Significance of Insensitivity of *Sclerotinia minor* to Iprodione in Control of Sclerotinia Blight of Peanut

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ABSTRACT

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Four hundred isolates of *Sclerotinia minor* were obtained from diseased peanut plants (*Arachis hypogaea*) in field plots untreated or treated three times with iprodione (1.12 kg/ha) in 1987. On glucose-yeast extract agar containing iprodione at 2 μg/ml, only 6% of the isolates grew. These isolates produced more mycelial growth than corresponding isolates not previously exposed to the fungicide in agar, indicating that insensitivity developed in vitro. In field microplots artificially infested with sclerotia of the insensitive isolates and planted to peanut for 7 yr, iprodione provided significant disease suppression even though insensitive isolates could still be recovered. In a separate 3-yr microplot study, the pathogenicity of an isolate (B-83-T2) of *S. minor* suspected of possessing field resistance to dicarboximide fungicides was compared to a sensitive isolate (S-2) after treatment of peanuts with dicarboximide fungicides (iprodione or vinclozolin), aromatic hydrocarbon fungicides (dicloran or pentachloronitrobenzene [PCNB]), and an experimental fungicide (fluazinam). Without fungicide treatment, disease incidence (stem lesions per microplot) at harvest averaged 19.9 in plots infested with isolate S-2 and 18.3 in plots infested with isolate B-83-T2 (no statistical difference), but yields were significantly lower (10%) in microplots infested with isolate S-2. When averaged across isolates, disease incidence was suppressed 96, 63, 42, 41, and 20% by fluazinam, vinclozolin, iprodione, PCNB, and dicloran, respectively. Insensitivity in *S. minor* to the dicarboximide fungicides appears low but persistent and does not pose a threat to the continued use of dicarboximides or related fungicides in peanut production.

Sclerotinia blight, caused by *Sclerotinia minor* Jagger (12,13), is the most destructive disease of peanut (*Arachis hypogaea* L.) in Virginia. Recent annual losses have ranged from 4 to 12% as a result of the limited efficacy of recommended fungicides (P. M. Phipps, *unpublished data*). The fungicidal properties of the dicarboximides were reported in 1971 (9), and soon thereafter procymidone was shown to be effective against *S. minor* on peanut in field trials (20). Registration of dicarboximide fungicides occurred after the release of iprodione in 1974 (14) and vinclozolin in 1975 (19). Iprodione was approved for use on peanut for control of Sclerotinia blight in 1985. The dicarboximides have been used extensively in viticulture in Europe as a replacement for the benzimidazole fungicides where benzimidazole resistance by *Botrytis cinerea* was detected in 1978 (11).

Iprodione and pentachloronitrobenzene (PCNB) are approved for control of Sclerotinia blight of peanut in Virginia (17). Prior to these registrations, dicloran was used for control of the disease pursuant to section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act. Both dicloran and PCNB contain a benzene ring structure similar to the dicarboximides. Isolates of *S. minor* possessing in vitro insensitivity to one dicarboximide were reported to be insensitive to other dicarboximides as well as to certain aromatic hydrocarbon fungicides in laboratory assays, including dicloran and PCNB (1,4,22). Although field isolates of *S. minor* have been screened, no field resistance to the dicarboximides was reported (2,21). However, isolates of *S. minor* developed in vitro insensitivity to the dicarboximide fungicide procymidone (0.5 μg/ml) at a frequency of 2.3% (21). A similar frequency of in vitro insensitivity to iprodione and vinclozolin has been reported (2).

In 1983, a study was initiated to compare the efficacy of iprodione and other fungicides for control of Sclerotinia blight of peanut in field microplots infested with a dicarboximide-sensitive (S-2) isolate and dicarboximide-insensitive isolates (R-2B and R-2C) that developed in vitro. Over a 3-yr period (1983–1985), iprodione was equally effective in suppressing disease caused by all isolates (4). The conclusion was that the insensitivity of *S. minor* to dicarboximides and related fungicides in vitro would not affect disease control. However, the effect of long-term exposure of *S. minor* to dicarboximide fungicides in the field has not been adequately assessed.

One dicarboximide-insensitive isolate (B-83-T2) was isolated from a microplot in 1986 infested with a sensitive isolate (S-2) and subjected to three continuous years of peanut culture and iprodione exposure. This isolate possessed a unique form of resistance to iprodione that differed from that of previous isolates with in vitro insensitivity (3). Using iprodione-treated excised peanut stems (5), isolate B-83-T2 was found to be pathogenic on stems treated at fungicide concentrations that effectively inhibited isolates possessing insensitivity that originated in vitro. The appearance of this isolate with enhanced insensitivity to iprodione on peanut stems renewed concerns about future problems in disease control.

The objectives of this study were to: 1) further assess the occurrence and characteristics of isolates of *S. minor* that are insensitive to iprodione; 2) determine the long-term effect of repeated applications of iprodione on control of Sclerotinia blight caused by dicarboximide-insensitive isolates; and 3) characterize the efficacy of other fungicides in combating disease caused by a dicarboximide-insensitive isolate believed to possess field resistance.

