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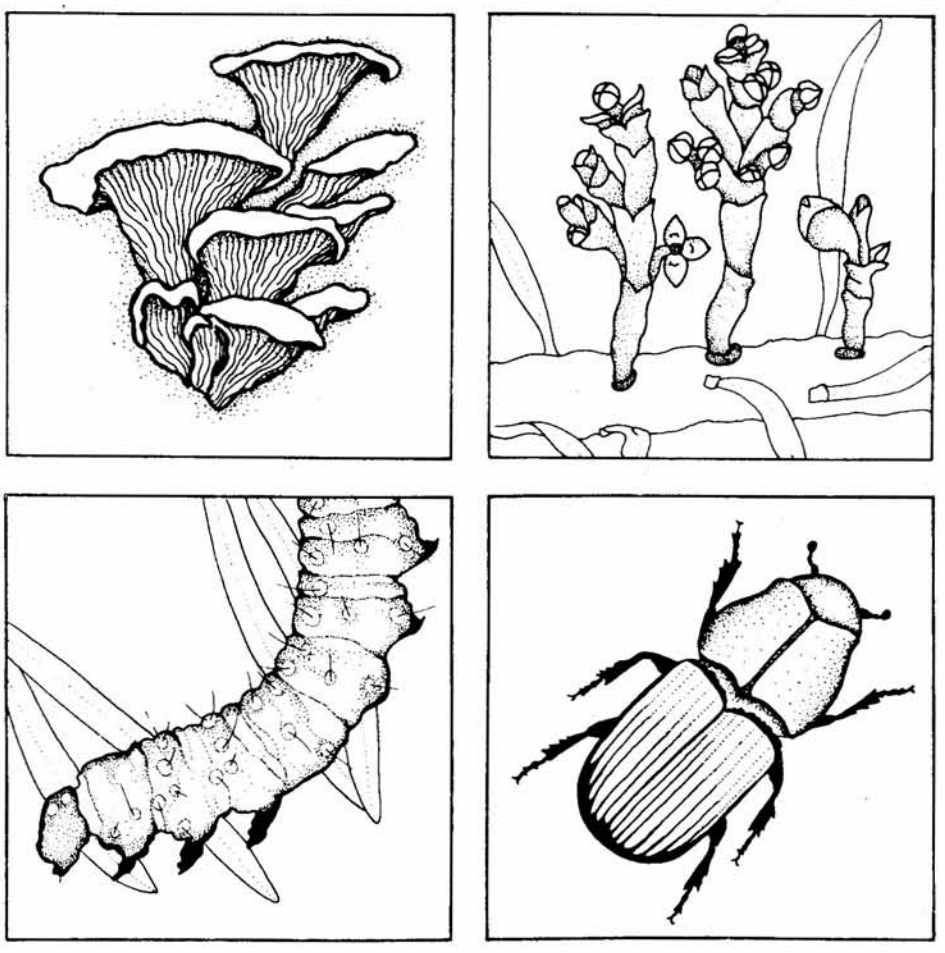

FUNGICIDAL TOLERANCE OF *BOTRYTIS CINEREA* FROM THE FLATHEAD INDIAN RESERVATION GREENHOUSE, RONAN, MONTANA

by
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ABSTRACT

Tests were conducted to determine levels of tolerance of 10 Botrytis cinerea isolates from the Flathead Indian Reservation greenhouse (Ronan, MT) to six fungicides. In vitro growth and conidial germination on fungicide-amended potato dextrose agar were used as criteria for assessing tolerance. All tested isolates were tolerant to benomyl and chlorothalonil, the fungicides routinely used in this greenhouse prior to the test. Most isolates displayed slight tolerance to captan; four were slightly tolerant to vinclozolin. Dicloran and iprodione were the most effective fungicides in limiting growth and spore germination of the Botrytis isolates tested. Field performance tests are needed to evaluate control efficacy of vinclozolin and iprodione. A rotating fungicide spray schedule coupled with good cultural practices such as sanitation, adequate air circulation, and limiting irrigation should provide adequate control for this disease.

