ABSTRACT

CANNON, MICHAEL DAVID. Sensitivity of *Sclerotinia minor* to common peanut fungicides. (Under the direction of Dr. Barbara B. Shew).

Sclerotinia blight caused by *Sclerotinia minor* is an important disease of peanut. Currently, fluazinam, marketed by Syngenta Crop Protection as “Omega 500F®,” is widely used for control of Sclerotinia blight because of its consistent performance. However, Omega 500F can cost over $50 per application and is only labeled for control of Sclerotinia blight. Products that are more affordable perform inconsistently. In this research, we evaluated the *in vitro* efficacy of five common peanut fungicides (fluazinam, boscalid marketed by BASF Crop Protection as Endura®, the mixture of benzovindiflupyr [marketed as Solatenol® fungicide] and azoxystrobin marketed by Syngenta as Elatus®, penthiopyrad marketed by DuPont™ as Fontelis® and the mixture of fluopyram and prothioconazole marketed by Bayer CropScience as Propulse®) against 44 isolates of *Sclerotinia minor*. Averaged across isolates, Omega and Elatus caused the greatest inhibition of growth in radial hyphae *S. minor*, with EC$_{50}$ values of 0.0133 ± 0.0048 µg/ml for Omega and 0.0134 ± 0.0082 µg/ml for Elatus. Propulse was least inhibitory at an effective concentration (EC$_{50}$) of 0.060 ± 0.0314 µg/ml. With Omega, 100% inhibition was reached at 0.316 µg/ml, while near total inhibition was obtained with Elatus at 1.0 µg/ml, Endura at 5.623 µg/ml, Propulse at 1.0 µg/ml, and Fontelis at 5.623 µg/ml.

Endura did not perform as well, with an EC$_{50}$ value of 0.051 ± 0.0396 µg/ml. While Endura is generally effective for control of Sclerotinia blight in the field, a few isolates tested appeared to be much more insensitive to Endura, while the rest were sensitive with low variability. Similarly, Fontelis had an EC$_{50}$ value of 0.017 ± 0.0245 µg/ml, with isolates W-10, VA3, FA-14 and K6 showing increased insensitivity. Propulse had an EC$_{50}$ value of
0.060 ± 0.0314 µg/ml with W-28, VA3 and RM9 showing much more insensitivity than other S. minor isolates. These results suggest that isolate differences may be the cause of variation in some inconsistency in fungicide performance. Products with a high-risk of resistance development should be applied with caution, to not select for fungicide resistance. Omega, containing fluazinam, a fungicide with a low risk of resistance development, exhibited consistent performance across all isolates, validating the observed performance consistency in the field.
DEDICATION

This thesis is dedicated to my grandparents, Joan E. and William V. Cannon.
BIOGRAPHY

Michael David Cannon was born in Newark, Delaware. He first came to love agriculture while growing up in New Castle County as an active member of Blackbird 4-H and Middletown FFA. He participated in horticultural science classes and greenhouse projects before deciding to pursue plant science in college. Michael graduated from the University of Delaware in Newark with Bachelor of Science degrees in Plant Protection and Entomology. While in college, he worked in the UD Plant Diagnostic Clinic and UD Cooperative Extension Service and cultivated his interests in plant protection and pest management. He began his graduate studies in plant pathology in July 2014 under the direction of Dr. Barbara B. Shew.
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LITERATURE REVIEW

Section I. Sclerotinia minor (Jagger) Kohn

Taxonomy and Importance of Sclerotinia minor

Sclerotinia minor Jagger is a member of the Family Sclerotiniaceae in the Order Helotiales, Class Leptomycetes and Phylum Ascomycota. Members in the Sclerotiniaceae produce inoperculate asci from stipitate apothecia that arise from sclerotial stroma (Bolton et al., 2006). The species Sclerotinia minor was first described in 1920, when it was found on lettuce in the northeastern United States and Florida (Jagger, 1920).

S. minor is characterized by production of small sclerotia (0.5-2.0 mm long). In culture, sclerotia are produced throughout the colony, unlike other species of Sclerotinia that produce larger, irregularly shaped sclerotia at the growing colony margin. Apothecia arise singly from a sclerotium, are cinnamon to umber in color, with a stipe of 1 to 4 mm long and 1 to 2 mm wide and receptacle 2 to 9 mm broad. Asci are cylindrical and 8-spored (Kohn, 1979a).

Sclerotinia minor is one of three economically important plant pathogens in the genus Sclerotinia. Sclerotinia sclerotiorum (Lib.) de Bary is the most common species, with a host range of over 400 plant species in 60 families (Farr and Rossman, 2017), whereas Sclerotinia trifoliorum Erikss. is limited to primarily forage legumes (Kohn, 1979b; Boland and Hall, 1994). Economically important hosts of S. minor include peanut, lettuce, green bean, chicory and sunflower (Melzer et al., 1997). At least 173 known plant species are reported as hosts (Farr and Rossman, 2017).
Mycelial compatibility

Genetic variability in populations of Sclerotinia spp., including S. minor, can be characterized by mycelial compatibility grouping (MCG; Shafer and Kohn, 2006). Mycelial compatibility or incompatibility (self-recognition) is determined macroscopically by pairing isolates on agar media (Kohn et al., 1991). Populations with few dominant MCG’s indicate a clonal population structure, whereas high variability in the number and frequency of MCG may indicate outcrossing (Cubeta et al., 1997). MCG was not correlated with aggressiveness of S. minor isolates from peanut in North Carolina (Hollowell et al., 2006). The relationship between MCG and fungicide sensitivity in isolates sampled from peanut in North Carolina has not been determined (B. Shew, pers. comm.)

Section II. The peanut production system

Peanut biology and cultivation

The cultivated peanut (Arachis hypogaea L.) or groundnut, is an annual legume that forms fruit underground. There are two subspecies of A. hypogaea. The widely-cultivated runner and virginia market-types belong to A. hypogaea subsp. hypogaea var. hypogaea. Spanish market-types belong to A. hypogaea subsp. fastigiata var. vulgaris. Valencia market-types belong to A. hypogaea subsp. fastigiata var. fastigiata (Isleib and Wynne, 1992). The peanut originated in South America, in southern Brazil and northeastern Paraguay. Human trade likely moved Arachis hypogaea to China and Africa. Later, humans moved, cultivated, and selected peanut in Africa and Asia. This modified germplasm probably returned to the Americas from Africa on slave ships (Moss and Rao, 1995; Simpson et al., 2001).
Agronomic importance to North Carolina

Peanut cultivation in the Virginia-North Carolina region is dominated by the virginia-type peanut. This peanut type is a market designation for large-seeded peanut, distinct from runner-type peanut grown throughout the southeastern United States (Georgia, Florida, Alabama). North Carolina farmers harvested 99,000 acres of peanut in 2016, yielding 3,450 pounds per acre for a total of 341.6 million pounds (USDA, 2017; NCDA, 2017a). Additionally, North Carolina peanut accounted for 16.6% of peanut production in the United States in 2016, sixth in production by state (USDA, 2017). While North Carolina is not the largest peanut producer in the United States, production is important to the state economy. In 2016, peanut production accounted for 96.6 million dollars in value added to the North Carolina economy (NCDA, 2017b).

