Control of Pineapple Disease of Sugarcane with Propiconazole

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ABSTRACT

Comstock, J. C., Ferreira, S. A., Ching, S. A., and Hilton, H. W. 1984. Control of pineapple disease of sugarcane with propiconazole. Plant Disease 68: 1072-1075.

In culture, propiconazole inhibited the growth of *Ceratocystis paradoxa*, the major pathogen that rots vegetative cuttings of sugarcane (*Saccharum* spp.) in Hawaii. Percent germination of the lateral buds of propiconazole-treated vegetative cuttings was greater than that of vegetative cuttings treated with benomyl and thiophanate-methyl, the two fungicides registered for use in Hawaii, and considerably greater than for untreated controls. Propiconazole is water-soluble at the recommended concentrations, and hence compatible with a 20-min immersion in water at 52 C, which is used in Hawaii to stimulate lateral bud germination of vegetative cuttings. At 8 or 12 wk, whole plants that developed from treated vegetative cuttings had residues less than 0.01 ppm based on the use of radiolabeled fungicide. Once registered, propiconazole will be useful in controlling an important sugarcane disease.

In pineapple disease of sugarcane, the pathogen *Ceratocystis paradoxa* (Dade) C. Moreau rots the vegetative cuttings of sugarcane (interspecific hybrids of *Saccharum* spp.) before the lateral buds germinate, causing germination failure. The disease was named pineapple disease because of the pineapple-like odor produced by the rotted cuttings after they are infected (4).

Pineapple disease of sugarcane has been reported in almost all sugarcane-growing countries (2) and is usually controlled by immersing vegetative cuttings in or spraying them with a fungicide solution before planting. Mercury compound MEMC is used in Australia (3) and India (1). Triadimefon and benomyl are also used in Australia. In Hawaii, vegetative cuttings are immersed in one of two fungicides, benomyl (Benlate) or thiophanate-methyl (Topsin-M), to protect the cut ends from infection. In addition, the cuttings are given a heat treatment either before or simultaneously with fungicide treatment to stimulate germination. Because benomyl and thiophanate-methyl settle out of suspension with cane debris and soil during seed treatment, this work was initiated to find an effective, more soluble fungicide. This paper presents data on the effectiveness of propiconazole (Tilt) in controlling pineapple disease.

Published with the approval of the director as paper 569 in the Journal Series of the Experiment Station, Hawaiian Sugar Planters' Association.

Accepted for publication 18 May 1984.

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MATERIALS AND METHODS

Fungicidal activity in vitro. The fungicidal activity of benomyl, thiophanate-methyl, and propiconazole against *C. paradoxa* was determined by incorporating the fungicides into Fries agar and measuring the diameters of colonies developing from mycelial plugs placed on the agar surface. Fungicidal activity is expressed as the concentration that inhibits growth by 50%.

Inoculum concentration. In field trials, natural inoculum levels of *C. paradoxa* vary and do not always cause sufficient disease for fungicide evaluation. Therefore, an inoculum level high enough to allow disease expression but low enough to allow fungicide control had to be determined. Vegetative cuttings of variety H50-7209 were treated at 52 C for 20 min in 150 mg a.i./L of benomyl suspension or in water and stored overnight. Before they were planted, the cuttings were inoculated by spraying 0.5-ml spore suspensions ranging from 10 to 1×10^7 *C. paradoxa* chlamydospores per milliliter on each end. Undamaged lateral buds were counted on each vegetative cutting. The amount of disease developing from each inoculum level was expressed as the percentage of undamaged lateral buds that failed to germinate. In Figure 1, the relationship between the amount of disease

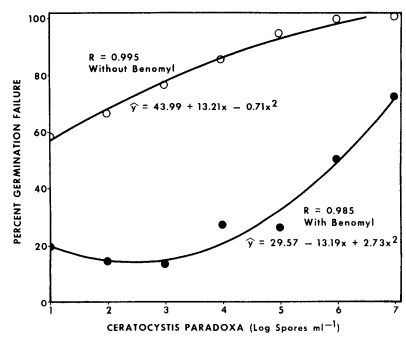

Fig. 1. Effect of *Ceratocystis paradoxa* inoculum concentration on pineapple disease as measured by germination failure.

