

Techniques for Screening Chemicals for Fire Blight Control

J. L. NORELLI and J. D. GILPATRICK, Department of Plant Pathology, New York State Agricultural Experiment Station, Cornell University, Geneva 14456

ABSTRACT

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Two *in vivo* methods are described for screening chemicals for control of fire blight of apple caused by *Erwinia amylovora*. One method uses immature pear fruit tissue and the other, germinating apple seedlings. The immature pear fruit tissue test predicted the field activity of all chemicals tested. The germinating apple seedling test predicted the activity of the antibiotics, copper compounds, and fungicides tested but failed to predict the activity of some synthesized organic compounds.

In vitro and greenhouse evaluations of chemicals for fire blight control have been unreliable for selecting chemicals that are effective in the orchard. Growth

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of *Erwinia amylovora* (Burr.) Winslow et al *in vitro* may be inhibited by chemicals, such as maneb and mancozeb (6), that do not control fire blight in the orchard. Tests conducted in the greenhouse in which chemicals are applied to vegetative apple shoots followed by inoculation with *E. amylovora* are not useful in screening chemicals for the control of blossom blight (3; *personal observation*), which is an important phase of the disease in the orchard. Potted blooming trees

have been used in greenhouse tests (4,5), but these trees are so difficult and expensive to maintain that only a limited number of chemicals can be tested. Blossoms on cut branches have also been used as test material in the greenhouse, but variable blossom vigor makes accurate disease evaluation difficult (4). Field techniques have been developed (1), but they are limited by cost and seasonality.

There is a need for inexpensive, rapid, and reliable laboratory and greenhouse methods for preliminary evaluation of experimental chemicals. We now describe two new methods for evaluation of chemicals for the control of fire blight.

MATERIALS AND METHODS

Chemical compounds. Three bactericides that are known to control fire blight in the orchard (9) were evaluated: cupric hydroxide (Kocide 101, 83W; Kocide Chemical Corp., Houston, TX 77045),

streptomycin (Agrimycin 17, 17W; Pfizer Chemical Co., New York, NY 10017, and Agri-Strep 17W; Merck and Co., Rahway, NJ 07065), and MBR-10995 3S (Minnesota Mining and Manufacturing, St. Paul, MN 55101). Three fungicides that have no proven effect against fire blight in the field were also evaluated: captan (50W; Rohm and Haas Co., Philadelphia, PA 19105), benomyl (Benlate, 50W; E.I. du Pont de Nemours & Co., Wilmington, DE 19898), and mancozeb (Dithane M-45, 80W; Rohm and Haas Co.). These six chemicals served as standards to test the reliability of the new test methods evaluated. Oxytetracycline (Mycoshield, 17W; Pfizer Chemical Co.), RE-20615 [(2-chloro-N-(2,6-dimethylphenyl)-N-(tetrahydro-2-oxo-3-furanyl)acetamide, 50W; Chevron Chemical Co., Fresno, CA 94105], and the three experimental bactericides KL-496 (Kalo Laboratories, Kansas City, MO 64114), A-16886B (Lilly Research Laboratories, Indianapolis, IN 46140), and CGA-78039 50W (Ciba-Geigy, Greensboro, NC 27409) were evaluated by the new test methods.

Field test. Field tests were conducted in Geneva, NY, on Idared apple trees on M.7 rootstock. Dilute sprays were applied to runoff to selected branches. A protective spray was applied at 25–50% bloom. Twenty-four hours later, or at about 50–70% bloom, treated branches were inoculated with a suspension of *E. amylovora*. A suspension of the bacterium containing 10^6 – 10^7 colony-forming units (CFU) per milliliter was sprayed into blossoms using a nitrogen-pressurized atomizer. Three days after inoculation, a second spray (eradicator application) was applied to treated branches. Three to four weeks later, the proportion of blighted blossom clusters on treated limbs was recorded. Field plots were designed as completely randomized blocks with five blocks per treatment and one single tree replicate per block.

Immature pear fruit tissue test (IPFT). The method of Koike et al (8) for screening chemicals for the control of soft rot diseases was modified for fire blight

by substituting cubes of immature pear fruit tissue for carrot tissue plugs. Immature pear fruits were harvested 4–8 wk before normal harvest date when fruits were 25–50 mm in diameter. Fruits were stored at 0–1 C until used. Fruits were cut into cubes approximately 1 cm³, placed in a solution of test chemicals for 4–5 min, and drained. Five identically treated cubes were placed equidistantly apart on moist filter paper in a petri dish. The cubes were then inoculated by placing a 10- μ l drop of an 18-hr-old Kado 523 broth (7) culture of *E. amylovora* containing approximately 10^9 – 10^{10} CFU/ml on top of each cube. The inoculated cubes were incubated at 28 C for 48 hr and then evaluated for symptoms of disease according to the following scale: 0 = no symptoms, 1 = slight ooze or water-soaking, and 2 = much ooze and discoloration (Fig. 1). The mean rating obtained for five cubes in one petri dish constituted one replicate.

