Control of Sclerotinia Blight of Peanut: Sensitivity and Resistance of *Sclerotinia minor* to Vinclozolin, Iprodione, Dicloran, and PCNB

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ABSTRACT

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Five isolates of *Sclerotinia minor* from different peanut fields were evaluated for sensitivity to dicloran, iprodione, PCNB, and vinclozolin. Tests were conducted with fungicide-amended glucose yeast-extract agar (GYEA). ED_{50} values showed vinclozolin (0.07 μg/ml) to be the most inhibitory, followed by iprodione (0.11 μg/ml), dicloran (0.91 μg/ml), and PCNB (1.27 μg/ml). ED_{50} concentrations of vinclozolin suppressed sclerotium production, whereas iprodione and dicloran increased it compared with GYEA alone. Sclerotial size increased significantly in the presence of ED_{50} concentrations of dicloran and vinclozolin. Nine strains of *S. minor* were isolated from fungicide-resistant growth sectors on GYEA amended with either iprodione or vinclozolin but not dicloran or PCNB. All resistant strains were capable of growth at up to 1,000 μg/ml of iprodione or vinclozolin. Eight of these retained resistance for 23 mo when repeatedly cultured on unamended GYEA. After 36 mo, only seven strains were resistant to fungicides. A total of 763 isolates from plants in fields treated with these fungicides were tested over 3 yr, and no resistance was found. All four products significantly increased peanut yield and value in replicated field trials. Disease incidence at harvest was reduced by an average of 37.1, 41.6, 49.0, and 72.0% by PCNB, dicloran, iprodione, and vinclozolin, respectively, over a 3-yr period.

Sclerotinia blight of peanut (*Arachis hypogaea* L.) caused by *Sclerotinia minor* (Jagger) Kohn (8) was first reported in Virginia in 1971 (12). It has since become an important peanut disease in Virginia, North Carolina, and Oklahoma. In 1982, farm income losses in Virginia alone were estimated to be $8.6 million. Various cultural practices, which include planting the partially resistant cultivar Virginia 81 Bunch, have reduced the severity of Sclerotinia blight, but fungicides are necessary to control the disease in problem fields.

Until more effective measures are found, fungicides will continue to play a key role in the management of Sclerotinia blight. Early screenings indicated that procymidone (DPX-4424) had excellent activity against the fungus and gave excellent disease control (11). Unfortunately, development of this fungicide was terminated before registration. Dicloran was used to control Sclerotinia blight (3) in Virginia from 1978 to 1984 (pursuant to section 18 approval by the EPA). PCNB, primarily used against *Sclerotium rolfsii* on peanut, has also been used for suppression of Sclerotinia blight in Virginia and North Carolina. The dicarboximide fungicides, iprodione and vinclozolin, have been effective in preliminary field studies (6,9), and vinclozolin was used by growers in Virginia during the 1984 season by section 18 approval. Iprodione gained full registration for use on peanut in 1985, whereas registration for vinclozolin continues to be sought. These fungicides will likely play a key role in future management strategies for this disease.

A factor of concern has been the reported in vitro development of resistance to dicarboximide fungicides by *Sclerotinia minor* (4,13,14), *S. homoeocarpa* (5), and several other fungi including *Alternaria alternata, Penicillium expansum, Ustilago maydis, Monilinia fructicola,* and *Botrytis cinerea* (2). Resistant variants of *M. fructicola* and *B. cinerea* have developed under field conditions, and loss of disease control has been reported (1,2,7). Porter and Phipps (13) surveyed several peanut fields treated with procymidone and failed to detect resistant strains of *S. minor* even though they developed in vitro. Such isolates from laboratory studies maintained resistance as a stable trait and were cross-resistant to dicloran, iprodione, and vinclozolin (14).

The objectives of this study were to determine 1) the effects of dicloran, iprodione, PCNB, and vinclozolin on mycelial growth and sclerotium formation

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by *S. minor*; 2) the incidence, level, and stability of in vitro resistance; 3) the field performance of these fungicides in control of Sclerotinia blight of peanut; and 4) if in vivo resistance develops after field application.

