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Effect of Oxytetracycline and Benzimidazole Treatments on Blight-affected Citrus Trees[1]

R. F. Lee, L. W. Timmer, and L. G. Albrigo[2]

University of Florida, IFAS, Agricultural Research and Education Center, Lake Alfred, FL 33850

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Abstract. **Citrus blight is a vascular wilt disease of unknown etiology. Blight-affected 'Pineapple' sweet orange [*Citrus sinensis* (L.) Osb.] trees on rough lemon (*C. jambhiri* Lush.) rootstock in early stages of decline were treated with benzimidazole fungicides or with oxytetracycline (OTC) by trunk injection, soil drench, or injection plus drench. The distribution and persistence of the materials were monitored by bioassay using *Bacillus cereus* var. *mycoides* for OTC and *Penicillium expansum* L. for benzimidazoles. Both materials were well-distributed in the canopy following trunk injections, in the root systems following soil drenches, and though most of the tree after injection plus drench treatments. Benzimidazoles and OTC persisted within the trees for several months and relatively high levels of activity were maintained for over a year by 3 applications. OTC persisted in soil for more than 6 months; whereas, benomyl disappeared from the soil in 3 months or less. Although high levels of bactericidal or fungicidal activity were maintained in the treated trees, most treated trees declined as rapidly as the untreated control trees. The injection plus drench group treated with OTC showed a slight improvement after 2 years. None of the treatments increased tree growth or resulted in increased water uptake. High levels of zinc in trunk wood, an internal symptom of blight, were unaffected by the benzimidazole treatments, but injection plus drench treatment with OTC significantly reduced zinc levels in trunk wood. Since neither OTC nor benzimidazoles completely reversed symptoms of blight, we were unable to conclusively confirm or refute proposed bacterial or fungal etiologies for citrus blight.**

Citrus blight is a serious decline of unknown etiology which results from a dysfunction in the vascular system (20, 28). Blight-affected trees characteristically have reduced water uptake (3), and a high concentration of zinc in the xylem (27) as compared to healthy trees. Circumstantial evidence has been presented indicating that blight may be caused by a xylem-limited, fastidious bacterium (XLB) (7, 11, 12), but other work suggests that a *Fusarium* sp. may be involved (16).

Trunk injections or soil drenches of tetracycline antibiotics have brought about the remission of symptoms of citrus diseases such as greening, likubin, and stubborn which are caused by phloem-limited procaryotes (14, 18, 19, 21, 24). Tetracycline applied as foliar sprays or soil drenches has also caused remission of symptoms of Pierce's disease of grape which is caused by an XLB (10, 13). In our previous studies (15, 22), trunk injections of oxytetracycline (OTC) in blight-affected trees failed to affect symptom development. However, since trunk injections introduce OTC primarily into the tree canopy and not into the roots (22), they would not be expected to control XLB, such as the phony peach bacterium which occurs predominately in the roots (25). In fact, tetracycline applied as a soil drench appeared to induce recovery of 3 of 5 treated blight trees (23).

Benzimidazole fungicides applied by trunk injection or soil drench have been used to control vascular wilt diseases such as Dutch elm disease and fusarium and verticillium wilts (4, 5). In limited trials, blight-affected trees did not appear to respond to trunk injection with low rates of benzimidazole fungicides (15). However, soil-applied benomyl appeared to induce temporary recovery of blight-affected trees (2).

Previous work on the effect of benzimidazole fungicides and tetracycline antibiotics on blight-affected citrus trees has not been completely conclusive because the distribution of the material in the tree was limited or not known. In this study, we attempted to attain the best possible distribution by applying the materials as soil drenches, trunk injections, or both. The treatments were observed for their effects on the development of blight symptoms. Distribution and persistence of the materials in the twigs, roots, and soil were monitored by bioassay.