INTRODUCTION

Botrytis cinerea Pers. ex. Fr. is an important pathogen of many different crops, including nursery-grown conifers in the western United States. Although the fungus is especially damaging in greenhouses (Gillman and James 1980; Kadow et al. 1938; Miller and Fletcher 1974), it can also cause disease of conifers in outside seedbeds (James 1980). Losses may be especially severe in winter greenhouse crops because the cool, wet conditions in greenhouses during the winter are ideal for the pathogen to spread and infect susceptible plants rapidly (Jarvis 1980a). Although most conifers are susceptible, western larch (Larix occidentalis Nutt.) and lodgepole pine (Pinus contorta Dougl.) are often severely damaged (James et al. 1982).

Botrytis is usually controlled in greenhouses by regulating environmental condition when possible and applying fungicides (Cooley 1981). Fungicides are generally applied through overhead irrigation systems when seedlings are most susceptible to infection. This occurs after the seedling canopy is closed and dense, and air circulation among trees is poor. This condition results in moisture remaining on foliage longer, providing ideal conditions for infection.

Botrytis has developed tolerance to several of the fungicides commonly used. Tolerance is especially common when only one fungicide is applied repeatedly in a greenhouse (Dekker 1976; Ogawa et al. 1976). Tolerance to the benzimidozole fungicides has been especially common (Gillman and James 1980; Maude 1980; Miller and Fletcher 1974). Other fungicides for which Botrytis tolerance has been shown include vinclozolin (Cooley 1981), zineb (Gillman and James 1980), dicloran (Cooley 1981; Webster et al. 1970), captan, chlorothalonil, and mancozeb (Cooley 1981; Gillman and James 1980).

Recent attempts to control Botrytis at the Flathead Indian Reservation (Bureau of Indian Affairs) greenhouse at Ronan, Montana with benomyl (Benlate®) and chlorothalonil (Exotherm®) were unsuccessful. Losses were especially severe on western larch and lodgepole pine grown during

the winter. To test our hypothesis of possible fungicidal tolerance, several isolates of Botrytis from this greenhouse were evaluated for their response to selected fungicides.

MATERIALS AND METHODS

Ten isolates of B. cinerea were obtained at random from the Flathead Reservation greenhouse and tested to represent a cross section of possible strains present. All tested isolates were from infected lodgepole pine. Nine isolates were obtained during 1982, just prior to the fungicide evaluations. The other isolate (81-33) was obtained in 1981 and maintained on potato dextrose agar (PDA) for approximately 9 months prior to testing.

Six fungicides were evaluated for their effects against Botrytis (table 1). Four of these (benomyl, dicloran, captan, and chlorothalonil) are commonly used to control Botrytis on conifers in greenhouses (James et al. 1982; McCain and Smith 1978; Smith et al. 1973). The other two, vinclozolin and iprodione, are newer fungicides. Vinclozolin is an organic contact fungicide with specific action against Sclerotinia and Botrytis and has been used on herbaceous and woody ornamental plants (Maude 1980; Powell 1982). Iprodione is a systemic fungicide developed for turf diseases (Danneberger and Vargas 1982; Sanders et al. 1978), but also controls Botrytis on containerized conifers (James et al. 1982).

Tolerance to fungicides was determined by measuring radial mycelial growth and assessing conidial germination on the surface of PDA amended with 50 ppm (active ingredient) of the fungicides. PDA with no fungicide served as a check. All these fungicides were wettable powders and, with the exception of benomyl, were added to liquid autoclaved PDA while at 45-55°C. Benomyl was added to PDA before autoclaving to increase solubility (Gillman and James 1980). Circular 8 mm plugs of mycelium from 4-day-old Botrytis cultures growing on PDA were placed onto the edge of petri dishes (100 mm diameter) containing 25 ml of test media. Each treatment and the check was replicated five times for each isolate. Inoculated dishes were incubated in the dark at 24°C for 6 days, after which diameters of the fungal colonies were measured. Data were analyzed using an analysis of variance. Significant treatment differences were located with Duncan's Multiple Range Comparison Test.

