Activity of Benomyl for Control of Postbloom Fruit Drop of Citrus Caused by *Colletotrichum acutatum*

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ABSTRACT

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Postbloom fruit drop (PFD) of citrus caused by *Colletotrichum acutatum* produces orange-brown lesions on petals and induces the abscission of young fruitlets and the retention of the calyces. Despite the fact that *C. acutatum* is not highly sensitive to benomyl in culture, this fungicide provides good control of the disease under field conditions. This study was undertaken to determine the effect of benomyl on various stages of disease development to understand the basis for its effectiveness in the field. We found that benomyl at 1.0 µg/ml reduced colony area of *C. acutatum* by about 75% and completely inhibited growth of *C. gloeosporioides*. Benomyl did not prevent conidial germination even at 100 µg/ml, but reduced germ tube elongation at 10 and 100 µg/ml. When benomyl was applied to flower clusters on screen-house-grown plants before inoculation, disease severity was greatly reduced. Applications at 24 and 48 h, but not at 72 h, after inoculation reduced PFD severity. Application of benomyl to symptomatic petals not bearing conidia did not prevent or reduce production of inoculum. Application to petals bearing conidia reduced viability of these fungal propagules by only about 50%. The viability of appressoria on mature leaves was not affected by benomyl application. Even when appressoria on mature leaves were stimulated to germinate by treatment with flower extracts, subsequent application of benomyl did not reduce propagule numbers below original levels. Benomyl appears to act by preventing infection and early development of the fungus in petals. However, once symptoms have developed, this fungicide has only minimal effects on further disease development and spread.

Postbloom fruit drop (PFD), caused by *Colletotrichum acutatum* J. H. Simmonds, produces orange-brown lesions on petals of citrus flowers and induces the abscission of fruitlets (20,21). Calyces from these flowers, commonly called buttons, persist for the life of the twig and are diagnostic for the disease. Conidia are produced in acervuli on infected petals and are dispersed by wind-blown rain during the bloom period (20,21). Conidia deposited on leaves and other vegetative tissues germinate and form thick-walled, pigmented appressoria which serve as survival structures between flowering periods. Unlike appressoria of *C.*

gloeosporioides (Penz.) Penz. & Sacc. in Penz. that germinate and infect senescent tissues (21,22), appressoria of *C. acutatum* germinate and produce a few conidia on the leaf surface (28). These conidia are then splashed onto the first flowers of the new season to continue the cycle.

The PFD pathogen is widely distributed in the Americas (20). Serious losses occur sporadically in Florida and Brazil when rains occur during the bloom period (15). The disease is endemic in the humid tropics of the Americas, where trees may flower any time of year, and has an important effect on yields annually in Central America, eastern Mexico, and the Caribbean.

PFD was reported first in Belize in 1979 and early studies on control indicated that benomyl and captafol were the most effective fungicides for control of the disease (7,8). In Florida, benomyl has been highly effective for control of PFD in the field (15,23,25). Benomyl applications have reduced flower infection, the number of buttons, and increased yields up to 600% compared with the nontreated control (25). Nevertheless, *C. acutatum* isolates from citrus are not highly sensitive to benomyl in culture (11,16). Colony diameter of *C. acutatum* isolates from citrus is generally reduced to about 50% of the control at 1

µg/ml, but higher concentrations do not reduce growth much further. On the other hand, growth of *C. gloeosporioides* is completely inhibited by benomyl at 1 µg/ml.

Isolates of *C. acutatum* from other crops are not very sensitive to benomyl. Growth of isolates of *C. acutatum* from deciduous fruits was reduced by 20 to 50% with benomyl at 2 µg/ml, whereas *C. gloeosporioides* isolates were completely inhibited (4). Peach and almond isolates of *C. acutatum* in California were insensitive to benomyl in a fungicide disc assay (1). In a recent review, Freeman et al. (9) considered all *C. acutatum* isolates to be insensitive to benomyl.

