Abstract

Smith, Damon Lee. Biology and Epidemiology of *Sclerotinia minor* on Peanut (*Arachis hypogaea* L.). (Under the direction of Dr. Barbara Shew)

Sclerotinia blight caused by the fungus *Sclerotinia minor* is a serious disease of cultivated peanut (*Arachis hypogaea* L.) in North Carolina. Laboratory and field experiments were conducted to gain a better understanding of how environment affects sclerotial germination, mycelial growth, and plant infection in soil. Furthermore, quantitative relationships between disease incidence, severity, environmental factors, and yield loss were examined in order to improve disease forecasting systems in North Carolina. Laboratory and field experiments also were conducted to gain a better understanding of the biology of both the host and fungus. Experiments were conducted to evaluate the relative importance of tissue resistance vs. plant architecture in high-performing cultivars.

In the laboratory, soil matric potential (Ψ_M) and temperature effects were measured for germination of sclerotia, mycelial expansion, and lesion expansion on detached leaflets. Temperature effect on the production of oxalic acid by mycelium of *S. minor* was also examined. Maximum sclerotial germination occurred at a Ψ_M of –7.2 kPa and a temperature of 30 C. Rate of mycelial expansion and lesion development and expansion on detached leaflets were maximal at temperatures of 18-22 C. Lesions on detached leaflets developed slowly at temperatures above 26 C, and failed to develop at temperatures of 29 C or greater. Lesions formed if inoculated leaflets were moved from 29 C to a cooler temperature of 18 or 22 C. Oxalic acid production at temperatures above 29 C was negligible.

In the field, incidence of Sclerotinia blight was measured at three sites in 2002 and 2003. A gradient of disease levels was established by utilizing one partially resistant cultivar, two susceptible cultivars and the fungicides fluazinam and boscalid at various rates. Weather data were collected in 2003 and modeled in both years. Disease incidence was highest on the susceptible cultivars with no fungicide treatment. Disease incidence of the partially resistant cultivar and of one susceptible cultivar in conjunction with various weather parameters were used as the dependent and independent variables, respectively, in a regression analysis. The following disease prediction model was created:

$$\text{Total Disease} = -79.52 + 3.04 \text{ relative humidity} - 0.020 \text{ relative humidity}^2 - 0.47 \text{ soil temperature} - 5.30 \text{ leaf wetness}.$$

In other laboratory tests, leaflets, pegs, lateral branches, and main stems of two susceptible cultivars and two partially resistant breeding lines were detached from plants grown in the greenhouse, inoculated with mycelial plugs, and placed in moisture chambers. Lesion development and severity on each part were measured for 7 days. In the field three cultivars and one breeding line were planted in replicated plots. Destructive samples of randomly selected plants were made weekly. Numbers of lesions on the four plant parts of interest were counted. Laboratory studies indicated that leaflets and pegs were the most susceptible plant tissues. Main stems and lateral branches were resistant to infection. In the field, however, lesions were found most frequently on lateral branches. Inconsistencies between laboratory and field studies indicate that there are other mechanisms of resistance operating in the field besides physiological resistance. Management strategies should focus on protecting lateral branches from infection by *S. minor*. Those management strategies may be improved further with the adoption of a

Sclerotinia blight disease forecasting system that utilizes remote site-specific weather data with no need for onsite sensors.

Biology and Epidemiology of *Sclerotinia minor* on Peanut (*Arachis hypogaea* L.)

By

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APPROVED BY:

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Dedication

I dedicate this thesis to my father and mother, David and Sally Smith. I have learned so much from them, and their love and support have made me the person I am today. Whatever weird scheme I came up with, they fully supported me. Even when I said "I'm going to pack everything I own in a truck, and head south to live on peanuts for two years, just for the fun of it," they still gave me support, though it killed them to see me move so far away. And I will never forget this piece of information that was recited to me over and over: 'It's a great life if you don't weaken.' Thank you so much and I love you both.

BIOGRAPHY

Damon Smith was born on April 13, 1979 in Canandaiguia, New York. He was raised in the heart of the Finger Lakes Region in Honeoye, New York. After graduating from high school as the salutatorian of his class, Damon attended the State University of New York at Geneseo (SUNY Geneseo). At SUNY Geneseo Damon concentrated his efforts in the study of plant sciences while he conducted undergraduate research entitled "Effects of Light Emitting Diodes (LED) on Plant Growth." In addition, he was actively involved in the biology program and was a member of the Beta Beta Beta Biology Honor Society. In May of 2001 he graduated from SUNY Geneseo with a Bachelor of Science Degree in Biology. In August of 2001 Damon accepted a graduate research assistantship from the Department of Plant Pathology at North Carolina State University where he pursued a Master's of Science degree in Plant Pathology. He completed his degree in the spring of 2004 under the direction of Dr. Barbara Shew.

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Literature Review

Section I. *Sclerotinia minor*

Distribution, importance, and host range. *Sclerotinia minor* Jagger, the fungus that causes Sclerotinia blight of peanut, can infect a wide range of host plants. *S. minor* occurs worldwide, but especially in cool, moist regions where it can be a major problem of many important flower and vegetable crops (Melzer, 1997). Common names of diseases caused by this fungus on different hosts include cottony rot, blight, white mold, and drop, among others (Agrios, 1997). At least 94 plant species belonging to 21 families and 66 genera are hosts for *S. minor* (Melzer, 1997). The hosts that have been documented are from the branch Angiospermae. Most hosts of *S. minor* are in the class Dicotyledonae, but Melzer (1997) listed two documented occurrences of *S. minor* on hosts belonging to the class Monocotyledonae. In addition, yellow nutsedge (*Cyperus esculentus* L.) recently was reported as a host of *S. minor* (Hollowell, 2001). Some common plants that are susceptible to *S. minor* include chicory, caraway, baby's breath, carnation, and other popular ornamentals. The weed species *Eclipta prostrata* also has been reported as a host of *Sclerotinia minor* in Oklahoma (Melouk et al., 1992b). Economically important crops include soybean (Phipps and Porter, 1982), sunflower (Sedun and Brown, 1989), common bean, lettuce (Beach, 1921; Sereni, 1944), and peanut (Porter and Beute, 1974), among others (Melzer, 1997). In lettuce production areas, crop losses can range from 10% to 50% and some growers may experience losses as high as 75%.

Taxonomy. *S. minor* is a member of the family Sclerotiniaceae*,* which encompasses inoperculate discomycetous fungi of the order Helotiales within the phylum Ascomycota (Alexopolous et al., 1996). Among the species within the genus *Sclerotinia,* four are considered plant pathogens. Taxonomy is well understood in three species: *S. minor* Jagger, *S. sclerotiorum* (Lib.) de Bary, and *S. trifoliorum* Erikss (Kohn, 1979b). A fourth species is currently known as *S. homeocarpa* F.T. Bennett with synonyms for the genus including either *Lanzia* Sacc. or *Moellerodiscus* Henn., which are both included in the complex *Rustroemia.* This species is not well understood and future molecular DNA studies will help clarify whether *S. homeocarpa* belongs in *Sclerotinia* or another genus (Smiley et al., 1992). Kohn (1979a) used cultural, biological, macroscopic, and microscopic characteristics to separate species in the genus *Sclerotinia*. The host range of *S. minor* is smaller than that of *S. sclerotiorum*, while more extensive than the host range of *S. trifoliorum,* which is limited to forage legume species (Kohn, 1979a). *Sclerotinia* species form apothecia that are stipitate and ascospores that are hyaline and ellipsoidal. Apothecia are pale orange to white and have concave or flat tops measuring 6 mm or more in diameter (Backman et al. 1997; Hao et al., 2003). The ectal expiculum is composed of globose cells, which are oriented perpendicular to the apothecial surface. Ascospores of *S. minor* are typically tetranucleate, and measure 8-17 x 5-7 μm. Sclerotia range in size from 0.5 mm to 2 mm (Backman et al. 1997; Hao et al., 2003). Ascospores of *S. trifoliorum* are tetranucleate, and have a length:width ratio of <2.0. In addition, the ascospores exhibit dimorphism in size and are so segregated within the ascus. Sclerotia of *S. trifoliorum* are large (2-20mm) as compared to those of *S. minor. S. sclerotiorum* produces large (2-20 mm) sclerotia as well, but the ascospores produced are binucleate.

These fungi also produce sclerotia that are tuberoid in shape and incorporate no host tissue (Kohn, 1979a).

Nomenclature. Madame M.A. Libert described the first "Sclerotinia-like" organism in 1837 and named it *Peziza sclerotiorum* (Purdy, 1979). Circa 1870, L. Fuckel moved the fungus to the new genus *Sclerotinia* and changed the species name to *libertiana.* Later, Anton de Bary named the fungus *Sclerotinia sclerotiorum* as it remains today (Purdy, 1979). In 1920, I.C. Jagger described a new *Sclerotinia* species isolated from *Lactuca sativa* L. in areas of upstate New York, Massachusetts, Pennsylvania, and Florida. Having found a distinct species, Jagger named the fungus *Sclerotinia minor* n. sp. (Jagger, 1920). In 1945, H.H. Whetzel erected the family *Sclerotiniaceae* and cited the type species as *Sclerotinia sclerotiorum* (Lib.) de Bary. Along with the formation of the new family, 12 species were named in the genus *Sclerotinia,* including *S. minor* (Kohn, 1979b). Korf and Dumont (1972) renamed the genus *Whetzelinia* with the type species name changed to *Whetzelinia sclerotiorum* (Kohn, 1979a). Finally, after much controversy in the scientific community, *Whetzelinia* was rejected in favor of the currently accepted genus of *Sclerotinia,* typified by the species *Sclerotinia sclerotiorum* (Kohn, 1979b).

Importance of oxalic acid production in *Sclerotinia spp.* Mode of pathogenesis by many fungi has intrigued mycologists even before the development of the field of plant pathology. Anton De Bary studied pathogenesis by *S. sclerotiorum* extensively. In 1886

he identified the importance of oxalic acid in the pathogenesis of hosts by this group of fungi (Lumsden, 1979).

Oxalic acid can be directly toxic to plant cells. Drastic pH changes in the cell and the chelation of cations caused by oxalic acid can contribute to cell death (Lumsden, 1979). Wilting symptoms that are observed in relation to infection by *Sclerotinia spp.* and oxalic acid production may result from the formation of oxalate crystals. Oxalate crystals are hypothesized to cause wilting in two ways; they may directly block xylem vessels and they may play a role in water transport within xylem elements (Lumsden, 1979). In addition to oxalic acid, pectinases, cellulases, and hemicellulases have been associated with the infection and parasitism of host tissue by *Sclerotinia spp.* (Lumsden, 1979). By influencing pH, oxalic acid can act in a synergistic manner with these cell wall degrading enzymes to cause maceration of host tissue. As the pH of the intercellular region decreases due to the production of oxalic acid by the fungus, the activity of these cell wall degrading enzymes has been shown to increase, resulting in the formation of larger lesions (Marciano et al., 1983). At higher pH levels, the effectiveness of cell wall degrading enzymes is inhibited.

Section II. The cultivated peanut (*Arachis hypogaea* L.)

***Arachis hypogaea:* origin and growth habits.** The cultivated peanut belongs to the genus *Arachis* of which there are between 80 and 100 named species (Simpson et al., 2001; Stalker and Simpson, 1995). *Arachis* belongs to the legume family and is native to South America. The cultivated peanut *Arachis hypogaea* L. is grown in tropical and subtropical regions of the world (Shokes, 1995). The cultivated peanut is thought to be

indigenous to the Rio de la Plata basin as this area contains both wild annuals of *Arachis,* the group *A. villosa,* which shows affinity toward *hypogaea,* and is a center for variability of the sub-species *hypogaea.*

A. hypogaea is divided into the subspecies *hypogaea* and *fastigiata* Waldron. Members of the subspecies *hypogaea* undergo seed dormancy after seed maturity and have alternate branching and a spreading or bunching growth habit. Within this subspecies are two botanical varieties *hypogaea* (virginia and runner market types) and *hirsuta* Köhler (Chinese dragon types or peruvian runner market types). The subspecies *fastigiata* has an erect growth habit, relatively few and curved upright branches, short maturity time, and no seed dormancy (Moss and Rao, 1995). The four botanical varieties encompassed in this subspecies are *fastgiata* (valencia market types), *vulgaris* Harz. (spanish market types), *peruviana* Krapov. and W.C. Gregory, and *aequatoriana* Krapov. and W.C. Gregory (Krapovickas and Gregory, 1994; Moss and Rao, 1995; Shokes, 1995).

Cultivated peanut grows upright to a height of approximately 15-60 cm with a well-defined taproot, many lateral roots and sparse to no hairs on the above ground parts of the plant (Moss and Rao, 1995). Peanut leaves are alternate and compound with four opposing leaflets (2-10 cm long) on each leaf. *Arachis hypogaea* susp. h*ypogaea* var. *hypogaea* and *A. hypogaea* susp. h*ypogaea* var. *hirsuta* have no floral axes on the main stem. Flowers form in leaf axils of lateral branches 4-6 weeks after planting. *Arachis hypogaea* susp. *fastigiata* have floral axes on the main stem and sequential floral axes on lateral branches (Moss and Rao, 1995). Peanut flowers self-pollinate; a week later a carpophore or gynophore (commonly known as a peg) forms. The gynophore is

geotropic and grows to the ground surface. Pods form horizontally to the pegs underground (Shokes, 1995).

Peanut production in the United States and North Carolina. Early explorers from Spain and Portugal found the native peoples of South America cultivating peanuts and helped to disseminate peanut to Europe, Africa, Asia, and the Pacific Islands. Once established in the early trade routes, peanut was brought to the southeastern United States (Hammons, 1982).

In the United States, harvested acreage was estimated at 550,000 ha in 2001 (Brown, 2003). Most of this production is concentrated in the southeast (Georgia, Florida, and Alabama) as well as in the southwest (New Mexico, Oklahoma, and Texas). A significant area of production includes the Virginia-Carolina region, which encompasses southern Virginia, North Carolina, and South Carolina. Production in this region is mainly of virginia-type peanuts. In North Carolina, average production yield for the period 1995-1999 was approximately 160 million kg/year (anonymous, 2003). In 2002, dry weather conditions and tomato spotted wilt virus (TSWV) devastated the crop in the Virginia-Carolina region (Brown, 2003). Harvested acreage in 2002 was down 8,900 ha from that in 2001 and yield was 100 million kg as compared to 161 million kg in 2001 (Brown, 2003). Despite the loss of a quota system for peanut growers due to significant changes that took place to the Farm Bill of 2002, peanut production remained about the same as in 2002 with 40,500 ha planted in 2003 and statewide yield was estimated at 3,360 kg/ha. Peanut production areas may shift within the state over the next few years as the new economic system takes shape in North Carolina.

Section III. Epidemiology of Sclerotinia blight on peanut.

***Sclerotinia minor* as a peanut pathogen.** *Sclerotinia minor* was first identified as a pathogen of peanut in Australia in 1948. By 1960, reports of *S. minor* causing root and pod rot were published in Argentina. The first reports of *S. minor* in the United States were from Virginia in 1971 and North Carolina in 1972 (Porter et al., 1982). Since these early reports in the United States, the disease has become widespread. Losses can be severe in Oklahoma and Texas as well as in Virginia and North Carolina (Porter and Melouk, 1997). In general, peanuts are very susceptible to Sclerotinia blight. *Sclerotinia minor* spreads rapidly within the peanut canopy and sclerotia have great persistence in the soil.

Disease cycle of *S. minor* on the cultivated peanut. The mycelium of *S. minor* attacks lower branches and rapidly invades succulent tissues, causing cells to collapse. Initially, leaves and stems have a water-soaked appearance. As the disease progresses, mycelium often appears as a white, fluffy mass on the surface of colonized tissue. Later, lesions become bleached and necrotic. Eventually, stems are severely shredded and die (Porter et al., 1982). Sclerotia are produced in abundant numbers on and in the dead tissue. Sclerotia may be shed from the plant tissue into the soil or retained on or in dead plant tissue as overwintering inoculum (Porter and Melouk, 1997). Following severe infection of a crop of peanuts, the top 2.5 cm of soil can be heavily infested with sclerotia. At the end of the growing season, sclerotial densities of ca. 50 sclerotia per 100 g of soil are possible (Porter et al., 1982). Some evidence suggests that sclerotia undergo a period of dormancy before germination is possible (Imolehin et al., 1980; Wymore and Lorbeer,

1987). When conditions are favorable, sclerotia can germinate into either mycelium (anamorph stage) or apothecia (teleomorph stage of the fungus). If sclerotia give rise to apothecia, then asci and ascospores (sexual spores) are formed (Porter and Melouk, 1997). Apothecia of *S. minor* are rarely seen in the natural environment, but may be present during February and March or in mid-fall. Carpogenic germination is not epidemiologically important on peanut crops because it occurs during winter fallow in peanut production areas (Porter and Melouk, 1997). More commonly, sclerotia germinate myceliogenically to initiate infections on peanut in mid-to-late summer. Following myceliogenic germination, *S. minor* is capable of infecting without a food base (Porter et al., 1982). Mycelium can infect pods, pegs, leaves, stems, and roots to begin a new disease cycle.

Environmental influences on *S. minor*. Favorable conditions for sclerotial germination and infection by *S. minor* include temperatures of 17-21 C and relative humidities of greater than 95 percent (Porter and Melouk, 1997). In addition, soil pH near 6.5 is favorable for the germination of sclerotia in the field (Porter et al., 1982). Presence of volatile materials from moist undecomposed plant tissue stimulates sclerotial germination (Hau et al., 1982). As reported for *Sclerotium rolfsii,* substances stimulatory to germination include alcohols, aldehydes, esters, and halogenated hydrocarbons (Beute and Rodriguez-Kabana, 1979).

Most of the information about effects of temperature and moisture on *S. minor* is derived from tests done on agar media (Dow et al., 1988, Woodward and Simpson, 1993). Effects of soil temperature and moisture were tested on lettuce isolates from

California in soils that differ markedly from those found in peanut-producing areas (Hao et al., 2003). In those studies, germination was optimal at approximately 15 C in soils with a water content of –0.1 MPa. As was predicted, *S. minor* sclerotia rarely germinated carpogenically.

Production practices greatly influence severity of Sclerotinia blight. Mechanically injured peanut tissue is very susceptible to colonization by *S. minor*. Plants injured by tractor tires during pesticide application showed a greater incidence of Sclerotinia blight than non-injured plants (Porter and Melouk, 1997). Yield losses were about twice as great on injured plants as on non-injured plants (Porter et al., 1982). In addition, frequent applications of the foliar fungicide chlorothalonil enhance Sclerotinia epidemics (Porter et al., 1982; Hau and Beute, 1983).

Resistance to *S. minor* in cultivated peanut. **I**n the Virginia-Carolina region, moderately resistant peanut cultivars are available to growers who must manage areas infested with *S. minor.* In 1994, the partially resistant cultivar VA 93B was the first virginia type cultivar to be registered for use by growers (Coffelt, et al., 1994). This was followed by the release of the partially resistant cultivars VA 98R (Mozingo et al., 2000) and Perry (Isleib et al., 2003). While VA 98R produces higher yields than VA 93B, resistance to Sclerotinia blight is marginally lower (Mozingo et al., 2000). Resistance to Sclerotinia blight in Perry was rated comparable to both VA 93B and VA 98R, but pod size, kernal size, and quality attributes are inferior to the standard cultivar NC 12C (Isleib et al., 2003). Various mechanisms may be responsible for the partial resistance observed in these cultivars. The cultivar VA 93B has a bunch type growth habit, while Perry

favors the runner type growth habit. All of these cultivars have somewhat prostrate lateral branches with an erect main stem (Coffelt et al., 1994; Mozingo et al., 2000; Isleib et al., 2003). This growth habit has been considered an avoidance mechanism for this disease (Coffelt and Porter, 1982; Chappell et al., 1995;), because the microclimate within the canopy is not favorable for disease development and foliage to ground contact is limited (Coffelt et al., 1982; Chappell et al., 1995). Pruning the plant canopy to alter the canopy microclimate also reduced disease incidence (Bailey and Brune, 1997; Butzler et al., 1998). Avoidance mechanisms have also been noted in other crops such as bean and alfalfa (Pratt, 1996; Arahana et al., 2001). Physiological resistance may also be present in cultivars resistant to infection by *S. minor*. Certain plant structural characteristics and defense responses have been noted as important in resistance to this fungus in bean and peanut. These characteristics include phytoalexin induction, waxy cuticles, and thickened cortical cells (Lumsden, 1979; Coffelt et al., 1982; Pratt, 1996).

Screening peanut cultivars for resistance to *S. minor.* The ability to screen for heritable resistance to plant pathogenic fungi is a valuable tool for a plant breeding programs. Cultivars such as VA 93B and VA 98R were not only screened for seed quality traits, but also for resistance to various pathogens including *S. minor* (Coffelt et al., 1994; Mozingo et al., 2000). Germplasm typically done in field plots, under varying levels of disease, over many years to determine the performance of each breeding line (Coffelt et al., 1982). Field screening results can be highly variable due to patchy pathogen distribution and environmental variation. Other methods have been sought in order to expedite the screening process so that many breeding lines can be screened at

one time, in limited amounts of space, and in a short period of time (Brenneman, et al., 1988).

Most alternatives to field trials involve some sort of green house or laboratory experiment (Melouk et al., 1992a; Chappell et al., 1995; Cruickshank et al., 2002). Laboratory screening methods have been attempted with other hosts of *Sclerotinia spp.* In alfalfa, excised leaf tissue of breeding lines were placed on various agar media at the margin of actively growing colonies of *S. trifoliorum*. The leaves were wounded or not and various leaf stages were examined (Pratt and Rowe, 1998). This method proved more efficient and as accurate as previous alfalfa excised leaflet screens in which leaflets were directly inoculated with mycelia of the fungus (Pratt, 1996). Detached leaf have been adapted for use in screening soybean lines for resistance to *S. sclerotiorum* (Arahana et al., 2001; Kim et al., 2000). Correlations between greenhouse/laboratory studies and field studies were identified (Kim et al., 2000). Leaf screens were used to identify putative quantitative trait loci in partially resistant breeding lines of soybean (Arahan et al., 2001).

Similar screening methods have been attempted with peanut. Whole plants were inoculated on the main stem with bean (*Phaseolus vulgais* L.) pods colonized with *S. minor* (Cruickshank et al., 2002). In other tests, cut plant shoots were placed in Hoagland's solution, inoculated with mycelial plugs, and lesion length measured. Low rates of lesion expansion denoted physiological resistance to S. *minor* (Melouk et al., 1992a). Still other tests used detached peanut stems inoculated with mycelial plugs taken from cultures of the fungus (Brenneman et al., 1988). A variation of this system included wounding or not wounding the detached stem and using an oat (*Avena sativa* L.) grain

infested with *S. minor* for the inoculation (Chappell et al., 1995). Lesion development and expansion on detached peanut leaflets placed in moist chambers and inoculated with mycelial plugs taken from actively growing cultures of *S. minor* have also been used in reistance screens (Hollowell et al., 2003b). For all laboratory and greenhouse screening methods, only physiological resistance is identified. Results of laboratory screening were not always predictive of field performance, probably due to avoidance mechanisms seen in the field (Chappell et al., 1995; Cruickshank et al., 2002). Thus, coupling field trials that identify overall levels of resistance with laboratory screens that identify physiological resistance may be the best approach to identifying highly resistant lines. (Chappell et al., 1995).

Weed species as alternative hosts of *S. minor* in peanut fields

A relatively recent area of research involves the identification of *S. minor* as a pathogen of weed species that may be found in or near peanut fields. This is becoming a focus of research as it is believed that the weeds may act as a source of overwintering inoculum of *S. minor*. Weeds that are found in fallow peanut fields and have been shown to be hosts for *S. minor* include: *Lamium aplexicaule* L. (henbit), *Cardamine parviflora* L. (smallflower bittercress), *Stellaria media* (L.) (common chickweed), *Cerastium vulgatum* L. (mouse-ear chickweed), *Coronopus didymus* (L.) (swinecress), *Oenothera laciniata* Hill (cutleaf eveningprimrose), *Conyza canadensis* (L.) (horseweed), *Brassica kaber* (D.C.) L. (wild mustard), and *Arabidopsis thaliana* (L.) Heynhold (mouse-ear cress) (Hollowell et al., 2003a). If such weeds support populations of *S.*

minor, it may be necessary to manage weed populations around peanut fields to reduce sources of primary inoculum.

