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Use of Tetracycline Antibiotics to Control Yellows Diseases

The 1967 discovery that mycoplasmas were the presumptive causal agents of the yellows group diseases opened a new chapter in plant pathology. These diseases, formerly thought to be of viral etiology, were shown to respond to applications of tetracycline antibiotics but not to penicillin, thus further implicating the wall-less prokaryotes as pathogens (6). While Koch's postulates have not been demonstrated for the majority of the yellows diseases, their response to differential chemotherapy provides evidence of the highest order in corroborating the evidence afforded by electron microscopic observations of mycoplasmalike organisms (MLOs) in diseased plants.

With the demonstration that yellows-diseased plants could produce symptom-free new growth after treatment with tetracyclines, a number of investigations into their use as practical control agents were initiated. In consequence, the antibiotic oxytetracycline hydrochloride (OTC) has been registered for use in control of lethal yellowing of coconut palm, pear decline, X-disease of peaches and cherries, and peach yellow leaf roll in the United States. Additional registrations are pending. The successful development of OTC as a practical disease control agent has not been without its problems and limitations. These are the subject of this paper.

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Remission of Yellows Diseases

Treatment of yellows-diseased plants with tetracycline has, in general, produced a marked improvement in subsequent growth. The degree of plant response may vary with plant age, symptom severity, method of treatment, and type of tetracycline used. This response is also of limited duration; repeated doses are necessary to maintain a plant in a state of remission. The duration of remission varies but is generally quite short in herbaceous plants, on the order of a few weeks, while it may be up to 2 years or longer for some woody plants. In general, higher doses of antibiotic will induce longer periods of remission. Also, plants with advanced symptoms are less likely to show remission and the duration of remission may be shorter.

Remission of disease is characterized by new growth that is free from symptoms. Distorted tissues do not recover their normal shape, although newly developing flowers, leaves, and stems appear normal. Previously yellowed leaves may or may not regain a normal green coloration. Mycoplasmalike organisms are seen to degenerate during remission and are not seen in new symptom-free growth. However, the MLOs reappear at the end of the remission period.

The short duration of remission in herbaceous plants limits antibiotic application as a practical control measure, but its value as a diagnostic aid remains. The much longer remission periods obtained in certain diseases of woody plants have allowed the development of field control measures for several of these maladies.

In addition to its therapeutic use, OTC is registered for prophylactic use to protect healthy palms in areas of high lethal yellowing incidence in Florida and Texas (Fig. 1). In fact, this constitutes the major use of OTC for practical field disease control in palms. Prophylactic treatment of peaches for yellow leaf roll has also been demonstrated to be of significant protective value in tests in California (G. Nyland, *personal communication*).

Treatment Methods

Methods used for treating plants with antibiotics have included foliar sprays, direct injection or infusion (Fig. 2), and hydroponic uptake. Soil drenches have been largely ineffective because tetracyclines bind to soil (4). Remission has been induced by foliar sprays, but systemic distribution has been limited and response generally poor in relation to the amount of antibiotic used (10,11). Uptake by immersion of roots in hydroponic solutions of antibiotic has given excellent systemic distribution and is practical on a laboratory or greenhouse scale but not for field use.

Full-scale field treatment programs

Fig. 1. (Right) Coconut palms 8 months after start of an oxytetracycline hydrochloride injection program to prevent lethal yellowing disease, compared with (left) untreated palms. (Courtesy Florida Department of Agriculture and Consumer Services)

have generally depended on pressure injection of aqueous antibiotic solutions into trees. Gravity infusion has been used on a limited scale and in preliminary testing. The rapidity of pressure injections has made these the methods of choice for large-scale commercial use, although smaller scale treatment of orchards has been practical with the pipette infusion method of McIntyre and Lacy (12). Pressure injections may be actuated by air (Fig. 3) or hydraulically and greatly increase the speed of uptake. These methods have been reviewed recently (10,11,13).