Due to the lack of sensitivity of *C. acutatum* to benomyl in culture, this fungicide has not been widely tested in the field against diseases caused by this pathogen. When tested against ripe rot of blueberry, benomyl significantly reduced disease severity on one of two cultivars tested (6). Benomyl is considered ineffective for control of anthracnose on leatherleaf fern caused by *C. acutatum* (17) and for control of anthracnose fruit rot on strawberries (12).

C. acutatum is not highly sensitive to benomyl in culture and benomyl performs poorly for control of diseases caused by *C. acutatum* on other crops; therefore, it is of interest to determine why benomyl is so effective for control of PFD on citrus. This study was undertaken to determine the effect of benomyl on the various stages of disease development to ascertain the basis for its effectiveness.

MATERIALS AND METHODS

The isolates of *C. acutatum* used in this study were recovered from infected petals of sweet orange flowers in various areas of Florida. *C. gloeosporioides* isolates were recovered from dead leaves and twigs. All *C. acutatum* isolates were maintained as dried cultures on sterile filter paper in sealed plastic containers with desiccant at -20°C. Cultures were grown on potato dextrose agar (PDA) at 27°C for production of conidia and mycelial plugs.

Sensitivity to benomyl in vitro. Cultures of *C. acutatum* (isolates CS-1, Maran, and Mayo) and *C. gloeosporioides* (isolates GND-1, TH-4, and Dil-Ark-1) were grown on PDA for 1 week and 0.5-cm-diameter mycelial plugs were cut from

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the margins of the colony. These plugs were transferred to three petri dishes each of PDA containing benomyl (as Benlate 50WP, DuPont Crop Protection, Wilmington, DE) at 0, 0.1, 1.0, 10, 100, and 1,000 µg/ml. Cultures were incubated for 1 week at 24°C with a photoperiod of 12 h light and 12 h dark. Colony radius was measured and the area calculated and expressed as a percentage of the control.

The effect of benomyl concentrations on conidial germination and germ tube growth was determined for isolates CS-1, Maran, and Mayo. Conidia of each isolate were harvested from cultures on PDA in sterile distilled water and added to potato dextrose broth (PDB) at a final concentration of 10⁴ conidia/ml. Three replicate petri dishes with 20 ml of PDB each were used for each isolate–benomyl concentration combination. Benomyl was added to dishes to make a final concentration of 0, 0.1, 1.0, 10, and 100 µg/ml immediately after conidia were added. Conidia were incubated at 24°C for 24 h and the percentage of conidia germinated determined microscopically. The length of the germ tube on the longest axis was measured using an eyepiece micrometer for 10 germinated conidia per petri dish.

The effect of the fungicide on mycelial growth from conidia was assessed in another experiment. Conidia were allowed to germinate overnight and form a short germ tube before benomyl was added. The germ tube length was determined 12 h later.

Pre- and post-infection activity of benomyl. The efficacy of pre- and post-inoculation applications of benomyl was evaluated on 4- to 8-year-old potted citrus trees in four separate experiments in the screenhouse. Experiments 1 and 2 were conducted on Valencia sweet orange (*Citrus sinensis* (L.) Osb.), experiment 3 on Orlando tangelo (*C. paradisi* Macf. × *C. reticulata* Blanco), and experiment 4 on tangerines (*C. reticulata*). The treatments used were benomyl as Benlate 50WP (1.2 g a.i./liter) applied 24 h prior to inoculation and at 24, 48, and 72 h after inoculation. For each treatment, six clusters with a few open flowers, some buds, and some pin-head flowers were selected. Conidia of isolate SGR-FTP-1 were washed from the surface of cultures on PDA and diluted in distilled water to a concentration of 10⁵ conidia/ml. Flower clusters were spray inoculated with conidia, covered with plastic bags, and maintained at ambient temperature (18 to 25°C) for 16 h. The plastic bags were removed and the plants maintained in the screenhouse. The number of symptomatic and total flowers was counted after 1 week and the data expressed as the percentage of infected flowers. About 1 month after inoculation, the number of persistent calyces was counted for each cluster.