Management of *S. minor* on the cultivated peanut. The most effective measures currently being used for control of Sclerotinia blight are rotation with non-hosts, planting resistant cultivars, and application of fungicides. Rotation with crops such as corn, cotton, sorghum, and small grains reduces the amount of initial inoculum of *S. minor* as well as other peanut pathogens such as *Cylindrocladium parasiticum* (C.A. Loos) D.K. Bell & Sobers, the causal agent of Cylindrocladium black rot (Shew, 2003). Because available resistance is moderate to low, fungicides such as fluazinam and boscalid often are needed to maintain a healthy crop (Shew, 2003). Minimizing mechanical injury to peanut plants is highly desirable (Porter et al., 1982). The use of the leaf spot fungicide chlorothalonil should be avoided in fields with a history of *S. minor* incidence, as it may enhance pathogenesis by *S. minor* (Hau and Beute, 1983). Soil pH should be considered when planting peanut to maximize yield and reduce the incidence of disease. In addition, fungicide seed treatments are effective in controlling seed transmission of *S. minor* (Bowen et. al., 2000). Possible biocontrol agents include such antagonistic organisms as *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp., *Talaromyces* spp., and *Sporodesmium* spp. (Sherwood et al., 1995). These organisms attack sclerotia of many fungal pathogens, and produce β-1, 3-glucanases and chitinases that can degrade fungal cell walls. The biocontrol agent *Coniothyrium minitans* has been produced for commercial application (Huang et al., 2000). *Coniothyrium minitans* interrupts the life cycle of *Sclerotinia* spp. by parasitizing sclerotia, thereby lowering inoculum density.

Current research suggests that control is inconsistent and may need to be supplemented with host resistance or fungicide use (D.E. Partridge, *personal communication*).

Sclerotinia blight advisories. Implementation of partially resistant cultivars and fungicides effective in controlling Sclerotinia blight have made it possible for growers to grow peanuts under low to moderate disease pressure and obtain average or above average yields. Cultivar choice is limited, however, and fungicides are very costly for growers to apply. Furthermore, fungicides must be applied at crucial periods, taking into consideration prevailing weather conditions, plant growth stage, and the biology of *S. minor* (Shew, 2003). Fungicide applications are typically made on demand, with first fungicide application made at first observation of signs and/or symptoms of Sclerotinia blight, and subsequent applications made as directed by the chemical label (Bailey, 2000). This was standard practice until models based on weather thresholds proved useful in predicting when the fungus would become active (Bailey et al, 1993). Sclerotinia blight advisories were fashioned after the longest running and best documented peanut disease forecasting system, the early leaf spot advisory system (Phipps et al., 1997). In North Carolina, the Sclerotinia blight advisory was based on several computer algorithms that took into consideration environmental thresholds of various parameters (rainfall, air temperature, soil temperature etc.) after canopy closure. Fungicide use based on the algorithms provided control comparable to, and in some cases better than, that obtained with the conventional method (Bailey et al., 1993). Research for a period of 16-yr was also examined in Virginia peanut production regions and confirmed that canopy closure was significant in predicting initial outbreaks of

Sclerotinia blight (Phipps, 1995). The inclusion of rainfall, as well as soil and air temperature in algorithms for disease prediction models was also confirmed. The algorithms have since been modified in Virginia and they now consider a five-day risk index (FDI), which is a sum of the five previous daily risk indices (DRI). The DRI is the product of the environmental index (product of the moisture and soil temperature thresholds) and host index (product of the vine growth and canopy thresholds) (Phipps et al., 1997). Once the FDI is favorable for disease development, a fungicide application is advised and the index is reset to zero for a 2 to 3 week period based on the protection offered by the applied fungicide. After fungicide protection expires, the FDI is monitored and fungicides are reapplied when thresholds are again met (Langston et al., 2002). Evaluations of the FDI algorithm have improved the timing of fungicide application when compared to the traditional method in Virginia peanut production (Langston et al., 2002). A similar Sclerotinia blight advisory has been implemented and is under evaluation by the North Carolina Cooperative Extension Service in the North Carolina peanut production region (Shew, 2003).

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Environmental influences on the epidemiology of Sclerotinia blight of peanut (*Arachis hypogaea* L.)

Abstract

Sclerotinia blight caused by the fungus *Sclerotinia minor* is a serious disease of cultivated peanut (*Arachis hypogaea* L.) in North Carolina. Laboratory and field experiments were conducted to gain a better understanding of how environment affects fungal germination, growth, and spread in soil. This research sought to develop quantitative relationships between disease incidence, severity, environmental factors, and yield loss in order to improve disease forecasting systems in North Carolina. In the laboratory, germination of sclerotia, mycelial expansion, and lesion expansion on detached leaflets were examined on the surface of field soil adjusted to various temperatures and soil matric potentials (Ψ_M). Production of oxalic acid by mycelium of *S. minor* was also examined at various temperatures. In the field, disease incidence was measured at three sites in 2002 and 2003. Weather data were modeled in both years and also were collected using an on-site weather station in 2003. A gradient of disease levels was established by utilizing one partially resistant cultivar, two susceptible cultivars and applications of the fungicides fluazinam and boscalid at various rates. In the laboratory, maximum sclerotial germination occurred at a Ψ_M –7.2 kPa at 30 C. Rate of mycelial expansion and lesion development were maximal at temperatures of 18-22 C. Lesions on inoculated detached leaflets developed slowly at temperatures above 26 C, and failed to develop at temperatures of 29 C or greater. Lesions formed if inoculated leaflets were

moved from 29 C to a cooler temperature of 18 or 22 C. Oxalic acid production at temperatures above 29 C was negligible. In the field, disease incidence was highest on the susceptible cultivars with no fungicide treatment. Disease incidence of the partially resistant cultivar and one susceptible cultivar were used as the dependent variables, while various weather parameters were used as the independent variables in a regression analysis. The following disease prediction model was created: Total Disease = -79.52 + 3.04 mean relative humidity – 0.020 mean relative humidity ^{2} –0.47 mean soil temperature – 5.30 leaf wetness. Our field research marks the first documented report of modeled weather data use in a Sclerotinia blight advisory. Site-specific weather data inputs with no on-site sensors, and a better understanding of factors contributing to germination and growth of the fungus, were useful in the derivation of a disease prediction model for Sclerotinia blight. The model may improve current Sclerotinia blight advisories being evaluated in peanut production areas of North Carolina.

Introduction

Sclerotinia minor was first identified as a pathogen of peanut in Australia in 1948. In the United States, *S. minor* was first identified in Virginia in 1971 and North Carolina in 1972 (Porter et al., 1982). The disease has become widespread in other peanut production areas including Oklahoma and Texas (Porter and Melouk, 1997).

The mycelium of *Sclerotinia minor* attacks all parts of peanut plants and rapidly invades succulent tissues, causing cells to collapse. At the end of the growing season, sclerotial densities of ca. 50 sclerotia per 100 g of soil are possible (Porter et al., 1982). Research on lettuce isolates suggests that sclerotia undergo a period of dormancy before

germination is possible (Imolehin et al., 1980; Wymore and Lorbeer, 1987). When conditions are favorable, sclerotia can germinate into either mycelium or apothecia. Apothecia of *S. minor* are rarely seen in the natural environment, but may be present during February and March or in mid-fall. Carpogenic germination is not epidemiologically important on peanut crops because it occurs during winter fallow in peanut production areas (Porter and Melouk, 1997). More commonly, sclerotia germinate myceliogenically to initiate infections on peanut in mid-to-late summer. Favorable conditions for sclerotial germination and infection include temperatures of 17-21 C and relative humidities of greater than 95 percent (Porter and Melouk, 1997). In addition, soil pH near 6.5 is favorable for the germination of sclerotia in the field (Porter et al., 1982). Presence of volatile materials from moist undecomposed plant tissue stimulate sclerotial germination (Hau et al., 1982). As reported for *Sclerotium rolfsii,* substances stimulatory to germination include alcohols, aldehydes, esters, and halogenated hydrocarbons (Beute and Rodriguez-Kabana, 1979).

Most of the information about effects of temperature and moisture on *S. minor* is derived from tests done on agar media (Dow et al., 1988, Woodward and Simpson, 1993). Work dealing directly with soil temperature and moisture was conducted in California with soils that differ markedly from those found in peanut-producing areas (Hao et al., 2003). In those studies, sclerotial germination of lettuce isolates was optimal at approximately 15 C in soils with a water potential of –0.1 MPa. As was predicted, *S. minor* sclerotia rarely germinated carpogenically.

Mode of pathogenesis in *Sclerotinia spp.* has been studied in great detail (Lumsden, 1979). Pathogenesis of peanut by *S. minor* appears to involve similar

processes (Hau et al., 1982). Anton De Bary showed that *S. sclerotiorum* produces oxalic acid, which is directly toxic to plant cells (Lumsden, 1979). In addition to oxalic acid, pectinases, cellulases, and hemicellulases have been associated with the infection and parasitism of host tissue by *Sclerotinia spp* (Lumsden, 1979). By influencing pH, oxalic acid can act in a synergistic manner with these cell wall degrading enzymes to cause maceration of host tissue. As the pH of the intercellular region decreases due to the formation of oxalic acid by the fungus, the activity of these cell wall degrading enzymes increases, resulting in the formation of larger lesions (Marciano et al., 1983). At higher pH levels, the effectiveness of cell wall degrading enzymes is inhibited.

Control of Sclerotinia blight requires an integrated approach that includes rotation with non-hosts (such as corn, cotton, sorghum, and small grains), planting resistant cultivars, and applications of fungicides. Currently, moderately resistant peanut cultivars include VA 98R (Mozingo et al., 2000) and Perry (Isleib et al., 2003). Because resistance in these cultivars is moderate to low, fungicides such as fluazinam and boscalid often are needed to maintain a healthy crop (Shew, 2003).

These practices have made it possible to grow peanuts under low to moderate disease pressure and still obtain average to above average yields. Cultivar choice is limited though, and fungicides are very costly for growers to apply. Furthermore, fungicides must be applied at crucial periods, taking into consideration prevailing weather conditions, plant growth stage, and the biology of *S. minor* (Langston et al., 2002; Phipps et al. 1997). Fungicide applications are typically made on demand, with the first fungicide application made at first observation of signs and/or symptoms of Sclerotinia blight and subsequent applications made as directed by the chemical label

(Bailey, 2000). This was standard practice until models based on weather thresholds proved useful in predicting when the fungus would become active (Bailey et al., 1993). Sclerotinia blight advisories were fashioned after the widely adopted early leaf spot advisory system (Phipps et al., 1997). In North Carolina, the Sclerotinia blight advisory was based on several computer algorithms that took into consideration environmental thresholds of multiple parameters (rainfall, air temperature, soil temperature etc.) after canopy closure. Fungicide use based on the algorithms provided control comparable to, and in some cases better than, that obtained with the conventional method (Bailey, et al., 1993). Research for a period of 16 years was also examined in Virginia peanut production regions and confirmed that canopy closure was significant in predicting initial outbreaks of Sclerotinia blight (Phipps, 1995). Rainfall, soil temperature, and air temperature were included in algorithms for disease prediction models. The algorithms have since been modified in Virginia and use a five-day risk index (FDI), which is a sum of the five previous daily risk indices (DRI). The DRI is the product of the environmental index (product of the moisture and soil temperature thresholds) and host index (product of the vine growth and canopy thresholds) (Phipps et al., 1997). Once the FDI is favorable for disease development, a fungicide application is advised and the index is reset to zero for a 2 to 3 week period based on the protection offered by the applied fungicide. After fungicide protection fades, the FDI is monitored and fungicides are reapplied when thresholds are again met (Langston et al., 2002). Evaluations of the FDI algorithm have improved the timing of fungicide application when compared to the traditional method in Virginia peanut production (Langston et al., 2002). A similar

Sclerotinia blight advisory has been implemented and is under evaluation by the North Carolina Cooperative Extension Service in the North Carolina peanut production region.

The objective of this project was to determine how selected environmental parameters affect fungal germination, growth, and spread in soil so that quantitative relationships could be defined between disease incidence, severity, environmental factors, and yield loss. Results will be used to improve disease forecasting systems for Sclerotinia blight in North Carolina.

Materials and Methods

Soil release curve. Soil from a field used for growing peanut was collected from the Upper Coastal Plains Research Station in Rocky Mount, North Carolina. The soil was a Norfolk loamy sand. The soil was air-dried, ground, passed through a sieve (No. 10, 2-mm openings). A 15 bar ceramic soil pressure plate (Soil Moisture Equipment Corporation, Santa Barbara, CA) was used to determine the water holding capacity of the soil at four matric potentials (Ψ_M): 30 kPa, 100 kPa, 300 kPa, and 1500 kPa. For each Ψ_M measured, five Plexiglas® rings filled with soil were placed on the appropriate saturated ceramic plate and equilibrated to the desired Ψ_M for 24 hours. Soil disks were removed from the apparatus and wet weight was obtained. Soil was dried at 105 C for 48 hours, and dry weight determined. Soil water content at each Ψ_M was determined and fitted to a moisture release curve. To determine the water holding capacity at Ψ_M of 0 kPa, 1 kPa, 5 kPa, and 10 kPa, soil was placed in 150 ml fritted-glass Büchner funnels. A water column was established at the proper distance below the funnel to obtain the desired Ψ_M. Soil was equilibrated to the desired Ψ_M for 24 hours. Soil was removed from the

apparatus and the water content was determined in the same manner as previously described. Using the soil moisture release curve, water content was adjusted to obtain three Ψ_M (-7.2 kPa, -10 kPa, -100 kPa) for use in experiements. The Ψ_M of -7.2 kPa represented of a Ψ_M near saturation, while -10 kPa represented a Ψ_M at field capacity, and -100 kPa represented a Ψ_M where water stress occurred in the sandy soil. For each $\Psi_{M,}$ 800g of soil was placed in plastic bags, the appropriate volume of water was added, and the soil was allowed to equilibrate for 24 hr. Once the soil was adjusted to the desired water content, it was placed in jars (6 cm diameter, 7 cm height) to a depth of approximately 2 cm.

Sclerotial Germination. Sclerotia of an aggressive isolate of *S. minor* (Shew Laboratory collection # 13) were cultured on sterile oat grains (Chappell, 1992) and stored at 4 C until harvest. Sclerotia > 1-mm and < 2-mm were surface disinfested (5% sodium hypochlorite solution), dried in a laminar flow hood, and placed in jars (four sclerotia per jar) on the surface of the soils adjusted to the three $\Psi_{M.}$ Lids from 6-cm-diam petri dishes were placed over the openings of the jars and sealed with paraffin film. Jars were placed in incubators at temperatures of 18 C, 22 C, 26 C, and 30 C and incubated under complete darkness for 10 days.

Mycelial Expansion. Mycelium from isolate #13 of *S. minor* was grown on one-half strength potato dextrose agar (PDA) (Difco, Detroit MI). The agar medium was made by adding 19.5g of dehydrated PDA to 1 L de-ionized water, plus 7.5g of bacto-agar (Difco) and then autoclaved. Two mycelial plugs (5-mm-diam) were taken from two-day-old

cultures and placed on soil adjusted to the three $\Psi_{M.}$ Jars were sealed and placed in incubators as described previously and colony diameter measured daily for seven days.

Leaflet Lesion Expansion. Leaflets of the second fully expanded leaf on the main stem were detached from three peanut lines. The lines included NC 12C (susceptible cultivar), Perry (moderately resistant cultivar), and N96076L (resistant breeding line). Leaflets were placed individually in jars containing soil at the three water potentials. The abaxial side was touching the soil surface. The adaxial side was inoculated with a mycelial plug (5 mm diam) from a two-day-old culture of *S. minor* isolate #13 grown on half-strength PDA. Jars were sealed and placed in incubators at temperatures of 18 C, 22 C, 26 C, and 30 C in the dark. Lesion length along the mid-rib was measured daily for seven days.

High temperature threshold for leaflet lesion expansion. Leaflets were detached from fully expanded leaves, placed in jars, and inoculated in the same manner as the previous procedure. The jars were placed in incubators at temperatures of 26 C, 27 C, 28 C, and 29 C in the dark. Lesion length along the mid-rib was measured daily for seven days.

Effect of temperature change on leaflet lesion expansion. Leaflets were detached, placed in jars, and inoculated using the same procedure as above. The jars were arranged in incubators at either 28 C or 29 C. After two days, half of the jars from each temperature were placed in an incubator at 18 C, while the remaining jars were placed in an incubator at 22 C. Lesion length along the mid-rib was measured for six days after inoculation.

Experimental design. Three jars (observations) were included for each combination of matric potential and temperature. The experimental design was a split-plot with temperature treatments as whole plots and moisture treatments as sub-plots. Five replicates were conducted over time.

Oxalic acid studies. Potato dextrose broth (PDB) (Difco) was prepared by adding 24g of dehydrated broth to 1 L de-ionized water. Broth was then transferred to 200 ml flasks in 25-ml aliquots, and flasks were plugged with a foam plug. The autoclaved flasks were allowed to cool, and one 5-mm agar plug from a two-day-old culture of *S. minor* (isolate #13) was placed in each flask. Flasks were then placed in incubators at temperatures of 26 C, 27 C, 28 C, and 29 C. Cultures were allowed to incubate under complete darkness for three days, when mycelial mats were removed from the flasks, weighed, dried at 35 C for two days, and reweighed. A kit manufactured for enzymatic determination of oxalate in urine (Sigma Diagnostics) was used to measure oxalic acid production. A 2-ml sample was taken from each flask and mixed in a polystyrene tube with 3 ml of sterile de-ionized water. A 5-ml aliquot of the sample diluent supplied with the kit was added to each tube. Each sample solution was adjusted to a pH between 6 and 7 with 1 N hydrochloric acid or 1 N sodium hydroxide. The initial pH and final pH of each sample were recorded. Oxalate reagent A (1-ml aliquot; supplied with the oxalic oxalate kit) was transferred to an Eppendorf tube (one tube for each sample). To each Eppendorf tube, 50 µl fractions of each sample were added. Finally, 100 µl of oxalate reagent B (supplied with the oxalic oxalate kit) was added to each tube. Standard oxalate reagents in the concentrations of

0.25 mmol, 0.5 mmol, and 1.0 mmol were mixed according to the instructions included with the oxalic oxalate kit. The total volume of the resulting sample solutions in each Eppendorf tube was transferred to cuvettes (one for each sample). The light absorbance of each sample was recorded by a light spectrophotometer (λ=590 nm). Three flasks (observations) were included for each temperature. Four replicates were conducted over time.

Field Studies. Studies were conducted in the 2002 and 2003 growing seasons in different 0.40 ha fields located in Perquimans County, NC. Planting dates were 15 May 2002, and 20 May 2003. Plots consisted of four 91-cm rows and were 7.6 m long and 4 rows wide with row spacing of 0.9 m. Planted borders between plots were 3 m long. Plots were planted with NC 12C or Perry at a seed spacing of 6 cm. Standard production practices for peanuts in North Carolina, including application of fungicides for foliar disease control, were followed throughout the growing season (Shew, 2003). Fluazinam was applied at three rates to establish different levels of disease within the field. Sprays treatments included a full rate (0.87 kg ai/ha), half rate (0.43 kg ai/ha), and no fungicide. The experimental design was six replicated split-plots with cultivars as whole plots and spray applications as subplots. Weekly ratings began on 12 Jul 2002 and continued for 12 wk. Weekly ratings began on 21 Jul 2003 and continued for 9 wk. In both years the center two rows for each plot were parted by hand and plants with new signs and/or symptoms of *S. minor* were marked with surveyor's flags. Weather data were modeled hourly in both years using the Skybit service by ZedX, Inc. (Bellafonte, PA). In 2003 an onsite micrologger weather station (CR10X, Campbell Scientific, Logan, UT, 84321-

1784) also was used to collect weather data. Leaf wetness sensors (Campbell Scientific Inc., Logan, UT, 84321-1784) were placed at 42 cm height, near the top of the mature canopy. The 15 unpainted sensors were placed in five canopy positions corresponding to top, middle left side (north), middle right side (south), middle and bottom of the canopy. Each sensor position was replicated three times. Rain was measured with a tipping bucket rain gauge (TE525, Texas Electronics, Dallas, TX 75235-7309). Wind speed, solar and net radiation were measured at the 3-meter level with a wind set (Wind Sentry, R.M. Young, Traverse City, MI, 49684), Li 200x pyranometer (Li 200x, Li-Cor, Lincoln, NE, 68504) and a net radiometer (Q-7.1, REBS, Seattle, WA, 98115-0152). Net radiation was also collected just below canopy level allowing canopy net radiation to be calculated from the difference of the two heights. Air temperature and relative humidity were measured with four sensors (HMP35C, Campbell Scientific, Logan, UT, 84321-1784) 1 meter above the canopy, at the top of the canopy, mid canopy height and at the bottom of the canopy. The average of the bottom three of these sensors was used as input into the model. Soil temperature sensors were placed at depths of 2.5 cm and 20 cm. Soil flux sensors were placed at a depth of 5-cm. Peanuts were dug on 4 Oct and harvested on 11 Oct 2002, and on 9 and 20 Oct 2003. Samples were dried and yields and market quality were determined.

A third field site in Chowan County, NC with a history of Sclerotinia blight was also included in this research in 2003. Plots consisted of four 91-cm rows, 15.25 m long, with 3 m alleys between plots. The cultivar planted was NC-V 11. The experimental design was a randomized complete block consisting of six replicates. Peanuts were planted on 20 May with a two-row tractor mounted planter set to dispense a minimum of

five seeds per ft. Temik 15G at 7.4 kg/ha was applied in-furrow at planting for insect control. Standard production practices for peanuts in NC, including application of fungicides for foliar disease control, were followed throughout the growing season (Shew, 2003). The six treatments consisted of calendar or advisory treatments of boscalid (Endura; BASF) at 0.45 kg ai/ha; fluazinam (Omega 500F; Syngenta Crop Protection, Inc.) at 0.87 kg ai/ha; advisory treatments only of boscalid at 0.32 kg ai/ha; and an untreated control. Calendar spray treatments were planned for 60, 80, and 100 days after planting (DAP) for boscalid at 0.45 kg/ha and 60 and 90 DAP for fluazinam. First actual calendar spray treatments were applied on 21 Jul, 62 DAP. On 8 Aug disease was observed and all advisory treatments were applied, as well as the 80 DAP calendar treatment of boscalid at 0.45 kg ai/ha. The scheduled 90 DAP fluazinam treatment was applied on 19 Aug. The 100 DAP boscalid treatment at 0.45 kg ai/ha and the remaining advisory treatments were applied on 2 Sep (105 DAP). Weekly disease ratings were taken on the two center rows of each plot beginning on 4 Aug and continued for 10 weeks. Weather data were modeled as in the previous sites. Peanuts were dug on 9 Oct and combined on 20 Oct. Samples were dried and yields and market quality were determined.

Weather Data Analysis. Modeled weather data were obtained for all sites in all years from ZedX, Inc. Data for the 2003 Perquimans County site were taken from the micrologger and any missing data were supplemented with modeled data. All hourly data were then pared to daily averages for each parameter. The parameters included: air temperature, leaf wetness, precipitation, relative humidity, soil moisture, and soil

temperature. The daily means, maximums, and minimums of each of those parameters were calculated, with the exception of soil temperature and soil moisture for which only means were calculated. Two additional parameters were also calculated. They included: 1) mean air temperature during leaf wetness; 2) hours that leaves were wet. The 5-da moving averages for the following parameters were calculated using SAS statements: mean, maximum, and minimum air temperature; mean, maximum, and minimum leaf wetness; temperature during leaf wetness; leaf wetness hours; total, mean, and maximum precipitation; mean, maximum, and minimum relative humidity; soil temperature and moisture. Results of principal components analysis of the moving averages were used to select parameters to input in a stepwise regression analysis with incremental disease incidence as the dependent variable. These data were from the no fungicide/Perry treatments at sites one and two, and the no fungicide treatment at site three. A general linear model procedure (PROC GLM) was then used to test the need for quadratic and interaction terms. The resulting equation was used to predict disease incidence at both sites, for all observations in 2002 and 2003 and, was graphed along with actual disease incidence for visual comparison.

