EVALUATION OF FUNGICIDE RESISTANCE IN *SCLEROTINIA MINOR* AND STRATEGIES FOR CHEMICAL CONTROL OF SCLEROTINIA BLIGHT OF PEANUT

by

Frisby Davis "Tad" Smith

Dissertation submitted to the Faculty of the

Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Plant Pathology

APPROVED:

Patrick M. Phipps, Co-chairman

R. Jay Stipes, Co-chairman

Anton B.A.M. Baudoin

Charles Hagedorn

Keith S. Yoder

December, 1990

Blacksburg, Virginia

EVALUATION OF FUNGICIDE RESISTANCE IN *SCLEROTINIA MINOR* AND STRATEGIES FOR CHEMICAL CONTROL OF SCLEROTINIA BLIGHT OF PEANUT

by

Frisby Davis "Tad" Smith

Patrick M. Phipps, Co-chairman

R. Jay Stipes, Co-chairman

Plant Pathology, Physiology and Weed Science

(ABSTRACT)

Testing several registered and experimental fungicides in the laboratory and field has resulted in the identification of two compounds possessing high levels of fungitoxicity to *Sclerotinia minor*, the causal agent of Sclerotinia blight of peanut. The two fungicides, ASC-66825 and RH-3486, are thought to have a different chemistry than the dicarboximide fungicides. The ED_{50} value (dose required for 50% inhibition of mycelial growth) of ASC-66825 and RH-3486 was 0.004 μg/ml and they were 45 times more fungitoxic to mycelial growth on glucose-yeast extract agar (GYEA) than iprodione. At simulated field rates (1.12 kg/ha), none of the experimental fungicides effectively inhibited sclerotial formation in soil-plate assays, whereas all the dicarboximide fungicides (chlozolinate, iprodione and vinclozolin) significantly inhibited sclerotial formation. No cross-resistance was detected between the dicarboximide fungicides and ASC-66825 or RH-3486. During three years of field tests, RH-3486 controlled Sclerotinia blight of peanut significantly better than iprodione. The spray adjuvant, pinolene (Nu-Film-17®), significantly improved the performance of iprodione (Rovral®) over 5 years of field tests. Average yields from plots treated with iprodione and pinolene were 365 kg/ha

higher and disease incidence 15% lower than plots treated with iprodione alone. Applying fungicides to experimental microplots infested with a pathogenic, dicarboximide-resistant isolate of *S. minor* (B-83-T2) indicated that fungicides still provided disease control in a field situation. Disease incidence was suppressed 96, 55, 62, 25 and 20% in microplots infested with isolate B-83-T2 and 97, 83, 33, 67 and 30% in plots infested with a sensitive isolate (S-2), following treatments with RH-3486, vinclozolin, iprodione, PCNB and dicloran, respectively. Sclerotia of *S. minor* from peanut fields treated with dicarboximides and other fungicides for leafspot control did not show field resistance to iprodione in spite of an *in vitro* resistance rate of 6.3% in GYEA tests containing 2 μg/ml of iprodione. Field resistance to iprodione does not seem to be a major threat to control of Sclerotinia blight of peanut with iprodione in Virginia. The use of chlorothalonil for leafspot control has been correlated with an increase in the incidence of Sclerotinia blight. Excised peanut stems obtained from plots exposed to field applications of chlorothalonil produced larger lesions after inoculation with *S. minor*, than stems from untreated plots. Treatment of excised stems with chlorothalonil just prior to inoculation did not enhance lesion development. However, cultures of *S. minor* conditioned on GYEA containing chlorothalonil at 10 μg/ml were more pathogenic on excised stems than unconditioned cultures. Chlorothalonil may increase the aggressiveness of *S. minor* by enhancing organic acid production without greatly inhibiting fungal growth.

ACKNOWLEDGEMENTS

The successful completion of this Ph.D. program could not have been obtained without guidance and direction from my major professor, Dr. Patrick M. Phipps, plant pathologist at the Tidewater Agricultural Experiment Station. Participating in an organized and well-funded research program contributed immensely to my success as a graduate student. The willingness of Dr. Phipps to intensively advise and guide my research aided in my transition from a graduate student to a plant pathologist.

During my stay in Blacksburg at Virginia Tech, I was fortunate to have had the opportunity to conduct research in the laboratory of Dr. R. Jay Stipes. His insights into the nature and responsibilities of life, the history of Virginia's magnificent trees and the role of plant pathology in our society gave my education a liberal arts flair. Many thanks go to Jean Ratliff for frequently and effectively fighting the bureaucracy of Virginia Tech on my behalf. Other committee members, Drs. Anton Baudoin, Charles Hagedorn and Keith Yoder, also contributed effort to this research program and have my sincere gratitude.

Sincere appreciation is due to all of the staff and faculty at the Tidewater Agricultural Experiment Station. In particular, Mike Arrington, Barron Britt and Steve Byrum deserve recognition for technical assistance and moral support during my graduate studies. Ramon Cu, a fellow graduate student, willingly provided assistance with statistical analyses of field data. Dr. Phipps graciously contributed the results of 1985 and 1986 spray adjuvant field data used in Chapter 2.

Finally, without the encouragement and financial support from my parents, I never could have survived the rigors of graduate studies.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

INTRODUCTION AND OBJECTIVES OF DISSERTATION

Description of research problem. Sclerotinia blight of peanut, caused by *Sclerotinia minor* (Jagger) Kohn (9), was first observed in Virginia in 1971 and one year later in North Carolina (13). Subsequently, the disease has become more severe and spread to peanut production areas in Oklahoma (19), New Mexico and Texas (17). During the 3-year period of this research (1987-1989), peanut diseases in Virginia claimed an average of 18.8% of the potential peanut crop each year (Figure 1A). Sclerotinia blight represented the biggest component of disease loss (Figure 1B) and was responsible for destroying approximately 6% of the peanut crop each year (P.M. Phipps, personal communication). Each percentage unit of yield loss equaled about a $1 million loss in potential farm income for Virginia peanut growers. Fungicides used to control Sclerotinia blight (mainly iprodione) represent a major production cost and currently provide only partial disease control. The heavy yield loss attributed to Sclerotinia blight and the lack of highly effective control strategies make the disease the most serious problem in peanut production for Virginia peanut growers.

Chemical control of Sclerotinia blight. Due to the absence of a high level of disease resistance in cultivars with acceptable market qualities, fungicides have been needed for control of Sclerotinia blight of peanut. In 1975 the chlorinated aromatic fungicides, dicloran and PCNB, were reported to give some control of the disease in field trials (2). Dicloran was used to control Sclerotinia blight as a result of eight emergency-use permits granted by the Virginia Department of Agriculture and the Environmental Protection Agency (EPA) from 1977 to 1984. PCNB now has a special local-need registration (Section 24-c, FIFRA) for

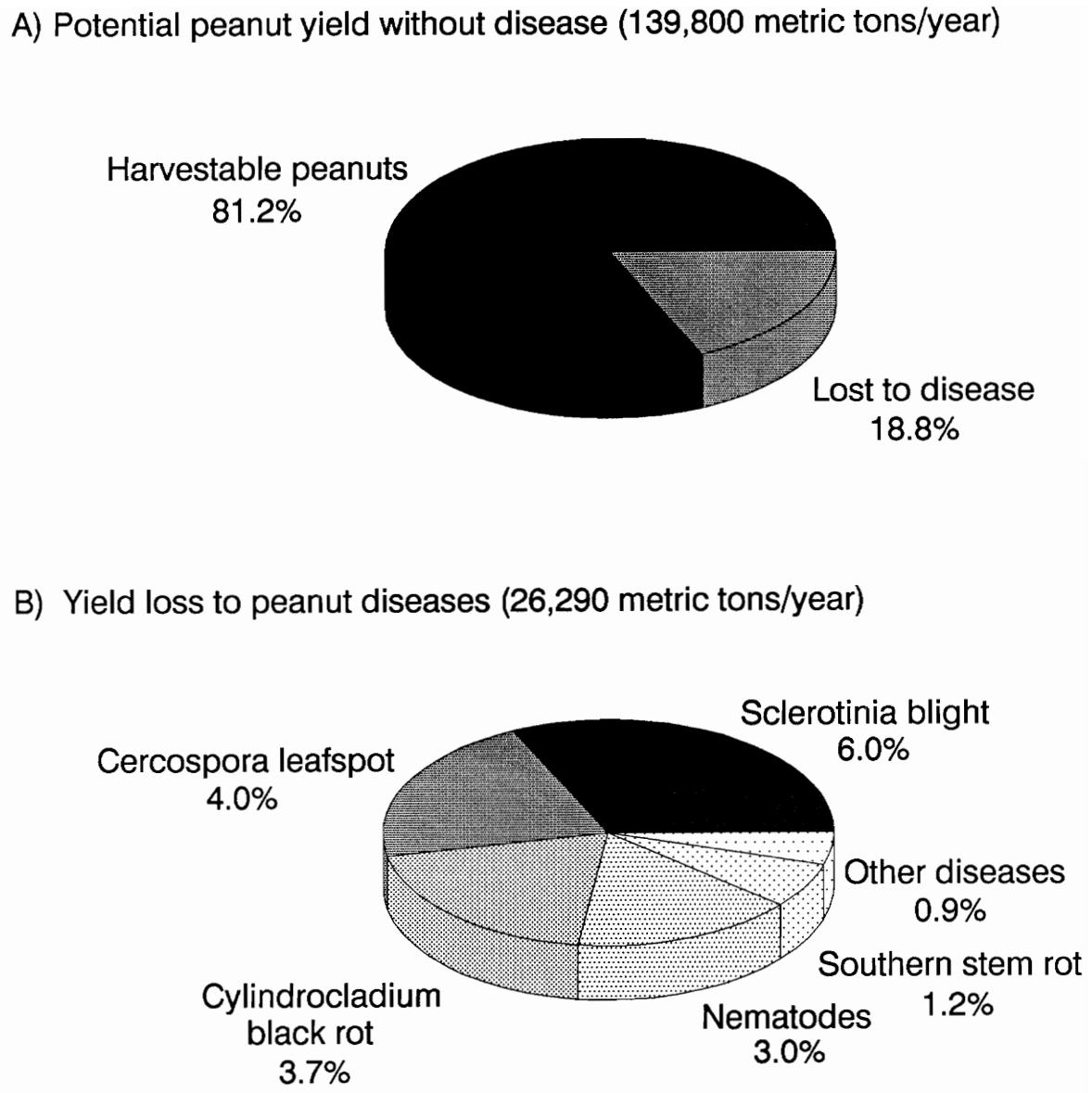

Figure 1. A) Percentage of harvestable peanuts and yield loss attributed to disease in Virginia during the period of 1987-1989. B) Specific diseases responsible for peanut yield loss expressed as the percentage of potential peanut yield claimed by each disease.

suppression of Sclerotinia blight in Virginia.

Much interest has been shown in a newer group of dicarboximide fungicides since their initial synthesis in Japan in the late 1960's. The older group of dicarboximide fungicides consisted of folpet, captafol and captan and are now known as phthalimide fungicides. Members of the new class of dicarboximide fungicides are particularly fungitoxic to *Sclerotinia* spp. and include iprodione and vinclozolin, compounds registered for use on several crops in the United States. After the efficacy and safety of certain of the dicarboximides were demonstrated, vinclozolin was used by peanut growers in Virginia during 1984 with Section-18 approval by the EPA. Iprodione received registration for use on peanut in 1985 and this fungicide has become the principal option for management of Sclerotinia blight. Registration of vinclozolin for disease control in peanuts still awaits EPA approval.

Current EPA regulations no longer require submission of efficacy data in addition to toxicology data for registration of fungicides. The idea behind this strategy is that the marketplace, and not government regulation, will determine the use of agrichemicals. In 1988, Tribasic® $CuSO_4$, and Tenn-Cop® 5E were labeled for suppression of Sclerotinia blight of peanut. Replicated field trials have not demonstrated the efficacy of copper compounds for control of the disease (10). Whether these copper compounds become competitive in the marketplace in light of their performance characteristics, remains to be determined.

Use of adjuvants to enhance fungicide performance. Spray adjuvants have been used to improve the physical characteristics of pesticides and performance in the field. Iprodione provides only partial control of Sclerotinia blight of peanut in spite of having a relatively high *in vitro* fungitoxicity to *S. minor* (3). Research on adjuvants may provide a much needed means of improving Sclerotinia blight control with registered fungicides. Pinolene appeared

to be an ideal adjuvant to examine as it is relatively non-toxic to animals (5) and has been shown to enhance the performance of fungicides for control of Cercospora leafspot on peanut (D.H. Smith, unpublished data). The active ingredient in pinolene is a polymer of di-1-p-menthene. The material has been promoted by its manufacturer as an extender-sticker-spreader which surrounds and holds the pesticide on sprayed areas of the plant, while reducing physical degradation by sunlight and high temperatures.

The threat of resistance to fungicides by *S. minor*. Several concerns have been raised regarding the use of dicarboximide fungicides for control of Sclerotinia blight. Poor disease control by iprodione has been reported from several sites in southeastern Virginia. A study of 763 isolates from 19 diseased fields failed to detect the development of iprodione- or vinclozolin-resistant strains (3). Isolates of *S. minor* obtained from three fungicide trials and two locations in 1986 where growers complained of poor disease control also did not show evidence of field resistance to iprodione (16). Thus, no dicarboximide-resistant isolates of *S. minor* have been reported from commercial fields in Virginia. The failure of iprodione to provide acceptable disease control may involve timing and methods of fungicide application.

When *S. minor* was exposed to the dicarboximides *in vitro*, a low frequency of resistant sectors developed on fungicide-amended agar. A resistance rate of 1.8% was observed when 300 cultures of *S. minor* were exposed to iprodione and vinclozolin (3). In spite of the ability of *S. minor* to develop *in vitro* resistance to dicarboximide fungicides, the threat posed by these resistant isolates may not be as great as previously thought. In microplots infested with two *in vitro* resistant isolates of *S. minor*, the dicarboximide fungicides were still able to control Sclerotinia blight of peanut (4). In the case of *Botrytis cinerea*, the acquisition of dicarboximide-resistance confers a loss of fitness on the fungal strain and may result in loss

of pathogenic abilities (1). Most isolates of *B. cinerea* with field resistance to the dicarboximides were reported to possess low-level resistance and were still controlled with applications of the dicarboximides.

To complicate the resistance situation, an *in-vivo* resistant isolate that was pathogenic to peanut was isolated from an iprodione-treated microplot in 1985 (4). This dicarboximide-resistant isolate, designated B-83-T2, has been a cause for concern as to the future of disease control with the dicarboximide fungicides. The continued appearance of isolates of *S. minor* with resistance to dicarboximide fungicides could result in widespread occurrence of resistant strains and greater problems in disease control. If high-level dicarboximide-resistant, pathogenic isolates of *S. minor* became established in a field, there would be no fungicides available to control the disease as the dicarboximides and chlorinated aromatic fungicides are remarkably similar in their mode of action (15). Isolates of *S. minor* with *in vitro* resistance to iprodione are capable of growth on media amended with iprodione, vinclozolin, dicloran and PCNB (3).

Sclerotinia blight enhancement by chlorothalonil. Peanuts are susceptible to many other plant pathogens besides *S. minor*. Control of early leafspot of peanut, caused by *Cercospora arachidicola* Hori, is necessary to produce a profitable crop in southeast Virginia. The favored means of leafspot control is the use of chlorothalonil, which is usually applied three to four times a year according to the Virginia Peanut Leafspot Advisory program (6,11). Unfortunately, applications of chlorothalonil at recommended rates for control of leafspot can significantly increase the severity of Sclerotinia blight (12). Chlorothalonil may enhance the disease by maintaining a dense peanut canopy which forms a microclimate conducive for Sclerotinia blight. The incidence and severity of Sclerotinia blight was reduced in peanut

plots that were manually defoliated (7).

Chlorothalonil may also have a direct effect on physical processes in *S. minor*. Inoculum of *S. minor* produced in the presence of 0.2 to 0.4 μg/ml of chlorothalonil for 5 days produced larger stem lesions on peanut plants after 24 hours than did inoculum grown in the absence of the fungicide (8). The observed lesions were not significantly larger on days 2, 3 and 4; however, the four-day-old cultures of *S. minor* exposed to chlorothalonil yielded filtrates containing more oxalic acid than similar cultures without fungicide. Oxalic acid has been implicated as having a role in the pathogenicity of the fungus. Cultures of *S. minor* are relatively insensitive to chlorothalonil *in vitro*; the fungus has an ED_{50} value of 25 μg/ml (14). Sectors insensitive to chlorothalonil developed in amended agar containing 1 and 2 μg/ml of fungicide at a frequency of 10 to 40%, but this ability to tolerate chlorothalonil ceased when the fungus began to differentiate sclerotia. Field resistance to chlorothalonil appears to be uncommon, probably due to its ability to interfere with numerous biological processes of the fungi by binding to sulfhydryl groups present on fungal enzymes (18).

Dissertation objectives. Chapters 1 and 2 deal with field performance of agrichemicals for control of Sclerotinia blight. Chapter 1 focuses on the performance characteristics of three dicarboximide fungicides and three experimental compounds in soil-plate, agar-plate and field tests for activity against *S. minor*. Chapter 2 concentrates on the performance of the dicarboximide fungicide iprodione, when used with various spray adjuvants for control of Sclerotinia blight of peanut. The objective was to identify compounds that might be useful for control of Sclerotinia blight of peanut.

Chapters 3 and 4 examine the threat of resistance following repeated dicarboximide usage for control of Sclerotinia blight. A 3-year field microplot study was conducted to

evaluate the effectiveness of iprodione, vinclozolin, dicloran, PCNB and RH-3486, to control disease caused by a dicarboximide-resistant isolate, B-83-T2, and its sensitive parent, isolate S-2 (Study I). Microplots previously established in 1983 and infested with two *in vitro* resistant isolates (R-2B and R-2C) of *S. minor* were maintained an additional 3 years (Study II) to observe the effect of repeated applications of fungicides (dicloran, iprodione and vinclozolin) on disease control. Thus, the goal of chapter 3 was to determine the pathogenicity of resistant isolates in a field situation. Chapter 4 reports the results of an assay of 1,200 sclerotia for resistance to iprodione. These sclerotia were collected from peanut plots exposed to heavy fungicide usage for control of both Cercospora leafspot and Sclerotinia blight. The purpose of chapter 4 was to determine whether exposure to leafspot fungicides induced cross-resistance to iprodione in *S. minor* and if field exposure to iprodione triggered the development of field resistance to this fungicide.

Chapter 5 investigates Sclerotinia blight enhancement in chlorothalonil-treated fields and the effect of chlorothalonil on the aggressiveness of *S. minor* using excised peanut stems. Both excised-stem and agar studies were used to examine a potential interaction between chlorothalonil and iprodione and the effect of the fungicides on pathogenicity of *S. minor*. Bromophenol blue in glucose-yeast extract agar (GYEA) was used to estimate acid production by the fungus during exposure to various concentrations of fungicides. The goal was to determine the mechanism of Sclerotinia blight enhancement by chlorothalonil.

LITERATURE CITED

1. Beever, R.E., and Brien, H.M.R. 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. New Zealand J. Agr. Res. 26:391-400.

2. Beute, M.K., Porter, D.M., and Hadley, B.A. 1975. Sclerotinia blight of peanut in North Carolina and Virginia and its chemical control. Plant Dis. Rep. 59:697-701.

3. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.

4. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Sclerotinia blight of peanut: Relationship between in vitro resistance and field efficacy of dicarboximide fungicides. Phytopathology 77:1028-1032.

5. Clarke, A.R. 1969. Pinolene - Toxicology. Pages 1-8 in: Pinolene - Toxicological and Biological Properties. Miller Chem. and Fert. Corp., Hanover, PA.

6. Cu, R.M., Phipps, P.M., Steele, J.L., and Stipes, R.J. 1988. Field performance of computerized advisories for fungicide application to control early leafspot of peanut. Proc. Am. Peanut Res. Educ. Soc. 20:32.

7. Dow, R.L., Powell, N.L., and Porter, D.M. 1988. Effects of modification of the plant canopy environment on Sclerotinia blight of peanut. Peanut Sci. 15:1-5.

8. Hau, F.C., and Beute, M.K. 1983. Effects of chlorothalonil on the virulence and physiology of a nontargeted pathogen, *Sclerotinia minor*. Phytopathology 73:475-479.

9. Kohn, L.M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.

10. Phipps, P.M. 1988. Control of Sclerotinia blight of peanut with experimental fungicides, 1987. Fungic. Nematicide Tests 43:221.

11. Phipps, P.M., and Powell, N.L. 1984. Evaluation of criteria for the utilization of peanut leafspot advisories in Virginia. Phytopathology 74:1189-1193.

12. Porter, D.M. 1980. Increased severity of Sclerotinia blight in peanuts treated with captafol and chlorothalonil. Plant Dis. 64:394-395.

13. Porter, D.M., and Beute, M.K. 1974. Sclerotinia blight of peanuts. Phytopathology 64:263-264.

14. Porter, D.M., and Lankow, R.K. 1981. Growth of *Sclerotinia minor* on media containing chlorothalonil and benomyl. Plant Dis. 65:591-594.

15. Sijpesteijn, A.K. 1982. Mechanism of action of fungicides. Pages 33-45 in: Fungicide Resistance in Crop Protection. J. Dekker and S.G. Georgopoulos, eds. Centre for Agriculture Publishing and Documentation, Wageningen, Netherlands.

16. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1987. Effect of field application of iprodione on Sclerotinia blight of peanut and the causal fungus, *Sclerotinia minor*. (Abstr.) Phytopathology 77:1748.

17. Sturgeon, R.V. 1986. Peanut disease loss estimates for major peanut producing states in the United States for 1984 and 1985. Proc. Am. Peanut Res. Educ. Soc. 18:24-26.

18. Vincent, P.G., and Sisler, H.D. 1968. Mechanism of antifungal action of 2,4,5,6-tetrachloroisophthalonitrile. Physiol. Plant. 21:1249-1264.

19. Wadsworth, D.F. 1979. Sclerotinia blight of peanuts in Oklahoma and occurrence of the sexual stage of the pathogen. Peanut Sci. 6:77-79.

AGAR PLATE, SOIL PLATE AND FIELD EVALUATION OF FUNGICIDES FOR CONTROL OF *SCLEROTINIA MINOR* ON PEANUT

ABSTRACT

The sensitivity of *Sclerotinia minor* to three dicarboximide fungicides (chlozolinate, iprodione and vinclozolin) and three experimental fungicides (ASC-66825, MON-13108 and RH-3486) was tested on fungicide-amended, glucose-yeast extract agar (GYEA). ED_{50} values (dose for 50% inhibition of mycelial growth) of a field isolate (S-2) were 0.004, 0.004, 0.025, 0.08, 0.18 and 0.38 μg/ml for ASC-66825, RH-3486, MON-13108, vinclozolin, iprodione and chlozolinate, respectively. An *in vivo*, dicarboximide-resistant isolate (B-83-T2) exhibited enhanced mycelial growth on GYEA with either iprodione or vinclozolin at 1.0 μg/ml, or chlozolinate at 5 μg/ml, but 1 μg/ml of RH-3486, ASC-66825 or MON-13108 suppressed growth by 92, 90 and 54%, respectively. At 100 μg/ml, vinclozolin, iprodione and chlozolinate inhibited growth of B-83-T2 by 93, 83 and 74%, respectively. When cultures of *S. minor* on a soil-corn meal medium were sprayed with fungicides to simulate an application rate of 1.12 kg a.i./ha, MON-13108 was the only treatment that did not greatly inhibit mycelial growth of S-2. ASC-66825 and RH-3486 significantly suppressed mycelial growth of B-83-T2 in soil plate tests. In replicated field trials, ASC-66825 and RH-3486 limited disease incidence and increased yield of peanuts more than other fungicides.

INTRODUCTION

Sclerotinia blight of peanut was reported in Virginia in 1971 and North Carolina in 1972 (17). The pathogen, *Sclerotinia minor* (Jagger) Kohn (8), is a soilborne fungus overwintering as sclerotia. In recent years, the disease has become more severe and spread to peanut production areas in Oklahoma (25), New Mexico and Texas (24). In 1987, a dry year and relatively unfavorable for Sclerotinia blight, yield suppression due to the disease was estimated to be 4% in Virginia (P.M. Phipps, personal communication). During 1988, a year with average precipitation and a cooler than normal September and October, conditions were extremely favorable for Sclerotinia blight, and the disease claimed 7% of the peanut crop. Heavy rains and above average temperatures during the summer of 1989 were conducive for early leafspot, caused by *Cercospora arachidicola* Hori, and the resulting defoliation limited damage attributed to Sclerotinia blight to only 7%; a value lower than expected. Average losses in farm income to Sclerotinia blight for the 3-year period were estimated to be $5.7 million/year.