Conidial production and viability. Sweet orange flowers on plants in the

screenhouse were inoculated as in the previous experiment. Petals affected by PFD were collected to measure the effect of benomyl on conidial production in two experiments. In experiment 1, the infected petals collected had not yet formed conidia. In experiment 2, petals with conidia were collected and vortexed in distilled water to remove conidia. Petals were selected for uniformity with about 50 to 60% of the surface area showing necrosis in experiment 1 and about 75 to 80% in experiment 2. Petals then were placed in petri dishes with moist filter paper. In all, 5 petals per treatment were used in experiment 1 and 10 in experiment 2. Petals were sprayed with benomyl at 1.2 g a.i./liter or left unsprayed. Treated and untreated petals were incubated for 16 h on the laboratory bench at room temperature (22 to 25°C). Each petal was vortexed in 10 ml of distilled water and the resultant conidial suspension diluted and counted on a hemacytometer. Data were expressed as the mean number of conidia per petal.

Similarly, symptomatic petals with abundant conidia were collected from inoculated sweet orange trees to assess the effect of benomyl on conidial viability. Three experiments were conducted using 10 petals for each treatment. Petals were placed in petri dishes with moist filter paper and treated with benomyl as above or left unsprayed. Petals were incubated for 16 h and conidial numbers per petal counted as above, except sterile distilled water was used to collect conidia. Conidia were diluted to 100 and to 1,000/ml, and 0.1 ml of each suspension was spread onto each of three petri dishes of PDA per flower petal. The plates were incubated for 3 days at 27°C and the number of colonies counted. Data were expressed as the number of viable conidia per petal.

Effect of benomyl on pathogen survival. The effects of benomyl on appressoria on the leaf surface and on propagules produced after appressoria were stimulated to germinate were evaluated. Appressoria were stimulated to germinate by application of flower extracts, after which the benomyl was applied in an attempt to eliminate residual inoculum on the leaves. The flower extract was prepared by macerating flower petals in four parts water to

one part petal tissue. Conidia of the Maran, Mayo, and CS-1 isolates of *C. acutatum* were produced on PDA and washed from the surface of the culture in water, after which the concentration was adjusted to 10⁵/ml using a hemacytometer. Ten 1-year-old sweet orange seedlings (two plants per treatment) growing in potting mix in 2.7-liter pots were used for each experiment. The experiment was conducted four times. All plants were sprayed to run-off with the conidial suspension. Plants were placed in a dew chamber at 25°C for 48 h to allow conidia to germinate and form appressoria. Plants then were transferred to a greenhouse and leaves maintained dry for about 30 days to allow ungerminated conidia to die, leaving only appressoria (28). Two plants each were subjected to the following treatments: (i) sprayed with water followed by benomyl at 1.2 g a.i./liter 2 days later, (ii) sprayed with water followed by water 2 days later, (iii) sprayed with flower extract followed by benomyl 2 days later, (iv) sprayed with flower extract followed by water 2 days later, and (v) untreated control. All plants then were placed in the greenhouse under dry conditions for 3 days. Ten leaves were collected from each treatment before the first application and 3 days after the last application and propagules were removed using the procedure of Agostini and Timmer (3). Briefly, 10 to 15 leaf discs were cut from each leaf, and leaf discs were placed in a test tube with 10 ml of water. Tubes were vortexed for 10 min at high speed to remove propagules from the leaf surface. Aliquots of 0.1 ml from each tube were plated on five petri dishes of the selective medium for *Colletotrichum* spp. (2). Propagules were incubated for 4 days at 18°C and for 1 day at 27°C. The number of colonies of *C. acutatum* was counted and expressed as propagules per unit of leaf surface area.