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Materials and Methods

Tree selection. Two groves of 'Pineapple' sweet orange trees on rough lemon rootstock near Lake Alfred, Fla., were selected for the study. Trees were rated for visual symptoms of blight on a scale of 0 = healthy; 1 = mild—leaves small with zinc-deficiency symptoms, internodes short, slight wilt, but little thinning of foliage; 2 = moderate—leaves small, often flaccid, with zinc-deficiency symptoms, canopy sparse with some twig dieback; 3 = severe—canopy thin, twig dieback substantial, trunk sprouts common. Trunk wood samples were taken for zinc analysis (27) from all trees with mild but distinct symptoms, i.e., those rated from 0.5 to 1.5, and from 4 to 5 healthy trees from each grove for comparative purposes. In each grove, 24 trees with mild symptoms were selected for use and 6 trees were assigned to each of the injection, drench, injection plus drench, and control treatments, such that each treatment had trees which were as comparable as possible with regard to symptom severity and zinc level. Rings were painted on the trunk and 7 scaffold branches of each tree and the circumference of each was measured.

Application of materials. The trunk injection apparatus used was developed and modified from the equipment described by Reil and Beutel (17). Modifications of the apparatus were described by Lee et al. (15) and the injection methods were those described by Timmer et al. (22). A new force-fit injector tap (9), which has proven more effective in introducing oxytetracycline into blighted citrus trees, was used throughout the present study.

OTC (Agricultural Terramycin, 20% active, supplied by V. J. Carroll, Pfizer, Inc., New York, NY 10017) for trunk injections was prepared at 250 g of the formulated material per liter. Since this material was viscous, it was difficult to inject and the time required to inject a single tree varied from 12 to 24 hr. Thus, it was difficult to accurately control the amount injected. All OTC-injection treatment trees were injected twice from April to May 14, 1980, and from Oct. 20 to Nov. 5, 1980. Trees in the injection-only group received an average of 23.6 g of actual OTC (range 11.0 to 45.0 g) per tree on the first date and 15.3 g of OTC (range 4.0 to 30.0 g) per tree on the second date. Trees in the injection plus drench treatment received an average of 28.5 g of actual OTC (range 6.3 to 62.5 g) per tree on the first date and 18.8 g OTC (range 10.0 to 26.3 g) per tree on the second date.

Since in previous trunk injection work with benomyl (15) a formulation with only 0.7% active ingredient was used, it was difficult to inject sufficient quantities into blighted trees to obtain good distribution and persistence. For the present study, a formulation containing 4.8% methyl 2-benzimidazolecarbamate (MBC) and 5.7% MBC·HCl in a solution of 40% methanol and 1.25% HCl was supplied by C. J. Delp (E. I. DuPont de Nemours & Co., Wilmington, DE 19898). For injection, this formulation was mixed with an equal volume of water. Since the material was not viscous, it required only about 4 to 5 hr to inject a tree. Trees in the injection-only group received an average of 26.3 g MBC (range 18.4 to 31.5 g) per tree and those in the injection plus drench group received an average of 25.6 g of MBC (range 19.7 to 32.8 g) per tree applied from Oct. 29 to Nov. 3, 1980. Control trees in both groves were injected with water only on the first injection date in each grove.

Soil drench applications were made by forming a ring of soil 30- to 40-cm-high around each tree at about 2.5 m from the trunk. Materials were mixed with water in a large tank and applied using 1500 to 2000 liters per tree. OTC was applied to all OTC-drench-treatment trees at 2.0 kg of the 20% active material per tree on April 18, July 25, and Nov. 14, 1980. Benomyl [methyl 1-(butylcarbamoyl) benzimidazole-2-yl carbamate] was applied to all benomyl drench treatment trees in the second grove at 0.45 kg of Benlate 50 WP per tree on the same dates.

Tissue sampling, bioassays, and tree evaluation. Tissue samples from the OTC experiment were bioassayed using *Bacillus cereus* var. *mycoides*. Antibiotic medium #3 (Difco Laboratories, Detroit, MI 48201) plus 1.5% agar was autoclaved, cooled to 50°C, 1.2 ml of a commercial spore suspension (*Bacillus cereus* spore suspension, Difco Laboratories) per liter of media was added, and plates were poured.