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

Much interest has been shown in the newer class of dicarboximide fungicides since

their initial synthesis in the late 1960's. These fungicides are particularly active against species of *Botrytis* and *Sclerotinia* (6). Procymidone (DPX-4424) was reported to be extremely effective in controlling Sclerotinia blight of peanut (16), but research on this compound was discontinued in the U.S. in 1979. Vinclozolin was the first dicarboximide fungicide to be used by peanut growers in Virginia with Section-18 approval by the Virginia Department of Agriculture and EPA in 1984. Iprodione gained full registration for use on peanut in 1985. Since 1986, iprodione and PCNB have been the only recommended fungicides for control of Sclerotinia blight of peanut in Virginia and North Carolina.

Cross-resistance to fungicides increases the importance of any potential resistance problems with *S. minor* in peanuts. Isolates of *S. minor* that originally exhibited *in vitro* resistance to iprodione were capable of growth on media amended with either vinclozolin, dicloran or PCNB (5). The dicarboximides and chlorinated aromatic fungicides are remarkably similar in their mode of action (19), although they differ in field performance characteristics (4). Unfortunately, all available fungicides for control of Sclerotinia blight of peanut fall into these two classes. The threat of cross-resistance and reports of marginal disease control with iprodione have intensified the search for effective fungicides with an alternate mode of action. In 1985, a pathogenic isolate of *S. minor* (B-83-T2) with resistance to iprodione was found in an experimental microplot that had been treated with iprodione at the Tidewater Agricultural Experiment Station, Suffolk, VA (5). The microplot had been artificially infested with a native, field isolate (S-2) of *S. minor* in the spring of 1983. The occurrence of B-83-T2 has been a cause for concern as to the future of disease control with the dicarboximide fungicides.

The purpose of this study was to compare the fungitoxicity of three dicarboximides

and three new experimental compounds to *S. minor* using agar- and soil-plate tests, and assess fungicide performance under field conditions. A second goal was to further characterize the resistance of *S. minor* to dicarboximide fungicides and to test for cross-resistance to other fungicides. Two preliminary reports have been published (21,22).

MATERIALS AND METHODS

Glucose-yeast extract agar (GYEA) consisting of agar, 20 g; dextrose, 20 g; yeast extract, 2.0 g; KH_2PO_4, 1.0 g; $MgSO_4$-$7H_2O$, 0.5 g and 1,000 ml of distilled water was utilized throughout this study as the basal medium for agar-plate tests as well as maintenance of *S. minor* in culture. Two isolates of *S. minor*, S-2 and B-83-T2, were maintained on agar slants at 5 C. Mycelium was transferred from the slant cultures to petri plates containing GYEA. After 3 days of incubation at 22 C, 6-mm-diameter plugs of agar and actively growing mycelium were removed from the periphery of the culture. These agar plugs served as the inoculum source for agar- and soil-plate tests of fungicide activity.

Suspensions of fungicides were prepared in sterile distilled water and added to the agar medium at 70 C in a waterbath to yield concentrations of 0.0005, 0.002, 0.01, 0.05, 0.2, 1, 5, 20 and 100 μg/ml. Four replicates of each concentration were used to determine the sensitivity of each isolate of *S. minor*. Two separate tests were performed. All fungicide concentrations are expressed as active ingredient. The following fungicides were evaluated: ASC-66825 (experimental chemistry 50WP, Fermenta Agricultural Specialty Chemicals, Mentor, OH); chlozolinate (SDS-65311 50WP, Fermenta ASC); iprodione (Rovral® 50WP, Rhône-Poulenc Inc., Research Triangle Park, NC); MON-13108 (experimental chemistry 3F, Monsanto Agr. Prod. Co., St. Louis, MO); RH-3486 (experimental chemistry 50WP, Rohm

and Haas Co., Philadelphia, PA); and vinclozolin (Ronilan® 50WP, BASF Wyandotte Corp., Parsippany, NJ). After mixing, the amended GYEA was dispensed into 9-cm-diameter petri plates and allowed to cool.

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

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

Suspensions of fungicides were prepared in distilled water to obtain standard concentrations of 0.70 mg/ml. Each suspension was then placed in an airbrush sprayer (Badger Air-Brush Co., model 200-1, Franklin Park, IL) and sprayed over the mycelium and

soil for 5 seconds. This technique delivered 1.0 ml of the solution uniformly over the soil-plate surface (63.5 cm^2) and simulated a field application of 1.12 kg/ha of fungicide. Treatments were replicated five times and the entire test was performed twice.

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

Field trials were conducted on land naturally infested with *S. minor* and having a history of severe Sclerotinia blight of peanut. The land was prepared by moldboard plowing and disking prior to the planting of peanuts. Florigiant peanuts were planted in 1987 and NC 9 was planted in 1988 and 1989. Field plot design followed recommended research practices (14). All tests were managed according to standard practices for peanut production in Virginia (13), and chlorothalonil at 1.26 kg/ha was used for control of Cercospora leafspot according to the Virginia Peanut Leafspot Advisory Program (15).

All fungicides for control of Sclerotinia blight were applied with one 8008LP nozzle centered at a level over each row to provide complete coverage of plants. Nozzles were calibrated to deliver 335 l/ha at 165 kPa and a ground speed of 4.39 km/hr. Fungicide treatments in 1987 were applied as follows: RH-3486 on 15 Jul, all other treatments on 31 Jul, 28 Aug and 25 Sep. In 1988, treatments were applied on 3 Aug and 1 Sep, and treatments in 1989 were applied on 19 Jul and 16 Aug. Sclerotinia blight incidence was monitored monthly and recorded as the number of infection centers in the two center rows of each plot (14). Occurrence of other diseases was also recorded at monthly intervals.

Yields were based on weight of harvested peanuts from the two center rows of each plot at a moisture content of 7% (w/w).

RESULTS

Agar-plate tests. In tests using fungicide-amended GYEA, the dose-response lines for a typical field isolate (S-2) of *S. minor* to the dicarboximide fungicides (chlozolinate, iprodione and vinclozolin) were very steep when compared to the lines obtained for the three experimental fungicides (ASC-66825, MON-13108 and RH-3486) (Fig. 2). Vinclozolin was the most active of the dicarboximides, followed by iprodione and then chlozolinate. Complete inhibition of mycelial growth was maintained for 4 days on plates amended with 1.0 μg/ml of vinclozolin, 5.0 μg/ml of iprodione or 20 μg/ml of chlozolinate. No inhibition of growth was detected at 0.05 μg/ml of chlozolinate, 0.01 μg/ml of iprodione or 0.002 μg/ml of vinclozolin. The three experimental compounds were much more fungitoxic to isolate S-2 at low concentrations in GYEA plate tests than the dicarboximides. ASC-66825 and RH-3486 inhibited growth by 33% at 0.002 μg/ml, and MON-13108 inhibited growth by 10% at 0.002 μg/ml. In contrast to the dicarboximides, complete inhibition of growth did not occur even at 100 μg/ml of ASC-66825, MON-13108 or RH-3486. On unamended GYEA, isolate S-2 grew approximately 19 mm/day during the first 4 days and this value was used as the 0% inhibition standard. A comparison of ED_{50} values for the fungicides showed that ASC-66825 and RH-3486 had identically low ED_{50} values of 0.004 μg/ml followed by MON-13108 at 0.025 μg/ml. The ED_{50} values of the dicarboximide fungicides were 0.08, 0.18 and 0.38 μg/ml for vinclozolin, iprodione and chlozolinate, respectively.

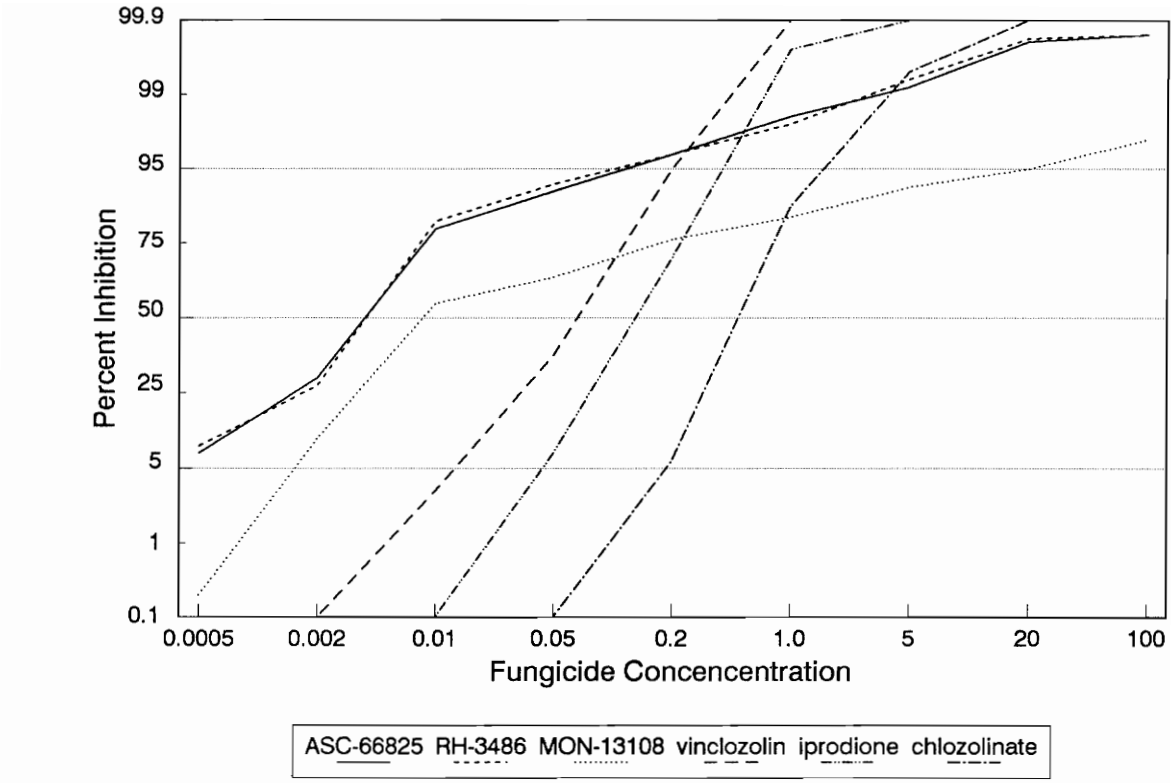

Figure 2. Dose-response of a typical field isolate (S-2) of *Sclerotinia minor* to various fungicides in glucose-yeast extract agar.

A comparison of the growth rate (mm/day) of isolate S-2 (Fig. 3) and B-83-T2 (Fig. 4) indicated that S-2 grew more vigorously on unamended GYEA than B-83-T2. Isolate B-83-T2 had an average growth rate of 11 mm/day which was 42% less than S-2. When isolate S-2 was exposed to the dicarboximides, a sharp decrease in growth rate occurred between the 0.05 and 1.0 μg/ml. This contrasted with the growth rate produced by isolate B-83-T2 when exposed to moderate concentrations of the dicarboximides. Growth of B-83-T2 was enhanced in the presence of 1.0 μg/ml of iprodione or vinclozolin, or 5.0 μg/ml of chlozolinate. Higher concentrations of the dicarboximide fungicides were partially inhibitory to isolate B-83-T2, but this isolate still exhibited some growth at 100 μg/ml of the dicarboximides. No growth of isolate S-2 was detected at 100 μg/ml of any of the dicarboximides. Due to the growth enhancement triggered by the dicarboximides only selected data were used in linear regression analyses were to determine ED_{50} values for isolate B-83-T2. The ED_{50} values were estimated by using growth values obtained at the highest fungicide concentration that enhanced growth and growth values at higher fungicide concentrations that inhibited growth. ED_{50} values were 4.0, 4.4 and 18.8 μg/ml for vinclozolin, iprodione and chlozolinate, respectively. These values were between 24 and 50 times larger than ED_{50} values obtained for isolate S-2. No enhancement of growth by either isolate occurred at any concentration of ASC-66825, MON-13108 or RH-3486 and increased concentrations produced greater growth inhibition. However, isolate B-83-T2 was more tolerant to the three experimental fungicides than isolate S-2. ED_{50} values were 0.013, 0.015 and 1.2 μg/ml for RH-3486, ASC-66825 and MON-13108, respectively. These values were 3.3, 3.8 and 48 times larger, respectively, than corresponding ED_{50} values for isolate S-2.

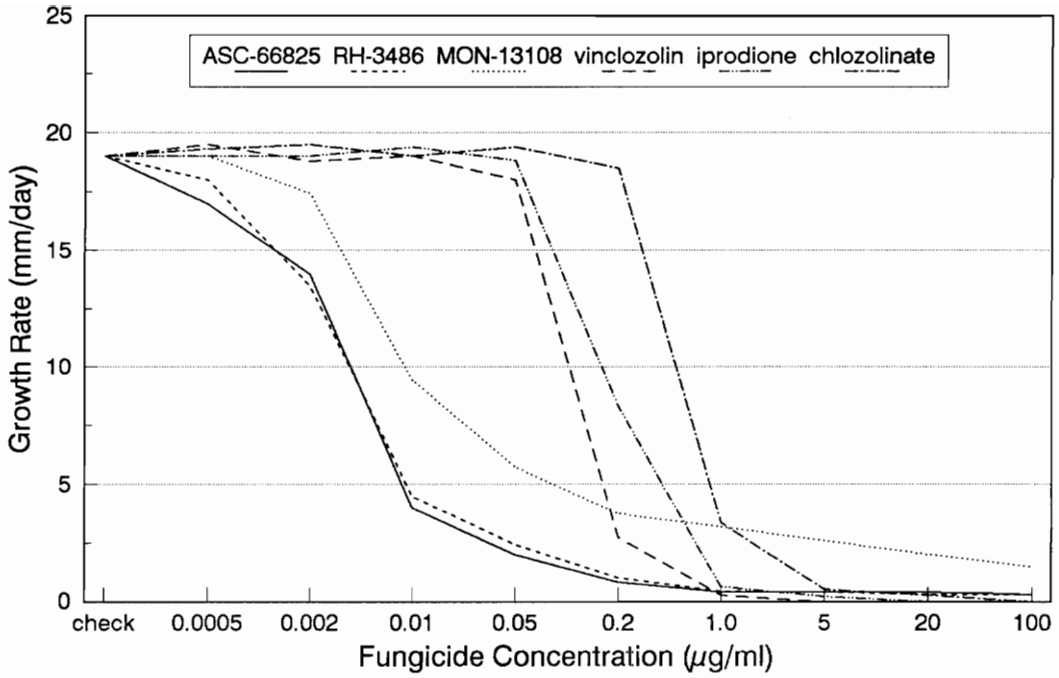

Figure 3. Growth rate of a typical field isolate (S-2) of *Sclerotinia minor* on glucose-yeast extract agar amended with various fungicides.

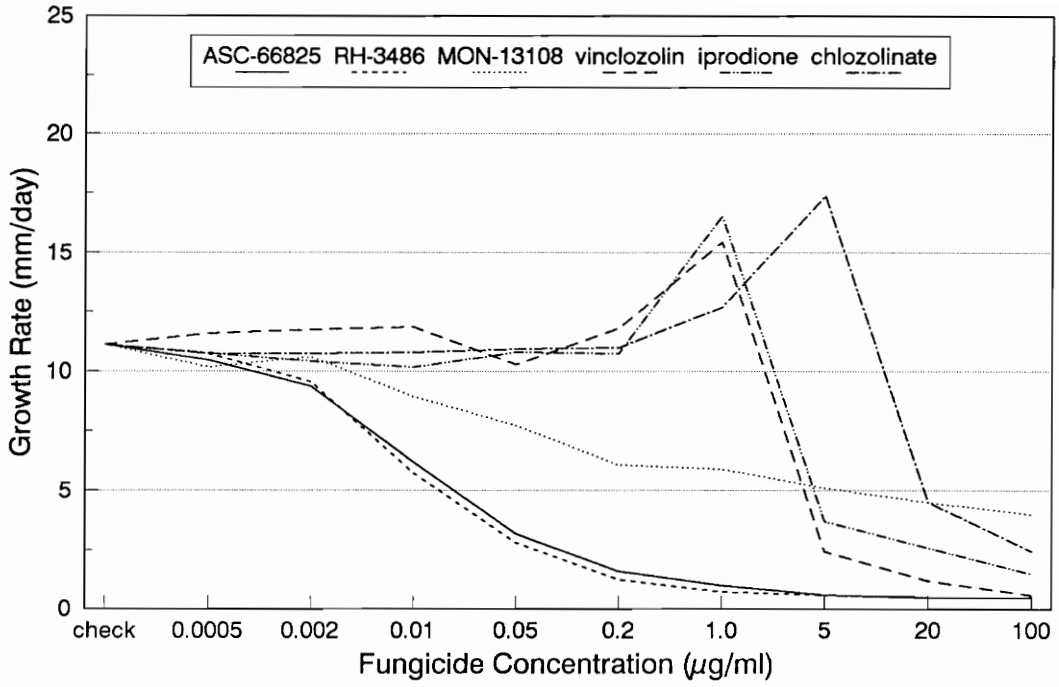

Figure 4. Growth rate of a dicarboximide-resistant isolate (B-83-T2) of *Sclerotinia minor* on glucose-yeast extract agar amended with various fungicides.

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

Sclerotial production by *S. minor* in soil plates was not inhibited as much as mycelial growth by the fungicide treatments. Only the dicarboximides were effective in significantly limiting sclerotial production by isolate S-2. Chlozolinate, vinclozolin and iprodione limited sclerotial production by 64, 51 an 40%, respectively. None of the fungicides significantly influenced sclerotial formation by B-83-T2. Without treatment, an average of 106.0 and 51.7 sclerotia were obtained from soil-plate samples originally inoculated with isolates S-2 and B-83-T2, respectively.

Field evaluation of fungicides. Weather conditions during the three growing seasons of field evaluation of fungicides varied greatly. Yields in untreated check plots were very high in 1987, moderate in 1989 and low in 1988. Sclerotinia blight pressure was greatest in 1988 due to cool temperatures in September. Peanut yields were high across all treatments in 1987 as dry weather suppressed the disease. During all years of testing, Sclerotinia blight was the only disease thought to significantly suppress yield, because other diseases were not detected or were successfully controlled.

Table 1. Mycelial growth (mm) and sclerotial production of a typical field (S-2) and dicarboximide-resistant (B-83-T2) isolate of *Sclerotinia minor* on fungicide-treated soil plates containing 5% corn meal[1]

Treatment[2]	Mycelial growth[3] (mm at 4 days)		Sclerotial production[4] (at 14 days)	
	S-2	B-83-T2	S-2	B-83-T2
vinclozolin	10.8 c	67.7 a	51.7 b	51.6 a
RH-3486	11.2 c	34.8 b	97.0 a	55.5 a
iprodione	11.8 c	75.8 a	63.1 b	62.4 a
ASC-66825	15.5 c	37.0 b	90.3 a	55.6 a
chlozolinate	31.4 b	68.1 a	38.3 b	55.6 a
MON-13108	67.9 a	64.5 a	114.9 a	57.8 a
water check	77.3 a	78.1 a	106.0 a	51.7 a

[1]Values are the means of five replications. The entire test was performed twice. Means in a column followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range test.

[2]Fungicides were tested at a rate equivalent to 1.12 kg a.i./ha.

[3]Mycelial growth represents colony diameter.

[4]Values are number of sclerotia recovered from a 10 cm^3 plug of soil from soil plates.

RH-3486 at 2.24 kg/ha was the most effective treatment in 1987, as one application at pegging suppressed disease incidence by 74% and increased yield by 922 kg/ha (Table 2). One application of RH-3486 at 1.12 kg/ha significantly limited disease incidence by 39%, but did not significantly enhance yield. Three applications of MON-13108 at 2.24 and 1.12 kg/ha provided significant disease control as disease incidence was suppressed by 45 and 51%, and yield was increased by 810 and 648 kg/ha, respectively. Similar applications of MON-13108 at 0.56 kg/ha did not provide significant disease suppression or a yield increase. Vinclozolin, applied three times at 0.84 kg/ha was the only dicarboximide treatment to significantly limit disease incidence in 1987. Plots treated with vinclozolin had 46% less disease than the untreated plots. Iprodione and chlozolinate did not significantly suppress disease. None of the treatments with the dicarboximides resulted in a significant yield increase.

In 1988, chlozolinate and MON-13108 were withdrawn from further field testing by their respective sources. The remaining fungicides were applied twice and all treatments significantly suppressed the incidence of Sclerotinia blight and increased yields. Two applications of RH-3486 at 1.12 and 0.56 kg/ha, vinclozolin at 0.84 kg/ha and iprodione at 1.12 kg/ha, suppressed disease incidence by 81, 69, 47 and 42%, and increased yields by 2246, 2193, 1671 and 1288 kg/ha, respectively.

A new fungicide, ASC-66825, was entered into the Sclerotinia blight control program in 1989. During this year, only ASC-66825 and RH-3486 significantly suppressed disease and increased yield. Two applications of ASC-66825 at 0.28 kg/ha and 0.56 kg/ha, and RH-3486 at 0.56 kg/ha suppressed disease incidence by 56, 67 and 68%, and increased yields by 1359, 1080 and 1206 kg/ha, respectively. Vinclozolin at 0.84 kg/ha suppressed disease incidence by 65%, but failed to significantly increase yield.

Table 2. Disease incidence and yield of peanuts from plots treated with fungicides for control of Sclerotinia blight[1]

Year and treatment	Rate (kg/ha)	Number of applications	Disease[2] incidence	Yield[3] (kg/ha)
1987				
iprodione	1.12	3	21.0 ab	5112 b-d
vinclozolin	0.84	3	16.0 bc	5254 a-d
RH-3486	1.12	1	18.0 b	5192 a-d
RH-3486	2.24	1	7.8 c	5690 a
chlozolinate	1.12	3	23.8 ab	5005 cd
MON-13108	0.56	3	22.5 ab	5042 cd
MON-13108	1.12	3	14.5 bc	5416 a-c
MON-13108	2.24	3	16.3 bc	5578 ab
untreated check	-----	--	29.5 a	4768 d
1988				
iprodione	1.12	2	27.5 b	3202 b
vinclozolin	0.84	2	25.3 b	3585 b
RH-3486	0.56	2	15.0 c	4107 a
RH-3486	1.12	2	9.0 c	4160 a
untreated check	-----	--	47.8 a	1914 c
1989				
iprodione	1.12	2	20.3 ab	3920 cd
vinclozolin	0.84	2	10.5 b	4042 b-d
RH-3486	0.56	2	9.5 b	4658 ab
ASC-66825	0.28	2	13.0 b	4811 a
ASC-66825	0.56	2	9.8 b	4532 a-c
untreated check	-----	--	29.8 a	3452 d

[1]Means in a column for each year followed by the same letter(s) are not significantly different at P=0.05 according to Duncan's multiple range test.

[2]Disease incidence represents counts of infection centers in the two center rows of each plot at harvest. An infection center was a point of active growth by *Sclerotinia minor* and included 15.2 cm of row length on either side of that point.

[3]Yields are based on weight of peanuts with moisture content of 7% (w/w).

DISCUSSION

In agar-plate tests, the dicarboximides produced steep dose-response lines that were similar in slope and indicative of a related mode of action against *S. minor.* Similar steep dose-response lines were reported for the dicarboximide fungicides against *S. sclerotiorum* (9) and *S. minor* (3). ASC-66825, MON-13108 and RH-3486 appeared to have a different mode of action than the dicarboximides, based on their comparatively flat dose-response lines. The dose-response lines suggested that the dicarboximides were fungicidal to *S. minor*, whereas ASC-66825, MON-13108 and RH-3486 were strong fungistats. The experimental compounds were active at extremely low concentrations, which was reflected in their low ED_{50} values in agar-plate tests. ASC-66825, RH-3486 and MON-13108 had ED_{50} values that were 45, 45 and 7.2 times lower than iprodione, respectively. The ED_{50} values obtained for iprodione and vinclozolin are similar to data obtained in an earlier study of iprodione and vinclozolin and two chlorinated aromatic fungicides (3). The high level of activity shown by ASC-66825 and RH-3486, compared to iprodione, is important because iprodione has become the competitive standard in industry for fungitoxicity against *S. minor.*

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

(10,18). Resistance to this class of fungicides may require the alteration of numerous cellular events, which could likely reduce the pathogenicity of the fungus. Even though B-83-T2 was capable of causing disease (21), its growth rate on unamended GYEA was less than that of its parent isolate, which suggests a partial loss of fitness as a saprophyte. ASC-66825 and RH-3486 effectively suppressed growth of B-83-T2 on fungicide-amended GYEA, although the ED_{50} values for these fungicides were slightly higher than values obtained for S-2, a typical field isolate of *S. minor*. The low ED_{50} values for ASC-66825 and RH-3486 indicated that no cross-resistance occurred between the dicarboximides and these two fungicides. MON-13108 suppressed growth of B-83-T2 at all fungicide concentrations on GYEA, but the ED_{50} value of the fungicide was 48 times higher against isolate B-83-T2 than S-2, which suggested that B-83-T2 had some cross tolerance to MON-13108. Dicarboximide resistance appears to convey increased tolerance to some classes of fungicides.