Results

Soil release curve. The Norfolk loamy sand used for the laboratory studies produced a steep moisture release curve. Water content ranged from approximately 40% at 0 kPa to 5% at –300 kPa (Fig. 1.1). Due to histeresis, the wetting curve used to determine a desired Ψ_M varied slightly among replicates and from the drying moisture release curve.

Sclerotial Germination. Soil temperature, Ψ_M, and their interaction significantly affected sclerotial germination after 10 days of incubation (Table 1.1). Germination was highly suppressed at –100 kPa, and completely inhibited at 26 C and –100 kPa. Sclerotia germinated at all other temperature and Ψ_M combinations. Maximum germination was recorded at 30 C and Ψ_M of –7.2 kPa (Fig. 1.2). Under these conditions, 90% of sclerotia germinated. Germination decreased with decreasing temperature at Ψ_M of –7.2 kPa and –10kPa (Fig. 1.2).

Mycelial expansion. Soil temperature and Ψ_M significantly affected mycelial expansion (Table 1.1). Mycelial growth was greatest at 18 C to 22 C (Fig 1.3). Temeprature effects were consistent across Ψ_M and colony diameters decreased with decreasing Ψ_M (Fig. 1.4).

Leaflet Lesion Expansion. The main effect of soil temperature on leaflet lesion size was highly significant, but effects of Ψ_M were not significant at $P \leq 0.5$ (Table 1.1). Leaflet lesion size was greatest at 18 C and 22 C (Fig. 1.5). Lesion development was slowed somewhat at 26 C (Fig. 1.5). At 30 C, no lesions developed, but mycelial growth was observed on the surface of the adaxial side of the leaflet. Leaflet lesions developed at similar rates at all Ψ_M tested (Fig. 1.6).

High temperature threshold for leaflet lesion expansion. As in the leaflet lesion-expansion study, soil temperatures of 26 C – 29 C significantly affected lesion development, but Ψ_M and temperature by Ψ_M interaction were not significant (Table 1.1).

Lesions formed at 26, 27, and 28 C, but not at 29 C. As at 30 C in the leaflet lesion expansion study, no lesions developed at 29 C (Fig. 1.7).

Effect of temperature change on leaflet lesion expansion. Initial temperature of incubation significantly affected lesion development, whereas final temperature and interaction effects were not significant (Table 1.2). Lesion development did not occur at 29 C, but when the inoculated leaflets were moved to temperatures of 18 C or 22 C, lesions formed. Mycelial growth on the surface of the leaflet was again observed at 29 C as in previous experiments. Mycelial growth and lesion expansion occurred at 28 C and accelerated when leaflets were moved to final temperatures of 18 C or 22 C (Fig. 1.8).

Oxalic acid studies. Temperature significantly affected the ability of *S. minor* to produce oxalic acid ($P<0.0001$). As temperature increased from 26 C to 29 C, oxalic acid production decreased. At 26 C, 4.19 ± 0.50 mmol of oxalic acid were detected by the assay, whereas $0.22 \pm .07$ mmol of oxalic acid were detected at 29 C (Fig. 1.9).

Field Studies.

Site one (2002) and site two (2003). Weather in 2002 was much dryer and warmer than in 2003. Disease was first observed on 12 July 2002 and on 21 July in 2003. Incremental increases in disease incidence in 2002 were small until week nine (6 Sep) when disease began to increase rapidly until harvest (Fig. 1.10). In contrast, incremental increases in disease incidence were observed consistently throughout the entire 2003 season (Fig. 1.11). Hurricane Isabel ravaged the field on 17 September 2003 and no rating was

possible after week nine. Cumulative disease incidence on the final rating date ranged from 39% to 82% in 2002 and from 9% to 65% in 2003 (Figs. 1.12 and 1.13).

In both years, fungicide effects were highly significant. Plots treated with the full rate (0.87 kg ai/ha) of fluazinam had the lowest total disease; the half rate (0.43 kg ai/ha) also reduced disease compared to the untreated control. The untreated check had the greatest total disease in both years (Figs. 1.14 and1.15). The cultivar effect was significant in 2002, with Perry plots having lower total disease incidence than NC 12C (Fig. 1.14). A similar trend was observed in 2003, but the cultivar effect was not significant (Fig. 1.15). Cultivar by treatment interactions were not significant in either year (Figs. 1.14 and 1.15).

Fungicide treatments also affected yield. Yields were greatest in the full rate treatment, while the half spray treatments and untreated checks yielded nearly the same (Fig. 1.16). Cultivar and interaction effects where not significant in 2002. Fungicide and cultivar main effects were both significant in 2003. As in 2002, the full spray treatment yielded the highest with the half spray treatment not significantly different from the full spray treatment. Yield in the untreated check was significantly lower than the other treatments. The moderately resistant cultivar Perry yielded higher than the susceptible cultivar NC 12C across all treatment combinations (Fig. 1.17).

Site Three. Modeled weather conditions at site three in 2003 where very similar to those of site two in 2003 (Appendix I). Incremental increases in disease incidence were relatively small until week seven (15 Sep) when disease began to increase rapidly as at sites one and two (Figs. 1.18 and 1.19). Fluazinam treatments and the two boscalid

treatments at 0.45 kg ai/ha did not differ significantly from each other, and offered significant control of Sclerotinia blight in the field (Fig. 1.20). The boscalid treatment at 0.32 kg ai/ha did not control *S. minor* as well as other treatments, but offered significantly better control than the untreated check (Fig. 1.20). All fungicide treatments yielded better than the untreated check but only the fluazinam treatments were significant (Fig. 1.21).

Weather Data Analysis. Four principal components accounted for 84.6% of the variability in the data set (Table 1.3). The first principal component accounted for 30.5% of the variability. Variables with high loadings on this component were associated with temperature, including mean, maximum, and minimum air temperature and mean soil temperature. Mean leaf wetness (negative), maximum leaf wetness (negative) and minimum leaf wetness (positive) also had high loadings on this component. The second principal component accounted for an additional 23% of variability in the data and was strongly associated with precipitation and temperature variables. Soil moisture, minimum relative humidity, and wet hours had high loadings on the third principal component, which accounted for 16.5% of the variability in the data. The fourth principal component, accounting for 14.6% of the variability, was associated with mean and maximum relative humidity, and maximum precipitation (negative). No other component accounted for more than 5% of the variability in the data. Based on the results of the principal components analysis, the following six variables were selected for stepwise regression: mean soil temperature, mean air temperature, mean relative humidity, mean precipitation, mean leaf wetness, and soil moisture. The backwards elimination option in the

STEPWISE procedure of SAS was used to perform the analysis. The analysis yielded three parameters: mean relative humidity (RH), mean soil temperature (ST), and mean leaf wetness (LW). The GLM procedure of SAS was used to test each term and interactions to identify the following model: Total Disease=[-79.52 + 3.04RH – 0.02RH^2 –0.47ST – 5.30LW]; P=0.0006, R^2=0.51, CV=52.92 (Table 1.4 and Fig. 1.22).

Discussion

Optimal sclerotial germination at 30 C and Ψ_M of –7.2 kPa differed from reports in the literature. In a recent study of sclerotia from isolates of *S. minor* from *Lactuca spp*. in California, optimal temperature and Ψ_M were 15 C and -100 kPa (Hao et al., 2003). The soils used in that study were sandy loams and differ from soils used in the southeastern United States for peanut production. In addition, we provided a stimulant to germination (acetone), which resulted in up to 90% gerimation by 10 days of incubation. In the lettuce isolate research, germination in the absence of a stimuant took up to 12 weeks. The discrepancy in optimal germination conditions could also be explained by a difference in ecotypes of the same fungus. Ecotypes may have originated from adaptations that have occurred due to differences in environments and available host species.

Optimal temperature for colony growth, and parasitism of peanut leaflets on the surface of soil was 18 C and 22. Other studies using peanut isolates of *S. minor* found that temperatures within, or slightly above this range, were optimal for mycelial growth and sclerotial formation on various agar media (Dow et al., 1988; Woodward and Simpson, 1993). No consistent Ψ_M effect was observed in our studies. This may reflect

similarities in humidity above the soil surface rather than differences in Ψ_M. Relative humidities of ≥95% have been shown to significantly affect germination of sclerotia in the laboratory (Dow et al., 1988). Furthermore, the largest numbers of viable, infectious sclerotia are found in the top 2-cm of the plow layer of natural soil (Adams, 1986). Sclerotia in this layer may be completely covered or partially exposed to the atmosphere. In our studies, sclerotia were partially exposed; therefore, the atmosphere immediately above the soil most certainly influenced germination of sclerotia. The soil contained in the jars provided enough moisture to the atmosphere to meet or exceed the relative humidity of 95% or higher. Therefore, our observations of Ψ_M effects may actually represent the affects of atmospheric relative humidity.

As temperature increased above 22 C, colony growth on the surface of soils, as well as lesion development and parasitism of leaflets, was minimal at all Ψ_M. Lesion development on leaflets did not occur at 30 C. When inoculated leaflets were moved from a high temperature (29 C) to a low temperature (18 C or 22C), lesions developed at the lower temperature. This indicates that the fungus was not killed at extreme temperatures but was merely rendered non-pathogenic. This differs from observations of Woodard and Simpson (1993) who reported that growth at 30 C was limited and peaked at 24-26 C. This difference is most likely due to growth on media versus growth on peanut leaflets. Large amounts of nutrients are readily available on agar media. In contrast, the fungus must produce oxalic acid and cell wall degrading enzymes to break down complex tissues in peanut leaflets. We showed that oxalic acid production also was minimal at 29 C. As temperature decreased from 29 C to 26 C, oxalic acid production

increased markedly. The lack of lesion development and parasitism of leaflets at high temperatures could be linked to the lack of oxalic acid production at those temperatures.

In field studies, disease incidence was lower when a fungicide was applied. Yield was affected at all sites, with significant increases in yield coinciding with fungicide application. While not offering a significant increase in yield at site one, the management strategy utilizing a combination of partially resistant cultivars (such as Perry) and application of a fungicide reduced disease incidence and increased yield. This is consistent with Lemay et al. (2002), in which the combination of partially resistant cultivars and fungicide resulted in the lowest levels of disease. Some physiological resistance may have accounted for this, but the upright, open canopy stature of these plants may have promoted better fungicide coverage and improved protection against fungal infection as in previous studies (Lemay et al., 2002). At sites one (2002) and two (2003) the half rate treatment of fluazinam produced nearly the same level of disease and yield as the full rate. This suggests that a reduced rate fungicide treatment may be beneficial in controlling Sclerotinia blight. Reduced fungicide use translates to reduced cost as well as a reduction in effects on other organisms. Using a reduced rate of fluazinam may have a negative effect on resistance management, however, by increasing populations of resistant individuals of *S. minor.*

At site three, the fungicide boscalid gave control of *S. minor* similar to the fungicide fluazinam. Reduced rates of boscalid did not control disease incidence as well as the full rate treatments of both fungicides. Yields were significantly larger with the use of fluazinam. Fluazinam may only be applied twice a season at full rate, with a third application at a reduced rate. In addition, fluazinam has a 30-day pre-harvest interval

(PHI). Boscalid on the other hand, may be applied three times a season at the full rate with a 14-day PHI. The epidemic at site three increased drastically 136 DAP. This was 14 days before the peanuts were dug. At sites one and two, the same late season increase in disease was noted 3-4 weeks before digging the peanuts. Late season protection against infection by *S. minor* may be critical 2-4 weeks before digging to limit the effects of diseae increase during this period. Final applications of boscalid appear to control the epidemic late at this critical time in the growing season, while fluazinam protection was not nearly as good. Boscalid may be preferred as a late season application in order to provide an acceptable level of control without delaying harvest. Based on yields, efficacy, and persistence, fluazinam would be a better early season fungicide. At site three, new weekly infections were lower for fluazinam treatments until 122 DAP. After this point, marginally improved control of new infections was observed with the 0.45 kg ai/ha treatments of boscalid. The use of partially resistant cultivars in combination with a fungicide program that include early season applications of fluazinam and late season applications of boscalid would be an adequate program for growing peanuts in an area with low to moderate disease pressure without increasing the resistant population of *S. minor.*

Total disease in the field was higher in 2002 (site one) than in 2003 (sites two and three). Incremental disease incidence was much smaller at site one than at sites two and three. Weather conditions were more favorable for disease throughout the season at sites two and three than at site one. Air and soil temperature were higher at site one than at sites two and three. Temperature and humidity were closer to the optima for growth of *S. minor* at sites two and three, than at site one based on observations by Dow et al. (1988)

and results from our laboratory studies. Correlations made between disease incidence, severity, environmental factors, and yield loss at all sites were consistent with correlations that have been used to develop currently implemented Sclerotinia blight advisories. The studies at all sites in this test confirm that relative humidity and soil temperature contribute significantly to the disease prediction model. Leaf wetness should also be considered for inclusion into the model as it made a significant contribution to disease development based on the data from all sites tested in the 2002 and 2003 growing seasons. Sclerotial germination may also have some relevance in a prediction model. Sclerotial germination studies in the laboratory suggest that germination is inhibited at low temperatures. Therefore, the soil temperature parameter in a Sclerotinia blight advisory should account for lack of germination at reduced temperatures.

Since the implementation of Sclerotinia blight advisories, the algorithms have been modified in Virginia and now consider a five-day risk index (FDI), which is a sum of the five previous daily risk indices (DRI). The DRI is the product of the environmental index (product of the moisture and soil temperature thresholds) and host index (product of the vine growth and canopy thresholds) (Phipps et al., 1997). Once the FDI is favorable for disease development, a fungicide application is advised and the index is reset to zero for a 2 to 3 week period based on the protection offered by the applied fungicide. After fungicide protection fades, the FDI is monitored and fungicides are reapplied when thresholds are again met (Langston et al., 2002). Evaluations of the FDI algorithm have improved the timing of fungicide application when compared to the traditional method in Virginia peanut production (Langston et al., 2002). Use of the FDI in 2002 and 2003 for these studies confirmed that the FDI was still valid for use in

Sclerotinia blight advisories. An advisory similar to that used in Virginia for Sclerotinia blight has been implemented and is under evaluation by the North Carolina Cooperative Extension Service in the North Carolina peanut production region (Shew, 2003).

Our Sclerotinia blight advisory evaluations can improve this forecasting system. Our field research marks the first documented report of modeled weather data use in a Sclerotinia blight advisory. Site-specific weather data inputs with no on-site sensors can be useful in disease prediction models of this sort (Magarey et al., 2001). This offers several advantages. There is no equipment that has to be placed in the field and maintained. Growers do not have to avoid large onsite weather stations with field equipment. Furthermore, the parameters that are utilized are easy to measure and can be manipulated using typical computer software.

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Table 1.1. Results of analyses of variance of soil temperature and moisture effects on germination, growth, and infection of peanut leaflets by *Sclerotinia minor*

Source[a]	**Sclerotial germination (%)**[b]		**Mycelial expansion (mm)**[c]		**Leaflet lesion expansion at 18, 22, 26, and 30 C (mm)**[d]		**Leaflet lesion expansion at 26, 27, 28, 29 C (mm)**[e]	
	F value	**Pr>F**	**F value**	**Pr>F**	**F value**	**Pr>F**	**F value**	**Pr>F**
Temperature (T)	4.49	0.0344	55.97	<0.0001	716.71	<0.0001	73.21	<0.0001
Soil matric potential (Ψ_M)	62.75	<0.0001	3.85	0.0243	2.49	0.0869	0.37	0.6892
T X Ψ_M	4.92	0.0002	0.83	0.5482	0.90	0.4946	0.78	0.5873
	R^2=0.73	C.V.=72.90	R^2=0.69	C.V.=36.44	R^2=0.93	C.V.=19.06	R^2=0.82	C.V.=44.39

[a] All statistics calculated from final observations for all time course studies.

[b] Percentage of sclerotia that germinated after 10 days of incubation at Ψ_M of –7.2 kPa, -10 kPa, –100 kPa and soil temperatures of 18, 22, 26, and 30 C.

[c] Diameter of mycelial mat measured daily for seven days at Ψ_M of –7.2 kPa, -10 kPa, –100 kPa and soil temperatures of 18, 22, 26, and 30 C.

[d] Length of leaflet lesions measured daily for seven days at Ψ_M of –7.2 kPa, -10 kPa, –100 kPa and soil temperatures of 18, 22, 26, and 30 C.

[e] Length of leaflet lesions measured daily for seven days at Ψ_M of –7.2 kPa, -10 kPa, –100 kPa and soil temperatures of 26, 27, 28 and 29 C.

Table 1.2. Results of analyses of variance of soil temperature effects on peanut leaflet infection by *Sclerotinia minor*

Source[a]	Leaflet lesion expansion at 29/22, 29/18, 28/22, and 28/18 C	
	F value	**Pr>F**
Initial Temperature (InT)	42.0	0.0029
Final Temperature (FT)	0.80	0.4214
InT X FT	1.95	0.2355
	R^2=0.75	C.V.=40.66

[a] All statistics calculated for final observations only for all time course studies.

Table 1.3. Principal components analysis of weather parameters collected or estimated for site one in 2002 and sites two and three in 2003

Parameter[a]	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
Mean air temp (C)	0.31174	0.33994	-0.03464	0.07967	0.11543	-0.04853	0.01796
Mean LW (mm)	-0.31549	0.17589	-0.07917	0.28325	-0.13096	-0.38255	0.08103
Mean prec. (mm)	-0.21665	0.31443	0.23887	-0.26898	0.14988	0.01271	-0.10796
Total prec. (mm)	-0.21668	0.31265	0.24333	-0.26341	0.16008	0.00903	-0.10433
Mean wet temp. (C)	0.19580	0.30292	-0.11794	0.18953	-0.49410	0.15355	0.20030
Mean RH (%)	-0.15659	0.03063	0.28450	0.46334	0.21452	0.05747	-0.22788
Mean soil temp. (C)	0.34303	0.28517	-0.01926	0.10571	0.10847	-0.15659	-0.14739
Max. air temp. (C)	0.27081	0.34349	-0.15333	0.05248	0.20069	0.15867	0.15454
Max. LW (mm)	-0.34152	0.19518	-0.16444	0.20074	-0.13595	-0.15721	-0.02847
Max prec. (mm)	-0.20852	0.32591	0.14102	-0.31123	0.08442	0.06702	-0.10190
Max. RH (%)	-0.01210	-0.13171	0.12109	0.49754	0.51904	0.19812	0.00447
Min. air temp. (C)	0.27773	0.34556	0.09390	0.11990	0.03128	-0.22844	-0.14644
Min. LW (mm)	0.31993	-0.10075	0.32699	-0.10797	-0.00247	0.23525	0.23791
Min. RH (%)	0.10944	-0.04525	0.45611	0.05155	-0.38856	0.27516	-0.42121
Sum wet temp. (C)	-0.28144	0.23094	-0.03197	0.05584	0.04617	0.53636	0.49264
wet hours (h)	-0.12034	0.08333	0.43221	0.27974	-0.33551	-0.07272	0.22077
Mean soil moist (%)	0.10058	-0.10651	0.42936	-0.11129	0.13255	-0.47939	0.52422
Eigenvalue	5.18921	3.91738	2.79602	2.47273	0.81866	0.77034	0.44440
Proportion	0.30520	0.23040	0.16450	0.14550	0.04820	0.04530	0.02610
Cumulative	0.30520	0.53570	0.70020	0.84560	0.89380	0.93910	0.96520

[a] Data analyzed were moving 5-da averages of the variables indicated. Disease incidence data were taken from plots planted with the cultivar Perry and no fungicide application.

Table 1.4. Output from SAS PROC GLM regression of three environmental parameters on disease incidence

The GLM Procedure

Dependent Variable: Disease Incidence

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	235.5783061	58.8945765	6.94	0.0006
Error	26	220.7247149	8.4894121		
Corrected Total	30	456.3030210			

R-Square	Coeff Var	Root MSE	Disease Incidence Mean
0.516276	52.92425	2.913660	5.505339

Source	DF	Type I SS	Mean Square	F Value	Pr > F
MeanRH	1	19.2891281	19.2891281	2.27	0.1438
MeanRH*MeanRH	1	64.7721807	64.7721807	7.63	0.0104
soiltemp	1	113.0288858	113.0288858	13.31	0.0012
MeanLW	1	38.4881116	38.4881116	4.53	0.0429

Source	DF	Type III SS	Mean Square	F Value	Pr > F
MeanRH	1	38.6528880	38.6528880	4.55	0.0425
MeanRH*MeanRH	1	34.5878063	34.5878063	4.07	0.0540
soiltemp	1	140.0537320	140.0537320	16.50	0.0004
MeanLW	1	38.4881116	38.4881116	4.53	0.0429

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-79.52013778	56.68445082	-1.40	0.1725
MeanRH	3.04432364	1.42671969	2.13	0.0425
MeanRH*MeanRH	-0.01860658	0.00921815	-2.02	0.0540
soiltemp	-0.47304894	0.11646557	-4.06	0.0004
MeanLW	-5.29740280	2.48793198	-2.13	0.0429

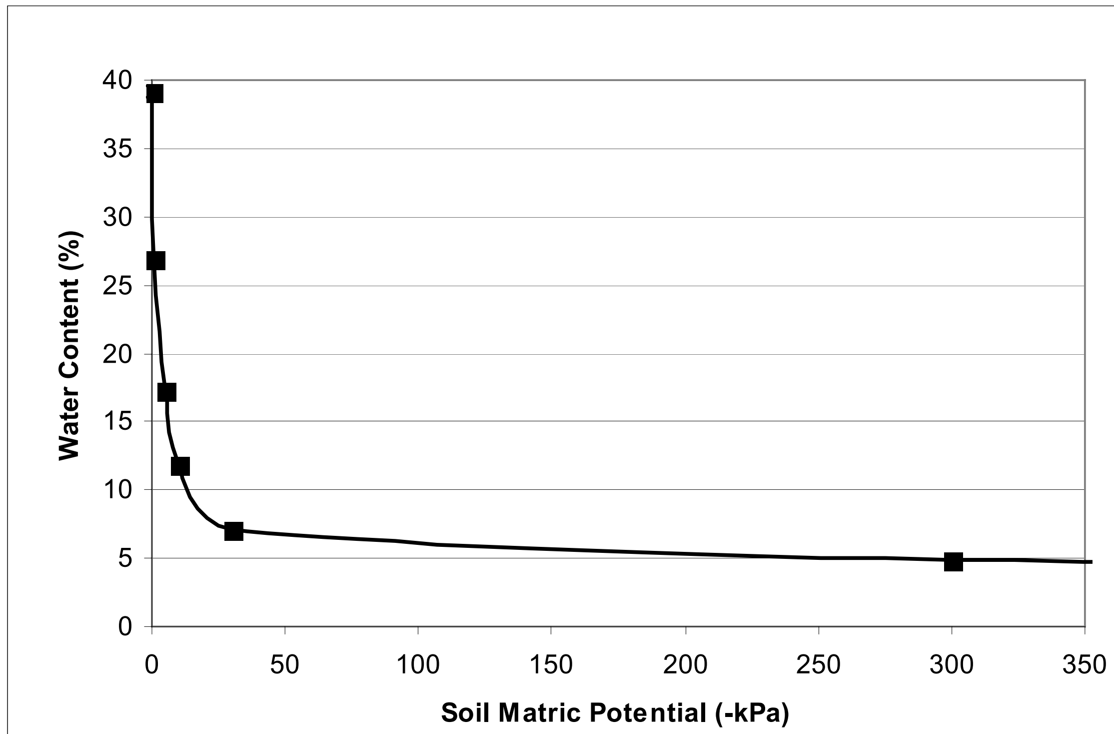

Figure 1.1. Moisture release curve of soil used in all experiments. The Norfolk loamy sand was taken from a field used for peanut production at the Upper Coastal Plains Research Station, Rocky Mount, NC.