Tissue samples from the benzimidazole experiment were bioassayed using *Pencillium expansum*. Spores produced on Difco potato dextrose agar were suspended in sterile distilled water plus 1 drop of Tween 20, counted in a hemacytometer, and the spore concentration adjusted to 1 to 5 $\times$ 10^6 conidia/ml. One-tenth ml of the spore suspension was spread on each PDA plate.

For bioassays, 4 twigs about 3 to 5 mm in diameter were collected 1.0 to 2.5 m above the ground at the cardinal points around each tree. Four roots 2- to 6-mm-in-diameter were collected 5 to 20 cm deep at 0.5 to 2.5 m from the trunk on 4 sides of the trees. The bark and cambium were removed from all root samples to assure that only OTC or benomyl actually taken up by the roots was measured. Three pieces, about 4 mm long, were cut from each twig or root and plated directly on the bioassay plates. Cores of soil, 2.5-cm-in-diameter, were collected about 1 m from the tree trunk from 4 sides of the tree at the 0 to 15, 15 to 30, and 30 to 45 cm depths. The 4 cores from each depth for a single tree were composited and mixed thoroughly. For bioassay, about 5 g of soil from each sample were mixed with 5 ml water and allowed to stand for about 1 hr. Six sterile paper discs, 6-mm-in-diameter, were soaked in the sample, blotted lightly, and plated directly on the bioassay plates. *B. cereus* plates were incubated for 24 hr and *P. expansum* plates for 40 hr at 22 to 26°C. The number of samples with activity was counted and the radii of the zones of inhibition of each sample were measured.

The amount of plugging in xylem of OTC-injected and drench trees, control trees, and healthy trees was determined by light microscopy in May 1982. A core sample was obtained about 50 cm above the bud union from 3 trees in each group using a Haglof 5 mm Swedish increment borer (Forestry Suppliers, Inc., Jackson, MS 39204). Cores were fixed and stored in 3% glutaraldehyde in 0.066 M sodium phosphate buffer, pH 7.0. At a depth of 2 cm from the cambium, longitudinal sections 40–50-μm-thick were cut using a cryostat; 10 sections from each core were mounted on slides and viewed under a light microscope. The number of xylem vessels and number of plugs in each section were counted. Data are reported as average number of plugs per xylem vessel. There were an average of 13.5 vessels in each section.

In June 1981, symptoms were rated on the scale described above, trunk wood samples were collected and analyzed for zinc content, and the circumferences were measured on marked limbs and trunks. In April 1982, symptoms were rated again, wood samples were collected for zinc analysis, and water uptake measurements were made using the procedure of Cohen (3).

Results

Distribution and persistence of OTC and benomyl. Trees receiving trunk injections of OTC had a high percentage of the twigs with activity, especially following the second injection treatment (Fig. 1). However, no more than 25% of the roots from the injection-only trees had activity at any time. Trees receiving drench treatments had a high percentage of the roots with activity. A substantial percentage of the twigs from the drench-only trees had activity only following the July 1980 treat-

