Botrytis Blight of *Exacum affine* and Its Control

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ABSTRACT

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Botrytis cinerea caused a severe blight of potted exacum. Girdling stem cankers, wilting, and death were the most severe symptoms. Isolates of *B. cinerea* from geranium, zinnia, and tomato caused similar and characteristic disease symptoms on exacum. A disease index based on percentage of plant blighted is presented. Crowded spacing significantly increased disease severity. Benomyl at 0.60 g active ingredient (a.i.) per liter did not control the disease in the greenhouse. Tolerance of *B. cinerea* to benomyl was indicated in vivo and in vitro. Chlorothalonil plus benomyl at 0.56 + 0.15 g a.i./L, respectively, and chlorothalonil at 1.4 g a.i./L, controlled the disease without phytotoxicity. Bitertanol at 0.08 g a.i./L, iprodione at 0.90 g a.i./L, triadimefon at 0.01 g a.i./L, and vinclozolin at 0.30 g a.i./L were phytotoxic. Iprodione and vinclozolin were most toxic to *B. cinerea* in vitro, followed by chlorothalonil + benomyl and chlorothalonil alone. Triadimefon, mancozeb, bitertanol, and benomyl were less toxic in vitro. Vinclozolin at 100 μg a.i./ml incorporated in synthetic medium was fungicidal and a lower concentration was fungistatic. Mycelial growth occurred at 4–36 C, but was scant at 30–36 C on potato-dextrose agar (PDA), acidified PDA, glucose-asparagine (GA) agar, and oat grain medium. After 4 days of growth on liquid GA, optimum temperature for mycelial growth was 16–28 C; after 14 days of growth, 16–24 C was optimum.

Additional key words: benomyl resistance, gray mold.

Blight of Arabian violet or exacum (*Exacum affine* Balf.) caused by *Botrytis cinerea* Pers. was described recently (22). It is a serious threat (11,13) to the commercial production of this plant, which is increasing in popularity as a flowering pot or bedding plant (4,8,15).

When environmental conditions are optimal for disease development, plant death may result 3–5 days after infection (22). Cankers near the base of the main stem are the most commonly observed symptom, but branches, leaves, and flowers are also affected. Sporulation is frequently observed on infected plant parts. Disease development is favored by cool humid conditions (22).

Current recommendations for control of *B. cinerea* on exacum are cultural manipulations and foliar applications of chlorothalonil or benomyl (11) or a tank mix of these fungicides (J. Sweet; Earl J. Small Growers, Inc., *personal communication*). Reports of tolerance of *B. cinerea* to the benzimidazole fungicide benomyl (3,18) and the dicarboximide fungicides iprodione, procymidone, and vinclozolin (7,20) indicate that chemical control tactics need to be reevaluated.

This paper reports symptomatology of Botrytis blight of exacum, spacing and watering studies, efficacy of fungicides in vitro against *B. cinerea* and as protectants against Botrytis blight in the greenhouse, and the effect of temperature on growth and development of the fungus in culture.

MATERIALS AND METHODS

Isolation and culture of *B. cinerea*. Cultures on potato-dextrose agar (PDA) in petri plates were incubated in cool-white fluorescent light at 21 ± 1 C. The fungus isolated was identified as *B. cinerea*, based on the descriptions of Ellis (6) and Hennebert (9).

Plant materials. Seedlings or rooted cuttings of exacum cultivars Blue Champion or Jill were transplanted (one plant per 10-cm-diameter clay pot) to unamended Metro-Mix 220 potting medium (W. R. Grace and Co., Cambridge, MA). Temperatures ranged from 17–33 or 15–25 C, respectively, when the greenhouse was unshaded October through January or was shaded with whitewash and cooled by an evaporative system during other months. Plants were fertilized 1 wk after transplanting with 3 g of 19-6-12 (N-P-K) Osmocote controlled release fertilizer (Sierra Chemical Co., Newark, CA) per pot and weekly, unless indicated otherwise, with a 20-20-20 fertilizer (Peters Fertilizer Products, Allentown, PA 18104) mixed at 26.4 g/L of water (to yield 5,280 ppm N) and applied at 15 ml per pot. All plants were watered with an overhead sprinkler as needed.

Disease severity scale. A disease severity scale was developed based on visual assessment of disease incidence on stems, leaves, and flowers (Fig. 1).

Effects of temperature and medium on cultural growth. Cultures (six replicates for each temperature, medium, and isolate combination) were incubated at 4, 8, 16, 20, 24, 28, 30, 32, 34, and 36 C in total darkness on PDA, acidified PDA (aPDA, 2 ml 50% lactic acid per liter), oat grain medium (OGM; 7 g oats + 25 ml distilled water per 9-cm-diameter petri dish), and glucose-asparagine (GA) agar prepared as basal medium (14) with glucose (2.5%) and agar (2.5%) added.

Cultures, unless otherwise indicated, were seeded with 5-mm-diameter agar disks cut with a sterile cork borer from the advancing edge of 2-day-old cultures of *B. cinerea*. Isolates tested in this part of the study were EXA-0 from exacum, and EXA-8 from exacum grown as previously described and treated with benomyl (0.30 g a.i./L) weekly for 8 wk.

Cultures were observed for mycelial growth, sporulation, and for sclerotial formation after 2 mo. Visual ratings were made noting the absence (−) or presence (+) of mycelial growth, sporulation, or

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formation of sclerotia. Exact counts of conidia and sclerotia were not made.

Effects of temperature on growth in liquid culture. Three cultures of isolates EXA-0 and EXA-8 in each of three tests were used for dry weight measurement after incubation for 4 and 14 days in each of 13 temperatures in darkness on liquid GA medium. Twenty-five milliliters of medium were dispensed into 250-ml Erlenmeyer flasks stoppered with cotton or foam stoppers. The pH of the medium was 5.7 after sterilization. Each flask was seeded with a single mycelial disk from an agar culture as described previously.

