Agar Plate, Soil Plate, and Field Evaluation of Fluazinam and Other Fungicides for Control of *Sclerotinia minor* on Peanut

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ABSTRACT

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The sensitivity of *Sclerotinia minor* to three dicarboximide fungicides (chlozolinate, iprodione, and vinclozolin), fluazinam (ASC-66825 and RH-3486), and MON-13108 was tested on fungicide-amended, glucose-yeast extract agar (GYEA). ED_{50} values (dose for 50% inhibition of mycelial growth calculated from a dose-response line) for a field isolate (S-2) were 0.004, 0.004, 0.025, 0.08, 0.18, and 0.38 μg/ml for ASC-66825, RH-3486, MON-13108, vinclozolin, iprodione, and chlozolinate, respectively. An in vivo, dicarboximide-resistant isolate (B-83-T2) exhibited enhanced mycelial growth on GYEA with either iprodione or vinclozolin at 1 μg/ml or chlozolinate at 5 μg/ml, but 1 μg/ml of RH-3486, ASC-66825, or MON-13108 suppressed growth by 92, 90, and 54%, respectively. At 100 μg/ml, vinclozolin, iprodione, and chlozolinate inhibited growth of isolate B-83-T2 by 93, 83, and 74%, respectively. When cultures of *S. minor* on a soil-cornmeal medium were sprayed with fungicides to simulate an application rate of 1.12 kg a.i./ha, MON-13108 was the only treatment that did not greatly inhibit mycelial growth of isolate S-2. ASC-66825 and RH-3486 significantly suppressed mycelial growth of isolate B-83-T2 in soil plate tests. In replicated field trials, ASC-66825 and RH-3486 limited disease incidence and increased yield of peanuts (*Arachis hypogaea*) more than other fungicides.

Sclerotinia blight was reported on peanut (*Arachis hypogaea* L.) in Virginia in 1971 and North Carolina in 1972 (18). The pathogen, *Sclerotinia minor* Jagger (9), is a soilborne fungus overwintering as sclerotia. In recent years, the disease has become more severe and spread to peanut production areas in Oklahoma (27), New Mexico, and Texas (25). In Virginia, Sclerotinia blight is currently the major disease problem for peanut growers. The disease has claimed an average of 6% of the potential peanut crop each year during the period from 1987 to 1990, in spite of current control recommendations (P. M. Phipps, *unpublished*). Average losses in farm income attributed to Sclerotinia blight were estimated to be $6.6 million per year.

Because no commercially acceptable peanut cultivars have a high level of resistance to Sclerotinia blight, fungicides are the primary method for disease control. In 1975, the aromatic hydrocarbon fungicides, dicloran and PCNB, were reported to give some control of the disease in field trials (1). Dicloran was used to control Sclerotinia blight of peanut in Virginia, based on emergency-use permits granted by the Environmental Protection Agency (EPA) from 1977 to 1984. PCNB is now used to control southern stem rot, caused by *Sclerotium rolfsii* Sacc., and the fungicide has a special local-need registration for suppression of Sclerotinia blight.

Much interest has been shown in the newer class of dicarboximide fungicides since their initial synthesis in the late 1960s. An older class of dicarboximide fungicides consisted of folpet, captafol, and captan and are now known as phthalimide fungicides. Members of the new class of dicarboximides are particularly active against species of *Botrytis* and *Sclerotinia* (7). Procymidone (DPX-4424) was reported to be extremely effective in controlling Sclerotinia blight of peanut (17), but research on this compound was discontinued in the United States in 1979. Vinclozolin was the first dicarboximide fungicide to be used by peanut growers in Virginia with Section 18 approval by the Virginia Department of Agriculture and EPA in 1984. Iprodione gained full registration for use on peanut in 1985. Since 1986, iprodione and PCNB have been the only recommended fungicides for control of Sclerotinia blight of peanut in Virginia and North Carolina.

Cross-resistance to fungicides increases the importance of any potential resistance problems with *S. minor* in peanuts. Isolates of *S. minor* that originally exhibited in vitro resistance to iprodione were capable of growth on media amended with either vinclozolin, dicloran, or PCNB (6). The dicarboximides and aromatic hydrocarbon fungicides are remarkably similar in their mode of action (20), although they differ in field performance characteristics (5). Unfortunately, all available fungicides for control of Sclerotinia blight of peanut fall into these two classes. The threat of cross-resistance and reports of marginal disease control with iprodione have intensified the search for effective fungicides with an alternate mode of action. In 1985, a pathogenic isolate of *S. minor* (B-83-T2) with resistance to iprodione was found in an experimental microplot that had been treated with iprodione at the Tidewater Agricultural Experiment Station, Suffolk, VA (4). The microplot had been artificially infested with a native field isolate (S-2) of *S. minor* in the spring of 1983. The occurrence of B-83-T2 has been a cause for concern as to the future of disease control with the dicarboximide fungicides.