Effects of fungicides on germination of Botrytis conidia were tested in vitro. Conidia were obtained from 9-day-old cultures grown at 24°C. The cultures were exposed to black light for the final 5 days of the growth cycle to stimulate spore formation (Epton and Richmond 1980). Conidia were harvested by flooding petri dishes with 10 ml sterile distilled water and agitating with a sterile camel's hair brush. Spore suspensions were passed through double layers of cheesecloth to remove mycelial fragments, and 1:10 dilutions were made with sterile distilled water. Approximately 0.5 ml of the diluted spore suspension from each tested isolate was placed on PDA amended with 50 ppm (active ingredient) of the test fungicide. PDA with no fungicide served as a check. There

Table 1.--Fungicides evaluated for tolerance of Botrytis cinerea.

Fungicide	Trade name	Chemical name	Manufacturer
vinclozolin	Ornalin®	3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1, 3-oxazolidin-2,4-dion	Mallinckrodt
iprodione	Chipco 26019®	Diethyl 4, 4'-O-phenylenebis 3-thioallophanate	Rhone-Poulenc
benomyl	Benlate®	Methyl-1-(butylcarbamoyl)-benzimidazole carbamate	Dupont
dicloran	Botran®	2,6-dichloro-4 nitroaniline	Tuco (Upjohn)
captan	Captan	N-(Trichloromethylthio)-4-cyclohexene-1, 2-dicarboximide	Stauffer
chlorothalonil	Exotherm®	Tetrachlorosiophthalonitrile	Diamond-Shamrock

were two replications for each isolate. After 24 hours incubation in the dark at 24°C, 200 randomly selected spores on each plate were examined for germination under a compound microscope (100-450X). Percentage germination on PDA and fungicide-amended media was compared using an analysis of variance. Significant treatment differences were located with Duncan's Multiple Range Comparison Test.

RESULTS

Iprodione and dicloran were the only fungicides that all 10 Botrytis isolates tested showed no tolerance for on radial growth tests (table 2). Four of the isolates displayed slight tolerance to vinclozolin. However, growth of tolerant isolates was slow and the mycelium was appressed to the agar surface (fig. 1). All tested isolates were tolerant to benomyl and chlorothalonil. Botrytis often produced irregular growth patterns with much aerial mycelium on benomyl media (fig. 2). Growth on chlorothalonil media was usually more uniform with an extensive aerial mycelium (fig. 3). Nine of the ten isolates were also tolerant to captan. However, their growth was irregular with abundant aerial mycelium (fig. 4). Sporulation of colonies on captan media appeared greater than on any other fungicide, including the PDA check.

Conidial germination of most tested isolates was reduced by all fungicides except benomyl (table 3). None of the isolates germinated on iprodione-amended media. Only one isolate (82-28) germinated on media containing vinclozolin and dicloran. However, this isolate did not display tolerance to these fungicides in the mycelial growth test (table 2). Only three isolates germinated on media containing captan and only a few spores of each isolate germinated. Benomyl did not inhibit conidial germination of any Botrytis isolates tested; i.e., there were no significant differences between germination percentage on benomyl-amended media and PDA checks. Chlorothalonil likewise did not greatly inhibit conidial germination, although some isolates responded more than others.

Most of the fungicides affected germ tube morphology of germinating spores. For example, spores germinating on vinclozolin (isolate 82-28) produced short, branched, multi-septate germ tubes (fig. 5). On the other hand, germ tubes less inhibited by fungicides such as those on chlorothalonil, often grew over the agar surface in a normal manner (fig. 6). Not all germinated spores of a particular isolate responded with similar germ tube growth habits on the same fungicide medium. For example, some germ tubes on chlorothalonil were rapid growing, branched and appeared normal. However, others on the same medium were small and bud-like (fig. 6). These differences may be due to differential responses of individual conidia to the fungicide.