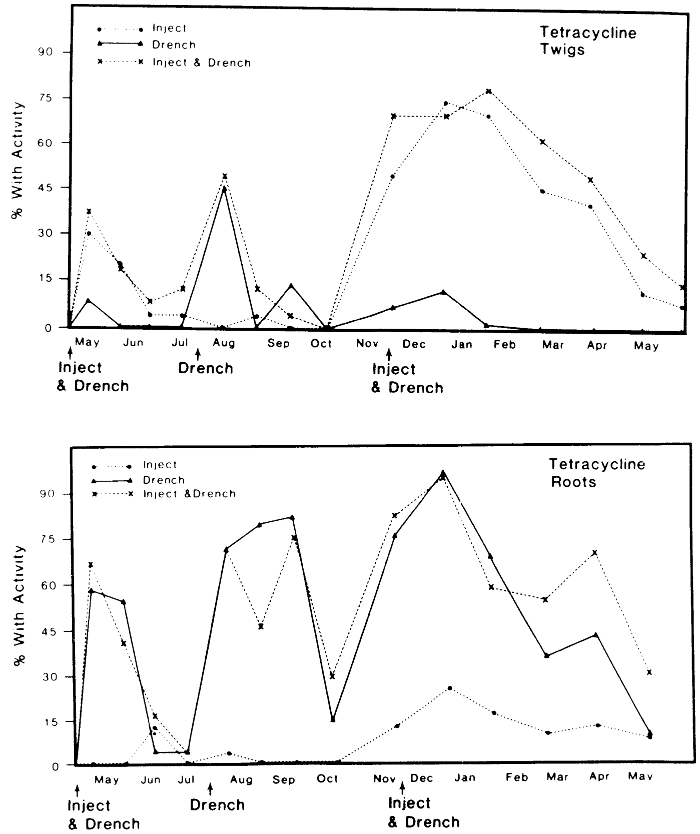

Fig. 1. Effect of tetracycline soil drench and trunk injection treatments on the percentage of twigs and roots with antibiotic activity against *Bacillus cereus* var. *mycoides*.

ment. The injection plus drench treatment produced a high degree of activity in the canopy and in the roots throughout most of the experiment (Fig. 1).

Drench-applied OTC penetrated the soil well and activity was detectable to the 45 cm depth (Table 1). Activity in the surface 15 cm was greatest on the first assay date after treatment and decreased with time. Activity at lower depths peaked later. The peak at the 30- to 45-cm depth occurred at the second assay after treatment following application during higher rainfall periods in late spring and summer, but not until the fourth assay following application during a lower rainfall period in winter. OTC was highly persistent in soil and was still detectable after 6 months.

OTC activity in the tree canopy decreased more rapidly in summer, but this trend was less obvious in roots and soil.

Drench treatments with benomyl produced a high percentage of roots with fungitoxic activity, but relatively little activity was detected in the twigs (Fig. 2). A high proportion of the twigs had activity following trunk injection of MBC and activity was detectable for up to 6 months. As with OTC, a low percentage of the roots had activity following trunk injection with MBC. A high degree of fungitoxic activity was maintained throughout much of the experimental period with the injection plus drench treatment.

Fungitoxic activity following benomyl soil drenches was greatest at the surface, but activity was also high at lower soil depths (Table 2). Benomyl did not persist for long periods in soil especially at lower depths. Disappearance was particularly rapid during the summer.

Small zones of inhibition (about 1 mm) were observed occasionally in soil or root bioassays on *B. cereus* plates from control trees. When this occurred, the average zone radius in controls was subtracted from that of the treated trees. No zones of inhibition were observed in twig, root, or soil bioassays on *P. expansum* plates from control trees or in twig bioassays on *B. cereus* plates from control trees.

Tree condition. None of the OTC treatments improved tree condition by June 1981. However, by April 1982, tree condition in the injection plus drench treatment had improved slightly (Table 3). Significant differences existed in the zinc levels in trunk wood among the different treatments at the beginning of the experiment. Trees in the drench-only treatment had the lowest levels and those in the injection-only treatment had the highest. In June 1981, the zinc levels in all OTC treatments had

Table 1. Effect of time and soil depth on antibiotic activity in the soil against *Bacillus cereus* var. *mycoides* following soil drench treatment with oxytetracycline. Data from the drench and injection plus drench treatments were combined and averaged since there was no difference between the 2 treatments.

