SENSITIVITY AND RESISTANCE OF *SCLEROTINIA MINOR* TO FUNGICIDES FOR CONTROL OF SCLEROTINIA BLIGHT OF PEANUT

by

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(ABSTRACT)

Sclerotinia blight, caused by *Sclerotinia minor*, is a severe disease of peanut in Virginia. Vinclozolin (V), iprodione (I), dicloran (D), and pentachloronitrobenzene (PCNB) were evaluated for their fungitoxicity to *S. minor*. The mean ED_{50} values for five isolates were found to be 0.07, 0.11, 0.91, and 1.27 µg/ml, for V, I, D, and PCNB, respectively, on fungicide-amended glucose yeast-extract agar (GYEA). Fungicide-resistant growth sectors developed on media amended with I or V. Nine such strains occurred; they were capable of growth on GYEA amended with up to 1000 µg/ml of I or V, and were cross-resistant to D or PCNB. Resistance was maintained in all but two strains after repeated culture in the absence of fungicide for 3 yr. In field microplots, two resistant strains were pathogenic to peanut and survived as well as a fungicide-sensitive field isolate. D, I and V were applied to peanuts in the microplots for 3 yr at total annual rates of 8.41, 3.36, and 2.52 kg/ha, respectively. Disease severity caused by the resistant strains was suppressed 19, 33, and 87% by D, I, and V, respectively, as compared to 15, 24, and 76% for the

sensitive isolate. Isolates recovered from tissue biopsies still grew on fungicide-amended GYEA indicating that *in vitro* and *in vivo* resistance are not equivalent in this case. Fungicide treatments reduced sclerotial populations of all strains, and reduced the viability of sclerotia from sensitive but not resistant strains. Fungicide-resistant strains were capable of surviving and competing pathogenically in microplots infested with equal numbers of sclerotia from a sensitive and a resistant strain; this trend was enhanced by fungicide applications. A survey of 763 isolates from fields treated with these fungicides failed to detect resistant strains. One fungicide-resistant isolate was recovered from an iprodione-treated microplot originally infested with a sensitive field isolate. A technique utilizing excised peanut stems was devised to evaluate isolate pathogenicity, cultivar resistance to the disease, susceptibility of different age peanut tissues, and fungicide persistence on peanut stems in the field. The method was also used to screen fungicides; results verified previous findings which indicated that *in vitro* resistance is not equivalent to *in vivo* resistance. Resistance to these fungicides may eventually become a field problem, but with correct management they should provide years of disease control.

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INTRODUCTION AND OBJECTIVES

The peanut (*Arachis hypogaea*) is a major field crop in Virginia even though production is limited primarily to eight counties in the southeastern part of the state. Approximately 100,000 acres of peanuts are grown in Virginia annually with a farm value in excess of 70 million dollars.

In 1971, a disease of peanut resembling Botrytis blight was reported in Virginia and North Carolina. The causative agent was tentatively identified as *Sclerotinia sclerotiorum* and the disease was named Sclerotinia blight (16). The primary pathogen was later found to be *Sclerotinia minor* (Jagger) Kohn (8). By 1978, the disease was reported throughout the Virginia production area and was becoming quite destructive. Aerial infrared photography showed actual yield losses to be directly proportional to the severity of infestation as determined by ground truth (19). Yield suppression was estimated at 7% for the 1978 crop, nearly double the loss from any other disease. In 1981, this figure rose to 10% which represents approximately 10 million dollars in farm income (11). Soybean has also been reported as a host for this pathogen and the occurrence of Sclerotinia blight of soybean may increase as a result of continued intercropping with peanuts (13).

S. minor survives in the soil as sclerotia that may remain viable for over four years (20). Under cool, moist conditions, sclerotia germinate myceliogenically. Mycelia formed may invade host tissues at points near the soil surface; volatile stimulants from peanut leaves and

soil pH are also factors influencing sclerotial germination (7). As the fungus colonizes plant tissues, light tan lesions develop which turn dark brown over time. After infection, stem tissues develop a shredded appearance and pods may be rotted. White, fluffy mycelia are evident at the advancing lesion margins, and small black sclerotia form both on the surface of, and within decaying tissues (16).

The rapid spread and destruction caused by this fungus has prompted researchers to evaluate various methods of control. Morphological and physiological resistances to S. minor have been demonstrated in several lines of peanuts with 'Chico', 'VGP 1', and 'NC 3033' being recommended sources of germplasm for breeding (4). The recent introduction of 'VA 81 Bunch' incorporates moderate disease resistance into a commercially acceptable cultivar. Foliar-applied nutrients, particularly zinc and copper, have provided some suppression of symptoms (6). Cultural practices, such as the wider spacing of rows to improve air movement and reduce humidity may help suppress fungal growth. Plant injury dramatically increases infection incidence and reduces yields (18).

Numerous fungicides have been evaluated as potential control agents for S. minor. Captafol and chlorothalonil, fungicides used to control Cercospora leafspot, both significantly increased severity of Sclerotinia blight (14). Early tests indicated that pentachloronitrobenzene (PCNB) and dicloran had some disease suppressive activity (2). Dicloran has been given eight emergency use labels by the Environmental Protection Agency for the control of Sclerotinia blight.

Certain dicarboximide fungicides have been found to have fungitoxic activity superior to dicloran and their low mammalian toxicity levels make

them especially attractive (21). Procymidone (15), iprodione and vinclozolin suppressed fungal growth *in vitro* (9) and suppressed the disease under field conditions (10,11,12). Unfortunately procymidone is no longer being developed, but registration for use on peanuts has been obtained for iprodione and will hopefully be forthcoming for vinclozolin as well. One cause for concern is that fungi exhibiting resistance to the dicarboximides have been detected. This resistance has been reported in a number of genera (1,3,22) and even in *S. minor* (17). Although resistance was detected primarily by studies *in vitro*, and was sometimes accompanied by a loss of pathogenicity, its existence still raises questions about the practical application of these fungicides. Compounding the problem is the fact that these fungi exhibit cross-resistance among the dicarboximides as well as to certain aromatic-hydrocarbon fungicides such as dicloran.

The mode of action of the dicarboximide fungicides is still unclear and further insight in this area may provide insight into the above problems (5,21). They do not appear to affect the physiological processes of respiration, RNA synthesis, protein synthesis or membrane permeability (1). It has been suggested that at least one active site involves nuclei (5) which may provide heterokaryotic species such as *S. minor* a mechanism of escape and possiblities for resistance.

This dissertation focuses on the use of dicloran, iprodione, PCNB, and vinclozolin in the management of Sclerotinia blight. Chapter one describes research to quantify the *in vitro* toxicity of these fungicides to *S. minor* and their efficacy in control of the disease. The occurrence of strains of *S. minor* that developed fungicide resistance during labo-

ratory studies is reported as well as results of surveys for resistant strains in field peanuts treated with fungicides. The degree and stability of resistance were also investigated.

Chapter two focuses on characterization of resistant strains of the fungus *in vitro* and *in vivo*. Cross-resistance patterns, pathogenicity, response to fungicides, and survival were also investigated. Additional research included documenting sclerotial populations in the soil and their viability, as well as populations of mycoflora associated with sclerotia in non-treated and fungicide-treated plots.

Chapter three reports a new technique developed for evaluation of isolate pathogenicity, efficacy of fungicides, and physiological resistance of peanut cultivars to colonization by the fungus. Chapter four utilizes yet another modification of the technique to quantitatively analyze the persistence and other performance characteristics of dicloran, iprodione, and vinclozolin under field conditions. This method afforded determination of differences in fungicides with respect to their ability to suppress infection and/or lesion elongation. The persistence of the fungicides on field treated peanuts was also correlated with several environmental parameters.

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THE EFFICACY OF FUNGICIDES FOR CONTROL OF SCLEROTINIA BLIGHT OF PEANUT AND THE IN VITRO DEVELOPMENT OF RESISTANCE

INTRODUCTION

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, losses from it in Virginia alone were estimated to be $8.6 million in farm income. Various cultural practices, which include planting the partially resistant cultivar VA 81 Bunch, have reduced the severity of Sclerotinia blight, but have not negated the need for fungicides to control the disease in problem fields.

Until more effective measures are found, fungicides will 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 prior to registration. Dicloran gave control of Sclerotinia blight (3) and was used in Virginia from 1978 to 1984 pursuant to section 18 approval by the EPA. Pentachloronitrobenzene (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 the field in preliminary 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 while 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. homeocarpa* (5), and a number of other fungi including *Alternaria alternata*, *Penicillium expansum*, *Ustilago maydis*, *Monilinia fructicola*, and *Botrytis cinerea* (2). Resistant variants of *Monilinia fructicola* and *Botrytis 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 isolates 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 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 resistance develops after routine field application.

MATERIALS AND METHODS

FUNGICIDES AND *IN VITRO* BIOASSAY. The following fungicides were used: dicloran, iprodione, PCNB, and vinclozolin (Appendix F). 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 (Appendix G). Flask contents were stirred during addition of fungicide and for 60 sec thereafter to insure uniform mixing. The medium was then dispensed at 23 ml/petri dish (85-mm dia.).

Five field isolates of S. minor were utilized in this study (Appendix H). Colonized stems were surface sterilized for 60 seconds in 10% Clorox bleach (.5% NaOCl) and placed on GYEA amended with 100 µg/ml each of chloramphenicol and chlortetracycline HCl to inhibit growth of bacteria. Actively growing colonies of S. minor were then subcultured on 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. Petri plates with fungicide-amended or -unamended medium were inoculated on the perimeter with a 5-mm-dia. agar plug with mycelium from the periphery of an actively growing colony of S. minor on GYEA. Plates were incubated at 25 C in darkness and linear growth (mm) measured at 24-hr intervals for a period of 17 days. Treatments were replicated five times and the test was repeated twice. 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 nonamended 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). Sclerotial counts and measurements were made from 3-cm-dia. samples of agar from 30-day-old cultures on GYEA.

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 nonamended media, these strains were tested for resistance by subsequent transfer 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 afford reference back to the sensitive isolate from which they originated.

The sensitivity of resistant strains was determined on GYEA amended with 1, 100, 500 and 1000 µg/ml of the fungicide to which resistance originated. Such strains were maintained on GYEA slants at 10 C and were transferred to fresh GYEA every 8 weeks. Every 12 months for a period of 3 years, the strains were checked for their ability to grow on GYEA amended with 2 µg/ml of either iprodione or vinclozolin.

FIELD PERFORMANCE. A total of five tests were conducted during the three-year period (1982-84) in fields with past histories of Sclerotinia blight. '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 leafspot advisory program to control Cercospora leafspot (10). Fungicides for control of Sclerotinia blight were applied to the two center rows of four row plots, each 12.2 m long, at the following rates 1) dicloran, 3.37 kg/ha followed by two applica-

tions of 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 with the second and third applications following about 4 and 8 weeks, respectively. All wettable fungicides were applied with a carbon dioxide back-pack sprayer, utilizing a single 8008LP nozzle centered over each row delivering 335.4 L spray/ha 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 weeks later. These use patterns have proven to be the most effective during preliminary studies. A randomized complete block design was utilized with four replications 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 dried to 8% moisture 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 obtained from diseased peanut plants in 19 different fields in Virginia and North Carolina; 13 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 which 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. Isolates collected were screened for resistance to dicloran (8 µg/ml), iprodione (2 µg/ml), PCNB (8 µg/ml), or vinclozolin (2 µg/ml).

RESULTS

MYCELIAL GROWTH AND SCLEROTIUM FORMATION. Although the degree of sensitivity varied among isolates, the order of sensitivity to the four fungicides was consistent (Table 1 on page 14). 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. These were taken from the dosage-response curves shown in Figure 1 on page 15.

Numerous small, black and irregular sclerotia developed over the surface of GYEA in the absence of fungicide. Some fungicide-resistant isolates produced more sclerotia than did their sensitive parent strains. Four isolates exposed to ED_{50} concentrations of these fungicides produced sclerotia that were larger than those formed on nonamended media (Table 2 on page 16).

The average size per isolate ranged from 1.02 to 2.23 mm on fungicide-amended GYEA as compared to 0.90 to 1.60 mm on nonamended GYEA. ED_{50} concentrations of dicloran and iprodione induced formation of greater numbers of sclerotia per plate, whereas vinclozolin caused a significant reduction in sclerotial numbers (Table 2 on page 16). 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 de-

Table 1. Growth of *Sclerotinia minor* on GYEA amended with dicloran, iprodione, PCNB or vinclozolin.

	ED_{50} (µg/ml)			
Isolate	PCNB	dicloran	iprodione	vinclozolin
S-1	1.29[1]	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	--	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

[1] Mean of two tests, each with 5 replications.