and inoculum concentration for benomyl-treated and untreated vegetative cuttings is given. Disease increased with inoculum concentration for both benomyl-treated and untreated cuttings. The slight drop in disease in the inoculum curve with benomyl probably reflects random variation of natural inoculum present in the soil. A concentration of 10^4 chlamydospores per milliliter was selected as the appropriate inoculum level for fungicide evaluation because at that level, benomyl gave adequate control, whereas more than 80% germination failure occurred in the untreated control.

Effect of propiconazole concentration on growth. Disease-free (uninoculated), single-bud vegetative cuttings were treated with a range of propiconazole concentrations in water by two treatment methods: 1) simultaneous fungicide and hot-water treatment for 20 min at 52 C and 2) a 1-min fungicide dip at ambient temperature without prior hot-water treatment. Vegetative cuttings were incubated at 28 C after treatment and shoot and root measurements were taken 7 days after treatment.

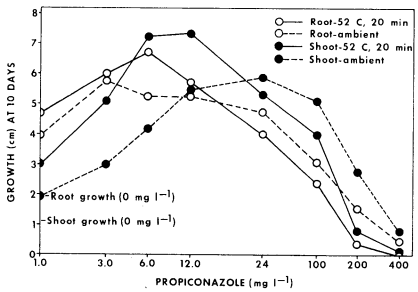

Fig. 2. Effect of propiconazole, applied in a 20-min soak at 52 C or in a 1-min dip at ambient temperature, on sugarcane shoot and root growth.

Field efficacy. Field trials were conducted at three high-elevation locations where cool temperatures favor disease development. The purpose of these tests was to determine the propiconazole concentration that effectively controls pineapple disease. In a test at The Lihue Plantation Company on the island of Kauai, variety H62-4671 was treated in two ways: 1) with fungicide in water (52 C) for 20 min and 2) with a 1-min immersion in a fungicide solution after a 20-min hot-water (52 C) treatment. Other tests were conducted at Mauna Kea Sugar Company (variety H70-144) and Ka'u Sugar Company (variety H56-4848) on the island of Hawaii, where a third treatment, a 1-min cold fungicide dip without any hot-water treatment, was evaluated along with the two treatments used in The Lihue Plantation Company test. Three concentrations of propiconazole were used. Controls in all tests were subjected to the appropriate water treatment but without fungicide. Each test had six replicates of 16 vegetative cuttings per treatment. Seed pieces were treated, inoculated with 1.0×10^4 *C. paradoxa* spores per milliliter, stored overnight, and planted. Determination of disease control was based on the percentage of germination of undamaged lateral buds identified at planting.

In a subsequent field trial, a factorial experiment was conducted to compare the responses of five sugarcane varieties (H56-4848, H62-4671, H68-2235, H70-0144, and H70-6957) to the effective propiconazole rate using the three methods of treating vegetative cuttings listed earlier. A control dipped in water only was included for each of the three treatment methods. The test was conducted similarly to the previous tests. Six replicates of 16 three-bud vegetative cuttings each were used per treatment. Fungicide efficacy was judged on the basis of the germination per undamaged lateral bud planted.