Harvests for dry weight measurement were made onto tared Whatman 50 (2–3 μm retention, Fisher Scientific, Pittsburgh, PA 15219) 9-cm-diameter filter paper placed in a Büchner funnel attached to an aspirator flask. A rubber spatula was used to scrape mycelium from flasks. Before being tared, the filter papers were wetted in sterile distilled water and dried 8–12 hr at 100 C in a drying oven in the same manner that the mycelial samples were treated. Filter paper and samples were always placed on aluminum foil sheets in the drying oven, removed immediately to a desiccator (silica gel), and weighed on an analytical balance directly from the desiccator.

Pathogenicity test. Five isolates of *B. cinerea*, EXA-0, EXA-8, ZIN from zinnia, GER from geranium, and TOM from tomato, were grown on PDA in petri plates. Inoculum was applied to 15- to 17-wk-old plants as dry conidia tapped from inverted plastic petri plate cultures supported in the top of an inverted 121.1-L plastic container used as a portable settling tower. One 9-day-old culture was tapped over six plants placed at the bottom of the settling tower. Each culture was tapped 30 times after which the six plants in the bottom of the settling tower were exposed for 30 min to the dry conidia. Conidial concentration was $\sim 10^4$ conidia per milliliter when conidia were counted in six spore traps (150 × 25 mm petri dishes, approximately the size of the drip area of foliage of 15- to 17-wk-old exacum plants; each plate contained 25 ml of 1.2% Tween-80 solution and was placed in the same position as a test plant). Inoculations for this and all following tests were made at night. Three plants were inoculated for each isolate. All plants in this study were inoculated in the manner described. Unless otherwise indicated, all plants were misted with water just prior to inoculation, covered with plastic bags immediately after inoculation to ensure conditions of high humidity, and incubated under the greenhouse bench for 48 hr. The plastic bags were then removed and the plants were placed on the greenhouse bench in a

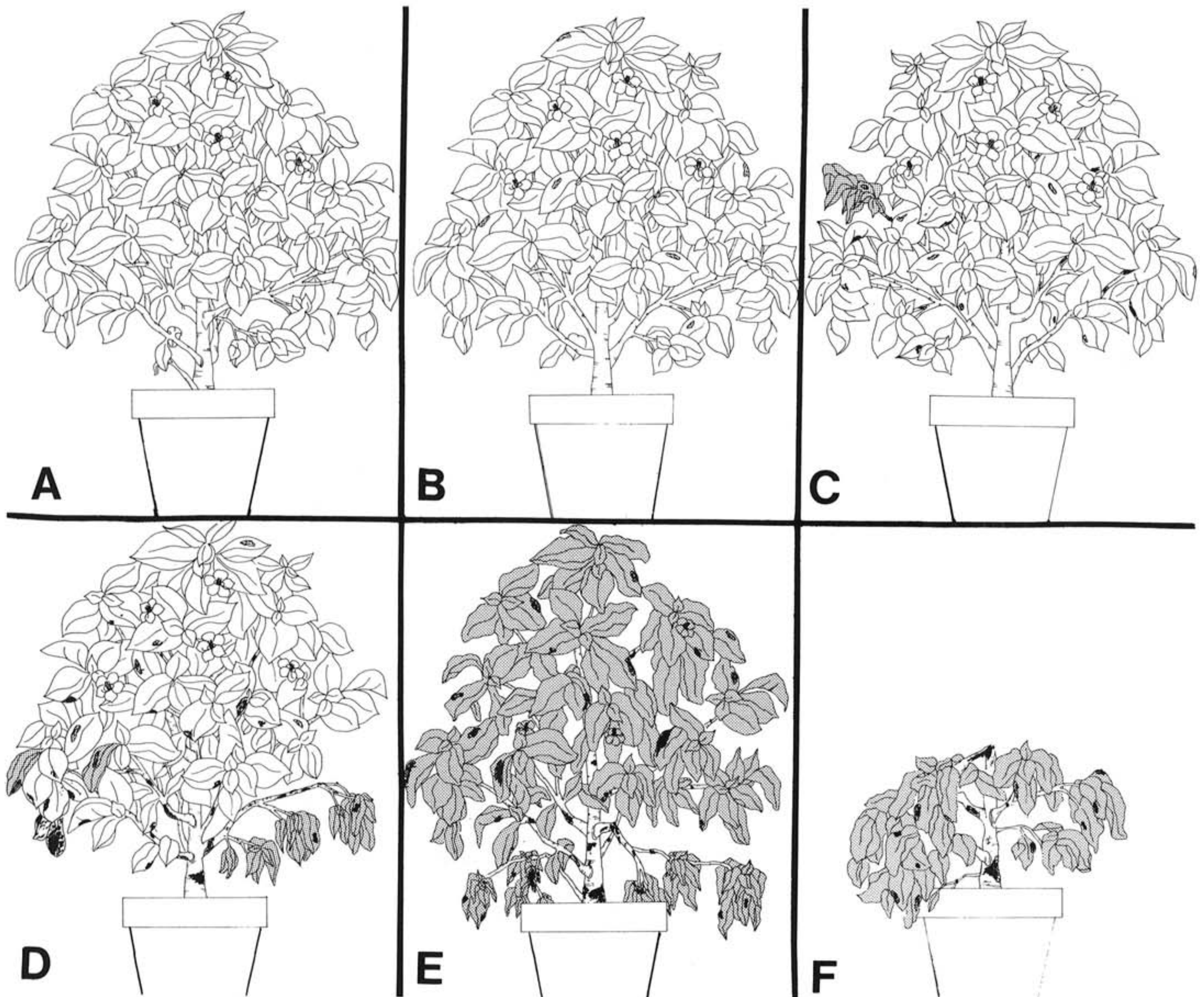

Fig. 1. Disease index for Botrytis blight of exacum. Approximate percent blighted plant determined by number and location of stem cankers. **A,** 0 = No disease. **B,** 1 = Trace, 1–25% blight or no cankers. **C,** 2 = Light, 26–50% blight or cankers on small lateral branches. **D,** 3 = Moderate, 51–75% blight or cankers on large lateral branches. **E,** 4 = Severe, 75% blight or canker on main stem. **F,** 5 = Dead plant. Blight includes stems, leaves, and flowers.

completely randomized design. Temperatures ranged from 8 to 30 C with an average low of 14 C and average high of 21 C. Symptoms were recorded 1, 2, and 7 days after inoculation.