		Avg radius of inhibition zone (mm)		
			Soil depth	
Application date	Bioassay date	0–15 cm	15–30 cm	30–45 cm
April 18, 1980	May 12, 1980	4.8	3.4	2.2
	June 3, 1980	4.4	3.2	4.2
	June 23, 1980	4.0	1.3	0.8
	July 14, 1980	3.8	1.6	1.9
July 25, 1980	Aug. 11, 1980	7.0	3.4	1.9
	Sept. 3, 1980	6.0	4.4	3.7
	Sept. 26, 1980	1.7	1.2	0.6
	Oct. 20, 1980	4.1	3.3	1.9
Nov. 14, 1980	Dec. 2, 1980	3.7	2.5	2.1
	Jan. 7, 1981	3.0	2.9	1.9
	Feb. 5, 1981	3.0	2.8	2.7
	Mar. 11, 1981	3.8	4.6	4.0
	April 13, 1981	2.3	1.1	0.9
	May 18, 1981	1.8	0.8	0.5
	June 23, 1981	1.1	0.3	0.2

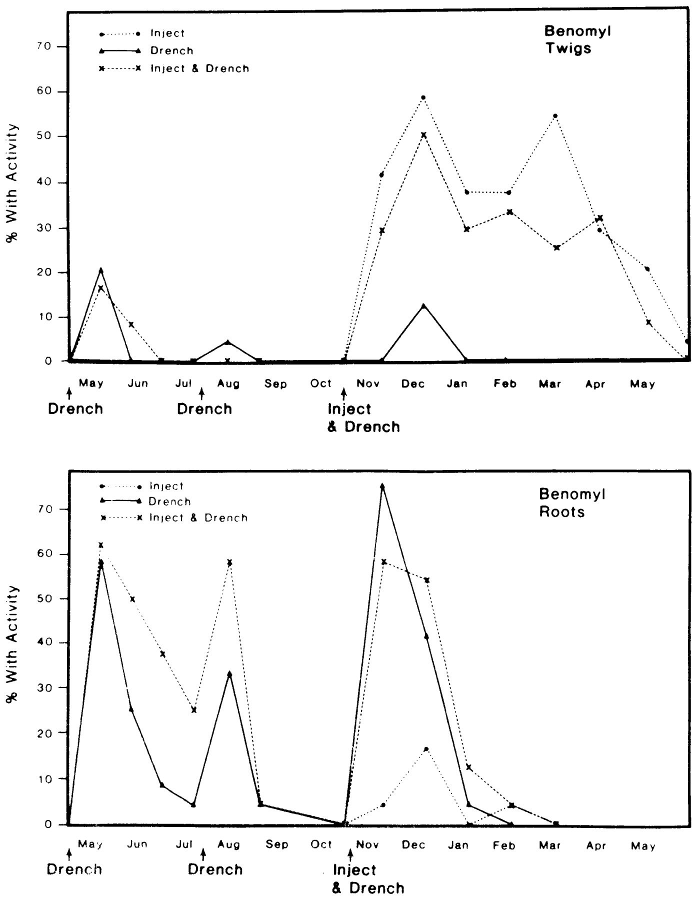

Fig. 2. Effect of benomyl soil drench and trunk injection treatments on the percentage of twigs and roots with activity against *Penicillium expansum*.

declined and the average zinc level in the injection plus drench treatment was significantly lower than in control trees. In April 1982, the zinc level of the injection and drench treatment remained low and was comparable to healthy trees in the same grove, whereas the zinc levels increased for the injection-only and drench-only treatments (Table 3). The zinc levels in the control trees remained fairly constant throughout the experiment. Growth increases in scaffold limbs and trunks were not significantly different among treatments. There were no significant differences in water uptake among treatments at the conclusion of the experiment. There was no significant difference in the amount of plugging in xylem vessels between nontreated control trees and injection plus drench treatment trees which averaged 0.98 and 0.87 plugs per xylem vessel, respectively. This amount of plugging was significantly different, however, from the average of 0.01 plugs per vessel for healthy trees in the same grove.

Trees in all benzimidazole treatments continued to decline (Table 4). Zinc levels in trunk wood increased in June 1981 and remained high through April 1982, and there were no significant differences among treatments. Treated trees were not significantly different from the untreated controls in trunk and branch growth or in water uptake (Table 4).

Except for a few strap-shaped leaves on the new growth produced on trees injected with OTC, no phytotoxicity was observed with any treatment.