Growth of the dicarboximide-resistant isolate, B-83-T2, was enhanced in the presence of dicarboximides at concentrations between 1 and 5 μg/ml in GYEA. These concentrations were highly fungitoxic to a sensitive isolate, S-2. Higher concentrations of dicarboximide fungicides inhibited growth of B-83-T2, indicating that resistance may be of a low-level type. Three continuous years of tests with *in vitro* dicarboximide-resistant isolates in confined field microplots indicated that disease control could be maintained by application of dicarboximide fungicides (5). Continued dicarboximide fungicide pressure may result in the selection of resistant isolates with increased virulence, but resistance to the dicarboximides has not been reported nor detected in commercial peanut fields treated with iprodione. A 3-year study of 763 isolates from 19 infested fields failed to detect any iprodione- or vinclozolin-resistant isolates (3). Screenings of 360 isolates of *S. minor* from plots in fungicide trials and

commercial fields treated with iprodione did not show evidence of *in vitro* resistance in 1986 (20). Another screening of 947 isolates from plots treated with various leafspot fungicides and plots treated with iprodione also did not detect any evidence of field resistance (23).

All of the dicarboximide fungicides were inhibitory to mycelial growth and sclerotial formation by isolate S-2 in soil-plate tests. An earlier study indicated that procymidone also suppressed mycelial growth and sclerotial formation (11). The entire group of dicarboximides appears to be highly active in a soil medium against sensitive isolates. Based on soil mobility tests (7), the dicarboximides may persist in an active state for periods longer than the recommended spray interval of 4 weeks. However, the dicarboximides were not effective in limiting mycelial growth or sclerotial production by isolate B-83-T2. Even though applications of dicarboximide fungicides in microplot studies suppressed the development of above-ground disease by *in vitro* resistant isolates (5), results from soil-plate tests suggested that the dicarboximides will not be effective in limiting sclerotial production by dicarboximide-resistant isolates. Thus, low-level dicarboximide resistance could lead to high inoculum build-up with continued use of these fungicides for disease control.

MON-13108 was not effective against either isolate of *S. minor* in soil-plate tests. In field tests during 1987, MON-13108 was comparable to the dicarboximide fungicides in performance, which indicated that its fungistatic properties were not dependant on soil activity. No conclusion can be made regarding cross-resistance of B-83-T2 to this fungicide on the basis of soil-plate tests. Unlike MON-13108, both ASC-66825 and RH-3486 were effective in suppressing mycelial growth of isolate S-2 and B-83-T2 in soil-plate tests. B-83-T2 did not appear to be cross resistant to ASC-66825 or RH-3486 according to these tests. However, ASC-66825 and RH-3486 did not suppress sclerotial development by either isolate

in soil-plate tests. These results suggest that these fungicides may have limited activity in soil, and further distinguishes their mode of action from the dicarboximides. The high efficacy of ASC-66825 and RH-3486 for field control of Sclerotinia blight of peanut was apparently not dependant on soil activity.

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

A ranking of the ED_{50} values for the six fungicides according to agar-plate tests was a better predictor of field performance than results from soil-plate tests. However, actual ED_{50} values did not correspond well to field disease incidence ratings. Soil-plate tests did not detect the ability of MON-13108 to control disease in the field. The soil assay also indicated that chlozolinate might be an effective fungicide against Sclerotinia blight. Subsequent field tests showed chlozolinate to be relatively ineffective against the disease. Thus, no single laboratory test could accurately predict the performance of a fungicide in the field. However, compounds possessing a low ED_{50} value in agar-plate tests and activity in soil-plate tests, such as ASC-66825 and RH-3486, can be quickly identified for field research. Few compounds apparently fit the above criteria (12), which justifiably limits the number of compounds for labor-intensive field tests on control of Sclerotinia blight.

This study identified two new two fungicides that were more fungitoxic to *S. minor*

than currently available dicarboximide fungicides. ASC-66825 and RH-3486 had an extremely high level of activity against *S. minor* in the laboratory and field. These two fungicides appear to have a different mode of action and different performance characteristics than the dicarboximides. If dicarboximide resistance results in the failure of iprodione to provide disease suppression, fungicides with chemistry similar to ASC-66825 or RH-3486 may prove essential for Sclerotinia blight management.

LITERATURE CITED

1. Beute, M.K., Porter, D.M., and Hadley, B.A. 1975. Sclerotinia blight of peanut in North Carolina and Virginia and its chemical control. Plant Dis. Rep. 59:697-701.
2. Bliss, C.I. 1935. The calculation of the dosage mortality curve. Ann. Appl. Biol. 22:135-167.
3. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.
4. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Performance characteristics of dicloran, iprodione, and vinclozolin for control of Sclerotinia blight of peanut. Plant Dis. 71:546-548.
5. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Sclerotinia blight of peanut: Relationship between in vitro resistance and field efficacy of dicarboximide fungicides. Phytopathology 77:1028-1032.
6. Burgaud, L., Chevrel, J., Guillot, M., Marechal, G., Thiolliere, J., and Cole, R.J. 1975. The hydantoin 26,019 RP, a new polyvalent fungicide. Proc. 8th Brit. Insecticide and

Fungicide Conf. 645-652.

7. Elmer, W.H., and Stipes, R.J. 1985. Movement and disappearance of dicloran, iprodione, and vinclozolin in peanut and nonpeanut soils. Plant Dis. 69:292-294.
8. Kohn, L.M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
9. Matheron, M.E., and Matejka, J.C. 1989. In vitro and field comparison of six new fungicides with iprodione and vinclozolin for control of leaf drop of lettuce caused by *Sclerotinia sclerotiorum*. Plant Dis. 73:727-730.
10. Pappas, A.C., and Fisher, D.J. 1979. A comparison of the mechanisms of action of vinclozolin, procymidone, iprodione and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10:239-246.
11. Phipps, P.M. 1980. Soil plate and field evaluation of fungicides for control of Sclerotinia blight of peanut. (Abstr.) Phytopathology 70:692.
12. Phipps, P.M. 1984. New products for control of Sclerotinia blight of peanut. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 16:67.
13. Phipps, P.M. 1990. Diseases of peanuts. Pages 55-67 in: 1990 Peanut Production Guide. Publ. MA197. Va. Coop. Ext. Ser., VPI&SU, Blacksburg, VA.
14. Phipps, P.M., and Porter, D.M. 1986. Evaluating chemicals to control soilborne diseases of peanut. Pages 217-220 in: Methods for Evaluating Pesticides for control of plant pathogens. K.D. Hickey, ed. APS Press, St. Paul, MN.
15. Phipps, P.M., and Powell, N.L. 1984. Evaluation of criteria for the utilization of peanut leafspot advisories in Virginia. Phytopathology 74:1189-1193.
16. Porter, D.M. 1980. Control of Sclerotinia blight of peanut with procymidone. Plant

Dis. 64:865-867.

17. Porter, D.M., and Beute, M.K. 1974. Sclerotinia blight of peanuts. Phytopathology 64:263-264.

18. Reilly, C.C., and Lamoureux, G.L. 1981. The effects of the fungicide, iprodione, on the mycelium of *Sclerotinia sclerotiorum*. Phytopathology 71:722-727.

19. Sijpesteijn, A.K. 1982. Mechanism of action of fungicides. Pages 33-45 in: Fungicide Resistance in Crop Protection. J. Dekker and S.G. Georgopoulos, eds. Centre for Agriculture Publishing and Documentation, Wageningen, Netherlands.

20. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1987. Effect of field application of iprodione on Sclerotinia blight of peanut and the causal fungus, *Sclerotinia minor*. (Abstr.) Phytopathology 77:1748.

21. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1988. Agar plate, soil plate and field evaluation of fungicides for activity against *Sclerotinia minor*. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 20:29.

22. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1988. Effect of RH-3486 on dicarboximide-resistant isolates of *Sclerotinia minor*. (Abstr.) Phytopathology 78:863.

23. Smith, F.D., Phipps, P.M., and Stipes. R.J. 1989. Assessment of resistance to iprodione in sclerotial populations of *Sclerotinia minor* from fungicide-treated peanuts. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 21:18.

24. Sturgeon, R.V. 1986. Peanut disease loss estimates for major peanut producing states in the United States for 1984 and 1985. Proc. Am. Peanut Res. Educ. Soc. 18:24-26.

25. Wadsworth, D.F. 1979. Sclerotinia blight of peanuts in Oklahoma and occurrence of the sexual stage of the pathogen. Peanut Sci. 6:77-79.

EFFECT OF PINOLENE AS AN ADJUVANT TO IPRODIONE FOR CONTROL OF SCLEROTINIA BLIGHT OF PEANUT

ABSTRACT

Pinolene (Nu-Film-17®), a pine resin derivative containing 96% di-1-p-menthene, improved the performance of iprodione (Rovral®) for control of Sclerotinia blight of peanut, caused by *Sclerotinia minor*. Average yields during 1985-1989 from test plots treated on demand with iprodione (1.12 kg/ha) and pinolene at 0.18% (v/v) in a spray volume of 335 l/ha were 365 kg/ha higher and disease incidence 15% lower than plots treated with iprodione alone. Treatments were applied three times in 1985, 1986 and 1987; twice in 1988 and 1989. The mean additional value of peanuts obtained per year with the addition of pinolene to iprodione was $298/ha, whereas the cost of using pinolene was $7.22/ha. These results were significant (P=0.05) when examined over the 5-year period.

INTRODUCTION

The purpose of spray adjuvants is to improve the physical properties of a pesticide mixture, thereby enhancing the efficacy of the spray. Because of renewed environmental interest and increasing concerns over the alleged health effects of agrichemical residues in the food supply, many fungicide applications are being made at the lowest effective dose and only when conditions are conducive for disease development. Adjuvants have the potential to increase the effectiveness of agrichemicals, enabling the reduction of application rates or

number of sprays.

Sclerotinia blight, caused by *Sclerotinia minor* (Jagger) Kohn (4), currently claims 4 to 8% of the peanut crop in Virginia each year (P.M. Phipps, personal communication). Iprodione is a dicarboximide fungicide and has been labeled for control of Sclerotinia blight since 1985. Currently, use of this fungicide is the favored method of controlling Sclerotinia blight of peanut in Virginia. The dicarboximides function as protectant fungicides with activity against representatives of the following genera of fungi: *Botrytis*, *Sclerotinia*, *Monilinia*, *Alternaria*, *Sclerotium* and *Phoma* (10). Control of Sclerotinia blight with iprodione commonly averages only 45-55% and there remains a need for more efficacious control strategies (1). The disease is first detectable at the soil surface and under the dense peanut canopy. The well-concealed infections are often not detected in time for the effective use of fungicides. Applications of iprodione should commence when the disease becomes active and thereafter at 4-week intervals for a total of not more than three times (8). During the 4-week spray interval, Sclerotinia blight can become active if the weather remains cool and wet for an extended time.

Sclerotinia blight was first detected in the Virginia-North Carolina area in 1971 (11). Since that time, the disease has been reported in other peanut-producing areas of the country, such as Oklahoma (14), New Mexico and Texas (13). The rapid spread of the disease has the potential to substantially reduce national peanut yields unless current control measures are improved. Since pinolene was non-toxic to animals at tested rates (2), and was reported to enhance the performance of chlorothalonil for control of peanut foliar diseases (D.H. Smith, unpublished data), it was included in a test of adjuvants with iprodione for control of Sclerotinia blight.

Chemically, pinolene contains 96% di-1-p-menthene. It is derived from pine resin and forms a terpenic polymer after application. The material has been promoted as an extender-sticker-spreader which surrounds and holds the pesticide on sprayed areas of the plant. Pesticide life is extended by negating the effects of environmental damage, such as rainfall, and wind erosion, photodecomposition, volatilization and heat destruction, thus prolonging the active life of most pesticides by 25 to 50%. Pinolene has been claimed as effective when used with fungicides such as anilazine, captafol, chlorothalonil, maneb and zineb (5).

The use of spray adjuvants does not always enhance plant disease control. Although adjuvants usually lack fungicidal properties, adjuvants do have the potential to alter the plant cuticle which forms the major barrier against biotic and non-biotic assaults. The application of some spray adjuvants without a fungicide significantly increased the development of disease in grapes caused by *Botrytis cinerea* (6). There was a significant correlation between water loss from grapes and disease development which indicated that the increase in disease was due to disruption of the normal function of the epicuticular waxes on the berry. Most of the adjuvants that enhanced disease development contained petroleum oils. These oils may have contributed to the removal of protective waxes from the grapes. Pinolene lacks additional oils and did not affect disease development on treated grapes.

Since Sclerotinia blight has proved to be a difficult disease to control with registered fungicides, research on adjuvants may provide a much-needed means for improved disease management. The development and registration of fungicides with greater efficacy than iprodione against *S. minor* (12), as well as biological control agents will take years of additional research. Adjuvants may help to lessen the need for compounds that are effective in controlling Sclerotinia blight by improving the performance of currently available fungicides.

MATERIALS AND METHODS

Adjuvants and fungicides. A group of ten adjuvants, representing a wide range of ingredients, were evaluated in 1985 for their activity with iprodione (Rovral®, Rhône-Poulenc Inc., Research Triangle Park, NC). Pinolene (Nu-Film-17®) and Spray-Aide® were obtained from Miller Chem. and Fert. Corp., Hanover, PA. Spray-Aide® is an acidifying surfactant containing 70% alkylaryl polyoxyethylene glycol phosphate ester. Acetic and hydrochloric acids were obtained as technical grade chemicals and were tested to determine the effects of lowered pH on performance of iprodione. Chem-Oil 83®, ChemWett Plus® and SoyOil 937® were obtained from Coastal Chemical Co., Greenville, NC. Chem-Oil 83® is 83% paraffin base petroleum oil with surfactants and is thought to function mainly as a surfactant. ChemWett Plus® contains 80% alkylaryl polyethylene glycols and organic solvents, and is classified as a spreader and activator. SoyOil 937® contains 93% soybean oil and 7% emulsifier. Agri-Dex®, Buffer P.S.® and Penetrator-3® were obtained from Helena Chem. Co., Memphis, TN. Agri-Dex® is a mixture of heavy range paraffin base petroleum oil, polyol fatty acid esters and their polyethoxylated derivatives that function as spreaders, stickers and/or penetrants. Buffer P.S.® contains 30% alkylaryl polyethoxy ethanol phosphates and organic phosphatic acids and is classified as a spreader and buffering agent. Penetrator-3® is a 98% mixture of paraffin based petroleum oil, polyol fatty acid esters and their polyethoxylated derivatives and functions mainly as a penetrant.

Field trials. Peanuts (cvs. Florigiant in 1985-1987 and NC 9 in 1988-1989) were planted and managed according to standard practices for peanut production in Virginia (8). Treatments to evaluate fungicide sprays with and without adjuvants were applied to the two center rows of four-row plots using a CO_2-pressurized backpack sprayer. The adjacent outer

rows of each plot functioned as guard rows. The experimental design consisted of four randomized complete blocks with 12.2-m rows spaced 0.9 m apart. Each block was separated by a 2.1-m alleyway. Disease incidence was monitored monthly and recorded as the number of infection centers in the center rows of each plot (9). Yields were based on weight of harvested peanuts from the two center rows and a moisture content of 7% (w/w). Values were determined from a 500-g composite sample from each treatment in accordance with Federal-State Inspection Service methods. Statistical analyses on disease incidence, yield and value were determined by Duncan's new multiple range test using a probability value of 0.05.

Ten adjuvants were individually evaluated with iprodione (1.12 kg/ha) as Rovral® 50WP in 1985 for disease control in a field having a history of severe Sclerotinia blight. Treatments were applied three times (18 Jul, 14 Aug, 12 Sep) using two different spray methods. High pressure, low volume sprays were delivered at 140 l/ha with three $D_2$13 (disk-core combination) nozzles per row and a pressure of 345 kPa. Low pressure, high volume sprays were applied at 335 l/ha with one 8008LP nozzle per row at 165 kPa. As commonly recommended by manufacturers of spray adjuvants, rates are expressed as percent of spray volume. At 140 l/ha, adjuvants and rates (v/v) included: 0.83 N acetic acid, 1.0%; Agri-Dex®, 0.83%; Buffer P.S.®, 0.13%; Chem-Oil 83®, 0.83%; ChemWett Plus®, 0.83%; 1 N hydrochloric acid, 0.75%; pinolene, 0.42%; Penetrator-3®, 0.42%; SoyOil 937®, 1.0%; and Spray-Aide®, 0.06%. At 335 l/ha, adjuvants and rates were: 0.83 N acetic acid, 1.0%; Buffer P.S.®, 0.13%; 1 N hydrochloric acid, 0.70%; pinolene, 0.18%; SoyOil 937®, 0.42%; and Spray-Aide®, 0.06%.

Subsequent tests during the next four years (1986-1989) focused on the use of pinolene with iprodione. Due to formulation changes by the manufacturer, iprodione was used as Rovral® 50WP in 1986, 1987 and 1988, and as Rovral® 4F in 1989. Treatments were

applied using only 8008LP nozzles calibrated to deliver 335 l/ha at 165 kPa. Iprodione was applied at 1.12 kg/ha with and without pinolene at 0.18% (v/v) at 4-week intervals after Sclerotinia blight became active in the field. Three applications were made in 1986 (10 Jul, 7 Aug, 4 Sep) and 1987 (31 Jul, 28 Aug, 25 Sep). Two applications were made in 1988 (3 Aug, 1 Sep) and 1989 (20 Jul, 16 Aug).

RESULTS

Preliminary evaluation of adjuvants. The application of iprodione alone with $D_2$13 nozzles suppressed disease incidence by only 30%, a result that was not significant in 1985 (Table 3). However, the addition of several different spray adjuvants to iprodione resulted in significant disease suppression. Disease incidence was suppressed by 49, 48, 48, 36 and 33% in plots treated with iprodione containing the adjuvants Spray-Aide®, ChemWett Plus®, pinolene, 1 N hydrochloric acid and Chem-Oil 83®, respectively. When 8008LP nozzles were used, iprodione alone significantly suppressed disease incidence by 33%. The addition of spray adjuvants to iprodione improved the performance of the fungicide as disease incidence was suppressed by 47, 40, 37 and 37% in plots treated with iprodione containing pinolene, SoyOil 937®, Buffer P.S.® and Spray-Aide®, respectively.

All applications of iprodione with and without various adjuvants produced significant yield increases in peanut as compared to untreated peanuts, with the exception of Agri-Dex®. Although not significantly better than iprodione alone, pinolene was the best-performing spray adjuvant based on peanut yield, regardless of the application method. Peanuts treated with iprodione and pinolene using $D_2$13 and 8008LP nozzles yielded 264 kg/ha and 454 kg/ha more, respectively, than peanuts similarly sprayed with iprodione alone.

Table 3. Comparison of spray adjuvants used with iprodione for control of Sclerotinia blight of peanut in 1985.[1]

Treatment and adjuvant rate (v/v)[2]	Disease incidence[3]	Yield (kg/ha)[4]
untreated	49.0 a	2875 c
Three $D_2$13 nozzles per row		
iprodione (1.12 kg/ha) alone	34.5 a-c	3847 ab
+ 0.83 N acetic acid, 1.0%	39.5 a-c	3758 ab
+ Agri-Dex, 0.83%	44.3 ab	3405 bc
+ Buffer P.S., 0.13%	37.0 a-c	3783 ab
+ Chem-Oil 83, 0.83%	32.8 bc	3682 ab
+ ChemWett Plus, 0.83%	25.3 c	3922 ab
+ 1 N hydrochloric acid, 0.75%	31.5 bc	3960 ab
+ pinolene, 0.42%	25.5 c	4111 ab
+ Penetrator-3, 0.42%	36.0 a-c	3607 ab
+ SoyOil 937, 1.0%	37.5 a-c	3758 ab
+ Spray-Aide, 0.06%	24.8 c	3783 ab
One 8008LP nozzle per row		
iprodione (1.12 kg/ha) alone	33.0 bc	3884 ab
+ 0.83 N acetic acid, 1.0%	37.3 a-c	3720 ab
+ Buffer P.S., 0.13%	30.8 bc	3821 ab
+ 1 N hydrochloric acid, 0.70%	36.8 a-c	3884 ab
+ pinolene, 0.18%	26.0 c	4338 a
+ SoyOil 937, 0.42%	29.3 bc	4035 ab
+ Spray-Aide, 0.06%	31.0 bc	3809 ab

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

[2]Three applications were made (18 Jul, 14 Aug, 12 Sep). Spray volumes were 140 l/ha with $D_2$13 nozzles or 335 l/ha with 8008LP nozzles.

[3]Disease incidence represents the number of infection centers in two 12.2-m rows at harvest. An infection center was a point of active growth by *Sclerotinia minor* and included 15.2 cm of row length on either side of that point.

[4]Yield based on weight of peanuts adjusted to 7% moisture (w/w).

Evaluation of pinolene with iprodione from 1985 to 1989. During tests of similar adjuvants in 1986, pinolene was the best performing fungicide adjuvant based on peanut yield (7). Since pinolene showed a trend of enhancing performance of iprodione using two different methods of spray application in 1985 and performed well during two seasons, pinolene was chosen for continued evaluation.

During the 5-year period, iprodione alone suppressed disease incidence an average of 32% and increased yield by 631 kg/ha compared to the untreated check. Similarly, the use of iprodione and pinolene resulted in an average of 42% disease suppression and an increase in yield of 996 kg/ha.

Application of iprodione alone did not significantly suppress Sclerotinia blight during two of five years: 1986 and 1987 (Table 4). The addition of pinolene to iprodione resulted in significant disease control during all 5 years of evaluation. Disease incidence at harvest was 21, 20, 13 and 15% less in plots treated with iprodione and pinolene in comparison to treatment with iprodione alone in 1985, 1986, 1987 and 1989, respectively. Addition of pinolene to the fungicide spray did not improve disease control in 1988. Yearly differences in disease incidence in plots treated with iprodione and pinolene or iprodione alone were not significant. Analysis of the combined results of field research over the 5-year period indicated a significant improvement in disease control by the use of pinolene as an adjuvant with iprodione. During this period, plots treated with both iprodione and pinolene had 15% less disease compared to plots treated only with iprodione.

Use of iprodione alone increased yields significantly during only 2 of 5 years, whereas use of iprodione and pinolene significantly increased yields during 4 of 5 years, compared to untreated plots. Yields from plots treated with both iprodione and pinolene averaged 454,

Table 4. Control of Sclerotinia blight of peanut with and without pinolene as an adjuvant to iprodione.[1]

Year and treatment[2]	Disease incidence[3]	Yield[4] (kg/ha)	Value[5] ($/ha)
1985			
iprodione + pinolene	26.0 b	4338 a	3002 a
iprodione	33.0 b	3884 a	2654 a
untreated	49.0 a	2875 b	1938 b
1986			
iprodione + pinolene	30.0 b	3176 a	2227 a
iprodione	37.5 ab	2745 ab	1847 a
untreated	44.8 a	1932 b	1281 b
1987			
iprodione + pinolene	18.3 b	5441 a	3714 a
iprodione	21.0 ab	5112 ab	3489 a
untreated	29.5 a	4768 b	3247 a
1988			
iprodione + pinolene	30.3 b	3093 a	2175 a
iprodione	27.5 b	3202 a	2257 a
untreated	47.8 a	1914 b	1316 b
1989			
iprodione + pinolene	5.5 b	4411 a	3109 a
iprodione	6.5 b	4284 a	2891 a
untreated	15.5 a	4253 a	2958 a
Five-Year Average			
iprodione + pinolene	21.6 c	4144 a	2880 a
iprodione	25.3 b	3779 b	2582 b
untreated	37.3 a	3148 c	2148 c

[1]Means followed by the same letter(s) within a given period are not significantly different at P=0.05 according to Duncan's new multiple range test.

[2]Three applications using one 8008LP nozzle/row at 335 l/ha were made in 1985, 1986 and 1987; two similar applications were made in 1988 and 1989. Iprodione was applied at 1.12 kg/ha and pinolene was applied at 0.18% (v/v).

[3]Disease incidence represents the number of infection centers in two 12.2-m rows at harvest. An infection center was a point of active growth by *Sclerotinia minor* and included 15.2 cm of row length on either side of that point.