Sclerotinia blight of peanut

*Sclerotinia minor* causes Sclerotinia blight of peanut. Sclerotinia blight was first detected on peanut in the Virginia-North Carolina growing region in 1971 (Porter and Beute, 1974). Currently, Sclerotinia blight of peanut is found in the Virginia, North Carolina, Texas, Oklahoma, and Louisiana (Farr and Rossman, 2017). Most recently, it has been reported in Arkansas in 2014 (Faske et al., 2014).

*S. minor* spreads through mechanical means and by plant to plant spread, with epidemics initiated via mycelial germination of sclerotia (Porter and Melouk, 1997; Hao and Subbarao, 2005). Additionally, canopy structure has been implicated in creating a favorable environment for Sclerotinia blight epidemics. Denser canopies have the higher humidity required for sclerotia germination, increasing severity of Sclerotinia blight (Smith et al.,
Pruning the canopy was shown to decrease severity of Sclerotinia blight (Butzler et al., 1998; Brune and Bailey, 1992).

Shredding of stems, pegs and pods is characteristic of Sclerotinia blight (Porter and Melouk, 1997). Signs include the presence of fluffy white mycelium or small, black, irregularly-shaped sclerotia. Under cool, moist conditions, sclerotia near the soil surface undergo myceliogenic germination and mycelium begins to infect the crown, limbs, pods and pegs of peanuts. The invading mycelium quickly kills host tissue, causing pale-colored lesions on affected plant parts. Bleached and blighted stems become apparent. Pathogenesis in *Sclerotinia* spp. is attributed to the production of oxalic acid, which results in the characteristic bleached appearance of host tissue (Hau and Beute, 1983; Godoy et al., 1990). Cell-wall degrading enzyme activity also assists the invading fungus in colonizing host tissue (Marciano et al., 1983). As disease progresses, light brown sclerotial initials form in and on plant tissue. Sclerotia turn black as the outer rind becomes melanized. Sclerotia survive in host debris and soil until conditions are once again favorable for germination (Wu and Subbarao, 2008; Lumsden, 1979).

Soil temperature, soil moisture and depth of burial affect sclerotia germination and viability. The optimal temperature for germination is 20 to 30° C, with constant high humidity. Isolates of *S. minor* from peanut in North Carolina have a higher optimal germination temperature than those from other regions and hosts, perhaps due to selection under hot, humid temperatures of North Carolina (Smith et al., 2006; Dow et al., 1988). Prolonged cold temperatures and wet soils decrease survival of sclerotia (Matheron and Porchas, 2005; Smith et al., 2006; Wu and Subbarao, 2008). While survival varies due to
differences in soil composition, sclerotia of *S. minor* can remain viable for many years (Wu and Subbarao, 2008). Soil pH is an important factor for sclerotia germination. A soil pH of 6.0 to 6.5 is highly correlated with sclerotia germination and disease incidence in the field (Hau and Beute, 1982). Soil-surface applications of calcium hydroxide to raise soil pH above 8 have been shown to inhibit sclerotia germination in lettuce fields (Wilson et al., 2005).

Sclerotia of *S. minor* may germinate carpogenically to form apothecia. However, ascospore infection is uncommon on most economic hosts of *S. minor* (Wu and Subbarao, 2008; Bolton et al., 2006) and ascospore infection occurs rarely on peanut in North Carolina (B. Shew, *pers. comm.*).

**Sclerotinia blight and host resistance**

Early fungicide studies on the highly susceptible cultivar Florigiant showed over 50 percent incidence of pod rot and correlated the number of diseased plants with decreasing yield (Beute et al., 1975). VA 93B, with an erect growth habit, exhibited more tolerance than other virginia-type cultivars on the market at the time (Coffelt et al., 1994). The growth habit (canopy size, height, density, etc.) of peanut has been implicated as a factor for *S. minor* epidemics, with some apparently resistant varieties exhibiting a more upright and open canopy than susceptible cultivars (Coffelt and Porter, 1982). Subsequently, VA 98R also exhibited partial resistance to Sclerotinia blight (Mozingo et al., 2000). The cultivar NC 12C was bred for partial resistance to CBR but was highly susceptible to Sclerotinia blight, especially when planted in high density (Isleib et al., 1997). The popular large-seeded cultivar Gregory, a 1999 release, also was susceptible to Sclerotinia blight (Isleib et al. 1999). Perry was released in 2003 and exhibited partial resistance to Sclerotinia blight similar to VA
98R and VA 93B (Isleib et al., 2003). The N96076L peanut germplasm line, developed for resistance to leaf spots, also exhibited high partial resistance to Sclerotinia blight (Isleib et al., 2006). More recently, Sclerotinia blight resistance was conferred in peanut transformed with a barley oxalate oxidase gene (Partridge-Telenko et al., 2011), but to date no cultivars with this transformation have been released.

The virginia-type cultivar Bailey was released by the North Carolina Agricultural Research Service in 2011. It has partial resistance to five diseases found in the area: early leaf spot, late leaf spot, Cylindrocladium black rot caused by *Cylindrocladium parasiticum* Crous, M.J. Wingfield, & Alfenas, Sclerotinia blight, and tomato spotted wilt virus caused *Tomato spotted wilt tospovirus* (Isleib et al., 2011). This cultivar has since become the most planted in the growing region because of its resistance traits and high yield. The cultivar Sugg was released concurrently with Bailey. Like Bailey, Sugg is relatively high yielding and has partial resistance to Sclerotinia blight and several other diseases (Isleib et al., 2015).

Although these cultivars have good partial disease resistance, application of fungicides is necessary to obtain good disease control. Weather-based diseases advisories are used to more accurately predict when fungicides should be applied (Langston et al., 2002). It has been demonstrated that a fixed calendar strategy was not consistently effective and one well-timed spray can be more effective that two or three sprays applied on a fixed schedule (Smith et al., 2007).
Section III. Fungicide use in Sclerotinia and related species

Dicarboximides

Dicarboximides are among the first fungicides used to control Sclerotinia blight in peanut production. They belong to Fungicide Resistance Action Committee (FRAC) Code 2, in Mode of Action Group E, and disrupt signal transduction. They have a medium to high risk of resistance development (FRAC, 2017). Dicarboximides used on peanut include procymidone (Porter, 1980), vinclozolin, procymidone and iprodione (Brenneman et al., 1987a).

Vinclozolin was used by growers in Virginia under section 18 in 1984 and iprodione was registered for use on peanut the following year (Brenneman et al., 1987a). Concerns quickly developed when resistance was shown to occur in Botrytis cinerea Pers. and other fungi (Brenneman et al., 1987b). In vitro studies showed that dicarboximide-resistance-induced isolates of S. minor sampled from peanut can compete with sensitive isolates at no perceived fitness cost (Brenneman et al., 1987b). Subsequent in vitro studies with lettuce isolates of S. minor demonstrated induction of dicarboximide resistance (Hubbard et al., 1997). Isolates of S. minor resistant to procymidone were also resistant to vinclozolin and iprodione (Porter and Phipps, 1985). Later, resistance was reported in isolates originating from field-collected sclerotia, but loss of disease control was not observed (Smith et al., 1995). Loss of efficacy in iprodione and vinclozolin has also been attributed to an increased rate of microbial degradation of the chemicals over time (Slade et al., 1992). Even in the absence of fungicide resistance, applications of iprodione do not always enhance yields or
disease control in peanut (Damicone and Jackson, 1996). Dicarboximides have since declined in use in favor of more effective fungicides.