The purpose of this study was to compare the fungitoxicity of three dicarboximides and three experimental compounds to *S. minor* using agar- and soil-plate tests and assess fungicide performance under field conditions. A second goal was to further characterize the resistance of *S. minor* to dicarboximide fungicides and to test for cross-resistance to other fungicides. Two preliminary reports have been published (22,23).

MATERIALS AND METHODS

Preparation of agar plates. Glucose-yeast extract agar (GYEA) consisting of 20 g of agar, 20 g of dextrose, 2 g of yeast extract, 1 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, and 1 L of distilled water was used throughout this study as the basal medium for agar-plate tests as well as maintenance of *S. minor* in culture. Two isolates of *S. minor*, S-2 and B-83-T2, were maintained on agar slants at 5 C. Mycelium was transferred from the slant cultures to petri dishes containing GYEA. After 3 days of incubation

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at 22 C, 6-mm-diameter plugs of agar and actively growing mycelium were removed from the periphery of the culture. These agar plugs served as the inoculum source for agar- and soil-plate tests of fungicide activity.

Suspensions of fungicides were prepared in sterile distilled water and added to the agar medium at 70 C in a water bath to yield concentrations of 0.0005, 0.002, 0.01, 0.05, 0.2, 1, 5, 20, and 100 μg/ml. Four replicates of each concentration were used to determine the sensitivity of each isolate of *S. minor*. Two separate tests were performed. All fungicide concentrations are expressed as active ingredient. The following fungicides were evaluated: chlozolinate (SDS-65311 50WP, ISK-Biotech, Mentor, OH), fluazinam (ASC-66825 50WP, ISK-Biotech, and RH-3486 50WP, Rohm and Haas Co., Philadelphia, PA), iprodione (Rovral 50WP, Rhone-Poulenc Inc., Research Triangle Park, NC), MON-13108 (experimental chemistry 3F, Monsanto Agricultural Products, St. Louis, MO), and vinclozolin (Ronilan 50WP, BASF Wyandotte Corp., Parsippany, NJ). After mixing, the amended GYEA was dispensed into 9-cm-diameter petri dishes and allowed to cool.

Agar plugs from cultures of *S. minor* on GYEA were placed with the surface mycelium face down on the medium at the edge of the petri dishes. The plates were incubated at 20 C. Mycelial growth (mm) across the agar surface was measured at 24-hr intervals. After 4 days of growth, the percent inhibition of linear growth was transformed into probability units (probits) and fungicide dosage was converted to logarithms (2). These transformations tended to straighten the sigmoid dosage-response curve. Linear regression analyses were then used to determine the ED_{50} values (estimated dose for 50% inhibition of mycelial growth) for each fungicide and isolate.

Preparation of soil plates. Field soil, classified as a Nansemond coarse-loamy, siliceous thermic Aquic Hapludult, was collected from areas untreated with chemicals and air-dried on a greenhouse bench. Soil was then sifted through a 5-mesh screen (4-mm openings) to remove debris. Commercial cornmeal was added to the soil to achieve a level of 5% (w/w). After thorough mixing, 50 cm^3 of the amended soil was placed in 9-cm-diameter glass petri dishes. The soil was moistened with 20 ml of distilled water and autoclaved for 40 min at 121 C under 103 kPa. After cooling, an agar plug with mycelium of *S. minor* was inverted in the center of each dish. The soil dishes were incubated for 3 days at 20 C, after which time the colony of *S. minor* was approximately 2.5 cm in diameter.

Suspensions of fungicides were prepared in distilled water to obtain standard concentrations of 0.70 mg/ml. Each suspension was placed in an airbrush sprayer (model 200-1, Badger Air-Brush Co., Franklin Park, IL) and sprayed over the mycelium and soil for 5 s. This technique delivered 1 ml of the solution uniformly over the soil-plate surface (63.5 cm^2) and simulated a field application of 1.12 kg/ha of fungicide. Treatments were replicated five times and the entire test was performed twice.