Chemicals were tested at four concentrations at fivefold increments, and each concentration was replicated four times. Each chemical was tested at one concentration above the expected rate required for fire blight control in the orchard and at three concentrations below that rate. Disease rating was plotted against log₁₀ chemical concentration, and the least squares estimated regression line was calculated. From the regression line, the expected chemical concentration for a disease rating of 1 was calculated. A positive test was indicated if the calculated chemical concentration for a disease rating of 1 was below the manufacturer's suggested rate of application.

Germinating apple seedling test (GAST). Apple seeds were stratified, germinated, and allowed to develop until the seed coat could be removed easily. Four seedlings were placed into holes in the lid of a petri dish so that the roots were submerged in water and the cotyledons were above the plate lid (Fig. 2). Seedlings were grown under a combination of fluorescent and incandescent lamps (14-hr photoperiod) until at

least two and preferably three true leaves developed. The seedlings were sprayed with solutions of test chemicals and incubated for 24 hr under lights at approximately 25 C. Seedlings were then inoculated by bisecting the youngest unfolded leaf with scissors that had been dipped in an 18-hr Kado 523 broth culture of *E. amylovora*.

Four days after inoculation, the proportion of diseased plants per dish was recorded. One dish of plants constituted one replicate. Plants were considered diseased if discoloration of the inoculated leaf had progressed into the vascular tissue of the leaf petiole. Chemicals were tested at four concentrations at fivefold increments, and each concentration was replicated four times. Two concentrations were above the expected rate required for fire blight control in the orchard, and two concentrations were below that rate. Regression analysis of the proportion of infected plants versus log₁₀ chemical concentration was done. The null hypothesis that the slope of the regression line was equal to 0 was tested using an *F* statistic. Rejection of the null hypothesis indicated a positive test.

RESULTS

Field test. Control of fire blight in 1979 orchard trials with the six standard chemicals applied at recommended field rates is given in Table 1. Streptomycin at 100 mg/L, cupric hydroxide, and MBR-10995 significantly reduced blossom infection relative to the water check. The three materials did not differ significantly from each other. In contrast, the fungicides mancozeb, captan, and benomyl did not differ significantly from the water control.

In orchard tests in 1979, a year with high disease incidence, oxytetracycline significantly reduced the amount of fire blight blossom infection but was less effective than streptomycin, whereas RE-20615 and KL-496 gave no control. In

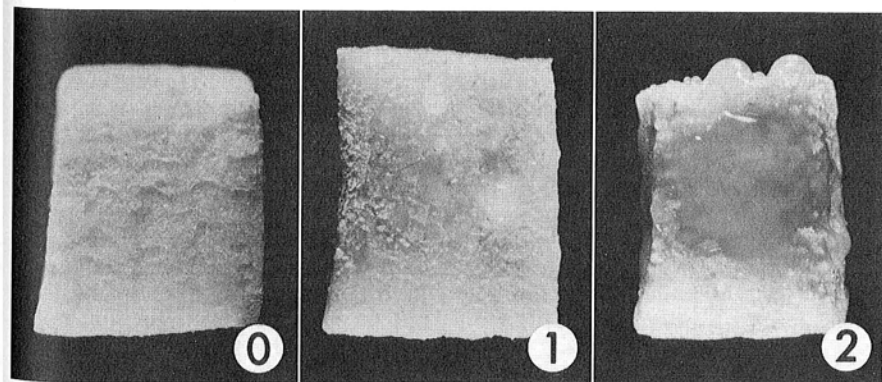


Fig. 1. Disease ratings of pear cubes based upon symptoms resulting from inoculation with *Erwinia amylovora* in immature pear fruit tissue test. Rating scale: 0 = no symptoms, 1 = slight ooze or water-soaking, 2 = much ooze and discoloration.

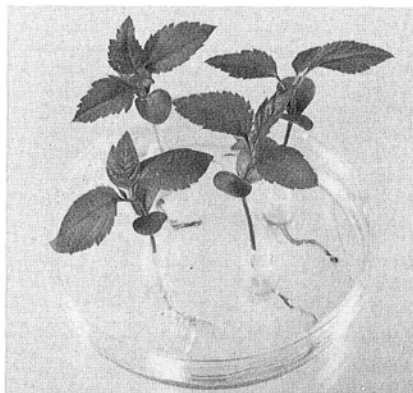


Fig. 2. Germinating apple seedling test. Four seedlings placed in a petri dish with roots submerged in water and cotyledons extending above the plate lid. Illustrated plate is ready for treatment with chemicals and inoculation with *Erwinia amylovora*.