MATERIALS AND METHODS

Fungicides and in vitro bioassay. The following fungicides were used: dicloran (Botran 75W), iprodione (Rovral 50W), PCNB (Terraclor 75W and 10G), and vinclozolin (Ronilan 50W). Fungicide suspensions of various concentrations were prepared in sterile distilled water and pipetted into flasks containing autoclaved glucose yeast-extract agar (GYEA) cooled to 70 C. The medium was stirred during addition of fungicide and for 60 sec thereafter to ensure uniform mixing. The medium was then dispensed at 23 ml per petri dish (85 mm diameter). The GYEA consisted of dextrose, 20 g; yeast extract, 2.0 g; KH_2PO_4, 1.0 g; $MgSO_47H_2O$, 0.5 g; agar, 20 g; and distilled water, 1,000 ml.

Five isolates of *S. minor* were obtained from naturally infected peanut plants in different fields within three counties of Virginia and were designated S-1 through S-5. Colonized stems were surface-sterilized for 60 sec in 0.5% NaOCl and placed on GYEA amended with 100 μg/ml each of chloramphenicol and chlorotetracycline HCl to inhibit growth of bacteria. Actively growing colonies of *S. minor* were then subcultured to GYEA in tubes. Cultures were incubated at 25 C and stored at 10 C after sclerotia formed. These stock cultures were transferred at 10-wk intervals and used to produce inoculum for all tests.

All isolates of *S. minor* were screened for sensitivity to selected concentrations of dicloran, iprodione, PCNB, and vinclozolin in GYEA. A 5-mm-diameter agar plug with mycelium from the periphery of an actively growing colony of *S. minor* was placed on the perimeter of petri plates with fungicide-amended and unamended GYEA. Plates were incubated at 25 C in darkness, and linear growth (mm) was measured at 24-hr intervals for 17 days. Treatments were replicated five times and the test was repeated. Growth curves were constructed with data for individual isolates and fungicide dosage levels. Percent inhibition was found by comparing growth rates on fungicide-amended media with those on unamended media. Levels of inhibition were plotted on log-probit graphs as a function of fungicide concentration. Linear regression analyses were used to determine dosage levels for 50% inhibition of growth (ED_{50} values). Counts and measurements of sclerotia were made from 3-cm-diameter samples of medium from 30-day-old cultures.

In vitro resistance to fungicides. Strains of *S. minor* suspected of being resistant to a fungicide were subcultured from rapidly growing sectors on fungicide-amended media. After transfer to unamended GYEA, these strains were tested for resistance by subsequent transfers to slant tubes containing GYEA amended with dicloran (8 μg/ml), iprodione (2 μg/ml), or vinclozolin (2 μg/ml). These concentrations of fungicides inhibited growth of fungicide-sensitive strains of *S. minor* but permitted recognition of strains with even low levels of fungicide resistance. Such strains were given code names to provide reference to the sensitive isolate and fungicide-amended medium from which they originated.

The sensitivity of resistant strains was determined on GYEA amended with 1, 100, 500, and 1,000 μg/ml of the fungicide to which resistance originated. At 10-wk intervals, the strains were cultured on GYEA at 25 C, then stored at 10 C. Every 12 mo for 3 yr, the strains were tested for fungicide resistance as described previously.

Field performance. Five tests were conducted during the 3-yr period (1982–1984) in fields with histories of Sclerotinia blight. Cultivar Florigiant peanut was planted in all tests, and recommended management practices were followed. Chlorothalonil (500 g/L) at 2.3 L/ha was applied according to the Virginia leaf spot advisory program to control Cercospora leaf spot (10). Fungicides for control of Sclerotinia blight were applied to the two center rows of four-row plots, each spaced 0.9 m apart and 12.2 m long, at the following rates: 1) dicloran, 3.37 kg/ha followed by two applications at 2.52 kg/ha; 2) iprodione, three applications at 1.12 kg/ha; 3) PCNB, two applications at 5.61 kg/ha; and 4) vinclozolin, three applications at 0.84 kg/ha. Where three applications of fungicide were used, the first treatments were applied about the second week of July and the second and third treatments were applied about 4 and 8 wk later, respectively. All wettable fungicides were applied with a CO_2-pressurized backpack sprayer with a single 8008LP nozzle centered over each row to deliver 335 L of spray per hectare at 152 kPa. PCNB granules were applied in a 40.6-cm band over the row with a Gandy applicator about the second week of July and again 6 wk later. These use

Table 1. Sensitivity of five field isolates of *Sclerotinia minor* to dicloran, iprodione, PCNB, and vinclozolin

Isolate	ED_{50} (μg/ml)			
	PCNB	Dicloran	Iprodione	Vinclozolin
S-1	1.29[a]	1.34	0.10	0.09
S-2	0.96	1.12	0.13	0.06
S-3	1.65	0.88	0.19	0.09
S-4	...[b]	0.75	0.07	0.06
S-5	1.18	0.47	0.07	0.03
Mean	1.27 ± 0.29	0.91 ± 0.34	0.11 ± 0.05	0.07 ± 0.03

[a] Mean of two tests, each with five replicates.
[b] Isolate not evaluated on PCNB.