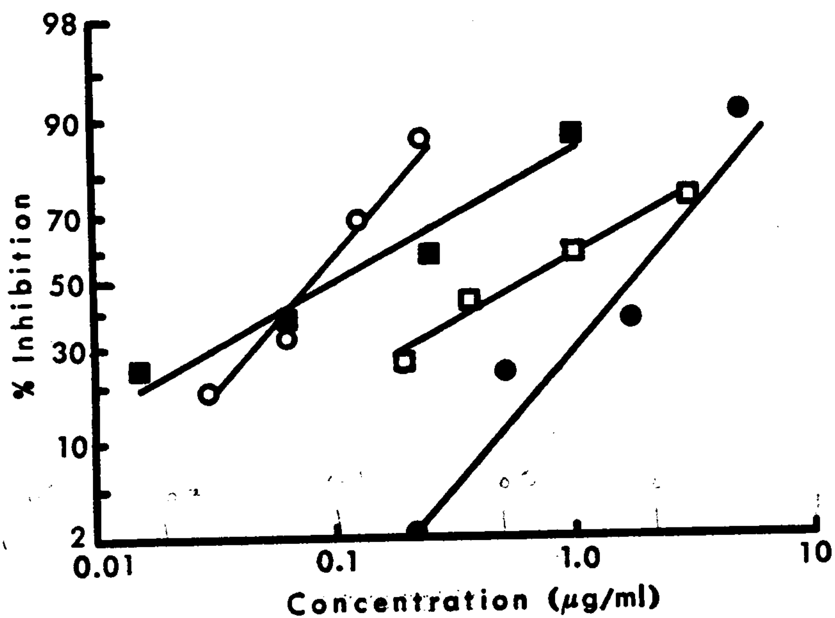

Figure 1. Dosage-response curves of Sclerotinia minor (S-3) to four fungicides. : Treatments are vinclozolin (○), iprodione (■), dicloran (□), and PCNB (●).

Table 2. Effects of dicloran, iprodione, and vinclozolin on size and number of sclerotia produced by *Sclerotinia minor*.[1]

Treatment	Size (mm)[2]	Number per plate[3]
Dicloran (0.91 µg/ml)	1.54 A	806.6 A
Iprodione (0.11 µg/ml)	1.27 B	824.4 A
Vinclozolin (0.07 µg/ml)	1.46 A	533.3 C
Check	1.20 B	719.9 B

[1] Mean values for four sensitive isolates with five replications. Mean separation by Duncan's multiple range test (P=0.05).

[2] Mean values for 240 sclerotia (60 per isolate).

[3] Plastic petri plates (85-mm-dia.) containing GYEA.

veloped 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 the _in vitro_ sensitivity testing, 33 strains of _S. minor_ were subcultured from growth sectors on fungicide-amended medium. After repeated transfers on nonamended GYEA, nine of these were capable of growth on media amended with high concentrations of fungicide. Four of these nine resistant strains were subcultured 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 prior to appearance of fungicide-resistant growth sectors varied from 6 to 29 days. These nine strains were isolated from 500 cultures growing on iprodione- or vinclozolin-amended media, 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 months. Twenty-three months after they were selected, eight of the nine were still resistant, and by 36 months only seven retained fungicide resistance. Resistant strains were capable of growing on media amended with 1, 100, 500 and 1000 µg/ml of the fungicide to which they originally developed resistance (Table 3 on page 19). Resistance to vinclozolin appeared to be independent of fungicide concentration with the three vinclozolin-resistant strains being inhibited no more at 1000 µg/ml than at 1 µg/ml. While this was also true for one iprodione-resistant strain, the other showed progressively less growth with increasing concentrations of iprodione (Table 3 on page 19).

Table 3. Growth of fungicide-sensitive and -resistant isolates of Sclerotinia minor on GYEA amended with iprodione or vinclozolin[1]

Fungicide and Isolate	Fungicide concentration (µg/ml)				
	0	1	100	500	1000
Vinclozolin					
Sensitive-1	80.0 A[2]	0 B	0 B	0 B	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 C	0 C	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 C	0 C	0 C
Resistant-5B	80.0 A	51.8 C	60.0 C	57.4 BC	52.6 BC
Iprodione					
Sensitive-1	80.0 A	0 B	0 B	0 B	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 C	0 C	0 C
Resistant-2C	68.6 A	30.8 B	34.6 B	18.0 C	13.4 D

[1] Growth (mm) after 6 da incubation. Data are the mean of five replications.

[2] Values followed by the same letters in each row are not significantly different at P=0.05 according to Duncan's multiple range test.

FIELD PERFORMANCE. Plots treated with fungicides exhibited Sclerotinia blight, but disease incidence was significantly lower than in untreated controls (Table 4 on page 20). Vinclozolin was the most effective fungicide followed by iprodione, dicloran and PCNB, respectively. The effect of the fungicides became more evident in the latter part of the growing season when disease pressure was heaviest. Decreased disease incidence in treated plots resulted in correspondingly higher crop yield and value (Table 4 on page 20).

SURVEY FOR FIELD RESISTANCE. Although resistance was found *in vitro*, it was not found in farmers' fields or replicated field plots of peanuts treated with these fungicides. Of the 763 isolates collected during the 1982, 1983 and 1984 growing seasons, none exhibited fungicide resistance. This indicates that disease loci in fungicide-treated plots were not caused by 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* with the dicarboximide fungicides being more active at low concentrations. 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 11.23 kg/ha, dicloran 8.42 kg/ha, iprodione 3.36 kg/ha and vinclozolin 2.52 kg/ha) relate well to

Table 4. Control of Sclerotinia blight with fungicides in field trials surveyed for fungicide-resistant strains of *Sclerotinia minor*.[1]

Fungicide treatment	Disease incidence[2]			Yield[3]	Value[4]
and concentration	1 Aug	1 Sep	1 Oct	(kg/ha)	($/ha)
Untreated check	4.9 A	18.8 A	35.3 A	2878 C	1705 C
PCNB (5.61 kg/ha 2X)	3.2 A	10.6 B	22.2 B	3666 B	2184 B
Dicloran (3.37 kg/ha + 2.52 kg/ha 2X)	2.8 A	9.3 BC	20.6 B	3436 B	1984 B
Iprodione (1.12 kg/ha 3X)	3.1 A	9.8 BC	18.0 B	3745 B	2211 B
Vinclozolin (0.84 kg/ha 3X)	3.0 A	5.6 C	9.9 C	4341 A	2613 A

[1] Data are the mean of five field trials during a 3-yr period with four replicates per treatment. Column mean separation by Duncan's multiple range test (P=0.05).

[2] Number of infection centers in the two center rows of each plot or a total of 24.4 m of row. 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.

[3] Yields are based on cured weight of peanuts (8% moisture).

[4] Value was determined from a 500 g sample from each replicate in accordance with Federal-State Inspection Service methods.

the ED_{50} values established in this study (PCNB 1.27 µg/ml, dicloran 0.91 µg/ml, iprodione 0.11 µg/ml and vinclozolin 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 variants. The threat of this occurring certainly exists, 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_ also showed resistance developed at a similar frequency (2.3%) to procymidone, another dicarboximide, but not dicloran (13). However, work with _Monilinia 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. If such an increase were to occur under field conditions, the higher densities of sclerotia present in the soil could result in heavier disease pressure in future growing seasons. Vinclozolin on the other hand significantly reduced the number of sclerotia formed. Also, 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 was enlarged upon exposure to these compounds may provide some clues as to the metabolic changes involved. Unfortu-

nately the metabolic pathways associated with sclerotium development and mechanisms for regulation have yet to be 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. With the exception of dicloran, these fungicides are new inputs for control of Sclerotinia blight. 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 likely occur with future use in problem fields. Increased selection pressure may increase greatly the probability that fungicide-resistant populations develop. Fungicide-resistant strains of *S. minor* obtained here and in earlier work (13) maintain resistance as a relatively stable trait, although it can be reversible over extended periods of time. The potential dangers of the sudden appearance of resistance in a pathogen population are well known. 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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CHARACTERIZATION OF SCLEROTINIA MINOR STRAINS WITH IN VITRO RESISTANCE TO IPRODIONE AND VINCLOZOLIN.

INTRODUCTION

The development of fungi with resistance to fungicides is a problem of increasing importance to modern agriculture. This is a relatively recent phenomenon and usually involves one of the more selective fungicides that have a single-site mode of action, although the problem is not limited to this class of compounds (20). Due to the potentially disastrous effects of fungicide resistance, it has become a major consideration prior to the registration of new compounds. Such determinations are generally based on laboratory studies or early monitoring results combined with more theoretical considerations (20).

Sclerotinia blight of peanut was first reported in 1971 (14) and has since become a major disease of peanuts in Virginia. Early testing showed the dicarboximide fungicides to be effective in controlling the disease (3,7,11,13). However, *in vitro* studies with the dicarboximide fungicide procymidone showed that actively growing mycelium of the pathogen, *Sclerotinia minor* (Jagger) Kohn (8), developed resistance to this fungicide at a rate of 2.3% (15). Resistance was maintained in the absence of the fungicide and strains were found to be cross-resistant to iprodione and vinclozolin as well as to dicloran. Although procymidone exhibited excellent potential for the control of Sclerotinia blight (13), work to gain registration was terminated in 1980. Since 1985, iprodione

has been the only fungicide with full registration for use on peanut to control Sclerotinia blight. Similar registration for vinclozolin continues to be sought. Previous studies (Chapter 1) showed that *in vitro* resistance to iprodione and vinclozolin occurred at a rate of 1.8% (3). Fungicide-resistant strains were capable of growing at up to 1000 µg/ml of the fungicide to which resistance originated.

The appearance of these strains of *S. minor* is cause for concern since dicarboximide-resistant strains of numerous other fungi have been reported (2). Although such studies involving selection for resistance have usually been conducted in the laboratory, there are also reports of field resistance and a loss of disease control (6,10). Much work has been done with *Botrytis cinerea* on a variety of crops, and results have indicated that resistant strains could still be controlled by regular fungicide applications (8). It has been suggested that resistant strains may be less ecologically fit than sensitive strains or that the level of resistance was too low for expression under field conditions (1,8). Such information is essential to the development of appropriate use patterns for fungicides. This is particularly true for control of Sclerotinia blight of peanut, if the widespread use of dicarboximides is adopted.

The objectives of this study were to characterize the dicarboximide-resistant strains of *S. minor* obtained in earlier studies (Chapter 1) as follows: 1) pathogenicity to peanuts treated and not treated with fungicides, 2) survival and regeneration of sclerotia in soils cropped to peanuts over a period of three years, 3) competition with fungicide-sensitive field isolates both in the presence and absence of

fungicides, and 4) cross-resistance to other fungicides with utility in Sclerotinia blight control.

MATERIALS AND METHODS

PATHOGENICITY AND RESPONSE TO FUNGICIDES. Field studies were conducted in microplots constructed of fiberglass barriers (0.3-cm thick, 60-cm high, 77-cm dia.) inserted into the soil to a depth of 45 cm. Sclerotia of *S. minor* for infestation of soil were produced in a sterilized soil and corn meal medium (Appendix G). After 2-weeks incubation at 25 C, sclerotia were washed on a 325-µm sieve to remove the growth medium. Standardized quantities of 1800 sclerotia were mixed into the upper 8-cm of soil in each microplot, resulting in a density of approximately 4 sclerotia per 100 g soil. The soil type was a Dragston fine sandy loam. Infestation was done just prior to planting 'Florigiant' peanut seed. Plant densities were standardized at three plants per plot after emergence to simulate field densities. Standard management practices were followed with the exception of weed control which was primarily manual. These practices are given in more detail in Appendix E along with information on irrigation which was applied as needed to reduce plant stress and promote disease development. Applications of chlorothalonil or benomyl plus sulfur were applied according to the Virginia leafspot advisory program to control Cercospora leafspot.

One sensitive isolate (S-2) and two dicarboximide-resistant isolates (R-2B and R-2C) were used to infest soil in microplots in the spring of 1983. A randomized complete block design was employed with four rep-

lications of the following treatments per isolate of S. minor ; 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) vinclozolin, three applications at 0.84 kg/ha, and 4) untreated. The first treatments were applied about the second week of July with subsequent applications at about 4-week intervals. A carbon dioxide back-pack sprayer utilizing a single D2-13 (disk-core) nozzle and 50 psi pressure was used to deliver sprays at a volume of 375 L/ha.

Disease data were recorded three times during the growing season. The first two involved counts of the number of active infection centers per plant in late July and August. At harvest in early October, the severity of disease for each plant was rated on a scale of 0(no disease) to 10(death of plant). Samples of diseased tissue were also collected from each plot at harvest. After surface sterilization in 10% Clorox bleach (0.5% NaOCl) for 60 seconds, tissue was placed on GYEA amended with antibiotics (Appendix G). Actively growing colonies of S. minor were then tested for their ability to grow on GYEA amended with dicloran (8 µg/ml), iprodione (2 µg/ml), and vinclozolin (2 µg/ml).

Peanut plants were dug and inverted to dry about the first week of October each year. Yields of pods per microplot were determined about 2-weeks later and expressed on a basis of 4-6% moisture (w/w).

Microplots were also used to determine the effect of long-term fungicide usage on disease control and resistance. For this purpose, microplots were maintained for three consecutive years. Each year they received the same fungicide treatment and were rated, sampled for resistance, and harvested to determine yields.

COMPETITION OF SENSITIVE AND RESISTANT STRAINS UNDER FIELD CONDITIONS. Microplots for this study were established in the spring of 1984 and managed as described in Appendix E. Nine hundred sclerotia from both a sensitive and resistant strain of S. minor were used to infest soil as described previously. The soil type at this location was a Goldsboro fine sandy loam. Three pairs of isolates were utilized (S-2 & R-2C, S-5 & R-5B, and S-1 & R-1D). Plots were either not treated or received three applications of vinclozolin (0.84 kg/ha) annually according to the treatment schedule described previously. Again a randomized complete block design was employed with four replications. Ratings of disease severity, sampling for resistance, and harvesting were done as described previously.

DETERMINATION OF SCLEROTIAL POPULATIONS. Populations of sclerotia in the soil within microplots were determined in 1985 for both tests. A quantitative technique using a semi-automatic elutriator to recover sclerotia (18) was used (6.75 minute elutriation period) and a binocular dissecting microscope (10 X) was employed to count sclerotia. Twelve soil cores (2 cm x 8 cm) were taken from each microplot. After thorough mixing, samples were screened over 3-mm mesh screen to remove large fragments of plant debris. Samples equivalent to 100 g dry weight of soil were then used for elutriation.