Residue analysis. Propiconazole residues were determined in sugarcane plants that developed from treated single-bud vegetative cuttings. Propiconazole-^{14}C) (Triazole) was provided by the Ciba-Geigy Corporation (specific activity 59.5 μCi/mg). Single-bud cuttings 10 cm long were immersed for 1 min in water containing two treatment levels, 27.1 and 57.2 mg a.i./L of radiolabeled plus unlabeled propiconazole. The radioactivity was adjusted so that residues could be determined to at least 0.01 μg/g. Six vegetative cuttings were immersed in a 50-ppm unlabeled propiconazole solution and used as controls to determine radioactive background. Two cuttings of each treatment plus a control served as 0-day samples and were not planted. The remaining vegetative cuttings were planted immediately after treatment. Plants from the field were sampled 4, 8, 12, and 16 wk after planting. All samples were either chopped manually or processed in a Cuisinart food chopper, freeze-dried, and ground in a Wiley Mill until able to pass through a 20-mesh screen. A 100-mg portion was combusted in the Harvey biological material oxidizing instrument for quantitation of $^{14}CO_2$ by liquid scintillation counting (Beckman LS 7500). Subsampling combustion and counting were done in triplicate and averaged for results. Another 1-yr-old plant sample was obtained and processed in a mini-factory to obtain samples of fiber (bagasse), sugar, and molasses for residue analysis.

Table 1. Analysis of variance effect of fungicide treatments on percentage of germination at three sugarcane plantations

	Mauna Kea		Ka'u		Lihue	
	df	Mean square	df	Mean square	df	Mean square
Replicates	5	59.2	5	193.2	5	40.9
Fungicides (F)	2	4,047.0*[a]	2	4,626.6*	1	83.4
Methods (M)	3	1,591.3*	3	9,260.2*	3	3,662.7*
F × M	6	817.5*	6	1,289.8*	3	662.0*
Error	55	56.1	55	113.2	35	67.3

[a] An asterisk indicates significance at $P < 0.01$.

Table 2. Effect of propiconazole on percentage of germination at three sugarcane plantations

	Percentage of germination with indicated methods of application							
	Mauna Kea			Ka'u			Lihue	
Treatment[a]	HWT[b]	HWT + fungicide dip	Fungicide dip	HWT	HWT + fungicide dip	Fungicide dip	HWT	HWT + fungicide dip
Benomyl	34.8	16.8	35.1	37.4	26.9	43.5	40.8	44.8
Thiophanate-methyl	27.2	12.9	16.6	31.7	24.8	47.1	40.1	44.5
Propiconazole	47.4	43.9	47.9	54.1	53.7	50.0	57.3	50.9
Control	23.1	19.3	26.1	29.6	28.8	38.8	40.3	34.0
FLSD 0.05		12.28			8.65		9.47	
FLSD 0.01		16.39			11.40		12.66	

[a] Rates for benomyl: 150 mg a.i./L for HWT and 300 mg/L for HWT + fungicide dip and fungicide dip alone. Thiophanate-methyl: 210 mg a.i./L for HWT and 420 mg a.i./L for HWT + fungicide dip and fungicide dip alone. Propiconazole: 24 mg a.i./L for all treatments at Mauna Kea and Ka'u and 25 mg a.i./L at Lihue.

[b] Hot-water treatment.

RESULTS

Fungicidal activity in vitro. Propiconazole inhibited growth in culture by reducing colony diameter 50% at a concentration of 0.01 mg/L, whereas inhibitory concentrations of benomyl and thiophanate-methyl were 50 and 100 mg/L, respectively.

Effect of propiconazole concentration on growth. As levels of propiconazole treatment increased from 1 to about 25 mg/L, both shoot and root growth of uninoculated vegetative cuttings were increasingly stimulated over that of untreated controls (Fig. 2). Further increases of the fungicide above 25 mg/L resulted in less stimulation of growth until inhibition became apparent at 200 mg/L. The stimulation of growth by

Table 3. Effect of propiconazole and treatment method on lateral bud germination from vegetative cuttings of five sugarcane varieties