DISCUSSION

Control of Botrytis blight is often very difficult, especially on greenhouse crops where environmental conditions are usually conducive to infection by and proliferation of the pathogen. Also, it is difficult because the pathogen is capable of attacking all plant parts at almost any stage of their growth and in storage (Maude 1980).

Table 2.--Effects of selected fungicides on radial growth of Botrytis cinerea isolates. 1/

	Isolate									
Fungicide	81-33	82-24	82-25	82-26	82-27	82-28	82-29	82-30	82-31	82-32
Check	100.0A	100.0A	100.0A	100.0A	100.0A	100.0A	100.0A	100.0A	100.0A	100.0A
vinclozolin	0.2E	0 D	4.0D	6.7C	3.2D	0 D	15.1D	0 C	0 C	0 E
iprodione	0 E	0 D	0 D	0 D	0 D	0 D	0 E	0 C	0 C	0 E
benomyl	53.7B	81.1B	129.3B	61.2B	59.8B	72.7B	47.9B	90.5A	101.4A	85.4B
dicloran	0 E	0 D	0 D	0 D	0 D	0 D	0 E	0 C	0 C	0 E
captan	26.5D	35.4C	39.6C	57.3B	15.8C	15.7C	28.0C	0.1C	23.5B	24.2D
chlorothalonil	36.0C	31.1C	46.7C	53.9B	47.2B	19.3C	35.8C	31.0B	22.8B	44.0C

1/ Fungicide concentration of 50 ppm active ingredient; growth expressed as percent of control. Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Duncan's Multiple Range Comparison Test.

Figure 1.--Growth of Botrytis cinerea (isolate 82-27) on PDA amended with 50 ppm vinclozolin (Ornalin®) after 6 days. Colonies grew slowly with the mycelium appressed to the agar surface.

Figure 2.--Growth of Botrytis cinerea (isolate 82-24) on PDA amended with 50 ppm benomyl (Benlate®) after 6 days. Colonies grew rapidly with irregular patterns and extensive aerial mycelium.

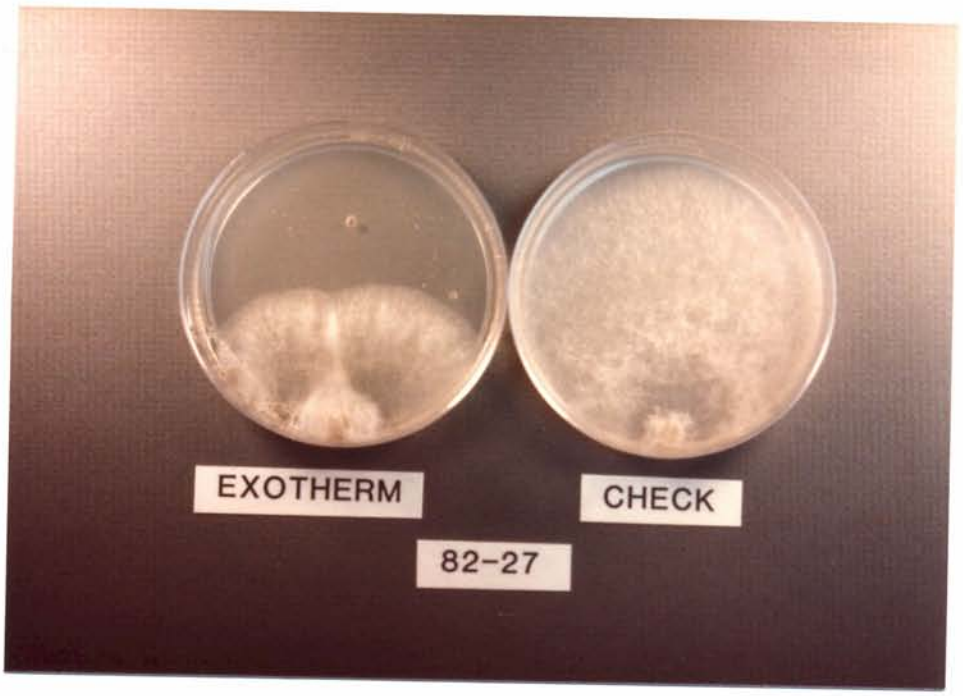

Figure 3.--Growth of Botrytis cinerea (isolate 82-27) on PDA amended with 50 ppm chlorothalonil (Exotherm®) after 6 days. Colonies were fast growing, uniform with abundant aerial mycelium.