[4]Yields based on weight of peanuts adjusted to 7% moisture (w/w).

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

431, 329 and 127 kg/ha more for years 1985, 1986, 1987 and 1989, respectively, than plots treated with iprodione alone. No increase in yield was attributed to use of pinolene in 1988. The effects on yield and crop value by the addition of pinolene to iprodione were not significant when analyzed for each individual year, but were significant when examined over the 5-year period. During 1985-89, average yields were increased by 365 kg/ha which represented an additional value in peanuts of $298/ha.

DISCUSSION

The use of pinolene as a spray adjuvant significantly improved peanut yields by increasing the efficacy of iprodione for control Sclerotinia blight of peanut during a 5-year test period. The addition of pinolene to iprodione also resulted in more consistent performance of this fungicide. During each year, plots treated with iprodione and pinolene had significantly less disease than untreated plots. These findings have resulted in the recommendation for Virginia peanut growers to use pinolene (Nu-Film-17®) with iprodione (Rovral®) for control of Sclerotinia blight (8). The average cost of using pinolene with iprodione was $2.77/ha for each application. During the period, the average seasonal cost associated with use of pinolene was $7.22/ha, and the additional value of peanuts was $298/ha. Thus, the use of pinolene was cost effective.

Iprodione with pinolene performed well during 1985 through 1987 with yield improvements attributed to use of the adjuvant ranging from 329 to 454 kg/ha. However, no yield improvement was obtained with the addition of pinolene to iprodione in 1988 and only a small improvement of 127 kg/ha was seen in 1989. During 1988 and 1989, only two applications of iprodione were made; three treatments were made in previous years. The

reduction in fungicide treatments may have limited availability of iprodione during critical periods for disease control.

Varying the application method of fungicides by using $D_2$13 nozzles at a spray rate of 140 l/ha or 8008LP nozzles at 335 l/ha did not significantly change the performance of iprodione alone or iprodione and pinolene during 1985, based on peanut yield. Thus, either spray technique appeared to be effective in delivering iprodione. Once the fungicide reaches the site of deposition, pinolene apparently functions more as an extender and sticker than as a spreader. The large droplets produced by 8008LP nozzles would limit the functioning of pinolene as a spreader, whereas the fine droplet produced by $D_2$13 nozzles would maximize the functioning of the adjuvant as a spreader. If pinolene was functioning mainly as a spreader, greater differences in performance would have been expected between the two methods of spray application.

According to publicized claims by Miller Fertilizer and Chemical Co., pinolene functions at first as a sticker to prevent losses of fungicide from rainfall. This occurs after polymerization of di-1-p-menthene, which is the active ingredient in pinolene. The active ingredient is also thought to act as an extender since the polymerized pinolene suppresses the oxidation and hydrolysis reactions of fungicide degradation. This role of pinolene may be important because the heterocyclic ring structure of iprodione is susceptible to base-catalyzed reactions and rearrangement with loss of fungicidal activity (3). Information supplied by the manufacturer indicated that iprodione will begin to degrade in an aqueous suspension above pH 7 after 12 hours. Depending on the weather and growth stage of peanuts, fungicides can be exposed to high levels of UV light and high temperatures that catalyze undesirable chemical reactions.

Pinolene improved the performance of iprodione so that the iprodione and pinolene treatments always had significantly less disease incidence than the untreated check. This improvement makes the use of the fungicide somewhat more environmentally tolerable because iprodione with pinolene has a higher ratio of benefit to amount of applied fungicide. The use of other spray adjuvants did not greatly alter the performance of iprodione for control of Sclerotinia blight. The acidifying agents, 0.83 N acetic acid, 1 N hydrochloric acid and Spray-Aide®, reduced the pH of tank mixes of iprodione from 7.6 to 5.6, 5.5 and 6.5, respectively. The change in pH had no significant effect on the performance of iprodione for control of Sclerotinia blight of peanut, suggesting that decomposition of iprodione in mildly alkaline water was not a serious problem. The two adjuvants containing petroleum oils and classified as penetrants, Agri-Dex® and Penetrator-3®, were the poorest performing adjuvants, based on peanut yield. Disruption of the plant cuticle may be responsible for increased susceptibility to infection (6). In addition, a damaged cuticle may have allowed the fungicide to wash off more easily.

LITERATURE CITED

1. Brenneman, T.B., Phipps, P.M., and Stipes. R.J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.
2. Clarke, A.R. 1969. Pinolene - Toxicology. Pages 1-8 in: Pinolene - Toxicological and Biological Properties. Miller Chem. and Fert. Corp., Hanover, PA.
3. Cooke, B.K., Loeffler, R.S.T., and Pappas, A.C. 1979. The structural rearrangement of iprodione in ethanolic solution. Pestic. Sci. 10:393-398.

4. Kohn, L.M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.

5. Nesmith, W.C. 1980. List of materials available for testing in 1980. Fungic. Nematicide Tests 35:254-261.

6. Marois, J.J., Bledsoe, A.M., Bostock, R.M., and Gubler, W.D. 1987. Effects of spray adjuvants on development of *Botrytis cinerea* on *Vitis vinifera* berries. Phytopathology 77:1148-1152.

7. Phipps, P.M. 1987. Evaluation of fungicides for control of Sclerotinia blight of peanut, 1986. Fungic. Nematicide Tests 42:127.

8. Phipps, P.M. 1990. Disease control. Pages 55-67 in: 1990 Peanut Production Guide. Publ. MA197., Va. Coop. Ext. Ser., VPI&SU, Blacksburg, VA.

9. Phipps, P.M., and Porter, D.M. 1986. Evaluating chemicals to control soilborne diseases of peanut. Pages 217-220 in: Methods for Evaluating Pesticides for Control of Plant Pathogens. K.D. Hickey ed. APS Press, St. Paul, MN.

10. Pommer, E.H., and Lorenz, G. 1987. Dicarboximide fungicides. Pages 91-106 in: Modern Selective Fungicides. H. Lyr ed. John Wiley & Sons, Inc., New York.

11. Porter, D.M., and Beute M.K. 1974. Sclerotinia blight of peanuts. Phytopathology 64:263-264.

12. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1989. Effects of fungicide RH-3486 on Sclerotinia blight of peanut in field and microplot tests. (Abstr.) Phytopathology 79:1170.

13. Sturgeon, R.V. 1986. Peanut disease loss estimates for major peanut producing states in the United States for 1984 and 1985. Proc. Am. Peanut Res. Educ. Soc. 18:24-26.

14. Wadsworth, D.F. 1979. Sclerotinia blight of peanuts in Oklahoma and occurrence of the sexual stage of the pathogen. Peanut Sci. 6:77-79.

DISEASE CONTROL IN FIELD MICROPLOTS INFESTED WITH IPRODIONE-RESISTANT ISOLATES OF *SCLEROTINIA MINOR*

ABSTRACT

A 3-year study in field microplots from 1987 to 1989 allowed for evaluation of fungicidal control of Sclerotinia blight of peanut caused by two isolates of *Sclerotinia minor*: a normal field isolate (S-2) and a pathogenic, dicarboximide-resistant isolate (B-83-T2). Mature sclerotia were incorporated into the upper 8 cm of soil in June each year to provide a density of four sclerotia/100 g soil for S-2 or two sclerotia/100 g soil for B-83-T2. The performance of two dicarboximide fungicides (iprodione and vinclozolin), two aromatic fungicides (dicloran and PCNB), and one experimental fungicide (RH-3486) were evaluated. Disease incidence (lesions/plot) at harvest in untreated plots averaged 21.9 for S-2 and 20.5 for B-83-T2. Disease incidence was suppressed 97, 83, 33, 67, and 30% in plots infested with isolate S-2, and 96, 55, 62, 25 and 20% in plots infested with isolate B-83-T2, by treatments with RH-3486, vinclozolin, iprodione, PCNB and dicloran, respectively. No significant differences (P=0.05) in disease incidence or yield, due to isolates, were found in plots treated with the same fungicide. All fungicide treatments significantly increased yields in plots infested with S-2, but only RH-3486 and iprodione increased yields significantly in plots infested with B-83-T2. Isolate B-83-T2 appeared to possess a low level of resistance to all fungicides, except RH-3486. A long-term microplot study indicated that iprodione and vinclozolin, but not dicloran, provided significant disease suppression of *in vitro* dicarboximide-resistant isolates (R-2B and R-2C) of *S. minor* after 6 years of fungicide treatments.

INTRODUCTION

Sclerotinia blight of peanut, caused by *Sclerotinia minor* (Jagger) Kohn (9), is currently the major disease problem facing Virginia peanut growers. The disease typically reduces state yields by an average of 6% each year, in spite of current disease control recommendations (P.M. Phipps, personal communication). The fungicidal properties of the dicarboximide fungicides were reported in 1971 (7), and soon thereafter procymidone was shown to be effective against *S. minor* on peanut (15). Commercial registration for dicarboximide fungicides did not occur until the release of iprodione in 1974 (10) and vinclozolin in the following year (14). Iprodione was approved for use on peanut for control of Sclerotinia blight in 1985. The dicarboximides have been used extensively in viticulture in Europe as a replacement for the benzimidazole fungicides, where benzimidazole-resistance in *Botrytis cinerea* Pers. ex Fr. had become a problem. The first dicarboximide-resistant isolate of *B. cinerea* was reported in 1978 (8). The search for effective and safe dicarboximides was slowed by early reports of resistance, but there are continued efforts to gain registration for vinclozolin on peanut.

Current choices of recommended fungicides for control of Sclerotinia blight of peanut in Virginia consist of iprodione and PCNB (12). PCNB belongs to the chlorinated aromatic group of organic fungicides. This group also includes dicloran, which is used in Oklahoma and Texas for Sclerotinia blight suppression. PCNB and dicloran each contain an aromatic ring structure similar to that in the dicarboximides. Isolates of *S. minor* possessing *in vitro* resistance to one dicarboximide fungicide were reported to be cross-resistant to other dicarboximides as well as certain chlorinated aromatic fungicides (4,17). The occurrence of cross resistance has posed a serious threat to the future of current fungicides used for control

of Sclerotinia blight of peanut. Several researchers (3,16,19,21) have screened field isolates of *S. minor* and failed to detect field resistance to the dicarboximides. However, *S. minor* was found to develop *in vitro* resistance to the dicarboximide fungicide, procymidone (0.5 μg/ml), at a frequency of 2.3% (16). A similar rate for occurrence of *in vitro* resistance to iprodione and vinclozolin was reported (3).

In 1983, a study was initiated to compare the field efficacy of iprodione, vinclozolin and dicloran for control of Sclerotinia blight of peanut in field microplots infested with a dicarboximide-sensitive (S-2) and two *in vitro* dicarboximide-resistant (R-2B and R-2C) isolates. Both of the resistant isolates were derived from culturing isolate S-2 on an agar medium amended with iprodione. During the 3-year period (1983-1985) disease caused by isolates with *in vitro* resistance was suppressed an average of 87, 33, and 19% by vinclozolin, iprodione, and dicloran, respectively, compared with 76, 24, and 15% for the sensitive isolate, indicating that disease control was still maintained by fungicide treatments (4). This microplot study suggested that the threat of field resistance in *S. minor* appeared to be less severe than originally thought. Unfortunately, one dicarboximide-resistant isolate was found in a microplot originally infested with S-2 that was treated with iprodione each year (4). This dicarboximide-resistant isolate was designated B-83-T2 and was later shown to be pathogenic on peanut (T.B. Brenneman, personal communication) using an excised peanut stem test (5).

The objectives of this study were two-fold: 1) to characterize the utility of fungicides for control of Sclerotinia blight of peanut caused by B-83-T2 and determine the stability of fungicide resistance in field microplots, and 2) to determine the effect of repeated applications of fungicides on disease control and the ecology of fungicide-resistant isolates of *S. minor* in field microplots.

MATERIALS AND METHODS

Recovery of *S. minor* from field-collected sclerotia. To determine the most effective means of surface-disinfesting sclerotia for optimal recovery of *S. minor*, sclerotia were plated on glucose-yeast extract agar (GYEA) after 0, 1, 2, 5, 10, 20, 50 and 100 minutes in 1.0% NaOCl. Each treatment included 20 sclerotia and was replicated three times. Sclerotia of *S. minor* were collected from diseased peanut stems in a field at the Tidewater Agricultural Experiment Station, Suffolk, VA, in October 1987.

Microplot Study I. Field microplot studies to evaluate the pathogenicity of a dicarboximide-resistant isolate (B-83-T2) and its original sensitive parent isolate (S-2) of *S. minor* were begun in 1987 and continued for two more years. The following fungicides were evaluated: dicloran (Botran® 75WP, Nor-Am Chemical Co., Wilmington, DE), iprodione (Rovral® 50WP, Rhône-Poulenc Ag Co., Research Triangle Park, NC), PCNB (Terraclor® 10G, Uniroyal Chemical Co., Inc., Middlebury, CT), RH-3486 (experimental chemistry 50WP, Rohm & Haas Co., Philadelphia, PA) and vinclozolin (Ronilan® 50WP, BASF Corp., Parsippany, NJ). The microplots consisted of 76-cm-diameter fiberglass barriers that extended 15 cm below and above the soil surface. The distance between centers of microplots was 1.8 m. The soil type was a Nansemond coarse-loamy, siliceous thermic Aquic Hapludult that had been fallow for two years and not previously planted to peanut.

Sclerotia for infesting soil in each microplot were produced in sterile soil amended with corn meal to a level of 5% (w/w). After 6 weeks of incubation at 22 C, the mature sclerotia were collected on a 40-mesh screen (425-μm openings). Water was used to rinse the sclerotia from the medium. In June of each year, the sclerotia were distributed over the surface of the previously planted microplots and incorporated into the upper 8 cm of soil.

Inocula equal to four sclerotia/100 g of soil for S-2 or two sclerotia/100 g for B-83-T2 were added to each microplot every year. The mass of inoculum for each isolate was estimated to be similar since sclerotia of the dicarboximide-resistant isolate were larger. The microplots were planted with seeds of peanut, cv. Florigiant, in May and thinned to three plants/plot after emergence to simulate plant density in commercial fields.

Fungicide treatments were applied to the microplots using a randomized complete block design with six treatments per isolate and four replications per treatment. Wettable powder formations of iprodione (1.12 kg/ha), vinclozolin (0.84 kg/ha) and RH-3486 (0.84 kg/ha) were applied three times at 4-week intervals in 1987 and 1988, and twice in 1989. A 10% granular formulation of PCNB (5.6 kg/ha) was applied twice each year at a 6-week interval. The initial fungicide treatment was made during the last week of July. All fungicide treatments, except PCNB, were applied using a CO_2 backpack sprayer with a single $D_2$13 nozzle which delivered the sprays at 375 l/ha at a pressure of 345 kPa. PCNB was broadcast by hand. Untreated plots served as the check for each isolate. The peanuts were managed according to standard practices, which included applications of chlorothalonil for control of Cercospora leafspot as indicated by the Virginia peanut leafspot advisory program (13). The number of stems with symptoms and signs of Sclerotinia blight in each microplot were recorded during the first week of August, September and October each year.

Prior to harvest in October 1987 and 1988, sclerotia were removed from diseased peanut stems in untreated plots and plots treated with iprodione. In addition, sclerotia were collected from plots treated with RH-3486 and vinclozolin in 1989. The sclerotia were stored in capped plastic tubes at 5 C until assayed for fungicide resistance. Sclerotia were surface disinfested in a 1.0% NaOCl solution for 10 minutes and placed on GYEA containing

chloramphenicol and chlortetracycline at 100 μg/ml. Upon recognition, isolates of *S. minor* were transferred to slants of GYEA in tubes. After collection, all isolates were transferred to unamended GYEA in 9-cm-diameter petri plates. During the phase of maximum mycelial growth (3 days at 22 C), a 5-mm-diameter agar plug, containing mycelia from the periphery of the colony was transferred to GYEA with and without iprodione at 2 μg/ml. Mycelial growth was recorded daily until the colony reached the opposite margin of the agar plate, which generally occurred after 4 days at 22 C on GYEA. Growth measurements on GYEA containing iprodione were terminated after 14 days.

Peanut plants were manually dug and inverted in mid to late October of each year. Pods were removed by hand after one week and allowed to air dry in a greenhouse for 2 weeks. The yield of peanuts was adjusted to 7% moisture (w/w).

Data were analyzed using nonparametric tests because of large differences between years in the coefficient of variance for disease incidence and yield. The Kruskal-Wallis test was used to compare the effect of fungicide treatments on disease incidence and yield for each year and during the 3-year period. Each isolate was analyzed separately. The Wilcoxon rank sum test was used to compare the effect of isolate S-2 and B-83-T2 on disease incidence and yield of peanuts in plots exposed to the same fungicide treatment. Isolate comparisons were made for each year and for the 3-year period.

Microplot Study II. Microplots established in 1983 were used during 1987-1989 to determine changes in pathogenicity and fungicide sensitivity of two *in vitro*, dicarboximide-resistant (R-2B and R-2C) and a fungicide-sensitive (S-2) isolate of *S. minor*. Treatments were the same as in 1983-1985 (4) and included no treatment, dicloran, iprodione and vinclozolin. All management practices were identical to those previously described. No

fungicides were applied to peanuts in these microplots during 1986. Since disease was well established in each microplot, no additional inoculum was added. The initial application of fungicides was made in the last week of July and thereafter at 4-week intervals in the same manner as described for Study I. In 1987 and 1988, the initial application of dicloran was 3.36 kg/ha, followed by two applications at 2.52 kg/ha. Iprodione was applied three times at 1.12 kg/ha, and vinclozolin was applied three times at 0.84 kg/ha. In 1989, the third fungicide treatment was not applied as weather conditions were not conductive for disease. Sclerotia of *S. minor* were collected from untreated, iprodione-treated and vinclozolin-treated plots in 1989 to assess any changes in the occurrence of dicarboximide-resistant isolates.

The Kruskal-Wallis test was used to compare the effect of fungicide treatments on disease incidence and yield during the 3-year period. Each isolate was analyzed separately. The same test was used to compare the effects of each isolate (S-2, R-2B and R-2C) on disease incidence and yield of peanuts in plots exposed to the same fungicide treatment for the 3-year period.

RESULTS

Effect of NaOCl on recovery of *S. minor* from sclerotia. Assays of 60 sclerotia without exposure to NaOCl yielded 37 colonies of *S. minor* and 29 colonies of other fungi (Fig. 5). As surface disinfesting time in 1.0% NaOCl was increased to 20 minutes, the number of colonies of *S. minor* increased and the number of other fungi decreased. Exposure to NaOCl for 10 minutes limited recovery of contaminating fungi, and 52 of 60 sclerotia yielded *S. minor*. Treatments of 50 and 100 minutes were detrimental to the survival of *S. minor*. All subsequent studies with field-collected sclerotia used a 10-minute surface-

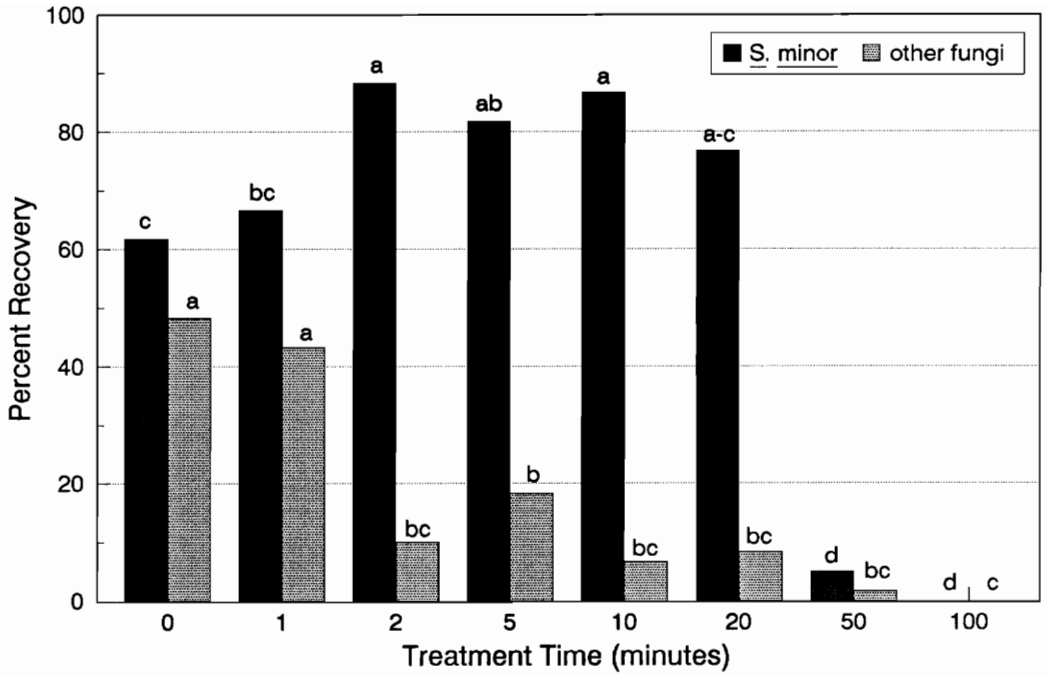

Figure 5. Recovery of *Sclerotinia minor* and other fungi from field sclerotia following treatment in 1.0% NaOCl. Bars represent the mean of three replications, each containing 20 sclerotia. The same letter(s) above bars of a given type denote the absence of significant differences at P=0.05 according to Duncan's multiple range test.

disinfesting time in 1.0% NaOCl.

Disease control in field microplots, Study I. Disease incidence was high in 1988, moderate in 1989 and low in 1987 (Table 5). Concomitantly, overall peanut yields were highest in 1987 and lowest in 1988. In plots infested with isolate B-83-T2, RH-3486 provided significant suppression of disease during all 3 years. Plots treated with RH-3486 had significantly greater yield in 1988 than untreated plots. In plots infested with isolate S-2, applications of RH-3486, PCNB and dicloran resulted in significant yield increases in 1987. During 1988, RH-3486 and vinclozolin significantly suppressed disease and enhanced yields. Only RH-3486 provided significant disease suppression caused by S-2 in 1989. Applications of dicloran resulted in significant enhancement of peanut yields, in spite of a disease incidence rating which approached the untreated check. No significant yearly differences in disease incidence or yield were detected in a comparison of plots infested with isolate S-2 or B-83-T2 and exposed to the same treatment.

Over the 3-year period, disease incidence (lesions/plot) in untreated plots averaged 21.9 for S-2 and 20.5 for B-83-T2 according to counts just prior to harvest in October (Fig. 6). Disease incidence was suppressed 97, 83, 33, 67 and 30% in plots infested with isolate S-2 and 96, 55, 62, 25, 20% in plots infested with B-83-T2 when treated with RH-3486, vinclozolin, iprodione, PCNB and dicloran, respectively. RH-3486, iprodione and vinclozolin gave significant control of disease in plots infested with isolate B-83-T2. RH-3486, vinclozolin and PCNB suppressed disease significantly in plots infested with isolate S-2. No significant differences in disease incidence over the 3-year period were found between isolate S-2 and B-83-T2 when exposed to the same treatment.

Table 5. Effect of fungicides on Sclerotinia blight incidence (lesions/plot) and yield (g/plot) of peanut in microplots infested with either a dicarboximide-resistant isolate (B-83-T2) or a field isolate (S-2) of *Sclerotinia minor*[1].

Isolate and treatment[2]	1987		1988		1989	
	Disease incidence[3]	Yield[4]	Disease incidence	Yield	Disease incidence	Yield
B-83-T2						
untreated	14.0 a	564 a	31.5 a	203 b	14.7 a	388 a
dicloran	3.5 ab	714 a	30.3 a	185 b	15.0 a	401 a
PCNB	11.5 ab	621 a	25.0 a	264 b	9.3 a	413 a
iprodione	1.5 ab	702 a	13.0 ab	359 b	8.5 ab	466 a
vinclozolin	1.5 ab	611 a	14.0 ab	274 b	14.7 a	461 a
RH-3486	0.0 b	723 a	1.0 b	486 a	1.8 b	498 a
S-2						
untreated	19.0 a	343 b	34.3 a	157 c	13.3 ab	252 b
dicloran	9.0 a	602 a	26.3 a	194 c	13.0 a	455 a
PCNB	0.0 a	722 a	16.8 ab	333 bc	5.0 ab	308 ab
iprodione	5.5 a	573 ab	27.8 a	227 bc	10.5 ab	327 ab
vinclozolin	0.8 a	600 ab	6.8 b	394 ab	3.5 bc	378 ab
RH-3486	0.5 a	749 a	0.0 c	530 a	1.3 c	433 ab

[1]Means followed by the same letter(s) within a column and under each isolate are not significantly different at P=0.05 according to the Kruskal-Wallis test.