Sclerotia recovered from soil were surface-sterilized (2 minute in 0.5% NaOCl), placed on GYEA amended with 100 µg/ml each of chloramphenicol and chlortetracycline HCl, and incubated at 20 C to determine viability. Colonies of S. minor resulting from germinating sclerotia were transferred to GYEA amended with fungicides as previously described to test

for resistance. Other fungi were also found associated with the sclerotia after surface sterilization. These were isolated and genera identified.

CROSS-RESISTANCE. The following fungicides were used: dicloran, iprodione, PCNB, and vinclozolin (Appendix F). Fungicide suspensions were prepared in sterile-distilled water and pipetted into flasks containing autoclaved glucose yeast-extract agar (GYEA) cooled to 70 C (Appendix G). The final concentration of all fungicides was 100 µg/ml with the exception of PCNB which was evaluated in a similar but separate experiment. The medium was stirred during addition of fungicide and for 60 seconds thereafter, and then aliquots dispensed at 23 ml/petri dish (85-mm-dia.).

The nine strains of S. minor with resistance to dicarboximide fungicides obtained during earlier in vitro sensitivity testing (Chapter 1) were evaluated along with the three sensitive field isolates from which they originated. Petri plates with fungicide-amended or nonamended medium were inoculated at the perimeter with a 5-mm-dia. agar plug with mycelium from the periphery of an actively growing colony of S. minor on GYEA. Plates were incubated at 25 C in darkness and linear growth (mm) measured at 24-hour intervals. From these data, linear growth rates in mm/day were calculated for each isolate.

RESULTS

PATHOGENICITY AND RESPONSE TO FUNGICIDES. Figure 2 on page 32 illustrates the mean disease severity rating at harvest of control and fungicide-treated plots for the three years of the study. Comparison of

disease severity in control plots indicated no differences in pathogenicity of the fungicide-sensitive and -resistant strains. Similar results with these and other isolates were obtained in growth chamber studies (Appendix D). Disease caused by all three strains was suppressed about equally by fungicides, regardless of a strains _in vitro_ sensitivity. This trend was also evident in disease incidence ratings taken earlier in the season. Yield data indicated no apparent differences in strains with consistent increases resulting from use of the dicarboximide fungicides (Table 5 on page 33). In spite of these trends, isolates recovered in tissue biopsies at harvest retained their original sensitivity or resistance on fungicide-amended GYEA. These results indicate that _in vitro_ dicarboximide resistance in _S. minor_ is not equivalent to _in vivo_ resistance.

The numbers of sclerotia recovered from soil revealed some differences in the biology of isolates (Table 6 on page 34). Numbers of sclerotia in soil of nontreated plots infested with one resistant strain differed significantly from those in soil infested with the sensitive isolate. Numbers of sclerotia of the other resistant strain were not statistically different from the sensitive isolate. Both sensitive and resistant strains formed fewer sclerotia in plots treated with fungicides. These differences were similar in magnitude to those for symptom suppression.

Most sclerotia maintained their original fungicide sensitivity or resistance (Table 6 on page 34). Resistance was detected in one isolate in a plot infested with a sensitive strain and treated with iprodione for three years. Not all sclerotia recovered from plots infested with re-

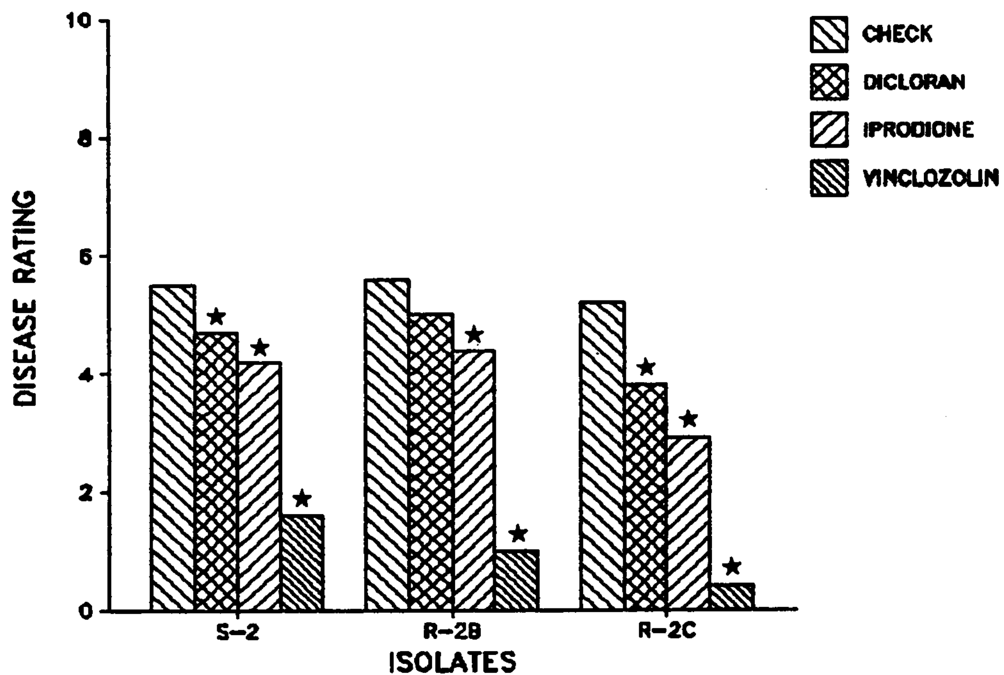

Figure 2. Disease severity ratings in fungicide-treated microplots infested with either a sensitive or resistant strain of Sclerotinia minor : [Mean of 3 years, each with 4 replications. (★) indicates significant difference from the control at P=0.05 according to Duncan's multiple range test].

Table 5. Effect of fungicides on peanut yields from microplots infested with fungicide-sensitive or -resistant strains of *Sclerotinia minor*.

	Pod yields (g/microplot)[1]			
Isolate	vinclozolin	iprodione	dicloran	check
S-2	385 A[2]	283 AB	235 B	198 B
R-2B	368 A	281 AB	234 B	224 B
R-2C	399 A	323 B	250 B	280 B

[1] Mean of three years, each with 4 replications. Cumulative annual rates of fungicides were dicloran (8.4 kg/ha), iprodione (3.4 kg/ha, and vinclozolin 2.5 kg/ha).

[2] Means in rows followed by the same letter(s) are not significantly different (P=0.05) according to Duncan's multiple range test.

Table 6. Populations of sclerotia of *Sclerotinia minor* in microplot soils after 3 yr of peanut culture and fungicide treatment.

Isolate and treatment[1]	sclerotia/100 g soil	% resistant
S-2		
untreated	52 A[2]	0
dicloran (8.4 kg/ha)	28 BCD	0
iprodione (3.4 kg/ha)	30 BC	4
vinclozolin (2.5 kg/ha)	10 DE	0
R-2B		
untreated	44 AB	96
dicloran (8.4 kg/ha)	25 CD	96
iprodione (3.4 kg/ha)	22 CDE	92
vinclozolin (2.5 kg/ha)	6 E	85
R-2C		
untreated	29 BCD	96
dicloran (8.4 kg/ha)	25 CD	100
iprodione (3.4 kg/ha)	11 CDE	95
vinclozolin (2.5 kg/ha)	6 E	80

[1] Cumulative annual rates of fungicide applied.

[2] Values followed by the same letters are not significantly different at P=0.05 according to Duncan's multiple range test.

sistant isolates were still resistant. This may be due to a loss of this trait over a period of years as is sometimes seen in the laboratory (3), although the great majority of sclerotia did retain their resistance.

Figure 3 on page 36 illustrates the percent viability of sclerotia according to isolate and treatment. Both resistant isolates behaved similarly and results for them are combined. It is apparent that the fungicides decreased the viability of sclerotia from sensitive strains of S. minor but had no apparent effect on viability of resistant strains. This effect was particularly evident with vinclozolin.

Numerous fungi were found associated with sclerotia after surface sterilization. Species of Fusarium, Trichoderma, and Verticillium were most commonly isolated. An analysis of population changes in these genera showed that Trichoderma spp. were associated with 8.9, 9.5, and 8.1% of sclerotia in plots treated with dicloran, iprodione or vinclozolin, respectively as compared to 3.0% in the nontreated plots. The incidence of other fungi did not appear to be affected by fungicide treatments.

COMPETITION OF DICARBOXIMIDE-SENSITIVE AND -RESISTANT STRAINS UNDER FIELD CONDITIONS. Disease severity in microplots infested with equal numbers of sclerotia from sensitive and resistant strains was always less than in microplots at the other location. Applications of vinclozolin were quite effective and resulted in disease suppression of 73-93% as compared to the nontreated plots.

Tissue biopsies revealed differences in behavior of isolate pairs in terms of competitive pathogenicity. Where no fungicide was applied, the percent recovery of resistant isolates varied from 0-64% (Table 7 on page 38). In plots treated with vinclozolin, the percent recovery in-

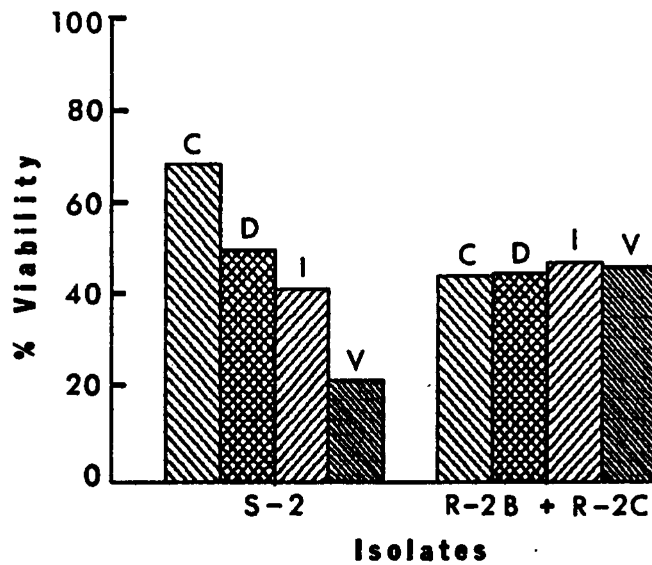

Figure 3. Effect of fungicides on the viability of sclerotia from fungicide-sensitive and -resistant strains of _Sclerotinia minor_ : (Treatments are C=check, D=dicloran, I=iprodione, and V=vinclozolin. Data for the resistant strains are given as the means of R-2B and R-2C).

creased dramatically and ranged from 67-100%. Vinclozolin suppressed disease symptoms to levels resulting in a low recovery of isolates for assay. This was especially true in 1985 when the disease was less severe than in 1984.

Populations of sclerotia in plots were quite low, even where fungicide was not applied (Table 8 on page 39). Neither isolate S-1 nor R-1D appeared to be adapted to survival as only 0.8 sclerotia per 100 g soil were recovered in untreated plots and none of these were viable. Higher numbers of sclerotia were recovered from soil infested with the other isolate pairs, but populations were low considering an initial infestation level of four sclerotia/100 g soil only two years earlier. The percent viability of sclerotia from plots varied widely among isolates (Table 8 on page 39), but on the average it was not too different from the viability of sclerotia in the other microplots (Figure 3 on page 36).

The resistant strains were quite competitive with sensitive strains, and in two instances accounted for greater than 50% of sclerotia recovered from soil (Table 8 on page 39). A shift toward greater recovery of resistant than sensitive strains where fungicides were applied was not evident here as it was with the biopsy samples, however, detection of such a shift may require an increase in sample size.

CROSS-RESISTANCE. Fungicide-resistant strains of S. minor were capable of growth on media amended with D, I, or V (Table 9 on page 40). A similar but separate test indicated that cross-resistance existed to PCNB as well. Dicloran was the most inhibitory to resistant strains followed by iprodione and vinclozolin, respectively. This is somewhat

Table 7. Summary of the fungicide sensitivity of isolates recovered from diseased plants in mixed inocula microplots

Treatment and	1984		1985	
Isolate Pair	# Recovered	% Resistant	# Recovered	% Resistant
check				
S-2 + R-2C	22	64	8	63
S-5 + R-5B	21	43	10	20
S-1 + R-1D	10	0	2	0
vinclozolin[1]				
S-2 + R-2C	1	100	2	100
S-5 + R-5B	6	100	0	--
S-1 + R-1D	6	67	1	100

[1] Three applications of vinclozolin, 0.84 kg/ha each.

Table 8. Populations of sclerotia in microplots two years after infestation with a sensitive and a resistant strain of *Sclerotinia minor*.

Treatment and Isolate Pair	Total # recovered	#/100 g soil	% viable	% resistant
check				
S-2 + R-2C	11	2.8	82	67
S-5 + R-5B	24	6.0	44	70
S-1 + R-1D	3	0.8	0	--
vinclozolin[1]				
S-2 + R-2C	1	0.3	100	0
S-5 + R-5B	4	1.0	25	100
S-1 + R-1D	1	0.3	100	0

[1] Three applications of vinclozolin, 0.84 kg/ha each.