Mycelial growth was measured at 24-hr intervals until growth in the water-sprayed check plates reached the edge of plates. After 14 days, sclerotial counts were made by removing a 10-cm^3 sample of soil equidistant from the center and margin of the soil plate. The sample was washed on a 40-mesh screen (425-μm openings), and sclerotia retained on the sieve were counted with a dissecting microscope.

Field trials. Field trials were conducted on land naturally infested with *S. minor* and having a history of severe Sclerotinia blight of peanut. The land was prepared by moldboard plowing and disking before the planting of peanuts. Florigiant peanuts were planted in 1987 and NC 9 was planted in 1988, 1989, and 1990. Field plot design followed recommended research practices (15). All tests were managed according to standard practices for peanut production in Virginia (14), and chlorothalonil at 1.26 kg/ha was used for control of Cercospora leaf spot according to the Virginia Peanut Leafspot Advisory Program (16).

All fungicides for control of Sclerotinia blight were applied with one 8008LP nozzle (TeeJet Spraying Systems Co., Wheaton, IL) centered at a level over each row to provide complete coverage of plants. Nozzles were calibrated to deliver 335 L/ha at 165 kPa and a ground speed of 4.39 km/hr. Fungicide treatments in 1987 were applied as follows: RH-3486 on 15 July, other treatments on 31 July, 28 August, and 25 September. During other years, fungicides were applied on 3 August and 1 September in 1988, 19 July and 16 August in 1989, and 26 July and 22 August in 1990. Sclerotinia blight incidence was monitored monthly and recorded as the number of infection centers in the two center rows of each plot (15). Occurrence of other diseases was also recorded at monthly intervals. Yields were based on weight of harvested peanuts from the two center rows of each plot at a moisture content of 7% (w/w).

RESULTS

Agar-plate tests. In tests using fungicide-amended GYEA, the dose-response lines for a typical field isolate (S-2) of *S. minor* to the dicarboximide fungicides (chlozolinate, iprodione, and vinclozolin) were very steep when compared with the lines obtained for the three experimental fungicides (ASC-66825, MON-13108, and RH-3486) (Fig. 1). Vinclozolin was the most active of the dicarboximides, followed by iprodione and then chlozolinate. Complete inhibition of mycelial growth was maintained for 4 days on plates amended with 1 μg/ml of vinclozolin, 5 μg/ml of iprodione, or 20 μg/ml of chlozolinate. No inhibition of growth was detected at 0.05 μg/ml of chlozolinate, 0.01 μg/ml of iprodione, or 0.002 μg/ml of vinclozolin.

The three experimental compounds were much more fungitoxic to isolate S-2 at low concentrations in GYEA plate tests than the dicarboximides. ASC-66825 and RH-3486 inhibited growth by 33% at 0.002 μg/ml, and MON-13108 inhibited growth by 10% at 0.002 μg/ml. In contrast to the dicarboximides, complete inhibition of growth did not occur even at 100 μg/ml of ASC-66825, RH-3486, or MON-13108. On unamended GYEA, isolate S-2 grew approximately 19 mm/day during the first 4 days, and

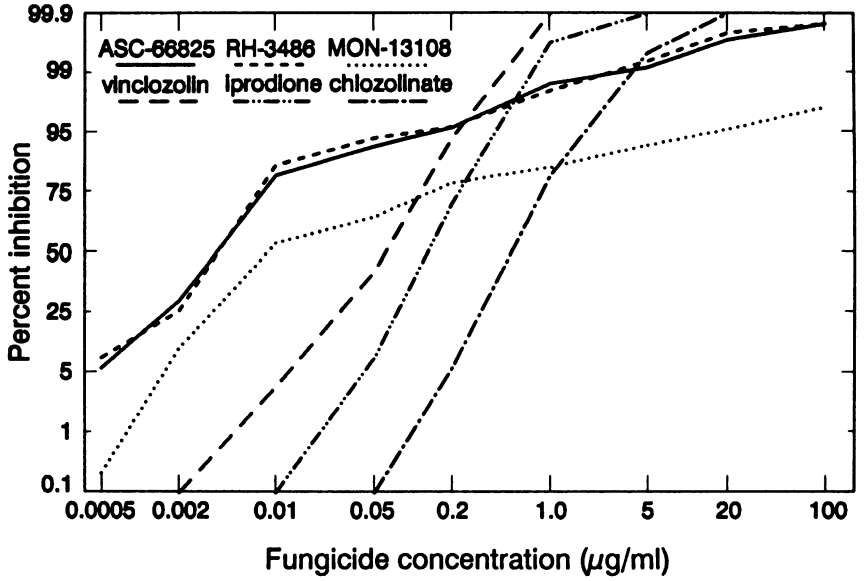

Fig. 1. Dose-response of a typical field isolate (S-2) of *Sclerotinia minor* to various fungicides in glucose-yeast extract agar.