MATERIALS AND METHODS

Isolation of *S. minor* from field-collected sclerotia. To determine the most effective method of surface-disinfesting field sclerotia for optimal isolation of *S. minor*, sclerotia from diseased peanut stems were collected from fields at the Tidewater Agricultural Experiment Station, Suffolk, VA, during October 1987. Three replicate samples of 20 sclerotia were treated for 0, 1, 2, 5, 10, 20, 50, or 100 min in 1% NaOCl. Sclerotia were placed on glucose-yeast extract agar (GYEA) in 9-cm-diameter petri plates (four sclerotia per plate) and

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incubated at 18 C for 6 days. After this time, fungal colonies were identified.

Assays of field sclerotia for response to iprodione. Sclerotia were collected during October 1987 from field plots of peanuts at the Tidewater station, where iprodione had been used in alternating years of a peanut-corn rotation since 1981. In 1987 the plots were untreated or treated three times with iprodione (Rovral 50WP) at 1.12 kg/ha for control of Sclerotinia blight. All fungicide rates were expressed as active ingredient. Chlorothalonil (Bravo 720) at 1.26 kg/ha was applied four times for control of early leaf spot (*Cercospora arachidicola*) according to the Virginia Peanut Leaf Spot Advisory Program (18). Two groups, consisting of 25 sclerotia, were obtained from each of four replications of iprodione-treated and untreated plots in a randomized complete-block design.

All sclerotia were surface-disinfested with 1% NaOCl for 10 min, and one group was placed on GYEA containing chloramphenicol and chlortetracycline at 100 μg/ml (GYEA-CC) amended with iprodione at 2 μg/ml (direct assay), and the second group was placed on GYEA-CC without iprodione (indirect assay). Colonies of *S. minor* obtained by the indirect assay were cultured in slant tubes containing GYEA for 2 wk at 22 C. One sclerotium from each isolate was transferred to petri plates containing GYEA amended with iprodione at 2 μg/ml. If colonies of *S. minor* were larger than 30-mm in diameter and formed sclerotia after 2 wk of incubation, they were classified as insensitive.

Insensitive isolates from the indirect assay were subsequently compared to the parent culture previously stored on slants by transferring both onto unamended GYEA and incubating for 3 days at 22 C. At that time, 5-mm-diameter mycelial plugs were transferred to petri plates containing GYEA amended with iprodione at 0, 1, 10, and 100 μg/ml. After 4 days of incubation, radial growth (in millimeters) was measured.

Long-term effects of iprodione on insensitive isolates. Iprodione-treated and untreated microplots were used to evaluate changes in pathogenicity during a 3-yr period (1987–1989). These microplots were established in 1983 and were infested with sclerotia from one of two insensitive isolates (R-2B and R-2C) of in vitro origin or from a fungicide-sensitive isolate (S-2) of *S. minor* (4). Each microplot consisted of a 76-cm-diameter fiberglass barrier that extended 15 cm below and above the soil surface, and the plots were equally spaced on 1.8-m centers. The soil type was a Nansemond coarse-loamy siliceous thermic Aquic Hapludult that had been fallow in 1981 and 1982 and not previously planted to peanut. The microplots were seeded to Florigiant peanut during May of each year. Seeds were commercially treated with captan plus dicloran (Botec at 2.1 g/kg of seed) and hand-selected to avoid transmission of seedborne fungal pathogens. Only undamaged and unblemished seeds were planted. Seedlings were thinned to three per plot after emergence to allow development of a foliar canopy structure similar to commercial fields. Implements used in the culture and harvest of peanuts were cleaned after working in each microplot. Plant debris was removed from the microplots at the end of the season. These sanitation procedures were followed to reduce the chance of cross-contamination of isolates between microplots.

Treatments applied to individual microplots remained constant throughout the study, except none were applied in 1986. A completely randomized factorial design (two treatments × three isolates) was used with four replications. Disease was prevalent in each microplot, and no additional inoculum was added. The initial spray of iprodione at 1.12 kg/ha was applied during the last week of July in 1987 and 1988 and twice thereafter at 4-wk intervals according to recommendations for control of Sclerotinia blight (17). In 1989, the final application was not made because weather conditions were not conducive for disease development. Sprays were applied using a CO_2-backpack sprayer with a single $D_2$13 nozzle (TeeJet spray nozzles, Spraying Systems Co., Wheaton, IL) that delivered 375 L/ha at 345 kPa. The microplots were managed to simulate standard peanut production practices, which included applications of chlorothalonil for control of early leaf spot. The number of stems with symptoms and signs of Sclerotinia blight in each microplot was recorded during the first week of October each year.