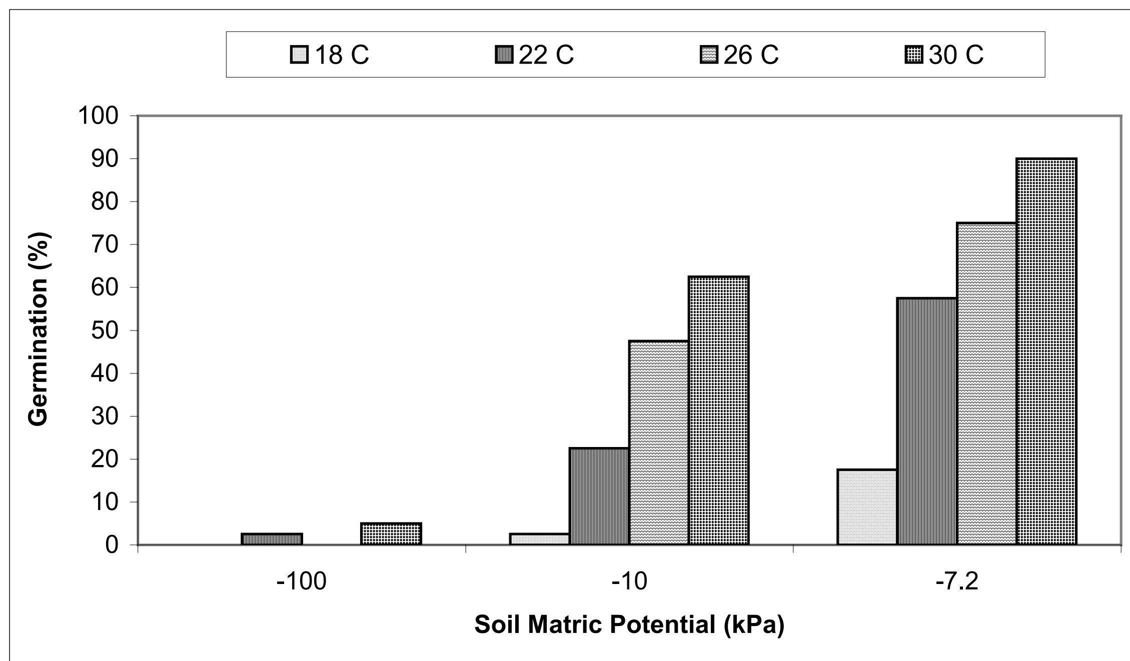

Figure 1.2. Percentage of sclerotia of *S. minor* (isolate #13) that germinated after incubation for 10 days at three soil matric potentials and four temperatures in the presence of acetone. Sclerotia were produced on sterile oat grains.

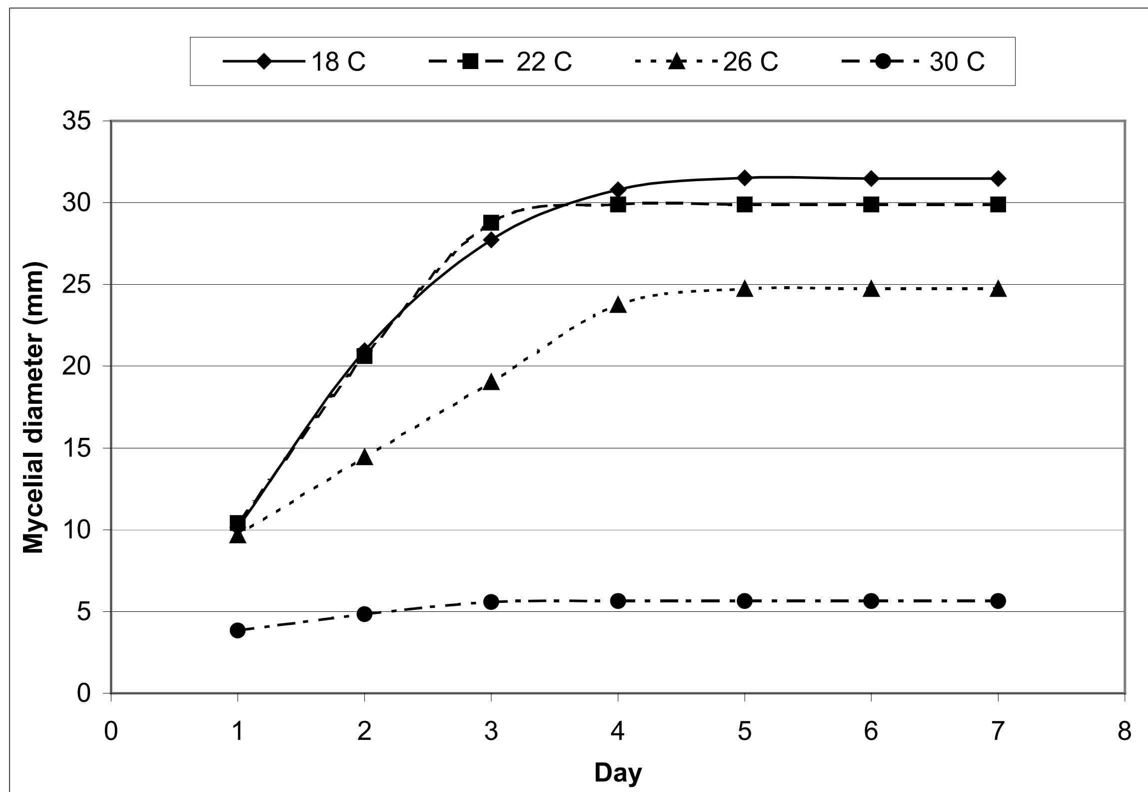

Figure 1.3. Effect of soil temperature on mycelial expansion by *S. minor* (isolate #13) from mycelial plugs taken from cultures grown on half-strength PDA. Values are averaged over three matric potentials.

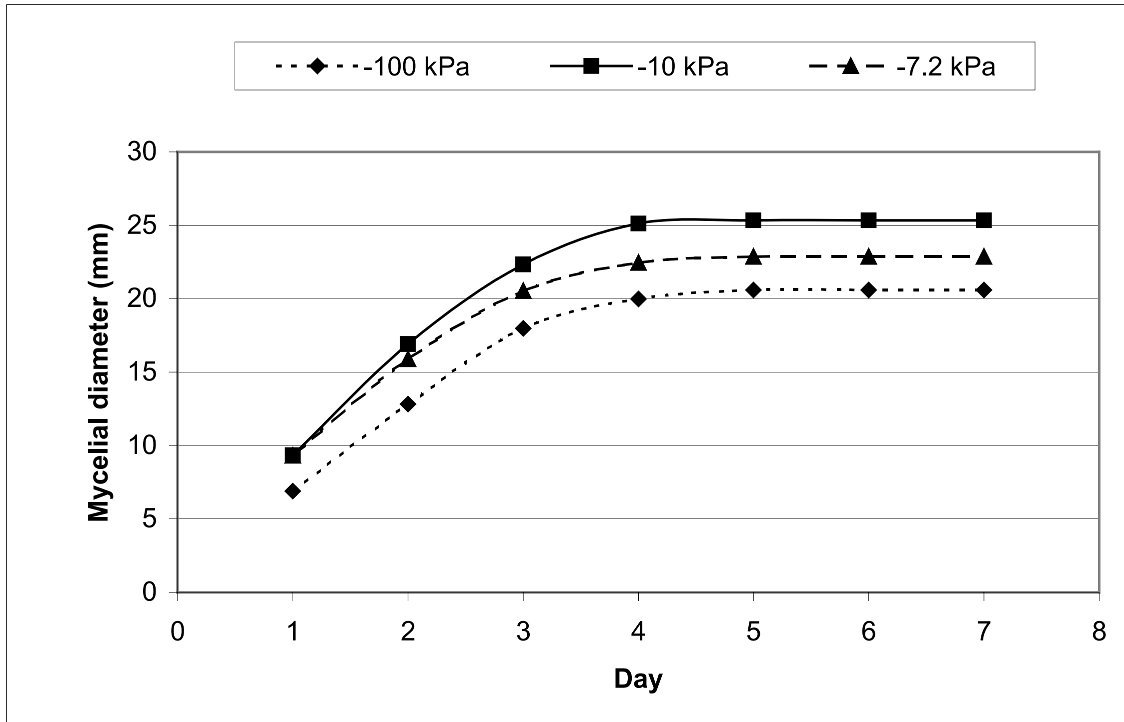

Figure 1.4. Effect of soil matric potential on mycelial expansion by *S. minor* (isolate #13) from mycelial plugs taken from cultures grown on half-strength PDA. Values are averaged over four temperatures.

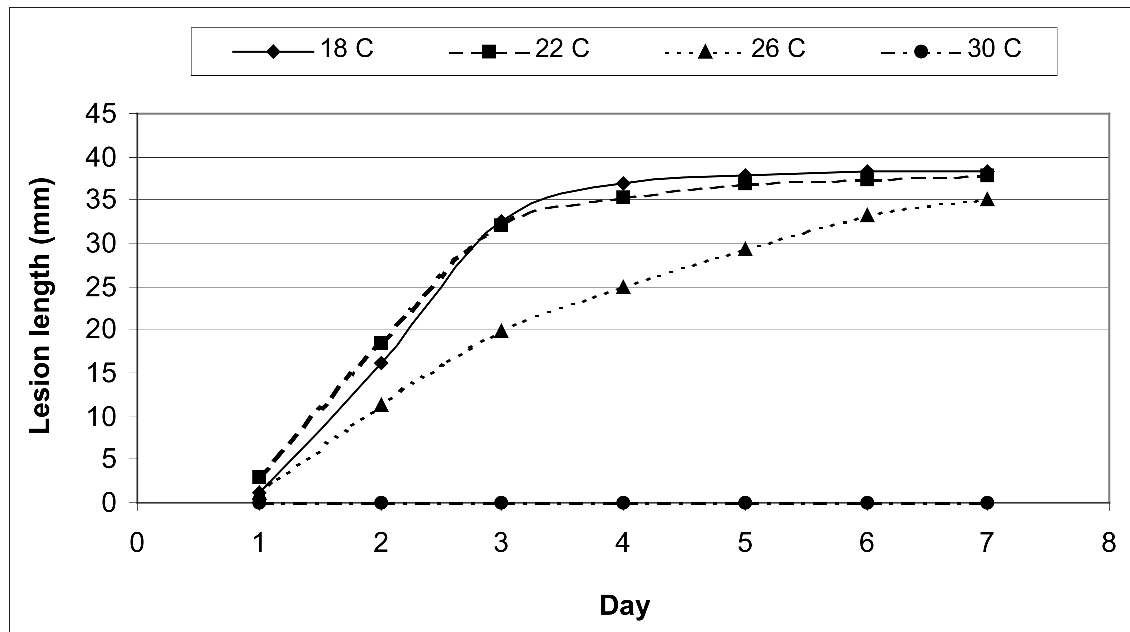

Figure. 1.5. Effect of soil temperature on development of lesions on detached peanut leaflets inoculated with *S. minor* (isolate #13) from mycelial plugs taken from cultures grown on half-strength PDA. Values are averaged over three matric potentials.

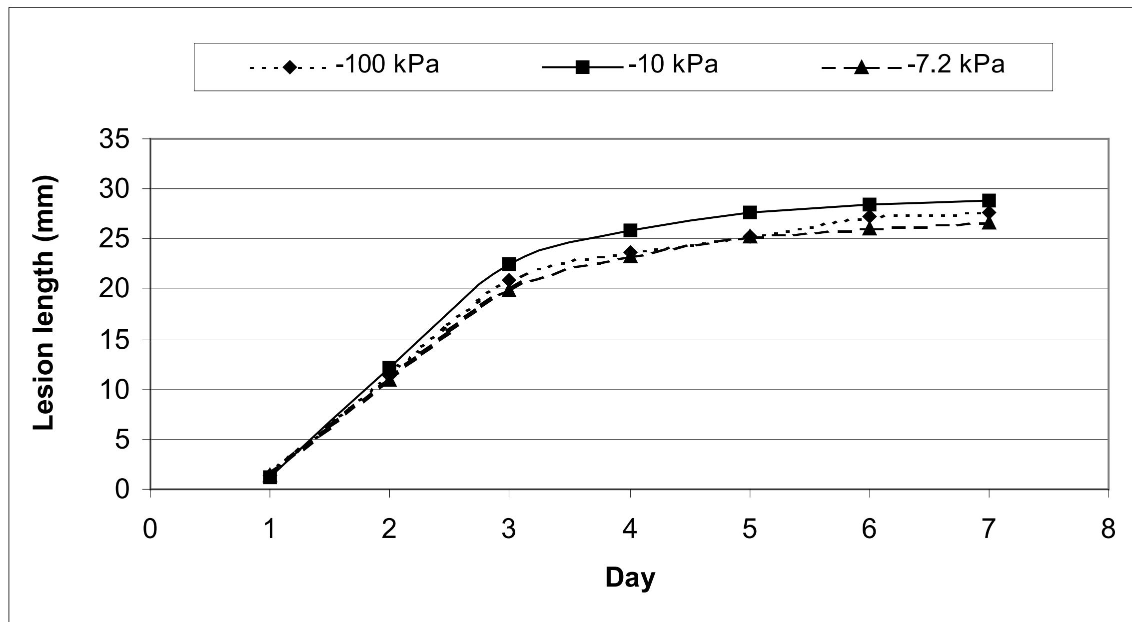

Figure 1.6. Effect of soil matric potential on lesion expansion on peanut leaflets inoculated with *S. minor* (isolate #13) from mycelial plugs taken from cultures grown on half-strength PDA. Values are averaged over four temperatures.

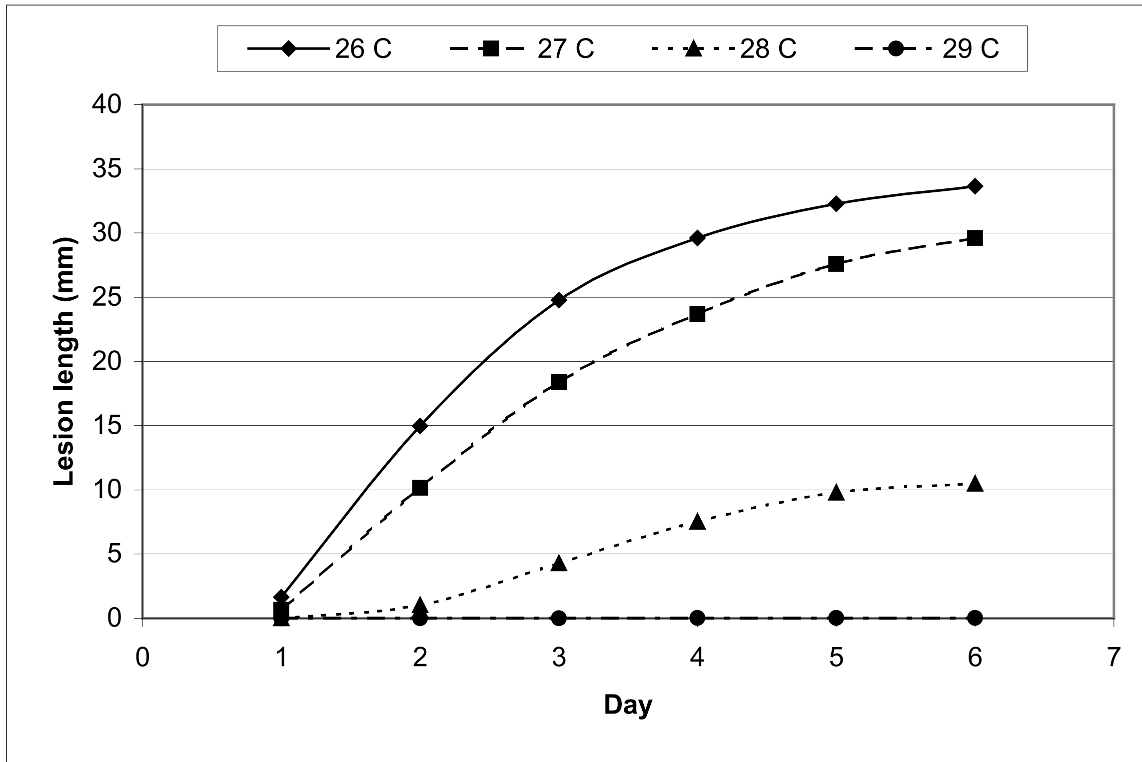

Figure 1.7. Effect of temperatures between 26 C and 29 C on lesion expansion on peanut leaflets inoculated with *S. minor* (isolate #13) from mycelial plugs taken from cultures grown on half-strength PDA. Values are averaged over three matric potentials.

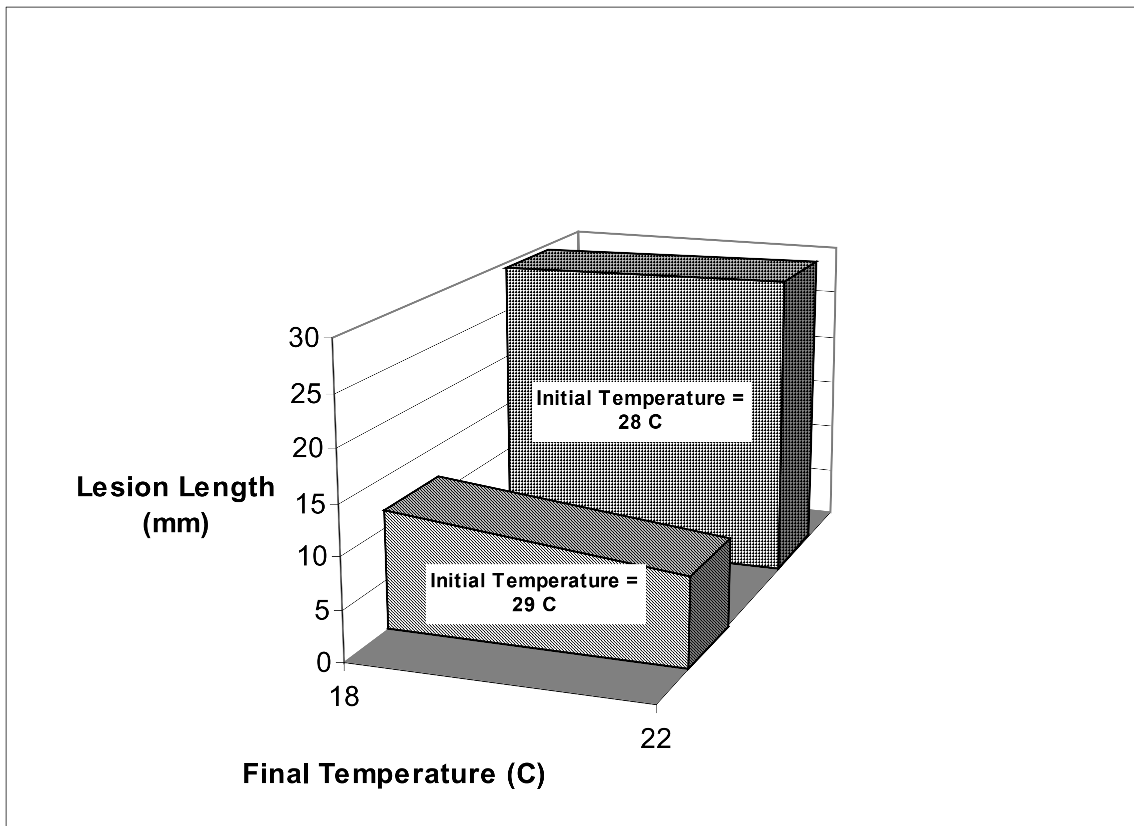

Figure 1.8. Effect of warm initial temperature and cool final temperature on lesion lengths produced on peanut leaflets by *S. minor* (isolate #13) from mycelial plugs taken from cultures grown on half-strength PDA. Leaves were incubated at 28 or 29 C for 2 da and then moved to 18 or 22 C for an additional 4 da. Values are averaged over three matric potentials.

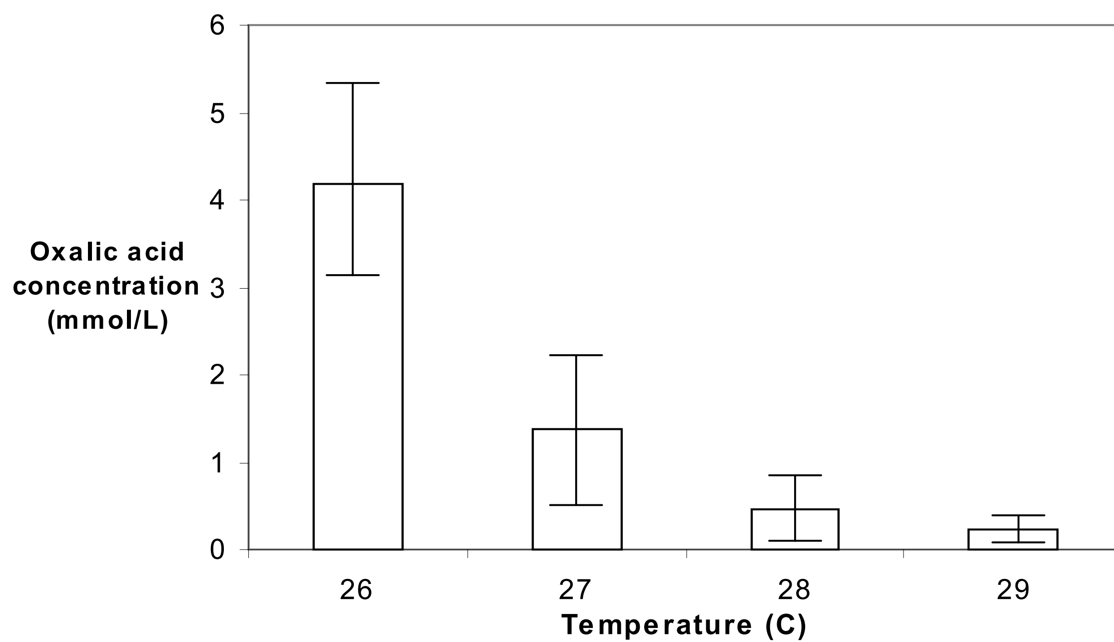

Figure 1.9. Oxalic acid production by *S. minor* (isolate #13) after three days of incubation in potato dextrose broth at four temperatures. Error bars indicate standard deviations.