this value was used as the 0% inhibition standard. A comparison of ED_{50} values for the fungicides showed that ASC-66825 and RH-3486 had identically low ED_{50} values of 0.004 μg/ml, followed by MON-13108 at 0.025 μg/ml. The ED_{50} values of the dicarboximide fungicides were 0.08, 0.18, and 0.38 μg/ml for vinclozolin, iprodione, and chlozolinate, respectively.

A comparison of the growth rate (mm/day) of isolate S-2 and B-83-T2 (Fig. 2) indicated that isolate S-2 grew more vigorously on unamended GYEA than B-83-T2. Isolate B-83-T2 had an average growth rate of 11 mm/day, which was 42% less than isolate S-2. When isolate S-2 was exposed to the dicarboximides, a sharp decrease in growth rate occurred between the rates of 0.05 and 1 μg/ml. This contrasted with the growth rate produced by isolate B-83-T2 when exposed to moderate concentrations of the dicarboximides. Growth of isolate B-83-T2 was enhanced in the presence of 1 μg/ml of iprodione or vinclozolin or 5 μg/ml of chlozolinate. Higher concentrations of the dicarboximide fungicides were partially inhibitory to isolate B-83-T2, but this isolate still exhibited some growth at 100 μg/ml of the dicarboximides. No growth of isolate S-2 was detected at 100 μg/ml of any of the dicarboximides.

Because of the growth enhancement triggered by the dicarboximides, only selected data were used in linear regression analyses to determine ED_{50} values for isolate B-83-T2. The ED_{50} values were estimated by using growth values obtained at the highest fungicide concentration that enhanced growth and growth values at higher fungicide concentrations that inhibited growth. ED_{50} values were 4.0, 4.4, and 18.8 μg/ml for vinclozolin, iprodione, and chlozolinate, respectively. These values were between 24 and 50 times larger than ED_{50} values obtained for isolate S-2. No enhancement of growth by either isolate occurred at any concentration of ASC-66825, RH-3486, or MON-13108, and increased concentrations produced greater growth inhibition. However, isolate B-83-T2 was more tolerant to the three experimental fungicides than isolate S-2. ED_{50} values were 0.013, 0.015, and 1.2 μg/ml for RH-3486, ASC-66825, and MON-13108, respectively. These values were 3.3, 3.8, and 48 times larger, respectively, than corresponding ED_{50} values for isolate S-2.