RESULTS

Sensitivity to benomyl in vitro. Isolates of *C. acutatum* from our collection were not highly sensitive to benomyl (Table 1). This fungicide reduced colony area by 60 to 70% of the control at 0.1 µg/ml and to about 25% of the control at 1.0 µg/ml, but increases in the fungicide concentration did not reduce growth much

Table 1. Effect of benomyl at different concentrations on the growth of isolates of *Colletotrichum acutatum* and *C. gloeosporioides* on potato dextrose agar

Species, isolate	Colony area as % of control per benomyl concentration (µg/ml) ²				
	0.1	1.0	10	100	1,000
<i>C. acutatum</i>					
CS-1	70.4 ± 1.3	27.5 ± 0.4	24.0 ± 1.8	22.6 ± 0.9	10.4 ± 0.4
Mayo	62.7 ± 2.9	24.6 ± 0.3	18.5 ± 0.3	19.9 ± 0.6	12.4 ± 0.6
Maran	60.5 ± 3.9	26.1 ± 0.6	21.9 ± 1.6	21.9 ± 1.0	11.0 ± 0.4
<i>C. gloeosporioides</i>					
GND-1	12.7 ± 1.8	0.0	0.0	0.0	0.0
TH-4	16.5 ± 3.6	0.0	0.0	0.0	0.0
Di1-Ark-1	32.0 ± 0.7	0.0	0.0	0.0	0.0

² Mean ± SE of three replicate plates.

further. In contrast, the colony area of *C. gloeosporioides* was 12 to 32% of the control at 0.1 µg/ml, and no growth occurred at higher concentrations.

The addition of benomyl to conidia of *C. acutatum* did not prevent spore germination of *C. acutatum* even at the highest concentration tested (100 µg/ml). Benomyl at 0.1 µg/ml did not reduce the hyphal growth of any of the isolates, even when added before germination (Table 2). Benomyl at 1.0 µg/ml reduced growth of the Maran isolate by 34%, but did not reduce growth of the CS-1 or Mayo isolates. At 10 and 100 µg/ml, hyphal growth of all isolates was reduced by about 70 to 80%.

Results were similar when benomyl was added to conidia which had already germinated (Table 2). Concentrations of 0.1 and 1.0 µg/ml only slightly reduced growth, but 10 and 100 µg/ml reduced growth by 55 to 80%.

Pre- and post-infection activity of benomyl. Benomyl applied to flowers 24 h prior to inoculation reduced the number of infected flowers by 70 to 100% and the number of persistent calyces formed on the inflorescences by 75 to 80% (Table 3). Applications at 24 and 48 h after the inoculation reduced the flower infection and button formation by 50 to 75%. However, applications 72 h after inoculation only reduced symptom development in one experiment where disease was not severe.

Conidial production and viability. Applications of benomyl to infected, symptomatic petals without conidia failed

to prevent the production of conidia (Table 4). Conidial numbers were the same or higher on benomyl-treated petals as on nontreated controls. Application of benomyl to conidia already present on petals appeared to reduce their viability by about 40%.

Effect of benomyl on survival. Application of benomyl to sweet orange leaves with appressoria present did not reduce the number of propagules recovered from those leaves compared with the water treatment or the nontreated control (Table 5). The number of propagules recovered was not significantly different from the number recovered from the same plants prior to treatment. Application of the flower extract tripled the number of propagules recovered from leaves of the same plants prior to treatment and increased the number of propagules as much or more compared with nontreated and water-sprayed controls. However, application of benomyl following the flower extract treatment failed to eliminate propagules. The flower extract followed by benomyl treatment reduced propagules to the same level observed prior to treatment, and numbers were similar to those in the controls.

DISCUSSION

The ability of benomyl to control PFD caused by *C. acutatum* on citrus appears to be unique. Our results show that *C. acutatum* isolates from citrus are not highly sensitive to benomyl in vitro, confirming results of other studies on citrus (11,16) and other crops (1,4,9). Nevertheless, in

early studies in Belize (7,8) and subsequent work in Mexico (14) and Florida (15,24,25), benomyl has proven as effective or more effective than other fungicides in controlling PFD.

Although *C. acutatum* is not highly sensitive to benomyl, low concentrations inhibit substantially growth in vitro. Colony diameter of *C. acutatum* is usually reduced by about 50% at 1.0 µg/ml compared with the unamended control (11,16). However, when growth was measured as colony area, we found that growth is reduced by 50% by 0.1 µg/ml. If growth were measured as mycelial dry weight, the concentration to achieve to 50% inhibition might be even lower. Germination of conidia even in the presence of high concentrations of benomyl was virtually 100%. The effect of benomyl on the growth of germ tubes was similar to the effect on growth of mycelium in culture, except that the effect of low concentrations was not apparent. Thus, despite the fact that benomyl does not kill or provide 100% inhibition, even at high concentrations, it substantially reduced the growth of *C. acutatum* in petals of citrus.