[2]Microplots were infested with sclerotia of a dicarboximide-resistant isolate (B-83-T2) or a field isolate (S-2) each June. Iprodione (1.12 kg/ha), vinclozolin (0.84 kg/ha) and RH-3486 (0.84 kg/ha) were applied three times at 4-week intervals in 1987 and 1988, and twice in 1989. PCNB (5.6 kg/ha) was applied twice each year at 6-week intervals.

[3]Disease incidence ratings were made in October by determining the number of stem lesions caused by *S. minor*.

[4]Yields are weight (g) of peanuts at 7% moisture (w/w).

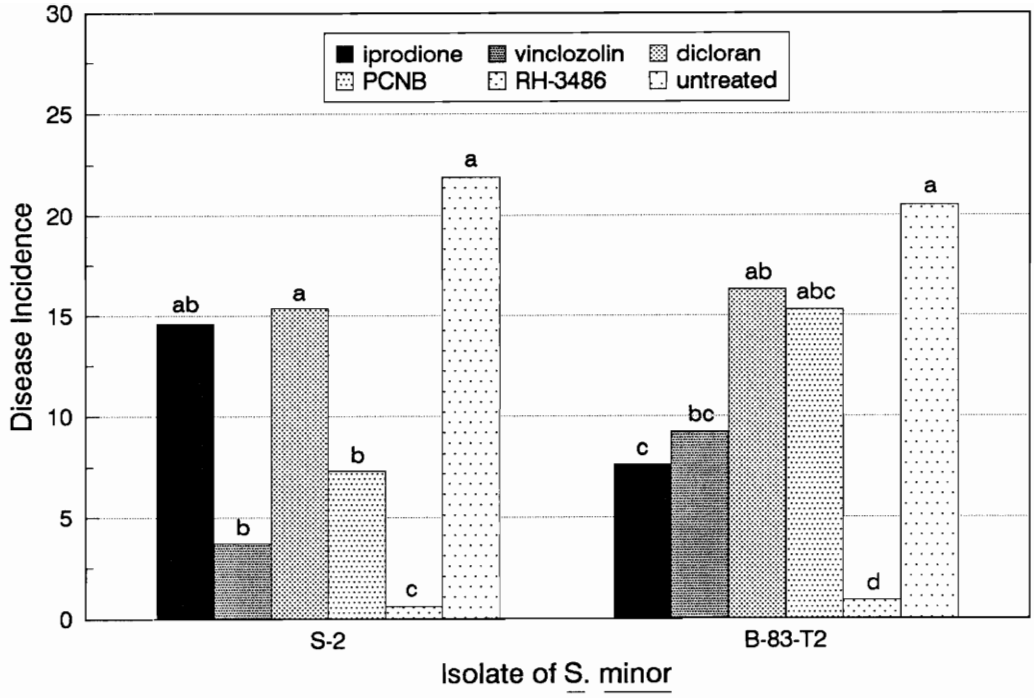

Figure 6. The effect of fungicide treatments on disease incidence (stem lesions/plot) in microplots infested with a dicarboximide-sensitive isolate (S-2) or a dicarboximide-resistant isolate (B-83-T2) of *Sclerotinia minor*. Bars represent the mean of 3 years (1987-1989). Bars within a group having the same letter(s) are not significantly different at P=0.05 according to the Kruskal-Wallis test.

Yields from untreated plots containing isolate B-83-T2 averaged 385 g and microplots containing S-2 averaged 260 g over the 3-year period (Fig. 7). Yields were 184, 80, 124, 48 and 48 g greater in plots infested with B-83-T2 and 310, 197, 116, 194 and 175 g greater in plots infested with S-2 when treated with RH-3486, vinclozolin, iprodione, PCNB and dicloran, respectively. Only RH-3486 and iprodione significantly increased yields in plots infested with B-83-T2, whereas all fungicides significantly increased yields in plots infested with S-2. No significant differences in yield over the period were found between isolate S-2 and B-83-T2 when exposed to the same treatment.

Recovery of *S. minor* isolates from microplots. Screening isolates of *S. minor* on iprodione-amended GYEA failed to detect any iprodione-resistant isolates in 1987 from microplots infested with either B-83-T2 or S-2 (Table 6). In 1988, a greater number of sclerotia were recovered from the microplots. Untreated and iprodione-treated microplots infested with B-83-T2 yielded 30% and 50% resistant isolates, respectively. All of the resistant isolates from untreated microplots were obtained from sclerotia collected in two microplots. Two microplots were also the source of all resistant isolates obtained from microplots treated with iprodione. No resistant isolates were detected in microplots infested with S-2. In 1989, the percentage of resistant isolates from microplots did not change greatly from the preceding year. Untreated and iprodione-treated microplots infested with B-83-T2 yielded 17.6 and 52.9% resistant isolates, respectively. All resistant isolates were recovered from the same microplots as in 1988. Untreated and iprodione-treated microplots infested with S-2 did not contain resistant isolates. However, one microplot infested with S-2 and treated with vinclozolin contained only resistant isolates. These 13 resistant isolates resulted in an overall resistance rate of 68.4% from the microplots originally infested with isolate S-2

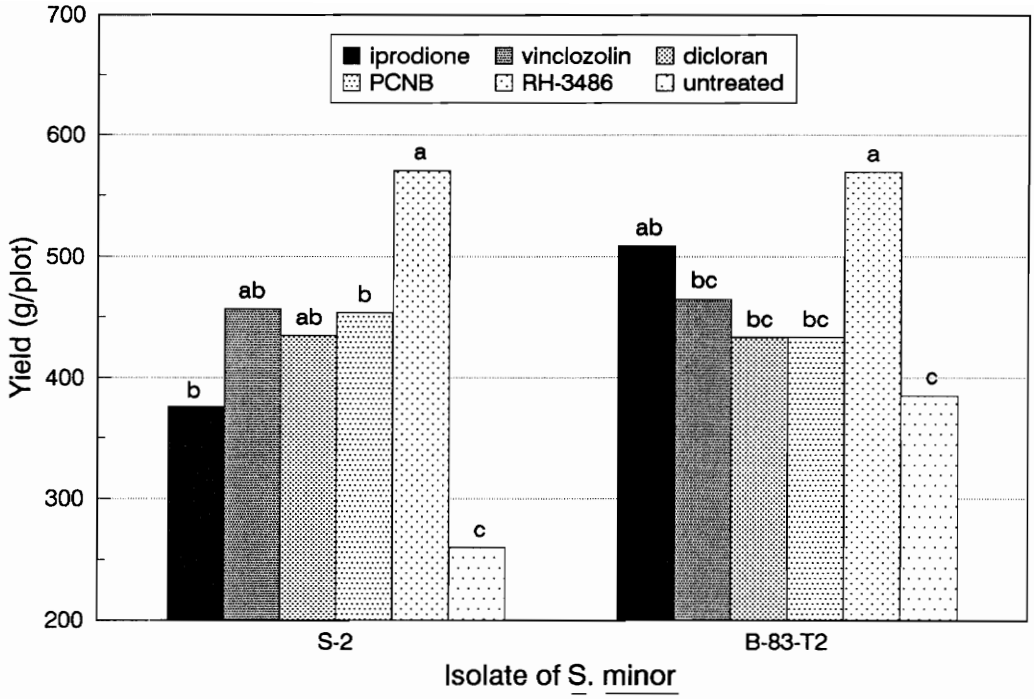

Figure 7. The effect of fungicide treatments on yield (g/plot) in microplots infested with a dicarboximide-sensitive isolate (S-2) or a dicarboximide-resistant isolate (B-83-T2) of *Sclerotinia minor*. Bars represent the mean of 3 years (1987-1989). Bars within a group having the same letter(s) are not significantly different at P=0.05 according to the Kruskal-Wallis test.

Table 6. Sensitivity and resistance to iprodione of *Sclerotinia minor* from microplots infested with a dicarboximide-sensitive (S-2) or -resistant (B-83-T2) isolate.

Year, treatment and isolate[1]	No. isolates recovered	Resistant[2] (%)
1987		
untreated		
S-2	6	0
B-83-T2	12	0
iprodione		
S-2	7	0
B-83-T2	16	0
1988		
untreated		
S-2	27	0
B-83-T2	30	30
iprodione		
S-2	33	0
B-83-T2	36	50
1989		
untreated		
S-2	13	0
B-83-T2	34	18
iprodione		
S-2	33	0
B-83-T2	34	53
vinclozolin		
S-2	19	68
B-83-T2	42	48

[1]Iprodione (1.12 kg/ha) and vinclozolin (0.84 kg/ha) were applied three times at 4-week intervals in 1987 and 1988, and twice in 1989. Microplots were infested with sclerotia of a dicarboximide-resistant isolate (B-83-T2) or a field isolate (S-2) each June.

[2]Colonies were classified resistant if growth on GYEA containing iprodione at 2 μg/ml was twice that of the sensitive isolate after 8 days and if colonies produced sclerotia after 2 weeks of growth.

and treated with vinclozolin. Microplots infested with B-83-T2 and treated with vinclozolin contained resistant isolates in two microplots for an overall resistance rate of 47.6%, similar to the rate for microplots treated with iprodione.

The rate of mycelial growth of isolates on unamended GYEA was not greatly affected by year or fungicide treatment. As the percent of recovery of resistant isolates increased, the average rate of mycelial growth on GYEA containing iprodione at 2 μg/ml also increased. Most of the resistant isolates produced colonies averaging 60 mm of radial mycelial growth after 8 days of incubation on iprodione-amended GYEA, whereas sensitive isolates averaged 18.2 mm.

Disease control in field microplots, Study II. Disease caused by the dicarboximide-sensitive isolate (S-2) was suppressed less by fungicide treatments than disease caused by two *in vitro*, dicarboximide-resistant isolates (R-2B and R-2C) during the 3-year period (Fig. 8). In microplots infested with S-2, dicloran provided no suppression of disease. Vinclozolin and iprodione suppressed disease incidence by 37 and 31%, respectively. These results were not significantly different from that of the untreated check. Vinclozolin, iprodione and dicloran suppressed disease incidence caused by isolate R-2B by 63, 66 and 42%, and isolate R-2C by 61, 48 and 17%, respectively. All three fungicide treatments suppressed disease incidence significantly in plots infested with isolate R-2B. Vinclozolin and iprodione significantly suppressed disease caused by R-2C.

Yields during the same period were not affected significantly by fungicide applications in microplots infested with isolate S-2 (Fig. 9). Vinclozolin resulted in a significant yield increase of 89 g/plot in microplots infested with isolate R-2B; likewise, applications of dicloran resulted in a significant yield increase of 102 g/plot in microplots infested with isolate R-2C.

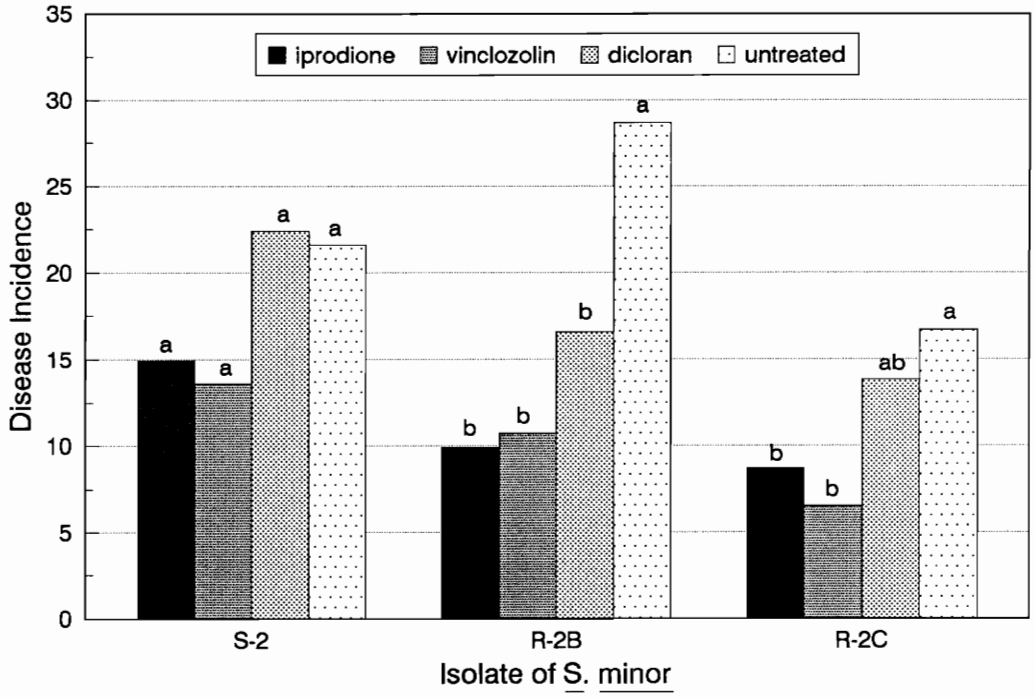

Figure 8. The effect of fungicide treatments on disease incidence (stem lesions/plot) in microplots infested in 1983 with a sensitive field isolate (S-2) and two *in vitro*, dicarboximide-resistant isolates (R-2B or R-2C) of *Sclerotinia minor*. Bars represent the mean of 3 years (1987-1989). Bars within a group having the same letter(s) are not significantly different at P=0.05 according to the Kruskal-Wallis rank sum test.

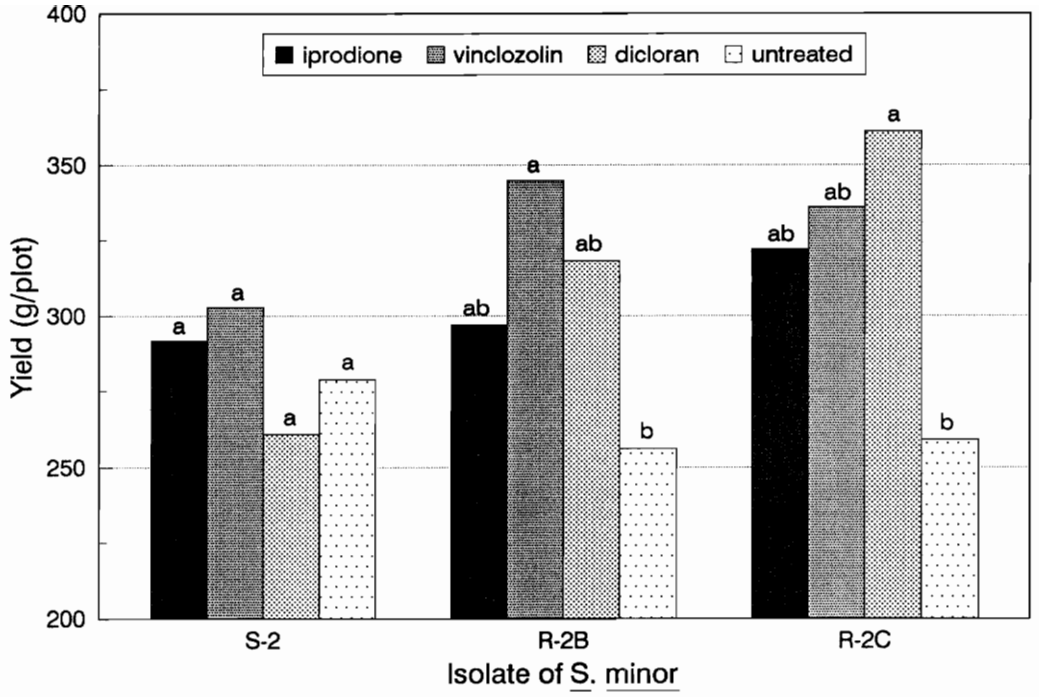

Figure 9. The effect of fungicide treatments on yield (g/plot) in microplots infested in 1983 with a sensitive field isolate (S-2) and two *in vitro*, dicarboximide-resistant isolates (R-2B or R-2C) of *Sclerotinia minor*. Bars represent the mean of 3 years (1987-1989). Bars within a group having the same letter(s) are not significantly different at P=0.05 according to the Kruskal-Wallis rank sum test.

Comparisons of results between the three isolates detected only two significant differences when the same fungicide treatment was used. After applications of vinclozolin, microplots infested with isolate S-2 had a significantly higher incidence of disease than microplots containing isolate R-2C. Microplots treated with dicloran and infested with isolate S-2 yielded significantly less than similarly treated microplots infested with isolate R-2C.

Fungicide applications to microplots infested with isolate S-2 did not result in an increase in recovery of resistant isolates. Twelve isolates of *S. minor* were obtained from each microplot. Plots treated with iprodione and vinclozolin yielded 6.3 and 4.2% resistant isolates, respectively. Plots originally infested with isolate R-2B contained the greatest percentage of resistant isolates. Assays showed that 43.8% of sclerotia from untreated plots were still resistant to iprodione. Exposure to fungicides increased the percentage of resistant isolates as plots treated with iprodione and vinclozolin contained 93.8 and 75.0% resistant isolates, respectively. Untreated, iprodione-treated and vinclozolin-treated microplots infested with isolate R-2C contained 10.4, 39.6 and 33.3% resistant isolates, respectively.

DISCUSSION

Surface-disinfesting sclerotia with a 1.0% solution of NaOCl resulted in a high recovery of *S. minor* from field sclerotia and provided for a representative sample of isolates in assays for resistance to iprodione. The 10-minute treatment in 1.0% NaOCl resulted in a bleached appearance of some sclerotia. Most of the sclerotia that turned completely white were viable, indicating that sclerotia were remarkably tolerant to this treatment. The standard surface-disinfesting treatment of 1 minute in 0.5% NaOCl may be too mild to allow for optimum recovery of *S. minor*. Exposure to stronger solutions for longer periods eliminated

more undesired fungi without damaging the viability of sclerotia.

The 3-year study of disease incidence and yield in microplot Study I indicated that all fungicides provided some control of isolate B-83-T2. Even though B-83-T2 was originally resistant to the dicarboximide fungicides, applications of iprodione significantly limited disease and increased yields. There was no evidence of failure of any fungicide against isolate B-83-T2. Overall, the chlorinated aromatic fungicides were least effective in controlling either isolate of *S. minor*. The dicarboximides were only moderately effective. Applications of RH-3486 virtually prevented disease development in spite of re-infesting the soil with sclerotia each spring. There was no evidence of cross-resistance between RH-3486 and the dicarboximide fungicides (20). Thus, fungicides with a mode of action equal or similar to RH-3486 may prove to be valuable in limiting the development of fungal resistance to the dicarboximides.

Disease pressure in microplot Study I varied greatly from year to year due to different precipitation patterns and the incidence of Cercospora leafspot. In spite of re-infesting the microplots with the appropriate isolate of *S. minor*, the increased inocula did not translate into subsequent higher disease levels during each additional year of the study. Relative disease pressure was lower in 1989 than in 1988. Heavy Cercospora leafspot pressure caused moderate defoliation of the peanut plants in 1989 which may have limited the activity of *S. minor*. Chlorothalonil applied according the leafspot advisory program did not control leafspot in the confined environment of the microplots which contained large amounts of inoculum due to continuous cropping of peanut.

Sclerotial assays indicated that infesting microplots with isolate B-83-T2 in 1987 did not result in the recovery of isolates with resistance to iprodione at the end of the season.

In 1988 and 1989, resistant isolates were recovered, but the frequency of occurrence was not distributed uniformly in microplots exposed to a given treatment. There was no correlation between the percentage of resistant isolates in a microplot and disease incidence or peanut yield. However, applications of the dicarboximides apparently increased the percentage of resistant isolates recovered from microplots infested with B-83-T2. After 3 years of continuous exposure to the dicarboximides, the percentage of isolates with resistance only reached a maximum of 53% in plots treated with iprodione, indicating that some of the disease pressure was caused by isolates that had reverted to a sensitive condition. Therefore, the applications of fungicides did not select for a population of *S. minor* with a high percentage of resistant isolates in dicarboximide-treated microplots.

In one microplot originally infested with isolate S-2 and treated with vinclozolin, all isolates collected were cross-resistant to iprodione. Disease incidence in the plot was not greater than the average of the other three plots which contained sensitive isolates. The occurrence of resistance in this vinclozolin-treated microplot after 3-years exposure to a dicarboximide fungicide was similar to occurrence of isolate B-83-T2, which was found by Brenneman in 1985 in an iprodione-treated microplot. Although fungicide exposure may trigger the development of dicarboximide-resistant isolates, the influence of these isolates on disease control with fungicides appeared to be minor.

In microplots established in 1983 (Study II), fungicide performance from 1983-1985 showed that dicarboximide fungicides were still effective against *in vitro* resistant isolates in a field microplot situation (4). With continuation of tests in these microplots (1987-1989), disease incidence caused by the two *in vitro* resistant isolates was suppressed an average of 62 and 59% by vinclozolin and iprodione, respectively. Yield data did not show significant

differences in isolates due to resistance of *S. minor* to the dicarboximides. These results indicated that no major changes in control of Sclerotinia blight by the dicarboximides occurred even after 7 years of continuous peanut production and 6 years of repeated use of fungicides.

Sclerotial assays indicated that fungicide applications tended to increase the percentage of resistant isolates in microplots. Previous assays of field-collected sclerotia from peanuts treated with various fungicides for control of Cercospora leafspot and Sclerotinia blight indicated that resistance to iprodione developed in an average of 6.3% of field isolates, irrespective of the fungicide-exposure history of the test plot (21). Approximately 6% of the isolates tested in the microplot studies may have originally been sensitive to dicarboximides and resistance developed *in vitro*.

Field resistance to iprodione or the other dicarboximides was not detected in peanut fields managed by current commercial production practices, including experimental fields treated periodically with iprodione during the period 1979 to 1986 (19). Several factors may play a role in preventing the occurrence of field resistance against the dicarboximide fungicides. In a farm situation, populations of sclerotia are not normally exposed to dicarboximides on a yearly basis. Moldboard plowing buries numerous sclerotia, and crop rotation with a non-host plant, such as corn, reduces the number of viable sclerotia. The microplot studies detected a large percentage of resistant isolates in plots infested with a sensitive isolate only after 3 years of continuous exposure to the dicarboximides. In a commercial situation, this would require 6 to 9 years to obtain an equivalent fungicide exposure depending on the rotation scheme. Iprodione has only been used commercially since 1985. Assuming some fungicide-resistant sclerotia may succumb to parasitism or be removed from the upper layer of the soil, resistant isolates may not readily become

established in a commercial field. The life cycle of *S. minor* may not be conducive for the rapid development of resistance to fungicides. A single resistant isolate of *S. minor* can only produce a limited number of sclerotia as compared to the millions of conidia that could be produced by a resistant isolate of *B. cinerea*.

The lack of a single-site mode of action of the dicarboximides may also reduce the likelihood of resistance development and the subsequent failure of a fungicide as occurred with the benzimidazoles. Iprodione does not directly affect energy production or act directly on biosynthetic processes related to DNA synthesis; however nuclear division was disrupted by iprodione (18). A common chemical moiety of the dicarboximides may allow for penetration into the fungal cell (11). A high level of resistance to the dicarboximides would require several genetic changes, perhaps both at the nuclear and cell membrane levels. Dicarboximide-resistance in *S. minor* appears similar to *B. cinerea*. Breakdown in control of *B. cinerea* has not occurred with the dicarboximides for two main reasons: the dicarboximide-resistant strains found in the field possess a low level of resistance and acquisition of dicarboximide resistance confers some loss of fungal fitness (1). The low-level resistant isolates, as typified by B-83-T2 which has an ED_{50} (fungicide dose for 50% inhibition of mycelial growth) to iprodione of less than 5.0 μg/ml (20), were still pathogenic to peanut. These isolates did not possess a level of resistance to overcome the inhibitory effects of normal application rates of dicarboximide fungicides.

Although these studies and previous reports (2,4) did not show failure of the dicarboximide fungicides in microplots infested with resistant isolates, the potential for field resistance remains. Adequate disease control of dollar spot of turf, caused by an iprodione/benomyl-resistant isolate of *S. homoeocarpa*, was not obtained with field

applications of iprodione (6). However, iprodione applications still suppressed disease incidence by 57%. With applications of benomyl, no disease control was obtained. Thus, it is likely that only a gradual loss in disease control would occur if an iprodione-resistant isolate of *S. minor* became established in a peanut field. Based on results obtained from microplot studies, detecting the occurrence of dicarboximide-resistant isolates of *S. minor* by observing changes in pathogenicity or fungicidal control of the disease would not be possible. Since iprodione applications maintained disease control against resistant isolates of *S. minor*, it appears that the dicarboximides will remain effective against Sclerotinia blight of peanut for the short-term future. The search and development of new fungicides with a superior level of activity against *S. minor* should continue because of the limited level of control as well as the potential of selecting isolates with higher levels of resistance to the dicarboximides.