Figure 4.--Growth of Botrytis cinerea (isolate 82-27) on PDA amended with 50 ppm captan after 6 days. Colonies were irregular with abundant aerial mycelium; sporulation was extensive (Note greyish sporulation zone behind white advancing margin).

Table 3.--Effects of selected fungicides on germination of Botrytis cinerea conidia. [1/]

Fungicide	Isolate									
	81-33	82-24	82-25	82-26	82-27	82-28	82-29	82-30	82-31	82-32
Check	100A	96A	98A	99A	100A	99A	98A	95A	85A	70A
vinclozolin [2/]	0C	0C	0C	0B	0C	51C	0C	0B	0C	0C
iprodione	0C	0C	0C	0B	0C	0D	0C	0B	0C	0C
benomyl [3/]	99A	97A	94A	95A	96A	99A	95A	93A	95A	58A
dicloran [4/]	0C	0C	0C	0B	0C	75B	0C	0B	0C	0C
captan [5/]	0C	2C	0C	1B	0C	0D	0C	1B	0C	0C
chlorothalonil [6/]	44B	65B	84B	90A	60B	89A	71B	88A	66B	17B

1/ Fungicide concentration of 50 ppm active ingredient. Figures in table are percent germination of randomly selected spores. Within each column, means followed by the same capital letter are not significantly different (P=0.05) using Duncan's Multiple Range Comparison Test.

2/ The one isolate (82-28) with germinating spores had multi-branched germ tubes with distinct septations.

3/ Germ tubes of some isolates (82-29, 82-32, and 82-24) were circular and curved around the spore; germ tubes of other isolates grew normally over agar surface.

4/ Germ tubes of isolate 82-28 were circular and wrapped around the spore.

5/ Germinated spores had short, bud-like germ tubes.

6/ Germ tubes of most germinated spores grew over the agar surface.

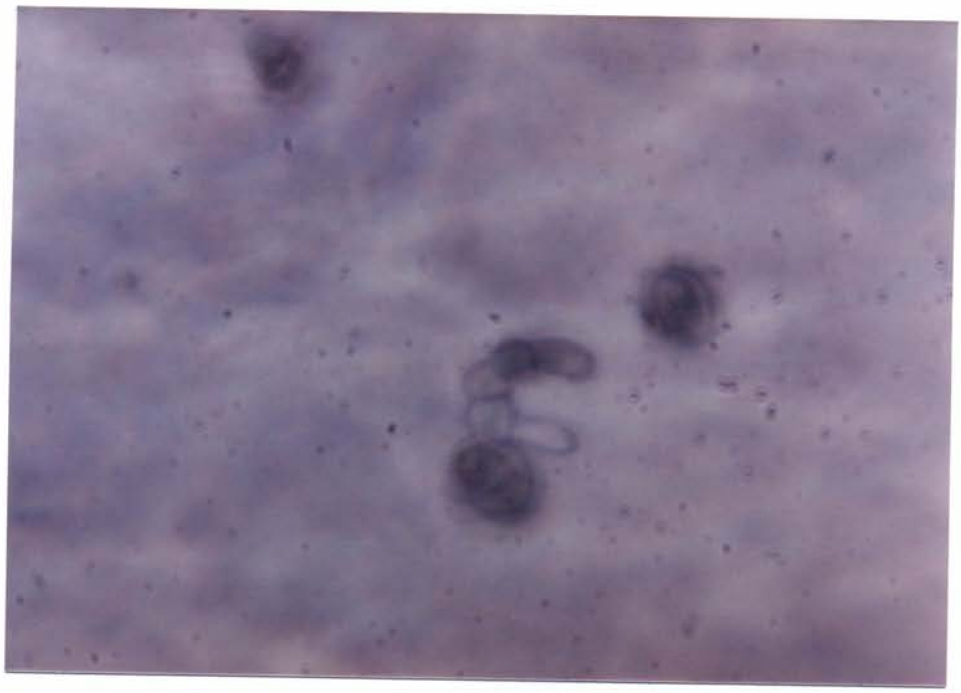

Figure 5.--Photomicrograph of Botrytis cinerea conidia germinating on PDA amended with 50 ppm (active ingredient) of vinclozolin (X450). Germ tubes are short, branched, and multi-septate.