**Succinate Dehydrogenase Inhibitors (SDHI)**

Several SDHI fungicides are used to control peanut diseases. SDHIs belong to FRAC Code 7, in Mode of Action Group C2, and act against respiration complex II (succinate-dehydrogenase). SDHI fungicides target the succinate dehydrogenase complex, also known as complex II, by blocking electron transport at ubiquinone sites, effectively stopping respiration (Avenot and Michailides, 2010). They are considered to have a medium to high risk of resistance development because of their single action site (FRAC, 2017). Some active ingredients used in peanut production include carboxin, flutalonil, benzovindiflupyr, boscalid, penthiopyrad, fluopyram and fluxapyroxad.

Several studies have established that boscalid provides effective control of Sclerotinia blight (Grichar and Woodward, 2016; Smith et al., 2007; Matheron and Porchas, 2004; Ryley et al., 2000). Penthiopyrad provided good control of Sclerotinia blight at higher concentration but inconsistent results at lower concentration (Grichar and Woodward, 2016). Fluopyram, currently a combination product with prothioconazole, is labeled for suppression of *S. minor*. The SDHI flutalonil is labeled for control of southern stem rot caused by *Sclerotium rolfsii* and Rhizoctonia pod and limb rot caused by *R. solani*. Benzovindiflupyr is currently labeled for peanut as a combination product with azoxystrobin and controls leaf spots, web blotch, southern stem rot, and Rhizoctonia limb rot (Shew, 2017).

Several mutations have been characterized that confer resistance to SDHI fungicides. The most common mutation occurs in a conserved region in the *sdhB* gene. It is found across
many species of fungal pathogens, in contrast with the more variably occurring SDHC and SDHD mutations (Avenot and Michailides, 2010). Boscalid resistance has been noted in several pathogens. Resistance to Pristine (boscalid + pyraclostrobin) was observed in Alternaria alternata (Fr.) Keissl., cause of late blight in pistachio. Resistance to boscalid, fluopyram, fluxapyroxad and penthiopyrad has been reported in B. cinerea isolated from strawberry (Fragaria spp.) grown in the eastern United States (Hu et al., 2016). However, resistance was not detected in New York isolates of S. sclerotiorum from snap bean (Phaseolus spp.) after years of exposure to boscalid, suggesting that reported control failures may have been due to other factors (Lehner et al., 2017). Only one case of resistance in S. sclerotiorum has been reported in France (Stammler et al., 2010). Mutations that lead to SDHI resistance have been characterized in vitro (Wang et al., 2015). Positive cross resistance within the group has been observed, namely with the SDH mutation in B. cinerea, between boscalid and penthiopyrad, and should be taken into consideration when developing a fungicide spray program (Avenot and Michailides, 2010). Additionally, studies have shown a negative cross resistance between boscalid and some fungicides of the QoI inhibitors and anilinopyrimidines (Zhang et al., 2007; Pasche et al., 2005). These results suggest that differences in isolate sensitivity may account for the variability of performance in controlling Sclerotinia blight among this group of fungicides.

**Fluazinam**

Fluazinam belongs to FRAC Code 29, Group C5 (uncouplers of oxidative phosphorylation) (FRAC, 2017). It is a phenylpyridylamine with low risk of resistance development because its action against oxidative phosphorylation does not target a specific
binding site in the mitochondria (Guo et al., 1991). Peanut growers quickly adopted fluazinam because of its superior performance against Sclerotinia blight (Smith et al., 2008). When applied correctly, it consistently provides a high level of disease control. However, fluazinam is expensive and has a narrow range of activity. It cannot be used in place of a leaf spot fungicide, but instead must be used in addition to routine fungicide applications. An application of Omega 500F (fluazinam) can cost over $50 per acre (B. Shew, pers. comm.). Alternatives, while promising, often perform inconsistently.

Fluazinam is much more active against *S. minor* than products labeled before it, such as iprodione (Smith et al., 1991; Damicone and Jackson, 1996). Reports of resistance are rare, but it has been documented in populations of *B. cinerea* (Tamura, 2000). Resistance to fluazinam has also been induced *in vitro* in strains of *Ustilago maydis* (Persoon) Roussel (Vitoratos, 2014).

**Demethylation Inhibitors (DMI)**

DMI fungicides belong to FRAC Code 3 in mode of action group sterol biosynthesis in the sterol biosynthetic pathway. They have a medium risk of resistance development (FRAC, 2017). Prothioconazole, tebuconazole and propiconazole are among the most common active ingredients used in peanut production. Several DMIs are active against leaf spots and effective in controlling other peanut diseases, such as southern stem rot, Rhizoctonia limb and pod rot, and Cylindrocladium black rot (Shew, 2017). However, DMI fungicides generally are not highly effective against *Sclerotinia* spp. No single ingredient DMI fungicide is labeled for Sclerotinia blight control on peanut (Shew, 2017).
**Quinone outside Inhibitors (QoI)**

QoI fungicides belong to FRAC Code 11 in Mode of Action Group C3 and affect respiration complex III [cytochrome bc1 (ubiquinol oxidase) at Qo site (cyt b gene)]. They halt respiration by binding to the Qo site of cytochrome b, which effectively blocks electron transport. They have a high risk of resistance development because of their single action site (FRAC, 2017). Some active ingredients used in peanut production include azoxystrobin, fluoxastrobins, and pyraclostrobin, and some are sold as mixtures with an SDHI partner. These fungicides are used to control leaf spots, web blotch, southern stem rot and Rhizoctonia limb and pod rot (Shew, 2017). Resistance to azoxystrobin and pyraclostrobin has been reported in *Monilinia fructicola* (G.Winter) Honey isolates from peach [*Prunus persica* (L.) Batsch] and nectarine [*P. persica* (L.) Batsch var. *nucipersica* (Suckow) C.K. Schneid] in South Carolina and Georgia (Amiri et al., 2010) and *Alternaria solani* Sorauer from the Midwest United States (Pasche et al., 2005). A single point mutation is strongly associated with QoI resistance in plant pathogenic fungi (Bartlett et al., 2002; Zhonghua and Michailides, 2005). No cross resistance has been reported between the QoI and SDHI groups (Amiri et al., 2010). Extreme care must be taken when incorporating these fungicides into spray programs because resistance development risk is high.

**Chlorothalonil**

Chlorothalonil is a broad-spectrum chloronitrile used to control of early leaf spot (ELL) [*Cercospora arachidicola* Hori] and late leaf spot (LLS) [*Cercosporidium personatum* (Berk & M.A. Curtis) Deighton] on peanut (Shew, 2017). It belongs to FRAC Code M5, in the multi-site contact mode of action group (FRAC, 2017). Fungi treated with chlorothalonil
have a low risk of resistance development because of its multiple modes of action. For this reason, it is commonly alternated or mixed with other products to reduce the risk of selection for fungicide resistance in populations of leaf spot fungi.

Chlorothalonil applications can increase the severity of Sclerotinia blight epidemics (Porter and Lankow, 1981; Porter, 1980a). Chlorothalonil promotes the production of oxalic acid by *Sclerotinia minor in vitro*. Also, Sclerotinia blight lesions release more electrolytes in the presence of low concentrations of chlorothalonil than in the absence of chlorothalonil (Hau and Beute, 1983). These results suggest that electrolyte leakage can be attributed to increases in oxalic production in the presence of chlorothalonil (Lumsden, 1979). Therefore, growers are advised to limit the frequency of chlorothalonil applications in peanut fields with known populations of *S. minor*.