Discussion

As in previous work (22), trunk injection of OTC resulted in uniform distribution of the material in the tree canopy, but distribution in the roots was limited. By applying OTC as a soil drench, uniform distribution of the material in the root system was achieved and with the injection plus drench treatment, the material was distributed through most of the tree. OTC was highly persistent in soil as reported previously (8).

No work has been published on the distribution and persistence of benzimidazoles in citrus trees. The distribution of this material in the tree was much like that of OTC in that activity was well-distributed in the canopy following trunk injections and was well-distributed in roots following soil drenches. Thus, the distribution of a material in the tree is probably as much a function of the method of application as the nature of the product. Benomyl persisted in elm trees for several years following soil injection of high rates (1), but, in the present study, activity was detectable in citrus trees for only a few months following drench or trunk-injection treatments. While benomyl persists for long periods in plants, it appears to break down rapidly in soil. Gupta and Chatrath (6) reported that benomyl was rapidly degraded in nonsterilized soil and that the degradation was accelerated by increasing temperatures up to 35°C. In our work, benomyl persisted in soil for up to 2 to 3 months at times, but disappeared more rapidly in summer.

Table 2. Effect of time and soil depth on activity in the soil against *Penicillium expansum* following soil drench treatment with benomyl. Data from drench and injection plus drench treatments were combined and averaged since there was no difference between the 2 treatments.

		Avg radius of inhibition zone (mm)		
			Soil depth	
Application date	Bioassay date	0–15 cm	15–30 cm	30–45 cm
April 18, 1980	May 20, 1980	11.6	6.1	8.4
	June 10, 1980	5.5	1.0	1.3
	July 1, 1980	0.7	0.0	0.0
	July 21, 1980	0.2	0.0	0.0
July 25, 1980	Aug. 14, 1980	2.3	0.2	0.2
	Sept. 4, 1980	0.0	0.0	0.0
Nov. 14, 1980	Nov. 25, 1980	8.5	6.1	5.9
	Dec. 23, 1980	4.0	1.0	0.8
	Jan. 21, 1981	4.3	1.0	1.0
	Feb. 19, 1981	0.1	0.1	0.0

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Table 3. Effect of oxytetracycline soil drench and trunk-injection treatments of trees affected with citrus blight on tree condition, zinc content of trunk wood, growth of trunks and scaffold branches, and water uptake.

	Tree condition rating[z]			Zn content of trunk wood (ppm)			Increase in circumf. (%)		Water uptake
Treatment	Mar. 1980	June 1981	Apr. 1982	Mar. 1980	June 1981	Apr. 1982	Branches	Trunk	(ml/24 hr)
Injection	1.2a	2.5a	2.7a	14.4a	6.1ab	9.8a	2.7a	2.0a	18b
Drench	1.0a	2.5a	2.4a	7.4b	6.0ab	13.2a	2.5a	1.5a	40b
Injection + drench	1.1a	2.3a	1.9a	9.7ab	3.9b	4.3b	2.0a	2.8a	52b
Control	1.2a	2.5a	2.5a	10.4ab	10.4a	10.1a	1.4a	2.3a	28b
Healthy	---	---	---	---	---	5.5b	---	---	295a

[z]Rated on a scale of 0 = healthy; 1 = mild; 2 = moderate; and 3 = severe blight symptoms.

Table 4. Effect of benzimidazole soil drench and trunk injection treatment of trees affected with citrus blight on tree condition, zinc content of trunk wood, growth of trunks and scaffold branches, and water uptake.

	Tree condition rating[z]			Zn content of trunk wood (ppm)			Increase in circumf. (%)		Water uptake
Treatment	Mar. 1980	June 1981	Apr. 1982	Mar. 1980	June 1981	Apr. 1982	Branches	Trunk	(ml/24 hr)
Injection	1.0a	2.0a	2.3a	9.7a	13.9a	13.2a	1.5a	1.8a	37b
Drench	1.0a	2.3a	2.6a	10.1a	11.8a	10.4a	1.2a	1.1a	10b
Injection + drench	1.0a	2.1a	2.3a	12.7a	15.1a	12.9a	1.3a	1.5a	20b
Control	1.0a	2.4a	2.5a	9.6a	10.1a	14.4a	1.4a	1.3a	42b
Healthy	---	---	---	---	---	3.1b	---	---	210a

[z]Rated on a scale of 0 = healthy; 1 = mild; 2 = moderate; and 3 = severe blight symptoms.