Benomyl has excellent postinfection activity against many diseases. This fungicide provides good control of apple scab and other diseases of deciduous fruit crops (13,18,19), even when applied as late as 3 to 5 days after infection. Bushong and Timmer (5) found that benomyl provided excellent control of citrus scab when applied up to 3 days after inoculation. On citrus flowers inoculated with *C. acutatum*, benomyl was effective when applied within 48 h after inoculation. However,

Table 2. Effect of benomyl on germ tube growth of conidia of *Colletotrichum acutatum*

Isolate	Time (h) ^z	Length (µm) per benomyl concentration (µg/ml) ^y				
		0.0	0.1	1.0	10	100
CS-1	0	1,360 ± 42	1,450 ± 35	1,520 ± 90	299 ± 15	328 ± 28
Maran	0	1,257 ± 26	1,265 ± 37	833 ± 39	334 ± 28	327 ± 10
Mayo	0	1,621 ± 31	1,770 ± 55	1,687 ± 24	478 ± 70	425 ± 23
CS-1	12	1,367 ± 18	1,033 ± 41	1,121 ± 30	431 ± 11	252 ± 12
Maran	12	1,302 ± 41	1,070 ± 8	903 ± 9	491 ± 10	281 ± 2
Mayo	12	1,356 ± 52	1,288 ± 21	1,182 ± 46	604 ± 25	304 ± 19

^y Mean ± SE of the three replicates.

^z Benomyl applied before (0) or 12 h after conidial germination.

Table 3. Effect of pre- and postinoculation applications of benomyl on the development of symptoms of postbloom fruit drop caused by *Colletotrichum acutatum*^w

Time (h) ^x	Experiment number									
	1		2		3		4		Mean	
	Flowers (%) ^y	Persistent calyces ^z	Flowers (%)	Persistent calyces	Flowers (%)	Persistent calyces	Flowers (%)	Persistent calyces	Flowers (%)	Persistent calyces
Control	5 a	7 a	16 a	12 a	42 a	15 a	57 a	3 a	30 a	9 a
-24	0 b	2 b	0 b	3 ab	1 b	2 cd	17 b	2 ab	6 c	2 b
+24	0 b	3 ab	10 ab	4 ab	4 b	1 d	12 b	2 ab	8 bc	3 b
+48	0 b	2 b	14 a	12 a	12 b	1 d	18 b	1 b	13 bc	4 ab
+72	0 b	2 b	10 ab	1 b	35 a	8 bc	37 ab	3 a	22 ab	4 ab

^w Mean separation by the Waller-Duncan *k*-ratio *t* test, *P* ≤ 0.05.

^x Application time after inoculation (h).

^y Average percentage of symptomatic flowers affected per cluster.

^z Number of persistent calyces remaining per cluster.

benomyl is able to prevent symptom development and, presumably, sporulation if applied soon after infection.

With many diseases, benomyl also is capable of preventing inoculum production on symptomatic tissue and eliminating inoculum. Benomyl prevents ascospore production by the apple scab fungus (10,18,19). This fungicide reduced pycnidial production on citrus twigs affected by melanose (27) and prevented development of pseudothecia and ascospores of *Mycosphaerella citri*, the causal agent of citrus greasy spot (26). With citrus scab, benomyl can eradicate fungal tissue in pustules and reduce sporulation on the surface (24). With PFD, benomyl did not reduce conidial production on infected symptomatic tissue and only marginally reduced the viability of existing inoculum. Thus, once the infection is well established in the petal, benomyl application probably does little to slow the epidemic.