Effects of cultural methods on disease severity. Botrytis blight development on cultivar Blue Champion was studied in the greenhouse. Treatment variables were: overhead irrigation versus subirrigation, crowded versus noncrowded spacing, weekly benomyl spray at 0.30 g a.i./L versus no spray, and inoculated versus uninoculated plants in each combination. Four treatment plants were surrounded by 12 border plants for each treatment combination with three replications, each replication on a different greenhouse bench. Treatment combinations were arranged in a complete randomized block design within each replication. In crowded spacing, leaves of neighboring plants overlapped; in wide spacing, there was a 5-cm distance between the leaves of neighboring plants. Three tests were made. Seedlings for the December–January test and the January–February test were 20–22 and 15–17 wk old, respectively, when inoculated. Rooted cuttings for the April–May test were 17 wk old. Greenhouse temperatures where 17–33 C for the winter tests and 15–20 C for the spring test. Relative humidity varied between 50 and 100%.

Benomyl at 0.30 g a.i./L (Benlate 50W, E. I. du Pont de Nemours & Co., Wilmington, DE 19898) was applied until runoff under fast-drying conditions with a compressed-air sprayer. The first application was allowed to dry 12 hr before inoculation with dry conidia. Isolate EXA-0 was used in both winter and spring tests. Subpopulations from benomyl-treated exacum plants previously described (EXA-1, EXA-2, EXA-3, EXA-4, EXA-5, EXA-6, EXA-7, EXA-8, and EXA-9) were used in the spring test. Plants were incubated on greenhouse benches for 48 and 72 hr in the spring and winter tests, respectively, prior to removal of the plastic bags. All plants were rated at 7-day intervals for 28 days.

Efficacy of fungicides. Fungicides were evaluated on nonflowering Jill (spring) and Blue Champion (fall) in the greenhouse. Rooted cuttings of Jill and seedlings of Blue Champion were 11–12 and 16 wk old, respectively, when inoculated. Prior to fungicide application, test plants were arranged on greenhouse benches in a complete randomized block design. Each block of three test plants was surrounded by border plants. Replications were as previously described. The treatments were: chemical application versus no chemical and inoculated versus uninoculated in all combinations.

Fungicides were applied as sprays at the recommended rate and at twice that rate. Materials were applied seven times at 7-day intervals to Jill plants in the spring and five times at 10-day intervals (except triadimefon, which was applied twice at a 30-day interval) to Blue Champion plants in the fall. Border plants were not sprayed directly since they were used to separate test plants. Rates of fungicides used are listed in Tables 1 and 2. Twelve hours after fungicide application (the fourth for Jill and the first for Blue Champion), plants were inoculated with dry conidia using all the isolates previously described from exacum in this study. Plants

TABLE 1. Efficacy of fungicides used as protectants in the spring against *Botrytis cinerea* on exacum cultivar Jill in the greenhouse 14 and 28 days after inoculation

		Disease ratings[a]			
		After 14 days		After 28 days	
Fungicide	Rate (g a.i./L)	Inoculated	Control	Inoculated	Control
Benomyl	0.30	1.6 CD[c]	1.3 C	2.4 B	1.8 BC
Bitertanol[b]	0.40	0.0 F	0.0 E	0.0 E	0.0 E
Chlorothalonil	1.4	0.1 F	0.0 E	0.2 E	0.0 E
Chlorothalonil plus benomyl	0.56 0.15	0.1 F	0.3 DE	0.1 E	0.6 DE
Iprodione[b]	0.90	0.0 F	0.0 E	0.0 E	0.0 E
Mancozeb	1.68	0.8 E	1.6 D	1.8 CD	1.3 CD
Triadimefon[b]	0.23	2.3 B	3.0 A	3.6 A	3.6 A
Vinclozolin[b]	0.75	0.0 F	0.0 F	0.0 E	0.1 E
None (control)		1.4 D	1.8 B	2.2 BC	2.1 B
LSD ($P = 0.05$)		0.39	0.55	0.57	0.71

[a] Disease index: 0 = no disease, 1 = trace (1–25% blight or no cankers), 2 = light (26–50% blight or cankers on small lateral branches), 3 = moderate (51–75% blight or cankers on large lateral branches), 4 = severe (>75% blight or canker on main stem), and 5 = dead plant; means of nine plants.
[b] These fungicides resulted in phytotoxicity and/or adverse growth effects after two to three applications.
[c] Means in same column followed by same letter are not different according to Fisher's least significant difference test.

TABLE 2. Efficacy of fungicides used as protectants in the fall against *Botrytis cinerea* on exacum cultivar Blue Champion in the greenhouse 10 and 40 days after inoculation

		Disease ratings[a]			
		After 10 days		After 40 days	
Fungicide	Rate (g a.i./L)	Inoculated	Control	Inoculated	Control
Benomyl	0.30	1.6 A[d]	0.8 AB	3.6 AB	3.0 A
Bitertanol[b]	0.08	1.0 ABCD	0.2 DE	2.6 CD	1.4 BC
Chlorothalonil	1.4	0.2 F	0.0 E	0.6 FG	0.8 CD
Chlorothalonil plus benomyl	0.56 0.15	0.5 CDEF	0.2 CDE	0.8 FG	0.4 D
Iprodione[b]	0.90	0.4 DEF	0.2 DE	1.1 EF	0.1 D
Mancozeb	1.68	0.8 ABCDE	0.3 CDE	1.8 DE	1.8 B
Triadimefon[c]	0.01	1.2 ABC	0.2 DE	2.4 CD	1.5 BC
Vinclozolin[b]	0.30	0.04 DEF	0.2 CDE	0.4 FG	0.2 D
None (control)		1.4 A	0.7 ABC	4.2 A	3.4 A
LSD ($P = 0.05$)		0.67	0.46	0.90	0.79