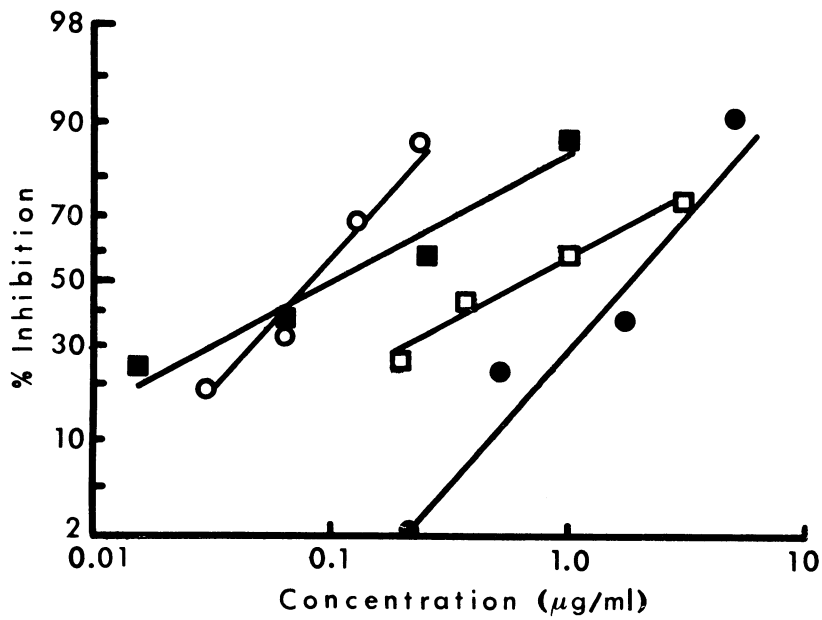

Fig. 1. Dosage-response curves of isolate S-3 to dicloran (□), iprodione (■), PCNB (●), and vinclozolin (O) on log-probit axes.

patterns conformed with current registrations and/or section 18 guidelines for each fungicide. A randomized complete block design was used with four replicates per treatment.

Disease incidence was assessed three times during the growing season. Incidence was taken to be the number of infection centers per two treated rows. An infection center was defined as a point of active growth by *S. minor* and included an area of row 15 cm on either side of that point. Yields were based on weight of peanuts at 8% moisture (w/w), and values were determined from a 500-g sample from each replicate in accordance with Federal-State Inspection Service methods.

Survey for field resistance. A total of 763 isolates of *S. minor* were isolated from diseased peanut plants in 19 fields in Virginia and North Carolina using the GYEA medium with antibacterial agents and the procedure described previously. Thirteen of these fields had been treated by farmers with either dicloran (4.21 kg/ha) or vinclozolin (0.84 kg/ha), but active mycelial growth was still present. The other six locations were replicated field trials that received the treatments described previously in this paper. An attempt was made to collect 24 isolates per treatment at all six replicated field tests. The PCNB treatment was sampled at only one location. The isolates obtained were screened for fungicide resistance by mycelial transfer to GYEA amended with either dicloran (8 μg/ml), iprodione (2 μg/ml), PCNB (8 μg/ml), or vinclozolin (2 μg/ml).

RESULTS

Mycelial growth and sclerotium formation. Although field isolates differed in sensitivity to specific fungicides, the order of sensitivity to the four fungicides was consistent (Table 1). Mean ED_{50} values for the five isolates were 1.27, 0.91, 0.11, and 0.07 μg/ml for PCNB, dicloran, iprodione, and vinclozolin, respectively. The slope of log-probit dosage response curves for iprodione and dicloran was not as steep as that for vinclozolin or PCNB and indicated that at least iprodione could be equally or possibly more inhibitory than vinclozolin at low concentrations (Fig. 1).