LITERATURE CITED

1. Beever, R.E., and Brien, H.M.R. 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. New Zealand J. Agr. Res. 26:391-400.
2. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1984. Characterization of *Sclerotinia minor* isolates with tolerance to dicloran, iprodione and vinclozolin. (Abstr.) Phytopathology 74:755.
3. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.
4. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Sclerotinia blight of peanut: Relationship between *in vitro* resistance and field efficacy of dicarboximide fungicides.

Phytopathology 77:1028-1032.

5. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1988. A rapid method for evaluating genotype resistance, fungicide activity, and isolate pathogenicity of *Sclerotinia minor* in peanut. Peanut Sci. 15:104-107.
6. Detweiler, A.R., Vargas, J.M., Jr., and Danneberger, T.K. 1983. Resistance of *Sclerotinia homoeocarpa* to iprodione and benomyl. Plant Dis. 67:627-630.
7. Fujinami, A., Ozaki, K., and Yamamoto, S. 1971. Studies on biological activity of cyclic amide compounds. Part I. Antimicrobial activity of 3-phenyloxazolidine-2,4-diones and related compounds. Agric. Biol. Chem. 35:1707-1719.
8. Holz, B. 1979. Uber eine Resistenzerscheinung von *Botrytis cinerea* an Reben gegen die neuen Kontaktbotrytizide im Gebiet der Mittelmosel. Weinberg, Keller 26:18-25.
9. Kohn, L.M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
10. Lacroix, L., Bic, C., Burgaud, L., Guillot, M., Leblanc, R., Riottot, R., and Sauli, M. 1974. Etude des proprietes antifongiques d'une nouvelle famille de derives de l'hydantoine et en particulier du 26 019 R.P. Phytiatrie Phytopharm. 23:165-174.
11. Pappas, A.C., and Fisher, D.J. 1979. A comparison of the mechanisms of action of vinclozolin, procymidone, iprodione and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10:239-246.
12. Phipps, P.M. 1990. Diseases of Peanuts. Pages 55-67 in: 1990 Peanut Production Guide. Va. Coop. Ext. Serv., Publ. MA 197, Suffolk, VA.
13. Phipps, P.M.,and Powell, N.L. 1984. Evaluation of criteria for the utilization of peanut leafspot advisories in Virginia. Phytopathology 74:1189-1193.

14. Pommer, E.H., and Mangold, E. 1975. Vinclozolin (BAS 352F), ein neuer wirkstoff zur bekampfung von *Botrytis cinerea*. Med. Fac. Landbouww. Rijksuniv, Gent 40:713-722.

15. Porter, D.M. 1980. Control of Sclerotinia blight of peanut with procymidone. Plant Dis. 64:865-867.

16. Porter, D.M., and Phipps, P.M. 1985. Effects of three fungicides on mycelial growth, sclerotium production, and development of fungicide-tolerant isolates of *Sclerotinia minor*. Plant Dis. 69:143-146.

17. Porter, D.M., and Phipps, P.M. 1985. Tolerance of *Sclerotinia minor* to procymidone and cross tolerance to other dicarboximide fungicides and dicloran. Peanut Sci. 12:41-45.

18. Reilly, C.C., and Lamoureux, G.L. 1981. The effects of the fungicide, iprodione, on the mycelium of *Sclerotinia sclerotiorum*. Phytopathology 71:722-727.

19. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1987. Effect of field application of iprodione on Sclerotinia blight of peanut and the causal fungus, *Sclerotinia minor*. (Abstr.) Phytopathology 77:1748.

20. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1988. Effect of RH-3486 on dicarboximide-resistant isolates of *Sclerotinia minor*. (Abstr.) Phytopathology 78:863.

21. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1989. Assessment of resistance to iprodione in sclerotial populations of *Sclerotinia minor* from fungicide-treated peanuts. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 21:18.

ASSESSMENT OF RESISTANCE TO IPRODIONE IN SCLEROTIAL POPULATIONS OF *SCLEROTINIA MINOR* FROM FUNGICIDE-TREATED PEANUTS

ABSTRACT

Isolates of *Sclerotinia minor* originating from 1200 sclerotia collected from symptomatic plants in field plots in 1987 did not exhibit stable resistance to iprodione at 2 μg/ml in glucose-yeast extract agar (GYEA), other than expected *in vitro* resistance. Plots were untreated or treated seven times with either benomyl (0.28 kg/ha) plus sulfur (3.36 kg/ha), chlorothalonil (1.26 kg/ha), or diniconazole (0.14 kg/ha) on a 14-day schedule for control of Cercospora leafspot. In a separate test, plots were untreated or treated three times with iprodione (1.12 kg/ha) for control of Sclerotinia blight. The frequency of *in vitro* resistance was not affected significantly by previous applications of fungicides, and resistance occurred in 6.3% of isolates. *In vitro* resistant isolates were significantly more tolerant to iprodione than their parent cultures, which were not previously exposed to iprodione in GYEA. In spite of *in vitro* resistance to dicarboximides being readily achieved by *S. minor*, the development and expression of a significant degree of *in vivo* resistance under field conditions has not been detected and does not appear to threaten continued use of iprodione for control of Sclerotinia blight of peanut.

INTRODUCTION

Sclerotinia blight of peanut, caused by *Sclerotinia minor* (Jagger) Kohn (4) has become a major problem in Virginia. The disease was first reported in the Virginia-North Carolina

area in 1971 (8). During recent years (1987-1989) the disease has reduced state yields of peanuts by an average of 6% each year, resulting in an average yearly loss of $5.8 million to Virginia peanut growers. One dicarboximide fungicide, iprodione, is labeled and recommended for control of Sclerotinia blight (6), but this fungicide is expensive and provides only partial control of the disease (1). Additional concerns have been reported about the continued use of iprodione. *In vitro* resistance to dicarboximide fungicides has been detected at a rate of 1.8% for iprodione and vinclozolin (1) and 2.3% for procymidone. The occurrence of resistant strains to a fungicide in the laboratory suggests a high potential for developing resistance in the field (3).

Assays of sclerotia have not detected resistance to iprodione in commercial peanut fields in Virginia (1,12). These results suggest that the occurrence of *in vitro* resistance may develop in a manner that is different from resistance in a field situation. However, assayed fields had not been exposed to iprodione for a long period of time. Repeated exposure to iprodione may allow for the build-up of fungicide-resistant isolates of *S. minor*, and necessitates continued assays to detect nascent resistance problems.

Other fungicides are routinely used to control Cercospora leafspot of peanut, caused by *Cercospora arachidicola* Hori. Chlorothalonil and benomyl differ in their activity *in vitro* against *S. minor* as they have ED_{50} values (dose required for 50% inhibition of mycelial growth) of 25 and 0.4 μg/ml, respectively (9). Field applications of either fungicide have not been reported to limit Sclerotinia blight. Use of chlorothalonil has been associated with increased severity of Sclerotinia blight (7). Chlorothalonil contains a similar chemical moiety to two chlorinated aromatic fungicides, dicloran and PCNB, which have activity against *S. minor*. Cross-resistance between iprodione and these chlorinated aromatic fungicides has

been reported for *S. minor* (2,11). Fungicides possessing dissimilar chemistry can trigger similar metabolic effects. Isolates of *Botrytis cinerea* exposed to prochloraz, a sterol biosynthesis inhibitor (SBI) fungicide, and isolates exposed to iprodione both showed evidence of interference with ergosterol biosynthesis (5). Other SBI fungicides, such as diniconazole, are being tested for efficacy against Cercospora leafspot of peanut.

The purpose of this research was to assay sclerotial populations of *S. minor* for evidence of resistance or increased tolerance to iprodione. Peanuts were previously exposed to benomyl plus sulfur, chlorothalonil or diniconazole for control of Cercospora leafspot, or iprodione for control of Sclerotinia blight. A preliminary report has been published (13).

MATERIALS AND METHODS

Collecting and assaying field sclerotia from diseased peanut plants. Sclerotia of *S. minor* were collected in October 1987 from symptomatic peanut plants in untreated plots and plots treated seven times at 2-week intervals for control of Cercospora leafspot with either benomyl (0.56 kg/ha) plus sulfur (3.36 kg/ha), chlorothalonil (1.26 kg/ha) or diniconazole (0.56 kg/ha) with SoyOil 937® (0.5% v/v) as an adjuvant. Plots were located at the Tidewater Agricultural Experiment Station Research Farm, Suffolk, VA. From each treatment, two sets of sclerotia were collected. Each set consisted of four replications of 25 sclerotia. A similar number of sclerotia were collected from a different test having plots untreated or treated three times with iprodione (1.12 kg/ha) for control of Sclerotinia blight. Chlorothalonil (1.26 kg/ha) was applied four times in this test for leafspot control according to the leafspot advisory program.

Two sclerotial assay procedures were performed simultaneously: a direct and indirect

means of evaluating resistance to iprodione. Sclerotia from both sets were surface-disinfested with 1.0% NaOCl for 10 minutes. One set of sclerotia was placed in 9-cm petri plates containing glucose-yeast extract agar (GYEA) amended with iprodione at 2.0 μg/ml as a direct assay of resistance. The other set was plated on unamended GYEA as an indirect assay. The isolates of *S. minor* obtained from the indirect assay were cultured on GYEA slant tubes and allowed to form sclerotia. After 14 days, an individual sclerotium from each isolate was transferred to GYEA amended with iprodione at 2 μg/ml. Colonies of *S. minor* larger than 30 mm in diameter after 2-weeks incubation at 22 C were classified as resistant.

Isolates of *S. minor* exhibiting resistance to iprodione in the indirect assay were subsequently compared to their original parent culture. Both the original and subsequent resistant isolates were transferred to unamended GYEA and incubated until actively growing mycelium was obtained (3 days at 22 C). At that time, an agar plug from each member of an isolate pair was transferred to the peripheral edge of a petri plate containing GYEA amended with iprodione at 0, 1, 10 and 100 μg/ml. After 4 days incubation at 22 C,, radial growth (mm) of mycelium was measured. Plates were maintained for 14 days.

RESULTS

Assay for resistance to iprodione in field-collected sclerotia. Isolates of *S. minor* derived from sclerotia collected in fields treated with various leafspot fungicides did not differ significantly in resistance to iprodione, regardless of assay procedure (Table 7). The direct assay procedure detected resistance to iprodione in 6.5, 7.6, 11.3 and 3.6% of sclerotia from plots treated with benomyl plus sulfur, chlorothalonil, diniconazole and untreated plots, respectively. Using the indirect assay, resistance to these fungicides was likewise detected in

Table 7. Effect of field fungicide treatments on incidence of resistance to iprodione in *Sclerotinia minor*[1].

Fungicide treatment	Direct Assay[2] No. resist. isolates	Total no. isolates	% resistant	Indirect Assay[3] No. resist. isolates	Total no. isolates	% resistant
Location A[4]						
untreated	3	83	3.6 a	4	90	4.4 a
benomyl + sulfur	5	77	6.5 a	3	75	4.0 a
chlorothalonil	6	79	7.6 a	8	83	9.6 a
diniconazole	9	80	11.3 a	4	85	4.7 a
total	23	319	7.2 a	19	333	5.7 a
Location B[5]						
untreated	2	43	4.7 a	3	70	4.2 a
iprodione	6	93	6.5 a	7	89	7.8 a
total	8	136	5.8 a	10	159	6.3 a

[1]Resistance values at a given location followed by the same letter are not significant at P=0.05 according to Duncan's multiple range test. Colonies with a radius exceeding 30 mm after 14-days growth were classified as resistant.

[2]Direct assay consisted of placing a sclerotium on glucose-yeast extract agar (GYEA) amended with 2.0 μg/ml iprodione.

[3]Indirect assay consisted of placing a sclerotium on GYEA and subsequently transferring a sclerotium from the resulting culture to GYEA amended with 2.0 μg/ml iprodione.

[4]Fungicides at location A were applied seven times (benomyl at 0.28 kg/ha plus sulfur at 3.36 kg/ha, chlorothalonil at 1.26 kg/ha or diniconazole at 0.14 kg/ha with SoyOil 937® at 0.5% v/v) on a 14-day schedule.

[5]Fungicides at location B were applied three times (iprodione at 1.12 kg/ha) on a 4-week schedule. In addition, four applications of chlorothalonil at 1.26 kg/ha were applied for control of Cercospora leafspot.

4.0, 9.6, 4.7 and 4.4% of cultures derived from sclerotia, respectively.

The frequency of resistant isolates was not influenced significantly by the assay procedure or applications of iprodione for control of Sclerotinia blight. The percentage of sclerotia from iprodione-treated and untreated plots having resistance to iprodione using the direct assay was 6.5 and 4.7%, respectively. This compares to 7.8 and 4.2% using the indirect assay. Sixty isolates of *S. minor* from both test locations out of a total of 947 isolates showed resistance to iprodione for an overall resistance frequency of 6.3%.

The original parent isolates collected from peanuts sprayed with fungicides for control of Cercospora leafspot or Sclerotinia blight were always more sensitive to iprodione in fungicide-amended GYEA plates than were the subsequent resistant isolates (Table 8). The sensitive parent isolates were inhibited an average of 97% by 1 μg/ml iprodione, and 100% by both 10 and 100 μg/ml iprodione after 4-days growth. The resistant isolates were inhibited an average of 38, 59 and 76% by 1, 10 and 100 μg/ml, respectively. The level of resistance to iprodione varied greatly, but no discernible trend between field fungicide exposure and tolerance to iprodione was observed. Some resistant isolates were able to make good growth on GYEA containing 100 μg/ml iprodione, whereas other isolates were completely inhibited. Resistant isolates capable of vigorous growth after 4 days on GYEA containing 100 μg/ml showed reduced mycelial growth on unamended GYEA compared to their parent isolates. Only one parent culture out of 24 paired cultures produced mycelial growth with a diameter in excess of 30 mm on iprodione-amended GYEA at all three concentrations after 14 days of growth. This isolate originated from an iprodione-treated field.

Table 8. Fungitoxicity of iprodione to field isolates of *Sclerotinia minor* and subsequent *in vitro* cultures having resistance to iprodione.

Fungicide treatment	No. of paired isolates	Isolate type[1]	Avg. growth (mm) on GYEA amended with iprodione (μg/ml) after 4 days[2] 0	1	10	100
Location A[3]						
untreated	3	parent	74.7	2.3	0.0	0.0
		resistant	61.4	45.7	32.7	23.3
benomyl + sulfur	2	parent	75.0	0.0	0.0	0.0
		resistant	55.0	14.0	7.0	3.5
chlorothalonil	6	parent	69.0	1.5	0.0	0.0
		resistant	62.2	38.8	15.5	6.3
diniconazole	3	parent	75.0	2.3	0.0	0.0
		resistant	62.3	49.0	34.7	19.0
Location B[4]						
untreated	3	parent	75.0	2.0	0.0	0.0
		resistant	42.3	19.0	15.3	9.0
iprodione	7	parent	75.0	4.8	0.0	0.0
		resistant	68.3	44.0	35.6	20.4
Total	24	parent	73.5	2.4	0.0	0.0
		resistant	60.8	36.5	28.8	17.0

[1]Parent isolates of *S. minor* originated from field-collected sclerotia. Resistant isolates showed evidence of resistance upon exposure to 2 μg/ml of iprodione in GYEA and were derived from the corresponding parent isolate.

[2]GYEA = glucose-yeast extract agar.

[3]Fungicides at location A were applied seven times (benomyl at 0.28 kg/ha plus sulfur at 3.36 kg/ha, chlorothalonil at 1.26 kg/ha or diniconazole at 0.14 kg/ha with SoyOil 937® at 0.5% v/v) on a 14-day schedule.

[4]Fungicides at location B were applied three times (iprodione at 1.12 kg/ha) on a 4-week schedule. In addition, four applications of chlorothalonil at 1.26 kg/ha were applied for control of Cercospora leafspot.

DISCUSSION

Assays of field-collected sclerotia from peanuts treated with various fungicides for control of Cercospora leafspot and Sclerotinia blight indicated that resistance to iprodione developed in an average of 6.3% of field isolates, irrespective of the isolate exposure to fungicides in the field. Treatments for control of Cercospora leafspot, representing fungicides from three different classes, had no significant effect on the sensitivity of *S. minor* to iprodione. Field exposure to leafspot fungicides did not induce detectable cross-tolerance to iprodione. Treatments with iprodione for control of Sclerotinia blight also had no significant effect on the sensitivity of the fungus or rate of resistance to iprodione. The incidence of resistance in GYEA containing iprodione at 2 μg/ml was greater than results previously reported for *in vitro* assays of dicarboximide fungicides (1,10). Evaluation of resistance was subjective as colonies displayed a wide range of growth patterns on iprodione-amended GYEA. Different growth conditions and methods of defining resistance may account for the variation in percentage of resistant isolates.

Parent isolates were always more sensitive to iprodione than their subsequent resistant isolates. This suggests that resistance detected in the laboratory assay was *in vitro* resistance and not a stable form of field resistance. After 14 days, only one parent isolate showed evidence of resistance to iprodione at 10 and 100 μg/ml. The occurrence of resistance in this one isolate out of 24, resulted in a resistance rate of 4.2% for parent isolates, similar to the rate of resistance for isolates exposed to iprodione for the first time in a laboratory assay. When assaying pathogens for fungicide resistance, it is important to determine the background level of *in vitro* resistance to a given fungicide. In this study of resistance to iprodione, *in vitro* resistance of a frequency as high as 11.3% did not reflect the presence of

field resistance.

The ability of *in vitro* resistant isolates to grow on GYEA containing high concentrations of dicarboximide fungicides has not resulted in loss of disease control with fungicides in field microplots infested with *in vitro* resistant isolates (2). Two *in vitro* resistant isolates of *S. minor*, R-2B and R-2C, were capable of growth on GYEA containing 1000 μg/ml of vinclozolin and iprodione, respectively. Should isolates of *S. minor* possessing this kind of *in vitro* resistance appear in a field, results from the microplot study suggest that recommended applications of iprodione would prove effective in disease control.

The inability of several researchers to detect field resistance to the dicarboximide fungicides coupled with fungicidal control of disease caused by *in vitro* resistant isolates suggests that resistance to these fungicides does not pose an immediate threat to the peanut industry. Annual surveys of populations of *S. minor* in fields treated with iprodione should be continued to monitor the background frequency of *in vitro* resistance. The sudden occurrence of a high percentage of iprodione resistant isolates in laboratory assays may be an indication of the establishment of field resistance to iprodione. Early detection of a resistance problem will be required for implementation of effective alternative strategies for sustaining use of the dicarboximides in the field.

LITERATURE CITED

1. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of *Sclerotinia minor* to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.

2. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Sclerotinia blight of peanut:

Relationship between *in vitro* resistance and field efficacy of dicarboximide fungicides. Phytopathology 77:1028-1032.

3. Dekker, J. 1985. The fungicide resistance problem: Will it get worse? EPPO Bull. 15:337-344.
4. Kohn, L.M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
5. Pappas, A.C., and Fisher D.J. 1979. A comparison of the mechanisms of action of vinclozolin, procymidone and prochloraz against *Botrytis cinerea*. Pestic. Sci. 10:239-246.
6. Phipps, P.M. 1990. Diseases of Peanuts. Pages 55-67 in: 1990 Peanut Production Guide. Va. Coop. Ext. Serv., Publ. MA 197, Suffolk, VA.
7. Porter, D.M. 1980. Increased severity of Sclerotinia blight in peanuts treated with captafol and chlorothalonil. Plant Dis. 64:394-395.
8. Porter, D.M., and Beute M.K. 1974. Sclerotinia blight of peanuts. Phytopathology 64:263-264.
9. Porter, D.M., and Lankow, R.K. 1981. Growth of *Sclerotinia minor* on media containing chlorothalonil and benomyl. Plant Dis. 65:591-594.
10. Porter, D.M., and Phipps, P.M. 1985. Effect of procymidone and other fungicides on mycelial growth, sclerotia production and development of fungicide-tolerant isolates of *Sclerotinia minor*. Plant Dis. 68:143-146.
11. Porter, D.M., and Phipps, P.M. 1985. Tolerance of *Sclerotinia minor* to procymidone and cross tolerance to other dicarboximide fungicides and dicloran. Peanut Sci. 12:41-45.
12. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1987. Effect of field application of iprodione

on Sclerotinia blight of peanut and the causal fungus, *Sclerotinia minor*. (Abstr.) Phytopathology 77:1748.

13. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1989. Assessment of resistance to iprodione in sclerotial populations of *Sclerotinia minor* from fungicide-treated peanuts. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 21:18.

MECHANISMS OF ENHANCEMENT OF SCLEROTINIA BLIGHT BY CHLOROTHALONIL

ABSTRACT

Peanut fields treated with chlorothalonil (1.26 kg/ha) according to the Virginia peanut leafspot advisory averaged 17% less incidence of Sclerotinia blight and yielded 870 kg/ha more than plots sprayed seven times on a 2-week calendar schedule. Plots treated on the advisory schedule were sprayed four times in 1987 and three times in 1988. All plots had a history of Sclerotinia blight. In glucose-yeast extract agar (GYEA) containing chlorothalonil at 2 to 1000 μg/ml, the addition of iprodione at 0.5 μg/ml increased the development of chlorothalonil-tolerant sectors of *S. minor*. On fungicide-treated excised stems, chlorothalonil did not affect the effectiveness of iprodione. Use of bromophenol blue in GYEA indicated increased organic acid production relative to mycelial growth in the presence of chlorothalonil and other fungicides. Cultures of *S. minor* conditioned on GYEA amended with chlorothalonil at 10 μg/ml were more pathogenic on excised stems than unconditioned cultures, but the increase in pathogenicity was not sustained after transfer to GYEA without fungicide. Treatment of excised stems with suspensions of chlorothalonil just prior to inoculation did not enhance lesion development. Field applications of chlorothalonil resulted in significantly larger lesions on inoculated excised stems indicating that time was required for changes in susceptibility of stems to *S. minor*.

INTRODUCTION

Sclerotinia blight, caused by *Sclerotinia minor* (Jagger) Kohn (8), is currently the most destructive disease of peanut in Virginia. Recent yield losses (1987-1989) to the disease have averaged 6% each year, in spite of increased use of iprodione (P.M. Phipps, personal communication). Control of Sclerotinia blight incidence with iprodione commonly averages 45-55% (2), and there remains a need for more efficacious control strategies. Losses occur as the disease attacks lateral limbs of the peanut at the soil surface. Decay of stems and pegs weakens the plant and results in a heavy loss of peanut pods remaining in the soil at harvest. In addition to Sclerotinia blight, foliar diseases must also be controlled in peanuts. Chemical control of Cercospora leafspot of peanut, caused by *Cercospora arachidicola* Hori., is required to produce an economical crop in the peanut production area of southeast Virginia. In spite of currently recommended control measures, early leafspot still reduces peanut yield by approximately 4% each year. Chlorothalonil is an effective fungicide for control of early leafspot, but its use has been demonstrated to increase the severity of Sclerotinia blight (16). To avoid this problem, growers have been advised to reduce the use of chlorothalonil and, in some instances, use less effective leafspot fungicides in fields with a history of severe Sclerotinia blight (13).

A reduction in the total number of fungicide applications for leafspot control resulted when the Virginia peanut leafspot advisory program was established in 1981 (15). Advisories to spray were issued to growers when peanut plants were vulnerable to leafspot infection. Growers following the advisory have reduced the number of spray applications an average of 3.5 per season (12). The incidence of Sclerotinia blight following applications of chlorothalonil according the advisory program was significantly less than applications at 2-

week intervals (11). A dense canopy has been implicated as a factor favoring Sclerotinia blight (5), but in peanut plots lacking significant differences in percent defoliation, plots sprayed more frequently with chlorothalonil still had a higher incidence of Sclerotinia blight (P.M. Phipps, unpublished). This suggested that frequent exposure to chlorothalonil was partially responsible for increased severity of Sclerotinia blight.

In agar studies, *S. minor* was relatively insensitive to chlorothalonil as the fungus had an ED_{50} value of 25 μg/ml (17). Sectors insensitive to chlorothalonil developed at a frequency of 10-40% on agar media containing the fungicide. Once the mycelia began to form sclerotia, the insensitivity to chlorothalonil was lost. The presence of 0.2 μg/ml chlorothalonil in potato dextrose agar (PDA) was reported to increase the amount of oxalic acid produced by *S. minor* (7). Isolates of *S. minor* grown in oat grains treated with 0.2-0.4 μg/ml chlorothalonil were also shown to produce larger stem lesions on peanut plants than similar inoculum grown without the fungicide.