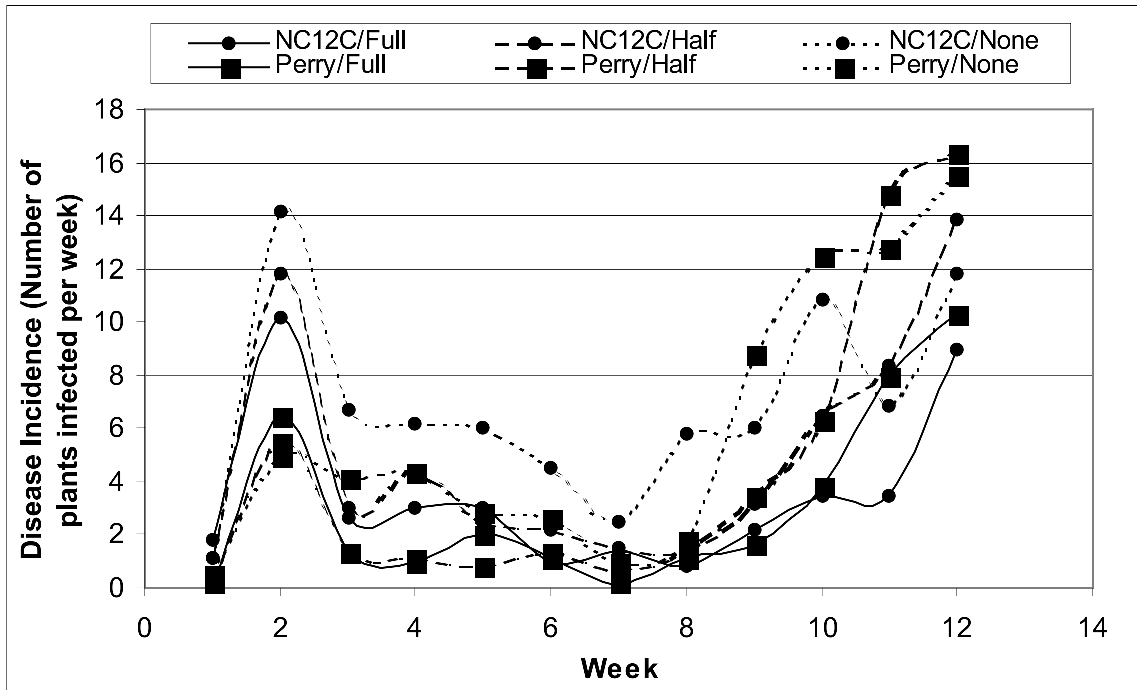

Figure 1.10. Weekly change in incidence of Sclerotinia blight in Perquimans County, 2002. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 15 Jul and 6 Aug. Weekly ratings began on 12 Jul and ended on 27 Sep.

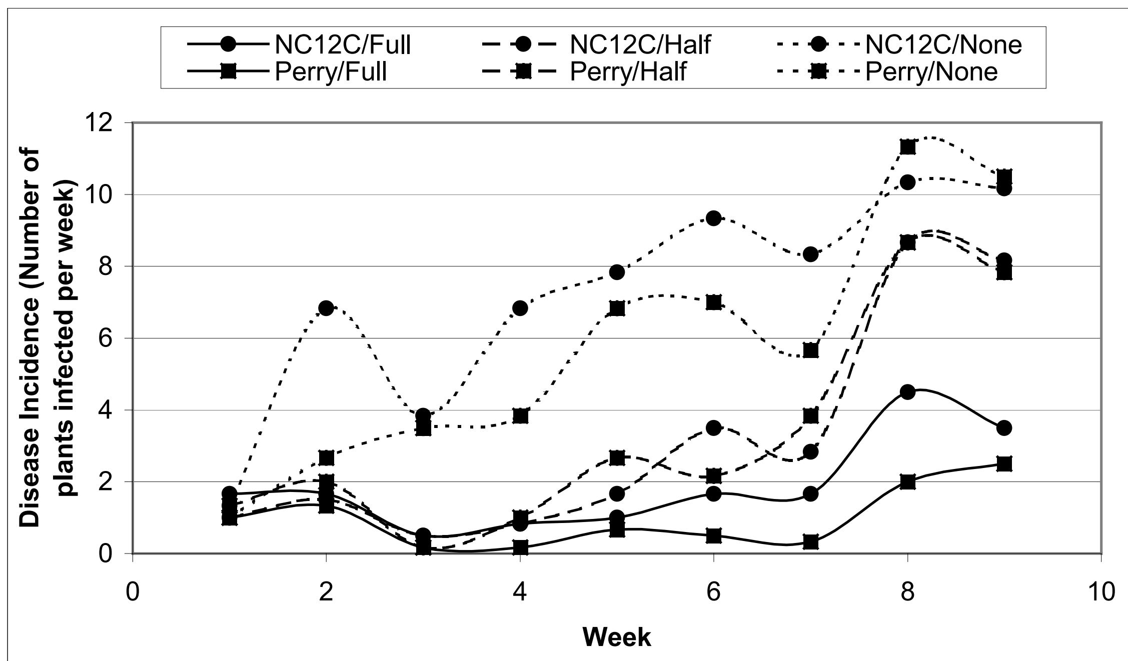

Figure 1.11. Weekly change in incidence of Sclerotinia blight in Perquimans County, 2003. Plots were treated with at 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 21 Jul and 18 Aug. Weekly ratings began on 21 Jul and ended on 15 Sep.

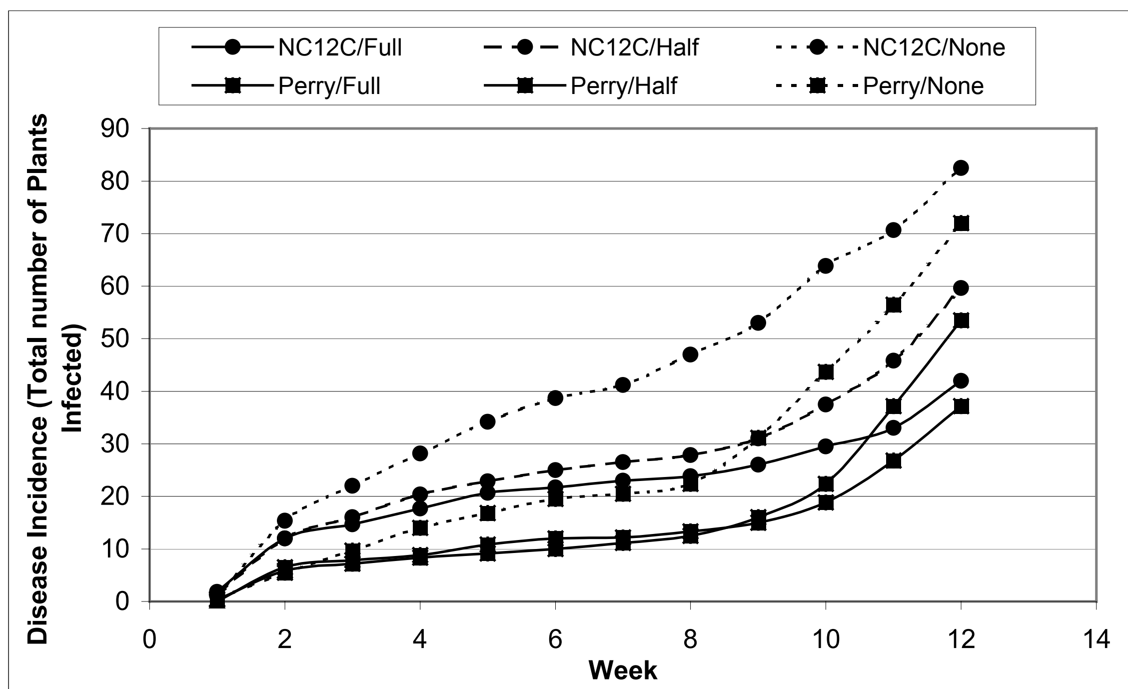

Figure 1.12. Cumulative progress of Sclerotinia blight on peanuts grown in Perquimans County, 2002. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 15 Jul and 6 Aug. Weekly ratings began on 12 Jul and ended on 27 Sep.

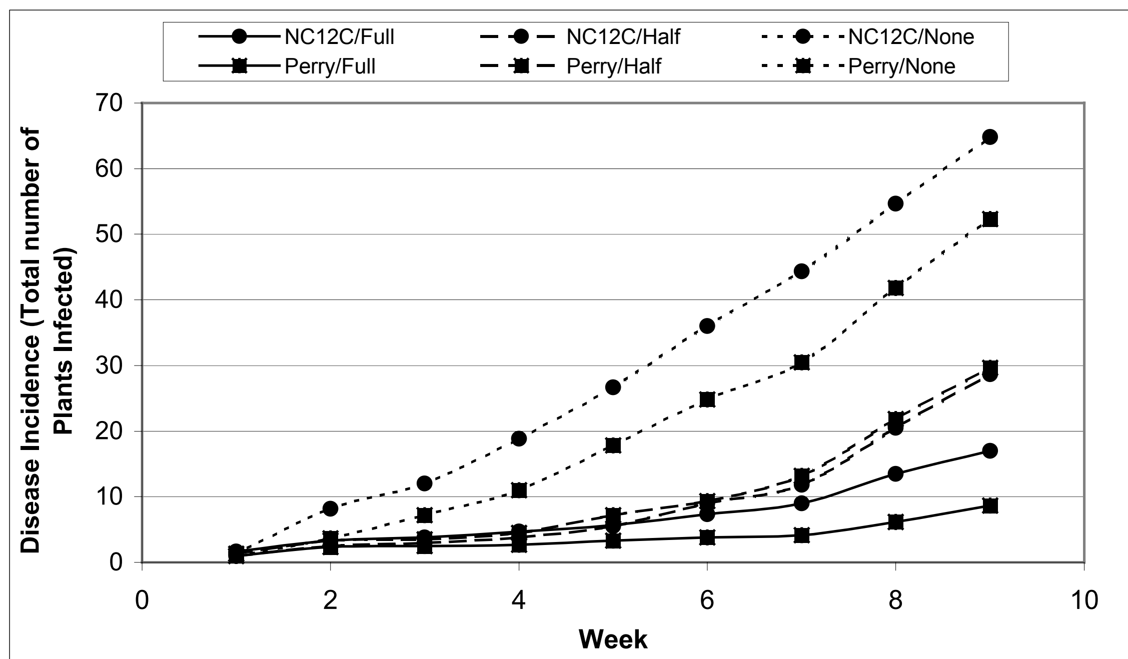

Figure 1.13. Cumulative progress of Sclerotinia blight on peanuts grown in Perquimans County, 2003. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 21 Jul and 18 Aug. Weekly ratings began on 21 Jul and ended on 15 Sep.

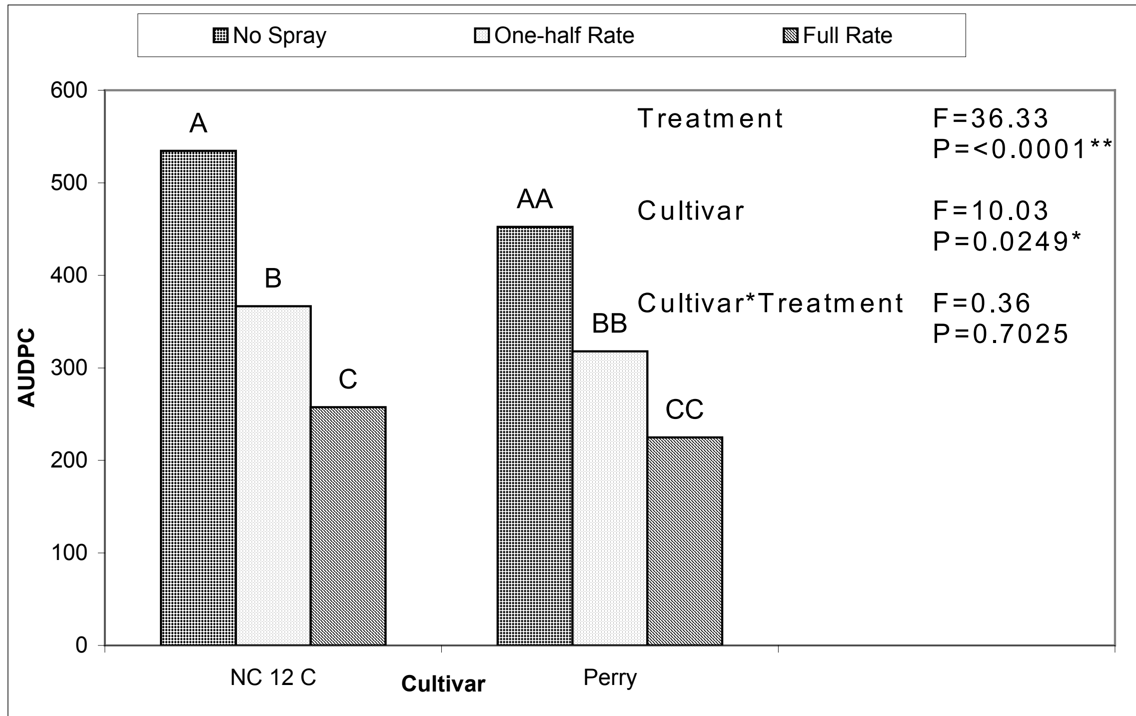

Figure 1.14. Area under the disease progress curve (AUDPC) of Sclerotinia blight on peanuts grown in Perquimans County, 2002. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 15 Jul and 6 Aug. Weekly ratings began on 12 Jul and ended on 27 Sep. Bars with different letter(s) are significantly different according to the Waller-Duncan K-ratio t test k=100. For the cultivar NC 12 C: MSD=96.06 R^2=0.85 CV=20.34. For the cultivar Perry: MSD=82 R^2=0.81 CV=20.16.

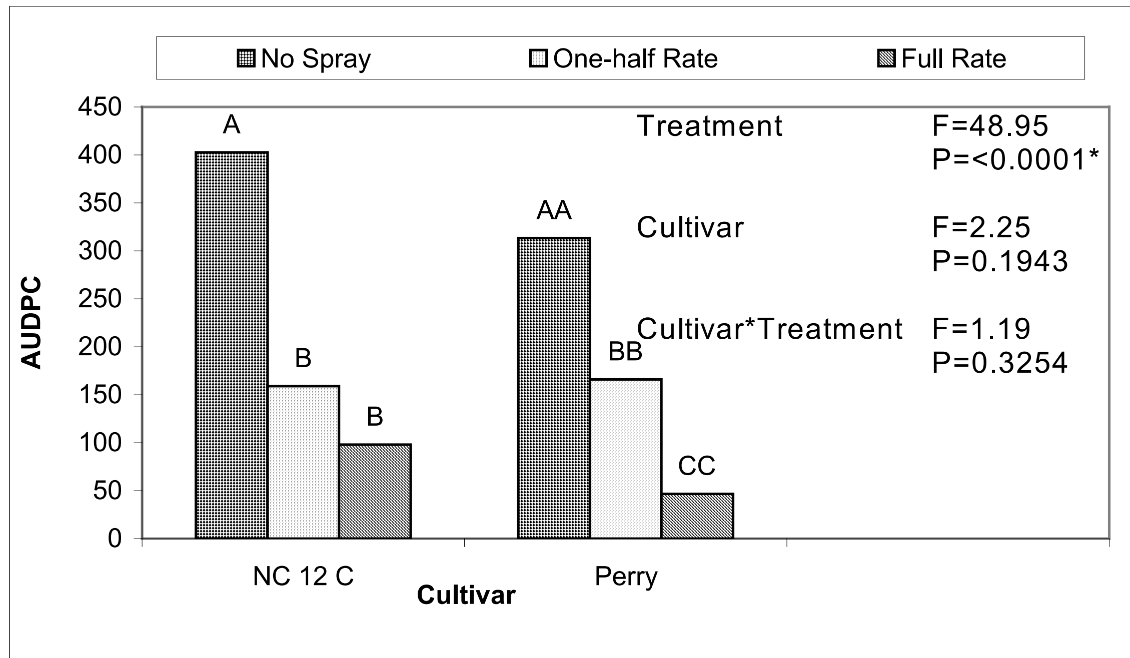

Figure 1.15. Area under the disease progress curve (AUDPC) of Sclerotinia blight on peanuts grown in Perquimans County, 2003. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 21 Jul and 18 Aug. Weekly ratings began on 21 Jul and ended on 15 Sep. Bars with different letter(s) are significantly different according to the Waller-Duncan K-ratio t test k=100. For the cultivar NC 12 C: MSD=66.93 R^2=0.91 CV=25.51. For the cultivar Perry: MSD=109.26 R^2=0.82 CV=50.24.

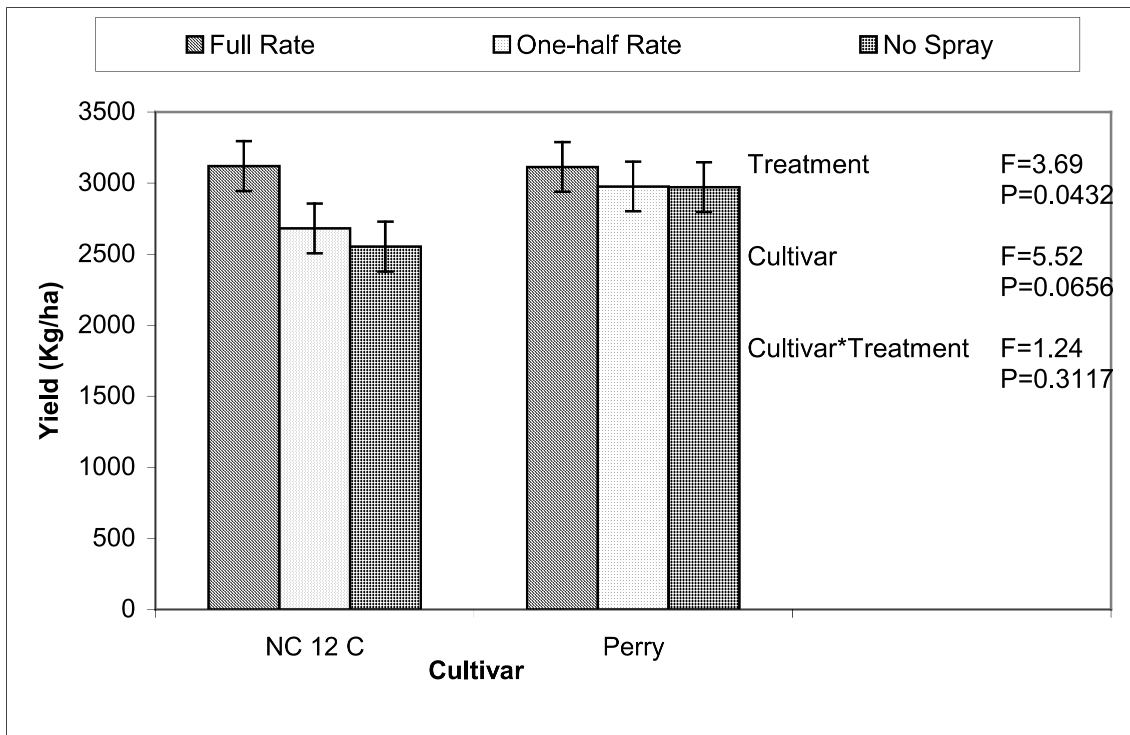

Figure 1.16. Dry yield of two peanut cultivars grown in Perquimans County, 2002. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 15 Jul and 6 Aug. Plots were dug on 4 Oct and harvested on 11 Oct. Error bars indicate standard deviations.

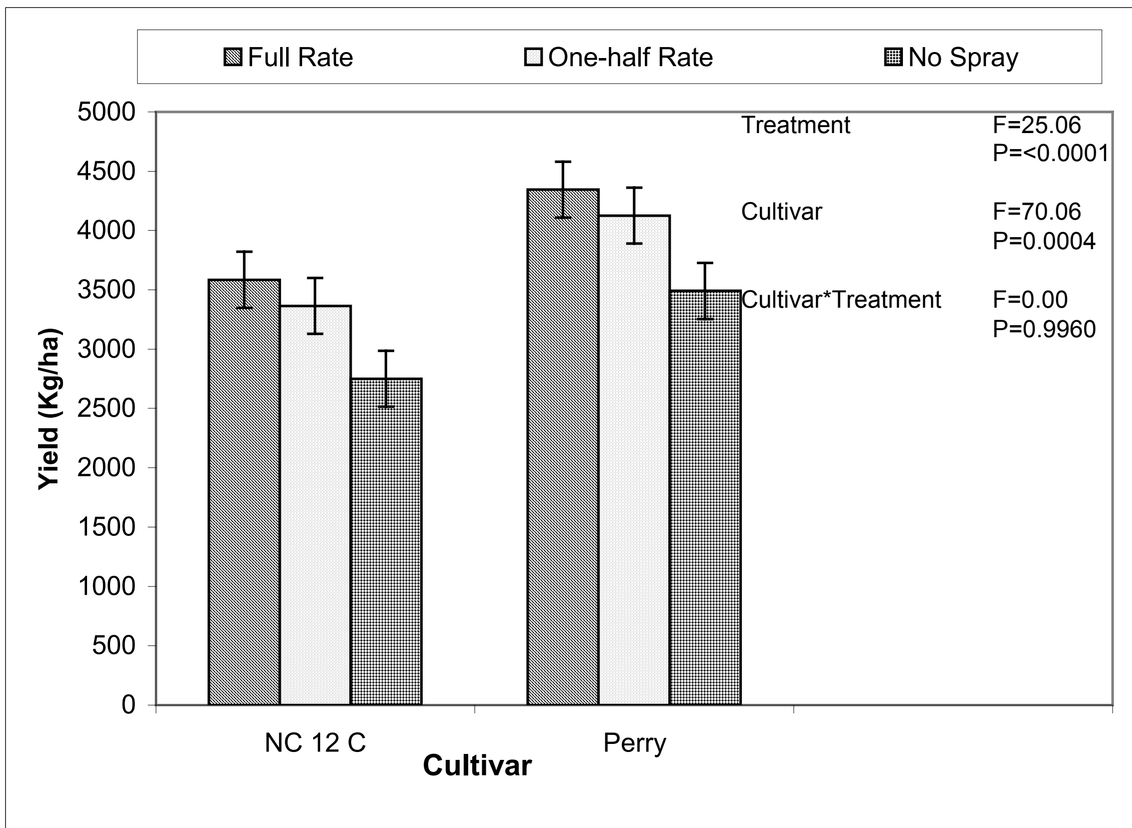

Figure 1.17. Dry yield of two peanut cultivars grown in Perquimans County, 2003. Plots were treated with 0.87 kg ai/ha (full), 0.43 kg ai/ha (half), or no fluazinam on 21 Jul and 18 Aug. Plots were dug on 9 Oct and harvested on 20 Oct. Error bars indicate standard deviations.

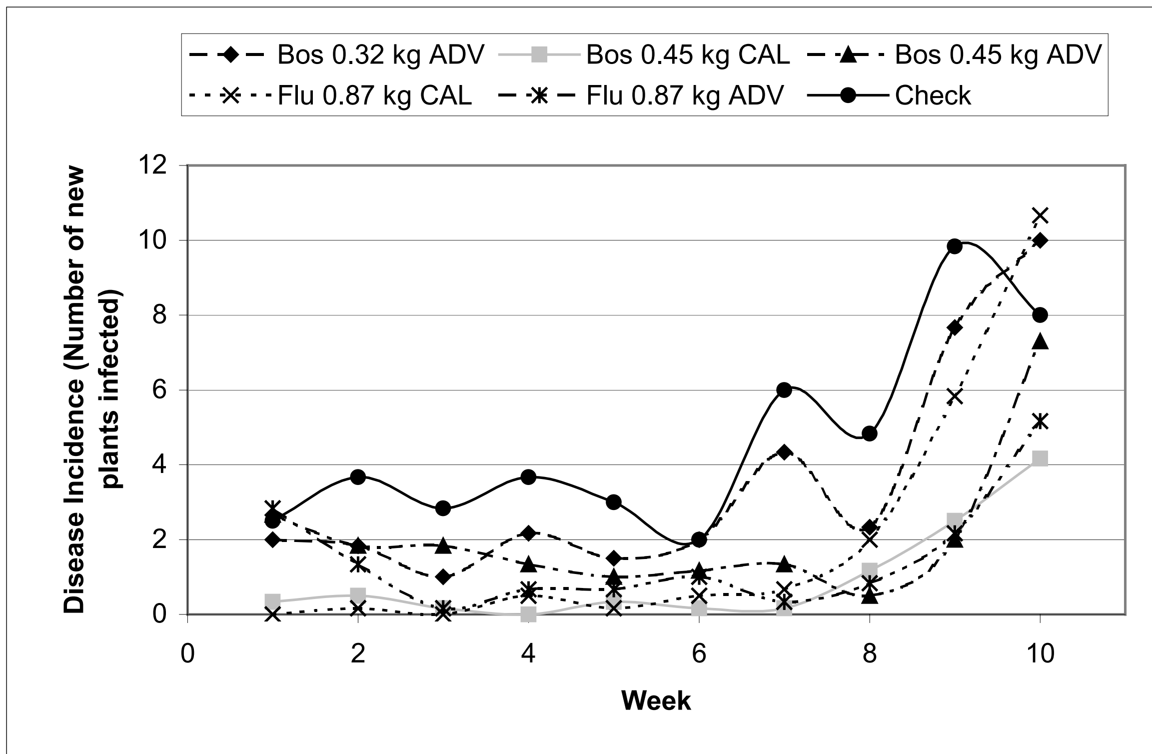

Figure 1.18. Weekly change in incidence of Sclerotinia blight in Chowan County, 2003. Bos indicates the fungicide boscalid; Flu indicates the fungicide fluazinam. Rates are indicated in kg ai/ha. Weekly ratings began on 4 Aug and ended on 9 Oct.