Numerous small, black, and irregular sclerotia developed over the surface of unamended GYEA. In the presence of ED_{50} concentrations of dicloran and vinclozolin, sclerotia were larger than those that formed on iprodione-amended and unamended medium (Table 2). The average size of sclerotium produced per isolate ranged from 1.02 to 2.23 mm on fungicide-amended GYEA compared with 0.90–1.60 mm on unamended GYEA. ED_{50} concentrations of dicloran and iprodione induced formation of greater numbers of sclerotia, whereas vinclozolin caused a significant reduction in sclerotial numbers. As the concentrations of all three fungicides were increased above ED_{50} levels, there were corresponding decreases in sclerotial numbers and increases in sclerotial size. Sclerotia often appeared fused together and/or developed in concentric rings around the point of inoculation. Fused or abnormally large sclerotia were viable upon transfer to GYEA.

In vitro resistance to fungicides. During in vitro sensitivity tests, 33 strains of *S. minor* were subcultured from growth sectors on fungicide-amended GYEA. After culture on unamended GYEA, nine strains were capable of growth and sclerotium production on GYEA amended with high concentrations of fungicide. Four of these strains originated from media amended with iprodione and five from media with vinclozolin, each at 0.25 to 4.0 μg/ml; no resistant strains developed on media amended with dicloran or PCNB. The length of exposure before appearance of fungicide-resistant growth sectors varied from 6 to 29 days. The nine strains were isolated from 500 cultures growing on iprodione- or vinclozolin-amended medium, indicating a 1.8% incidence of resistant sectors. All nine isolates of the fungus that originally appeared to be fungicide-resistant maintained their resistance in the absence of fungicides for 15 mo. Twenty-three months after they were selected, eight of the nine were still resistant, and by 36 mo, seven retained fungicide resistance. Resistant strains were capable of growth on media amended with 1, 100, 500, and 1,000 μg/ml of the fungicide to which they originally developed resistance (Table 3). Resistance to vinclozolin appeared to be independent of fungicide concentration; the three vinclozolin-resistant strains were inhibited no more at 1,000 that at 1 μg/ml. Although this was also true for one iprodione-resistant strain, the other strains showed progressively less growth with increasing concentrations of iprodione.

Field performance and survey for resistance. Plots treated with fungicides showed Sclerotinia blight, but disease incidence was significantly lower than in untreated controls (Table 4). Vinclozolin was the most effective fungicide in suppressing disease incidence and resulted in crop yields and values significantly higher than those attained with either iprodione, dicloran, or PCNB. Of 763 isolates collected during the 1982, 1983, and 1984 growing seasons from farmers' fields and replicated field plots of peanuts treated with these fungicides, none showed fungicide resistance. This indicated that disease loci in fungicide-treated plots were not caused by fungicide-resistant strains but rather may be attributed to incomplete fungicide coverage and/or the lack of persistence under field conditions.

DISCUSSION

All four fungicides showed good fungitoxicity in vitro; the dicarboximide

Table 2. Effects of ED_{50} concentrations of dicloran, iprodione, and vinclozolin on size and number of sclerotia produced by *Sclerotinia minor*[x]

Treatment (μg/ml)	Size[y] (mm)	No./plate[z]
Dicloran (0.91)	1.54 a	806.6 a
Iprodione (0.11)	1.27 b	824.4 a
Vinclozolin (0.07)	1.46 a	533.3 c
Check	1.20 b	719.9 b

[x]Mean values for four sensitive isolates with five replicates. Mean separation by Duncan's multiple range test ($P = 0.05$).
[y]Mean values for 240 sclerotia (60 per isolate).
[z]Plastic petri plates (85 mm diameter) containing glucose yeast-extract agar.

Table 3. Growth of three fungicide-sensitive and five fungicide-resistant isolates of *Sclerotinia minor* on glucose yeast-extract agar amended with iprodione or vinclozolin[y]

Isolate	Fungicide concentration (μg/ml) 0	1	100	500	1,000
Vinclozolin medium					
Sensitive-1	80.0 a[z]	0.0 b	0.0 b	0.0 b	0.0 b
Resistant-1A	80.0 a	27.8 c	40.4 b	16.2 c	24.6 c
Sensitive-2	65.0 a	3.4 b	0.0 c	0.0 c	0.0 c
Resistant-2B	69.8 a	36.8 bc	25.4 c	34.0 b	46.8 b
Sensitive-5	53.8 a	2.6 b	0.0 c	0.0 c	0.0 c
Resistant-5B	80.0 a	51.8 c	60.0 c	57.4 bc	52.6 bc
Iprodione medium					
Sensitive-1	80.0 a	0.0 b	0.0 b	0.0 b	0.0 b
Resistant-1B	80.0 a	80.0 a	10.8 b	12.4 b	11.6 b
Sensitive-2	65.0 a	6.4 b	0.0 c	0.0 c	0.0 c
Resistant-2C	68.6 a	30.8 b	34.6 b	18.0 c	13.4 d