**Section IV. Assessment of fungicides *in vitro***

**Methods of assessment**

Evaluation of *Sclerotinia* and related species for fungicide sensitivity *in vitro* has typically been conducted on fungicide amended agar dispensed in Petri plates (Porter and Phipps, 1985; Matheron and Porchas, 2004; Hubbard et al., 1997; Porter and Lankow, 1981; Smith et al., 1991; Lehner et al., 2017; Amiri et al., 2010). Similarly, evaluation of sclerotia germination typically uses surface sterilized sclerotia placed on media poured into Petri plates (Matheron and Porchas, 2004). In some cases, assays have been conducted in 24-well plates (Stammler et al., 2007; Hu et al., 2016). In one study, sterilized soil was dispensed in
Petri plates and sprayed with fungicide to evaluate mycelial growth in *S. minor* (Smith et al., 1991).

Sensitivity of mycelium to fungicides often is evaluated on potato dextrose agar (PDA) (Hubbard et al. 1997; Porter and Phipps 1985; Matheron and Porchas, 2004; Porter and Lankow, 1981; Lehner et al., 2017; Amiri et al., 2010). However, PDA is nutrient rich and may allow the fungus to grow too quickly for accurate assessment of sensitivity. In one study, growth of *Monilinia fructicola* on PDA was not appropriate for obtaining an EC$_{50}$ because it allowed for values greater than 100 µg/ml in some instances (Hu et al., 2011).

Other media that can be used include glucose yeast-extract agar, glycerol-yeast extract agar (Villani et al., 2016) and liquid yeast-bacto peptone agar (Stammler et al., 2007). Hu et al. 2011 compared different media for determining mycelial sensitivity to fungicides in *M. fructicola*. Among the media assessed, minimal medium (MM) (10g glucose, 1.5g K$_2$HPO$_4$, 2g KH$_2$PO$_4$, 1g (NH$_4$)$_2$SO$_4$, 0.5g MgSO$_4$•7H$_2$O, 1L H$_2$O) proved to be the best for mycelial growth studies. *M. fructicola* grew fastest on unamended MM and exhibited maximum sensitivity to SDHIs on amended MM. Additionally, the resistance phenotype on MM correlated with detached fruit assays.
LITERATURE CITED


CHAPTER 1. Sensitivity of Sclerotinia minor to common peanut fungicides.

ABSTRACT

Sclerotinia blight caused by Sclerotinia minor is an important disease of peanut. Currently, fluazinam, marketed by Syngenta Crop Protection as “Omega 500F®,” is widely used for control of Sclerotinia blight because of its consistent performance. However, Omega 500F can cost over $50 per application and is only labeled for control of Sclerotinia blight. Products that are more affordable perform inconsistently. In this research, we evaluated the in vitro efficacy of five common peanut fungicides (fluazinam, boscalid marketed by BASF Crop Protection as Endura®, the mixture of benzovindiflupyr [marketed as Solatenol® fungicide] and azoxystrobin marketed by Syngenta as Elatus®, penthiopyrad marketed by DuPont™ as Fontelis® and the mixture of fluopyram and prothioconazole marketed by Bayer CropScience as Propulse®) against 44 isolates of Sclerotinia minor. Averaged across isolates, Omega and Elatus caused the greatest inhibition of growth in radial hyphae S. minor, with EC\textsubscript{50} values of 0.0133 ± 0.0048 µg/ml for Omega and 0.0134 ± 0.0082 µg/ml for Elatus. Propulse was least inhibitory at an effective concentration (EC\textsubscript{50}) of 0.060 ± 0.0314 µg/ml. With Omega, 100% inhibition was reached at 0.316 µg/ml, while near total inhibition was obtained with Elatus at 1.0 µg/ml, Endura at 5.623 µg/ml, Propulse at 1.0 µg/ml, and Fontelis at 5.623 µg/ml.

Endura did not perform as well, with an EC\textsubscript{50} value of 0.051 ± 0.0396 µg/ml. While Endura is generally effective for control of Sclerotinia blight in the field, a few isolates tested appeared to be much more insensitive to Endura, while the rest were sensitive with low variability. Similarly, Fontelis had an EC\textsubscript{50} value of 0.017 ± 0.0245 µg/ml, with isolates W-
10, VA3, FA-14 and K6 showing increased insensitivity. Propulse had an EC<sub>50</sub> value of 0.060 ± 0.0314 µg/ml with W-28, VA3 and RM9 showing much more insensitivity than other S. minor isolates. These results suggest that isolate differences may be the cause of variation in some inconsistency in fungicide performance. Products with a high-risk of resistance development should be applied with caution, to not select for fungicide resistance. Omega, containing fluazinam, a fungicide with a low risk of resistance development, exhibited consistent performance across all isolates, validating the observed performance consistency in the field.

**INTRODUCTION**

Epidemics of Sclerotinia blight cause significant yield loss in peanut (Arachis hypogaea L.) in North Carolina and Virginia. Sclerotinia blight is caused by Sclerotinia minor, which was first detected in the Virginia-Carolina growing region in 1971 (Porter and Beute, 1974). Within the United States, Sclerotinia blight is found on peanut in Virginia, North Carolina, Texas, Oklahoma, and Louisiana (Farr and Rossman, 2017). Most recently, it has been reported in Arkansas in 2014 (Faske et al., 2014).

Crop rotation, use of resistant cultivars, treated seed, and fungicides applied to stems and foliage are used to control Sclerotinia blight. Rotation with non-host crops of S. minor, such as corn (Zea mays L.), cotton (Gossypium hirsutum L.), sorghum [Sorghum bicolor (L.) Conrad Moench] and small grains, can reduce populations in soil. Planting disease resistant cultivars such as Bailey and Sugg is an important means of disease control (Isleib et al., 2011, 2015; Porter and Melouk, 1997; Shew, 2017). Use of high quality peanut seed treated with chemical protectants can minimize the possibility of seed transmission of S. minor.
Growers are encouraged to manage soil pH since pH greater than 6.5 correlates with higher disease incidence in the field compared to lower pH (Hau and Beute, 1982). Growers rely on fungicides to avoid yield loss when cultural methods do not provide sufficient control of Sclerotinia blight.

The dicarboxamide iprodione was the first fungicide widely used to control Sclerotinia blight on peanut (Brenneman et al., 1987a). However, resistance was quickly detected in Botrytis cinerea and other fungi closely related to Sclerotinia (Brenneman et al., 1987b). Additionally, degradation by microbial activity was detected and use of the fungicide has declined as more effective products were introduced (Slade et al., 1992).

Fluazinam is the most popular active ingredient used for Sclerotinia blight control in peanut. It provides a high level of disease control and has a low risk of resistance development because it acts against oxidative phosphorylation, but does not target a specific binding site in mitochondria (Guo et al., 1991). Growers quickly adopted it because of its superior performance (Smith et al., 2008). However, a single application of Omega 500F (fluazinam) can cost over $50 per acre. Fluazinam is labeled for control of Sclerotinia blight, but not other common peanut diseases (Shew, 2017).