Oxalic acid plays an important role in the pathogenicity of *Sclerotium rolfsii* Sacc. (1) and *Sclerotinia* spp. (9). The production of oxalic acid is thought to create an acidic environment favorable for polygalacturonase activity resulting in the formation of calcium chelates and weakened pectates in the host plant. *S. sclerotiorum* secretes large amounts of oxalic acid during the initial phase of pathogenesis (10). The addition of oxalic acid to peanut plants has been reported to induce wilting similar to that caused by *S. minor* (7). Further evidence of the importance of oxalic acid was the demonstration that mutants of *S. sclerotiorum* deficient in the production of oxalic acid were not pathogenic to bean plants (6). The lack of oxalic acid production was detected by growing isolates of *S. sclerotiorum* on PDA amended with 50 μg/ml bromophenol blue. In the presence of oxalic acid, the pH sensitive

dye changed from blue to yellow.

The purpose of this research was to examine how chlorothalonil increases the severity of Sclerotinia blight of peanut and influences the performance of iprodione. The impetus for this project was the difficulty in obtaining good control of Cercospora leafspot of peanut without triggering increased levels of Sclerotinia blight in fields under pressure from both diseases.

MATERIALS AND METHODS

Field Tests. In 1987 and 1988, fungicide trials for control of early leafspot were conducted in fields with a history of severe Sclerotinia blight. A 2-week calendar schedule and the leafspot advisory program for application of chlorothalonil were compared for their effects on Sclerotinia blight. Treatments were replicated four times in randomized complete blocks, and plots consisted of four, 12.2-m rows. Fungicides were applied only to the two center rows of each plot using three $D_2$13 (disk-core combination) nozzles per row calibrated to deliver 140 l/ha at a pressure of 345 kPa. No fungicides were applied for control of Sclerotinia blight. Chlorothalonil at 1.26 kg/ha was applied seven times on a 2-week schedule that began on 25 July in 1987. According to the advisory program, applications were made on 6 Jul, 5 Aug, 18 Aug, 9 Sep and 22 Sep. In 1988, seven calendar sprays were made at 2-week intervals that began on 22 Jun. Advisory program sprays were applied on 22 Jul, 3 Aug, 22 Aug and 12 Sep. Incidences of Sclerotinia blight and Cercospora leafspot were monitored monthly in the two center rows. Severity of Sclerotinia blight was determined by counting number of infection centers in the two center rows of each plot (14). Leafspot and defoliation were rated as a percent of leaflets containing spots or leaflets which had abscised

from the plant. Arcsine transformation of leafspot data was made in analyses to determine statistical significance. Yields were based on weight of harvested peanuts and a moisture content of 7% (w/w). Statistical analyses included Duncan's new multiple range test using a probability value of 0.05. The final disease rating for Sclerotinia blight and Cercospora leafspot was made just prior to harvest on 12 Oct and 13 Oct during 1987 and 1988, respectively.

Agar-plate tests. The effect of chlorothalonil on fungicidal activity of iprodione was assessed in glucose-yeast extract agar (GYEA), consisting of agar, 20 g; glucose, 20 g; yeast extract, 2.0 g, KH_2PO_4, 1.0 g; $MgSO_4$-$7H_20$, 0.5 g and 1,000 ml distilled water. After autoclaving, the solutions of GYEA were maintained at 70 C in a waterbath. Chlorothalonil (Bravo® 720, Fermenta Agricultural Specialty Chemicals, Mentor, OH) was added to GYEA to obtain concentrations of 1000, 200, 50, 10, 2, 0.5 μg/ml. Replicate samples with each level of chlorothalonil were then amended with iprodione at 0.5 μg/ml (Rovral® 50WP, Rhône-Poulenc inc, Research Triangle Park, NC). GYEA not amended with either fungicide served as the check. After thorough mixing, the media were dispensed into 9-cm-diameter petri plates and allowed to cool. On GYEA containing 0.5 μg/ml iprodione, a typical field isolate of *S. minor* was able to make limited growth as this fungicide concentration was approximately 2.5 times higher than a previously reported ED_{50} value for iprodione (2). Any enhancement of growth or inhibition with the addition of chlorothalonil would be detectable. Chlorothalonil was used at concentrations known to include rates with and without fungitoxicity to *S. minor*.

Agar plugs, 6-mm in diameter, which contained rapidly-growing mycelia of a dicarboximide-sensitive, field isolate (S-2) of *S. minor* were placed face down on the agar

media in the center of the petri plate, and the plates were incubated at 22 C. Mycelial growth (maximum colony radius in mm), and the presence or absence of fungicide-tolerant sectors of *S. minor* were recorded at 24-hour intervals for a period of 2 weeks. Treatments were replicated 30 times and the experiment was performed twice.

In additional studies, bromophenol blue (BPB) (3',3",5',5"-tetrabromophenol-sulfonphthalein sodium salt, Sigma Chem. Co., St. Louis, MO) was used as an indicator of organic acid production in GYEA with and without fungicides. GYEA containing chlorothalonil at 0.1, 1, 10 and 100 μg/ml, iprodione at 0.01, 0.1, 1 and 10 μg/ml, and RH-3486 (50WP, Rohm and Haas Co, Philadelphia, PA) at 0.001, 0.01, 0.1 and 1 μg/ml were prepared as previously described. Just after addition of fungicide to GYEA, BPB was added to obtain a final concentration of 50 μg/ml. Two isolates of *S. minor* were used in this test: S-89 was a typical fungicide-sensitive isolate and B-83-T2 was a dicarboximide-resistant isolate. Each isolate was transferred to the periphery of each of the GYEA plates as previously described. Treatments were replicated three times and the entire experiment was performed twice. Radial growth (mm) of each isolate was recorded daily along with the radius of the zone of color change from blue to yellow. A ratio of the zone of color change to colony growth was calculated as follows: ratio = $\pi(r^2_{zone} - r^2_{growth})/(r_{growth})(100)$. This equation relates the area of color change outside the colony area to the radial growth of the colony.

Excised stem tests. During Aug 1988, stems of NC 9 peanut were collected from plants in the field that were not previously treated with chlorothalonil or iprodione. Uniform, lateral, prostrate stems containing several carpophores were cut from plants and placed in an ice-chest for transporting to the laboratory. Stems used in this test were 15-weeks old. Leaves were removed with a razor blade by cutting the petiole at a distance of 4 mm from

the stem. The stems were washed with tap water to remove soil and debris. Segments 95-mm long and containing the third, fourth and fifth nodes from the apex were selected. The prepared segments were rinsed again in tap water and then placed in the appropriate suspension of fungicide for 1 minute. After treatment, the stems were allowed to air-dry on paper towels.

Fungicide suspensions of chlorothalonil and iprodione were prepared in distilled water at rates to simulate a tank-mix concentration of fungicides used in commercial applications and a 1/100th rate. The full tank-mix rates of chlorothalonil and iprodione were 9000 and 3000 μg/ml, respectively. In order to detect an interaction of the fungicides, excised-stem segments were untreated, treated with iprodione or chlorothalonil, and treated with a mixture of both fungicides. Each experiment was performed twice and each treatment consisted of 12 stems.

Two isolates of *S. minor*, designated S-17 and S-69, were selected for inoculation of the excised stems. Both isolates were typical of normal field isolates of *S. minor* in terms of growth on GYEA and sensitivity to fungicides. Agar plugs of each isolate from GYEA without fungicides were removed from the periphery of the cultures with actively growing mycelium and used as inoculum. Prior to inoculating the stems, a circular wound was made on the stem by removing the remaining petiole segment at the fourth node with a sharp razor blade. The inoculum was placed on the stem so that the mycelium was in direct contact with the wound. The inoculated stems were incubated in humid boxes at 20 C (3). Lesion lengths (mm) were measured at 24-hour intervals.

To investigate the effect of field applications of chlorothalonil (1.26 kg/ha) on development of Sclerotinia blight of peanut, 15-week old stems of NC 9 peanut were

collected from plots untreated and treated with chlorothalonil on a 28-, 21- and 14-day spray schedule. At the time of stem collection on 13 Aug, the treated plants had been exposed to chlorothalonil a total of two, three and four times, respectively. In 1989, 18-week-old stems of NC 9 peanut were collected from untreated plots or plots sprayed a total of three, four, five and six times with chlorothalonil. Plots treated 6 times were sprayed every 2 weeks. Plots treated three, four and five times were sprayed according to various versions of a new advisory model for leafspot management (4). During both years, stems were processed as previously described. Isolates S-17 and S-69 were used in 1988, and isolate S-89 was used in 1989. No additional fungicides were applied to excised stems.

The effect of conditioning cultures of *S. minor* to chlorothalonil was investigated by growing isolate S-17 on GYEA with and without chlorothalonil at 10 μg/ml. The cultures were transferred twice on the media prior to being used as inoculum on excised stems from 15-week-old plants of NC 9 peanut. GYEA plates containing no fungicide were inoculated 3 days prior to removing an inoculum plug of *S. minor*, whereas plates containing chlorothalonil at 10 μg/ml were inoculated 5 days prior to use. This delay resulted in the diameter of mycelial growth being about equal in each agar plate at the time of collection of agar plugs for stem inoculation. In order to determine the stability of chlorothalonil-induced changes in pathogenicity, cultures of *S. minor* grown on chlorothalonil-amended GYEA were transferred to GYEA containing no fungicide. After 3-days growth, plugs from unamended GYEA plates were used to inoculate excised stems with either the culture of *S. minor* previously exposed to chlorothalonil or the culture never exposed to the fungicide. Excised stems used in this test were either untreated, treated with iprodione at 3000 μg/ml, chlorothalonil at 9000 μg/ml or both fungicides at the this tank-mix rate.

RESULTS

Effect of leafspot control with chlorothalonil on Sclerotinia blight. Peanuts sprayed according to the advisory program averaged 17% less Sclerotinia blight than plots sprayed on a 2-week spray schedule (Table 9). In the heavily defoliated plots that received no leafspot treatment, the incidence of Sclerotinia blight was 79% less than plots receiving chlorothalonil treatments on the 2-week schedule. These differences were significant when analyzed over the 2-year period.

The use of chlorothalonil in both programs of application significantly limited incidence of leafspot and defoliation when compared to untreated plots. However, no significant differences in levels of leafspot and defoliation occurred between plots treated with chlorothalonil on the 2-week or advisory program. Plots treated with the additional applications of chlorothalonil on the 2-week spray program had a significant increase in the incidence of Sclerotinia blight.

During the 2-year period, average peanut yields were greatest in plots treated according to the advisory program and least in plots treated on the 2-week spray schedule. The increased severity of Sclerotinia blight in plots treated on the 2-week calendar spray contributed to an average yield loss of 870 kg/ha of peanuts as compared to plots sprayed on the advisory schedule. Untreated peanut plots yielded 738 kg/ha more than plots treated on the 2-week schedule. In 1987 and 1988, leafspot pressure was not severe until late in the season, enabling untreated peanut plants to produce a moderate yield.

Interaction of chlorothalonil and iprodione in agar tests. Chlorothalonil alone had a limited effect on mycelial growth of *S. minor* (Table 10). At fungicide concentrations up to 10 μg/ml, no sectors were detected and growth was only inhibited 11%. The mycelium of

Table 9. Effect of chlorothalonil applied according to the leafspot advisory program and a 2-week schedule on peanut yields and incidence of Sclerotinia blight and Cercospora leafspot of peanut[1].

Year, treatment and no. of applications[1]	Sclerotinia blight[2]	Cercospora leafspot[3]		Yield[4] (kg/ha)
		% leafspot	% defoliation	
1987				
untreated	8.5 c	91.3 a	70.0 a	4589 a
chlorothalonil advisory (5x)	32.8 b	11.3 b	2.5 b	4829 a
chlorothalonil 14-day (7x)	40.5 a	2.5 b	1.0 b	3742 b
1988				
untreated	3.8 b	99.0 a	68.8 a	3403 a
chlorothalonil advisory (4x)	15.5 a	14.0 b	2.3 b	3428 a
chlorothalonil 14-day (7x)	17.5 a	2.8 b	8.8 b	2774 a
2-Year Average				
untreated	6.2 c	95.2 a	69.4 a	3996 b
chlorothalonil advisory (4.5x)	24.2 b	12.7 b	2.4 b	4128 a
chlorothalonil 14-day (7x)	29.0 a	2.6 b	4.9 b	3258 c

[1]Means within a column and group followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range test. Chlorothalonil was applied at 1.26 kg a.i./ha.

[2]Data are counts of infection centers of *Sclerotinia minor* in two 12.2-m rows on 12 Oct 1987 and 13 Oct 1988.

[3]Leafspot and defoliation were rated on 12 Oct 1987 and 13 Oct 1988. 0 = no spots or defoliation, 100 = spots on all leaflets or no leaflets on plants. Arcsine transformation of data was made in analyses to determine statistical significance.

[4]Yields are based on weight of peanuts with moisture content of 7% (w/w).

Table 10. Effect of chlorothalonil and iprodione on mycelial growth of *Sclerotinia minor* (S-2) on glucose-yeast extract agar (GYEA) after 5 days incubation at 22 C[1].

Treatment	Radial Growth (mm)[2]			% sectors[3]
	Plates with sectors	Plates without sectors	All plates	
chlorothalonil (μg/ml)				
0	--	40.0	40.0 a	0.0 d
0.5	--	40.0	40.0 a	0.0 d
2	--	35.7	35.7 b	0.0 d
10	--	35.8	35.8 b	0.0 d
50	ND[4]	ND	31.3 b	--
200	ND	ND	32.1 b	--
1000	ND	ND	27.7 c	--
chlorothalonil (μg/ml) + iprodione 0.5 μg/ml				
0	--	16.4	16.4 ef	0.0 d
0.5	--	18.7	18.7 de	0.0 d
2	16.3	4.9	8.3 g	30.0 c
10	11.6	5.0	8.8 g	56.7 b
50	19.3	5.4	13.8 f	60.0 b
200	18.7	4.3	15.3 f	76.7 a
1000	21.9	9.0	20.2 d	89.7 a

[1]Means within a column followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range test. Values are the average of two tests, each containing 30 replications.

[2]Radial growth measurements represent most distant mycelial growth from source of inoculum.

[3]Sectors exceeded normal colony margin by at least 1 cm.

[4]ND = Not discernible, because of highly irregular mycelial growth.

S. minor appeared to be more dense on the surface of GYEA containing low levels of chlorothalonil. Growth inhibition increased to 31% at rates up to 1000 μg/ml. Colony margins were irregular at concentrations of chlorothalonil at or above 50 μg/ml. The addition of iprodione at 0.5 μg/ml had a profound effect on the mycelial growth of *S. minor* on GYEA containing varying amounts of chlorothalonil. The presence of chlorothalonil at 0.5 μg/ml did not significantly affect growth of *S. minor* on GYEA containing 0.5 μg/ml iprodione. However, as concentrations of chlorothalonil were increased from 2 to 1000 μg/ml in the presence of iprodione, the frequency of sectoring also increased which resulted in an increase in mycelial growth. On iprodione-containing GYEA plates amended with chlorothalonil at 1000 μg/ml, the incidence of sectoring reached its highest rate of 89.7%. Radial growth of non-sectoring colonies averaged between 4.3 to 9.0 mm after 5 days, and was less than that on GYEA plates amended with either fungicide alone. These results indicated synergistic activity of the fungicides against non-sectoring colonies. Sectors were readily recognized by their radiating pattern of rapid growth. The average size of sectors was not greatly influenced by the concentration of chlorothalonil nor did the size of sectors differ greatly from non-sectoring mycelial growth on GYEA containing iprodione at 0.5 μg/ml. The overall increase in colony size, as concentrations of chlorothalonil increased, can be attributed to the higher rate of sectoring.

Use of bromophenol blue as an indicator of organic acid production by *S. minor* in agar-plate tests. BPB was an effective indicator of pH changes caused by growth of *S. minor* on GYEA. The GYEA medium containing BPB changed from blue to yellow in response to organic acid production by fungal mycelium. Growth of both isolates, S-89 and B-83-T2, was not significantly inhibited by the addition of BPB at 50 μg/ml to GYEA (Table 11). On

Table 11. Evaluation of acid production by two isolates of *Sclerotinia minor* in glucose-yeast extract agar (GYEA) containing bromophenol blue (BPB) at 50 μg/ml and fungicides[1].

Treatment	Isolate S-89			Isolate B-83-T2		
	growth (mm)[2]	acidification zone[3]	ratio[4]	growth (mm)[2]	acidification zone[3]	ratio[4]
unamended GYEA	61.7 a	--.-	-.--	36.0 a	--.-	-.--
BPB alone	60.3 a	65.7	0.35	34.0 a	41.7	0.54
chlorothalonil (μg/ml) and BPB						
0.1	54.7 a	60.7 a	0.39	32.0 a	43.0 a	0.81
1.0	25.0 b	34.3 b	0.69	21.7 b	35.0 b	1.09
10	12.7 c	22.0 c	1.16	17.0 c	30.7 c	1.21
100	12.3 c	21.7 c	1.17	17.7 c	33.7 b	1.46
iprodione (μg/ml) and BPB						
0.01	57.3 a	62.3 a	0.33	37.7 a	45.7 a	0.56
0.1	29.0 b	35.0 b	0.41	34.7 b	43.7 a	0.64
1.0	1.0 c	4.0 c	0.50	38.7 a	43.7 a	0.33
10	0.0 c	0.0 d	-.--	11.7 c	20.3 b	0.74
RH-3486 (μg/ml) and BPB						
0.001	44.7 a	52.3 a	0.51	29.7 a	39.3 a	0.70
0.01	13.3 b	28.7 b	1.52	16.0 b	34.0 b	1.77
0.1	4.0 c	17.3 c	2.22	5.0 c	26.7 c	4.32
1.0	0.7 c	4.3 d	0.85	2.0 d	18.3 d	5.20

[1]Means within a column and fungicide grouping followed by the same letter are not significantly different at P=0.05 according to Duncan's multiple range test. Values are the average of two tests, each containing three replications. Isolate S-89 is a common field isolate and B-83-T2 is a dicarboximide-resistant isolate.

[2]Radial growth (mm) measured at day 3.

[3]Zone measurements (mm) represent distance of color change of BPB from inoculum source.

[4]Ratio represents relationship of area of color change outside area of mycelial growth to colony radius, ratio = $\pi(r^2_{zone} - r^2_{growth})/(r_{growth})(100)$.

GYEA containing only BPB, the dicarboximide resistant isolate (B-83-T2) appeared to be an effective producer of organic acids, based on a 0.54 ratio of acidification zone to colony radius. This compared to a 0.35 ratio for field isolate S-89. Isolate B-83-T2 usually produced a higher ratio value than isolate S-89 on GYEA containing various concentrations of the three fungicides examined. The only exception occurred on plates amended with iprodione at 1.0 μg/ml. B-83-T2 showed a slight simulation in mycelial growth at this concentration of fungicide.

None of the tested fungicides was effective in suppressing organic acid production by either isolate of *S. minor*. Increasing the concentrations of all fungicides increased the acidification ratio until mycelial growth was suppressed. Iprodione had the least effect on acid production as ratio values were only slightly greater than obtained for the BPB check. Chlorothalonil was the only fungicide which triggered an increase in the acidification ratio over the tested range of concentrations without greatly inhibiting mycelial growth of *S. minor*. Exposure of *S. minor* to RH-3486 at 0.1 μg/ml and 1 μg/ml produced the highest acidification ratio for isolate S-89 and B-83-T2, respectively. However, all concentrations of RH-3486 greatly inhibited mycelial growth of *S. minor*.

Interactions of chlorothalonil and iprodione on excised peanut stems. Treatment of excised stems with chlorothalonil at 90 or 9000 μg/ml did not have a significant effect on the size of lesions produced by isolate S-17 or S-69 of *S. minor* (Table 12). Iprodione at 30 μg/ml significantly inhibited lesion development by approximately 20%. Lesion development was completely inhibited by iprodione at 3000 μg/ml. At either low or high rates of fungicides, the addition of chlorothalonil to iprodione did not significantly alter the fungicidal properties of iprodione according to measurements of stem lesions.

Table 12. Effect of chlorothalonil and iprodione on lesion length (mm) after 3 days following inoculation of excised peanut stems with *Sclerotinia minor*.

Treatment[1]	Lesion Length[2]	
	Isolate S-17	Isolate S-69
1/100th field rate		
untreated	67.3 a	70.1 a
chlorothalonil (90 μg/ml)	67.9 a	69.7 a
iprodione (30 μg/ml)	53.0 b	56.1 b
chlorothalonil + iprodione	52.9 b	53.2 b
Full field rate		
untreated	88.6 a	88.8 a
chlorothalonil (9000 μg/ml)	90.1 a	88.2 a
iprodione (3000 μg/ml)	7.5 b	13.2 b
chlorothalonil + iprodione	7.2 b	8.7 b

[1]Excised stems were soaked in fungicide suspensions for 1 minute. Stems were air dried prior to inoculation.

[2]Data are the mean of two experiments, each treatment containing 12 stems. Means followed by the same letter(s) are not significantly different at P=0.05 according to Duncan's multiple range test.

Lesion development on peanut stems from field plots treated with chlorothalonil. Stems collected in 1988 from peanut fields treated with chlorothalonil developed larger lesions than stems from untreated fields when inoculated with *S. minor*. Isolate S-69 produced lesions that were 14.6, 7.3 and 4.3 mm greater on stems exposed to four, three and two field applications of chlorothalonil, respectively, compared to lesions on untreated stems (Table 13). All increases in lesion length were significant. The relationship between chlorothalonil treatments and lesion length was not as distinct for isolate S-17. Field exposure to chlorothalonil resulted in significantly larger lesions after inoculation with S-17 in stems exposed to two and four chlorothalonil treatments. Leafspot had not become severe by 13 Aug. Approximately 10% of leaflets in untreated plots had leafspot lesions. The leafspot rating on plants sprayed according to the 3- and 4-week schedules were less than 2%, and for the 2-week schedule, less than 1%. Defoliation was not a detectable in any of the plots.

In 1989, stems were collected from peanut plots later in the season after plants had been sprayed more times with chlorothalonil. Stems from chlorothalonil-treated plots averaged larger lesions than stems from untreated plots, but the magnitude of differences was not as great as in 1988 (Table 13). Only stems treated five times with chlorothalonil had significantly larger lesions when inoculated with isolate S-89 of *S. minor*. Severe leafspot pressure in 1989 resulted in a leafspot rating of 85, 20, 15, 4 and 2% for untreated plots and plots treated three, four, five and six times with chlorothalonil at the time of stem collection.

Effect of conditioning cultures of *S. minor* to chlorothalonil. On excised peanut stems untreated and treated with chlorothalonil during tests *in vitro*, conditioning *S. minor* on GYEA amended with chlorothalonil at 10 μg/ml resulted in lesions approximately 40% larger in length than lesions produced by *S. minor* grown on GYEA containing no fungicide (Table

Table 13. Effect of field applications of chlorothalonil in 1988 and 1989 on lesion length (mm) after 3 days following inoculation of excised peanut stems with *Sclerotinia minor*[1].

Year and treatment[2]	No. of chlorothalonil field applications[3]	Lesion Size[3]		
		Isolate S-17	Isolate S-69	Isolate S-89
1988				
untreated	0	69.6 c	68.8 c	--[5]
4-week schedule	2	77.3 a	73.1 b	--
3-week schedule	3	70.0 bc	76.1 b	--
2-week schedule	4	76.6 ab	83.4 a	--
1989				
untreated	0	--	--	68.1 b
advisory version 120	3	--	--	71.7 ab
advisory version 72	4	--	--	69.3 ab
advisory version 48	5	--	--	72.6 a
2-week schedule	6	--	--	71.3 ab

[1]Stems were 15-week old in 1988 and 18-week old in 1989.
[2]Initial fungicide treatments were made on 22 Jun 1988 and 26 Jun 1989.
[3]Chlorothalonil was applied at 1.26 kg/ha.
[4]Data are the mean of two experiments, each treatment containing 12 stems. Means followed by the same letter(s) are not significantly different at P=0.05 according to Duncan's multiple range test.
[5]Isolate not tested.

14). These results were significant at P=0.05. Conditioning the fungus did not significantly affect lesion length on stems treated with iprodione alone or in combination with chlorothalonil as indicated by previous experiments, treatment of stems with chlorothalonil alone had no significant impact on lesion length. Once the conditioned culture of *S. minor* was transferred to GYEA containing no fungicides, it no longer showed evidence of increased pathogenicity compared to a culture never exposed to chlorothalonil.

