western-X little cherry. Three to six holes were drilled per tree, near the soil and aligned vertically with major branches. Six to 8 litres of 100 ppm solution were used per tree.

Filer (12) used a lightweight pressure injector for putting tetracycline into trees. He assembled apparatus from a 12-litre pressure tank designed to hold freon, a hose to pipe adaptor, a valve, two 3/8-inch nipples, a 3/8-inch tee, a bushing (3/8×1/4-inch) and a 1/4-inch Schrader tank valve. Lag bolts of 1/2-inch diameter were drilled with 1/4-inch holes and screwed into holes in the tree for injecting the chemicals. Materials cost was about \$ 12.00 and installations required less than 10 minutes.

Coconut palm trees with lethal yellowing were successfully injected with tetracyclines by McCoy (22), who used a pressurized air tank containing antibiotic solution. The tank was connected by a hose to a hollow lag screw inserted into a hole bored into the tree trunk. A quick coupling device connected the hose to the screw.

Schwarz and Van Vuuren (38) injected greening-affected citrus trees by a method described by Schreiber (34). Holes 6 mm in diameter and angled slightly downward were drilled into opposite sides of the trunk 5 cm above the bud union. Tapered plastic reduction pieces were hammered into these holes, which were then filled with antibiotic from a syringe. A plastic bottle of solution was suspended above the holes and connected to the reduction pieces by tubing, taking care to exclude air from the system. Schwarz (35) described a pressurized modification of this system using a bicycle valve secured to the plastic bottle with wire. Plastic tubes from the bottle to the reduction pieces were also wired in place. A bicycle pump was used to pressurize the system. About 10-20 strokes each half hour provided suitable pressure. Schwarz (35) described another system in which a plumber's blowtorch was substituted for the plastic bottle.

Cohen (11) devised a system similar to that of Schwarz and Van Vuuren (38) for diagnosing young tree decline of citrus in Florida. One hole, 1/4-inch diameter was bored in each tree, a tapered metal connector was hammered into the hole, and the hole was filled by syringe. Rubber tubing, filled from the bottom with a hypodermic syringe, was used to join the metal connector to the reservoir.

dOther apparatuses for injecting chemicals into trees have also been described (1, 13, 18).

METHODS OTHER THAN INJECTION

Application methods for antibiotics include conventional foliar spraying, root dip or drench, budwood dip, hydroponics, and deposition of antibiotic paste on or beneath the bark (2, 35).

ANTIBIOTIC TREATMENT OF GREENING DISEASE

Tetracyclines have been used for experimental treatment of greening in India (9, 10, 28, 32, 39), the Philippines (24), and in South Africa (35, 37, 38).

Greening-infected mandarin budsticks immersed for 25 min in 1000 ppm achromycin by Martinez *et al.* (24), in contrast to untreated budsticks, failed to transmit greening to healthy mandarin cuttings. They also noted remission of symptoms in small infected citrus plants whose foliage was repeatedly dipped in or sprayed with 100 ppm achromycin.

Remission of greening symptoms in potted, two-year-old sweet orange seedlings that received 10 weekly sprays of 500 ppm achromycin, chlortetracycline HCl (aureomycin) or dimethyl chlortetracycline HCl (ledermycin) was reported by Nariani *et al.* (28). Later, Raychaudhuri *et al.* (32) sprayed field-grown plants with achromycin or ledermycin at weekly intervals for 8 weeks. The sprayed trees showed varying degrees of recovery for about 3 months but later the symptoms of greening reappeared.

Capoor and Thirumalachar (9) and Capoor *et al.* (10) compared the effects of achromycin, ledermycin and a chemotherapeutant known as B. P.-101 applied as foliar sprays (500 ppm), as applications under the bark (0.25 g/plant), or as injections (500 ppm) in sloping holes drilled into the trunks of four-year-old trees of lime, mandarin, and sweet orange. Drill holes were sealed with polythene tape. Some of the sprayed trees improved temporarily but those sprayed with achromycin or ledermycin redeveloped symptoms within 60 days after discontinuation of treatment. B. P.-101 was slow in taking effect but remission of symptoms lasted longer than with the tetracyclines. Best results were obtained by trunk injection, especially with B. P.-101. B. P.-101-injected plants appeared healthy one year after injection even though indexing showed them to be greening infected. In a later experiment (10), trees of Kagzi lime, Mosambi, sweet orange, grape-fruit, and Nagpur mandarin, all on rough lemon rootstock, were injected three times at 30-day intervals with 500 ppm B. P. -101. All the treated plants recovered within 190 days but retained the greening

pathogen. Lime trees similarly treated in an orchard responded likewise.

