Effectiveness of New Systemic Fungicides for Control of Powdery Mildew of Begonia

J. A. QUINN, Graduate Research Associate, and C. C. POWELL, JR., Associate Professor, Department of Plant Pathology, Ohio State University, Columbus 43210, and the Ohio Agricultural Research and Development Center, Wooster, 44691

ABSTRACT

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The new systemic fungicides bitertanol, CGA 64251, fenarimol, triadimefon, and triforine were found to be effective preventive and eradicative mildewcides on begonia when applied as foliar sprays. Triadimefon and CGA 64251 were also found to give long-term prevention of powdery mildew when applied as dilute soil drenches. These fungicides were less phytotoxic to flowers than currently used fungicides. Even though applied before inoculation, these fungicides killed *Oidium begoniae* after haustorial formation and minimal hyphal growth had occurred, but before sporulation was initiated.

Only benomyl and dinocap are currently registered by the U.S. Environmental Protection Agency for control of powdery mildew on the highly susceptible Rieger begonias (*Begonia* × *hiemalis* Fotsek) and moderately susceptible wax begonia (*Begonia* × *semperflorens-culturum* Hort.) (2). In addition, lime sulfur and cycloheximide are registered for control of this disease on tuberous begonias. Dinocap is an effective preventive fungicide (9,11,12). However, the chemical is less effective as an eradicative fungicide because the fungus survives in buds and other locations that are difficult to reach with foliar sprays (5). Dinocap is also phytotoxic to flowers of Rieger begonias (9,11). Benomyl resistance in *O. begoniae* is widespread, causing this fungicide to be of limited use (4,10).

Several new systemic mildewcides that inhibit ergosterol synthesis in fungi have recently been released (3). This study reports on their efficacy, phytotoxicity, mode of action, and persistence in begonia and compares these results with those obtained for dinocap and benomyl.

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Present address of senior author: Rohm and Haas Research Laboratories, Spring House, PA 19477.

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MATERIALS AND METHODS

The fungicides tested were CGA 64251 (1-[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole; 0.8% EC or 10 WP, Ciba-Geigy, Greensboro, NC); bitertanol (Bay KWG 0599, Baycor 25 WP, Mobay Chemical Corp., Kansas City, MO); benomyl (Benlate 50 WP, Du Pont Chemical Co., Wilmington, DE); dinocap (Karathane 19.5 WD, Rohm and Haas Chemical Co., Philadelphia, PA); fenarimol (EL 222, 12.5% EC, Elanco, Indianapolis, IN); triadimefon (Bayleton 50 WP, Mobay Chemical Corp., Kansas City, MO); and triforine, (18.2% EC, Chevron Chemical Corp., San Francisco, CA). A sticker-extender (Exhalt 800, PBI Gordon Corp., Kansas City, KS) was added to all WP materials.

Protectant fungicides were tested on excised Schwabenland Red Rieger begonia leaves by dipping 30 leaves per treatment in fungicide suspensions for 10 sec, dipping the leaves for 10 sec in water 1 wk later to simulate watering, and inoculating them with conidia 2 wk later by shaking infected leaves over the treated leaves. The excised leaves were incubated at 21 C using a double petri dish chamber with the leaf petiole extended into water in the lower chamber (5–7). One week after inoculation the numbers of visible colonies and small, local lesions were counted. Germination of conidia on treated leaves and sporulation of resulting colonies were also noted.

Eradicative action of fungicides was tested by dipping 14 excised leaves per treatment in fungicide suspensions 1 wk after inoculation and incubating for 1 wk at 21 C and 90% relative humidity. The shriveled appearance or germinability of conidia from the previously treated colonies on these leaves was observed by shaking them over glass slides or uninfected begonia leaves. The appearance of the mycelium and haustoria was microscopically examined on leaf tissue mounted in water. In some instances, the tissue was stained by a malachite green-acid fuchsine mixture before microscopic examination (1).