[a] Disease index: 0 = no disease, 1 = trace (1–25% blight or no cankers), 2 = light (26–50% blight or cankers on small lateral branches), 3 = moderate (51–75% blight or cankers on larger lateral branches), 4 = severe (>75% blight or canker on main stem), 5 = dead plant; means of nine plants.
[b] These fungicides resulted in phytotoxicity and/or adverse growth effects after two to three applications.
[c] Only two applications of triadimefon.
[d] Means in same column followed by same letter are not different according to Fisher's least significant difference test.

were incubated under greenhouse benches for 15 and 48 hr in the spring and fall tests, respectively, prior to removing plastic bags. Warmer spring temperatures (33 C) required early removal of plastic. Fungicides tested were: benomyl; bitertanol (Baycor 25W—Mobay Chem. Corp., Kansas City, MO 64120); chlorothalonil (Daconil 2787 75W—Diamond Shamrock Corp., Cleveland, OH 44114); iprodione (Chipco 26019 50W—Rhone-Poulenc Chem. Co., Monmouth Junction, NJ 08852); mancozeb (Dithane M-45 80W—Rohm and Haas, Philadelphia, PA 19105); triadimefon (Bayleton 25W—Mobay Chem. Corp., Kansas City, MO 64120); and vinclozolin (Ornalin 50W—Mallinckrodt, Inc., St. Louis, MO 63147). The control spray was tap water applied in the same manner as the fungicides. All fungicides were mixed just prior to application. Plants were rated for disease and examined for phytotoxicity at 7- and 10-day intervals in the spring and fall tests, respectively. The fall test was repeated once. All plant heights were measured before any fungicide application and immediately after the last application.

Dosage-response relations. Mycelial growth of *B. cinerea* (EXA-8) from 5-mm-diameter agar disks in liquid GA medium amended with various concentrations of fungicides (those used in the efficacy test) was measured as dry weight after 11 days at 20 ± 1 C. Liquid medium was dispensed in 24-ml quantities into 250-ml Erlenmeyer flasks. Fungicides were added to cooled media.

All fungicides were prepared so that 1 ml of a stock solution added to 24 ml of culture media (23 ml of media were used in chlorothalonil + benomyl treatment) gave 1.0, 10.0, 100.0, 1,000.0, or 2,000.0 μg a.i./ml of each fungicide. Each fungicide concentration was aseptically blended in sterile water for 1 min at low speed, and added aseptically to each culture flask. Control flasks contained only culture medium with no fungicide. All flasks in each test were agitated and moved approximately every 12 hr to

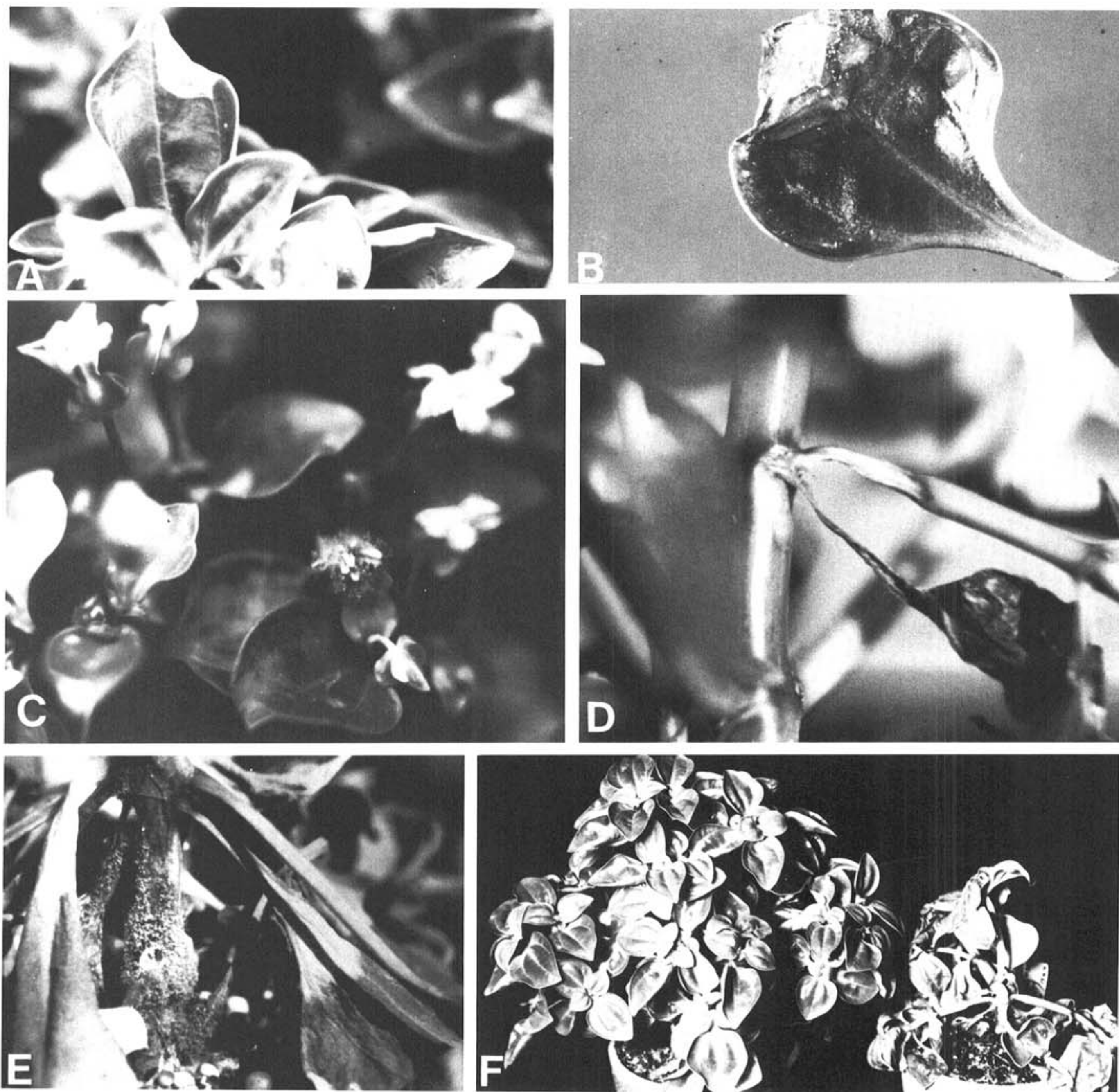

Fig. 2. Symptoms of Botrytis blight caused by *Botrytis cinerea* on *Exacum affine* 'Blue Champion' growing in the greenhouse. **A,** Leaf spots and bleached margins. **B,** Closer view of blighted leaf. **C,** Diseased blossom with sporulation. **D,** Canker on main and lateral stems. **E,** Basal stem canker with sporulation. **F,** Plant collapsed with basal stem canker beside a healthy plant.