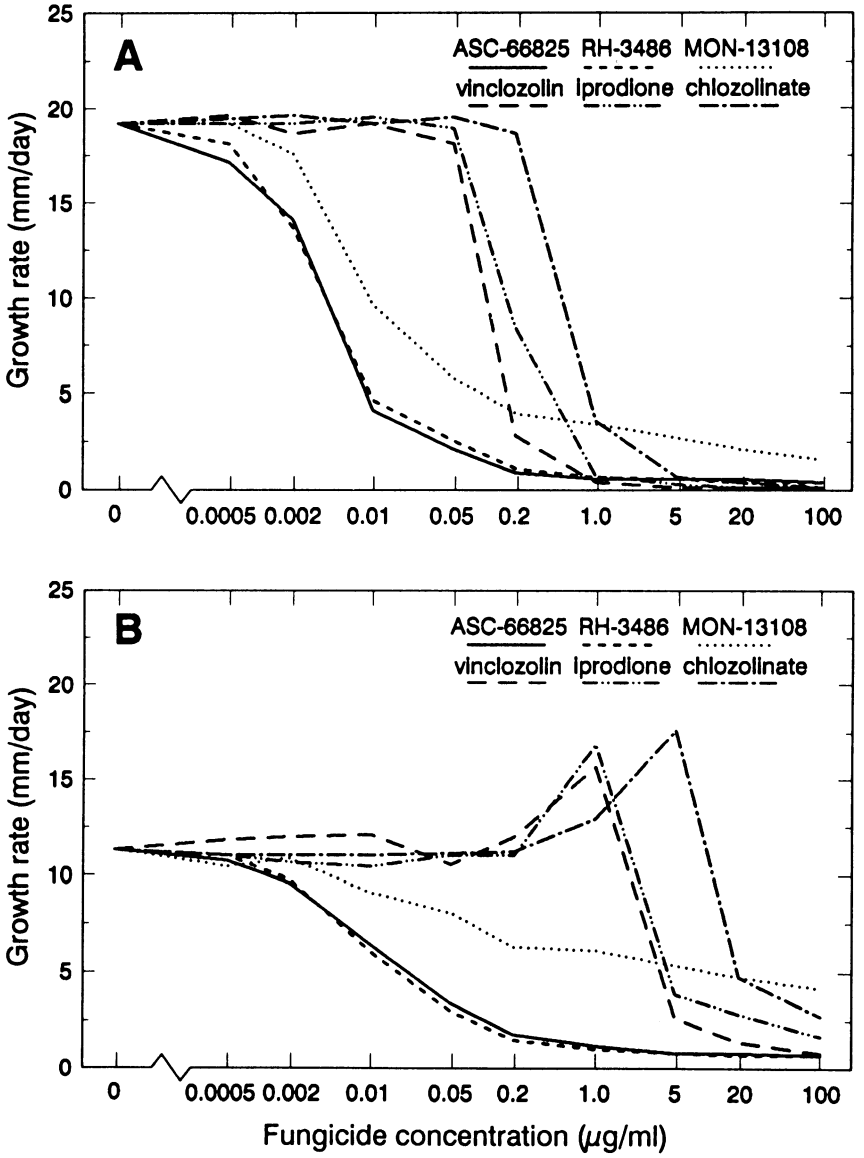

Fig. 2. Growth rate of **(A)** a typical field isolate (S-2) and **(B)** a dicarboximide-resistant isolate (B-83-T2) of *Sclerotinia minor* on glucose-yeast extract agar amended with various fungicides.

Soil-plate tests. The colony diameter of isolate S-2 and B-83-T2 on soil plates without fungicide treatments averaged 77.3 and 78.1 mm after 6 days of incubation, respectively. These values were used as the 0% growth inhibition level for each isolate. Vinclozolin, RH-3486, iprodione, and ASC-66825 significantly inhibited mycelial growth of isolate S-2 by 86, 86, 85, and 80%, respectively (Table 1). Chlozolinate was less fungitoxic to isolate S-2, inhibiting growth by 59%. Exposure to the dicarboximides did not significantly inhibit growth of B-83-T2 on soil plates. ASC-66825 and RH-3486 inhibited mycelial growth of B-83-T2 by 55 and 53%, respectively. MON-13108 was not effective in inhibiting growth of either isolate of *S. minor* in soil-plate tests.

Sclerotial production by *S. minor* in soil plates was not inhibited as much as mycelial growth by the fungicide treatments. Only the dicarboximides were effective in significantly limiting sclerotial production by isolate S-2. Chlozolinate, vinclozolin, and iprodione limited sclerotial production by 64, 51, and 40%, respectively. None of the fungicides significantly influenced sclerotial formation by isolate B-83-T2. Without treatment, an average of 106 and 51.7 sclerotia were obtained from soil-plate samples originally inoculated with isolates S-2 and B-83-T2, respectively.

Field evaluation of fungicides. Weather conditions during the four growing seasons of field evaluation of fungicides varied greatly. Yields in untreated check plots were high in 1987 and 1990, moderate in 1989, and low in 1988. The incidence of Sclerotinia blight was greatest in 1988 because of cool temperatures in September. Warm temperatures and long periods of low rainfall limited disease pressure in 1987 and 1990. Heavy precipitation and above-average temperatures in 1989 were conducive for early leaf spot, caused by *Cercospora arachidicola* S. Hori, and the resulting defoliation suppressed Sclerotinia blight. With the possible exception of 1989, Sclerotinia blight was the only disease thought to significantly suppress yield, because other diseases were not detected or were successfully controlled.

RH-3486 at 2.24 kg/ha was the most effective treatment in 1987; one application at pegging significantly suppressed disease incidence by 74% and increased yield by 922 kg/ha (Table 2). One application of RH-3486 at 1.12 kg/

ha significantly limited disease incidence by 39% but did not significantly enhance yield. Three applications of MON-13108 at 2.24 and 1.12 kg/ha also significantly suppressed disease incidence by 45 and 51% and increased yield by 810 and 648 kg/ha, respectively. Similar applications of MON-13108 at 0.56 kg/ha did not provide significant disease suppression or increased yield. Vinclozolin, applied three times at 0.84 kg/ha, was the only dicarboximide treatment to significantly limit disease incidence in 1987. Plots treated with vinclozolin had 46% less disease than the untreated plots. Iprodione and chlozolinate did not significantly suppress disease. None of the treatments with the dicarboximides resulted in a significant yield increase.