Triadimefon and CGA 64251 were further tested as systemic protectants by pouring 100 ml of fungicide suspension into ten 15-cm-diameter pots of 16- to 18-wk-old plants per treatment or 50 ml of suspension into ten 10-cm-diameter pots containing recently transplanted, 10- to 12-wk-old plants per treatment. The plants were randomly placed on a

Table 1. Preventive fungicides for control of powdery mildew caused by *Oidium begoniae* on begonias[w]

Chemical	Rate (g a.i./100 L)	Colonies/leaf (mean no.)[x]	Leaves with local lesions (%)[y]
Dinocap 19.5 WD + Exhalt 800	9 + 60	0 b[z]	6 c[z]
Fenarimol 12.5 EC	2	0 b	94 a
Fenarimol 12.5 EC	4	0 b	100 a
CGA 64251 0.8 EC	5	0 b	88 a
CGA 64251 0.8 EC	10	0 b	83 a
Bitertanol 25 WP + Exhalt 800	30 + 60	0 b	67 ab
Bitertanol 25 WP + Exhalt 800	60 + 60	0 b	72 ab
Triforine 20 EC	12	1 b	94 a
Benomyl 50 WP + Exhalt 800	30 + 60	26 a	90 a
Control	···	53 a	44 bc

[w] Leaves dipped in fungicides and inoculated 2 wk later by shaking infected leaves over treated leaves.
[x] Leaves observed 7 days after inoculation. There were 30 excised leaves per treatment.
[y] Local lesions (minute necrotic areas) observed with naked eye at time of count.
[z] Numbers followed by the same letter do not differ significantly ($P = 0.05$) using Duncan's new multiple range test.

Table 2. Effect of preventive mildewcide treatments on stages in development of *Oidium begoniae* on excised leaves of *Begonia* × *hiemalis*

Chemical	Rate (g a.i./100 L)	Germination (%)[t]	Hyphal length (μm)[u]	Sporulation/ colony[v]	Haustoria (%)	
					Shriveled	Encapsulated
Control	...	38 a[w]	1,430 a[w]	620	5	3
Dinocap 20 WD	9[x]	1 c	0 c	0	...[y]	...
Triforine 20 EC	18[x]	37 a	110 b	0	...	...
CGA 64251 0.8 EC	10[x]	13 b	116 b	0	100	98
Triadimefon 50 WP	0.6[z]	14 b	106 b	0	99	99

[t] Germination observed 48 hr after inoculation on epidermal peels. Two leaves per treatment and three peels per leaf were observed.
[u] Hyphal length observed 7 days after inoculation. Five leaves per treatment and 10 longest hyphae in 10 colonies per leaf were counted.
[v] Sporulation observed after 9 days of inoculation. Five leaves per treatment and 10 colonies per leaf were counted.
[w] Numbers followed by the same letters do not differ significantly ($P = 0.05$) using Duncan's new multiple range test.
[x] Treated 2 wk before inoculation as a foliar spray to runoff.
[y] Haustorial observations not carried out.
[z] Treated 2 wk before inoculation as a root drench (100 ml of solution per 15-cm pot).

greenhouse bench and allowed to become naturally infected. After 4, 6, 8, 10, and 12 wk, the number of colonies per leaf was counted. Plant height, leaf and flower number, and other phytotoxic symptoms were noted weekly.

Sclerotinia homoeocarpa F. T. Bennett was used as a bioassay organism for determining persistence of systemic fungitoxicant in leaves of the soil drench trials using 16- to 18-wk-old plants. Three leaf disks obtained from treated plants with a No. 5 (10-mm-diameter) cork borer were spray frozen with Cryokwik (Damon Corp., IEC Division, Needham Heights, MS) to rupture membranes and release fungicide from the tissues. The disks were placed near the edge of each of five potato-dextrose agar plates per treatment. Each plate contained disks from a separately treated plant. An agar plug of *S. homoeocarpa* was placed in the center of the plate, and the zone of inhibition was measured after 1–3 days growth when the fungus had grown to the control leaf disks.

RESULTS

All materials tested as preventive fungicides, except benomyl, were effective when applied as foliar dips (Table 1). In subsequent whole-plant sprays, bitertanol at 30 g a.i./100 L and CGA 64251 at 5 g a.i./100 L slightly discolored the margins of flowers, but less than did dinocap. Examination of inoculated leaves previously dipped in fungicides showed that dinocap prevented germination of conidia (Table 2). Triforine, CGA 64251, and triadimefon did not kill the fungus until after haustoria had been produced and some mycelium had formed local lesions (Tables 1 and 2). Haustoria, which stained red in malachite green acid fuchsine, were generally shriveled and surrounded by a wall-like encapsulation that stained green. Occasionally haustoria did not form, and a thickened, green-stained area (possibly a papilla) was seen on the inner cell wall opposite an appressorium. Hyphae usually stopped growing when 100 μm long or less and had swollen, club-shaped endings. No sporulation was observed on leaves treated with CGA 64251, triforine, triadimefon, or dinocap (Table 2). Triadimefon severely stunted plants and burned leaf margins at 0.6 g a.i./100 L.