[y]Growth (mm) after 6 days of incubation. Data represent the mean of five replicates.
[z]Values followed by the same letters in each row are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

Table 4. Control of Sclerotinia blight with dicloran, iprodione, PCNB, and vinclozolin in field trials surveyed for fungicide-resistant strains of *Sclerotinia minor*[w]

Fungicide treatment, rate, and no. of applications	Disease incidence[x]			Yield[y] (kg/ha)	Value[z] ($/ha)
	Aug.	Sept.	Oct.		
Untreated check	4.9 a	18.8 a	35.3 a	2,878.3 c	1,705.0 c
PCNB (5.61 kg/ha, 2×)	3.2 a	10.6 b	22.2 b	3,666.9 b	2,184.7 b
Dicloran (3.37 kg/ha + 2.52 kg/ha, 2×)	2.8 a	9.3 bc	20.6 b	3,436.1 b	1,984.7 b
Iprodione (1.12 kg/ha, 3×)	3.1 a	9.8 bc	18.0 b	3,745.1 b	2,211.9 b
Vinclozolin (0.84 kg/ha, 3×)	3.0 a	5.6 c	9.9 c	4,341.7 a	2,613.4 a

[w] Data represent the mean of five field trials during a 3-yr period (1982–1984) with four replicates per treatment. Column mean separation by Duncan's multiple range test ($P = 0.05$).
[x] Number of infection centers in the two center rows of each plot or a total of 24.4 m of row. Counts were made on or about the first day of each month. An infection center was defined as a point of active growth by *S. minor* and included an area of row 15 cm on either side of that point.
[y] Yields are weight of peanuts at 8% moisture (w/w).
[z] Value was determined from a 500-g sample from each replicate in accordance with Federal-State Inspection Service methods.

fungicides were more active at low concentrations (Fig. 1). The field trials reported here and previously (6) demonstrate the in vivo efficacy of these fungicides in controlling Sclerotinia blight of peanut. Rates shown to give control in the field (PCNB at 11.23 kg/ha, dicloran at 8.42 kg/ha, iprodione at 3.36 kg/ha, and vinclozolin at 2.52 kg/ha) relate well to the order of ED_{50} values from in vitro tests (PCNB at 1.27 μg/ml, dicloran at 0.91 μg/ml, iprodione at 0.11 μg/ml, and vinclozolin at 0.07 μg/ml).

Although dicloran and PCNB were the least inhibitory of the four fungicides, they did not appear to induce development of resistant pathogen variants. The threat of this occurring may exist, but these data indicate that dicloran and PCNB are less selective for resistant variants than the dicarboximide fungicides iprodione or vinclozolin. Other studies with *S. minor* showed that resistance developed at a similar frequency (2.3%) to procymidone, another dicarboximide, but not dicloran (13). However, work with *M. fructicola* showed no difference in selection for resistance between dicloran, iprodione, vinclozolin, and procymidone (15).

Sclerotia are the primary survival structures for *S. minor* in peanut soils and serve as the initial inoculum in future growing seasons. The fact that ED_{50} concentrations of dicloran and iprodione increase sclerotial production may have epidemiological significance. Vinclozolin, on the other hand, significantly reduced the number of sclerotia formed. Strains of *S. minor* resistant to vinclozolin and iprodione produced as many or more sclerotia than did sensitive strains.

The mode of action of these fungicides is not currently known. The fact that sclerotial size and number were increased after exposure to certain compounds and not others may provide some clues as to the metabolic changes involved. Unfortunately, the metabolic pathways associated with sclerotium development and mechanisms for regulation have not been defined (16).

Although this and a previous study (13) failed to detect the in vivo development of dicarboximide-resistant strains of *S. minor*, the potential for their developing must be recognized. Because of the relatively recent introduction of dicarboximide fungicides for disease control in peanuts, none of the fields sampled had been exposed to regular applications of vinclozolin or iprodione over a period of years. Repeated exposure to these fungicides will probably occur with future use in problem fields. Increased selection pressure may greatly increase the probability that fungicide-resistant populations will develop. Fungicide-resistant strains of *S. minor* obtained in this and in earlier work (13) maintained resistance as a relatively stable trait. Problems resulting from fungicide resistance in a pathogen population are well known (2). In light of this, and considering the major role that these fungicides will probably have in managing this disease, it is important that we understand and monitor this phenomenon carefully.

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