DISCUSSION

Field applications of chlorothalonil were found to increase the severity of Sclerotinia blight of peanut as reported previously (16). Stems collected from the field during August 1988 and inoculated with *S. minor* produced larger stem lesions if the original plant had been previously treated with chlorothalonil. However, an immediate effect of chlorothalonil on the fungus or the peanut stem did not appear to be responsible for this increase in lesion size as stems treated with chlorothalonil just prior to inoculation with *S. minor* did not show a chlorothalonil-induced change in lesion size. Since chlorothalonil applications appear to induce only a moderate increase in lesion length, other factors were also believed to be responsible for increased severity of Sclerotinia blight in chlorothalonil-treated fields. Stems collected in September 1989 from peanuts treated up to six times with chlorothalonil sprays, did not show a marked increase in lesion length after inoculation with *S. minor*. In addition, inoculated stems from heavily defoliated plants produced lesions that were not significantly different from stems collected from plots with an intact canopy. The age of the peanut stems collected late in the 1989 growing season probably masked the effect of additional chlorothalonil applications. Brenneman et al. (3) reported that lesion development was not

Table 14. Effect of conditioning *Sclerotinia minor* (S-17) to 10 μg/ml chlorothalonil added to glucose-yeast extract agar (GYEA) on lesion length following inoculation of excised peanut stems.

Stem Treatment[1]	Lesion length (mm) at day 3[2]	
	Culture exposed to chlorothalonil (10 μg/ml)	Culture not exposed chlorothalonil
Week 1.[4]		
untreated	89.0 a	64.1 b
chlorothalonil (9000 μg/ml)	89.5 a	64.1 b
iprodione (3000 μg/ml)	9.8 c	9.8 c
chlorothalonil & iprodione	8.9 c	9.7 c
Week 2.[5]		
untreated	74.5 a	69.5 a
chlorothalonil (9000 μg/ml)	71.9 a	70.3 a
iprodione (3000 μg/ml)	10.4 b	9.7 b
chlorothalonil & iprodione	9.8 b	9.5 b

[1]Excised stems were soaked in fungicide suspensions for 1 minute. Stems were air dried prior to inoculation.

[2]Means followed by the same letter under each week are not significantly different at P=0.05 according to Duncan's multiple range test. Data are the mean of two experiments, each containing 12 replications.

[3]Agar plugs served as the carrier for fungal mycelia to inoculate stems.

[4]During week 1, chlorothalonil-exposed culture was grown on GYEA amended with chlorothalonil, and unexposed culture was grown on GYEA.

[5]During week 2, chlorothalonil-exposed and unexposed cultures were grown on GYEA containing no fungicide.

as great on older stems, perhaps due to increased lignification and/or decreased sugar content. Heavy defoliation may also have contributed to low sugar content in stems collected from peanut plants treated infrequently with chlorothalonil.

Field results from 1987 and 1988 indicated that higher peanut yields were obtained from plots treated with applications of chlorothalonil according to the peanut leafspot advisory program. Plots treated on a 2-week schedule developed severe Sclerotinia blight. The increase in disease may be partially attributed to vine damage caused by frequent passes of tractor tires and the increased susceptibility of injured vines to Sclerotinia blight (18). The dense peanut canopy maintained by chlorothalonil applications also favored Sclerotinia blight (5). In fields subjected to disease pressure from both Cercospora leafspot and Sclerotinia blight, the increase of Sclerotinia blight attributed to increased sprays of chlorothalonil was more detrimental to peanut yields than failure to control Cercospora leafspot during seasons of moderate leafspot pressure. A 17% increase in Sclerotinia blight reduced peanut yields by 870 kg/ha when Cercospora leafspot was controlled with a 2-week schedule of chlorothalonil applications instead of the advisory program. Use of the advisory program for leafspot control increased yields by only 132 kg/ha compared to plots receiving no leafspot control. Use of the peanut leafspot advisory program appeared to limit damage due to Sclerotinia blight and resulted in significantly higher yields than where a 2-week spray schedule was utilized for leafspot control.

In agar-plate tests, iprodione and chlorothalonil appeared to act synergistically to inhibit mycelial growth of non-sectoring colonies. As the concentration of chlorothalonil was increased, the rate of sectoring increased. The development of sectors was responsible for the observed increase in mycelial growth of *S. minor* as chlorothalonil concentrations were

increased. The growth of sectors on GYEA amended with both fungicides did not exceed the mycelial growth obtained on GYEA amended only with 0.5 μg/ml of iprodione. Increased chlorothalonil concentrations did trigger the development of fungal sectors tolerant to chlorothalonil but not iprodione. Apparently, chlorothalonil did not affect the fungicidal properties of iprodione in GYEA.

In tests using excised stems treated with fungicides, chlorothalonil had no effect on the performance of iprodione. Field applications of chlorothalonil apparently do not impact on the performance of iprodione. In addition, no iprodione-tolerant isolates of *S. minor* were obtained from field plots treated frequently with chlorothalonil which indicated that chlorothalonil did not alter the sensitivity of *S. minor* to iprodione (19). Thus, use of chlorothalonil for leafspot control is unlikely to directly affect the fungicidal properties of iprodione for control of Sclerotinia blight.

As reported by Hau et al., the addition of chlorothalonil to an oat-grain nutrient source for the fungus increased the aggressiveness of *S. minor* on inoculated plant material. The addition of 10 μg/ml chlorothalonil to GYEA was observed to increase the density of mycelial growth. An agar plug from these cultures would provide more mycelium in contact with the wounded stem tissue and result in possibly a greater inoculum potential for rapid colonization of the inoculated stem. The presence of chlorothalonil in nutrient media apparently triggered increased pathogenicity of *S. minor*. However, the chlorothalonil-induced changes were not stable. Once the conditioned isolate of *S. minor* was grown on unamended GYEA, the increase in pathogenicity was no longer detected using the excised-stem technique. Since the fungicidal action of chlorothalonil has been attributed to inactivation of enzymatic thiol groups and the effect was reversible *in vitro* by addition of thiol compounds

(21), it was not unexpected that the chlorothalonil-induced response required the presence of the fungicide in the nutrient media.

Use of BPB-amended GYEA indicated that a sensitive isolate (S-89) and a dicarboximide-resistant isolate (B-83-T2) of *S. minor* exposed to fungicides in GYEA produced more organic acid compounds in relation to mycelial growth than cultures grown on GYEA containing no fungicides. Some of these organic acids are believed to be oxalic acid, according to Godoy et al. (6). Of three tested fungicides, chlorothalonil was most effective in triggering an increase in the acidification ratio, as mycelial growth was not greatly inhibited by the fungicidal properties of this compound. None of the fungicides tested was effective in inhibiting the production of organic acids by *S. minor*. A similar test using BPB-amended media may prove useful in identifying fungicides that prevent organic acid production. Such compounds might alter or reduce the pathogenic nature of acid-producing fungi, such as *S. minor*.

Use of BPB-amended media also indicated that isolate B-83-T2 was an effective producer of organic acids. In field microplot studies (20), B-83-T2 was shown to be pathogenic on peanut. Therefore, possession of dicarboximide resistance did not appear to affect *in vitro* production of organic acids or pathogenicity in the field.

In summary, the enhancement of Sclerotinia blight is likely due to a combination of factors. Chlorothalonil had no detectable fungicidal properties against *S. minor* on excised peanut stems nor did the fungicide alter the effectiveness of iprodione. In addition to maintaining a healthy canopy, chlorothalonil applications are thought to alter metabolic processes of the fungus without greatly inhibiting fungal growth. These changes in metabolism occur after an undefined period of conditioning and may enable the pathogen to

function more effectively in colonizing stems beneath the often dense foliar canopy resulting from good control of leafspot. The excised stem test indicated that field applications of chlorothalonil make peanut stems more susceptible to infection by *S. minor* after a period of time following application of the fungicide. GYEA tests with BPB suggested that chlorothalonil may augment the ability of *S. minor* to produce organic acid compounds, some of which are likely involved in pathogenesis.

LITERATURE CITED

1. Bateman, D.F. and Beer, S.V. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. Phytopathology 55:204-211.
2. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1987. Control of Sclerotinia blight of peanut: Sensitivity and resistance of Sclerotinia minor to vinclozolin, iprodione, dicloran, and PCNB. Plant Dis. 71:87-90.
3. Brenneman, T.B., Phipps, P.M., and Stipes, R.J. 1988. A rapid method for evaluating genotype resistance, fungicide activity, and isolate pathogenicity of *Sclerotinia minor* in peanut. Peanut Sci. 15:104-107.
4. Cu, R.M., Phipps, P.M., Steele, J.L., and Stipes, R.J. 1988. Field performance of computerized advisories for fungicide application to control early leafspot of peanut. Proc. Am. Peanut Res. Educ. Soc. 20:32.
5. Dow, R.L., Powell, N.L., and Porter, D.M. 1988. Effects of modification of the plant canopy environment on Sclerotinia blight of peanut. Peanut Sci. 15:1-5.
6. Godoy, G., Steadman, J.R., Dam, R., and Dickman, M. 1989. Selection and use of

oxalate minus mutants to study pathogenicity of *Sclerotinia sclerotiorum*, cause of bean white mold disease. (Abstr.) Phytopathology 79:1145.

7. Hau, F.C., and Beute, M.K. 1983. Effects of chlorothalonil on the virulence and physiology of a nontargeted pathogen, *Sclerotinia minor*. Phytopathology 72:475-479.
8. Kohn, L.M. 1979. A monographic revision of the genus *Sclerotinia*. Mycotaxon 9:365-444.
9. Lumsden R.D. 1979. Histology and physiology of pathogenesis in plant diseases caused by *Sclerotinia* species. Phytopathology 69:890-896.
10. Maxwell, D.P., and Lumsden, R.D. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. Phytopathology 60:1395-1398.
11. Phipps, P.M. 1986. A use pattern for chlorothalonil to control early leafspot of peanut without increased severity of Sclerotinia blight. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 18:52.
12. Phipps, P.M. 1989. The Virginia Peanut Leafspot Advisory Program. Va. Coop. Ext. Serv., Publ. 432-010, Suffolk, VA.
13. Phipps, P.M. 1990. Diseases of Peanuts. Pages 55-67 in: 1990 Peanut Production Guide. Va. Coop. Ext. Serv., Publ. MA 197, Suffolk, VA.
14. Phipps, P.M., and Porter, P.M. 1986. Evaluating chemicals to control soilborne diseases of peanut. Pages 217-220 in: Methods for Evaluating Pesticides for control of Plant Pathogens. K.D. Hickley, ed. APS Press, St. Paul, MN.
15. Phipps, P.M., and Powell, N.L. 1984. Evaluation of criteria for the utilization of peanut leafspot advisories in Virginia. Phytopathology 74:1189-1193.
16. Porter, D.M. 1980. Increased severity of Sclerotinia blight in peanuts treated with

captafol and chlorothalonil. Plant Dis. 64:394-395.

17. Porter, D.M. 1981. Growth of *Sclerotinia minor* on media containing chlorothalonil and benomyl. Plant Dis. 65:591-594.

18. Porter, D.M., and Powell, N.L. 1978. Sclerotinia blight development in peanut vines injured by tractor tires. Peanut Sci. 5:87-90.

19. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1989. Assessment of resistance to iprodione in sclerotial populations of *Sclerotinia minor* from fungicide-treated peanuts. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 21:18.

20. Smith, F.D., Phipps, P.M., and Stipes, R.J. 1990. Pathogenicity of a dicarboximide-resistant isolate of *Sclerotinia minor* to peanut in microplots treated with fungicides. (Abstr.) Proc. Am. Peanut Res. Educ. Soc. 22:(in press).

21. Vincent, P.G., and Sisler, H.D. 1968. Mechanism of antifungal action of 2,4,5,6-tetrachloroisophthalonitrile. Physiol. Plant. 21:1249-1264.

SYNOPSIS

Sclerotinia blight, caused by the soil-borne fungus *Sclerotinia minor*, is currently the most destructive disease of peanuts in Virginia. Recent losses to the disease have averaged 6% of the potential peanut crop each year. The sensitivity of *S. minor* to three dicarboximide fungicides and three experimental fungicides was tested on fungicide-amended glucose-yeast extract agar (GYEA). ED_{50} values (dose required for 50% inhibition of mycelial growth) by a common-type, field isolate (S-2) were 0.004, 0.004, 0.025, 0.08, 0.18 and 0.38 μg/ml for ASC-66825, RH-3486, MON-13108, vinclozolin, iprodione and chlozolinate, respectively. ASC-66825 and RH-3486 were effective in suppressing mycelial growth of a dicarboximide-resistant isolate (B-83-T2) on fungicide-amended GYEA and fungicide-treated soil plates. In soil plates, only the dicarboximide fungicides were effective in limiting sclerotial production. During replicated field trials in 1989, plots treated twice with ASC-66825 at 0.28 or 0.56 kg/ha, or RH-3486 at 0.56 kg/ha were the only treatments to result in significant suppression of disease incidence and increased yield compared to untreated plots. These field and laboratory tests have resulted in the identification of two fungicides with superior activity against *S. minor* than the dicarboximides.

The spray adjuvant pinolene significantly improved the performance of iprodione for control of Sclerotinia blight. During 1985-1989, plots treated with iprodione (1.12 kg/ha) and pinolene 0.18% (v/v) using a spray volume of 335 l/ha had yields that were 365 kg/ha higher and disease incidence 15% lower than plots treated with only iprodione. Pinolene (Nu-Film-17®) is now recommended as a spray adjuvant for iprodione (Rovral®).

The performance of two dicarboximide fungicides (iprodione and vinclozolin), two aromatic fungicides (dicloran and PCNB), and RH-3486 was evaluated in field microplots infested with isolate S-2 and B-83-T2 of *S. minor*. During the period 1987-1989, peanut plants in untreated plots averaged 21.9 and 20.5 lesions following growth in soil infested with isolate S-2 and B-83-T2, respectively. Microplots infested with S-2 had 97, 83, 33, 67 and 30% less disease incidence (lesions/plot) when treated with RH-3486, vinclozolin, iprodione, PCNB and dicloran, respectively, than untreated plots. Plots infested with B-83-T2 had 96, 55, 62, 25 and 20% less disease when similarly treated. All fungicide treatments significantly increased yields in plots infested with S-2, but only RH-3486 and iprodione significantly increased yields in plots infested with B-83-T2. Thus, application of iprodione still gave some control of B-83-T2 in microplot studies, which suggested that B-83-T2 possessed a low-level type of resistance to dicarboximide fungicides.

A long-term study of microplots infested in 1983 with *in vitro*, dicarboximide-resistant isolates (R-2B and R-2C) and their sensitive parent isolate (S-2), indicated that fungicides still gave disease suppression after 6 years of fungicide use. Disease incidence for the 3-year period (1987-1989) in plots infested with S-2 was suppressed 37, 31 and 0% by vinclozolin, iprodione and dicloran, respectively. Disease incidence was suppressed 63, 66 and 42% in plots infested with R-2B, and 61, 48 and 17% in plots infested with R-2C following treatment with vinclozolin, iprodione and dicloran, respectively. There was no evidence of failure of any fungicide in controlling Sclerotinia blight caused by dicarboximide-resistant isolates in these microplots.

A survey of sclerotia collected from field test plots treated with fungicides in 1987 failed to detect field resistance to the dicarboximide fungicides in spite of an *in vitro*

resistance frequency of 6.3% on GYEA amended with 2.0 μg/ml iprodione. Applications of fungicides for control of Cercospora leafspot or Sclerotinia blight did not affect the rate of *in vitro* resistance to iprodione by isolates of *S. minor*. Field resistance to iprodione has not been detected in commercial peanut fields in Virginia. Resistance problems associated with continued use of iprodione do not appear to be an immediate or major threat to peanut production in Virginia.

Peanut plots sprayed with chlorothalonil according to the leafspot advisory program averaged 17% less incidence of Sclerotinia blight and yielded 870 kg/ha more than plots sprayed more frequently according to a 2-week schedule. In GYEA, the addition of various concentrations of chlorothalonil (2 to 1000 μg/ml) to iprodione at 0.5 μg/ml increased the development of chlorothalonil-tolerant sectors but did not affect the fungicidal properties of iprodione. Addition of chlorothalonil to suspensions of iprodione did not influence the fungicidal properties of iprodione in GYEA or excised-stems tests. Use of bromophenol blue in GYEA indicated increased acid production, relative to mycelial growth, in the presence of chlorothalonil at concentrations that were not greatly fungitoxic to *S. minor*. Field applications of chlorothalonil did result in significantly larger lesion sizes on inoculated excised stems. Treatment of excised stems with suspensions of chlorothalonil just prior to inoculation did not affect lesion development, suggesting that time was required to induce changes in plant susceptibility to the pathogen. Cultures of *S. minor* conditioned on GYEA containing chlorothalonil at 10 μg/ml were more pathogenic on excised stems than unconditioned cultures. This increase in pathogenicity was not stable upon transferring the culture to GYEA containing no fungicide. Use of bromophenol blue in GYEA indicated increased organic acid production in the presence of chlorothalonil. The enhancement of Sclerotinia

blight by chlorothalonil is probably due to a combination of factors affecting both the plant and pathogen.

APPENDIX A. CROP MANAGEMENT PRODUCTS

Agrichemicals used in field, laboratory and microplot research are listed by trade name, common name and chemical name.

1. ASC-66825, Fermenta experimental fungicide, confidential chemistry
2. Botran, dicloran, 2,6-dichloro-4-nitroaniline
3. Bravo 720, chlorothalonil, tetrachloroisophthalonitrile
4. Landplaster, agricultural gypsum, calcium sulfate
5. Nu-Film-17, pinolene, di-1-p-menthene
6. MON-13108, Monsanto experimental fungicide, confidential chemistry
7. RH-3486, Rohm and Haas experimental fungicide, confidential chemistry
8. Ronilan, vinclozolin, 3-(3,5-dichlorophenyl)-5-ethenyl-5-methyl-2,4-oxazolidinedione
9. Round-up, glyphosate, isopropylamine salt of N-(phosphonomethyl) glycine
10. Rovral, iprodione, 3-(3,5-dichlorophenyl)-N-(1-methylethyl)-2,4-dioxo-1-imidazolidinecarboximide
11. SDS-65311, chlozolinate, ethyl-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-1,3-oxazolidine-5-carboxylate
12. Sevin, carbaryl, 1-naphthyl-N-methylcarbamate
13. Solubor, borax, sodium borate
14. Tecmangam, (none), manganese sulfate
15. Temik, aldicarb, 2-methyl-2-(methylthio)-propionaldehyde-O-(methylcarbamoyl)-oxime
16. Terraclor, PCNB, pentachloronitrobenzene

APPENDIX B. MANAGEMENT PRACTICES IN FIELD MICROPLOTS

The following is a list of crop management activities performed on field microplots. The older microplots (Study II) were established in 1983 by T.B. Brenneman and maintained through 1985. In 1987, previous fungicide treatments with dicloran, iprodione, and vinclozolin were resumed and continued through 1989. The newer microplots (Study I) were established in 1987, and they were treated with dicloran, iprodione, PCNB, RH-3486 and vinclozolin for three growing seasons. In addition, the new microplots were re-infested every spring with sclerotia of *Sclerotinia minor* (isolate S-2 or B-83-T2). Otherwise, both sets of microplots were managed similarly.

1987

May 5 . . . . . incorporated Temik 15 G (14.6 kg/ha) and planted 12 seeds of Florigiant peanut/microplot

Jun 9 . . . . . infested new microplots with four sclerotia/100 g soil in upper 4 cm of soil with isolate S-2 or two sclerotia/100 g soil for B-83-T2 and thinned plants to three/microplot

Jun 17 . . . . . sprayed Bravo 720 (1.75 l/ha) for leafspot control based on occurrence of disease

Jun 24 . . . . . applied Landplaster (1009 kg/ha)

Jun 25 . . . . . irrigated microplots (2.54 cm water)

Jul 6 . . . . . sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory)

Jul 23 ..... irrigated microplots (2.54 cm water)

Jul 28 ..... applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75WP (4.48 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) and Terraclor 10G (56.0 kg/ha) to new microplots

Jul 30 ..... irrigated microplots (2.54 cm water)

Aug 5 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory) and applied Solubor (1.4 kg/ha)

Aug 6 ..... applied Tecmangam (4.48 kg/ha)

Aug 19 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory), applied Solubor (1.4 kg/ha) and treated areas between microplot barriers with Round-up for weed control

Aug 25 ..... applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75WP (3.36 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) to new microplots for Sclerotinia blight control

Sep 8 ..... applied Terraclor 10G (56.0 kg/ha) to new microplots for Sclerotinia blight control

Sep 9 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory)

Sep 22 ..... applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75WP (3.36 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) to new microplots for Sclerotinia blight control

Nov 5-7 ..... harvested peanuts from microplots

1988

May 15-16 .... incorporated Temik 15 G (14.6 kg/ha), fertilized with 10-10-10 (4.48 kg/ha) and planted 12 seeds of Florigiant peanut/microplot

Jun 8 ..... treated areas between microplot barriers with Round-up for weed control, thinned peanut plants to three plants/microplot

Jun 9 ..... infested new microplots with four sclerotia/100 g soil in upper 4 cm of soil with isolate S-2 or two sclerotia/100 g soil for B-83-T2

Jun 20 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control based on occurrence of disease

Jun 30 ..... applied Landplaster (1009 kg/ha)

Jul 17 ..... irrigated microplots (2.54 cm water)

Jul 20 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory) and treated areas between microplot barriers with Round-up for weed control

Jul 21 ..... applied Tecmangam (4.48 kg/ha)

Jul 27 ..... applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75 WP (4.48 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) and Terraclor 10G (56.0 kg/ha) to new microplots

Aug 1 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory) and applied Solubor (1.4 kg/ha)

Aug 19 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory) and applied Solubor (1.4 kg/ha)

Aug 23 ..... applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran (3.36 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) to new microplots for Sclerotinia blight control

Sep 7 ..... applied Terraclor 10G (56.0 kg/ha) to new microplots for Sclerotinia blight control

Sep 13 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory)

Sep 21 ..... applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75WP (3.36 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) to new microplots for Sclerotinia blight control

Oct 24-25 .... harvested peanuts from microplots

1989

Apr 17 ..... treated areas between microplots with Round-up for weed control

May 8-10 .... incorporated Temik 15 G (14.6 kg/ha), fertilized with 10-10-10 (4.48 kg/ha) and planted 12 seeds of Florigiant peanut per microplot

May 26 ..... infested new microplots with four sclerotia/100 g soil in upper 4 cm of soil with isolate S-2 or two sclerotia/100 g soil for B-83-T2

Jun 1 ..... thinned plants to three/microplot and treated areas between microplots with Round-up for weed control

Jun 20 ..... sprayed Bravo 720 (1.75 l/ha) for leafspot control based on occurrence

of disease

Jun 30 . . . . . applied Landplaster (1009 kg/ha)

Jul 14 . . . . . sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory) and treated areas between microplots with Round-up for weed control

Jul 21 . . . . . applied Tecmangam (4.48 kg/ha)

Jul 28 . . . . . applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75WP (4.48 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) and Terraclor 10G (56.0 kg/ha) to new microplots

Aug 1 . . . . . sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory), applied Solubor (1.4 kg/ha)

Aug 19 . . . . . sprayed Bravo 720 (1.75 l/ha) for leafspot control (advisory), applied Solubor (1.4 kg/ha)

Aug 28 . . . . . applied Rovral 50WP (2.24 kg/ha), Ronilan 50WP (1.68 kg/ha) and Botran 75WP (3.36 kg/ha) to new and old microplots; and RH-3486 50WP (1.68 kg/ha) to new microplots for Sclerotinia blight control and treated areas between microplots with Round-up for weed control

Sep 11 . . . . . applied Terraclor 10G (56.0 kg/ha) to new microplots for Sclerotinia blight control and applied Bravo 720 (1.75 l/ha) for leafspot control

Oct 23-24 . . . . harvested peanuts from microplots

VITA

Frisby Davis "Tad" Smith III was born on September 1, 1960 in Charlottesville, Virginia. In 1964, his parents, Frisby and Adelaide Smith, moved to Wauwatosa, Wisconsin where the author attended public schools until graduating from Wauwatosa East High School in 1979. That fall, the author began attendance at Lawrence University in Appleton, Wisconsin. He graduated with honors in 1983 and received a Bachelor of Arts degree in biology. He began his graduate studies at the University of Minnesota in 1983. Under the direction of Dr. Ernest E. Banttari, the author completed his Master of Science degree in plant pathology in 1986. The thesis was entitled, "Dot-ELISA on Nitrocellulose Membranes for Detection of Potato Leafroll Virus." The author returned to Virginia to begin a Ph.D. program at Virginia Polytechnic Institute and State University under the directions of Dr. Patrick M. Phipps at the Tidewater Agricultural Experiment Station and Dr. R. Jay Stipes in the Department of Plant Pathology, Physiology and Weed Science. During his career at Virginia Tech, the author received a graduate student research award from the Potomac Division of the American Phytopathological Society and a student competition award from the American Peanut Research and Education Society in 1989. In addition, he received a $500 scholarship from the Virginia Ag-Chemical and Soil Fertility Association in 1990. He was awarded the Ph.D. degree December 15, 1990.