Bitertanol, fenarimol, and CGA 64251 were also as effective as dinocap in the trials where inoculation preceded fungicide application, although a few colonies developed with all fungicides tested (Table 3). As with the preventive treatments, eradicative treatments appeared to induce shriveling and encapsulation of haustoria as well as shriveling of conidia (Table 4).

Triadimefon and CGA 64251 gave long-term protection from mildew when applied as dilute soil drenches (Tables 5 and 6). These compounds had many side effects at rates higher than those needed for efficacious treatment, including darkening of foliage, shortening of internodes, and stunting (Table 5). Of the two compounds tested, triadimefon seemed preferable because it was more efficacious (Tables 5 and 6).

Leaf disk bioassays of drenched plants demonstrated that the amount of triadimefon was consistently lower in young leaves compared with older leaves, although those differences were insignificant (Table 6). Onset of signs of disease was delayed, and colony increase proceeded at lower rates on drenched plants (Tables 5 and 6). Treated plants were observed to have fewer mildew colonies on them even 6 mo after treatment. Conidial germination on

Table 3. Effectiveness of eradicant fungicides measured by the ability of *Oidium begoniae* colonies on treated begonia leaves to provide inoculum for formation of colonies on untreated bioassay leaves

Chemical	Rate (g a.i./100 L)	Colonies per leaf[y] (% of control)
Control	...	100.0 a[z]
Benomyl 50 WP + Exhalt 800	30 + 60	18.4 b
Dinocap 19.5 WD + Exhalt 800	9 + 60	0.9 c
Bitertanol 25 WP + Exhalt 800	60 + 60	0.4 c
Fenarimol 12.5 EC	4	0.3 c
CGA 64251 0.8 EC	10	0.2 c

[y] Colonies on the bioassay leaves were counted 7 days after fungicides were applied. There were 14 leaves per treatment.
[z] Numbers followed by the same letter do not differ significantly ($P = 0.05$) using Duncan's new multiple range test.

Table 4. Effect of eradicative mildewcide treatments on condition of haustoria and on shriveling of exposed *Oidium begoniae* conidia on begonia leaves

Chemical	Rate (g a.i./100 L)	Haustoria (%)[v]			Exposed conidia shriveled (%)
		Shriveled	Encapsulated	With browning	
Control	...	7	5	0	4
Dinocap 19.5 WD + Exhalt 800	9 + 60[w]	81	37	52	96[x]
Triforine 20 EC	18[w]	40	28	26	56[x]
Fenarimol 12.5 EC	0.4[w]	52	48	33	...[y]
Triadimefon 50 WP	0.6[z]	30	19	9	95[x]

[v] Four leaves per treatment with 100–200 haustoria per leaf observed.
[w] Sprayed to runoff 1 wk after inoculation.
[x] Very few recognizable conidia left; actual percentage of original number of conidia shriveled is thus probably higher. Two leaves per treatment and 400 (if possible) conidia per leaf were observed by appressing leaf to glass slide 7 days after treatment.
[y] Observation not made.
[z] One-hundred milliliters of this solution was added to 15-cm pots 1 wk after inoculation.

Table 5. Effect of triadimefon and CGA 64251 applied as soil drenches on number of *Oidium begoniae* colonies, of leaves and flowers, and heights of 10-wk-old *Begonia* × *hiemalis* 'Schwabenland Red' plants

Chemical[x]	Rate (g a.i./100 L)	Mildew colonies/plant					Height (cm/plant)	Leaves (no./plant)	Flowers (no./plant)
		22 Oct.[y]	29 Oct.	5 Nov.	12 Nov.	19 Nov.	19 Nov.	19 Nov.	19 Nov.
Control	···	30 a[z]	181 a	485 a	854 a	1,432 a	15.3 a	13.5 b	4.5 a
Triadimefon 50 WP	0.6	0 b	1.4 b	0.7 c	3.0 cd	3.6 cd	15.4 a	14.7 b	7.4 a
Triadimefon 50 WP	1.25	0 b	1.4 b	2.9 c	12.9 cd	24.0 cd	14.6 a	17.4 ab	11.0 a
Triadimefon 50 WP	2.5	0 b	0 b	0 c	0 d	0 d	13.6 a	15.9 ab	12.0 a
Triadimefon 50 WP	5	0 b	0 b	0 c	0 d	0 d	10.8 b	17.6 ab	9.1 a
CGA 64251 10 WP	0.75	2.1 ab	21 b	111 b	225 b	399 b	13.6 a	13.9 b	5.9 a
CGA 64251 10 WP	3	0 b	14 b	26 bc	91 bc	123 bc	11.0 b	20.1 a	7.8 a