Prior to harvest each year, sclerotia were collected from diseased stems in all microplots. An attempt was made to obtain 12 isolates of *S. minor* from each microplot. The sclerotia were stored in capped plastic tubes at 5 C for approximately 1 mo until they were assayed for fungicide response. Sclerotia were surface-disinfested in a 1% NaOCl solution for 10 min and placed on GYEA-CC. Isolates identified as *S. minor* were transferred to tube slants of GYEA in preparation for subsequent testing. For fungicide response evaluation, all isolates were transferred to GYEA in petri plates. After 3 days at 22 C, a 5-mm mycelial plug from the colony margin was transferred to GYEA with and without iprodione (2 μg/ml). Colony growth was recorded daily for up to 4 days on unamended GYEA and 14 days on GYEA containing iprodione. Isolates were classified as insensitive if growth on GYEA containing iprodione at 2 μg/ml exceeded 30 mm in diameter and sclerotia were formed after 14 days.

Peanut plants were dug and inverted during mid-October each year. Pods were removed by hand after 1 wk and air-dried in a greenhouse for 2 wk to achieve 7% moisture (w/w). An analysis of variance (ANOVA; SAS Institute Inc., Cary, NC) was performed on disease incidence and yield data to determine the significance of main effects and interactions of fungicide treatment, isolate, and year. Because of wide variation in disease incidence and yield between years, the data analysis treated the year effect as a component of a split-plot design in time. Mean separation, where appropriate, was determined by the LSD test at $P = 0.05$. To correct for imbalances in data caused by missing values, all disease incidence and yield averages were reported as least square means.

Effects of soil fungicides on isolate B-83-T2. Field microplot studies to evaluate the pathogenicity of a dicarboximide-insensitive isolate (B-83-T2) suspected of possessing field resistance to iprodione and its sensitive parent isolate (S-2) of *S. minor* were begun in 1987 and continued for 3 yr. Sanitation and planting procedures were the same as previously described. A completely randomized factorial design (two isolates × six treatments) was used with four replications. Sclerotia of both isolates were produced in glass petri plates containing sterile soil amended with corn meal at 5% (w/w). After 6 wk of incubation at 22 C, the average number of fully developed sclerotia per plate was determined by washing the culture on a 40-mesh sieve (425-μm pore size) and counting sclerotia. During the first week of June each year, sclerotia from an equal number of soil plates per isolate were distributed over the surface of each microplot. The sclerotia were incorporated into the upper 8 cm of soil without disturbing the peanut plants. Inoculum densities were four sclerotia per 100 g of soil for isolate S-2 or two sclerotia per 100 g for isolate B-83-T2. The mass of inoculum was similar because sclerotia of isolate B-83-T2 were approximately twice as large as those of isolate S-2.

In addition to iprodione the following fungicides were evaluated: dicloran (Botran 75WP), fluazinam (RH-3486 50WP and, subsequently, ASC-66825 50 WP), PCNB (Terraclor 10G), and vinclozolin (Ronilan 50WP). The initial treatment each year was applied during the last week of July. Using a CO_2-backpack sprayer, fluazinam (0.84 kg/ha), iprodione (1.12 kg/ha), and vinclozolin (0.84 kg/ha) were applied three times at 4-wk intervals in 1987 and 1988 and twice in 1989. The initial application of dicloran was 3.36 kg/ha, and subsequent applications consisted of 2.52 kg/ha on the same schedule as the other fungicides. The granular formulation of PCNB (5.6 kg/ha) was applied by hand at pegging and again 6-wk later each year. Un-

treated microplots served as checks for each isolate. Other management practices were identical to those previously described.

Prior to harvest during October of each year, sclerotia were removed from diseased peanut stems in untreated plots and plots treated with iprodione. Isolates of *S. minor* derived from sclerotia were assayed for response to iprodione. Disease incidence and yield data were analyzed by ANOVA as previously described.

RESULTS

Isolation of *S. minor* from field-collected sclerotia. Assays of 60 sclerotia not surface-disinfested yielded 37 colonies of *S. minor* and 29 colonies of other fungi (Fig. 1). As exposure time to 1% NaOCl reached 2–20 min, the recovery of *S. minor* was maximal and the occurrence of other fungal colonies was minimal. Exposure to NaOCl for 10 min provided the highest recovery with the least contamination. Exposures of 50 and 100 min were detrimental to the survival of *S. minor*. Approximately 15% of sclerotia treated with NaOCl for 20 min or less yielded colonies of bacteria, but they did not affect recovery of *S. minor*. Based on these results, a 10-min surface-disinfesting time in 1% NaOCl was used in all subsequent studies of field-collected sclerotia.

Assays of field sclerotia for response to iprodione. The number of insensitive isolates of *S. minor* derived from sclerotia collected in fields with or without iprodione ranged from 4 to 8% and did not differ significantly ($P = 0.05$) in comparisons of the direct and indirect assay procedures (Table 1). Overall, 18 isolates of 295 (6%) showed insensitivity to iprodione at 2 μg/ml: 13 isolates (8%) were from fields treated with iprodione, and five isolates (4%) were from fields without treatment.