Table 9. Cross-resistance of *Sclerotinia minor* to fungicides.[1]

Isolate[2]	Mycelial Growth (mm/day)			
	check	dicloran	iprodione	vinclozolin
S-1	24.0	0.6	0	0
R-1A	14.0	4.0	3.3	5.7
R-1B	13.8	4.4	3.5	5.3
R-1C	16.8	4.0	3.3	9.0
R-1D	16.6	4.0	1.8	5.3
S-2	8.6	0.8	0	0
R-2A	14.9	4.5	6.6	10.2
R-2B	15.1	4.5	6.2	5.6
R-2C	15.0	4.1	7.0	8.9
S-5	11.9	0.5	0	0
R-5A	14.1	3.7	3.0	7.6
R-5B	14.1	5.5	8.2	10.3
Mean of sensitive	14.8	0.6	0	0
Mean of resistant	14.9	4.3	4.8	7.5

[1] Glucose yeast-extract agar was used; the concentration of all fungicides was 100 µg/ml.

[2] 'S' indicates a sensitive field isolate and 'R' indicates a fungicide-resistant strain of *S. minor*.

surprising since dicloran is the least fungitoxic to sensitive field isolates (3). Similar results have been reported previously for *S. minor* (16) and *Monilinia fructicola* (19).

DISCUSSION

There has been concern about the possibility of dicarboximide-resistant strains of *S. minor* becoming a problem upon the widespread use of such fungicides in peanut culture (3,16). Speculation was severely limited, however, since there were no field data upon which to draw. Our findings indicate that a fairly stable change toward decreased sensitivity of the fungus occurs readily in the lab but seldom in the field. This *in vitro* resistance does not translate into *in vivo* resistance since such strains still responded to fungicide treatment in microplots. This phenomenon, while not fully understood, may be due to the profound differences between growth on a nutrient-rich agar medium and growth as a parasite on a living plant. Indeed, there are indications that nutrition of this fungus can influence the occurrence and expression of fungicide resistance (Appendix A).

Resistant strains were capable of surviving and competing pathogenically with sensitive strains. This trend occurred even in the absence of the fungicides, but was enhanced when they were applied. The genes for dicarboximide resistance do not appear to be detrimental to the survival of the fungus. In fact, a supplemental study with two resistant strains of *S. minor* showed them to be even more tolerant of high temperature stress than their sensitive parent isolate (Appendix C). This is

in contrast to the situation with dicarboximide-resistant strains of *Botrytis cinerea* which often have decreased vigor and rapidly revert to their sensitive state in the absence of the fungicides (12). Furthermore, such strains of *Botrytis cinerea* exhibit an unusual degree of sensitivity to osmotic stress (1). Although this was the case with some *S. minor* strains resistant to the dicarboximides, the trend was not as distinct as reported for other fungi (Appendix B). Considering these traits, as well as the stability of dicarboximide resistance in *S. minor*, such strains might persist in nature for a period of years.

A previous study reported that applications of iprodione and vinclozolin served to increase the longevity of *S. minor* sclerotia in the soil (5). This could have a detrimental effect on long term disease control by allowing higher populations of sclerotia to develop. The trend was reversible, however, if *Trichoderma* spp. were added to the soil. The data presented in this study demonstrate that the viability of sclerotia is either not affected or is reduced by applications of these fungicides (Figure 3 on page 36). This discrepancy might be explained by the high populations of *Trichoderma* naturally present in peanut field soil. The fact that fungicide applications substantially increased the incidence of *Trichoderma* recovery from sclerotia supports this theory.

Fungicides with an alternate mode of action might be employed to prevent the development of resistance (15). Unfortunately, such fungicides are not currently available and current evidence for cross-resistance in *S. minor* limits the selection of candidates. Without such alternatives, and considering the destructive potential of this pathogen, these products should be used only as part of an integrated control pro-

gram of several disease suppressive inputs. Such an integrated program should include measures to delay onset of the disease such as late planting, reduced seeding rates, use of 0.91-m single rows, and controlling plant growth with Kylar (P. M. Phipps, personal communication). Selection of a suitable cultivar (4) and avoiding unwarranted injury to vines (17) are also important factors. It would be advisable as well to maintain an active survey program to detect any changes in sensitivity of the fungus, particularly where a loss of disease control is suspected. Although the threat of field resistance in S. minor may appear to be less severe than originally thought, this is still a very real biological phenomenon that should not be ignored.

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A METHOD OF SCREENING PEANUT GERMPLASM AND FUNGICIDES FOR CONTROL OF SCLEROTINIA BLIGHT OF PEANUT.

INTRODUCTION

Sclerotinia blight of peanut (*Arachis hypogaea* L.), caused by *Sclerotinia minor* (Jagger) Kohn (5), has become a serious disease problem for peanut growers in Virginia, Oklahoma and northeastern North Carolina. Although not reported until 1971 (9), losses to this disease in Virginia alone have been estimated at 13% in years favorable for disease development (13).

Such losses have stimulated researchers to evaluate various disease management strategies. Current control recommendations include planting 'VA 81 Bunch', a cultivar with moderate resistance to Sclerotinia blight, and the use of iprodione, the only currently registered fungicide for control of this disease. Various laboratory, growth chamber and field tests have been used to evaluate the efficacy of these measures (4,7,10,12). Test procedures using detached plant parts have been developed to investigate a number of parameters in other diseases such as peanut leaf spot (6). Such techniques have the advantage of requiring little greenhouse space, thus making it possible to evaluate many genotypes. Results are usually obtained very quickly also. This paper reports on an excised stem technique that can be adapted to rapidly evaluate physiologic resistance in peanut genotypes, efficacy of fungicides and/or spray adjuvants, and pathogenicity of fungal isolates.

One preliminary report has been published on the method itself (2) and another on the use of the method to determine ED_{50} values for various fungicides (1).

MATERIALS AND METHODS

Uniformly developed lateral stems were cut from peanut plants growing in the field or greenhouse, rinsed in tap water and all leaves and pegs were cut off flush with the stem. Care was used in handling the stems to avoid injury at sites other than where they were cut. Stem segments 8.5-cm long were cut and thoroughly washed in distilled water. The segments were then placed in moist chambers consisting of 20 x 10 x 3.8 cm plastic boxes with hinged lids. The stems were supported at each end by a 18.5 x 1.3 x 1.3 cm pine slat and high humidity maintained by adding 30 ml water to a paper towel in each box.

Isolates of *S. minor* were grown on plates of glucose yeast-extract agar (GYEA; see Appendix G). Five-mm diameter plugs were taken from the periphery of actively growing cultures for inoculation of stems. These plugs were placed with the mycelium directly in contact with the stem either between nodes or at a node where the leaf or peg had been removed. Inoculations at internodes were made to assess the importance of host wounds in infection by *S. minor*. In this case, inoculum was applied either to an intact surface or to a 3-mm-long by 1-mm-deep puncture wound made with a needle. Separate segments were cut from the terminal, median, and basal areas of the stem. These were inoculated at leaf nodes to determine the relative susceptibility of different parts of the stem. In-

oculated stems were incubated in moist chambers at 18-20 C. Lesion size was measured daily to determine colonization rates.

EVALUATION OF ISOLATE PATHOGENICITY. Twelve-week-old 'Florigiant' peanut stems were used to compare the pathogenicity of *S. minor* isolates. One of the comparisons of interest was that of normal field isolates (S-1, S-2 and S-5) versus dicarboximide-resistant subcultures (Appendix H).

EVALUATION OF PHYSIOLOGIC RESISTANCE IN PEANUT CULTIVARS. Stem segments from six peanut cultivars were evaluated for susceptibility to colonization by *S. minor*. Median stem segments were cut from 10-week-old field-grown peanut plants, and inoculated at trimmed nodes as described previously with mycelial plugs from active cultures of isolate S-2. The test was repeated twice for five of the cultivars with greenhouse-grown plants.

ADAPTATION TO FUNGICIDE TESTING. The procedure was also adapted to evaluate fungicides for potential in control of Sclerotinia blight of peanut. Median stem segments were immersed in fungicide suspensions for one minute. Stems were then air-dried and inoculated at a node with *S. minor*. Again a comparison of interest was that of fungicide-sensitive and -resistant strains of the fungus.

A base suspension of 10 µg/ml iprodione in deep well water from Suffolk, VA was used. The following spray additives were evaluated alone and with iprodione: Spray-Aide (Miller Chemical and Fertilizer Corp., Hanover, PA), and household white vinegar, (5% acidity). The pH of all suspensions was determined immediately after mixing. Isolate S-2 was used for inoculum and each treatment was replicated four times.

RESULTS AND DISCUSSION

EFFECT OF WOUNDS AND HOST TISSUE DEVELOPMENT. Experiments demonstrated that wounding was necessary for infection to occur. Stems inoculated between nodes without injury at the site of inoculation rarely developed lesions. Similar results have been reported for *S. minor* on peanut and soybean seedlings in growth chamber tests (8). It is not known if the lesions that occurred on a few stems were the result of direct tissue penetration or the presence of superficial abrasions made inadvertently during collection and preparation of stems. (Table 10 on page 50).

Stems inoculated at nodes developed longer lesions of more consistent length than did stems inoculated at wounds between nodes. In a study utilizing greenhouse-grown 'Florigiant' peanut stems inoculated with isolate S-2, the mean length of lesions at 2.5 days was 34.2 ± 4.9 mm for node-inoculated segments. Similar stem segments inoculated at a wound in the internode had lesions with a mean length of 22.4 ± 8.0 mm. Since node inoculation resulted in lesions of longer, more consistent length, this method was used in all subsequent work.

Comparing colonization rates of various stem segments revealed differences in tissue susceptibility. The terminal portions of stems were the most rapidly colonized by the fungus followed by the median and basal segments, respectively (Table 10 on page 50). This trend was evident as early as 48 hours after inoculation. The physiologic basis for this difference in susceptibility is not known, but it may be related to increased lignification and/or decreased sugar content often associated

Table 10. The effect of wounding and tissue age on infection of excised stems by <u>Sclerotinia minor</u>.

	Lesion length (mm)	
Stem segment	48 hr[1]	72 hr
Inoculated at node (wound)		
Terminal	34.0 A[2]	63.2 A
Median	28.0 B	54.0 B
Basal	20.3 C	38.6 C
Inoculated at internode		
Terminal	0.3 D	0.3 D
Median	0 D	0 D
Basal	0 D	0 D

[1] Time elapsed betwen inoculation and lesion measurement.

[2] Average of 6 isolates, 4 replcates per isolate.

Means followed by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

with older tissues. From a disease control perspective, these findings reinforce the importance of broadcasting fungicide treatments over the entire width of the row as opposed to band applications over rows. Results also further support previous reports of increased incidence and severity of disease where vine tips have been injured by tractor tires (11).

EVALUATION OF ISOLATE PATHOGENICITY. The colonization rates of nine isolates of S. minor showed significant differences in pathogenicity of isolates (Table 11 on page 52). Although several of the in vitro fungicide-resistant strains had reduced pathogenicity, they were all capable of infecting stems and causing visible lesions. Colonization rates of isolates S-2 and R-2C were not significantly different in this test which corresponds to pathogenicity data obtained with these same isolates in field microplots (Chapter 2).

EVALUATION OF PHYSIOLOGIC RESISTANCE IN PEANUT CULTIVARS. The excised stem method proved suitable for rapid, preliminary evaluations of peanut genotypes for physiological resistance to Sclerotinia blight. The results from the test with field-grown plants are shown in Table 12 on page 54. Evaluations of these cultivars in a similar test with plants grown in the greenhouse gave comparable results with the exception that 'NC 7' was not as susceptible as it was in the test with field-grown plants. 'VA 81 Bunch' and 'AD 1', varieties known for their resistance to Sclerotinia blight, were in fact the most resistant cultivars. Although 'VA 81 Bunch' is a bunch type peanut and 'AD 1' is a runner type, both have a sparse branching habit. This produces an open canopy which is thought to suppress fungal growth by allowing sunlight penetration and

Table 11. Evaluation of pathogenicity of *Sclerotinia minor* isolates on excised peanut stems.

Isolate[1]	Lesion length (mm) at day 3
S-2	47.3 ABC[2]
R-2A	50.3 AB
R-2C	50.3 AB
S-5	56.5 A
R-5A	43.0 BCD
R-5B	33.0 D
S-1	49.7 AB
R-1A	37.5 CD
R-1D	53.2 AB

[1] Isolates designated as S=dicarboximide-sensitive field isolates and R=resistant strains of *S. minor* originating *in vitro*.

[2] Mean of 4 replications, each being a median stem segment. Means followed by same letters are not significantly different (P=0.05) according to Duncan's multiple range test.

efficient air circulation, thus lowering the relative humidity (4). The results of this study suggest that these cultivars possess some degree of physiological resistance as well. Earlier work showed 'Florigiant', the next least susceptible cultivar in this study, to also have some field resistance (12). This resistance, however, was not as great as might be predicted by the excised stem evaluation. This discrepancy might be due to the dense canopy characteristic of 'Florigiant' which promotes environmental conditions favorable for fungal growth.

FUNGICIDE TESTING. The degree of inhibition due to iprodione alone was not significant (P=0.05) according to Duncan's multiple range test (Table 13 on page 55). Although neither spray additive was fungitoxic alone, both resulted in a substantial increase in inhibition of lesion elongation when mixed with iprodione. The deep well water had a pH 7.6 which was lowered to 5.11 and 6.28 by the white vinegar and Spray-Aide, respectively. The addition of iprodione to all solutions resulted in a slight increase in their pH. Although iprodione provides good control of the disease in the field, it is known to hydrolyze under alkaline conditions. Since groundwater in the peanut growing region of Virginia is often alkaline as in the case of this study, this is a point of concern. The data reported here indicate that the addition of acidifying agents can increase the inhibition of S. minor by iprodione. The excised stem method proved to be a suitable tool for this type of fungicide evaluation and such information could prove beneficial in the field where spray mixtures may not be applied immediately after preparation.