The systemic distribution of tetracyclines in plants has been readily demonstrated by injection with radiolabeled antibiotic or bioassay of tissues after treatment (10,13). Foliar concentrations of tetracycline rise rapidly after injection or root immersion. The pattern of distribution after infusion or root immersion is typical of a xylem pathway, with rapid upward movement in the

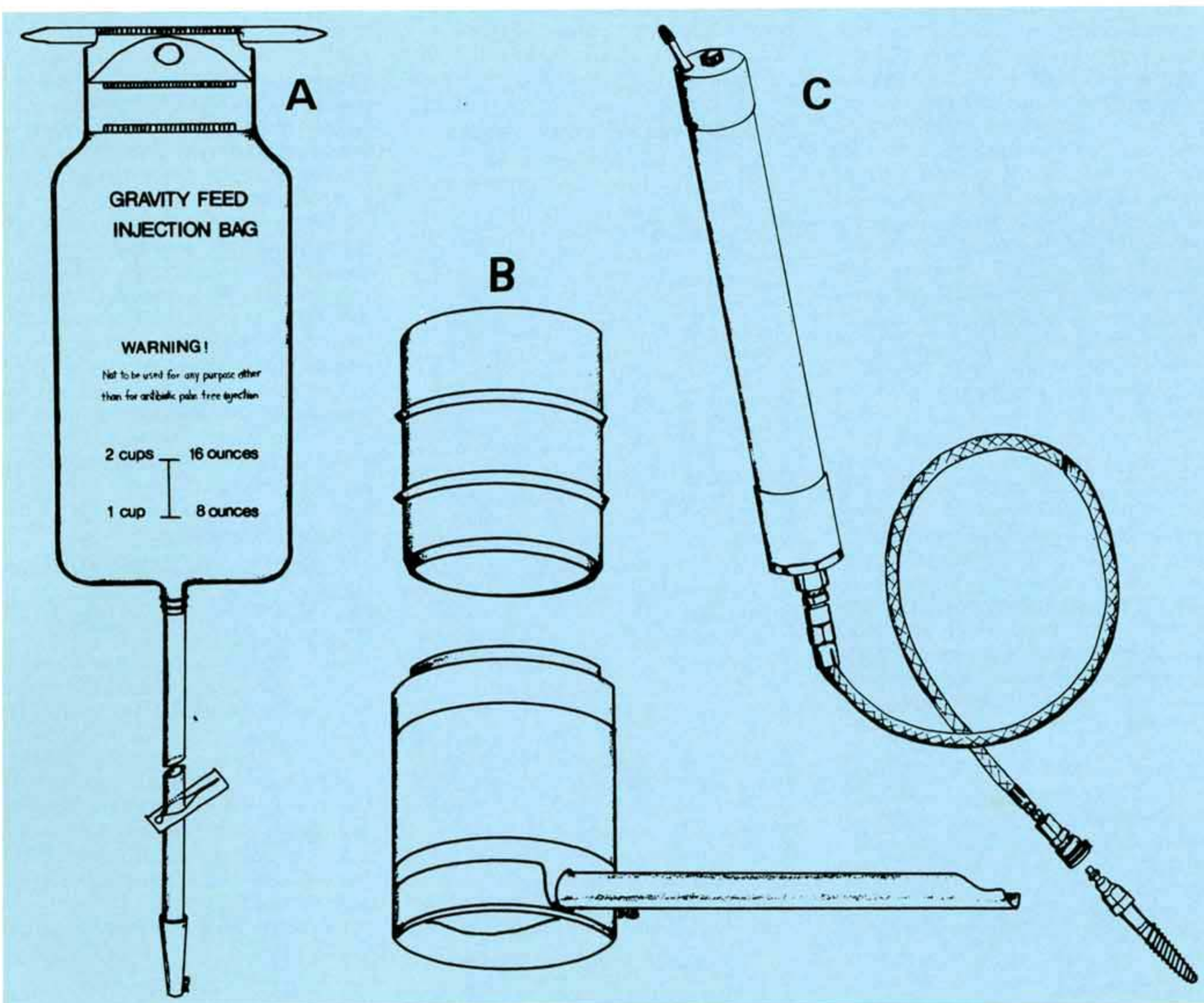


Fig. 2. Apparatus for injecting coconut palms with oxytetracycline: (A) Gravity infusion device made from a disposable enema bag is inexpensive and reusable. (B) Mauget injector (J. J. Mauget Co., Burbank, CA 91504) is simple, disposable, and highly efficient. The solution reservoir holding 14 ml of aqueous antibiotic is attached to the feeder tube inserted in a hole drilled into the tree trunk. (C) Air pressure-actuated injector is made from plastic pipe and fittings. A 0.5-L reservoir is pressurized by a tire pump. A quick-connect coupling allows rapid attachment to a hollow lag screw inserted in the injection hole.