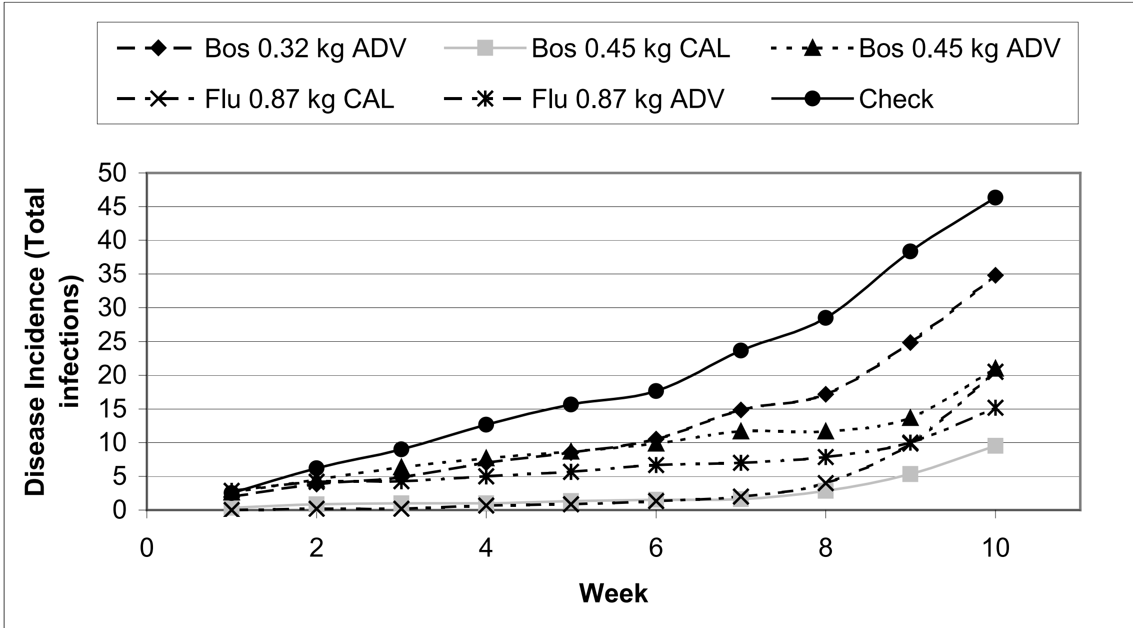

Figure 1.19. Cumulative progress of Sclerotinia blight on peanuts grown in Chowan County, 2003. Bos indicates the fungicide boscalid; Flu indicates the fungicide fluazinam. Rates are indicated in kg ai/ha. Weekly ratings began on 4 Aug and ended on 9 Oct.

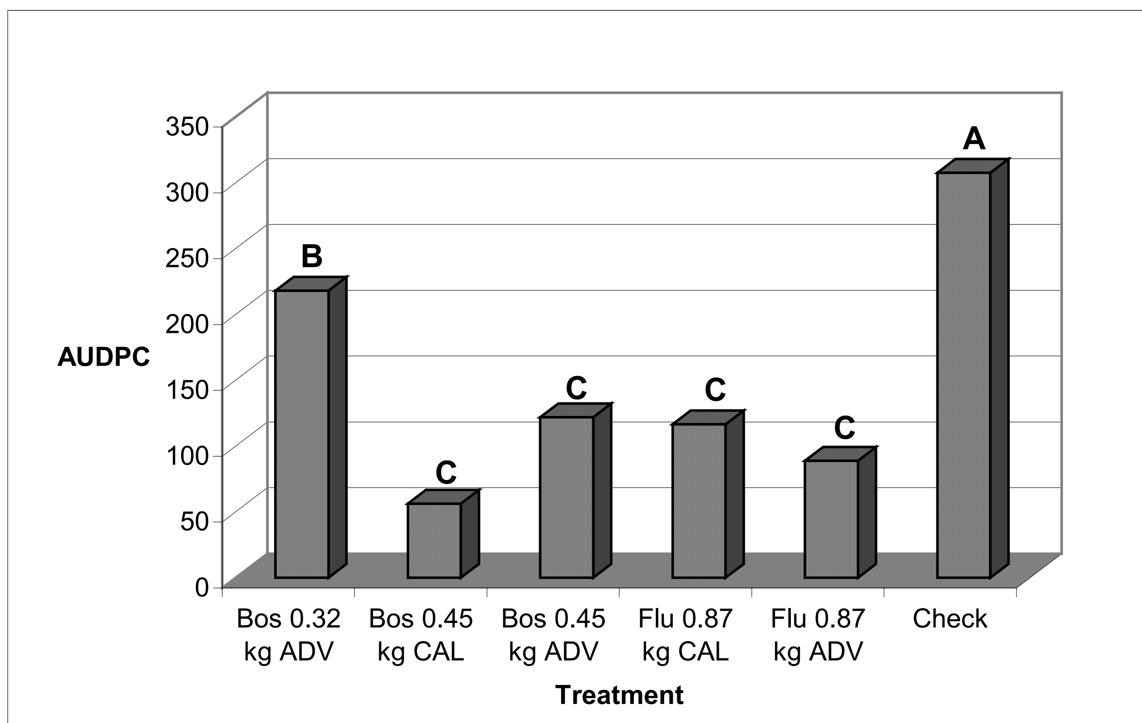

Figure 1.20. Area under the disease progress curve (AUDPC) of Sclerotinia blight on peanuts grown in Chowan County, 2003. Bos indicates the fungicide boscalid; Flu indicates the fungicide fluazinam. Rates are indicated in kg ai/ha. Weekly ratings began on 4 Aug and ended on 9 Oct. Bars with different letter(s) are significantly different according to the Waller-Duncan K-ratio t test k=100; MSD=76.53 R^2=0.78 CV=45.15.

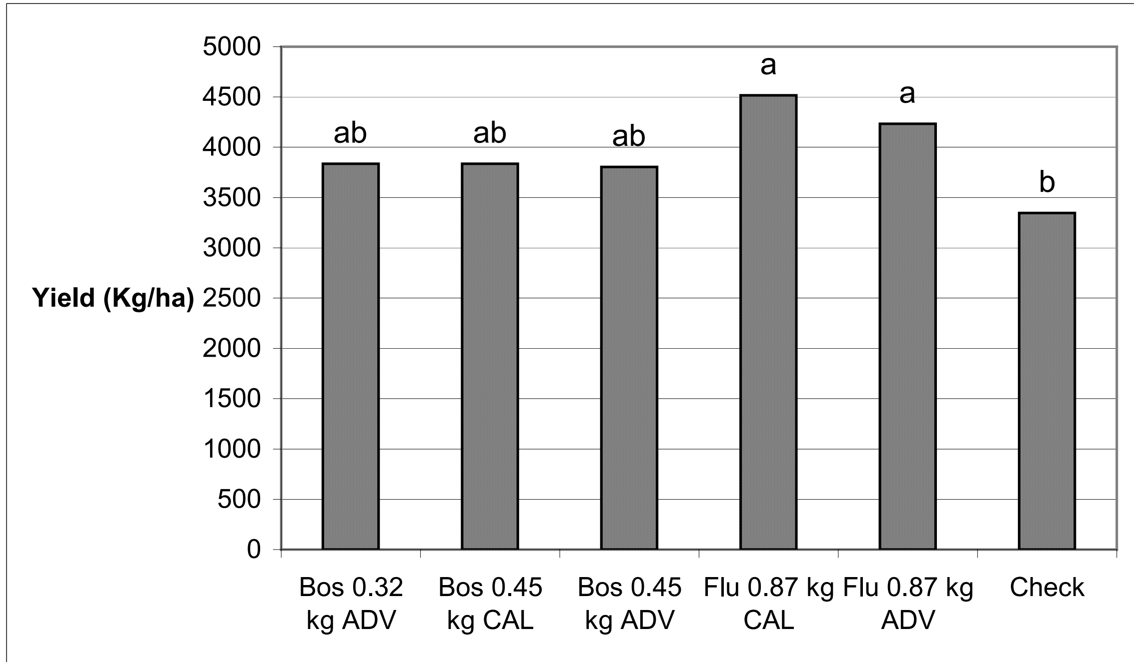

Figure 1.21. Dry yield of peanuts grown in Chowan County, 2003. Bos indicates the fungicide boscalid; Flu indicates the fungicide fluazinam. Rates are indicated in kg ai/ha. Plots were dug on 9 Oct and harvested on 20 Oct. Bars with different letter(s) are significantly different according to the Waller-Duncan K-ratio t test k=100; MSD=772 R^2=0.83 CV=14.65.

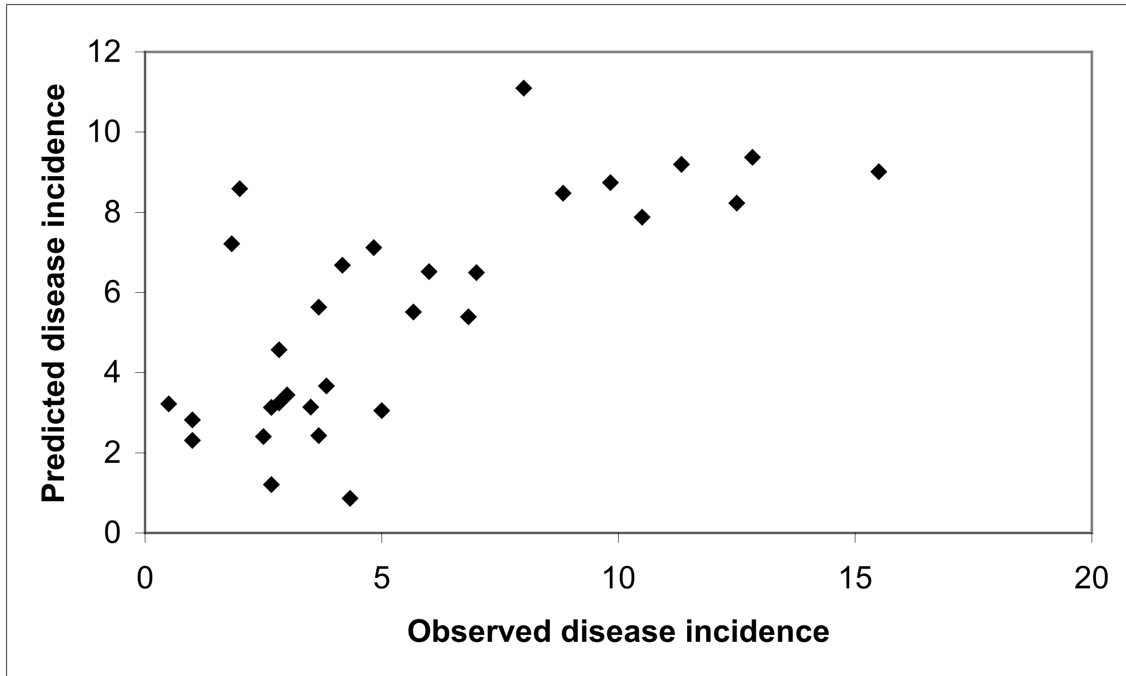

Figure 1.22. Scatterplot of observed and predicted disease incidence for sites in Perquimans and Chowan Counties in 2002 and 2003. Predicted disease incidence was based on the model $Y=[-79.52 + 3.04RH - 0.02RH^2 - 0.47ST - 5.30LW]$; where RH=mean relative humidity, ST=mean soil temperature, and LW=mean leaf wetness; P=0.0006, R^2=0.51, CV=52.92.

Susceptibility of detached peanut (*Arachis hypogaea*) plant parts to *Sclerotinia minor*

Abstract

Sclerotinia blight caused by the fungus *Sclerotinia minor* is a serious disease of cultivated peanut (*Arachis hypogaea* L.) in North Carolina. Laboratory and field experiments were conducted to evaluate the relative importance of tissue resistance vs. plant architecture in high performing cultivars. In laboratory tests, leaflets, pegs, lateral branches, and main stems of two susceptible cultivars and two partially resistant breeding lines were detached from plants grown in the greenhouse, inoculated with mycelial plugs, and placed in moisture chambers. Lesion development and severity on each plant part was measured for 7 days. In the field, three cultivars and one breeding line were planted in replicated plots. Destructive samples of randomly selected plants were made weekly once the start of an epidemic was observed. Numbers of lesions on leaflets, pegs, lateral branches, and main stems were counted. Laboratory studies indicated that leaflets and pegs were the most susceptible parts of the plant. Main stems and lateral branches were equally resistant to infection. In the field, lesions were most frequent on lateral branches. Inconsistencies between laboratory and field studies indicate that there are mechanisms of resistance operating in the field besides the physiological resistance observed in laboratory inoculations. Pegs are much smaller than lateral branches and are less likely to come in contact with a germinating sclerotium than lateral branches. Leaflets do not contact the soil surface as often as lateral branches and are not a frequent site of infection.

Management strategies should focus on protecting the lateral branches from infection by *S. minor*.

Introduction

Sclerotinia minor Jagger, the fungus that causes Sclerotinia blight of peanut, can infect a wide range of host plants. *Sclerotinia minor* was first identified as a pathogen of peanut in Australia in 1948. The first reports of *S. minor* in the United States were made in Virginia in 1971 and in North Carolina in 1972 (Porter et al., 1982). Since the early reports of Sclerotinia blight of peanut, the disease has become widespread in peanut production areas and losses can be severe.

The mycelium of *Sclerotinia minor* attacks all parts of peanut plants and rapidly invades succulent tissues, causing cells to collapse. Initially, leaves and stems have a water-soaked appearance. As the disease progresses, mycelium often appears as a white, fluffy mass on the surface of colonized tissue. Later, lesions become bleached and necrotic. Eventually, stems are severely shredded and die (Porter et al., 1982).

Cultivated peanut grows upright to a height of approximately 15-60 cm. Plants have a well-defined taproot, many lateral roots, sparse to no hairs on above ground plant parts, and have numerous lateral branches (Moss and Rao, 1995). *Arachis hypogaea* susp. *hypogaea* var. *hypogaea* have no floral axes on the main stem. Flowers form in leaf axils of lateral branches 4-6 weeks after planting. Peanut flowers self-pollinate; a week later a carpophore or gynophore (commonly known as a peg) forms. The gynophore is geotropic and penetrates the ground surface. Pods form horizontally to the pegs underground (Shokes, 1995).

In general, peanuts are very susceptible to Sclerotinia blight. *Sclerotinia minor* spreads rapidly within the peanut canopy and sclerotia can survive for long periods in the soil. The most effective control measures currently used include rotation with non-hosts (such as corn, cotton, sorghum, and small grains), application of fungicides, and panting resistant cultivars.

Currently, moderately resistant peanut cultivars are available to growers in the Virginia-Carolina region who need to manage Sclerotinia blight. The partially resistant cultivar VA 93B was registered for use by growers in 1994 (Coffelt, et al., 1994), followed by the partially resistant cultivars VA 98R (Mozingo et al., 2000) and Perry (Isleib et al., 2003). Various mechanisms may be responsible for the partial resistance that is observed in these cultivars. They have prostrate lateral branches with an erect main stem (Coffelt et al., 1994; Mozingo et al., 2000; Isleib et al., 2003). Growth habits of this type can act as an avoidance mechanism (Coffelt and Porter, 1982; Chappell et al., 1995). Physiological resistance may also be present in cultivars resistant to infection by *S. minor*. Certain structural and physiological characteristics have been noted as important in resistance to this fungus in bean and peanut, including phytoalexin induction, waxy cuticles, and thickened cortical cells (Lumsden, 1979; Coffelt et al., 1982; Pratt, 1996).

The ability to screen for heritable resistance to plant pathogenic fungi is a valuable tool for a plant breeding program. Evaluations have traditionally been done in field plots, under varying levels of disease, and over many years to determine the performance of each breeding line (Coffelt et al., 1982). Field trials do not identify physiological resistance alone, other mechanisms (avoidance) come into play in the field

(Chappell et al., 1995). Identifying breeding lines with physiological resistance is helpful before those lines are tested in the field. Therefore, other screening methods have been sought in order to expedite the selection process so that many breeding lines can be screened at one time, in limited amounts of space, and in a short period of time, to identify levels of physiological resistance (Brenneman, et al., 1988). Most alternatives to field trials involve some sort of greenhouse or laboratory experiment (Melouk et al., 1992; Chappell et al., 1995; Cruickshank et al., 2002).

Laboratory screening methods vary. In alfalfa (*Medicago sativum* L.), excised leaf tissue of breeding lines were placed on various agar media at the margin of actively growing colonies of *Sclerotinia trifoliorum* (Pratt and Rowe, 1998). This method proved more efficient and was as accurate as an earlier method in which leaflets were directly inoculated with mycelia of the fungus (Pratt, 1996). In peanut, similar screening methods have been attempted. Whole peanut plants were inoculated on the main stem with bean (*Phaseolus vulgais* L.) pods colonized with *S. minor* (Cruickshank et al., 2002). In other tests, cut plant shoots were placed in Hoagland's solution, inoculated with mycelial plugs, and lesion length measured. Low rates of lesion expansion denoted physiological resistance to S. *minor* (Melouk et al., 1992). Still other tests used detached peanut stems inoculated with mycelial plugs taken from cultures of the fungus (Brenneman etal., 1988). A variation of this system included wounding or not wounding the detached stem and using an oat (*Avena sativa* L.) grain infested with *S. minor* for the inoculation (Chappell et al., 1995). Recently, lesion development and expansion were compared on detached peanut leaflets of 12 peanut lines. Leaves were placed in moist chambers and inoculated with mycelial plugs taken from actively growing cultures of *S. minor*

(Hollowell et al., 2003). Results of these studies suggest that detached leaflet inoculation had utility for screening large populations for resistance to *S. minor.* However, results were not highly correlated with field trial results. Other plant parts (pegs, pods, lateral branches) may respond differently to *S. minor*, and these reactions should be considered in screening for resistance. A better understanding of the biology of both the host and fungus would help improve breeding and management efforts directed towards improving resistance in individual plant parts and in the entire plant. The objective of this project was to evaluate the relative importance of tissue resistance vs. plant architecture in high performing peanut cultivars.

Materials and Methods

Preparation of plant material for laboratory studies. Three peanut lines were planted and grown in the greenhouse for 8-10 wk before testing. In the first set of two runs, lines included the highly susceptible cultivar NC 12C, the moderately susceptible cultivar NC 7, and GP-NC WS12 (a germplasm line with high levels of partial resistance (Stalker et al., 2002; Hollowell et al., 2003). In the second set of three runs, the lines included NC 12C, NC 7, and N96076L, an advanced breeding line that exhibited high partial resistance to Sclerotinia blight in the field as indicated by the frequency of plants exhibiting symptoms (T.G. Isleib, *personal communication*). Seeds were pre-germinated for 2-3 days prior to planting. Two seeds were planted in 15-cm clay pots containing a 2:1 ratio of steamed sandy-loam soil and play sand. Forty-one pots of each line were planted for each run. Plants were fertilized at 42 days after planting (DAP) with 100 ml of a solution of water-soluble 20-20-20 fertilizer (Peter's All Purpose Plant Food).

Beginning at first flowering, a calcium treatment was applied to foliage of one-half of the pots. The rate of 5g $CaCl_2$ per liter of water was similar to that recommended for blossom end rot control in tomato (Averre and Shoemaker, 2000). The solution was applied to runoff with a backpack sprayer every 4 da.

Detached plant part inoculation. Plants grown as described were used to supply various plant parts for inoculation. A razor blade was used to detach peanut plant parts on the day of inoculation. Detached parts included a peg (gynophore) that had penetrated the soil surface, a single leaflet still attached to the petiole, the main stem, and two primary lateral branches. The detached parts were brought back to the laboratory and placed in humidity boxes (Pioneer Plastics, Inc., Dixon, KY) measuring 35 cm long, 27 cm wide, and 10 cm deep. The boxes contained 825 cm^3 of sterile washed play sand atop which was placed a galvanized 6-mm mesh screen. The detached plant parts were placed on the screen and inoculated. A 3-mm-diam agar plug was taken from a 2-da-old culture of an aggressive isolate of *Sclerotinia minor* (Shew Laboratory collection #13) originally obtained from a diseased peanut growing in northeastern NC. The main stem was inoculated in the center at the second branching node from the bottom of the stem; the peg was inoculated in the center; the leaflet was inoculated along the midrib on the adaxial side; one primary lateral branch was inoculated at a vegetative node; and one primary lateral branch was inoculated at a flowering node. The plant parts were sprayed until droplet formation with a mixture of Tween 20 and water (30 µl of Tween 20 to 100 ml of water) after inoculation. Plant parts were arranged randomly within boxes and boxes were arranged in randomized blocks in a growth chamber at 20 C. Thus, the

experimental design was a split-plot with lines as whole plots and parts as subplots. Each day for seven days, lengths of lesions were measured along the longest axis of each part and a ratio to the overall length of the plant part was calculated.

Plant part examination in the field. Four lines that varied in susceptibility to Sclerotinia blight were planted in a grower field in northeastern NC on 20 May 2003. The lines planted included NC 12 C (highly susceptible), VA 98R (moderately susceptible), Perry (moderately resistant), and N96076L (highly resistant breeding line). Typical growing practices and pesticide applications were used (Shew, 2003), but no fungicide for control of Sclerotinia blight was applied. The test was arranged in a randomized complete block design with six replications. The plots were 13 m long with alleys measuring 3 m, which separated each plot within the replicate. Four-row borders separated the six replicates making up the randomized complete block design. Sampling began on 28 Jul (69 DAP) and continued on a weekly basis for 7 weeks.

In each week, one of nine possible sampling locations was selected at random using the experimental plan procedure (PROC PLAN) of SAS (SAS Institute, Cary NC). A sample section (0.30 m) was examined in each of the center two rows of each plot. All the plants in a section were dug by hand with a pitchfork and inspected visually for signs and/or symptoms of the pathogen. Infections were counted on the main stem, primary lateral branches, pegs, and leaflets of each plant present.

Results

Detached plant part inoculation. Trends were consistent across all runs regardless of the breeding line that was included in the run. Calcium treatment did not affect lesion development as measured by the area under the disease progress curves (AUDPC) for severity (proportion of part covered by a lesion) in any run ($P>0.40$). Lesions formed and expanded on all parts that were examined in all runs (Figs. 2.1 and 2.2). Leaflets and pegs followed similar trends whereas lateral branches (inoculated at either a vegetative or reproductive node) and main stems were similar to each other (Figs 2.1 and 2.2). Lesions on leaflets and pegs expanded quickly and quickly consumed most of the part within a few days. Lesions developed more slowly on lateral branches and main stems (Figs. 2.3 and 2.4). Main effects of cultivar did not differ significantly in runs using GP-NC WS12 ($P=0.5749$; Fig. 2.5), but there was a cultivar by plant part interaction. AUDPCs differed significantly for leaflets, and marginally for pegs and lateral branches inoculated at a vegetative node (Fig. 2.6). Lesions on leaflets of GP-NC WS12 expanded at a slower rate than those of the other two cultivars ($P=0.0187$). Cultivar main effects were significant for runs using the breeding line N96076L (Fig. 2.5) and there was a cultivar by plant part interaction. The AUDPCs of N96076L were significantly smaller than those of the other cultivars with the exception of main stems (Fig. 2.7; $P\leq0.0001$).

Plant part examination in the field. Data were tested using a Chi square (X^2) test. Total numbers of lesions counted over the entire seven week rating period were not significantly different from the expected values for each cultivar examined ($P=0.850$).