In earlier testing, the excised stem method was beneficial for comparing the fungitoxicity of iprodione with other fungicides, specif-

Table 12. Evaluation of physiologic resistance of excised stems from six peanut cultivars to infection by *Sclerotinia minor*.

Cultivar	Lesion length (mm) at day 3[1]
NC 7	53.8 A[2]
NC 6	53.0 A
NC 8C	47.6 B
Florigiant	46.2 B
VA 81B	45.4 B
AD 1	43.2 B

[1] Mean values of two isolates, each replicated five times.

[2] Means followed by same letters are not significantly different ($P=0.05$) according to Duncan's multiple range test.

Table 13. Effect of acidifying agents on fungitoxicity of iprodione to *Sclerotinia minor*.

Treatment	pH	Lesion length (mm) at day 4
Check (Deep well water)	7.60	66.0 A[1]
Iprodione (10 µg/ml)	7.63	57.7 AB
White vinegar (10.0 ml/l)	5.11	64.3 A
White vinegar (10.0 ml/l) + iprodione (10 µg/ml)	5.30	47.0 B
Spray-Aide (0.63 ml/l)	6.28	66.5 A
Spray-Aide (0.63 ml/l) + iprodione (10 µg/ml)	6.49	44.3 B

[1] Means followed by same letters are not significantly different ($P=0.05$) according to Duncan's multiple range test.

ically dicloran and vinclozolin. Dosage response curves were calculated from those data which allowed comparison of ED_{50} values for each fungicide. The slopes of those curves varied considerably and that information proved beneficial in analyzing the persistence of these fungicides in the field (1). Fungicide ED_{50} values for *in vitro* resistant and sensitive isolates were found to be very similar in preliminary work (1). This correlates with findings in fungicide-treated microplots where such isolates were controlled to the same degree as were sensitive field isolates (3).

The strength of the excised stem technique is that many breeding lines, fungicides, or isolates can be evaluated rapidly with only a small amount of plant material. This is a major benefit during early screening procedures when compared to field studies which require a large investment of land, labor, and time. Another advantage of this method is that it more closely simulates field conditions than other *in vitro* tests such as mycelial growth on fungicide-amended agar. Data in these tests indicate that *in vitro* resistance expressed on synthetic media is not expressed in the parasitic phase of pathogen growth on peanut tissues.

As was pointed out by Melouk et. al. (6), these tests are not a substitute for field evaluation since reactions there may be different. But, considering the speed, simplicity and adaptability of this method, it should prove to be useful for research on Sclerotinia blight of peanut.

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PERFORMANCE CHARACTERISTICS OF DICLORAN, IPRODIONE AND VINCLOZOLIN FOR CONTROL OF SCLEROTINIA BLIGHT OF PEANUT.

INTRODUCTION

Sclerotinia minor (Jagger) Kohn (5) has become one of the most destructive pathogens of peanuts in several peanut producing areas. Growers in Virginia and northeastern North Carolina where the disease was first found (9), have experienced heavy losses to Sclerotinia blight in the last decade.

There is an urgent need for effective fungicides to reduce crop losses and slow the continued spread of the fungus. Among the products tested to date, the dicarboximide fungicides have shown the highest degree of fungitoxicity to *S. minor*. Although the efficacy of these fungicides has been demonstrated (Chapter 1), our understanding of their performance in the field is incomplete. For example, laboratory determinations of the fungitoxicity of dicloran, iprodione and vinclozolin to *S. minor* would indicate that considerably more dicloran would be required to achieve disease control equivalent to that of the other two fungicides (2). Higher rates of dicloran are recommended, but not to the degree that would be expected.

Another area of uncertainty is that of application strategy. Thorough coverage across the row and penetration to the soil are thought to be important factors in fungicide application to peanut (P. M. Phipps, personal communication). However, the question of fungicide persistence,

which influences treatment interval, has not been addressed quantitatively. Dicloran, iprodione and vinclozolin have been recommended for application at the initial onset of disease and then at 4-week intervals until harvest. The high cost per fungicide treatment coupled with the early appearance of disease symptoms in recent years have made this approach expensive. A better understanding of the activity of these fungicides and the factors associated with their persistence in the field would be useful in refining current recommendations.

The objectives of this study were to 1) determine the persistence of dicloran, iprodione and vinclozolin on peanut stems under field conditions, 2) identify the environmental factors associated with decreased fungicide activity over time, 3) quantify the toxicity of these fungicides on peanut stems, and 4) gain a better understanding of the behavior of these fungicides in the field.

MATERIALS AND METHODS

FUNGICIDES AND THEIR PERSISTENCE. The following products were used: dicloran, iprodione and vinclozolin (Appendix F). Glucose yeast-extract agar (GYEA) was used for culture maintenance and production of inoculum (Appendix G). S-2 was the isolate of *S. minor* used in this bioassay (Appendix H).

'Florigiant' peanuts about 15-weeks-old and planted in rows 0.9-m apart were divided into plots 2.4-m long for fungicide treatment. The upright stems on one side of the row were pulled back to expose the fairly uniform, prostrate, lateral stems. After removing loose debris, these

lateral stems were carefully rinsed with water using a hand-held plant sprayer (Terra Verde Plant and Garden Sprayer). Fungicide suspensions in tap water were then applied to the stems until runoff with a similar sprayer. Concentrations used were 10.0, 3.3, and 2.5 mg/ml for dicloran, iprodione and vinclozolin, respectively. These are the concentrations used currently for iprodione and used previously for dicloran and vinclozolin to achieve disease control in commercial fields.

The following bioassay was developed to quantify fungicide residues on the stems. Treated stems were randomly selected in each plot, cut at the basal end, and brought into the lab. All leaves and pegs were trimmed flush with the stem and an 85-mm segment was cut from the median portion. Each segment had a node in the center which served as the inoculation site. A 5-mm-dia. agar plug with mycelium from the periphery of an actively growing colony of S. minor on GYEA was placed at this node directly on the wound created when the stem was trimmed. Inoculated stem segments were incubated in moist chambers at 18 C and by day 2, distinct, water-soaked lesions were visible on stems not treated with fungicide. Lesions were measured daily to determine colonization rates. After 4 days incubation, the inhibition by each fungicide was determined by comparison of lesion lengths on treated stems and untreated stems. The ability of each fungicide to prevent infection, i.e. the fungicidal effect, was analyzed by comparing the number of non-zero values in each treatment via the Fisher's Exact Test (4). The Wilcoxon Rank Sum Test (4) was used to determine the ability of each fungicide to inhibit colonization once a lesion was initiated, i.e. the inhibition effect. This test compared only the nonzero values for lesion length within each treatment. A rank LSD

procedure based on the Kruskal-Wallis test was used to evaluate the experimentwise error rate of each data set (6).

The bioassays for fungicide activity were conducted immediately after treating the stems and then at 1-week intervals to measure change in fungicide activity over a period of 4 weeks. This test was conducted once in 1984 with six replications per treatment and twice in 1985 with eight and nine replications, respectively.

ENVIRONMENTAL FACTORS. Meterological conditions during the course of the experiment were recorded at the field site by a computerized weather monitoring unit of Virginia's Agro-Environmental Monitoring System (13).

Sunlight is thought to be one of the most important physical determinants of the fate of pesticides in nature (7). Therefore, solar radiation and rainfall, another factor of known importance, were two parameters of greatest interest. Cumulative data for both factors were obtained and the product moment (Pearson) correlation found between each and the decrease in fungicide activity over time. The correlation between time and the change in fungicide activity was also determined.

ED_{50} DETERMINATIONS. Uniform, lateral stems of 13-week-old 'Florigiant' peanut plants were cut in the field and trimmed in the laboratory as described earlier. The stem segments were rinsed in tap water, submerged in fungicide suspensions of varying concentration for 1 minute, and then inoculated as before with S. minor. Isolate S-2 was used along with isolate R-2C which originated as a fungicide-resistant subculture from S-2 during in vitro fungicide testing. Again lesion lengths were measured at day four and the percent inhibition for each fungicide con-

centration found by comparing lesion lengths on treated and untreated stems. Levels of inhibition were plotted as a function of fungicide concentration and linear regression analysis used to determine dosage levels for 50% inhibition of growth (ED_{50} values).

RESULTS

FUNGICIDE PERSISTENCE. None of the fungicide-treated stems assayed immediately after treatment developed lesions, but the activity of all fungicides decreased in subsequent assays during the course of the experiment (Figure 4 on page 64). Distinct differences in fungicides were recognized. The Fisher's Exact Test demonstrated that dicloran did not retain significant fungicidal activity 1 week after application (Table 14 on page 65). Iprodione was significantly fungicidal for 2 weeks and vinclozolin for 3 weeks. None of the fungicides prevented lesion development at 4 weeks. Data shown are for the 1984 test. Results obtained in both 1985 tests were very similar, the one exception being that iprodione and vinclozolin both remained significantly fungicidal all four weeks in one of those tests.

Dicloran was the least fungicidal compound, but was the most effective inhibitor of lesion expansion according to the Wilcoxon Rank Sum Test (Table 15 on page 66). This inhibition of lesion elongation was significant (P=0.05) through 3 weeks. By contrast, once lesions formed on stems treated with iprodione or vinclozolin, their rate of elongation was not significantly different from that on untreated stems. Again data shown are for the 1984 test. The same trends were evident in both 1985

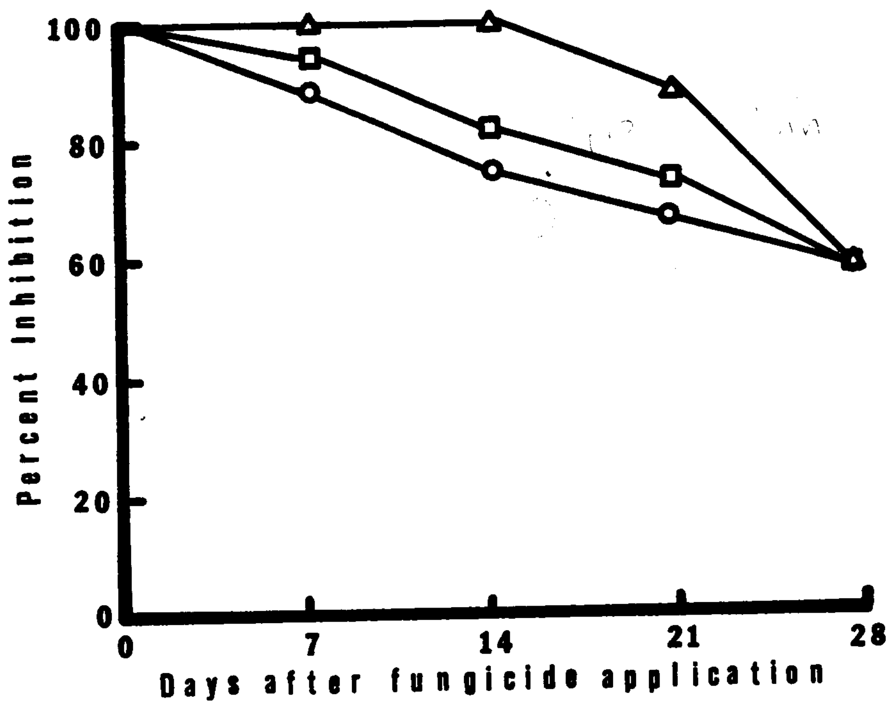

Figure 4. Persistence of fungicides on peanut stems in the field. [Data are the mean of three tests. Treatments are dicloran (O), iprodione (□) and vinclozolin (△)].

Table 14. Efficacy of dicloran, iprodione and vinclozolin in preventing lesion development on peanut stems (1984 test).

Treatment	Number of Bioassay Stems with Lesions				
	Day 0[1]	Day 7	Day 14	Day 21	Day 29
Check	6 A[2]	6 A	6 A	6 A	6 A
Dicloran (3.37 kg/ha)	0 B	3 AB	3 AB	6 A	4 A
Iprodione (1.12 kg/ha)	0 B	1 B	2 B	5 AB	5 A
Vinclozolin (0.84 kg/ha)	0 B	0 B	0 B	2 B	4 A

[1] Day of bioassay with *Sclerotinia minor* after fungicide application.

[2] Values in columns followed by the same letter(s) are not significantly different at P=0.05 according to Fisher's Exact Test. A total of six stems per treatment were bioassayed on each date.

Table 15. Efficacy of dicloran, iprodione and vinclozolin in suppressing lesion elongation on peanut stems (1984 test).

Treatment	Lesion Length									
	Day 0[1]		Day 7		Day 14		Day 21		Day 29	
Check	42.0	A[2]	60.2	A	57.7	A	41.0	A	56.3	A
Dicloran (3.37 kg/ha)	-[3]		8.0	B	7.0	B	16.8	B	49.5	A
Iprodione (1.12 kg/ha)	-		36.0	A	43.0	A	27.4	A	47.4	A
Vinclozolin (0.84 kg/ha)	-		-		-		22.5	A	46.8	A

[1] Day of bioassay with *Sclerotinia minor* after fungicide application.

[2] Means in columns followed by the same letter(s) are not significantly different according to the Wilcoxon Rank Sum Test and the Kruskal Wallis test, both at P=0.05.

A total of six stems per treatment were bioassayed on each date, but only stems with lesions were used to calculate means.

[3] '-' indicates that no lesions were formed.

tests except that dicloran remained significantly inhibitory all four weeks.