The parent isolates from the indirect assay were always more sensitive to iprodione in vitro than the derived insensitive isolates (Table 2). The parent isolates were inhibited an average of 95% by iprodione at 1 μg/ml and 100% by iprodione at 10 and 100 μg/ml after 4 days of growth. The insensitive isolates were inhibited an average of 43, 54, and 74% by iprodione at 1, 10, and 100 μg/ml, respectively. Insensitive isolates that had vigorous growth after 4 days on GYEA containing iprodione at 100 μg/ml showed reduced mycelial growth on unamended GYEA compared to their parent isolates. Some insensitive isolates grew well on GYEA containing iprodione at 100 μg/ml, whereas others were inhibited. The level of insensitivity to iprodione varied greatly, but no trend was observed between field exposure of the parent isolate to iprodione and subsequent sensitivity.

Long-term effects of iprodione on insensitive isolates. Disease incidence varied significantly between years and was high in 1988, moderate in 1989, and low in 1987. There was no significant interaction between years and other variables subjected to ANOVA in the experiment. Contrasts between the iprodione-sensitive isolate (S-2) and the insensitive isolates (R-2B and R-2C) and between R-2B and R-2C did not show a significant statistical effect of isolates on disease incidence (Table 3). The application of iprodione significantly limited disease incidence by 31, 63, and 47% in microplots infested with isolate S-2, R-2B, and R-2C, respectively. Overall, peanuts in microplots treated with iprodione had 48% less disease than those in untreated microplots.

Yields of peanuts ranged from 284 to 298 g per microplot and were not different in microplots infested with various isolates of *S. minor* (Table 4). Application of iprodione increased the average yield by 29 g per microplot, but this increase was not statistically significant. Yields varied significantly from year to year, but there were no interactions with year. Severe early leaf spot in 1989, associated with continuous peanut cropping, may have limited yield and lowered incidence of diameter by triggering defoliation and opening of the canopy.

Applications of iprodione to peanuts in microplots infested with isolate S-2 did not increase the recovery of insensitive isolates (Table 5). Sclerotia from untreated and iprodione-treated peanut plants exhibited an average of 8 and 6% insensitivity to iprodione, respectively, during the 3-yr period. Applications of iprodione resulted in greater recovery of insensitive isolates from microplots originally infested with either isolate R-2B or R-2C during all 3 yr. In the absence of iprodione, an average of 39 and 58% insensitive isolates was obtained from

Table 1. Effect of field applications of iprodione on incidence of in vitro insensitivity in *Sclerotinia minor*[w]

Fungicide treatment[x]	Direct assay[y]			Indirect assay[z]		
	No. of isolates recovered	No. of insensitive isolates	Percent insensitive	No. of isolates recovered	No. of insensitive isolates	Percent insensitive
Iprodione	93	6	7	89	7	8
Untreated	43	2	5	70	3	4
Total	136	8	6	159	10	6

[w] Colonies with a diameter exceeding 30 mm and forming sclerotia after 14-days growth on glucose-yeast extract agar (GYEA) containing iprodione at 2 μg/ml were classified as insensitive. Differences between numbers of insensitive isolates obtained from iprodione-treated or untreated fields were not significant at $P = 0.05$.

[x] Iprodione at 1.12 kg/ha was applied three times to peanuts on a 4-wk schedule beginning the last week of July. Four applications of chlorothalonil at 1.26 kg/ha were made for control of early leaf spot.

[y] Direct assay consisted of placing a sclerotium on GYEA containing iprodione at 2 μg/ml.

[z] Indirect assay consisted of placing a sclerotium on GYEA and subsequently transferring a sclerotium from the resulting culture to GYEA containing iprodione at 2 μg/ml for evaluation.

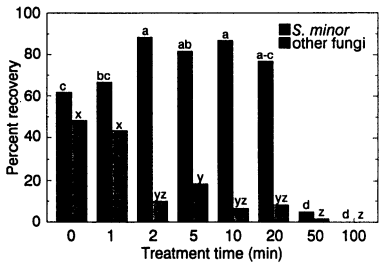

Fig. 1. Recovery of *Sclerotinia minor* and other fungi from field sclerotia after treatment with 1% NaOCl. Data are the mean of three replications, each containing 20 sclerotia. The same letter(s) above bars of a given type denote the absence of significant differences at $P = 0.05$.

Table 2. Sensitivity to iprodione of field isolates of *Sclerotinia minor* and their subsequent iprodione-insensitive isolates

Fungicide treatment[y]	No. of paired isolates	Isolate type[z]	Avg. growth (mm) on GYEA amended with iprodione after 4 days			
			0 μg/ml	1 μg/ml	10 μg/ml	100 μg/ml
Iprodione	7	Parent	75	5	0	0
		Insensitive	68	44	36	20
Untreated	3	Parent	75	2	0	0
		Insensitive	42	19	15	9

[y] Iprodione at 1.12 kg/ha was applied three times to peanut on a 4-wk schedule beginning the last week of July. Four applications of chlorothalonil at 1.26 kg/ha were made for control of early leaf spot.

[z] The parent isolate of *S. minor* originated from field-collected sclerotia. Isolates with insensitivity were identified on glucose-yeast extract agar (GYEA) containing iprodione at 2 μg/ml and were derived from the parent isolate.

microplots infested with isolates R-2B and R-2C, respectively. When treated with iprodione, recovery of insensitive isolates increased to an average of 75 and 74% from microplots infested with isolates R-2B and R-2C, respectively.