keep the fungicide suspended and to assure uniform environmental conditions within a block. Harvests were made as described previously in the study of effects of temperature on growth in liquid culture. Three flasks per concentration of each fungicide were used in each of three replicates. Each replicate was arranged in a complete randomized block design. This test was repeated once.

Semilogarithmic plots of the dosage response curves for *B. cinerea* for each fungicide were found by the methods of Benson (2) and Dimond et al (5). The percent inhibition was calculated by the following formula: Percent inhibition = [(mycelial dry weight from culture medium − mycelial dry weight from fungicide-amended culture medium)/mycelial dry weight from culture medium] × 100.

Effects of vinclozolin at eight concentrations on growth of *Botrytis cinerea*. Vinclozolin was incorporated at 0.0, 0.1, 0.25, 0.5, 1.0, 10.0, 100.0, 1,000.0, or 2,000.0 μg a.i./ml into liquid GA medium to investigate fungicidal or fungistatic effects on growth from agar disks of isolate EXA-8. Three replicate flasks per concentration per harvest were used for mycelial dry weight measurement as previously described after 3, 7, and 15 days of growth and an additional three flasks were used for transferring to fungicide-free media after the same growth periods. Each transferred culture was aseptically removed from the vinclozolin-amended flasks and rinsed three times in sterile distilled water rinses for 1 min each time and then transferred to a flask containing 25 ml of GA media without fungicide. Final harvests were made after 7 days of growth in the vinclozolin-free medium. Flasks were arranged in replicates as previously described. The test was repeated once.

Data for these studies, unless otherwise indicated, were analyzed using Fisher's least significant difference test. The Statistical Analysis System (24) was used.

RESULTS

Symptomatology. Symptoms of Botrytis blight of exacum on leaves were 3-mm-diameter tan flecks or marginal necrotic areas (Fig. 2A) which sometimes coalesced to form large, tan, zonate membranous (Fig. 2B) lesions. Pinpoint lesions less than 1 mm in diameter and bleached leaf margins were also observed. Symptoms on leaves varied from a very few pinpoint brown lesions to few to many brownish-tan lesions with concentric rings. Lesions often coalesced, resulting in completely blighted leaves covered with spores.

The initial symptoms on the flowers were water-soaked flecks or complete petal collapse. Petals became brown within 24 hr after inoculation and sporulation was noted in 36 hr. Infected flower blossoms were either covered with abundant sporulation (Fig. 2C) or were brown and shriveled. The pathogen was isolated from petals, sepals, peduncles, and stigmas, but not stamens. Blossoms were frequently entry points into the stem for the fungus. Blossom buds originating in the leaf axil were readily infected and from there the fungus moved down the peduncle and into the stem.

Initial stem symptoms were water-soaked areas, most frequently on the lower half of the plant. Tan to reddish brown cankers developed from these water-soaked areas. Sometimes cankers would be dried and sunken, especially when fungal spread was from an infected flower or leaf (Fig. 2D). Other cankers remained somewhat water-soaked in appearance, did not become sunken, and were surrounded by bleached areas. Basal stem cankers (Fig. 2E) were frequent, but cankers on lateral branches both close to and distant from the main stem were common. Loss of plant turgidity followed by loss of leaf glossiness were the first noticeable symptoms. Upon close examination, a stem canker was always discovered at the base of the stem in such cases. Basal cankers frequently occurred at a small lateral branch just above or below the soil line. Basal stem cankers were the most severe symptom (Fig. 2F) and plants often died within 1 wk after cankers were first observed. Rarely, plants were observed with small, shrunken, dried lesions at the base of the stem. Such plants remained turgid and the