transpiration stream and accumulation in mature foliage (15). After pressure injection of fruit trees, movement in the xylem is equidistant up the shoots and down into the roots, presumably because of the high pressures generated by the injection equipment. To be effective against phloem-delimited MLOs, however, the antibiotic must accumulate in the phloem. Injection of ^{3}H-labeled chlortetracycline revealed accumulation of activity in fine roots after 14 days, indicating redistribution of antibiotic with downward movement in the phloem (13). Injection of single mature leaves of lethal yellowing-affected palms resulted in remission of symptoms in other parts of the tree, again indicating export of tetracycline from treated leaves via a phloem pathway (10).

Since initial distribution of the tetracyclines in plants appears to be in the xylem, the development of injection techniques that maximize xylem uptake should be emphasized. Because in most woody plants only the outermost annual ring or rings are functional in xylem transport, trunk injections should be made as shallow as possible, preferably into wood just beneath the bark. Deep injection of the antibiotic into nonfunctional wood greatly reduces the efficacy of treatment. Sachs et al (13) reported that injected dye moved only in the outer two annual rings of fruit trees and that a substantial portion of the injected chemicals did not move away from the injection site. Although injection into ports 2.5, 5, or 7.5 cm deep gave similar results, it is probable that the outer annual rings were bypassed after insertion of the injection screw. Several methods for shallow injection have recently been reported (1,14), and further evaluations of their effectiveness are needed.

Distribution of the injected material is often unilateral in dicotyledonous plants; there is very little lateral movement of the antibiotic as it moves upward from the injection site. As a result, several injection sites are usually chosen equidistantly around the trunk of a woody tree. Placing injection ports beneath the major scaffold branches of fruit trees assists in gaining a more uniform distribution. Also, placing the injections in root flares has been found to aid systemic distribution in elm trees (7). On the other hand, the unique vascular system of palms allows excellent systemic distribution from only one injection site. The advantage of root-immersion feeding of antibiotics to smaller plants is that even systemic distribution is assured.

Dose-Response Tests

The timing of antibiotic treatments and the optimal dosages for producing disease remission without deleterious phytotoxicity must be determined individually for each disease and plant species tested. In general, the tetracyclines are highly phytotoxic. However, if a balance can be found between increased productivity on the one hand and phytotoxicity on the other, treatment may become economically feasible. Generally speaking, the dosage chosen is at or just below that inducing phytotoxicity, although in coconut palms, doses 20 times greater than required for therapeutic response have caused no foliar toxicity symptoms.

The timing of treatments is best determined from the length of the remission period. Remission in coconut palms lasts from 4 to 7 months and retreatment is recommended at 4-month intervals (9). In pear decline, remission lasts 2 to 3 years and injections are made at 2-year intervals. No particular season for treatment is possible for coconut palms because of their indeterminate growth. For woody fruit trees, however, treatments are timed to come after harvest so that all traces of the antibiotic will be degraded by the time of the following year's harvest. Although coconut palms must be treated more often than woody fruit trees, the treatment is considered to be a temporary measure to be carried out for the 5 years or so required to establish replacement palms resistant to lethal yellowing. There is no source of resistance to pear decline or peach X-disease, and treatments must be kept up indefinitely.

Fig. 3. Air pressure-actuated injection of coconut palms in Miami.

Formulations and Stability

To date, all registered uses of an antibiotic for control of yellows diseases have been for OTC. Tetracycline hydrochloride (TC) has also proved effective in field testing. Chlortetracycline hydrochloride (CTC) has been efficacious, but often not as much as OTC or TC. Since, contrary to common belief among phytopathologists, all xylem vessels are of finite length and have closed ends (15), solutions—not suspensions—of chemotherapeutants must be used for injection. Additionally, these compounds must not bind to the cellulose walls of the tracheary elements or they will be adsorbed before reaching the tops of trees. The hydrochloride moieties of the tetracyclines are water-soluble in acidic solution and thereby readily translocatable in the plant. Unlike streptomycin, which binds to cell walls and is poorly systemic (5), the tetracyclines move readily in the xylem and appear to be an excellent choice for systemic treatment of trees. In basic solution, the tetracyclines are hydrolyzed and rendered insoluble. Including an organic acid in the treatment solution helps keep these antibiotics soluble.