1980, a year with very low disease incidence, oxytetracycline and A-16886B significantly reduced fire blight blossom infection but not as effectively as streptomycin. In 1981, CGA-78039 significantly reduced fire blight blossom infection. The rate of CGA-78039 used was below that previously reported to give control comparable with streptomycin (2).

Immature pear fruit tissue test. The sensitivity of IPFT to low concentrations of streptomycin is illustrated in Figure 3. At the rate of streptomycin recommended for orchard application (100 mg/L), the

Table 1. Control of fire blight of apple blossoms in the orchard¹

Chemical treatment	Conc. (mg a.i./L)	Blossom clusters infected (%) ²
Results for 1979		
Streptomycin	100	21 a
Streptomycin	50	48 bcd
Cupric hydroxide	1,492	30 ab
MBR-10995	300	32 abc
Mancozeb	1,400	66 def
Captan	1,200	72 f
Benomyl	225	67 ef
Oxytetracycline	200	42 bc
Oxytetracycline	100	50 cde
RE-20615	2,100	67 def
KL-496	50	78 f
Water	10	65 def
Water	...	66 def
Results for 1980		
Streptomycin	100	01 a
Streptomycin	50	07 b
Oxytetracycline	200	04 b
Oxytetracycline	100	08 b
A-16886B	250	08 b
Water	...	30 c
Results for 1981		
Streptomycin	100	04 a
CGA-78039	100	11 a
Water	...	59 b

¹Blossoms had been inoculated with *Erwinia amylovora* using a standardized technique.

²Treatments with the same letter within a single year did not differ significantly ($P = 0.05$) according to Waller and Duncan's Bayesian multiple comparison procedure (10) performed on arcsin transformed data.

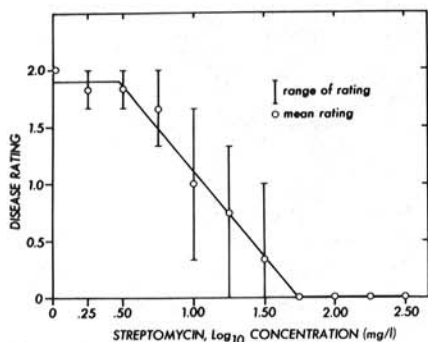


Fig. 3. Sensitivity of immature pear fruit tissue test to increasing concentrations of streptomycin. Disease rating plotted against \log_{10} streptomycin concentration. Disease rating is a measure of symptom development on fruit tissue resulting from inoculation with *Erwinia amylovora*.

disease rating was 0; at 10 mg/L, the disease rating was 1.

Graphs illustrating the activity of the six standard chemicals in IPFT are shown in Figure 4. Disease rating is plotted against \log_{10} chemical concentration with the calculated regression line. Streptomycin, cupric hydroxide, and MBR-10995 each caused a rapid decrease in disease rating with increasing chemical concentration. Mancozeb had little effect, and there was no response to increasing chemical concentration with captan and benomyl. The expected chemical concentrations for a disease rating of 1 calculated from the regression line are streptomycin, 12 mg/L; cupric hydroxide, 39 mg/L; MBR-10995, 6 mg/L; mancozeb, 115,000 mg/L; captan, $>10^6$ mg/L; and benomyl, $>10^6$ mg/L.

Oxytetracycline and CGA-78039 were assigned positive test ratings in IPFT based upon the expected chemical concentrations for a disease rating of 1

obtained from the calculated regression line. The concentrations were far below rates recommended by the suppliers (Table 2).

Germinating apple seedling test. The sensitivity of GAST to streptomycin is in the approximate range of rates recommended for orchard application (Fig. 5). At 100 mg/L, the proportion of infected seedlings was reduced to half, and there was a response to streptomycin concentrations in the range of 25–600 mg/L.

In GAST, streptomycin and cupric hydroxide caused a significant decrease in the percentage of infected seedlings with increasing chemical concentration (Table 3). With MBR-10995, there was a decrease in the percentage of infected seedlings with increasing chemical concentration, which was not significant at $P = 0.05$. Mancozeb, captan, and benomyl caused no response.