Treatment		Percentage of germination for indicated variety					
Method	Conc. (mg a.i./L)	H68-2235	H62-4671	H70-6957	H56-4848	H70-0144	Avg.[a]
1-Min cold dip, no HWT[b]	0 (check)	37.0	29.7	21.8	38.5	26.7	30.7
1-Min fungicide dip, no HWT	25	44.9	36.7	38.2	40.0	39.8	39.9
1-Min fungicide dip after HWT	0 (check)	44.2	46.5	30.2	20.5[c]	24.0	33.2
1-Min fungicide dip after HWT	25	68.0	57.6	51.0	40.6	37.4	50.9
20 Min at 52 C	0 (check)	46.7	49.2	29.6	42.5	27.0	39.0
20 Min at 52 C	25	53.1	67.6	57.9	40.7	46.8	53.0
Avg. for variety[d]		49.0	47.7	38.1	37.1	33.6	

[a] Average percentage of germination of all varieties for treatment.
[b] Hot-water treatment.
[c] Germination of this treatment of H56-4848 was unexplainably lower than the other 0-ppm propiconazole controls.
[d] Average percentage of germination of all treatments for variety.

propiconazole was similar under both treatment methods (52 C for 20 min or 1-min immersion at ambient temperature).

Field efficacy. Propiconazole effectively controlled pineapple disease in field tests. Analysis of variance of field tests separately by location showed fungicides, treatment methods, and the interaction of the two all significant except for fungicides in the Lihue test (Table 1). Treatment of vegetative cuttings with propiconazole controlled pineapple disease, as indicated by improved lateral bud germination over treatment with either benomyl or thiophanate-methyl in all tests and locations (Table 2). The lateral buds of the propiconazole-treated vegetative cuttings averaged 15% greater germination than vegetative cuttings treated with benomyl and thiophanate-methyl (Table 2). Among the propiconazole concentrations tested (0–25 mg/L), the higher the concentration the better the germination. A multiple-regression equation ($y = 32.4 + 1.94\,x - 1.51\,x^2$) was obtained between propiconazole concentration (x) and germination (y) with β_1 significantly greater than zero (0.05 level) and β_2 significantly less than zero (0.05 level) ($r = 0.6$). Furthermore, shoots developing from all levels of propiconazole-treated cuttings were more vigorous than shoots developing from vegetative cuttings receiving any of the other treatments. Plots in the Lihue test were rated using a scale of 1 = good, 2 = fair, and 3 = poor growth. The average rating of shoots from the propiconazole-treated vegetative cuttings was 1.3 compared with 2.0, 2.2, and 2.6 for the benomyl, thiophanate-methyl, and untreated vegetative cuttings, respectively.

Propiconazole at 25 mg/L, a concentration that effectively controls pineapple disease, was used to evaluate the responses of five varieties and three methods of fungicide treatment. Results showed the importance of considering variety, method of fungicide treatment, and the interaction of variety with method of fungicide treatment when deciding how to treat vegetative cuttings for pineapple disease control (Tables 3 and 4). This study showed the following: 1) Hot-water treatment alone increased germination of H68-2235, H62-4671, and H70-6957 by at least 6.8, 16.8, and 7.8% but not of H70-0144 or H56-4848. 2) Propiconazole treatment alone stimulated germination of all varieties except H56-4848. The results of variety H56-4848 were inconsistent with results obtained at Ka'u (Table 2). We do not know the reason. 3) Propiconazole treatment in addition to hot-water treatment further increased germination by about 13–17%, depending on the variety. 4) Propiconazole treatment (but not hot-water treatment) increased germination of variety H70-0144 by at least 13.1%.

Residue analysis. Immediately after treatment in a 27.1-mg/L propiconazole-^{14}C solution, two single-bud vegetative cuttings were analyzed and had residues of 0.48 and 0.37 μg/g on the basis of radiation counts. Remnants of two other vegetative cuttings sampled 12 wk after planting had residues of 0.045 and 0.051 μg/g. Developing plants absorbed only small amounts of propiconazole from the treated vegetative cuttings. No residues greater than 0.01 μg/g (detection limit) were detected in whole plants at 8 wk or in stalks, green leaves, and suckers at 12 wk.