The cultivars NC 12C and Perry had a greater number of lesions on lateral branches, leaves, and pegs than did either VA 98R or the breeding line N96076L (Table 2.1). Very few lesions were found on main stems of any line. The greatest number of lesions were found on lateral branches, followed by pegs and leaves.

Discussion

Calcium application did not influence disease development on any detached part. Some research suggests that applications of certain formulations of calcium may protect against Sclerotinia blight development in the field (Beam et al., 2002). This was not observed in our laboratory tests. Calcium may help prevent disease if applied in a different manner and in a different formulation. In these tests, calcium (formulation: $CaCl_2$) was applied in an aqueous solution to all above ground foliage. Absorption and systemic transport of the calcium throughout the plant probably did not occur with the $CaCl_2$ formulation and application method; therefore, no benefit from a calcium application was observed.

Leaflets and pegs showed similar susceptibility to parasitism by *S. minor.* Leaflets and pegs contain less woody tissue than lateral branches and main stems. These parts are, therefore, more vulnerable to the action of oxalic acid and cell-wall degrading enzymes. Parts detached from cultivars with partial resistance were susceptible to parasitism by the fungus. However, lesions expanded more slowly on parts from partially resistant cultivars as compared to susceptible cultivars. This is consistent with other screening methods in which rate of lesion development was used to assess physiological resistance (Brenneman et al., 1988; Melouk et al., 1992; Chappell et al.,

1995). Therefore, comparative studies of lesion size on any of the detached parts examined in these studies, except main stems, can be used to screen and rank cultivars based on their resistance. Leaflets and pegs are the best candidates as their size and speed of lesion formation and expansion make them easy to work with. Leaflet inoculations are an easy, clean, quick, and straightforward tool to determine resistance in peanuts cultivars. This finding is consistent with other recent studies where leaflets were used to conduct a resistance screen in various peanut cultivars and breeding lines (Hollowell et al., 2003). Excised leaf inoculations have also been successful in susceptibility screenings of other crops such as alfalfa (Pratt and Rowe, 1998). Furthermore, leaflet inoculations in the laboratory help to predict performance in field resistance screens. For this set of experiments, resistance ranking of cultivars in the laboratory was consistent with those made in the field study. These findings were consistent with observations by Hollowell et al. (2003) even though fewer lines were used in our study.

After adoption of many of the previous screening methods, it was apparent that peanut cultivars performed differently in the field compared to the laboratory. This was probably due to the presence of other mechanisms of resistance or avoidance not identified in the laboratory or greenhouse screens. Our findings are consistent with other studies in that field trials must also be utilized, in addition to laboratory and greenhouse screens, to identify other mechanisms of resistance (Chappell et al., 1995; Cruickshank et al., 2002).

It was evident from this study that lateral branches are the main infection court in the field. This outcome seems contradictory to the laboratory results. In field and

laboratory studies, tissue resistance was being examined, but in the field we must also account for avoidance due to plant architecture. Peanut plants have large numbers of leaves, pegs, and branches as compared to main stems. For this reason, only a few lesions were recorded on main stems because the odds were less that this part would come in contact with the fungus. Leaves were not often infected, because they usually were not in direct contact with the soil. An exception occurred after rain or windstorms, which pressed foliage in contact with the soil, thereby making leaves vulnerable to infection by a soilborne pathogen such as *S. minor.* Plant parts most commonly in contact with the soil were pegs and lateral branches. Because lateral branches have a larger surface area in contact with the soil, they are more likely than pegs to contact the fungus. Laboratory and field evaluations make it evident that lateral branches are composed of tissue that is more resistant than that of pegs; however, pegs may be able to avoid infection because of their reduced size and smaller area available for an infection court. The likelihood of a peg contacting a sclerotium in the field is also reduced because the surface area is much less than other plant parts. Few infections on pegs also may indicate that peg infections were difficult to identify, and our sampling and rating methods may require adjustment.

Management strategies may need adjustment in order to focus protection from *S. minor* infection on lateral branches. Using protectant fungicides such as fluazinam or boscalid in a spray program can protect all above ground parts of the plant. However, focusing spray treatments on parts lower in the canopy (especially parts touching the soil surface) by adjusting nozzle height or increasing spray pressure may improve protection of these parts. Bailey and Brune (1997) showed that pruning the plant canopy changed

the micrclimate near the soil and helped to reduce levels of disease. Minor pruning or thinning of the canopy may also help promote fungicide coverage of plant parts in contact with the soil while not have a significant effect on yield.

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Table 2.1. Total and mean lesion counts from destructive sampling of plants of four peanut cultivars for seven weeks, 2003.

		Main Stems		Lateral Branches		Pegs		Leaves	
	# of Plants examined	Total Lesions[a]	Mean Lesions[b]	Total Lesions[a]	Mean Lesions[b]	Total Lesions[a]	Mean Lesions[b]	Total Lesions[a]	Mean Lesions[b]
N96076L	237	1	0.0042	71	0.3000	4	0.0168	5	0.0210
NC 12C	222	1	0.0045	361	1.6261	19	0.0855	12	0.0540
Perry	287	0	0.0000	350	1.2069	27	0.0931	22	0.0758
VA 98R	279	1	0.0035	155	0.5556	9	0.0322	6	0.0215

[a] Total number of lesions counted for each part for entire seven week rating season beginning 8 Aug (86 DAP). Samples were taken on the center two rows of plots measuring 10.7 m long. Samples were 0.30 m in length and typically contained three to five plants.

[b] Mean of the number of lesions per plant, per part for a seven week rating season beginning 8 Aug (86 DAP).

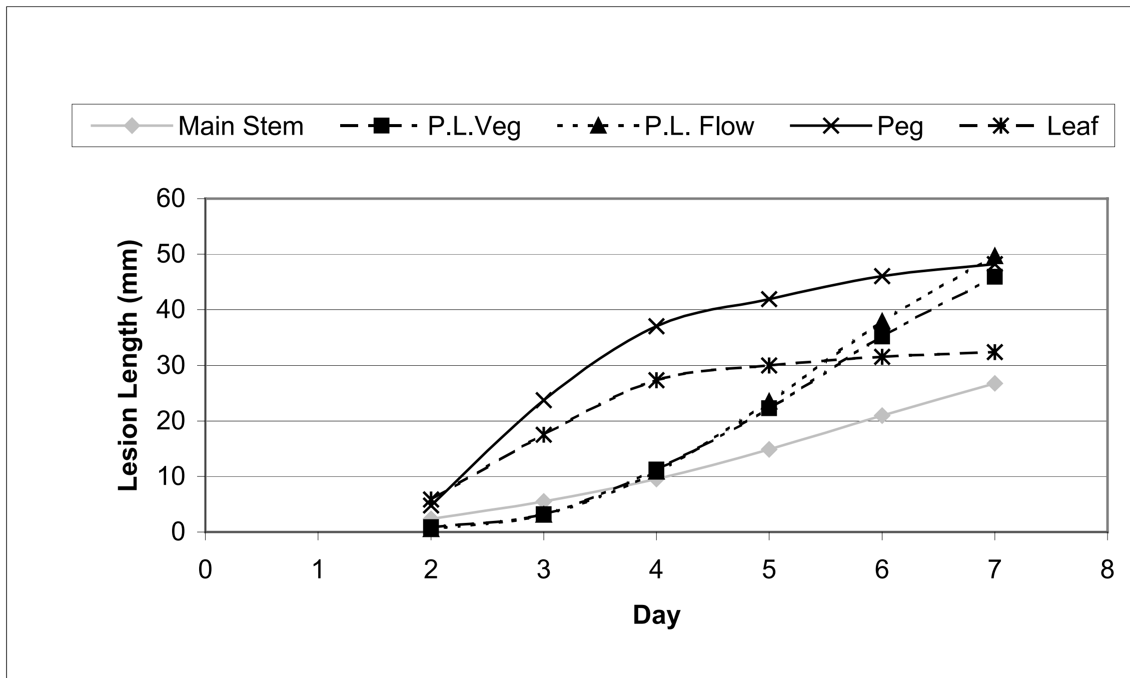

Figure 2.1. Actual lesion lengths observed on five plant parts over seven days after inoculation. All runs used the breeding line, GP-NC WS12. All inoculations were made with *S. minor* isolate #13.

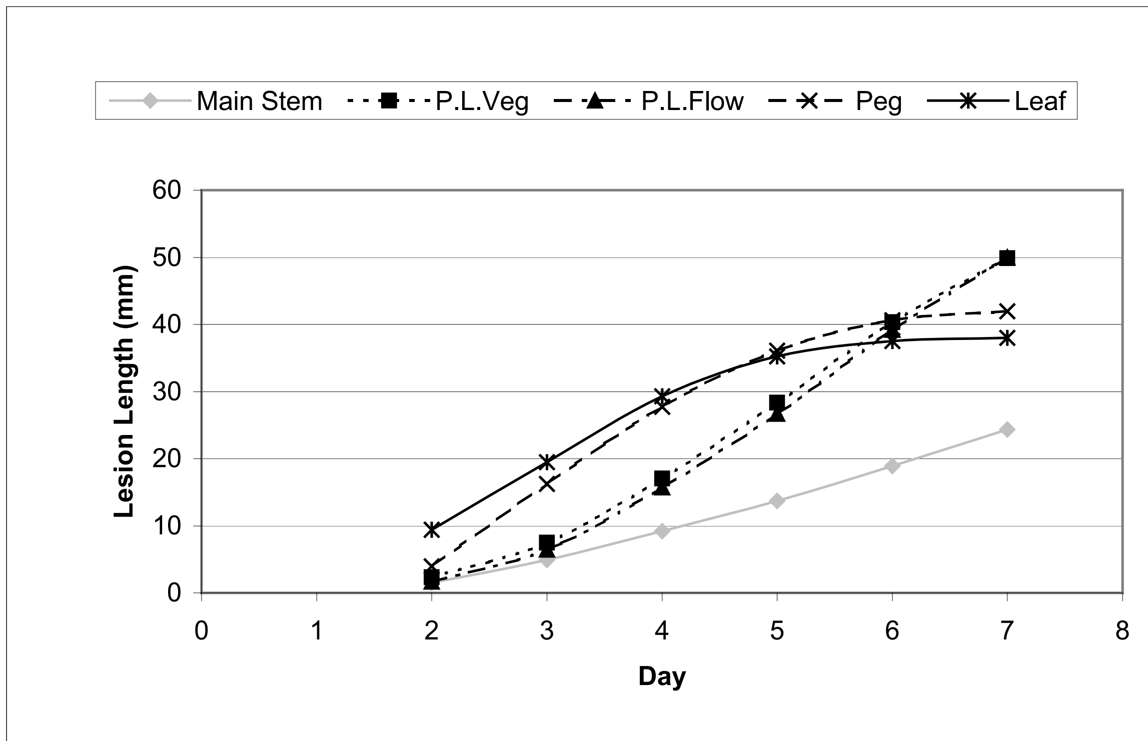

Figure 2.2. Actual lesion lengths observed on five plant parts over seven days after inoculation. All runs used the breeding line, N96076L. All inoculations were made with *S. minor* isolate #13.

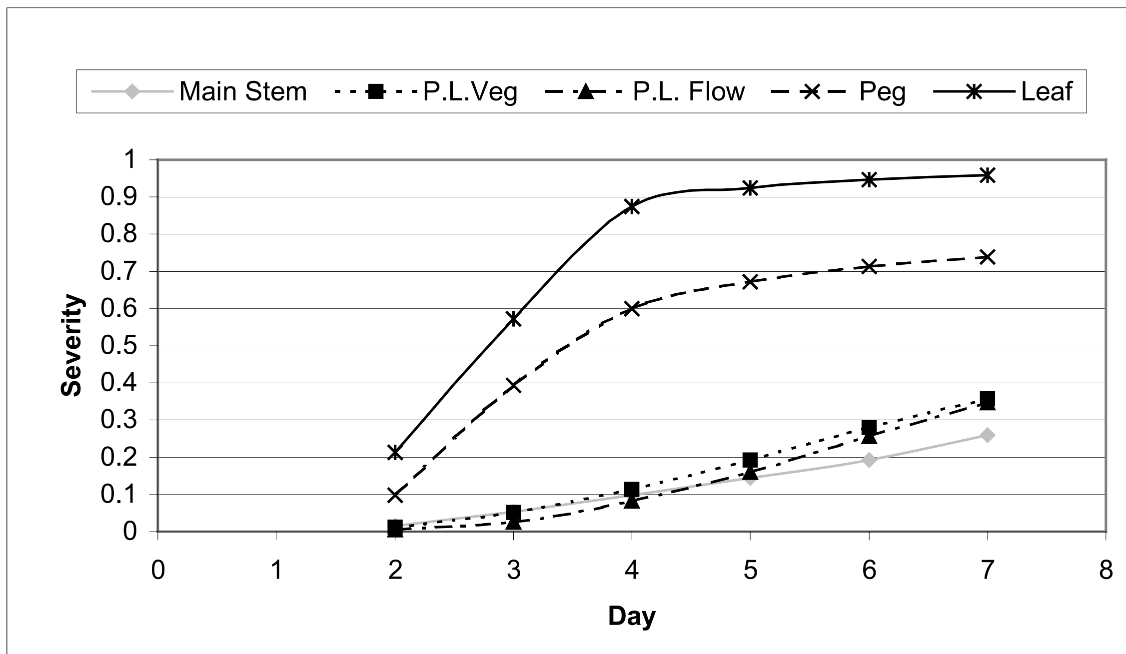

Figure 2.3. Daily proportions of five plant parts covered by a lesion for seven days after inoculation. Severity was measured as the proportion of part covered by a lesion. All runs used the cultivars, NC 7, NC 12C, and GP-NC WS12. Cultivar and breeding line susceptibility is as follows: NC 7 (susceptible), NC 12C (moderately susceptible), GP-NC WS12 (moderately resistant). All inoculations were made with *S. minor* isolate #13.

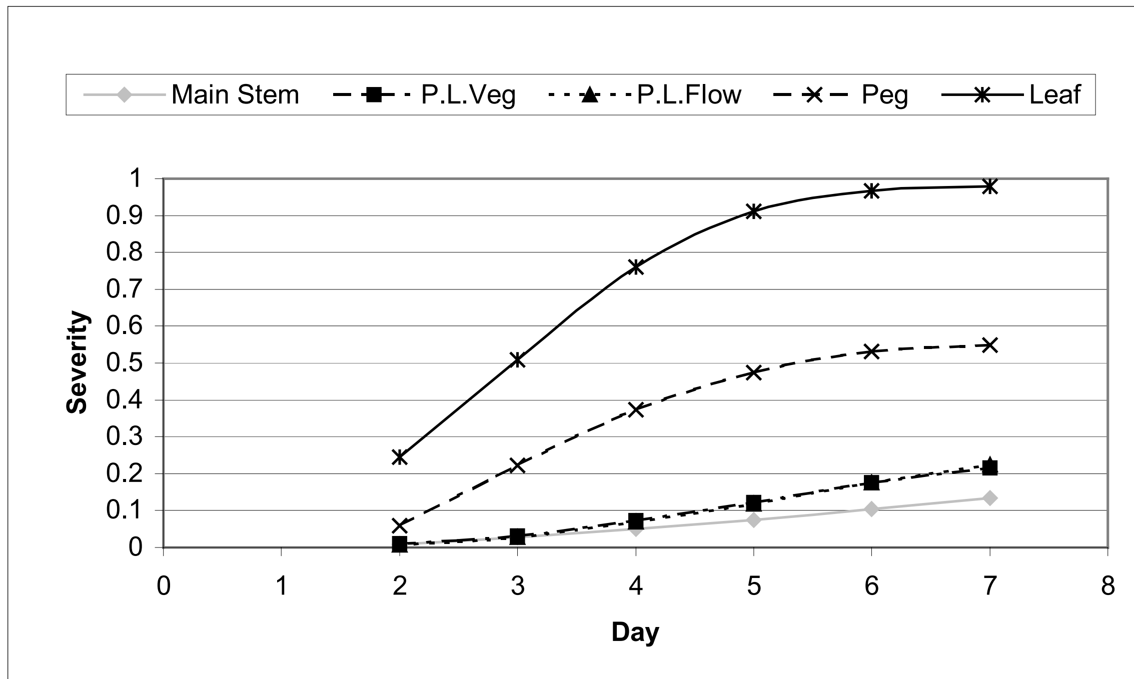

Figure 2.4. Daily proportions of five plant parts covered by a lesion for seven days after inoculation. Severity was measured as the proportion of part covered by a lesion. All runs used the cultivars, NC 7, NC 12C, and N96076L. Cultivar and breeding line susceptibility is as follows: NC 7 (susceptible), NC 12C (moderately susceptible), N96076L (moderately resistant). All inoculations were made with *S. minor* isolate #13.

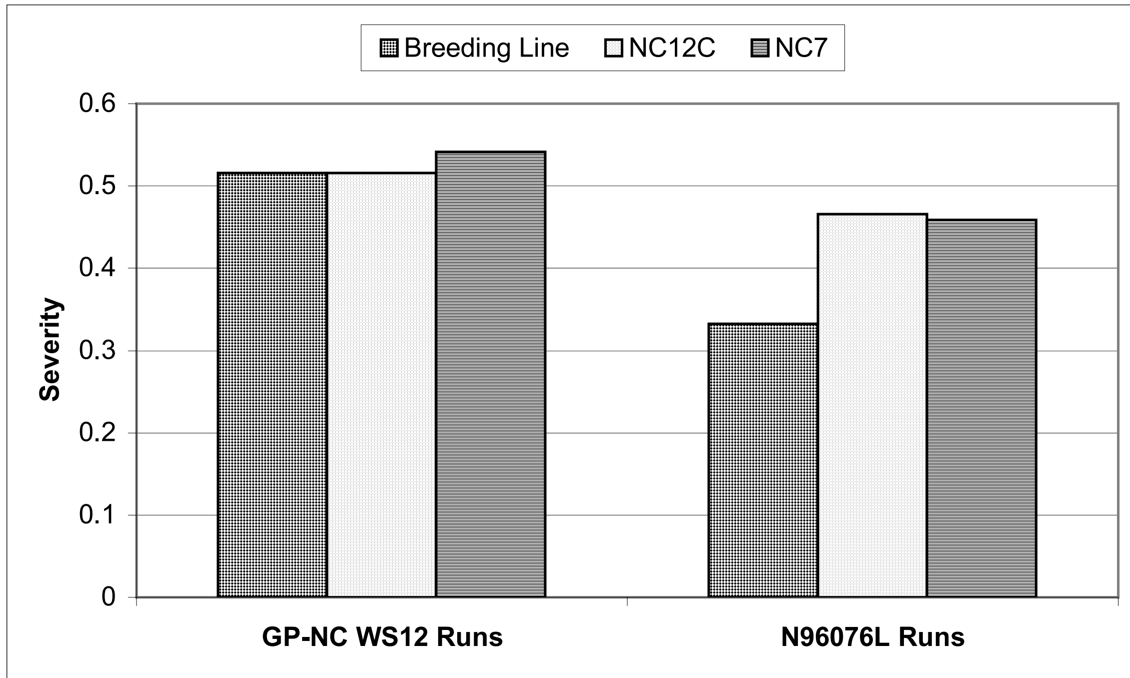

Figure 2.5. Main effects of cultivar on final severity ratings after leaflet inoculation with *S. minor* (isolate #13). Severity was measured as the proportion of part covered by a lesion. All runs used the cultivars NC 7 and NC 12C. Cultivar and breeding line susceptibility is as follows: NC 7 (susceptible), NC 12C (moderately susceptible), GP-NC WS12 and N96076L (moderately resistant).

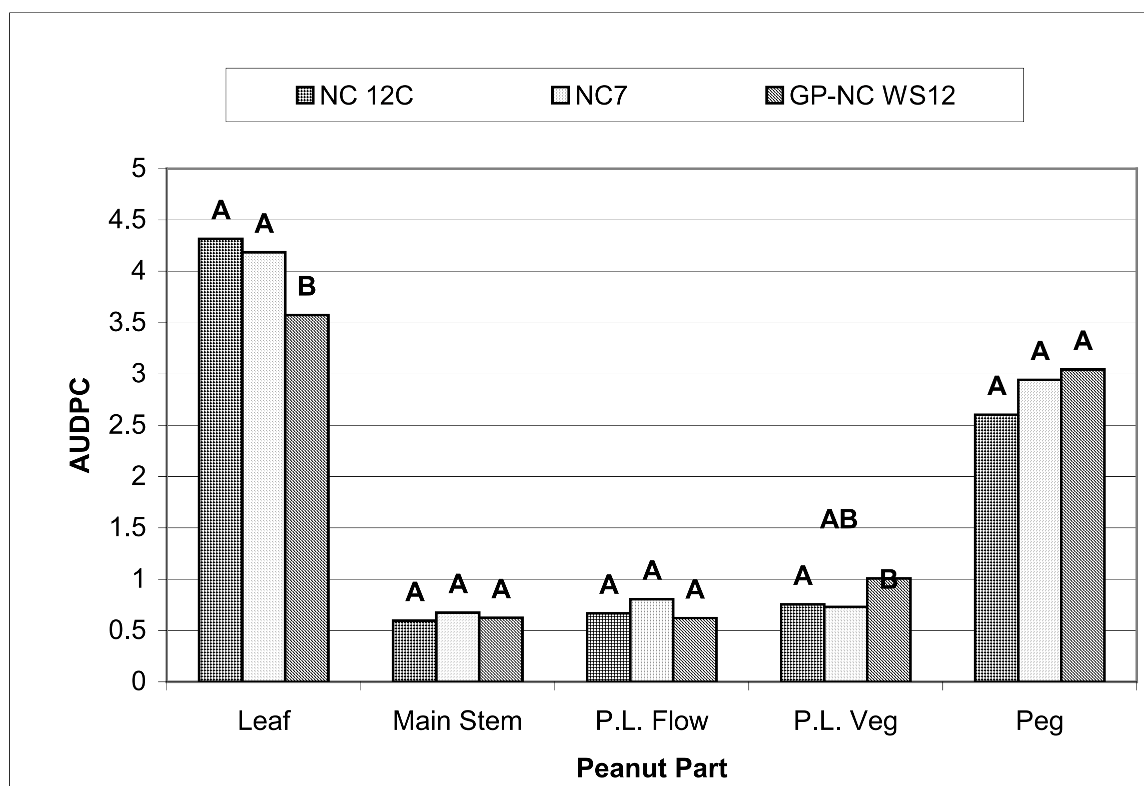

Figure 2.6. Area under the disease progress curve (AUDPC) and Waller-Duncan K-ratio t test for each plant part for two cultivars and the breeding line GP-NC WS12, for seven days after inoculation. Cultivar and breeding line susceptibility is as follows: NC 7 (susceptible), NC 12C (moderately susceptible), GP-NC WS12 (moderately resistant). All inoculations were made with *S. minor* isolate #13. Bars with different letter(s) are significantly different according to the Waller-Duncan K-ratio t test k=100. For leaf: MSD=0.35 R^2=0.52 CV=18.67. For main stem: MSD=0.34 R^2=0.42 CV=83.74. For P.L. Flow: MSD=0.31 R^2=0.30 CV=75.26. For P.L. Veg: MSD=0.26 R^2=0.45 CV=60.79. For peg: MSD=1.28 R^2=0.39 CV=59.41.