The tetracyclines are degraded by light and should be held in opaque containers or placed on the shaded side of a tree. These antibiotics are also excellent chelating agents and should never be used in steel containers or with iron-containing fittings. Brass, stainless steel, or plastic is recommended for injection apparatus. Steel fittings chemically plated with nickel may be used (electrolytic plating will not protect the bore). Without such protection, steel fittings rapidly corrode and the antibiotic solution loses activity.

Safety of Antibiotic Use

An important factor to be considered in the agricultural use of the tetracyclines is that the persons applying the material and, ultimately, the consumers of the treated plants should not be exposed to inordinate concentrations of these clinically important compounds. The toxicity of the tetracyclines is not a problem; they have been proved safe through years of clinical use (2). The average dose given a tree in a year is one-tenth that given a human during a 1-week therapeutic series. However, the exposure of human and environmental microflora to the tetracyclines must be minimized. Exposure of ubiquitous bacteria to these antibiotics will result in selection for resistant strains. It is feasible that these strains could transmit this resistance to pathogenic bacteria through plasmid transfer or interspecific conjugation and thus reduce the value of the compounds in human therapy.

Daniels (3) recently reported that tetracycline-injected bean plants exuded antibiotic from their roots and into hydroponic nutrient solutions. Treatment of field-grown beans increased the number of tetracycline-resistant bacteria on the rhizoplane and decreased *Rhizobium* nodulation. Although the tetracyclines are elaborated by several actinomycetes and have been recovered from naturally infested soil, their exudation from the roots of treated plants should not go unheeded. This aspect warrants further investigation.

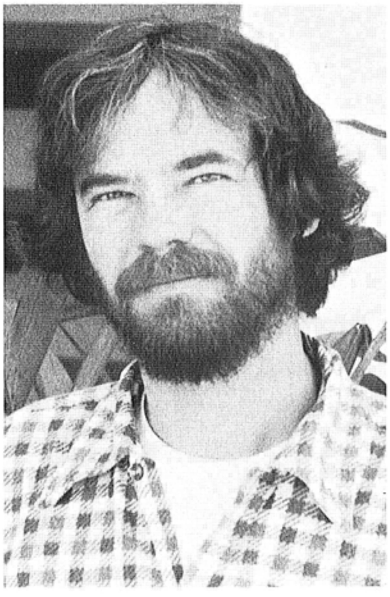
The hazards of phytotoxicity have already been mentioned. Generally, a level may be found at which remission may be observed without the development of toxicity symptoms. However, a few plants are sufficiently sensitive that the tetracycline dose inducing a toxic response is not adequate to induce remission. In such cases, it is very difficult to observe the effects of treatment.

The selection of resistant strains of the target pathogen must also be considered. Continued therapeutic use of OTC over a period of years could result in the selection of resistant strains and the breakdown of a treatment program. However, when laboratory selections of resistant strains of spiroplasmas were made by Liao and Chen (8), they found that strains resistant to OTC were not necessarily resistant to TC or CTC, and vice versa. Thus it would appear that alternative tetracyclines could be used should resistance to OTC show up in the field.

Conclusions

Research on the use of tetracycline antibiotics as practical control agents for the yellows diseases has yielded several recent registrations for tree crop uses. However, the short duration of remission and the potential for exposure of the environment to these clinically important antibiotics have limited their use on herbaceous crops. Even so, tetracycline is still useful as a diagnostic aid for determining possible MLO etiologies of new diseases of herbaceous plants. The tetracyclines are an excellent choice for treating the yellows diseases in that they not only have broad-spectrum activity against the mycoplasmas but also are readily systemic in plants.

The large-scale use of tetracyclines to control plant diseases must be carefully evaluated, particularly for safety. Environmental exposure to these antibiotics must be minimized through application techniques that limit them to the interior of plants. Exposure of common microflora, whether on the plant surface or in the humans applying the treatment or consuming the treated product, can result in selection of resistant strains. This could endanger the use of these agents in human medicine. The safety of applying oxytetracycline to trees through injection has been demonstrated (2), however, and, consequently, the antibiotic poses little risk to humans when prudently used according to label directions. The use of antibiotics in integrated programs with other effective measures, such as vector control, eradication of alternate MLO hosts, and replanting with resistant cultivars, is allowing higher degrees of control of MLO-associated tree diseases than ever thought possible.



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