Thirumalachar (39) made a new formulation of B. P., using methyl cellosolve for miscibility with water and surfactant action, and sprayed several varieties of orange trees with a 100 ppm solution at 15-day intervals for three months. He obtained complete remission of greening symptoms and good fruit production.

In South Africa, Schwarz and Van Vuuren (38) injected terramycin at 250 and 500 ppm; achromycin at 250, 500, and 750 ppm; and aureomycin at 750 ppm in bearing trees of sweet orange, obtaining mean uptakes of 456-1246 ml. Incidence of fruit greening was reduced by these treatments with 500 or 750 ppm providing the best control at about 50% reduction of greened fruits. Fruit greening incidence remained at a low level four years after treatment (36). Schwarz *et al.* (37) tested injections of ledermycin and tylan and conducted a large-scale trial with penicillin, achromycin, aureomycin, and terramycin, each at two dosage levels. Tylan and penicillin were ineffective. Best results were obtained from injections of one litre of 1,000 ppm achromycin during the September (spring) flush. Aureomycin and terramycin also reduced the percentage of greened fruit. They also assessed phytotoxicity of tetracyclines and insecticides injected into sweet orange trees. Insecticides used were: Azodrin, chlorphenamidone, dimethoate, Du Pont 1410, Foremëtomate, methomyl, and Phorate. All these insecticides reduced aphid and psylla population in 3-4 weeks with Azodrin appearing the most promising. No phytotoxicity was detected from any of the materials. Experiments for the control of South African greening have been summarized by Schwarz (35).

Although heat generated inside a plastic cover may cause temporary remission of greening symptoms in South Africa heat damage to the tree may be excessive (Schwarz, personal communication). Neither can the use of clean propagating materials, in the presence of rapid natural spread avoid greening. Therefore, the use of chemicals is the only feasible control for greening in areas where greening and its vectors are present.

TREATMENT OF STUBBORN DISEASE WITH ANTIBIOTICS

Igwegbe (14) grew small stubborn-infected sweet orange seedlings in hydroponic solutions containing an antibiotic. He compared the effects of achromycin, aureomycin, and penicillin at 10, 20, and 50 ppm in a warm room with forced ventilation. Stubborn symptoms were suppressed by achromycin or aureomycin at 50 and 20 ppm, but not by penicillin. Twenty eight of 32 plants treated at 50 ppm remained symptomless at the termination of the experiment 7 months later and may have been free of the pathogen (16). Drench treatments

of sweet orange seedlings planted in quartz sand with achromycin, aureomycin and penicillin had no apparent effect on development of stubborn symptoms. However, achromycin or aureomycin applied as 1000 ppm root dips for 14 hours suppressed stubborn symptoms for the 6-month period of the experiment. Achromycin was slightly phytotoxic in this treatment.

Calavan and Blue (unpublished) grew stubborn-diseased Valencia sweet orange/Troyer citrange plants in hydroponic solutions containing achromycin or erythromycin. Achromycin was used at 50, 100, and 150 ppm for 11 weeks, at 250 ppm for 8 weeks and at 500 ppm for 8 days. Erythromycin was used at 50, 150, 250, and 500 ppm for 11 weeks. After treatment all trees were planted in soil. Two of 5 plants treated with 50 ppm achromycin turned stubborn after treatment ceased but all 20 plants treated at 100-500 ppm remained healthy 20 months after treatment and appeared to be free of *S. citri*. Erythromycin did not suppress stubborn symptoms at any concentration and was phytotoxic at 250 and 500 ppm.

Calavan, Roistacher, and Blue (unpublished) injected field-grown and severely stubborn-diseased Valencia and Navel sweet orange trees, 6 years or more of age, with 100, 500, and 1,000 ppm terramycin by the method of Nyland and Moller (29). In April 1973 the uptake was 2-3 litres/tree. Four months after treatment the only visible reaction was a reduction in leaf mottle on all treated trees. There was no increase in fruit set. In March and April 1974, terramycin and achromycin were injected at 500, 750, and 1000 ppm but uptake was less than in 1973. The only response noted within 6 months was an improved growth flush in two Navel orange trees that received more than 2 litres each of terramycin. Terramycin at 500, 1000 and 1500 ppm was sprayed on stubborn-diseased Valencia and Navel orange trees monthly for 7 months beginning in the spring of 1973. Two percent kerosene was included as a spreading and penetrating agent. One plot was sprayed with 1000 ppm terramycin and 2% kerosene every 10 days for 13 applications. No reaction to any of these spray treatments was detected.