Several quinone-outside inhibitor (QoIs) fungicides are used to control leaf spots [caused by Cercospora arachidicola Hori and Cercosporidium personatum (Berk. & M.A. Curtis) Deighton], web blotch [caused by Didymella arachidicola (Khokhr.) Tomilin], southern stem rot (caused by Sclerotium rolfsii Sacc.), and Rhizoctonia limb (caused by Rhizoctonia solani J.G. Kühn) and pod rot (caused by Fusarium Link., Pythium myriotylum Drechsler, and Rhizoctonia solani) peanut, but are not used against Sclerotinia blight (Shew,
They have reduced activity against *S. minor*, but this is not well documented (B. Shew, unpublished). QoIs affect respiration complex III (FRAC, 2017). A single point mutation is associated with QoI resistance in plant pathogenic fungi and these fungicides should be used carefully to prevent resistance development (Bartlett et al., 2002; Zhonghua and Michailides, 2005).

Products containing succinate dehydrogenase inhibitors (SDHIs) have been used to control peanut diseases, including seedling diseases, leaf spots, web blotch, southern stem rot, Rhizoctonia limb rot and Sclerotinia blight. Endura, Fontelis, and Convoy (flutaloil) contain an SDHI as the only active ingredient, whereas others, including Propulse, Elatus, and Priaxor are a mixture of an SDHI and another active ingredient of a different chemistry, usually a QoI. SDHI fungicides target respiration complex II (FRAC 2017). Boscalid is effective against Sclerotinia blight in peanut (Smith et al., 2007; Grichar and Woodward, 2016), but has not gained widespread acceptance in the Virginia-Carolina production area (B. Shew, unpublished). Penthiopyrad, the active ingredient in Fontelis, controls Sclerotinia blight at high concentrations, but performance is inconsistent at low concentrations (Grichar and Woodward, 2016). Like penthiopyrad, several products with efficacy against species of *Sclerotinia* are labeled for control or suppression of Sclerotinia diseases (Shew, 2017), but results have been inconsistent in peanut (Shew, unpublished). The reasons for inconsistent performance are not clear but could include application method or timing, chemical degradation, or differences in sensitivity among *S. minor* isolates.

Evaluation of *S. minor* and related species for fungicide sensitivity *in vitro* typically has been conducted on fungicide-amended agar dispensed in standard Petri plates (Matheron 2017).
and Porchas, 2004; Hubbard et al., 1997; Porter and Lankow, 1981; Smith et al., 1991; Lehner et al., 2017; Amiri et al., 2010). In some cases, assays have been conducted in 24-well plates (Stammler et al., 2007; Hu et al., 2016). In one study, sterilized soil was dispensed in Petri plates and sprayed with fungicide to evaluate mycelial growth in S. minor (Smith et al., 1991).

Sensitivity of mycelium to fungicides is often evaluated on potato dextrose agar (PDA) (Hubbard et al., 1997; Porter and Phipps, 1985; Matheron and Porchas, 2004; Porter and Lankow, 1981; Lehner et al., 2017; Amiri et al., 2010). However, PDA is nutrient rich and the fungus may grow too quickly for accurate assessment of sensitivity (Hu et al., 2011). In culture, some fungi can bypass the site of action of QoIs by utilizing an alternate oxidase pathway (AOX) (Xu et al., 2013). The AOX pathway allows fungi to regulate growth and mitigate oxidative stress (Xu et al., 2012). Amending a medium with salicylhydroxamic acid (SHAM) blocks the AOX to more accurately assess fungicide activity.

The overall objective of this research was to determine the sensitivity of S. minor isolates to common peanut fungicides. Through this research, we hope to better understand observed inconsistent performance of some fungicides used for Sclerotinia blight control.

**MATERIALS AND METHODS**

*Sclerotinia minor* isolates

**Isolate collection and maintenance**

Eight contemporary isolates of S. minor were obtained from infested soil collected at the Upper Coastal Plain Research Station (UCPRS) in Rocky Mount, NC in 2016. Soil samples were taken from fields planted with peanut and were air-dried until assayed. Wet-
sieved soil samples were examined under a dissecting microscope and sclerotia were
removed from samples. Sample sclerotia then were surface-disinfested with a 0.6% (v/v)
NaOCl, dried with paper towels and plated on potato dextrose agar (PDA) to germinate. In
addition, three isolates were collected from peanut grown in research fields in Suffolk, VA.
These isolates were obtained directly from surface-disinfested peanut stems plated on PDA.
All isolates were maintained on PDA by transferring plugs of actively growing cultures onto
new plates of PDA.

Isolates collected before 2004 were maintained on colonized oat grain and stored in a
to 4 C° were also evaluated in this study. The grains were surface-disinfested,
refrigerator at 4 C° were also evaluated in this study. The grains were surface-disinfested,
dried with paper towels and plated on PDA. These historical isolates then were then
maintained on PDA for the duration of the study.

Isolate selection

Forty-four isolates of *Sclerotinia minor* used for this study were selected based on the
location and date of collection, host of isolation, and Mycelial Compatibility Group (MCG)
(Table 1). Among these isolates were 30 isolates from peanut, nine from weed hosts, three
isolates from field collected apothecia (Hollowell et al., 2003a, Hollowell et al., 2003b), and
two isolates from lettuce. Isolates collected prior to 2004 from multiple hosts accounted for
31 isolates and 11 isolates were collected in 2016. Isolates collected before 2004 were not
exposed to fungicides labeled after that year and this can be considered baseline isolates for
several of the fungicides tested, including Elatus, Propulse and Fontelis. However, it is
possible that they previously were exposed to other SDHIs, including carboxin (first
registered in the US in 1968), boscalid (2003), and flutalolinil (1985). The majority of isolates


were collected in North Carolina, and isolates collected in Virginia and California were also included. Ten MCGs were included, with the group proportions representative of the entire isolate collection.

**Selection of media**

In preliminary experiments, mycelial growth of *S. minor* was evaluated on glycerol-yeast extract agar (GLYE; 10g glycerol, 10g yeast extract, 15g agar, 6g NaNO₃, 1.5g KH₂PO₄, 0.5g MgSO₄, and 0.5g KCl in 1L H₂O), minimal medium (MM; 10g glucose, 1.5g K₂HPO₄, 2g KH₂PO₄, 1g (NH₄)₂SO₄ and 0.5g MgSO₄•7H₂O in 1L H₂O), and potato dextrose agar (PDA). Growth was fastest on PDA and was greatly inhibited on MM. Growth then was compared on PDA and GLYE amended with selected SDHIs (Hu et al. 2011; Villani et al. 2016). For example, growth of six *S. minor* isolates was compared on the GLYE and PDA amended with Endura at 0.001, 0.01, 0.1, 1.0 and 10 µg/ml. Growth was measured at 48 hours by taking two perpendicular colony diameter measurements. The mean colony diameter on was 18.0 on GLYE and 30.4 on PDA. At a concentration of 10 µg/ml, colony diameters were inhibited only 72% in PDA, while complete inhibition was observed on GLYE. Similar results were found in trials with Fontelis. We concluded that GLYE was suitable for evaluation of sensitivity to SDHIs in *S. minor* and used GLYE in all subsequent studies.

**Fungicide sensitivity**

Selected isolates were screened for sensitivity to commercial formulations of five fungicides commonly used in peanut production in the Virginia-Carolina region. The range of concentrations used for each product was selected based on the relative sensitivity of *S.
minor as determined in preliminary experiments. Omega 500F (fluazinam; Syngenta) was tested at 0.001, 0.003, 0.01, 0.03 and 0.316 µg/ml and Elatus (azoxystrobin + benzovindiflupyr; Syngenta) at 0.001, 0.006, 0.032, 0.178 and 1 µg/ml. Endura (boscalid; BASF Corporation), Fontelis (penthiopyrad; DuPont) and Propulse (fluopyram + prothioconazole; Bayer CropScience LP) were each tested at 0.006, 0.032, 0.178, 1.0 and 5.623 µg/ml. Serial dilutions of the fungicides were made in sterile deionized water to prepare the amended media. The study was conducted on GLYE in 100 mm-diam plastic Petri plates.