The experimental chemicals oxytetra-

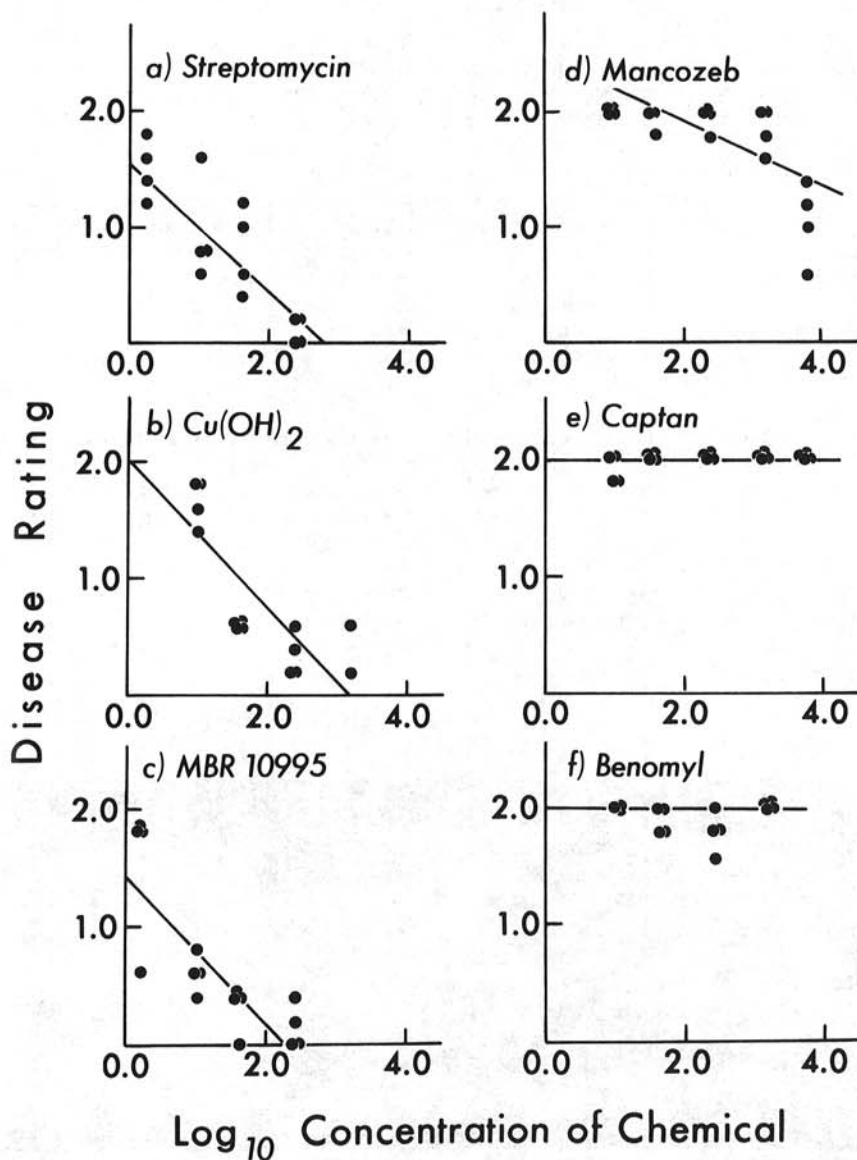


Fig. 4. Activity of six standard chemicals in immature pear fruit tissue test. Disease rating plotted against \log_{10} chemical concentration and calculated regression lines. Disease rating is a measure of symptom development on fruit tissue resulting from inoculation with *Erwinia amylovora*.

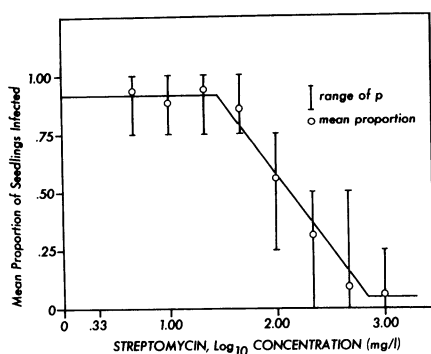


Fig. 5. Sensitivity of germinating apple seedling test to increasing concentrations of streptomycin. The mean proportion of seedlings having visible symptoms of fire blight infection is plotted against \log_{10} streptomycin concentration.

cycline and A-16886B were assigned positive test ratings in GAST based upon significant decreases in the percentage of infected seedling with increasing chemical concentration, whereas RE-20615, KL-496, and CGA-78039 were assigned negative ratings (Table 3).