Effects of soil fungicides on isolate B-83-T2. Over a 3-yr period, disease incidence at harvest averaged 10.0 for isolate S-2 and 11.5 for isolate B-83-T2, but these differences were not significant (Table 6). Disease incidence was suppressed an average of 96, 63, 42, 41, and 20% in plots treated with fluazinam, vinclozolin, iprodione, PCNB, and dicloran, respectively. Fluazinam and vinclozolin were the only two fungicides that provided significant disease control. There was a significant year effect because disease pressure was light in 1987 and heavy in 1988. No interactions of year with other variables were detected.

The average yield of peanut from microplots infested with isolate B-83-T2 across all fungicide treatments was significantly greater than that from microplots infested with isolate S-2 (Table 7). All fungicide treatments significantly improved yields, regardless of the sensitivity of the isolate. Overall, yields were increased by 78, 39, 38, 38, and 31% with fluazinam, vinclozolin, PCNB, iprodione, and dicloran, respectively. Again, there was a significant year effect as yields were low in 1988 due to favorable conditions for Sclerotinia blight and in 1989 due to heavy leaf spot pressure compared to 1987. There was a significant interaction of isolates and fungicide treatments, probably due to the different performance properties of the fungicides used in this trial.

Sclerotial assays in 1987 from microplots infested with isolate B-83-T2 showed a low percentage of isolates with detectable insensitivity to iprodione (Table 8). Recovery of insensitive isolates was higher each year from microplots treated with iprodione than from untreated microplots. The frequency of recovery of insensitive isolates was lowest in 1987 and highest in 1988. During the 3-yr period, iprodione-treated microplots yielded 58% insensitive isolates, and untreated microplots yielded 37% insensitive isolates. The percentage of isolates with detectable insensitivity did not approach 100% in any microplot. A low percentage of insensitive isolates was obtained from microplots infested with isolate S-2, regardless of iprodione applications. The overall frequency of insensitivity for isolate S-2 was approximately 10%.

Table 3. Disease incidence and summary of analysis of variance (ANOVA) for peanut microplots infested with sensitive (S-2) or in vitro iprodione-insensitive (R-2B or R-2C) isolates of *Sclerotinia minor* during a 3-yr period[x]

	Fungicide treatment[y]		
Isolate	**Iprodione**	**Untreated**	**Mean**
R-2B	9.9	26.9	18.4
R-2C	8.7	16.5	12.6
S-2	14.9	21.6	18.3
Mean	11.2 b	21.7 a	16.4

Source of variation	**df**	**Mean square[z]**	***F* value**	***P* > *F***
Whole plot				
Replication	3	256.5	1.41	0.2801
Fungicide (F)	1	1,878.4	10.29	0.0059
Isolate (I)	2	259.7	1.37	0.2847
F × I	2	182.3	1.00	0.3916
Error	15	182.5		
Split plot				
Year (Y)	2	1,960.2	20.52	0.0001
F × Y	2	134.3	1.41	0.2591
I × Y	4	101.2	1.06	0.3913
F × I × Y	4	83.4	0.87	0.4902
Error	34	95.5		

[x]Disease incidence was least square means of diseased stems in microplots prior to harvest during October. ANOVA used 70 of 72 possible observations. Fungicide means followed by a common letter are not significantly different (LSD = 7.0 at $P = 0.05$). Isolate means were not significantly different (LSD = 8.4 at $P = 0.05$).
[y]Iprodione (1.12 kg/ha) was applied to peanut using $D_2$13 nozzles at 140 L/ha three times during 1987 and 1988 and twice during 1989.
[z]Mean squares were derived from type III sums of squares for the general linear model.

Table 4. Yield and summary of analysis of variance (ANOVA) for peanut microplots infested with sensitive (S-2) or in vitro iprodione-insensitive (R-2B or R-2C) isolates of *Sclerotinia minor* during a 3-yr period[x]

	Fungicide treatment[y]		
Isolate	**Iprodione**	**Untreated**	**Mean**
R-2B	298	270	284
R-2C	322	274	298
S-2	292	279	286
Mean	304	275	289

Source of variation	**df**	**Mean square[z]**	***F* value**	***P* > *F***
Whole plot				
Replication	3	13,652.3	1.53	0.2468
Fungicide (F)	1	14,699.2	1.65	0.2183
Isolate (I)	2	1,276.8	0.14	0.8676
F × I	2	1,786.3	0.20	0.8204
Error	15	8,903.8		
Split plot				
Year (Y)	2	395,271.5	55.30	0.0001
F × Y	2	3,011.1	0.42	0.6596
I × Y	4	7,632.9	1.07	0.3875
F × I × Y	4	1,407.5	0.20	0.9383
Error	34	7,148.2		

[x]Yield was least square means (grams) of peanut from microplots harvested by hand during October and adjusted to 7% moisture (w/w). ANOVA used 70 of 72 possible observations. Fungicide means (LSD = 49 at $P = 0.05$) and isolate means (LSD = 58 at $P = 0.05$) were not significantly different.
[y]Iprodione (1.12 kg/ha) was applied to peanuts using $D_2$13 nozzles at 140 L/ha three times during 1987 and 1988 and twice during 1989.
[z]Mean squares were derived from type III sums of squares for the general linear model.