GLYE was prepared and autoclaved for 30 minutes at 15 KPa, then amendments were added to the autoclaved media before being poured into Petri plates. SHAM was added to Elatus at 25 µg/ml. Plugs (6 mm-diam) were cut from the leading edge of an actively growing culture and inverted in the middle of each agar plate. Plates were arranged on the laboratory bench at room temperature (25°C). Each fungicide was tested individually, with all concentrations and isolates. There were three replicates of each treatment arranging in a randomized complete block design. Isolates were grouped within blocks and fungicide concentration randomized within isolates. Colony growth (radial mycelial growth) was measured at 48 hours by taking two perpendicular measurements and averaging. The experiment was conducted twice.

Data collection and analysis

Percent inhibition was calculated by subtracting the test isolate diameter from the control (non-amended medium) isolate diameter, dividing that number by the control isolate diameter and multiplying by 100. Within each fungicide, effective concentration to inhibit
50% of radial colony growth (EC₅₀) values were calculated for each replicate, trial, and isolate combination by regressing percentage inhibition of mycelium growth (diameter) vs. log₁₀ fungicide dose in µg/ml of formulated fungicide. EC₅₀ values were analyzed within each fungicide with the mixed models procedure (PROC MIXED), of SAS 9.4 statistical software package, considering isolates to have fixed effects and replicate and experiment to have random effects (SAS Institute; Cary, NC). The correlation procedure (PROC CORR) was used to calculate Spearman product-moment correlations among isolate EC₅₀ values and ranks across fungicides. Means and standard deviations of EC₅₀ also were calculated for each fungicide.

RESULTS AND DISCUSSION

Mycelial compatibility group (MCG)

MCG is determined by paring a given isolate and a tester isolate on a Petri plate containing PDA and observing the hyphal interaction zone between isolates. Isolates in the same MCG group successfully anastomosed, creating a continuous colony. Non-compatible isolates did not anastomose, but create a zone of dead cells between the two colonies. This is observable as a line between the two colonies, or the creation of fluffy mycelium in the hyphal interaction zone. Isolates from MCGs 1, 4, 6, and 7 were represented among the most insensitive isolates.

Activity of fungicides against Sclerotinia minor

Averaged across isolates, Omega and Elatus caused the greatest inhibition of growth in S. minor, with EC₅₀ values of 0.0133 ± 0.0048 µg/ml for Omega and 0.0134 ± 0.0082
µg/ml for Elatus. Propulse was least inhibitory at an EC\textsubscript{50} of 0.060 ± 0.0314 µg/ml (Table 2; Figure 1). With Omega, 100% inhibition was reached at 0.316 µg/ml (Figure 2), while near total inhibition was obtained with Elatus at 1.0 µg/ml (Figure 3), Endura at 5.623 µg/ml (Figure 4), Propulse at 1.0 µg/ml (Figure 5), and Fontelis at 5.623 µg/ml dose (Figure 6).

In the context of this paper, the terms ‘sensitivity’ and ‘insensitivity’ are not indicative of susceptibility or resistance, but as a means to differentiate the responses of isolates to fungicides.

**Isolate differences in fungicide sensitivity**

Isolates varied in their response to all fungicides tested [Omega, Fontelis, Elatus and Propulse (P<0.0001); Endura (P=0.0023)]. However, range of isolate responses differed for the fungicides (Figures 7-11; Table 3). The smallest variation across isolates was observed in Omega (Figure 7; Table 3). Isolates of *S. minor* least sensitive to Omega included P-10, W-26, W-28, W-57, P-1, FA-14 and P-30. This high sensitivity and lack of variation across isolates could account for the consistent performance of Omega. Similarly, Elatus (Figure 8; Table 3), had a low mean EC\textsubscript{50} value and variability across isolates. The isolates least sensitive to Elatus included RM2, VA3, P-10, W-28, RM1, W-57, and RM6. Isolate response to Propulse (Figure 9; Table 3) and Fontelis (Figure 10; Table 3) was highly variable compared to Elatus and Omega. The isolates least sensitive to Propulse included W-28, VA3, RM9, W-57, RM7, P-10 and FA-14. The isolates least sensitive to Fontelis included W-10, VA3, FA-14, K-6, W-28, P-36 and W-17. Isolate response to Endura was highly variable (Figure 11), and isolate differences were not detected (Table 3). The least sensitive isolates
included K-6, FA-14, RM2, W-10, W-53, K-4, and W-37. The three most insensitive isolates, K-6, FA-14 and RM2, show a large difference from the rest of the isolates.

Elatus is a combination product containing azoxystrobin and benzovindiflupyr and currently is not labeled for use in Sclerotinia blight control on peanut. Elatus had one of the lowest EC$_{50}$ values of 0.0134 $\pm$ 0.0082 µg/ml. While there is little published information addressing control of Sclerotinia blight of peanut with benzovindiflupyr, the results suggest that the SDHI component of Elatus is inhibitory to S. minor and that Elatus could be suitable for Sclerotinia blight control in peanut.

Endura did not perform as expected, with an EC$_{50}$ value of 0.051 $\pm$ 0.0396 µg/ml. While Endura has been effective for control of Sclerotinia blight, a few of the isolates we tested appeared to be very insensitive to Endura, while variability was low among the other isolates (Figure 11). Similarly, Fontelis had an EC$_{50}$ value of 0.017 $\pm$ 0.0245 µg/ml, with isolates W-10, VA3, FA-14 and K6 showing the greatest insensitivity. Propulse had an EC$_{50}$ value of 0.060 $\pm$ 0.0314 µg/ml with W-28, VA3 and RM9 showing increased sensitivity. These results suggest that isolate differences may be the cause of some of the observed inconsistency in fungicide performance of these fungicides. Isolate differences may be caused by the overuse of SDHI fungicides. Resistance to boscalid has been observed in several closely related species (Avenot and Michailides, 2010; Hu et al., 2016), and has been attributed to the use of SDHI fungicides.

Ranks of isolate responses were correlated to growth on medium with Fontelis and Endura (r = 0.44; P = 0.0031), and with Elatus and Propulse (r = 0.55; P = 0.0001). These correlations indicate the ability to predict the sensitivity rank of an isolate in one fungicide
based on another, such that the rank of a particular isolate response to one fungicide is likely to be similar to the rank of that same isolate in another fungicide. Both of these significant combinations included SDHI products. Additionally, isolates sampled more recently were less sensitive to a given fungicide than those isolated 10 to 15 years ago. More recently collected isolates may have been exposed to SDHIs in the field and some selection may have occurred. Recently collected isolates, especially those collected at the UCPRS in Rocky Mount, came from test plots with a history of extensive testing with products for *Sclerotinia* control.