Spiroplasma citri, *in vitro*, is very sensitive to the tetracyclines and to doxycycline, methacycline, minocycline, erythromycin, tylosin, carbomycin, oleandomycin, filipin, chloramphenicol, and to thiabendazole in 5% dimethyl-sulfoxide but is less sensitive to kanamycin and streptomycin (4). Additionally, Bove *et al.* (3) found it sensitive to sodium polyanethol-sulfonate and digitonin and Saglio *et al.* (33) found it sensitive to neomycin, actinomycin D, azosulfamide, and amphotericin B. The possible effects of these chemicals on stubborn-diseased plants are unknown, except for the tetracyclines and erythromycin.

PREVENTION OF STUBBORN DISEASE BY CULTURAL MEASURES

The natural incidence of stubborn disease is highly variable in California, from 0 to 90 percent within 3 years in young sweet orange trees (7). Stubborn incidence in trees 6 years or more of age may occur but is rather uncommon except under desert conditions. Therefore, if infection can be prevented in the nursery and young orchard, practical control of stubborn might be achieved in most areas.

I determined 10 years ago that a 20-acre orchard of 5-year-old Valencia trees at the University of California, Riverside had about 50% incidence of stubborn. Trees for this orchard had been grown in an outdoor nursery by traditional methods. This orchard was replaced with trees propagated indoors from selected healthy parent trees. The new trees were planted when about one year old. At 6 years of age the new orchard has less than 5% stubborn diseased trees. This indicates the importance of using clean budwood and keeping trees protected from insect vectors during their first year even in a location where normal incidence of stubborn in unprotected young trees is high.

DISCUSSION AND CONCLUSIONS

South African greening disease can be partially controlled in some locations by injections of achromycin and this method is being used by some growers (Schwarz, R. E. and J. N. Moll, personal communication). Injection has the advantage of being more economical and apparently more effective than spraying but might possibly contribute to residue problems if used shortly before harvest. Asian greening is often more destructive than South African greening and may be more difficult to control. However, injection experiments with antibiotics in India have given promising results suggesting that commercial use of antibiotics may be practical. Recent spray experiments by Thirumalachar (39) indicate that Asiatic greening control is feasible by spraying with B. P. If vector control could be achieved by an effective insecticide or parasite, the success of antibiotic treatments would be considerably enhanced. Combined injections of insecticides and antibiotics are possible (35).

Budwood free of the greening pathogen should, of course, be used and nurseries should be indoors or in greening-free areas. Vector control is desirable, but not always economical, on young as well as on bearing trees. It is very important to avoid introduction of vectors, and greening-infected propagative materials or trees into noninfested areas.

Stubborn disease appears to be more difficult than greening to control, at least in severely affected field-grown trees. The fact that it can be controlled in small plants grown by hydroponics suggests that more chemical experimentation with orchard trees is needed. Unless fruit set of diseased trees is increased, however, treatment must be considered impractical. It is already obvious that antibiotic treatments of citrus trees of bearing age do not cause the spectacular remission of symptoms obtained in pear trees (29) or in peach and cherry trees (31).

At present the only practical control for stubborn disease is the use of clean propagating materials indoors or in an area practically free of leafhoppers that carry *S. citri* (5,20). Leafhopper control and roguing of stubborn-diseased trees in young trees also appear advisable.

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FUNGICIDAL UMBRELLA

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According to the conventional meaning, umbrella is a device to protect against inclement weather. This word, used in conjunction with others, connotes diverse meanings, such as, protective umbrella of air craft to give support to ground troops in army; protective umbrella for controlling prices in industry; a unifying, conditioning, stabilising or controlling factor, etc. In short, all usages indicate provision of protection against adverse factors by diverse means.

In so far as control of plant diseases is concerned, an umbrella can best be considered as a unifying, conditioning, stabilising or controlling factor because their controls/prevention is attempted by diverse means, such as, use of chemicals, resistant varieties, crop rotation, sanitation, etc. The above are listed in historical sequence and not necessarily on the relative importance of each, which has continued to change with pace of changes in agricultural practices in different countries in which socio-economic changes have played a dominant role. Of no less importance in this transformation has been progress in science and technology, which again has been uneven in different countries.

The ravages due to diseases to crop plants have been known and references to rusts and mildews have been made since Biblical times. Attempts to control these by use of chemicals are, however, of a more recent origin following the experimental evidence that fungi were the causative factor. At a much later date other living organisms like bacteria and viruses were also identified to be responsible for many ailments in crop plants. It is significant to mention that more instances are on record of famines caused by plant diseases than by insect pests; to cite a few examples, the late blight of potato in Ireland in mid-nineteenth century and brown leaf spot of paddy in Bengal in 1942/43. Equally noteworthy are recorded instances of large scale destruction of some commercial crops like coffee plantations by rust disease in Ceylon, the simultaneous widespread outbreak