DISCUSSION

Surface-disinfesting sclerotia with a 1% NaOCl solution for 10 min provided the highest recovery of *S. minor* from field populations, ensuring isolation of a representative sample of *S. minor*. Although this treatment resulted in a bleached appearance of some sclerotia, they were usually viable. Standard procedures for surface-disinfesting sclerotia for 1 (2) to 3 min (20) in 0.5% NaOCl may be too mild for optimum recovery of *S. minor* from field-collected sclerotia.

The detection of insensitivity to iprodione in isolates of *S. minor* originating from field-collected sclerotia was greater than previously reported for in vitro assays of dicarboximide fungicides (2,21). Different growth conditions, surface-

disinfesting techniques, and definitions of insensitivity or resistance may account for these reported differences. Evaluation of insensitivity was subjective because colonies displayed a wide range of growth patterns on iprodione-amended GYEA.

Parent isolates were always more sensitive to iprodione than were the subsequent insensitive isolates that developed on iprodione-amended medium, and the frequency of insensitivity did not appear related to use of iprodione in peanut fields. Much, if not all, of the insensitivity detected in laboratory assays appeared to have originated in vitro. When assaying pathogens for fungicide sensitivity, it is important to determine the frequency of in vitro insensitivity for a given fungicide. If the sample size is large, the expected rate of in vitro resistance to iprodione by *S. minor* using GYEA containing the fungicide at 2 μg/ml can vary between 4 and 8%.

In microplots infested with in vitro insensitive isolates of *S. minor* in 1983, fungicide performance from 1983 to 1985 showed that iprodione provided partial control of Sclerotinia blight regardless of in vitro insensitivity (4). Similar levels of disease control were observed between 1987 and 1989, in spite of continuous peanut cropping and an additional 3 yr of iprodione use. Long-term exposure to iprodione did not result in a significant loss of disease control. Although iprodione treatments produced a twofold decrease in disease incidence, a significant yield response was not observed. These results are similar to that reported in numerous field trials wherein iprodione provided only partial control of Sclerotinia blight. In addition, the lack of a significant yield response with iprodione also may be attributed to problems affiliated with peanut monoculture.

Isolate B-83-T2 was believed to possess greater fitness than previously tested insensitive isolates (3). In spite of the expected greater insensitivity of isolate B-83-T2, there was no evidence of a loss of disease control by any of the tested fungicides. A mix of iprodione-insensitive and -sensitive isolates of *S. minor* were recovered from microplots infested with isolate B-83-T2, indicating that some of the disease pressure was caused by isolates that had reverted to a sensitive condition. Even though isolate B-83-T2 exhibited insensitivity to the dicarboximide fungicides in agar-based (25) and excised peanut-stem assays (3), field applications of iprodione significantly increased yield in microplots infested with this isolate. Isolate B-83-T2 did not appear to possess a level of resistance or fitness to overcome the inhibitory effects of recommended application rate of iprodione. In vitro assays of isolate B-83-T2 showed that the calculated ED_{50} value of iprodione was 50 times greater than a typical sensitive isolate (25), suggesting that the insensitivity level was moderate. A detectable loss of disease control was not seen in this study, in spite of maximizing the potential for resistance problems by infesting microplots each year with sclerotia of the dicarboximide-insensitive isolate B-83-T2, planting continuous peanut, and applying the same fungicide to specific microplots.

The aromatic hydrocarbon fungicides were least effective in controlling disease caused by either isolate of *S. minor*, whereas the dicarboximides were only moderately effective. Applications of fluazinam almost completely prevented disease development. Performance of fungicides was similar to results from field trials (16,26). If registration is obtained for fluazinam (27) in the United States, it may reduce the dependency on the dicarboximides and related fungicides for control of diseases caused by

Table 5. Incidence of insensitivity to iprodione among isolates of *Sclerotinia minor* from peanut microplots infested with sensitive (S-2) or in vitro iprodione-insensitive (R-2B or R-2C) isolates during a 3-yr period[y]

	Isolate introduced into microplots					
	S-2		R-2B		R-2C	
Year Treatment[z]	No. of isolates recovered	Percent insensitive	No. of isolates recovered	Percent insensitive	No. of isolates recovered	Percent insensitive
1987						
Iprodione	34	9	27	63	36	86
Untreated	36	8	45	24	44	75
1988						
Iprodione	42	5	48	75	32	81
Untreated	40	10	45	51	46	61
1989						
Iprodione	48	6	16	94	18	39
Untreated	48	6	32	44	19	11
3-yr summary						
Iprodione	124	6	91	75	86	74
Untreated	124	8	122	39	109	58

[y] Colonies with a diameter exceeding 30 mm and forming sclerotia after 14-days growth on glucose-yeast extract agar containing iprodione at 2 μg/ml were classified as insensitive.
[z] Iprodione at 1.12 kg/ha was applied three times to peanuts at 4-wk intervals during 1987 and 1988 and twice during 1989. Chlorothalonil at 1.26 kg/ha was applied routinely for control of early leaf spot.