In spite of good distribution of both materials using injection plus drench treatments, there was no remission of symptoms in the benzimidazole treatments and only a slight response in the OTC treatments. With OTC treatments, 2 symptoms associated with blight were partially reversed. Tree condition improved for the injection plus drench treatment from an average rating of 2.3 in June 1981 to an average of 1.9 in April 1982, but differences among treatments were not statistically significant. The zinc content of trunk wood of injection plus drench treated trees, which was low in June 1981, was again significantly less than untreated trees in April 1982. Zinc levels fluctuate somewhat depending on the season (26) which could account partially for the general decline in zinc levels from March 1980 to June 1981 in some treatments (Table 3). However, zinc levels in the injection plus drench treatment trees declined sharply and remained low through April 1982. Thus, the combination treatment with OTC reversed one of the characteristic symptoms of blight—high zinc levels in trunk wood. This observation might be taken as an indication that OTC had controlled a pathogenic organism in the tree. However, reduced water uptake, lack of growth in trunk and major scaffolds (Table 3), and a high number of plugs in the xylem vessels indicated no improvement in the other symptoms associated with blight.

All of our chemotherapy work (15, 22) has failed to produce substantial reversal of blight symptoms and failed to conclusively confirm or refute proposed bacterial or fungal etiologies for blight. Whatever the cause of blight, we strongly suspect that once a tree has begun to show symptoms, permanent internal changes already have occurred which make it difficult to reverse further decline of the tree. Additional chemotherapy work is probably not justified until and unless a method is developed to reliably detect blight trees prior to expression of visible symptoms.

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Effectiveness of Fluidized Bed Material as a Calcium Source for Apples[1]

R. F. Korcak[2]

U.S. Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Beltsville, MD 20705

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Abstract. **Fluidized bed material (FBM), a dry, high Ca, alkaline waste product which results from combining coal and limestone, was used as the sole source of Ca for 'York Imperial' apples (*Malus domestica* Borkh.) grown in outdoor sand cultures over 3 growing seasons. FBM treatments were compared to gypsum, applied at similar rates based on apple tree Ca requirements, and to a no Ca amended control. Over 3 years, leaf Ca was significantly enhanced by increasing levels of FBM. FBM was a better Ca source compared to gypsum applied at similar rates only during the third year. Fruit flesh Ca and the incidence of cork spot were not significantly or consistently affected by treatments. There were no visual or nutrient deficiencies or toxicities noted from the FBM nor were yields and average fruit size affected.**

With the current increased utilization of coal as an energy source, there is a need to reduce SO_2 emissions from coal-fired plants. One of the newer, economical methods of reducing SO_2 emission from such plants is the utilization of the fluidized-bed combustion boiler design. This process involves mixing fine grain coal and limestone (or dolomitic limestone) in a furnace with a "fluid bed" achieved by injecting air. The limestone reacts with S from the coal during combustion. Unlike the waste sludge produced by conventional scrubber facilities, the fluidized bed material (FBM) is dry and easily transported. Ruth (7) estimated that a 1000 MW fluidized-bed power plant without regeneration of the bed material would produce about 1800 metric tons of FBM per day.

Terman et al. (11) utilized FBM both as a nutrient source for peanuts and corn and as a pH amendment for acid soils and coal mine spoils. They found that the material was satisfactory for both purposes. Since FBM contains about 30% Ca and since low Ca has been associated with many fruit disorders (8), FBM may also be beneficial as a Ca source or lime substitute for apples. Previous work (5) has shown that FBM, when applied at or near the lime requirement of a range of soils, increased apple seedling growth and Ca status in the greenhouse. This

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[2]Soil Scientist, Fruit Laboratory, Horticultural Science Institute.