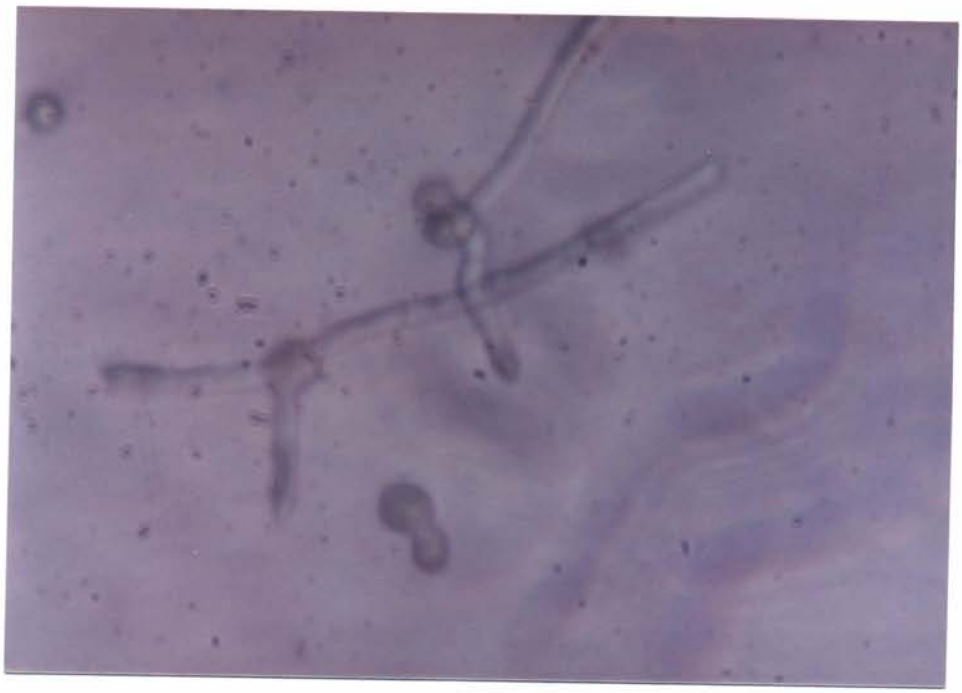

Figure 6.--Photomicrograph of Botrytis cinerea conidia germinating on PDA amended with 50 ppm (active ingredient) of chlorothalonil (X450). Some germ tubes are rapid growing and appear normal; others are small and bud-like (lower center).

Dependence on fungicides to control Botrytis blight has increased. Benomyl and chlorothalonil were commonly used fungicides to control Botrytis at the Flathead Reservation greenhouse in Ronan. However, recently these chemicals were unsuccessful in reducing losses to acceptable levels. Our tests indicated that all isolates of Botrytis from this greenhouse are tolerant to both of these fungicides. Spores readily germinated and fungal colonies grew rapidly in the presence of these chemicals. Continued use of these two fungicides apparently provided sufficient pressure on the pathogen to develop tolerant strains which proliferated and spread throughout the greenhouse.