Table 6. Disease incidence and summary of analysis of variance (ANOVA) for peanut microplots infested with sclerotia of a sensitive (S-2) or a dicarboximide-insensitive (B-83-T2) isolate of *Sclerotinia minor* and treated with or without fungicides during a 3-yr period[x]

	Fungicide treatment[y]						
Isolate	Iprodione	Vinclozolin	Dicloran	PCNB	Fluazinam	Untreated	Mean
B-83-T2	7.7	10.4	16.3	15.3	0.9	18.3	11.5
S-2	14.6	3.7	14.1	7.3	0.6	19.9	10.0
Mean	11.1 ab	7.0 bc	15.2 ab	11.3 ab	0.8 c	19.1 a	10.7

Source of variation	df	Mean square[z]	*F* value	*P* > *F*
Whole plot				
Replication	3	138.1	0.62	0.6057
Fungicide (F)	5	871.2	3.92	0.0067
Isolate (I)	1	66.3	0.30	0.5884
F × I	5	171.1	0.77	0.5777
Error	33	222.0		
Split plot				
Year (Y)	2	1,992.7	17.17	0.0001
F × Y	10	174.6	1.50	0.1584
I × Y	2	35.3	0.30	0.7388
F × I × Y	10	47.9	0.41	0.9358
Error	65	116.1		

[x] Disease incidence was least square means of diseased stems in microplots prior to harvest during October. ANOVA used 137 of 144 possible observations. Fungicide means followed by a common letter were not significantly different (LSD = 8.8 at $P = 0.05$). Isolate means were not significantly different (LSD = 5.5 at $P = 0.05$).
[y] Iprodione (1.12 kg/ha), vinclozolin (0.84 kg/ha), dicloran (3.36 kg/ha, initial, and 2.52 kg/ha, additional applications), and fluazinam (0.84 kg/ha) were applied to peanuts using $D_2$13 nozzles at 140 L/ha. Three applications at 4-wk intervals were made during 1987 and 1988 and two during 1989. Pentachloronitrobenzene (PCNB) (5.60 kg/ha) was applied twice each year by hand at 6-wk intervals.
[z] Mean squares were derived from type III sums of squares for the general linear model.

Sclerotinia spp. Significant control of isolate B-83-T2 by fluazinam indicated there was no cross-resistance between fluazinam and the dicarboximide fungicides (25). Fungicides with a mode of action similar to fluazinam may prove to be valuable in controlling Sclerotinia blight of peanut and limiting the potential for development of insensitivity to dicarboximides.

Assays of sclerotia from studies of long-term microplots suggested that iprodione insensitivity by *S. minor* was stable over a long period of time in the absence of continued selection pressure by fungicide applications. This situation may be similar to *B. cinerea* as insensitive strains did not disappear from the grape-growing area of Germany after the termination of dicarboximide use (15).

Table 7. Yield summary of analysis of variance (ANOVA) from peanut microplots infested with sclerotia of a sensitve (S-2) or a dicarboximide-insensitive (B-83-T2) isolate of *Sclerotinia minor* during a 3-yr period[x]

	Fungicide treatment[y]						
Isolate	**Iprodione**	**Vinclozolin**	**Dicloran**	**PCNB**	**Fluazinam**	**Untreated**	**Mean**
B-83-T2	509	439	433	433	569	391	462 a
S-2	376	457	407	454	570	250	419 b
Mean	442 b	447 b	420 b	443 b	570 a	321 c	441

Source of variation	**df**	**Mean square[z]**	***F* value**	***P* > *F***
Whole plot				
Replication	3	15,307.8	1.15	0.3448
Fungicide (F)	5	137,091.8	10.27	0.0001
Isolate (I)	1	58,300.5	4.37	0.0444
F × I	5	30,250.9	2.27	0.0708
Error	47	13,352.7		
Split plot				
Year (Y)	2	1,284,144.3	80.16	0.0001
F × Y	10	26,307.5	1.64	0.1144
I × Y	2	19,947.8	1.25	0.2946
F × I × Y	10	9,828.5	0.61	0.7968
Error	65	16,019.3		

[x]Yield (grams) was least square means of peanut harvested from microplots by hand during October and adjusted to 7% moisture (w/w). ANOVA used 137 of 144 possible observations. Fungicide means (LSD = 68 at $P = 0.05$) and isolate means (LSD = 42 at $P = 0.05$) followed by a common letter were not significantly different.

[y]Iprodione (1.12 kg/ha), vinclozolin (0.84 kg/ha), dicloran (3.36 kg/ha, initial, and 2.52 kg/ha, additional applications), and fluazinam (0.84 kg/ha) were applied to peanuts using $D_2$13 nozzles at 140 L/ha. Three applications at 4-wk intervals were made during 1987 and 1988 and two during 1989. Pentachloronitrobenzene (PCNB) (5.60 kg/ha) was applied twice each year by hand at 6-wk intervals.