In 1988, chlozolinate and MON-13108 were withdrawn from further field testing by their respective sources. The remaining fungicides were applied twice, and all treatments significantly suppressed the incidence of Sclerotinia blight and increased yields. Two applications of RH-3486 at 1.12 and 0.56 kg/ha, vinclozolin at 0.84 kg/ha, and iprodione at 1.12 kg/ha significantly suppressed disease incidence by 81, 69, 47, and 42% and increased yields by 2,246, 2,193, 1,671, and 1,288 kg/ha, respectively.

ASC-66825 was entered into the Sclerotinia blight control program in 1989. During this year, only ASC-66825 and RH-3486 significantly suppressed disease and increased yield. Two applications of ASC-66825 at 0.28 and 0.56 kg/ha, and RH-3486 at 0.56 kg/ha suppressed disease incidence by 56, 67, and 68% and increased yields by 1,359, 1,080, and 1,206 kg/ha, respectively. Vinclozolin at 0.84 kg/ha suppressed disease incidence by 65% but failed to significantly increase yield. Iprodione did not provide significant disease suppression or increase yield in 1989.

In 1990, RH-3486 was withdrawn from evaluation. All remaining fungicide treatments increased yield, but these increases were not significant because of variation in data. Late-season disease pressure was uneven and resulted in a minimal impact on yield. Only applications of ASC-66825 at the two highest rates resulted in significant disease control. Two applications of ASC-66825 at 0.28, 0.56, and 0.84 kg/ha suppressed disease incidence by 27, 50, and 38%, respectively. Applications of iprodione or vinclozolin provided only marginal suppression of disease incidence.

DISCUSSION

In agar-plate tests, the dicarboximides produced steep dose-response lines that were similar in slope and indicative of a related mode of action against *S. minor*. Similar steep dose-response lines have been reported for the dicarboximide fungicides against *S. sclerotiorum* (10) and *S. minor* (3). ASC-66825, RH-3486, and MON-13108 appeared to have a different mode of action than the dicarboximides, based on their comparatively flat dose-response lines. Growth inhibition of isolate S-2 at fungicide concentrations above 1 μg/ml suggested that the dicarboximides were fungicidal to *S. minor*, whereas ASC-66825, MON-13108, and RH-3486 were strong fungistats. The experimental compounds were active at extremely low concentrations, which was reflected in their low ED_{50} values in agar-plate tests. ASC-66825, RH-3486, and MON-13108 had ED_{50} values that were 45, 45, and 7.2 times lower than iprodione, respectively. The ED_{50} values obtained for iprodione and vinclozolin were similar to data obtained in an earlier study of iprodione and vinclozolin and two aromatic hydrocarbon fungicides (3). The high levels of activity shown by ASC-66825 and RH-3486, compared with iprodione, are important because iprodione has become the com-

Table 1. Mycelial growth (mm) and sclerotial production of a typical field isolate (S-2) and a dicarboximide-resistant isolate (B-83-T2) of *Sclerotinia minor* on fungicide-treated soil plates[v]

Treatment[w]	Mycelial growth[x] (mm)		Sclerotial production[y]	
	S-2	B-83-T2	S-2	B-83-T2
Vinclozolin	10.8 c[z]	67.7 a	51.7 b	51.6 a
RH-3486	11.2 c	34.8 b	97.0 a	55.5 a
Iprodione	11.8 c	75.8 a	63.1 b	62.4 a
ASC-66825	15.5 c	37.0 b	90.3 a	55.6 a
Chlozolinate	31.4 b	68.1 a	38.3 b	55.6 a
MON-13108	67.9 a	64.5 a	114.9 a	57.8 a
Water check	77.3 a	78.1 a	106.0 a	51.7 a

[v] Containing cornmeal at a level of 5% (w/w).
[w] Fungicides were tested at a rate equivalent to 1.12 kg a.i./ha.
[x] Mycelial growth represents colony diameter at 4 days.
[y] Values are number of sclerotia recovered from a 10-cm^3 plug of soil from soil-cornmeal medium at 14 days.
[z] Values are the mean of five replications. Means in a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 2. Disease incidence and yield of peanuts from plots treated with fungicides for control of Sclerotinia blight