The Kruskal-Wallis one-way ANOVA by ranks was significant at P=0.005 for all data sets.

ENVIRONMENTAL FACTORS. Accumulated solar radiation for days 7, 14, 21 and 28 varied from a low of 2248, 5600, 8849 and 11,900 langleys, respectively in the first 1985 test to a high of 2886, 6326, 9918 and 13,103 langleys in the second 1985 test. Accumulated precipitation for the same periods varied from a low of 1.76, 2.37, 2.37 and 2.37 cm, respectively in the second 1985 test to a high of 2.39, 3.13, 3.16 and 9.29 cm in the first 1985 test. The correlation coefficients relating these environmental factors to the length of lesions in bioassays were found. Although all coefficients were significant (P=0.01), none indicated a high degree of correlation. Also, there was excellent correlation between these factors which makes it difficult to hypothesize as to their individual effect.

ED_{50} DETERMINATIONS. Illustrated in Figure 5 on page 68 are the dosage-response curves for isolate S-2 to dicloran and vinclozolin with the ED_{50} values being 33.6 and 5.8 µg/ml, respectively. Isolate R-2C responded similarly with ED_{50}'s of 44.9 and 5.8 µg/ml for dicloran and vinclozolin, respectively. All curves fit a linear model with R^2 values between 0.92-0.99. Slopes were calculated and the mean slope for the two isolates was 1.4 and 10.1 for dicloran and vinclozolin, respectively. The test was repeated with similar results. Iprodione was evaluated in a similar but separate experiment with isolate S-2. ED_{50} and slope values for iprodione were 8.7 µg/ml and 5.3, respectively.

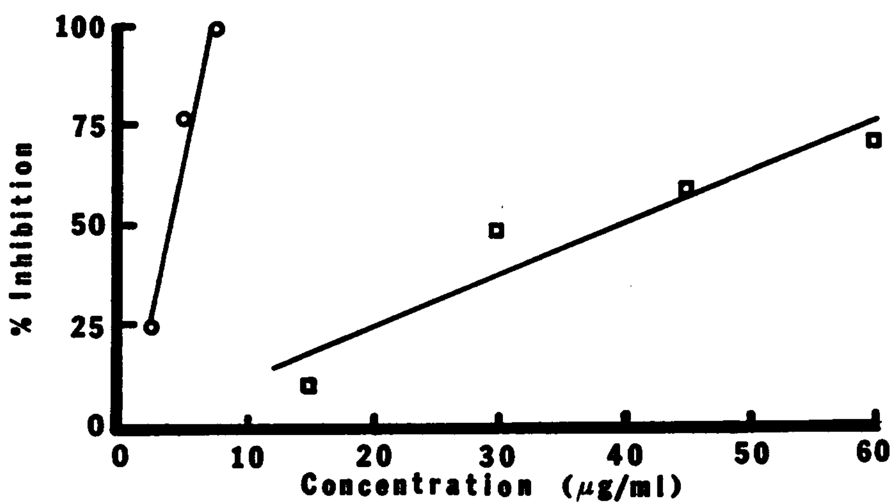

Figure 5. Sensitivity of Sclerotinia minor isolate S-2 to various concentrations of fungicides on peanut stems. : [Treatments are dicloran (▫) and vinclozolin (o)]

DISCUSSION

Although all three fungicides have been reported to suppress disease development in the field (3,8,Chapter 1), results of the current study suggest that dicloran acts by a mechanism distinctly different from that of either iprodione or vinclozolin. The activity of dicloran appears to be fungistatic in nature, as indicated by the fact that it significantly inhibited lesion expansion for 3 weeks and was fungicidal only immediately after application. These data are further supported by laboratory studies on fungicide-amended agar wherein up to 100 µg/ml of dicloran was not fungicidal to S. minor (unpublished data). Concentrations of less than 10 µg/ml of iprodione or vinclozolin were fungicidal in similar tests with fungicide-amended agar. Dicloran is an aromatic-hydrocarbon whereas iprodione and vinclozolin are both dicarboximides. All have a similar spectrum of microbial activity, but their primary mode of action has not been defined (10). Data from the current study suggest that there may be fundamental differences in the mode of action of these fungicides.

ED_{50} determinations with excised stems, although not completely analagous to the field situation, have quantified the relative toxicities of these fungicides and verified previous sensitivity studies on fungicide-amended GYEA (2). Of equal significance in these tests is the difference in slope of the dosage-response curves of the fungicides. The mean slope for vinclozolin was more than seven times steeper than that for dicloran. This means that to achieve a similar change in the percent inhibition by these fungicides, much larger changes in concentration are

required for dicloran than for vinclozolin. For example, a decrease from 60 to 30 μg/ml of dicloran resulted in about the same change in activity, ie. from 80% to 40% inhibition, as did a decrease from 6 to 3 μg/ml of vinclozolin (Figure 5 on page 68). This may explain the superior activity of dicloran in suppressing lesion elongation as opposed to the "all or nothing" phenomenon evident with iprodione and vinclozolin (Table 15 on page 66).

Although not of direct consequence to this study, it is interesting to note the similar ED_{50} values for isolate S-2 and isolate R-2C. This indicates that *in vitro* fungicide resistance is not equivalent to *in vivo* resistance. This conclusion is supported by experiments conducted in field microplots (3).

It should also be noted that this study evaluated only the effect of fungicide that remains on the plant. Since *S. minor* is a soilborne pathogen, it may be that the soil surface is also an important target for fungicide treatment. Indeed, studies with PCNB indicate that the soil-applied granular formulation is more effective in suppression of Sclerotinia blight than is the wettable powder (8).

Many factors are known to influence the persistence of a pesticide on a plant. Burchfield (1) lists 1) decomposition by hydrolysis, photolysis, microbes, etc., 2) volatility, 3) tenacity or resistance to displacement, and 4) plant growth. The current study does not address all aspects of this complex subject. A better understanding of the factors impinging upon a given fungicide in the field would be valuable in predicting the duration of disease control. Some of these factors are predictable and dependent on the physicochemical traits of a fungicide.

Examples include volatility, tenacity and decomposition. The effects of plant growth are also predictable and should not have had a major impact on this study since the plants were 15 weeks old at the time of fungicide application. Environmental influences, however, may vary widely but can also be monitored. This research has provided important information on persistence and activity of fungicides for control of Sclerotinia blight. Although some definite trends were established, more work is needed to construct models that predict fungicide persistence under field conditions.

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SYNOPSIS

Sclerotinia blight, caused by *Sclerotinia minor*, is a severe disease of peanut in Virginia. Vinclozolin, iprodione, dicloran, and pentachloronitrobenzene (PCNB) were evaluated for their fungitoxicity to *S. minor*. The mean ED_{50} values for five isolates were found to be 0.07, 0.11, 0.91, and 1.27 µg/ml, for vinclozolin, iprodione, dicloran, and PCNB, respectively, on fungicide-amended glucose yeast-extract agar (GYEA). Fungicide-resistant growth sectors developed on media amended with iprodione or vinclozolin. Nine such strains occurred; they were capable of growth on GYEA amended with up to 1000 µg/ml of I or V, and were cross-resistant to dicloran or PCNB. Resistance was maintained in all but two strains after repeated culture in the absence of fungicide for 3 years.

In field microplots, two resistant strains were pathogenic to peanut and survived as well as a fungicide-sensitive field isolate. Dicloran, iprodione and vinclozolin were applied to peanuts in the microplots for 3 years at total annual rates of 8.41, 3.36, and 2.52 kg/ha, respectively. Disease severity caused by the resistant strains was suppressed 19, 33, and 87% by dicloran, iprodione, and vinclozolin, respectively, as compared to 15, 24, and 76% for the sensitive isolate. Isolates recovered from tissue biopsies still grew on fungicide-amended GYEA indicating that *in vitro* and *in vivo* resistance are not equivalent in this case. Fungicide treatments reduced sclerotial populations of all strains, and reduced the viability of sclerotia from sensitive but not

resistant strains. Fungicide-resistant strains were capable of surviving and competing pathogenically in microplots infested with equal numbers of sclerotia from a sensitive and a resistant strain; this trend was enhanced by fungicide applications. A survey of 763 isolates from fields treated with these fungicides failed to detect resistant strains. One fungicide-resistant isolate was recovered from an iprodione-treated microplot originally infested with a sensitive field isolate.

A technique utilizing excised peanut stems was devised to evaluate isolate pathogenicity, cultivar resistance to the disease, susceptibility of different age peanut tissues, and fungicide persistence on peanut stems in the field. The method was also used to screen fungicides; results verified previous findings which indicated that *in vitro* resistance is not equivalent to *in vivo* resistance. Resistance to these fungicides may eventually become a field problem, but with correct management they should provide years of disease control.

Hopefully this research has increased our knowledge of several factors impinging upon the management of Sclerotinia blight with fungicides. Chapters one and two contribute specifically in the area of formulating use rates and patterns to achieve maximum efficiency while avoiding the development of fungicide resistance. Chapter four is also of significance in this area and serves to expand our understanding of how these fungicides can suppress disease. Finally, the method described in chapter three is quite adaptable and should find application in the future study of various aspects of this plant-pathogen interaction.

APPENDIX A. INFLUENCE OF SUBSTRATE ON EXPRESSION OF DICARBOXIMIDE RESISTANCE BY SCLEROTINIA MINOR IN VITRO.

In vitro resistance of *Sclerotinia minor* to the dicarboximide fungicide vinclozolin appeared to be related to the culture substrate utilized. Growth studies were conducted utilizing a common nutrient medium consisting of 0.2 mg iron, 0.2 mg zinc, 0.1 mg manganese, 1.0 g KH_2PO_4, 0.5 g $MgSO_47H_2O$, 100 µg thiamine HCl, 12.0 g agar and 1.0 l distilled water. Various carbon and nitrogen sources were used, each adjusted to give a constant C:N ratio of 24:1. Since glucose had been used in our previous *in vitro* resistance studies, this was used as the common carbon source for three sources of nitrogen. The nitrogen was either in a nitrate form (sodium nitrate), amino acid form (L-asparagine), or an ammonia form (ammonium sulfate). Casein hydrolysate was selected as the common nitrogen source since it contains all the common amino acids. Carbon was provided as cellulose or pectin, two polysaccharides that are constituents of plants. The third carbon source was rhamnose, a simple sugar.

Sensitive isolate S-5 grew significantly faster on some C:N combinations than on others (Table 16 on page 77). It did not grow on any of the media amended with vinclozolin at 2 µg/ml. Resistant strain R-5B also grew at different rates on different C:N combinations (Table 17 on page 78). It was capable of growth on all media amended with vinclozolin although the rate of growth again varied depending on the medium.

Sclerotia were formed by both isolates on all nonamended media (Table 18 on page 79). The resistant strain, although able to grow on all amended media, was unable to produce sclerotia on the base medium containing casein hydrolysate as a nitrogen source and either cellulose or rhamnose as a carbon source.

The substrate also influenced rates of mutation for resistance; resistant sectors developed only on media providing glucose as a carbon source with sodium nitrate, L-asparagine, or ammonium sulfate as a nitrogen source. Earlier studies showed that resistant sectors also could develop on glucose yeast-extract agar (Chapter 1). It is apparent that nutrition of the fungus may indeed play a role in the occurrence and expression of resistance.

Table 16. Effect of substrate on growth and fungicide sensitivity of *Sclerotinia minor* strain S-5.

Medium	Growth at Day 5 (mm)	
	Nonamended	Vinclozolin (2 µg/ml)
Casein hydrolysate		
+ Cellulose	52.2 A[1]	0.0
+ Pectin	32.3 B	0.0
+ Rhamnose	28.2 D	0.0
Glucose		
+ Sodium nitrate	32.0 B	0.0
+ L-Asparagine	29.8 CD	0.0
+ Ammonium sulfate	25.5 E	0.0
Glucose +		
Casein hydrolysate	31.3 BC	0.0

[1] Data are the means of five replicates. Means in columns followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P=0.05).

Table 17. Effect of substrate on growth and fungicide sensitivity of *Sclerotinia minor* strain R-5B.

Medium	Growth at Day 5 (mm)	
	Nonamended	Vinclozolin (2µg/ml)
Casein hydrolysate		
+ Cellulose	40.5 A[1]	22.2 BC
+ Pectin	41.8 A	17.0 D
+ Rhamnose	30.3 D	23.0 B
Glucose		
+ Sodium nitrate	41.3 A	31.3 A
+ L-Asparagine	37.3 B	28.7 A
+ Ammonium sulfate	34.2 C	17.8 D
Glucose +		
Casein hydrolysate	32.2 CD	19.2 CD

[1] Data are the means of five reps. Means in columns followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P=0.05).

Table 18. Effect of substrate on formation of sclerotia by *Sclerotinia minor* strains S-5 and R-5B.

	Sclerotia formed per 15.9 cm^2		
Medium	S-5 (check)	R-5B (check)	R-5B (vinclozolin)
Casein hydrolysate			
+ Cellulose	43.3 E[1]	45.7 E	0.0 D
+ Pectin	53.7 D	63.7 D	43.5 C
+ Rhamnose	134.7 A	119.3 B	0.0 D
Glucose			
+ Sodium nitrate	124.3 B	138.7 A	106.2 A
+ L-Asparagine	143.7 A	119.8 B	76.0 B
+ Ammonium sulfate	108.7 C	122.2 B	68.3 B
Glucose +			
Casein hydrolysate	114.3 C	107.5 C	38.5 C

[1] Data in columns followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P=0.05).