[z]Mean squares were derived from type III sums of squares for the general linear model.

Table 8. Incidence of insensitivity to iprodione among isolates of *Sclerotinia minor* from peanut microplots infested with sclerotia of a sensitive (S-2) isolate or a dicarboximide-insensitive (B-83-T2) isolate during a 3-yr period[y]

	Isolate introduced into microplots			
	S-2		B-83-T2	
Year Treatment[z]	**No of isolates recovered**	**Percent insensitive**	**No. of isolates recovered**	**Percent insensitive**
1987				
Iprodione	7	14	16	19
Untreated	6	0	12	17
1988				
Iprodione	33	9	33	70
Untreated	27	11	36	50
1989				
Iprodione	33	9	34	53
Untreated	13	8	34	29
3-yr summary				
Iprodione	73	10	83	58
Untreated	46	9	82	37

[y]Colonies with a diameter exceeding 30 mm and forming sclerotia after 14-days growth on glucose-yeast extract agar containing iprodione at 2 μg/ml were classified as insensitive.

[z]Microplots were infested with sclerotia of isolate B-83-T2 or S-2 each June. Iprodione at 1.12 kg/ha was applied to peanuts three times at 4-wk intervals beginning the last week of July. Three applications were made during 1987 and 1988 and two during 1989. Chlorothalonil at 1.26 kg/ha was applied routinely for control of early leaf spot.

Insensitive isolates of *S. minor* persisted in untreated microplots infested with isolates R-2B and R-2C at levels above the expected background rates, even after 7 yr without exposure to iprodione. In contrast, some factor, perhaps a higher level of virulence or greater fitness to edaphic conditions, also favored the maintenance of dicarboximide-sensitive isolates of *S. minor* even with continued exposure to dicarboximide fungicides. However, as observed in 1985 (4), the presence of dicarboximide-insensitive isolates did not result in a loss of efficacy of iprodione.

The reduced frequency of insensitive isolates in 1989 from microplots infested with isolates B-83-T2 may have been due to unusually severe early leaf spot pressure that partially defoliated peanut plants and provided conditions unfavorable for Sclerotinia blight. It also is possible that these isolates may have possessed greater fitness and greater fungicide sensitivity than isolates obtained in previous years that enabled them to cause disease under conditions that were only marginal for disease development.

Several factors may play a role in limiting the occurrence of field resistance to dicarboximide fungicides. In a cropping situation, numerous sclerotia remain buried in soil and are not exposed to fungicide treatments. Fungicide applications are unlikely to provide complete coverage of disease sites, so selection pressure is not as great against soilborne pathogens as against foliar pathogens. Iprodione persists for less than 4 wk when applications are made at the labeled rate (8). The dormant nature of sclerotia in soil also would minimize selection pressure by any fungicide residues. In addition, crop rotation with nonhosts, such as corn, reduces the number of viable sclerotia in soil between peanut crops (23).

The dicarboximides provide less opportunity for resistance in fungi compared to single-site fungicides. Dicarboximide-resistant isolates of fungi are not known to possess a specific change at a site of fungicide action, as occurred with benzimidazole-resistant isolates, and iprodione does not directly affect energy production or act directly on biosynthetic processes related to DNA synthesis (24). Instead, the dicarboximides appear to interact with the flavin enzyme cytochrome-c reductase to block normal electron flow (7). This blockage results in the generation of free radicals and the peroxidation of cellular membranes. The inner membranes of mitochondria are especially sensitive to damage. A high level of resistance to the dicarboximides probably would require more than one genetic change, perhaps to produce membranes less susceptible to membrane peroxidation, to increase antioxidation protective mechanisms, or to exclude the fungicide from the fungus.

Adequate reduction of dollar spot of turf, caused by an iprodione-benomyl–resistant isolate of *S. homoeocarpa*, was not seen after field applications of iprodione (6), although disease incidence was reduced by 57%. With applications of benomyl, no disease control was obtained. Resistance to benomyl is typical of a qualitative response to fungicides for which concentrations that are effective on sensitive isolates have no effect on resistant isolates. With iprodione, insensitivity appears to be a quantitative response, and complete loss of fungicide efficacy is infrequent (10). For control of Sclerotinia blight of peanut, 45–55% disease suppression by iprodione applications is normal. The marginal efficacy of iprodione under field conditions and the easy occurrence of induced in vitro insensitivity makes fungicide insensitivity very difficult to detect.

Applications of iprodione provided control of disease caused by an isolate of *S. minor* possessing the highest level of resistance and fitness known to date. In addition, even after 6 yr of iprodione use on peanut in microplots infested with insensitive isolates of *S. minor*, disease incidence of Sclerotinia blight was suppressed. The development of insensitivity of *S. minor* to iprodione appears to be low and quantitative. Should iprodione-insensitive isolates of *S. minor* become established in a peanut field, our results suggest that no detectable loss in disease control would occur, even over a long period of time.

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