As expected, higher concentrations of residues were detected in remnants of the planted vegetative cuttings that were treated with 57.2 mg/L of propiconazole-^{14}C. At 16 wk after planting, concentrations of 0.23 and 0.10 μg/g were detected in the remnants of two vegetative cuttings. However, no residues greater than 0.01 μg/g were detected at 12 and 16 wk in stalks, leaves, or suckers developing from vegetative cuttings treated with the higher propiconazole-^{14}C concentration. At 58 wk after treatment, about halfway through the normal Hawaiian crop cycle of 2 yr plus, stalks were sampled, chopped, and processed into bagasse, raw sugar, and molasses. No residues were detected by methods that detected 0.01 μg/g as a limit.

DISCUSSION

Propiconazole is effective against pineapple disease at lower rates than benomyl or thiophanate-methyl and appears to be compatible with the standard practice of hot-water treating vegetative cuttings at 52 C, benefiting germination when applied either with or subsequent to hot-water treatment. For commercial use, a propiconazole concentration of 25 mg/L (about 5.6 ml of formulated Tilt/100 L) is recommended. The optimal chemical application method, whether with or subsequent to hot-water treatment, is variety dependent.

Another advantage of propiconazole is its initial stimulation of early growth. Field tests showed that plants developing from propiconazole-treated cuttings inoculated with *C. paradoxa* were more vigorous than plants that developed from either benomyl- or thiophanate-methyl-treated cuttings inoculated similarly. Also, propiconazole-treated cuttings produced more shoots per undamaged lateral bud. This is due in part to the improved control of pineapple disease. However, propiconazole also stimulated the initial shoot growth from disease-free (uninoculated) vegetative cuttings in laboratory tests. This initial growth stimulation may help to establish sugarcane stands.

Maintaining a suspension of either benomyl or thiophanate-methyl fungicides in water has created serious problems because a tank mix may be used over a 4- to 6-wk period. Fungicide concentration in the tank is monitored daily and additional fungicide is added as required. Even with agitation, benomyl and thiophanate-methyl, both water-insoluble wettable powders, settle out of suspension with settling soil and debris. Propiconazole, on the other hand, is an emulsifiable concentrate, with the active ingredient soluble in water at 110 ppm at 20 C. This

Table 4. Analysis of variance of germination data of propiconazole-treated vegetative cuttings of five sugarcane varieties

Source	df	Mean square
Blocks	5	206
Varieties (V)	4	1,675*[a]
Treatment method (T)	2	1,745*
Propiconazole conc. (P)	1	8,342*
V × T	8	524*
V × P	4	279
T × P	2	273
V × T × P	8	162
Error	145	121

[a] An asterisk indicates significance at $P<0.01$.

concentration is considerably greater than the 25-mg/L recommended usage rate. Because propiconazole will not precipitate out of solution, less fungicide may be used by this factor alone, making it both more economical and easier to handle.

There was an average of 0.4 μg/g of residue per vegetative cutting immediately after treating. About 6.7 t of vegetative cuttings are planted per hectare, and only 2.47 g/ha of propiconazole would be applied in a commercial field. This estimate may be high because the 0.4-μg/g residues found in the residue test were determined using short, single-bud vegetative cuttings. We have concluded, on the basis of assay of radioactive-dipped cuttings that showed most fungicide uptake is via the cut ends, that a normally used three-bud vegetative cutting would accumulate less fungicide on a weight basis. Even assuming the maximum of 2.47 g/ha of propiconazole applied, unmetabolized residues would remain in the soil or in the remnant cutting because sugarcane is harvested at the soil line. No residues were found in any of the plant parts 12 wk after planting, and the crop is harvested at 2 yr. Likewise, no residues were found in bagasse, raw sugar, and molasses prepared from sugarcane harvested 58 wk after planting. Considering a yield of about 224 t/ha of sugarcane from which 24.5 t of sugar is extracted, no detectable residues would be expected in the fiber, sugar, or molasses when sugarcane is harvested at 2 yr.

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