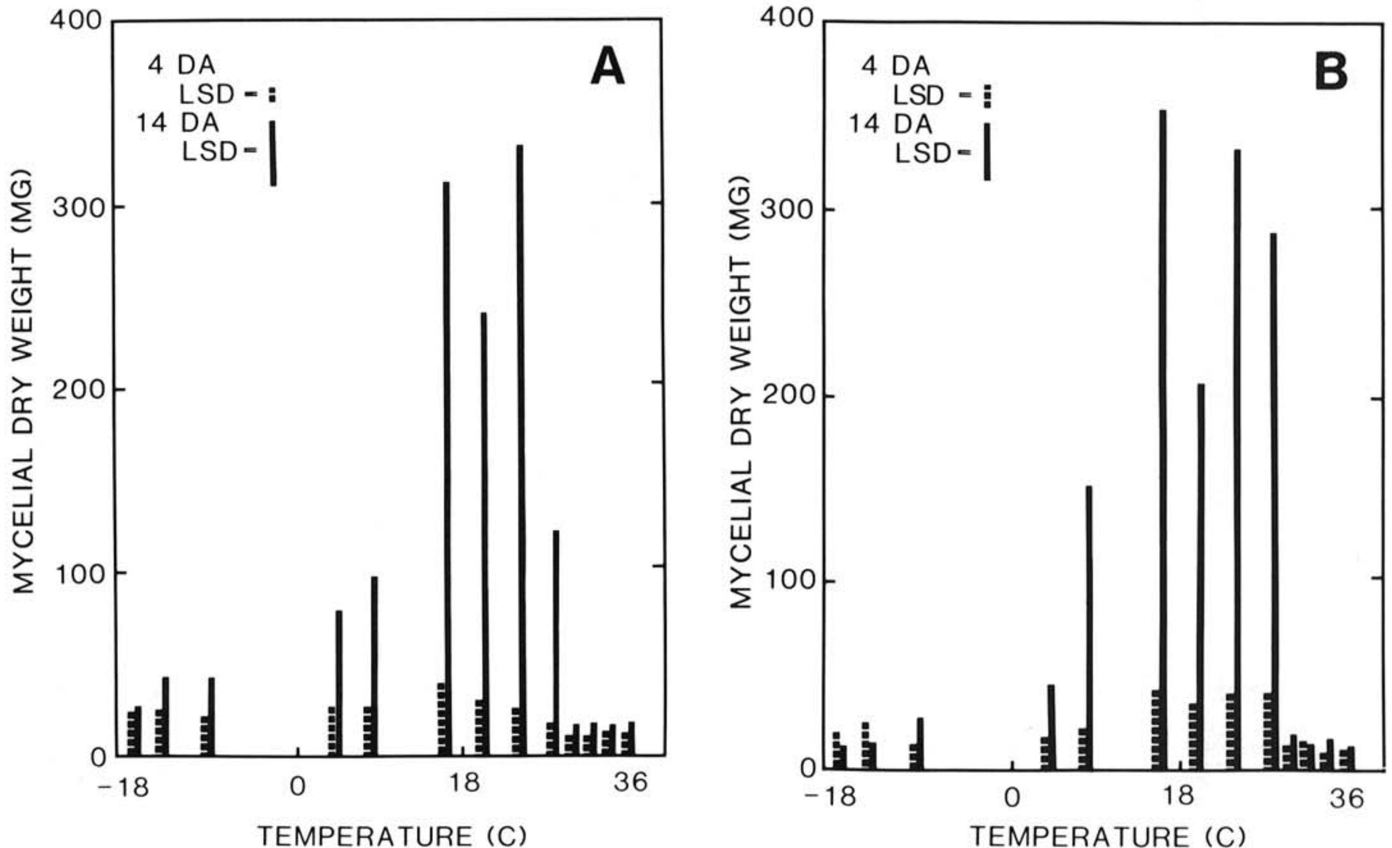

Fig. 3. Mycelial dry weight of *Botrytis cinerea* at 13 temperatures after 4 and 14 days of growth in liquid glucose-asparagine medium in total darkness; **A,** isolate EXA-0 from exacum and **B,** isolate EXA-8 from exacum receiving benomyl weekly. Dry weight of inoculum disks was 8–25 mg. Results are means of nine cultures per temperature per isolate (LSD at $P = 0.05$).

leaves glossy.

Isolation and culture of *B. cinerea*. Isolations were made from leaf spots and stem cankers on exacum after surface sterilization with 0.5% sodium hypochlorite. Leaves and stems were soaked for 1–2 and 5 min, respectively. Leaves, but not stems, were rinsed afterward in sterile distilled water. Infected leaf or stem pieces were placed on aPDA in petri dishes. Most isolates sporulated within 2–4 days after subcultures were made onto PDA in petri dishes. After 9 days mycelial growth with spores completely covered 9-cm-diameter plates.

Effects of temperature and medium on cultural growth. Growth characteristics for isolates EXA-0 and EXA-8 were similar. Mycelial growth occurred at 4–36 C, but was very limited at 30–36 C on PDA, aPDA, GA, and OGM. Sporulation occurred at 4–28 C, but was most abundant at 16–24 C on these same media. Large black sclerotia (1–4 mm diameter) were produced at 4–20 C, small black sclerotia (up to 1 mm diameter) at 24 C, but none at 28–32 C. The size of the sclerotia was consistent within a treatment.

Effects of temperature on growth in liquid culture. After 4 days, optimum temperature for mycelial growth (Fig. 3) was 16 C for EXA-0 and 16–28 C for EXA-8. Mycelial growth occurred at 4–28 C, sporulation at 24 and 28 C, and sclerotia failed to form at any temperature. After 14 days, optimum temperature for mycelial growth was 16–24 C for both isolates. Sporulation occurred at 16–28 C but was prolific at 20 C where mycelial mats were sparse. Mycelial dry weight at 20 C was less than weights at 16 and 24 C. No mycelial extensions from the inoculum disks were observed in cultures incubated at −18 to −10 C or at 30 to 36 C. Mycelial dry weights of agar disks similar to those used for seeding of liquid cultures were 8–25 mg.

Pathogenicity test. Isolates of *B. cinerea* from exacum, geranium, zinnia, and tomato caused similar and characteristic disease symptoms on exacum. Symptoms were indistinguishable from those caused by the original isolate of *B. cinerea* from exacum. All inoculated plants showed similar symptoms of bleached leaf margins, minute leaf spots, and bleached blossoms 1 day after inoculation; sporulation on the adaxial surface of spreading leaf lesions and on blossoms after 2 days; and cankers on lateral and main stems resulting in larger blighted areas after 7 days.

Botrytis cinerea was reisolated from water-soaked plant parts. When reisolations were made from plants inoculated with *B. cinerea* in this study, no evidence was found of *Fusarium solani*, which causes a stem rot and wilt of exacum in California (21).

Effects of cultural methods on disease severity. Crowding of plants caused more severe disease (Table 3). Results of the December–January test were similar to those of the January–February and April–May tests.

In control plants, crowding and overhead irrigation resulted in more severe disease by 14 days than did other treatments. Benomyl sprays did not control the disease; there were no differences for benomyl applications in any treatment combination.

Efficacy of fungicides. All fungicides tested except benomyl and triadimefon gave some disease control on both cultivars. Disease ratings for only the lower rates tested are in Tables 1 and 2 since disease severity was not different for the higher rate tested except for bitertanol and triadimefon. Both bitertanol and triadimefon were phytotoxic or affected growth. Chlorothalonil alone and in combination with benomyl at both concentrations gave protection without phytotoxicity or growth effects. Benomyl treatments were not different from the treatments with no fungicide. Triadimefon was phytotoxic after two applications, even at reduced rates and fewer applications used in the fall test. Leaf margins and blossoms were burned. Small brown circular lesions with concentric rings developed on both older and younger leaves. The fungus was consistently isolated from these leaves. Triadimefon-treated plants were stunted and had small leaves.