DISCUSSION

The efficacy of six standard and experimental chemicals in controlling fire blight in IPFT agreed completely with their ability to control disease in the orchard. The calculated chemical concentration for a disease rating of 1 in IPFT was far below recommended orchard rates for streptomycin, cupric hydroxide, and MBR 10995. These standard chemicals also gave excellent control of fire blight in the orchard (Table 1). Cupric hydroxide was phytotoxic in the orchard at the tested concentration.

Although mancozeb showed some activity in IPFT at the highest concentration tested (Fig. 4), the expected concentration for a disease rating of 1 calculated from the regression line was a value far in excess of recommended field rates. To consider the results from IPFT positive, the chemical concentration for a disease rating of 1 should be below a reasonable field rate. Based upon this criterion, mancozeb was given a negative rating by IPFT. In vitro, however, mancozeb shows significantly more activity than streptomycin (6; *personal observation*). IPFT demonstrates a higher level of selectivity for predicting orchard activity than traditional in vitro techniques.

Oxytetracycline and CGA-78039 gave strong positive results in IPFT and significantly reduced the amount of blossom infection in the orchard (Tables 1 and 2). A-16886B showed low activity in IPFT but significantly reduced the amount of blossom infection in the orchard. It should be noted that A-16886B was orchard tested in a year with low disease incidence and that its performance under higher disease

Table 2. In vivo activity of experimental chemicals in controlling fire blight in immature pear fruit test

Chemical	Calculated regression line	Conc. at disease rating of 1 (mg a.i./L) ²	Recommended field conc. (mg a.i./L)	Test rating
Oxytetracycline	$y = -0.40x + 0.865$	0.5	200	+
RE-20615	$y = -0.0004x + 1.99$	$>10^6$	2,000	-
KL-496	$y = -0.28x + 2.09$	7,800	10-50	-
A-16886B	$y = -0.44x + 2.06$	247	250-500	±
CGA-78039	$y = -0.64x + 1.29$	15	200-400	+

²Expected chemical concentration at a disease rating of 1 obtained from calculated regression line for in vivo activity where y = disease rating and x = \log_{10} chemical concentration. Disease rating is a measure of symptom development on fruit tissue following inoculation with *Erwinia amylovora*. Expected chemical concentration at a disease rating of 1 below the recommended field concentration (suggested by manufacturer) is considered a positive test rating.

Table 3. Activity of six standard and experimental chemicals in controlling fire blight in germinating apple seedling test

Chemical	Infected seedlings (%) at conc. (mg a.i./L)						Regression F value	Test rating ^y
	10	50	250	1,250	6,250	31,250		
Streptomycin	100	58	40	19	23.98 ^z	+
Cupric hydroxide	94	94	47	54	12.57 ^z	+
MBR-10995	...	85	69	52	60	...	3.95	-
Mancozeb	94	90	100	100	2.06	-
Captan	90	79	94	79	0.11	-
Benomyl	...	77	100	90	75	...	0.15	-
Oxytetracycline	100	58	63	13	16.37 ^z	+
RE-20615	88	88	81	94	0.15	-
KL-496	88	100	92	83	0.11	-
A-16886B	...	100	83	56	75	...	7.04 ^z	+
CGA-78039	94	81	94	94	0.19	-

^yA positive test rating is indicated by a significant decrease in the percentage of seedlings having visible symptoms of fire blight infection with increasing chemical concentration.

^zSignificance at $P = 0.05$.

pressure is not known. Both KL-496 and RE-20615 were inactive in IPFT, and neither was effective in the orchard. KL-496, a compound with strong in vitro activity against *E. amylovora*, behaved in a manner similar to mancozeb, causing a reduction in disease rating only at extremely high chemical concentrations.

Although IPFT accurately predicts which chemicals should be tested in the orchard, it is not reliable for determining the appropriate concentration of experimental chemicals to be used in the orchard. Both oxytetracycline and CGA-78039 were as active as streptomycin in IPFT, yet they required higher concentrations than streptomycin for equal control in the orchard.

GAST predicted the orchard activity of streptomycin, cupric hydroxide, oxytetracycline, and A-16886B and the lack of activity of captan, benomyl, mancozeb, RE-20615, and KL-496 (Tables 1 and 3). However, it did not detect the activity of the synthesized organic compounds MBR-10995 and CGA-78039, which control fire blight in the orchard.

Both IPFT and GAST are valuable preliminary screening techniques to evaluate new chemicals for their ability to control fire blight. IPFT is a simpler and faster test than GAST and is more reliable for predicting field activity. IPFT also responded to lower concentrations of test chemicals than GAST (Figs. 3 and 5) and can be conducted with very small

quantities of test chemicals. However, IPFT requires a supply of immature green pear fruits, which can be stored for only 4-5 mo.

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