Year and treatment	Rate (kg/ha)	Number of applications	Disease incidence[x]	Yield[y] (kg/ha)
1987				
Iprodione	1.12	3	21.0 ab[z]	5,112 b-d
Vinclozolin	0.84	3	16.0 bc	5,254 a-d
RH-3486	1.12	1	18.0 b	5,192 a-d
RH-3486	2.24	1	7.8 c	5,690 a
Chlozolinate	1.12	3	23.8 ab	5,005 cd
MON-13108	0.56	3	22.5 ab	5,042 cd
MON-13108	1.12	3	14.5 bc	5,416 a-c
MON-13108	2.24	3	16.3 bc	5,578 ab
Untreated check	...	...	29.5 a	4,768 d
1988				
Iprodione	1.12	2	27.5 b	3,202 b
Vinclozolin	0.84	2	25.3 b	3,585 b
RH-3486	0.56	2	15.0 c	4,107 a
RH-3486	1.12	2	9.0 c	4,160 a
Untreated check	...	...	47.8 a	1,914 c
1989				
Iprodione	1.12	2	20.3 ab	3,920 cd
Vinclozolin	0.84	2	10.5 b	4,042 b-d
RH-3486	0.56	2	9.5 b	4,658 ab
ASC-66825	0.28	2	13.0 b	4,811 a
ASC-66825	0.56	2	9.8 b	4,532 a-c
Untreated check	...	...	29.8 a	3,452 d
1990				
Iprodione	1.12	2	25.0 ab	5,229 a
Vinclozolin	0.84	2	21.3 ab	5,022 a
ASC-66825	0.28	2	18.8 ab	5,583 a
ASC-66825	0.56	2	12.8 c	5,730 a
ASC-66825	0.84	2	16.0 bc	5,916 a
Untreated check	...	...	25.7 a	4,912 a

[x] Disease incidence represents counts of infection centers in the two center rows of each plot at harvest. An infection center was a point of active growth by *Sclerotinia minor* and included 15.2 cm of row length on either side of that point.
[y] Yields are based on weight of peanuts with moisture content of 7% (w/w).
[z] Means in a column for each year followed by the same letter(s) are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

petitive standard in the industry for fungitoxicity against *S. minor*.

The dicarboximide-resistant isolate (B-83-T2) was cross-resistant to iprodione, chlozolinate, and vinclozolin. This was not unexpected, as previously characterized resistant isolates of *S. minor* were reported to be cross-resistant to other dicarboximide fungicides (3). Future registration of chlozolinate or vinclozolin may contribute to development of dicarboximide-resistance problems without providing substantial improvement in the control of Sclerotinia blight. The mode of action of the dicarboximides affects a wide range of metabolic functions resulting in morphological changes in growth and cell-wall synthesis. No specific metabolic step has yet been linked with the site of action of the dicarboximides (11,19).

Resistance to this class of fungicides may require the alteration of numerous cellular events, which could likely reduce the pathogenicity of the fungus. Even though isolate B-83-T2 was capable of causing disease (22), its growth rate on unamended GYEA was less than that of its parent isolate, which suggests a partial loss of fitness as a saprophyte. ASC-66825 and RH-3486 effectively suppressed growth of isolate B-83-T2 on fungicide-amended GYEA, although the ED_{50} values for these fungicides were slightly higher than values obtained for isolate S-2, a typical field isolate of *S. minor*. The low ED_{50} values for ASC-66825 and RH-3486 indicated that isolate B-83-T2 was not cross-resistant to this type of fungicide chemistry. MON-13108 suppressed growth of isolate B-83-T2 at all fungicide concentrations on GYEA, but the ED_{50} value of the fungicide was 48 times higher against isolate B-83-T2 than S-2, which suggested that B-83-T2 had some cross-tolerance to MON-13108. Dicarboximide resistance appears to convey increased tolerance to more than one class of fungicides.

Growth of the dicarboximide-resistant isolate, B-83-T2, was enhanced in the presence of dicarboximides at concentrations between 1 and 5 μg/ml in GYEA. These concentrations were highly fungitoxic to a sensitive isolate, S-2. Higher concentrations of dicarboximide fungicides inhibited growth of isolate B-83-T2, indicating that resistance may be of a low-level type. Three continuous years of tests with in vitro dicarboximide-resistant isolates in confined field microplots indicated that disease control could be maintained by the application of dicarboximide fungicides (6).