[x] Plants drenched 10 September when they were 10 wk old. Ten plants were drenched per treatment.
[y] First weekly reading at which mildew colonies were found.
[z] Numbers in columns followed by the same letter do not differ significantly ($P = 0.05$) using Duncan's new multiple range test.

Table 6. Effect of triadimefon and CGA 64251 applied as soil drenches on *Oidium begoniae* on 16-wk-old *Begonia* × *hiemalis* 'Schwabenland Red' plants and persistence in the leaves as measured by a *Sclerotinia homoeocarpa* leaf disk bioassay

Chemical	Rate (g a.i./100 L)[u]	Mildew colonies/plant[v]						Bioassay zones of inhibition on 19 Nov. (cm)[x]	
		15 Oct.[w]	22 Oct.	29 Oct.	5 Nov.	12 Nov.	19 Nov.	Old leaves	Young leaves
Control	···	29 a[y]	123 a	309 a	489 a	1,088 a	1,324 a	0.8 a[y]	0.3 a
Triadimefon 50 WP	0.6	0 b	0 b	0 c	0 b	1.2 cd	1.5 bc	···[z]	···
Triadimefon 50 WP	1.25	0 b	0 b	0 c	0 b	0 d	1.3 bc	···	···
Triadimefon 50 WP	2.5	0 b	0 b	0 c	0 b	0 d	0 c	1.9 a	0.3 a
Triadimefon 50 WP	5.0	0 b	0 b	0 c	0 b	0 d	0 c	4.9 b	3.9 b
CGA 64251 10 WP	0.75	5 a	15 a	85 ab	148 a	380 ab	536 a	···	···
CGA 64251 10 WP	3.0	0 b	0 b	0.3 bc	0.7 b	31 bc	43 b	1.3 a	0.8 a

[u] One-hundred milliliters of this solution was applied to each 15-cm pot on 10 September when plants were 16 wk old.
[v] Seven plants per treatment.
[w] First date at which any mildew was noted. Plants were inoculated on 11 September.
[x] Five plants per treatment and three disks per plant whose zone of inhibition was measured.
[y] Numbers in columns followed by the same letter do not differ significantly ($P = 0.05$) using Duncan's new multiple range test.
[z] Bioassay not conducted.

leaves from plants drenched with triadimefon was somewhat reduced (Table 2). Extension of hyphae and sporulation were lowered, and shriveling and encapsulation of haustoria were greatly increased by the triadimefon drench treatment (Table 2).

DISCUSSION

Preventive sprays are a necessary part of control of this disease. One good fungicide program suggested by these results is a weekly or biweekly spray program with dinocap before flowering, followed by a drench with triadimefon during flowering and just before sale to the consumer. Such a program is, of course, not yet legal. If triadimefon becomes properly labeled, the consumer will then have a plant with long-term protection from mildew.

The growth regulator effects of triadimefon drenches are not undesirable at the low rate. Tests should be conducted to see whether the darker foliage and more compact growth of the plants may allow growers to skip treatments with growth regulators during summer. If triadimefon is applied before flower buds have set, some inhibition of flowering may take place. We noted an increase in number of flowers on plants drenched after flowering had begun.

The reaction of the fungus to inhibition by resistance not specific to race (5), heat treatments (6), and the new fungicides that inhibit ergosterol synthesis is similar. All reactions had the following in common: conidial germination was reduced about 50%; hyphae grew sparingly and eventually stopped growing; haustoria became encapsulated, shriveled, and were associated with melaninlike substances; host local lesions formed; sporulation of existing colonies was totally inhibited; and rate of disease progress was slowed. However, the club-shaped hyphal endings were unique to the fungicide treatments. Tests of the ergosterol synthesis inhibition mode of action have mostly been made in vitro (2,8). The mode of action in vivo is likely to be more complicated and may be related to a general host-mediated resistance reaction.

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