Few necrotic lesions were noted on any of the Blue Champion or Jill plants treated with vinclozolin; however, there were pronounced growth regulating effects at both concentrations tested. Blossoms were faded purple to white for both cultivars. Jill was severely stunted while the typically small leaves were large and similar to the normally larger leaves of cultivar Blue Champion. Blue Champion was stunted, but leaf size was not affected until after three to four applications when new foliage failed to enlarge. Treated foliage of both cultivars was deeper green than untreated plants.

Disease symptoms were not detected on the Jill plants that had been treated with bitertanol, but Blue Champion plants were diseased. Plants of both cultivars were stunted and had deeper green foliage than control plants at both concentrations. Disease symptoms were noted on mancozeb-treated plants of both cultivars at both concentrations tested, but were less severe than those on control plants.

Few disease symptoms were noted on the plants treated with iprodione, although small necrotic spots developed on young and old leaves. The fungus was not isolated from these lesions. Phytotoxicity was noted on Blue Champion and Jill plants after the third and sixth fungicide applications, respectively, at both concentrations tested. Blossoms were not burned.

Dosage-response relations. Dosage-response curves were found for inhibition of mycelial growth of *B. cinerea* on liquid GA medium incorporated with benomyl, bitertanol, chlorothalonil, chlorothalonil + benomyl, iprodione, mancozeb, triadimefon, and vinclozolin (Fig. 4).

Effects of vinclozolin at eight concentrations on growth of

TABLE 3. Botrytis blight of exacum (cultivar Blue Champion) in the greenhouse 14, 21, and 28 days after inoculation as affected by spacing, irrigation practices, and weekly benomyl sprays at 0.30 g a.i./L in December–January tests

			Disease ratings[a]					
			After 14 days		After 21 days		After 28 days	
Plant spacing[b]	Irrigation system[c]	Benomyl	Inoculated	Control	Inoculated	Control	Inoculated	Control
Crowded	s	yes	2.5 A[d]	0.1 B	3.4 A	0.4 B	4.2 A	0.8 B
	s	no	2.2 A	0.0 B	3.2 A	0.0 B	4.2 A	0.4 B
	o	yes	2.9 A	1.2 A	4.1 A	2.1 A	4.4 A	3.0 A
	o	no	2.9 A	1.6 A	3.8 A	2.4 A	4.6 A	3.2 A
Widely spaced	s	yes	1.2 B	0.2 B	2.2 B	0.5 B	3.2 B	0.9 B
	s	no	0.6 B	0.2 B	1.6 B	0.3 B	2.5 B	0.6 B
	o	yes	0.8 B	0.1 B	1.5 B	0.2 B	2.5 B	0.6 B
	o	no	0.8 B	0.2 B	1.4 B	0.2 B	2.3 B	0.8 B
LSD ($P = 0.05$)			0.82	0.59	0.88	0.77	0.88	0.88

[a] Disease index: 0 = no disease; 1 = trace (1–25% blight or no cankers); 2 = light (26–50% blight or cankers on small lateral branches); 3 = moderate (51–75% blight or cankers on large lateral branches; 4 = severe (>75% blight or canker on main stem); 5 = plant dead; means of 12 plants.

[b] Crowded plants = leaves of neighboring plants overlapping. Widely spaced plants = 5 cm distance maintained between leaves of neighboring plants.

[c] s = Subirrigation, o = overhead sprinkler.

[d] Means in same column followed by same letter are not signifiantly different according to Fisher's least significant difference test.

B. cinerea. Growth was slight after 3 days on vinclozolin-amended medium. After 7 days on vinclozolin-amended medium, mycelial mats formed in the 0.1, 0.25, and 0.5 μg a.i./ml and the unamended GA medium. Essentially no growth occurred at other concentrations. After 15 days on vinclozolin-amended medium, large mycelial mats and heavy sporulation occurred at 0.1, 0.25, and 0.5 μg a.i./ml. Characteristic growth at 1 μg a.i./ml was small white balls of mycelium. When mycelium was washed and transferred to vinclozolin-free medium for 7 days, cultures from 3, 7, and 15 days on 0.1, 0.25, and 0.5 μg a.i./ml vinclozolin, were similar in mat formation and sporulation to that on unamended medium. Growth was much less in cultures from 1 μg a.i./ml after 3, 7, and 15 days. No growth occurred in cultures from 100 μg a.i./ml.

DISCUSSION

Loss of turgidity, loss of leaf glossiness, bleached leaf margins, and flecked petals were initial symptoms of Botrytis blight of exacum. Basal stem cankers were the most serious symptom, confirming the observations of Ploetz and Engelhard (22).

A disease index for Botrytis blight of floral crops based on the entire plant was developed for this study. Previously, disease severity had been judged by petal lesions (1), degree of flower infection (10), percentage flower infection (12), number of spots on bracts (16), and by the number of leaves and stipules infected or stems rotted (17). Botrytis blight symptoms on exacum are variable. The disease index developed in this study based on the most serious symptom, stem cankering, is useful when rating the entire plant.

The isolates of *B. cinerea* from exacum in the present study were fast-growing and sporulated abundantly, whereas those described by Ploetz and Engelhard (22) were slow-growing and sporulated sparsely. Optimum temperatures for mycelial growth of isolates of *B. cinerea* from exacum were different from those reported for mycelial growth on potato sucrose agar (20–28 C) (26) and on nutrient gelatin (20–22 C) (25). Optimum temperature for sclerotial production also differed from those previously reported (19). Temperatures favoring mycelial production in *B. cinerea* generally depress sclerotial production and vice versa (19); however, there was an overlap of these temperatures for exacum isolates. The prolific sporulation that occurred at 20 C in the current study falls within the 12–22 C optimum range previously reported (19).

Crowded spacing and overhead irrigation increased disease severity, as did the buildup of diseased plant parts noted in disease progress in the uninoculated plants. Wide-spacing and subirrigation practices, as well as crop hygiene, need to be considered in control tactics. Efficacious chemicals should always be coordinated with cultural control methods for more effective control.