Continued dicarboximide fungicide pressure may result in the selection of resistant isolates with increased virulence, but resistance to the dicarboximides has not been reported or detected in commercial peanut fields treated with iprodione. A 3-yr study of 763 isolates from 19 infested fields failed to detect any iprodione- or vinclozolin-resistant isolates (3). Subsequent screenings of 360 isolates of *S. minor* from plots in fungicide trials and commercial fields treated with iprodione did not show evidence of in vitro resistance in 1986 (21). Another screening of 947 isolates from plots treated with various leaf spot fungicides and plots treated with iprodione also did not detect any evidence of field resistance (24).

All of the dicarboximide fungicides were inhibitory to mycelial growth and sclerotial formation by isolate S-2 in soil-plate tests. An earlier study indicated that procymidone also suppressed mycelial growth and sclerotial formation (12). The entire group of dicarboximides appears to be highly active in a soil medium against sensitive isolates. Based on soil mobility tests (8), the dicarboximides may persist in an active state for periods longer than the recommended spray interval of 4 wk. However, the dicarboximides were not effective in limiting mycelial growth or sclerotial production by isolate B-83-T2. Even though applications of dicarboximide fungicides in microplot studies suppressed the development of aboveground disease by in vitro resistant isolates (6), results from soil-plate tests suggested that the dicarboximides will not be effective in limiting sclerotial production by dicarboximide-resistant isolates. Thus, low-level dicarboximide resistance could lead to high inoculum buildup with continued use of these fungicides for disease control.

MON-13108 was not effective against either isolate of *S. minor* in soil-plate tests. In field tests during 1987, MON-13108 was comparable to the dicarboximide fungicides in performance, which indicated that its fungistatic properties were not dependent on soil activity. No conclusion can be made regarding cross-resistance of isolate B-83-T2 to this fungicide on the basis of soil-plate tests. Unlike MON-13108, both ASC-66825 and RH-3486 were effective in suppressing mycelial growth of isolate S-2 and B-83-T2 in soil-plate tests. Isolate B-83-T2 did not appear to be cross-resistant to ASC-66825 or RH-3486 according to these tests. However, ASC-66825 and RH-3486 did not suppress sclerotial development by either isolate in soil-plate tests. These results suggest that these fungicides may have limited activity in soil and further distinguishes their mode of action from the dicarboximides. The high efficacy of ASC-66825 and RH-3486 for field control of Sclerotinia blight of peanut was apparently not dependent on soil activity.

Iprodione provided significant disease control during only of 4 yr of field testing, whereas ASC-66825 and RH-3486 provided excellent disease control during 4 yr of evaluation under dramatically different weather conditions. One application of RH-3486 (2.24 kg/ha) gave significant control of Sclerotinia blight throughout the 1987 growing season and resulted in significantly higher yields than two applications of iprodione at 1.12 kg/ha. Lower rates of RH-3486 (0.56 and 1.12 kg/ha in 1988) and ASC-66825 (0.28 kg/ha in 1989) applied twice also performed significantly better than similar applications of iprodione at 1.12 kg/ha.

A ranking of the ED_{50} values for the six fungicides according to agar-plate tests was a better predictor of field performance than results from soil-plate tests. However, actual ED_{50} values did not correspond well to field disease incidence ratings. Soil-plate tests did not detect the ability of MON-13108 to control disease in the field. The soil assay also indicated that chlozolinate might be an effective fungicide against Sclerotinia blight. Subsequent field tests showed chlozolinate to be relatively ineffective against the disease. Thus, no single laboratory test could accurately predict the performance of a fungicide in the field. However, compounds possessing a low ED_{50} value in agar-plate tests and activity in soil-plate tests, such as ASC-66825 and RH-3486, can be quickly identified for field research. Few compounds apparently fit the criteria (13), which justifiably limits the number of compounds for labor-intensive field tests on control of Sclerotinia blight.

This study is the first report on the use of fluazinam for control of Sclerotinia blight. In 1991, ISK-Biotech and Rohm and Haas Co. confirmed the chemistry of ASC-66825 and RH-3486, respectively, as fluazinam (26). Currently, ISK-Biotech is pursuing the development of fluazinam as an agricultural fungicide. Because of the high level of activity of fluazinam against Sclerotinia blight in field trials, registration of fluazinam on peanut has the potential to revolutionize strategies for control of Sclerotinia blight of peanut in the Virginia-North Carolina region. In addition, use of fluazinam could reduce the dependence on dicarboximide and aromatic hydrocarbon fungicides for control of Sclerotinia blight. If dicarboximide-resistant isolates of *S. minor* ever become a serious threat to the continued use of iprodione, fluazinam or related fungicides may prove essential for Sclerotinia blight management.

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