Tolerance of B. cinerea to benomyl and chlorothalonil has been reported many times (Cohen and Dennis 1975; Cooley 1981; Gillman and James 1980; Ogawa et al. 1976; and others). Systemic fungicides such as benomyl gave good control of Botrytis and related diseases when first introduced and as a result, growers quickly became dependent on these chemicals (Maude 1980). However, it did not take too long for Botrytis to become tolerant to benomyl after the fungicide was first introduced. Tolerance to Botrytis was first reported on cyclamen (Bollen and Scholten 1971) and later occurred on flower, vegetable, fruit, and conifer tree crops (Dekker 1976; Fletcher and Scholefield 1976; Miller and Fletcher 1974; Ogawa et al. 1976). Tolerance of Botrytis to benomyl is now common in conifer greenhouses throughout the West (Cooley 1981; Gillman and James 1980; James et al. 1982; McCain and Smith 1978). Performance of the fungicide against Botrytis is now so poor that most growers no longer use it.

Botrytis had the least tolerance for dicloran, vinclozolin, and iprodione. All three fungicides are chemically similar (Ritchie 1982). Although tolerance to dicloran has previously been reported (Cooley 1981; Lankow 1971), levels of tolerance are usually low and not as widespread as with some other fungicides such as benomyl and chlorothalonil (Cohen and Dennis 1975; Gillman and James 1980). Field performance of dicloran against Botrytis is usually satisfactory. Although relatively high levels of infection were reported on containerized western larch despite frequent dicloran applications, seedling survival after the test was good (James et al. 1982). Therefore, dicloran will probably provide effective control in most operations if rotated with other fungicides.

Tolerance of Botrytis to vinclozolin has also been reported (Cooley 1981). Several of the isolates tested in this study were slightly tolerant to the fungicide. However, field tests are needed to determine actual efficacy of vinclozolin in reducing losses to Botrytis in conifer greenhouses. Registration is also required before the fungicide can be used operationally.

None of the Botrytis isolates tested showed any level of tolerance to iprodione. This fungicide previously provided excellent control of Botrytis on containerized conifers in a greenhouse test (James et al. 1982) and has been recommended for general use against the pathogen (Powell 1982). However, registration of the fungicide is required before it can be used operationally.

Captan is commonly used to control several different plant diseases. Its protective, nonspecific action and low cost make it a valuable fungicide. Several of the Botrytis isolates we tested showed slight tolerance to captan. Other reports (Cooley 1981; Gillman and James 1980; Pepin and MacPherson 1982) indicated that tolerance to the chemical occurred and that some strains of the fungus may be very tolerant. Although field performance of captan on greenhouse conifers has generally been excellent (James et al. 1982), captan-resistant strains of Botrytis may result in disease control problems (Pepin and MacPherson 1982).

RECOMMENDATIONS

Botrytis cinerea has a wide host range, including over 200 species of plants (Jarvis 1980b). It infects weeds, plant debris, and also produces resting structures called sclerotia; all of these may serve as inoculum sources. Therefore, sanitation practices are very important in reducing the potential hazard to a new crop, especially in greenhouses. All greenhouse benches, floors, and walls should be surface sterilized with a solution of sodium hypochlorite (Clorox®) between crops. Plant debris should be removed and infected plants culled and disposed of during the growing season. Cull piles should not be located near greenhouses.

Other cultural operations that help reduce disease incidence include regulating stocking density to ensure adequate air circulation among plants (Cooley 1981). If small Ray Leach containers are used, individual tubes and trays can be spaced to improve air movement among plants. Less frequent irrigation to reduce foliage moisture retention will also help reduce disease incidence (Cooley 1981).

The following points should be considered when developing procedures and schedules for chemical control of Botrytis:

1. Several different fungicides should be applied in rotation so that fungal strains tolerant to one or more of these chemicals will not become dominant.

2. The lowest possible concentration of fungicides that will give adequate control should be used. This will reduce selection pressure for tolerant strains of the fungus.

3. Selected fungicides should have different modes of action. For example, systemic chemicals should be alternated with broad spectrum protectants.

4. Combine cultural practices (sanitation, adequate air circulation, irrigation reduction) with rotated use of several different fungicides. Cultural practices can reduce fungal inoculum and thus chances for infection while fungicides protect susceptible plant tissues from infection. Both procedures are needed for an effective control strategy.

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This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed have been registered. All uses of pesticides must be registered by appropriate State and/or Federal agencies before they can be recommended. CAUTION: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife--if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.

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