APPENDIX B. THE OSMOTIC SENSITIVITY OF FUNGICIDE-SENSITIVE AND -RESISTANT STRAINS OF SCLEROTINIA MINOR.

In the paper by Beever cited in chapter one of this dissertation, the criterion for a fungus being classified as abnormally sensitive to osmotic pressure was growth inhibition on a medium amended with 0.68 M sodium chloride. Such abnormal sensitivity is strongly correlated with resistance to dicarboximide fungicides in several other fungi. The study reported here utilized GYEA as the basal medium amended with 0.68 M or 0.34 M sodium chloride. Plates were inoculated, incubated, and the mycelial growth measured as described previously for the _in vitro_ fungicide testing.

Results are given (Table 19 on page 81) for the seven _S. minor_ isolates evaluated. Two fungicide-resistant strains (R-1B and R-5B) were inhibited by 0.34 M sodium chloride whereas none of the sensitive strains were. In fact, two sensitive strains actually showed increased growth on GYEA amended with the lower concentration of salt. However, at 0.68 M sodium chloride all strains of the fungus were significantly inhibited. Comparing the mean growth for all fungicide-sensitive isolates with that of fungicide-resistant strains demonstrates that while resistant strains may be somewhat more sensitive to osmotic stress, the correlation of osmotic sensitivity and dicarboximide resistance does not appear to be as distinct as reported in other fungi.

Table 19. Growth of *Sclerotinia minor* isolates on GYEA amended with sodium chloride.

	Mycelial Growth[1]		
Isolate[2]	Check	0.34 M NaCl	0.68 M NaCl
S-1	54.0 A[3]	55.2 A	39.0 B
R-1B	34.0 A	11.6 B	3.8 C
S-2	53.0 B	58.8 A	41.4 C
R-2B	48.6 A	48.0 A	32.6 B
R-2C	46.8 A	44.6 A	30.8 B
S-5	44.2 B	49.2 A	32.0 C
R-5B	38.6 A	27.0 B	17.4 C
Mean of sensitive	50.4 ± 5.4	54.4 ± 4.8	37.5 ± 4.9
Mean of resistant	42.0 ± 6.9	32.8 ± 16.9	21.2 ± 13.4

[1] Growth (mm) at day 3 after inoculation. Mean of five replications.

[2] 'S' indicates a sensitive field isolate and 'R' indicates a fungicide-resistant strain of *S. minor*.

[3] Mean separation within rows by Duncan's multiple range test (P=0.05).

APPENDIX C. THERMAL SENSITIVITY OF SCLEROTIA OF SCLEROTINIA MINOR.

The thermal sensitivity of sclerotia was another trait investigated in the characterization of fungicide-sensitive and -resistant strains of *S. minor*. It was intended to supplement evaluation of comparative survival traits but may have other applications such as indicating potential for use of solarization via plastic mulches to reduce inoculum levels in the soil.

The technique developed utilized glass test tubes (85 X 20 mm), each containing 10 ml of sterile distilled water, to hold the sclerotia during heating. Water was used in the tubes to facilitate heat transfer although preliminary tests indicated that heating sclerotia in dry tubes gave similar results. The tubes, each with 12 sclerotia, were immersed in a water bath for 10 minutes at various temperatures and then rapidly cooled on ice. The sclerotia were then removed aseptically and placed on GYEA plates. These plates were incubated at 20 C and the number that germinated myceliogenically recorded.

The first test compared sclerotia of one sensitive and two resistant isolates that had been produced on GYEA. Differences were found among isolates and the results three days after treatment are given in Figure 6 on page 87. It is evident that sensitive isolate S-2 was able to germinate much more rapidly than either resistant strain. However, Figure 7 on page 88 shows that by day seven after treatment, the trend was reversed and both resistant strains were in fact more heat tolerant than S-2 which was nearly eliminated at 47 C.

The next test evaluated sclerotia of isolate S-2 that had been produced on GYEA with sclerotia produced on autoclaved oats. Figure 8 on page 89 depicts the results for GYEA; in this case 47 C for 10 minutes was lethal and 45 C greatly delayed germination. In contrast, sclerotia produced on oats were more tolerant of thermal stress (Figure 9 on page 90). By day 7 over 50% of the sclerotia treated at 47 C had germinated.

The time of exposure to the heat was also lengthened to 12 hours and this dramatically lowered the temperature needed to achieve 100% kill. Both 41 C and 39 C were lethal (Table 20 on page 85); 37 C greatly slowed germination and ultimately only 33% of sclerotia treated at that temperature were viable. Even 35 C effectively delayed germination as compared to the untreated check.

The age of sclerotia at time of treatment was found to have significant impact on their thermal tolerance as well. This is evident from the data collected for five treatments over the course of seven sequential samplings at weekly intervals (Table 21 on page 86). Comparisons should be made within columns only in this table since a different number of days between treatment and determination of viability was used for each temperature; this was done in order to best illustrate the effect of each treatment.

Older sclerotia were found to germinate more rapidly. This is apparent when comparing sclerotia 1.5 weeks old with those 3.5 weeks old. At day two after treatment, the percent germination of untreated sclerotia was 25% for the former compared to 100% for the latter (Table 21 on page 86). Somewhat similar trends are seen at 41 C, 43 C and 45 C. The effect of sclerotial age was perhaps most striking at 47 C. None of the

sclerotia less than 5.5 weeks old remained viable after heat treatment. However, after 6.5 weeks 75% were viable and after 7.5 weeks 100% were viable. Although sclerotia are thought to mature much sooner, these data indicate that the ability to tolerate high temperatures continues to develop for at least 7.5 weeks.

Table 20. Effect of exposure to various temperatures for 12 hours on viablity of sclerotia.

	Percent Germination of Sclerotia[1]				
Temperature	2 Days[2]	4 Days	6 Days	8 Days	10 Days
41 C	0	0	0	0	0
39 C	0	0	0	0	0
37 C	0	8	17	33	33
35 C	17	58	83	83	92
Check	100	100	100	100	100

[1] Based on twelve sclerotia per treatment, isolate S-2.

[2] Number of days between treatment and determination of viability.

Table 21. Effect of sclerotial maturity on thermal sensitivity.

Age of sclerotia	Percent Germination of Sclerotia[1]				
	Check (2 D[2])	41 C (2 D)	43 C (3 D)	45 C (5 D)	47 C (8 D)
1.5 weeks	25	0	58	17	0
2.5 weeks	33	58	67	42	0
3.5 weeks	100	83	75	33	0
4.5 weeks	100	83	92	50	0
5.5 weeks	100	75	67	33	0
6.5 weeks	100	75	67	50	75
7.5 weeks	100	75	83	67	100

[1] Twelve sclerotia per treatment, isolate S-2.

[2] Number of days between treatment and determination of viability.

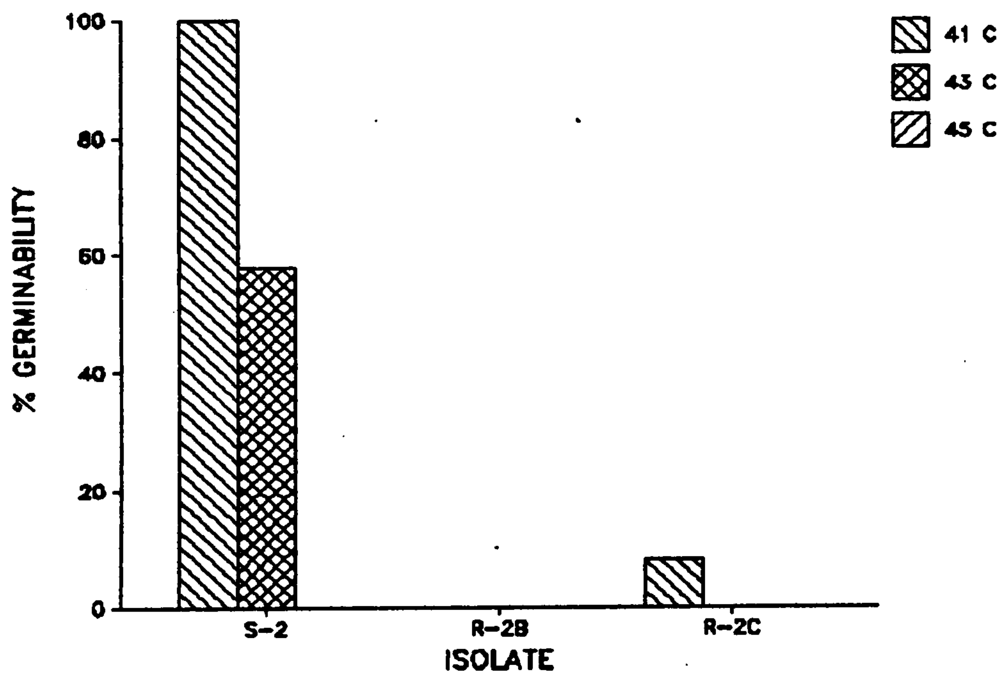

Figure 6. Percent germination of Sclerotinia minor sclerotia 3 days after heat treatment for 10 minutes.: (12 sclerotia per treatment produced on GYEA.)

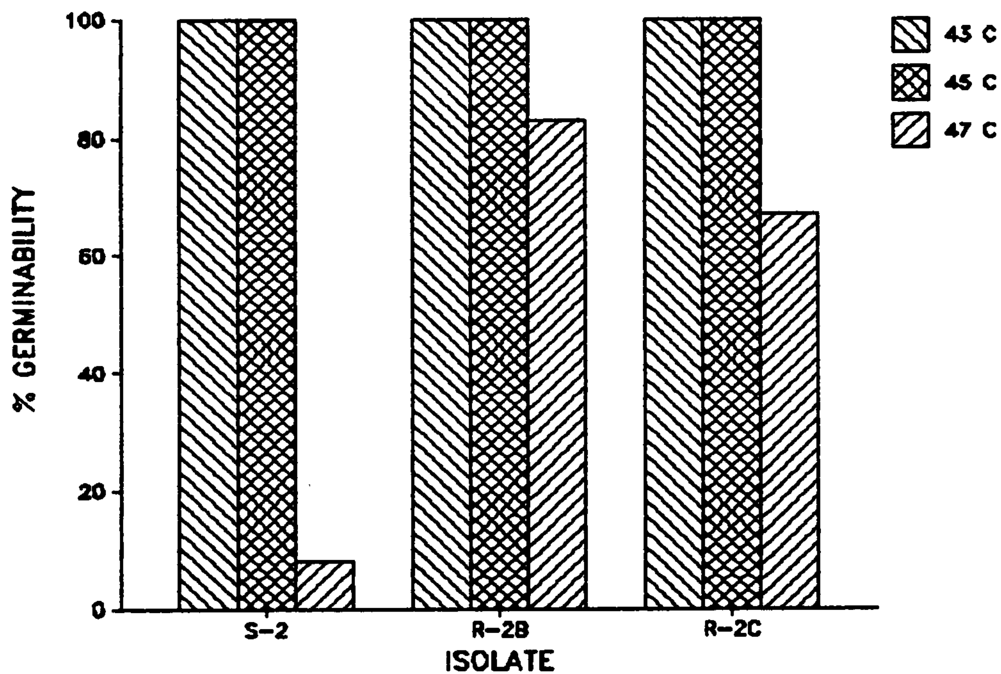

Figure 7. Percent germination of *Sclerotinia minor* sclerotia 7 days after heat treatment for 10 minutes.: (12 sclerotia per treatment produced on GYEA.)

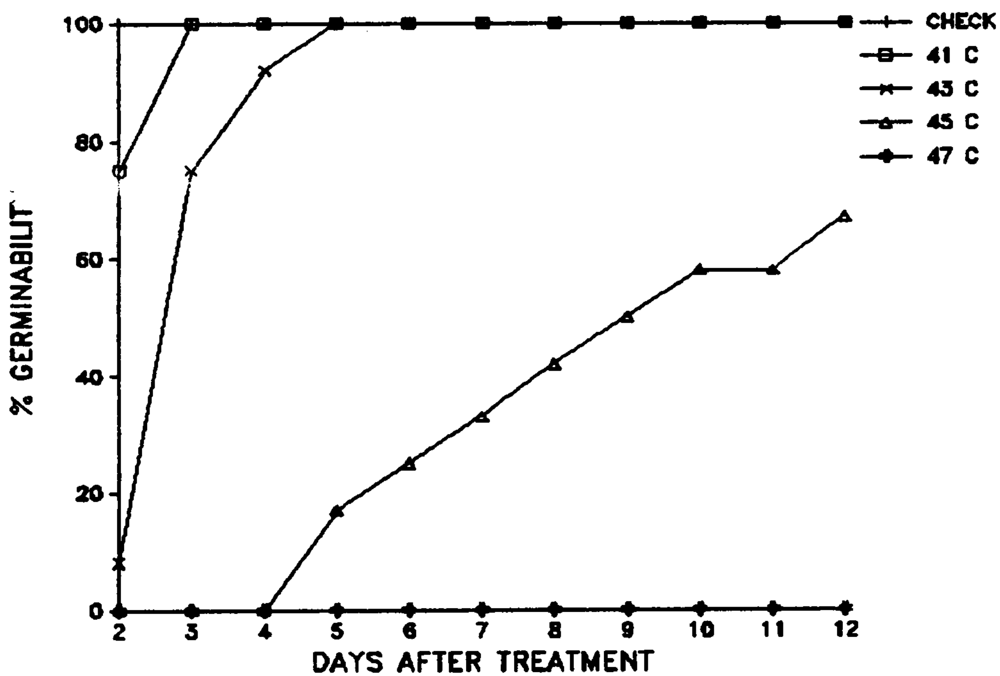

Figure 8. Thermal sensitivity of <u>Sclerotinia</u> <u>minor</u> sclerotia produced on GYEA.: (12 sclerotia per treatment, 10 min. at each temperature.)