Our results suggest resistance development is possible in *S. minor*, especially with Endura and Fontelis. Applying these products with a high-risk of resistance development should be applied with caution, to not select for resistant isolates of *S. minor*. Risk of resistance development can be lowered with the use of combination products with multiple active ingredients from different fungicide groups that have activity on the same organism. Omega was shown to have consistent performance across all isolates, validating the observed performance consistency in the field. Further studies, especially in the field, will complement this data and yield information that includes all the variables present in the field.
LITERATURE CITED


Table 1. Host, location collected, date collected, and MCG for isolates of *Sclerotinia minor* tested for sensitivity to five fungicides

<table>
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<td>2016</td>
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</tr>
<tr>
<td>VA3</td>
<td>Peanut</td>
<td>Suffolk, VA</td>
<td>2016</td>
<td>3</td>
</tr>
</tbody>
</table>
Table 2. Mean and standard deviation of EC₅₀ for inhibition of mycelial growth of 44 isolates of *Sclerotinia minor* by five fungicides tested *in vitro*

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Mean EC₅₀ᵃ</th>
<th>Standard Deviation</th>
<th>Minimum EC₅₀</th>
<th>Maximum EC₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omega</td>
<td>0.01325</td>
<td>0.0048</td>
<td>0.0067</td>
<td>0.027</td>
</tr>
<tr>
<td>Elatus</td>
<td>0.01336</td>
<td>0.0082</td>
<td>0.0035</td>
<td>0.044</td>
</tr>
<tr>
<td>Fontelis</td>
<td>0.0170</td>
<td>0.0245</td>
<td>0.0020</td>
<td>0.146</td>
</tr>
<tr>
<td>Endura</td>
<td>0.0512</td>
<td>0.0396</td>
<td>0.0211</td>
<td>0.272</td>
</tr>
<tr>
<td>Propulse</td>
<td>0.0601</td>
<td>0.0314</td>
<td>0.0168</td>
<td>0.174</td>
</tr>
</tbody>
</table>

ᵃEC₅₀ was calculated from linear regression of log₁₀ fungicide concentration vs. percentage inhibition of colony diameter. Values are means of 44 isolates, three replicates and two trials.
Table 3. Mean isolate EC$_{50}$ values for inhibition of mycelial growth of 44 isolates of *Sclerotinia minor* by five fungicides *in vitro*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Omega*</th>
<th>Elatus</th>
<th>Propulse</th>
<th>Fontelis</th>
<th>Endura</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-1</td>
<td>0.019</td>
<td>0.0093</td>
<td>0.0499</td>
<td>0.0057</td>
<td>0.0269</td>
</tr>
<tr>
<td>P-4</td>
<td>0.013</td>
<td>0.0119</td>
<td>0.0418</td>
<td>0.0091</td>
<td>0.0365</td>
</tr>
<tr>
<td>P-6</td>
<td>0.011</td>
<td>0.0171</td>
<td>0.0541</td>
<td>0.0084</td>
<td>0.0427</td>
</tr>
<tr>
<td>P-8</td>
<td>0.015</td>
<td>0.0071</td>
<td>0.0265</td>
<td>0.0020</td>
<td>0.0284</td>
</tr>
<tr>
<td>P-10</td>
<td>0.027</td>
<td>0.0322</td>
<td>0.0897</td>
<td>0.0104</td>
<td>0.0411</td>
</tr>
<tr>
<td>P-18</td>
<td>0.009</td>
<td>0.0126</td>
<td>0.0310</td>
<td>0.0092</td>
<td>0.0471</td>
</tr>
<tr>
<td>P-22</td>
<td>0.009</td>
<td>0.0060</td>
<td>0.0403</td>
<td>0.0136</td>
<td>0.0404</td>
</tr>
<tr>
<td>P-27</td>
<td>0.008</td>
<td>0.0106</td>
<td>0.0737</td>
<td>0.0074</td>
<td>0.0374</td>
</tr>
<tr>
<td>P-29</td>
<td>0.011</td>
<td>0.0072</td>
<td>0.0299</td>
<td>0.0076</td>
<td>0.0337</td>
</tr>
<tr>
<td>P-30</td>
<td>0.016</td>
<td>0.0057</td>
<td>0.0181</td>
<td>0.0032</td>
<td>0.0404</td>
</tr>
<tr>
<td>P-33</td>
<td>0.016</td>
<td>0.0112</td>
<td>0.0393</td>
<td>0.0033</td>
<td>0.0296</td>
</tr>
<tr>
<td>P-36</td>
<td>0.011</td>
<td>0.0092</td>
<td>0.0231</td>
<td>0.0246</td>
<td>0.0439</td>
</tr>
<tr>
<td>P-48</td>
<td>0.011</td>
<td>0.0035</td>
<td>0.0354</td>
<td>0.0159</td>
<td>0.0429</td>
</tr>
<tr>
<td>P-57</td>
<td>0.009</td>
<td>0.0106</td>
<td>0.0562</td>
<td>0.0064</td>
<td>0.0369</td>
</tr>
<tr>
<td>P-60</td>
<td>0.012</td>
<td>0.0138</td>
<td>0.0386</td>
<td>0.0100</td>
<td>0.0250</td>
</tr>
<tr>
<td>P-61</td>
<td>0.013</td>
<td>0.0094</td>
<td>0.0549</td>
<td>0.0093</td>
<td>0.0502</td>
</tr>
<tr>
<td>W-10</td>
<td>0.016</td>
<td>0.0061</td>
<td>0.0469</td>
<td>0.1458</td>
<td>0.0777</td>
</tr>
<tr>
<td>W-17</td>
<td>0.008</td>
<td>0.0094</td>
<td>0.0777</td>
<td>0.0205</td>
<td>0.0344</td>
</tr>
<tr>
<td>W-26</td>
<td>0.026</td>
<td>0.0117</td>
<td>0.0601</td>
<td>0.0066</td>
<td>0.0347</td>
</tr>
<tr>
<td>W-28</td>
<td>0.026</td>
<td>0.0282</td>
<td>0.1735</td>
<td>0.0319</td>
<td>0.0351</td>
</tr>
<tr>
<td>W-31</td>
<td>0.007</td>
<td>0.0047</td>
<td>0.0577</td>
<td>0.0072</td>
<td>0.0353</td>
</tr>
<tr>
<td>W-37</td>
<td>0.010</td>
<td>0.0080</td>
<td>0.0501</td>
<td>0.0149</td>
<td>0.0635</td>
</tr>
<tr>
<td>W-43</td>
<td>0.010</td>
<td>0.0099</td>
<td>0.0539</td>
<td>0.0034</td>
<td>0.0342</td>
</tr>
<tr>
<td>W-53</td>
<td>0.011</td>
<td>0.0101</td>
<td>0.0359</td>
<td>0.0104</td>
<td>0.0769</td>
</tr>
<tr>
<td>W-57</td>
<td>0.024</td>
<td>0.0215</td>
<td>0.0954</td>
<td>0.0187</td>
<td>0.0587</td>
</tr>
</tbody>
</table>
Table 3 Continued