Benomyl applications made to vegetative tissue prior to flowering could potentially reduce residual appressoria and postpone the onset of the epidemic. However, we found no indication that benomyl affected viability directly. Even when flower extracts were applied to stimulate germination of appressoria, benomyl applications did not eliminate viable propagules from the leaf surface. These results were consistent with previous work, indicating that viability of appressoria of *C. acutatum* on fruit is not affected by postharvest applica-

tion of the related fungicide, thiabendazole (22). In contrast, postharvest application of benzimidazole fungicides to fruit bearing appressoria of *C. gloeosporioides* strongly inhibits development of the fungus and prevents postharvest anthracnose (21,22).

The activity of benomyl in controlling PFD appears to lie in its ability to prevent infection, reduce mycelial development, and prevent sporulation on treated petals. Concentrations of fungicide as applied by sprayers in the field range from 250 to 1,000 g/liter, which should be more than adequate to prevent growth of *C. acutatum*. Once symptoms have developed, benomyl appears to have little effect on epidemic development. *C. acutatum* is not highly sensitive to benomyl in culture; therefore, few field tests have been conducted to evaluate its efficacy on diseases of other crops caused by *C. acutatum*. It could prove more effective than results in culture might indicate. *C. acutatum* is not highly sensitive to benomyl and apparently does not readily develop resistance to this fungicide (15; unpublished data). *C. gloeosporioides*, which is highly sensitive, can develop resistance to benomyl quickly. Germination of conidia of *C. acutatum* is not affected by benomyl; therefore, there may be no selection for mutations that occur for resistance.

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Table 4. Effect of benomyl application to detached, infected sweet orange petals on production of conidia of *Colletotrichum acutatum* symptomatic petals, and on the viability of conidia from symptomatic petals already bearing spores^x

Treatment	Conidia/petal (×10 ⁶) per experiment ^y			Viable conidia/petal (×10 ⁶) per experiment ^z			
	1	2	Mean	1	2	3	Mean
Control	5.30 a	5.43 b	6.68 a	23.2 a	5.3 a	10.6 a	13.0 a
Benomyl	4.06 a	7.99 a	5.39 a	14.6 a	3.7 b	5.5 b	7.9 b

^x Mean separation by the Waller-Duncan *k*-ratio *t* test, *P* ≤ 0.05.

^y Benomyl at 2.4 µg a.i./ml was applied to symptomatic petals without conidia; treated and nontreated petals were incubated for 16 h and the number of conidia produced counted.

^z Benomyl at 1.2 µg a.i./ml was applied to symptomatic petals with abundant conidia; viable conidia per petal was determined by dilution plating after 16 h of incubation.

Table 5. Effect of applications of citrus flower extracts (FE) or water followed by application of benomyl or water on the number of propagules/cm² of *Colletotrichum acutatum* produced on inoculated sweet orange leaves

Treatments ^y		Propagules/cm ² of leaf ^x									
		Experiment 1		Experiment 2		Experiment 3		Experiment 4		Mean	
Day 1	Day 3	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
None	None	8.7	1.5	14.2	12.1	9.4	8.1	5.8	3.8	9.5	6.4 b
Water	None	8.4	7.5	21.7	37.0	14.1	23.7	11.3	5.1	13.9	18.3 b
FE	None	21.4	52.9	23.2	82.8	15.1	52.1	2.8	11.7	15.6	49.9 a ^z
Water	Benomyl	11.3	5.1	26.0	17.4	17.1	11.3	3.1	2.7	14.4	9.1 b
FE	Benomyl	2.4	7.5	19.5	22.4	15.4	12.2	14.5	13.0	13.0	11.4 b

^x Pre = sampled immediately before treatment on day 1; post = sampled 3 days after treatment on day 3. Mean separation within columns by the Waller-Duncan *k*-ratio *t* test, *P* ≤ 0.05.

^y All plants sprayed with conidia 30 days prior to initiation of the experiment. Data are the means of 10 leaves collected from two different plants. Benomyl applied at 1.2 g a.i./liter.

^z The "Post" mean significantly different from the "Pre" mean according to the "t" test, *P* ≤ 0.05. All other across-column comparisons were not significant.

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