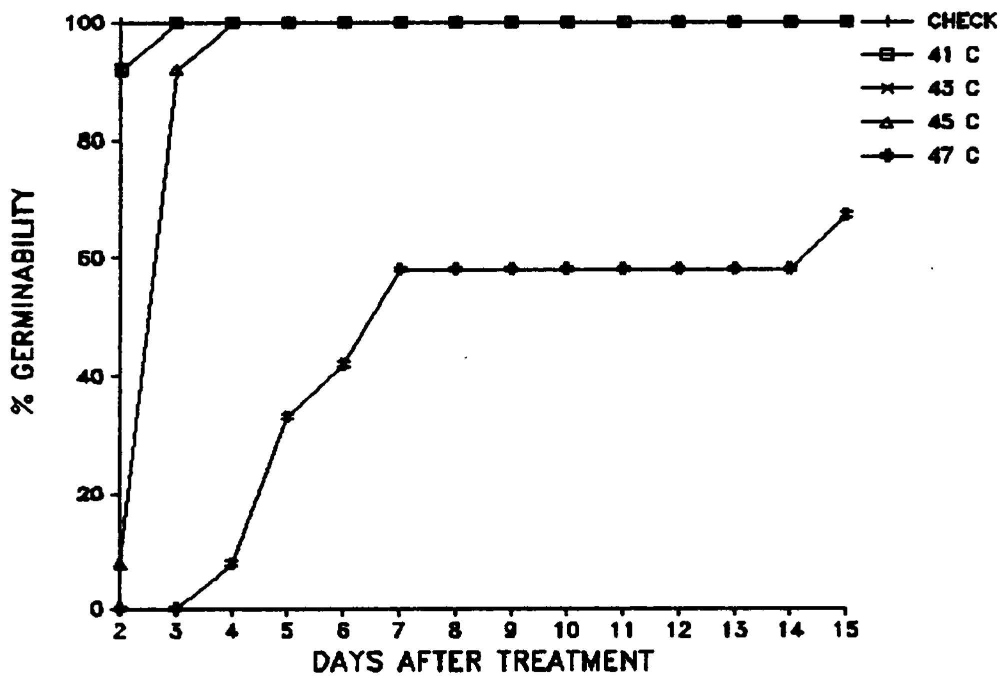

Figure 9. Thermal sensitivity of *Sclerotinia minor* sclerotia produced on oats.: (12 sclerotia per treatment, 10 min. at each temperature.)

APPENDIX D. PATHOGENICITY OF SCLEROTINIA MINOR TO PEANUT PLANTS IN A CONTROLLED ENVIRONMENT CHAMBER.

The pathogenicity of all nine resistant strains and the sensitive isolates from which they were derived was evaluated in a growth chamber study. Four-inch clay pots containing a 3:1 mix of sterilized soil and vermiculite were used, each containing three 'Florigiant' peanut seedlings. When the plants were four weeks old, they were wounded by making a uniform 1-cm slit at the base of each mainstem with a needle probe. A 6-mm-diameter plug of GYEA with actively growing mycelium of *S. minor* was placed directly on the wound and held in place by an additional 1.5 cm of moist vermiculite.

Pots were then placed in a controlled-environment chamber in a random order. Relative humidity near 100% was maintained by a water-mist system. Lights were on a 12-hour cycle and day temperatures set at 25 C (± 2 C) with night temperatures at 18 C (± 2 C).

Disease ratings were taken every other day for each plant using a 0-3 scale with 0=no disease and 3=collapse of the mainstem. A disease index was calculated for each isolate based on ratings taken 16 days after inoculation (Table 22 on page 93). These data show that all nine fungicide-resistant strains were capable of causing at least some disease. Although two of the sensitive isolates were approximately as pathogenic as their resistant subcultures, one (S-1) was unable to infect the stems in this test. S-1 had been isolated from a diseased peanut plant and kept in culture for 13 months prior to this test. The four

resistant strains obtained from it had also been kept in culture for about one year and expressed varying degrees of pathogenicity.

These results confirm that dicarboximide-resistant strains of *S. minor* are at least as pathogenic to peanut as are sensitive isolates.

Table 22. Disease indices of *Sclerotinia minor* isolates on peanut seedlings.[1]

Isolate	Disease Index[2]
S-2	68.9
R-2A	57.8
R-2B	68.9
R-2C	64.4
S-5	71.1
R-5A	62.2
R-5B	60.0
S-1	0.0
R-1A	11.1
R-1B	40.0
R-1C	53.3
R-1D	55.6

[1] Means of five pots with three plants each.

[2] Disease Index=100[Σ(class rating X class frequency)/total plants X 3].

APPENDIX E. MANAGEMENT PRACTICES IN FIELD MICROPLOTS

The following is a summary of the crop management practices applied to the two sets of microplots utilized in this study. Common names and chemical names of each product are given in Appendix F.

MIXED INOCULA MICROPLOTS

1984

- 5/11 Incorporated Temik 15G (14.6 kg/ha) at planting
- 7/1 Bravo 500 (2.3 kg/ha)
- 7/16 Bravo 500 (2.3 kg/ha)
- 7/24 Ronilan 50W (1.7 kg/ha) and landplaster (898 kg/ha)
- 8/3 Bravo 500 (2.3 kg/ha)
- 8/7 Sevin 80W (1.4 kg/ha) and Tecmangam (4.5 kg/ha)
- 8/21 Bravo 500 (2.3 kg/ha)
- 8/24 Ronilan 50W (1.7 kg/ha) and Sevin 80W (1.4 kg/ha)
- 8/31 Irrigated (2.54 cm water)
- 9/9 Irrigated (2.54 cm water)
- 9/10 Ronilan 50W (1.7 kg/ha)

1985

- 5/9 Incorporated Temik 15G (14.6 kg/ha) at planting
- 7/1 Bravo 500 (2.3 kg/ha), Sevin 80W (1.4 kg/ha), and landplaster (898 kg/ha)
- 7/7 Ronilan 50W (1.7 kg/ha)

7/30 Bravo 500 (2.3 kg/ha) and Tecmangam (4.5 kg/ha)

8/6 Ronilan 50W (1.7 kg/ha)

8/8 Sevin 80W (1.4 kg/ha) and Kylar 80W (1.12 kg/ha)

8/17 Irrigated (2.54 cm water)

8/23 Bravo 500 (2.3 kg/ha) and Lannate 1.7L (1.76 L/ha)

9/4 Ronilan 50W (1.7 kg/ha) and irrigated (2.54 cm water)

9/13 Sevin 80W (1.4 kg/ha), Kelthane 1.6EC (1.0 L/ha) and irrigated (2.54 cm water)

MICROPLOTS TREATED WITH DICLORAN, IPRODIONE OR VINCLOZOLIN

1983

5/14 Incorporated Temik 15G (14.6 kg/ha) at planting

6/23 Benlate 50W (.56 kg/ha) and Super Six (4.68 L/ha)

7/5 Landplaster (898 kg/ha)

7/7 Benlate 50W (.56 kg/ha) and Super Six (4.68 L/ha)

7/19 Botran 75W (4.49 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

8/5 Irrigated (2.54 cm water)

8/9 Kylar 80W (1.12 kg/ha)

8/17 Irrigated (2.54 cm water)

8/24 Botran 75W (3.37 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

8/25 Bravo 500 (2.3 kg/ha), Sevin 80W (1.4 kg/ha), and Kelthane 1.6EC (1.0 L/ha)

9/10 Irrigated (2.54 cm water)

9/12 Botran 75W (3.37 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

1984

5/11 Incorporated Temik 15G (14.6 kg/ha) at planting

7/1 Bravo 500 (2.3 kg/ha)

7/15 Botran 75W (4.49 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

7/16 Bravo 500 (2.3 kg/ha)

7/26 Landplaster (898 kg/ha)

8/3 Bravo 500 (2.3 kg/ha)

9/12 Botran 75W (3.37 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha). Also Sevin 80W (1.4 kg/ha) and Tecmangam (4.5 kg/ha)

8/21 Bravo 500 (2.3 kg/ha)

8/31 Lannate 1.8L (1.76 L/ha) and irrigated (2.54 cm water)

9/7 Botran 75W (3.37 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

1985

5/9 Incorporated Temik 15G (14.6 kg/ha) at planting

8/25 Bravo 500 (2.3 kg/ha), Sevin 80W (1.4 kg/ha), and landplaster (898 kg/ha)

7/7 Botran 75W (4.49 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

7/30 Bravo 500 (2.3 kg/ha) and Tecmangam (4.5 kg/ha)

8/6 Botran 75W (3.37 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

8/8 Kylar 80W (1.12 kg/ha) and Sevin 80W (1.4 kg/ha)

8/17 Irrigated (2.54 cm water)

8/23 Lannate 1.8L (1.76 L/ha) and Bravo 500 (2.3 kg/ha)

9/4 Botran 75W (3.37 kg/ha), Rovral 50W (2.25 kg/ha), or Ronilan 50W (1.7 kg/ha)

9/13 Sevin 80W (1.4 kg/ha) and Kelthane 1.6EC (1.0 L/ha)

9/15 Irrigated (2.54 cm water)

APPENDIX F. CROP MANAGEMENT PRODUCTS

The following crop management products were used during the course of this research. They are listed by trade name, common name, and chemical name.

1. Benlate, benomyl, methyl 1-(butylcarbamoyl)-2-benzimidazole-carbamate
2. Botran, dicloran or DCNA, 2,6-dichloro-4-nitroaniline
3. Bravo, chlorothalonil, tetrachloroisophthalonitrile
4. Kelthane, dicofol, 4,4'-dichloro-alpha-trichloro-methylbenzhydrol, or, 1,1-bis(chlorophenyl)-2,2,2-trichloroethanol
5. Kylar, daminozide, succinic acid 2,2-dimethyl hydraxide
6. Landplaster (bulk), gypsum, calcium sulfate
7. Lannate, methomyl, S-methyl-N-((methylcarbamoyl)oxy)-thioacetamide
8. Ronilan, vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione
9. Rovral, iprodione, 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboximide
10. Sevin, carbaryl, 1-napthyl-N-methylcarbamate
11. Spray-Aide, alkylaryl polyoxyethylene glycol phosphate ester
12. Super Six, sulfur
13. Tecmangam, manganese sulfate
14. Temik, aldicarb, 2-methyl-2(methylthio)propionaldehyde-O-(methylcarbamoyl)oxime
15. Terraclor, PCNB, pentachloronitrobenzene

APPENDIX G. MEDIA AND ANTIBIOTICS

Glucose yeast-extract agar (GYEA) was used throughout this study since it is composed of primarily synthetic components and is easily reproduced. This medium consisted of the following:

dextrose	20	g
yeast extract	2.0	g
KH_2PO_4	1.0	g
$MgSO_4 7H_2O$	0.5	g
agar	20	g
distilled water	1000	ml

The same medium was used for isolation of the fungus from diseased plant tissue with the exception that chloramphenicol and chlortetracycline HCl were each added at 100 µg/ml to minimize bacterial competition.

One part of this work where GYEA was not used was in the production of sclerotia for infestation of microplots. A soil and corn meal medium was utilized which was prepared as follows:

1. Screen air-dried field soil with a 20 mesh screen
2. Amend soil with corn meal (5% w/w)
3. Dispense 50 cc per 9-cm glass petri plate
4. Add 20 ml water per plate
5. Autoclave plates for 40 min. (15 psi and 120 C)

The influence of several other media on the occurrence and expression of dicarboximide resistance in _S. minor_ is discussed in Appendix A and the components of those media are listed there.

APPENDIX H. ORIGIN AND NOMENCLATURE OF ISOLATES

Five fungicide-sensitive field isolates of *Sclerotinia minor* were used in this study. Each was isolated from a diseased peanut plant from a different field in Virginia and designated as S-1 through S-5 to indicate their fungicide sensitivity. The origin of each isolate was:

S-1	Isolated 6/82	(M & L Farms, Sedley, VA)
S-2	Isolated 10/78	(Prince farm, Southampton Co., VA)
S-3	Isolated 7/82	(Jack Beale farm, Wakefield, VA)
S-4	Isolated 6/82	(Partridge farm, Dreweryville, VA)
S-5	Isolated 11/81	(Chappel's Store, Suffolk, VA)

During the course of *in vitro* fungicide sensitivity testing, nine strains of *S. minor* were subcultured from fungicide-resistant growth sectors on agar amended with iprodione or vinclozolin. These were labeled to afford reference back to the sensitive parent isolate by giving them the same number but preceeded by the prefix 'R'. They were also given another letter to allow differentiation where more than one resistant strain originated from a single sensitive isolate. These strains, and the fungicide concentration on which their formation occurred, are listed below:

R-1A	vinclozolin, 0.25 µg/ml
R-1B	iprodione, 1.0 µg/ml
R-1C	iprodione, 1.0 µg/ml
R-1D	iprodione, 0.25 µg/ml
R-2A	vinclozolin, 1.0 µg/ml

R-2B	vinclozolin, 2.0 µg/ml
R-2C	iprodione, 4.0 µg/ml
R-5A	vinclozolin, 4.0 µg/ml
R-5B	vinclozolin, 2.0 µg/ml

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