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Omega</th>
<th>Elatus</th>
<th>Propulse</th>
<th>Fontelis</th>
<th>Endura</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-4</td>
<td>0.012 e-l</td>
<td>0.0109 i-n</td>
<td>0.0734 c-i</td>
<td>0.0112 de</td>
<td>0.0752 b</td>
</tr>
<tr>
<td>K-6</td>
<td>0.010 h-l</td>
<td>0.0141 f-l</td>
<td>0.0527 d-j</td>
<td>0.0590 bc</td>
<td>0.2721 a</td>
</tr>
<tr>
<td>K-9</td>
<td>0.012 e-l</td>
<td>0.0129 g-m</td>
<td>0.0484 d-j</td>
<td>0.0022 de</td>
<td>0.0282 b</td>
</tr>
<tr>
<td>K-12</td>
<td>0.012 e-k</td>
<td>0.0121 g-n</td>
<td>0.0395 f-j</td>
<td>0.0132 de</td>
<td>0.0555 b</td>
</tr>
<tr>
<td>K-14</td>
<td>0.010 h-l</td>
<td>0.0091 i-n</td>
<td>0.0168 j</td>
<td>0.0061 de</td>
<td>0.0364 b</td>
</tr>
<tr>
<td>FA-7</td>
<td>0.015 c-h</td>
<td>0.0123 g-m</td>
<td>0.0678 c-j</td>
<td>0.0084 de</td>
<td>0.0301 b</td>
</tr>
<tr>
<td>FA-13</td>
<td>0.014 c-i</td>
<td>0.0061 k-n</td>
<td>0.0640 d-j</td>
<td>0.0049 de</td>
<td>0.0452 b</td>
</tr>
<tr>
<td>FA-14</td>
<td>0.018 cd</td>
<td>0.0098 i-n</td>
<td>0.0890 c-f</td>
<td>0.0617 b</td>
<td>0.1148 b</td>
</tr>
<tr>
<td>RM1</td>
<td>0.012 e-l</td>
<td>0.0241 c-e</td>
<td>0.0765 c-h</td>
<td>0.0092 de</td>
<td>0.0602 b</td>
</tr>
<tr>
<td>RM2</td>
<td>0.014 d-i</td>
<td>0.0441 a</td>
<td>0.0792 c-g</td>
<td>0.0185 de</td>
<td>0.1141 b</td>
</tr>
<tr>
<td>RM3</td>
<td>0.013 d-k</td>
<td>0.0104 i-n</td>
<td>0.0383 f-j</td>
<td>0.0096 de</td>
<td>0.0449 b</td>
</tr>
<tr>
<td>RM4</td>
<td>0.008 j-l</td>
<td>0.0152 f-j</td>
<td>0.0807 c-g</td>
<td>0.0064 de</td>
<td>0.0458 b</td>
</tr>
<tr>
<td>RM6</td>
<td>0.013 d-k</td>
<td>0.0204 d-g</td>
<td>0.0643 d-j</td>
<td>0.0151 de</td>
<td>0.0538 b</td>
</tr>
<tr>
<td>RM7</td>
<td>0.011 f-l</td>
<td>0.0136 f-l</td>
<td>0.0910 c-e</td>
<td>0.0068 de</td>
<td>0.0372 b</td>
</tr>
<tr>
<td>RM8</td>
<td>0.014 d-i</td>
<td>0.0202 d-h</td>
<td>0.0425 e-j</td>
<td>0.0068 de</td>
<td>0.0593 b</td>
</tr>
<tr>
<td>RM9</td>
<td>0.012 e-k</td>
<td>0.0144 f-k</td>
<td>0.1185 bc</td>
<td>0.0086 de</td>
<td>0.0334 b</td>
</tr>
<tr>
<td>VA1</td>
<td>0.012 e-l</td>
<td>0.0078 j-n</td>
<td>0.0588 d-j</td>
<td>0.0193 de</td>
<td>0.0211 b</td>
</tr>
<tr>
<td>VA2</td>
<td>0.011 g-l</td>
<td>0.0099 i-n</td>
<td>0.0453 d-j</td>
<td>0.0109 de</td>
<td>0.0402 b</td>
</tr>
<tr>
<td>VA3</td>
<td>0.015 c-g</td>
<td>0.0336 b</td>
<td>0.1467 ab</td>
<td>0.0647 b</td>
<td>0.0334 b</td>
</tr>
</tbody>
</table>

*a Means followed by the same letter are not significantly different at P ≤ 0.01 according to paired t-tests*
Table 4. Correlations of isolate EC$_{50}$ ranks between 44 isolates of *Sclerotinia minor* by fungicide *in vitro*

<table>
<thead>
<tr>
<th></th>
<th>Elatus</th>
<th>Endura</th>
<th>Fontelis</th>
<th>Omega</th>
<th>Propulse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Elatus</strong></td>
<td>1.0000</td>
<td>0.12911</td>
<td>0.15715</td>
<td>0.24386</td>
<td>0.54856</td>
</tr>
<tr>
<td><strong>Endura</strong></td>
<td>0.1291</td>
<td>1.0000</td>
<td>0.43566</td>
<td>-0.03172</td>
<td>0.06441</td>
</tr>
<tr>
<td><strong>Fontelis</strong></td>
<td>0.1571</td>
<td>0.43566</td>
<td>1.0000</td>
<td>0.04694</td>
<td>0.26794</td>
</tr>
<tr>
<td><strong>Omega</strong></td>
<td>0.2438</td>
<td>-0.0317</td>
<td>0.04694</td>
<td>1.0000</td>
<td>0.19347</td>
</tr>
<tr>
<td><strong>Propulse</strong></td>
<td>0.5486</td>
<td>0.06441</td>
<td>0.26794</td>
<td>0.19347</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Spearman Correlation Coefficients ($\hat{\rho}$), $n = 44$

Prob $> \mid r \mid$ under $H_0$: $\hat{\rho} = 0$
Figure 1. Means and standard deviations of EC$_{50}$ for fungicide inhibition of mycelial growth of *Sclerotinia minor in vitro*. Means represent 44 isolates, three replicates, and two experiments.
Figure 2. Fungicide concentration (µg/ml; logarithmic scale) vs. percent inhibition of *Sclerotinia minor* by Omega (fluazinam) *in vitro*. Means represent 44 isolates, three replicates, and two experiments.
Figure 3. Fungicide concentration (µg/ml; logarithmic scale) vs. percent inhibition of *Sclerotinia minor* by Elatus (azoxystrobin + benzovindiflupyr) *in vitro*. Means represent 44 isolates, three replicates, and two experiments.
**Figure 4.** Fungicide concentration (µg/ml; logarithmic scale) vs. percent inhibition of *Sclerotinia minor* by Endura (boscalid) *in vitro*. Means represent 44 isolates, three replicates, and two experiments.
Figure 5. Fungicide concentration (µg/ml; logarithmic scale) vs. percent inhibition of *Sclerotinia minor* by Propulse (fluopyram + prothioconazole) *in vitro*. Means represent 44 isolates, three replicates, and two experiments.
Figure 6. Fungicide concentration (µg/ml; logarithmic scale) vs. percent inhibition of *Sclerotinia minor* by Fontelis (penthiopyrad) *in vitro*. Means represent 44 isolates, three replicates, and two experiments.
Figure 7. Effective concentration (EC$_{50}$) of Omega (fluazinam) by isolate of *Sclerotinia minor*. EC$_{50}$ value represents three replicates and two experiments.
Figure 8. Effective concentration ($EC_{50}$) of Elatus (azoxystrobin + benzovindiflupyr) by isolate of *Sclerotinia minor*. $EC_{50}$ value represents three replicates and two experiments.
Figure 9. Effective concentration (EC$_{50}$) of Propulse (fluopyram + prothioconazole) by isolate of *Sclerotinia minor*. EC$_{50}$ value represents three replicates and two experiments.
Figure 10. Effective concentration (EC$_{50}$) of Fontelis (penthiopyrad) by isolate of Sclerotinia minor. EC$_{50}$ value represents three replicates and two experiments.
Figure 11. Effective concentration (EC$_{50}$) of Endura (boscalid) by isolate of *Sclerotinia minor*. EC$_{50}$ value represents three replicates and two experiments.