Phytotoxicity, noted as burning of foliage and blossoms or the fading of blossoms, and stunting were evident with some fungicides at both concentrations used in the current study. Triadimefon injured foliage and blossoms in both tests, resulting in the development of much necrotic tissue and causing these plants to be more vulnerable to the pathogen than control plants. Vinclozolin effectively controls Botrytis blight in the greenhouse (23), but its effects on growth of exacum at the rates tested precludes its use in the commercial production of exacum. Iprodione was efficacious, but some phytotoxicity was also observed. A lower rate of iprodione may achieve disease control without phytotoxicity.

The amount of disease in the plants treated with benomyl indicates that the isolate of *B. cinerea* used was tolerant to benomyl. To compare our isolate of *B. cinerea* with those of Bollen and Scholten (3) in their benomyl tolerance studies (using aqueous suspensions incorporated in PDA), we determined from their data ED_{50} values for benomyl of 127 μg/ml and 0.11 μg/ml for a resistant and sensitive isolate, respectively. The ED_{50} value we determined in vitro was over twice that of the resistant isolate used in Bollen and Scholten's study (3). The benomyl-tolerant isolate of *Botrytis* from exacum used in our study may have been selected for in the greenhouse as a result of weekly applications of benomyl. Our study did not compare sensitive and tolerant isolates. Other isolates of *B. cinerea* need to be screened for benomyl tolerance before the ED_{50} value can be determined for a sensitive isolate.

The isolate of *B. cinerea* used in the current study was sensitive to vinclozolin and iprodione. These fungicides gave the most growth suppression in vitro. The ED_{50} values determined by Pappas (20) were 0.2 and 0.1 μg a.i./ml for iprodione and vinclozolin, respectively, for sensitive isolates when acetone solvent was used. Isolates tolerant to iprodione and vinclozolin reported by Pappas (20) had ED_{50} values greater than 100 μg a.i./ml.

Vinclozolin at concentrations of 100 μg a.i./ml or greater incorporated in synthetic medium appeared to kill the mycelium of *B. cinerea*. The lower concentrations tested in this study were fungistatic since the washed mycelium placed in vinclozolin-free media resumed growth.

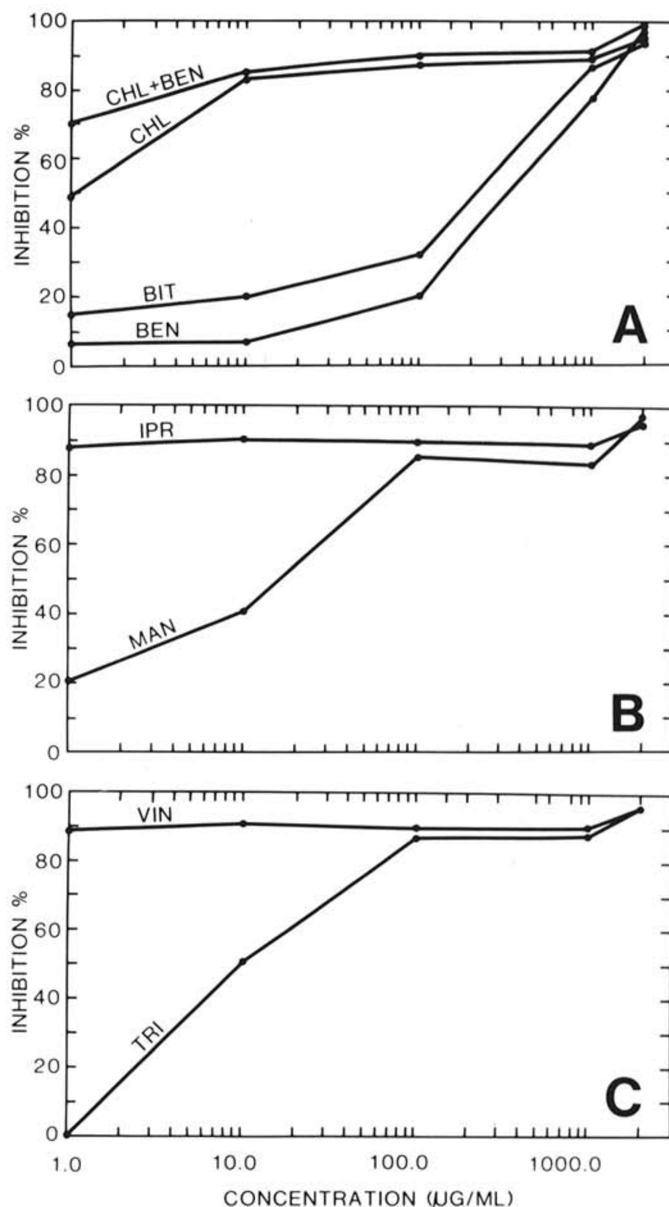

Fig. 4. Semilogarithmic plot of dosage response curve for *Botrytis cinerea*. Inhibition of mycelial growth in liquid glucose-asparagine medium incorporated with **A,** chlorothalonil (CHL) + benomyl (BEN), chlorothalonil, bitertanol (BIT), and benomyl; **B,** iprodione (IPR) and mancozeb (MAN); and **C,** vinclozolin (VIN) and triadimefon (TRI) as determined from comparison to growth in unamended media. Inhibition (%) = [(mycelial dry weight from culture medium − mycelial dry weight from fungicide-amended culture medium)/mycelial dry weight from culture medium] × 100.

Since chlorothalonil was effective in disease control, the rates used in the present study and lower rates should be evaluated. Chlorothalonil suppressed growth of *B. cinerea* in vitro the most after iprodione and vinclozolin. A wider range of concentrations of these fungicides used with the appropriate solvent would make the present in vitro studies more precise, but the relationships in growth inhibition are illustrated.

Extensive growth and sporulation of *B. cinerea* on bitertanol-amended medium was not consistent with greenhouse results in the spring test. Possibly bitertanol stimulates a resistance to *Botrytis* reaction in exacum. Further testing is necessary to determine the usefulness of this fungicide in control.

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