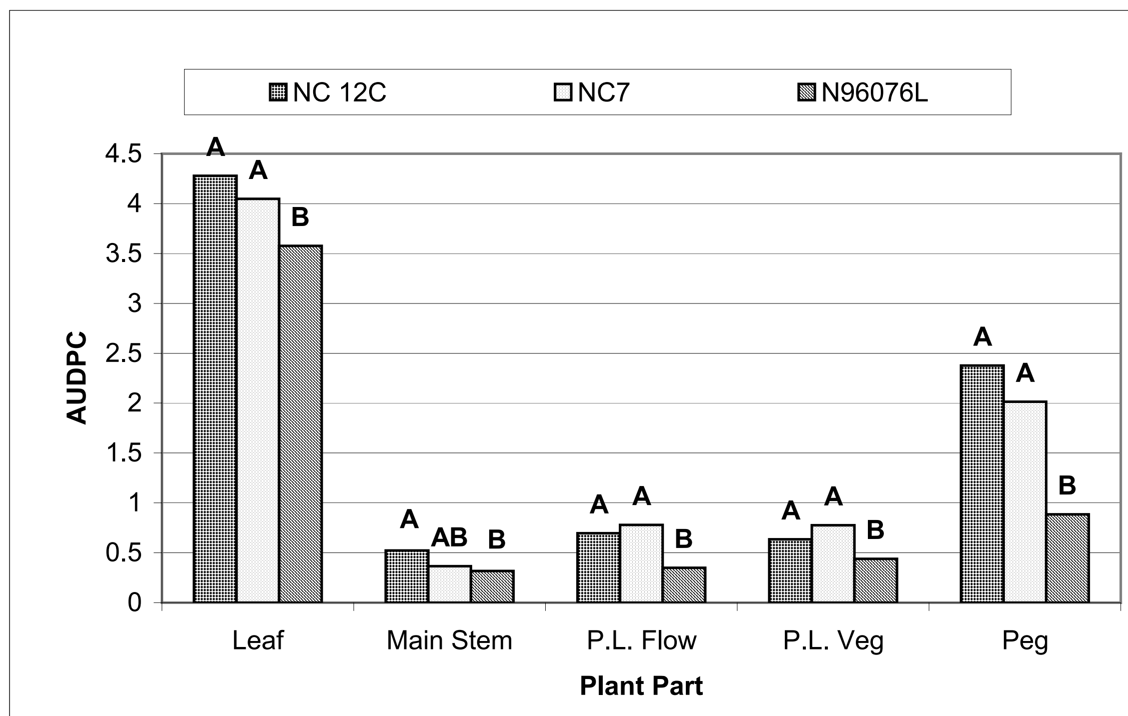

Figure 2.7. Area under the disease progress curve (AUDPC) and Waller-Duncan K-ratio t test for each plant part for two cultivars and the breeding line N96076L, for seven days after inoculation. Cultivar and breeding line susceptibility is as follows: NC 7 (susceptible), NC 12C (moderately susceptible), N96076L (moderately resistant). All inoculations were made with *S. minor* isolate #13. Bars with different letter(s) are significantly different according to the Waller-Duncan K-ratio t test k=100. For leaf: MSD=0.24 R^2=0.50 CV=16.74. For main stem: MSD=0.12 R^2=0.54 CV=95.94. For P.L. flow: MSD=0.12 R^2=0.64 CV=65.81. For P.L. veg: MSD=0.13 R^2=0.56 CV=67.82. For peg: MSD=0.59 R^2=0.58 CV=78.76.

Appendix I. Daily Air, Soil, and Plant Canopy Environment Parameters, 2002 and 2003.

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
1	2002	7	7	24.54	31.11	18.33	0.000	0.000	0.000	----	0	0.00	0.00	0.00	61.8	87.0	34.0	25.3	84.5
1	2002	7	8	26.04	32.22	20.56	0.000	0.000	0.000	----	0	0.00	0.00	0.00	59.2	85.0	32.0	27.0	68.2
1	2002	7	9	27.96	34.44	22.22	0.004	0.018	0.000	24.45	8	0.00	0.00	0.00	65.4	94.0	39.0	28.8	50.6
1	2002	7	10	29.05	32.78	26.67	0.003	0.024	0.000	28.78	5	5.08	0.21	3.56	67.3	80.0	50.0	29.7	100.0
1	2002	7	11	24.35	26.11	21.67	0.015	0.024	0.000	23.70	17	11.20	0.47	4.06	77.9	100.0	52.0	23.5	88.8
1	2002	7	12	21.55	27.22	15.00	0.000	0.000	0.000	----	0	0.00	0.00	0.00	62.6	87.0	40.0	21.6	74.5
1	2002	7	13	23.61	30.00	17.78	0.000	0.000	0.000	----	0	0.00	0.00	0.00	69.4	89.0	51.0	24.2	61.2
1	2002	7	14	25.44	27.78	23.33	0.017	0.024	0.000	25.59	21	19.80	0.83	4.32	84.9	96.0	65.0	26.7	100.0
1	2002	7	15	26.00	30.00	23.33	0.017	0.024	0.000	24.80	20	0.76	0.03	0.25	81.3	93.0	63.0	27.1	90.3
1	2002	7	16	28.26	34.44	23.33	0.008	0.024	0.000	25.16	12	0.00	0.00	0.00	72.1	97.0	37.0	28.9	74.7
1	2002	7	17	28.22	33.89	21.67	0.004	0.018	0.000	24.18	8	0.00	0.00	0.00	64.9	94.0	32.0	28.7	58.5
1	2002	7	18	28.84	35.00	23.89	0.000	0.000	0.000	----	0	0.00	0.00	0.00	67.5	88.0	45.0	30.1	42.5
1	2002	7	19	29.42	34.44	25.56	0.000	0.000	0.000	----	0	0.00	0.00	0.00	71.3	88.0	50.0	30.7	100.0
1	2002	7	20	27.52	31.11	23.89	0.018	0.024	0.000	26.62	22	18.80	0.78	6.35	78.0	93.0	60.0	28.9	100.0
1	2002	7	21	26.18	29.44	23.33	0.016	0.024	0.000	24.82	18	4.57	0.19	1.52	81.1	96.0	62.0	27.4	87.5
1	2002	7	22	26.62	32.22	21.11	0.009	0.024	0.000	23.82	13	0.00	0.00	0.00	77.9	97.0	54.0	27.0	72.7
1	2002	7	23	27.48	30.56	23.89	0.003	0.011	0.000	26.22	9	0.51	0.02	0.51	78.1	96.0	63.0	28.3	60.2
1	2002	7	24	26.25	28.33	24.44	0.010	0.024	0.000	26.30	20	15.50	0.65	6.86	82.3	89.0	73.0	27.6	100.0
1	2002	7	25	25.16	27.22	23.89	0.023	0.024	0.016	25.11	24	15.00	0.62	3.30	89.4	96.0	80.0	26.5	100.0
1	2002	7	26	24.88	27.78	23.33	0.023	0.024	0.021	24.86	24	13.50	0.56	4.57	87.6	92.0	79.0	26.2	100.0
1	2002	7	27	26.16	31.11	23.33	0.019	0.024	0.000	25.19	22	7.11	0.30	2.79	87.5	97.0	70.0	27.4	100.0
1	2002	7	28	28.01	35.00	23.89	0.013	0.023	0.000	24.85	17	0.00	0.00	0.00	77.3	91.0	53.0	29.6	84.1
1	2002	7	29	31.13	36.11	26.67	0.000	0.000	0.000	----	0	0.00	0.00	0.00	72.0	90.0	49.0	32.1	67.1
1	2002	7	30	31.41	36.11	26.67	0.000	0.000	0.000	----	0	0.00	0.00	0.00	66.8	87.0	44.0	32.3	50.2
1	2002	7	31	29.26	32.78	25.56	0.000	0.000	0.000	----	0	0.00	0.00	0.00	71.2	89.0	51.0	30.3	85.1
1	2002	8	1	28.98	33.89	23.89	0.002	0.011	0.000	25.22	7	0.00	0.00	0.00	67.8	92.0	40.0	29.5	70.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
1	2002	8	2	28.47	33.33	22.78	0.003	0.015	0.000	25.03	7	0.00	0.00	0.00	68.5	93.0	40.0	28.9	55.0
1	2002	8	3	27.41	32.22	22.22	0.002	0.010	0.000	23.99	7	0.00	0.00	0.00	69.8	92.0	48.0	28.2	40.5
1	2002	8	4	27.50	32.22	22.78	0.004	0.018	0.000	24.83	9	0.00	0.00	0.00	73.3	92.0	52.0	28.3	85.6
1	2002	8	5	27.62	32.78	22.22	0.007	0.024	0.000	24.57	12	0.00	0.00	0.00	73.2	96.0	50.0	28.2	71.0
1	2002	8	6	26.48	29.44	23.89	0.000	0.003	0.000	24.23	3	0.00	0.00	0.00	71.4	90.0	44.0	26.1	58.5
1	2002	8	7	22.87	26.67	18.89	0.000	0.000	0.000	----	0	0.00	0.00	0.00	55.8	75.0	36.0	22.7	44.0
1	2002	8	8	22.04	27.78	15.56	0.000	0.000	0.000	----	0	0.00	0.00	0.00	59.5	86.0	33.0	22.2	87.0
1	2002	8	9	22.45	28.89	15.56	0.000	0.004	0.000	15.94	4	0.00	0.00	0.00	60.6	90.0	33.0	22.7	74.2
1	2002	8	10	22.92	30.00	15.56	0.002	0.014	0.000	18.07	7	0.00	0.00	0.00	63.0	92.0	36.0	23.3	60.4
1	2002	8	11	24.03	32.22	16.11	0.002	0.011	0.000	18.36	6	0.00	0.00	0.00	65.2	94.0	36.0	24.6	46.2
1	2002	8	12	27.01	34.44	21.67	0.000	0.000	0.000	----	0	0.00	0.00	0.00	61.5	81.0	37.0	28.5	84.0
1	2002	8	13	28.87	36.11	23.33	0.000	0.000	0.000	----	0	0.00	0.00	0.00	60.1	82.0	33.0	30.3	67.0
1	2002	8	14	28.15	32.78	23.33	0.000	0.003	0.000	23.75	2	0.00	0.00	0.00	64.0	91.0	41.0	29.4	51.7
1	2002	8	15	27.82	31.67	24.44	0.002	0.024	0.000	29.67	4	2.79	0.12	2.79	73.5	89.0	59.0	29.1	88.0
1	2002	8	16	27.62	32.78	23.89	0.010	0.024	0.000	26.05	15	0.25	0.01	0.25	79.0	94.0	57.0	28.9	75.2
1	2002	8	17	28.87	34.44	25.00	0.008	0.019	0.000	26.03	14	0.51	0.02	0.51	72.5	87.0	49.0	30.3	91.4
1	2002	8	18	28.15	33.89	24.44	0.016	0.024	0.000	26.19	20	10.70	0.44	4.83	76.4	92.0	52.0	30.1	78.1
1	2002	8	19	28.80	35.00	23.33	0.009	0.022	0.000	25.95	16	0.00	0.00	0.00	72.3	94.0	44.0	29.8	63.6
1	2002	8	20	28.52	33.33	24.44	0.000	0.005	0.000	30.10	3	0.25	0.01	0.25	71.7	89.0	52.0	30.0	50.0
1	2002	8	21	26.81	30.56	23.89	0.008	0.024	0.000	25.25	13	0.00	0.00	0.00	76.8	92.0	56.0	28.2	86.8
1	2002	8	22	26.46	32.78	21.11	0.000	0.004	0.000	21.71	3	0.00	0.00	0.00	67.7	91.0	44.0	27.3	73.3
1	2002	8	23	29.54	35.56	24.44	0.000	0.000	0.000	----	0	0.00	0.00	0.00	66.1	90.0	40.0	30.4	57.9
1	2002	8	24	30.56	36.67	26.67	0.006	0.024	0.000	33.15	6	12.70	0.53	3.56	66.8	84.0	40.0	31.2	100.0
1	2002	8	25	26.97	31.67	23.33	0.015	0.024	0.000	24.97	17	4.32	0.18	2.29	77.6	93.0	55.0	29.2	87.6
1	2002	8	26	25.67	27.78	23.89	0.011	0.024	0.000	25.17	16	9.91	0.41	6.60	79.7	91.0	62.0	27.0	100.0
1	2002	8	27	24.42	26.67	22.78	0.023	0.024	0.018	24.40	24	17.00	0.71	2.79	85.3	91.0	74.0	25.7	100.0
1	2002	8	28	23.80	25.00	22.78	0.024	0.024	0.024	23.80	24	46.20	1.93	6.10	91.8	95.0	88.0	24.9	100.0
1	2002	8	29	23.38	25.00	22.22	0.023	0.024	0.018	23.35	24	22.10	0.92	4.57	92.5	97.0	84.0	24.3	100.0
1	2002	8	30	22.75	24.44	21.67	0.024	0.024	0.022	22.75	24	21.80	0.91	4.57	90.5	93.0	85.0	23.9	100.0
1	2002	8	31	22.73	24.44	21.67	0.024	0.024	0.022	22.72	24	20.30	0.85	2.79	91.9	96.0	84.0	23.8	100.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
1	2002	9	1	23.56	25.00	22.78	0.023	0.024	0.015	23.53	24	27.40	1.14	4.83	91.6	98.0	83.0	23.4	100.0
1	2002	9	2	21.97	24.44	20.56	0.019	0.024	0.006	21.56	24	0.00	0.00	0.00	85.6	92.0	74.0	22.2	95.7
1	2002	9	3	22.96	30.00	17.78	0.012	0.024	0.000	19.78	18	0.00	0.00	0.00	80.3	95.0	57.0	23.7	84.2
1	2002	9	4	27.15	33.33	22.78	0.009	0.024	0.000	24.20	14	0.00	0.00	0.00	78.2	96.0	54.0	28.3	71.0
1	2002	9	5	25.83	29.44	21.11	0.010	0.024	0.000	23.64	13	2.54	0.11	1.78	72.8	90.0	48.0	26.1	58.6
1	2002	9	6	22.82	27.22	18.33	0.007	0.024	0.000	20.76	11	0.00	0.00	0.00	76.2	95.0	54.0	23.9	47.8
1	2002	9	7	22.34	26.67	17.22	0.000	0.000	0.000	----	0	0.00	0.00	0.00	74.2	88.0	56.0	22.7	89.8
1	2002	9	8	23.70	27.78	20.56	0.006	0.021	0.000	22.12	12	0.00	0.00	0.00	79.5	93.0	62.0	24.7	80.7
1	2002	9	9	23.89	27.22	21.11	0.008	0.024	0.000	22.22	13	0.00	0.00	0.00	81.0	94.0	63.0	25.0	70.6
1	2002	9	10	25.21	26.11	24.44	0.023	0.024	0.000	25.22	23	20.10	0.84	2.79	89.4	97.0	80.0	25.9	100.0
1	2002	9	11	26.25	32.22	21.11	0.014	0.024	0.000	23.48	15	0.00	0.00	0.00	70.5	92.0	42.0	25.6	86.4
1	2002	9	12	21.34	25.56	15.00	0.000	0.000	0.000	----	0	0.00	0.00	0.00	56.6	79.0	42.0	22.1	75.4
1	2002	9	13	19.84	27.22	12.78	0.004	0.018	0.000	16.16	8	0.00	0.00	0.00	69.3	94.0	38.0	20.4	64.8
1	2002	9	14	23.45	28.33	20.00	0.006	0.022	0.000	23.22	11	0.00	0.00	0.00	82.0	95.0	68.0	24.7	55.9
1	2002	9	15	25.21	29.44	23.33	0.020	0.024	0.000	24.54	22	3.56	0.15	1.78	84.1	93.0	63.0	27.0	46.9
1	2002	9	16	24.68	26.11	23.33	0.024	0.024	0.022	24.68	24	29.20	1.22	6.35	91.5	97.0	86.0	25.5	100.0
1	2002	9	17	23.96	27.78	21.11	0.016	0.024	0.000	22.60	18	0.00	0.00	0.00	82.7	99.0	54.0	23.8	90.1
1	2002	9	18	22.04	27.22	17.78	0.010	0.024	0.000	19.78	14	0.00	0.00	0.00	79.4	96.0	54.0	23.3	80.7
1	2002	9	19	22.34	27.78	17.22	0.006	0.019	0.000	19.37	11	0.00	0.00	0.00	76.4	96.0	52.0	23.1	70.9
1	2002	9	20	23.36	28.33	18.89	0.009	0.024	0.000	21.21	14	0.00	0.00	0.00	78.6	97.0	54.0	24.2	61.8
1	2002	9	21	23.33	28.89	17.78	0.009	0.024	0.000	21.28	13	0.00	0.00	0.00	78.9	97.0	54.0	23.9	52.4
1	2002	9	22	23.94	29.44	18.33	0.007	0.024	0.000	21.63	12	0.00	0.00	0.00	80.5	96.0	58.0	24.6	90.9
1	2002	9	23	23.70	25.56	22.22	0.014	0.024	0.000	23.38	22	0.00	0.00	0.00	87.0	97.0	73.0	25.0	85.7
1	2002	9	24	22.66	26.67	19.44	0.000	0.000	0.000	----	0	0.00	0.00	0.00	70.2	82.0	50.0	23.3	75.8
1	2002	9	25	21.60	25.56	18.89	0.000	0.000	0.000	----	0	0.00	0.00	0.00	76.0	87.0	65.0	23.1	93.6
1	2002	9	26	22.48	25.00	20.56	0.023	0.024	0.000	22.49	23	26.70	1.11	3.81	91.5	96.0	78.0	23.6	100.0
1	2002	9	27	26.27	30.56	23.89	0.016	0.024	0.000	24.70	19	0.00	0.00	0.00	84.7	95.0	67.0	27.1	91.6
1	2003	7	7	24.58	31.11	18.89	0.000	0.000	0.000	----	0	0.00	0.00	0.00	61.3	86.0	0.0	30.4	53.0
1	2003	7	8	26.30	32.78	21.11	0.000	0.000	0.000	----	0	0.00	0.00	0.00	58.0	82.0	0.0	29.9	51.0
1	2003	7	9	28.22	35.00	22.78	0.000	0.000	0.000	23.80	0	0.00	0.00	0.00	64.2	91.0	0.0	30.9	48.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
1	2003	7	10	29.07	32.78	26.67	0.125	1.000	0.000	28.61	3	5.08	0.21	3.56	66.8	79.0	0.0	28.9	54.0
1	2003	7	11	24.26	26.11	21.67	0.667	1.000	0.000	23.64	16	11.20	0.47	4.06	77.5	99.0	0.0	29.2	54.0
1	2003	7	12	21.37	27.22	14.44	0.000	0.000	0.000	----	0	0.00	0.00	0.00	63.0	88.0	0.0	28.0	54.0
1	2003	7	13	23.47	30.00	17.22	0.000	0.000	0.000	----	0	0.00	0.00	0.00	69.3	89.0	0.0	27.0	54.0
1	2003	7	14	25.37	27.78	23.33	0.833	1.000	0.000	25.49	20	20.60	0.86	4.06	85.4	95.0	0.0	25.4	100.0
1	2003	7	15	25.90	30.00	23.33	0.750	1.000	0.000	24.47	18	0.51	0.02	0.25	81.1	94.0	0.0	25.8	85.0
1	2003	7	16	28.31	34.44	23.33	0.417	1.000	0.000	25.19	10	0.00	0.00	0.00	71.5	96.0	0.0	28.1	66.0
1	2003	7	17	28.17	33.89	21.67	0.208	1.000	0.000	24.12	5	0.00	0.00	0.00	64.8	94.0	0.0	27.7	54.0
1	2003	7	18	24.26	30.71	20.86	0.430	1.000	0.000	----	12	6.35	0.25	6.35	67.7	73.6	0.0	24.9	54.0
1	2003	7	19	24.08	28.44	21.02	0.380	1.000	0.000	22.15	12	0.00	0.00	0.00	67.0	73.0	0.0	24.2	54.0
1	2003	7	20	24.23	29.12	19.68	0.520	1.000	0.000	22.72	14	0.00	0.00	0.00	66.3	73.8	0.0	24.5	54.0
1	2003	7	21	25.47	30.23	19.97	0.370	1.000	0.000	21.20	9	0.00	0.00	0.00	65.5	73.5	0.0	25.1	53.0
1	2003	7	22	26.47	30.69	23.78	0.300	1.000	0.000	21.48	8	0.00	0.00	0.00	64.6	73.0	0.0	25.8	51.0
1	2003	7	23	23.67	27.92	21.12	0.300	1.000	0.000	23.99	8	768.00	33.30	574.00	67.3	73.9	0.0	23.8	58.0
1	2003	7	24	23.09	28.06	20.46	0.620	1.000	0.000	21.76	16	25.90	1.02	19.30	68.9	73.6	0.0	23.4	63.0
1	2003	7	25	23.32	28.02	18.94	0.450	1.000	0.000	21.32	12	0.00	0.00	0.00	67.3	73.8	0.0	23.5	54.0
1	2003	7	26	23.56	31.39	18.67	0.580	1.000	0.000	20.10	16	232.00	10.20	232.00	68.5	74.2	0.0	23.7	54.0
1	2003	7	27	25.68	30.36	21.58	0.380	1.000	0.000	20.64	10	0.00	0.00	0.00	66.1	73.9	0.0	24.9	53.0
1	2003	7	28	26.44	30.87	22.84	0.230	1.000	0.000	22.66	7	0.00	0.00	0.00	66.7	73.0	0.0	25.6	51.0
1	2003	7	29	24.93	29.68	22.38	0.400	1.000	0.000	23.54	11	70.90	3.30	70.90	70.1	73.2	0.0	24.9	48.0
1	2003	7	30	24.86	28.76	22.53	0.226	1.000	0.000	24.49	7	0.00	0.00	0.00	69.7	73.8	0.0	24.8	54.0
1	2003	7	31	24.73	29.93	21.41	0.213	1.000	0.000	22.80	6	0.00	0.00	0.00	68.6	73.1	0.0	24.7	54.0
1	2003	8	1	25.80	29.96	22.86	0.420	1.000	0.000	23.02	11	0.00	0.00	0.00	68.3	73.6	0.0	25.2	53.0
1	2003	8	2	26.07	30.48	22.64	0.298	1.000	0.000	23.60	8	0.00	0.00	0.00	66.8	73.6	0.0	25.5	51.0
1	2003	8	3	24.96	28.80	22.69	0.648	1.000	0.000	23.08	17	206.00	8.60	116.00	70.7	73.6	0.0	25.0	54.0
1	2003	8	4	24.64	29.05	22.48	0.668	1.000	0.000	24.08	18	71.00	2.96	32.30	70.7	73.7	0.0	24.7	54.0
1	2003	8	5	24.64	28.75	21.31	0.517	1.000	0.000	23.36	14	19.40	0.84	12.90	77.4	97.7	0.0	24.4	54.0
1	2003	8	6	24.19	29.74	20.37	0.492	1.000	0.000	22.66	14	45.20	1.88	32.30	93.3	99.5	0.0	24.3	89.0
1	2003	8	7	23.49	26.63	21.96	0.804	1.000	0.000	21.70	22	561.00	24.40	110.00	97.4	99.4	0.0	24.0	100.0
1	2003	8	8	23.59	28.12	21.23	0.620	1.000	0.000	23.05	17	394.00	17.10	316.00	97.3	100.0	0.0	23.8	100.0
1	2003	8	9	24.05	27.25	22.42	0.647	1.000	0.000	22.32	17	277.00	11.60	148.00	96.6	99.9	0.0	24.0	100.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
1	2003	8	10	23.73	26.12	22.16	0.547	1.000	0.000	23.28	16	58.10	2.42	51.60	96.3	99.5	0.0	24.0	95.0
1	2003	8	11	24.83	29.11	21.64	0.532	1.000	0.000	22.91	14	277.00	11.60	123.00	93.2	99.5	0.0	24.4	100.0
1	2003	8	12	25.25	30.73	20.61	0.542	1.000	0.000	22.67	14	0.00	0.00	0.00	90.8	99.5	0.0	24.7	85.0
1	2003	8	13	24.49	31.80	20.84	0.726	1.000	0.000	22.47	18	38.70	1.61	32.30	93.0	99.8	0.0	24.4	71.0
1	2003	8	14	24.71	31.01	20.64	0.544	1.000	0.000	22.67	14	0.00	0.00	0.00	92.6	99.8	0.0	24.4	85.0
1	2003	8	15	25.32	31.76	19.31	0.523	1.000	0.000	21.93	14	0.00	0.00	0.00	89.4	99.9	0.0	24.5	68.0
1	2003	8	16	23.89	29.91	21.46	0.580	1.000	0.000	21.38	15	6.45	0.29	6.45	93.2	99.4	0.0	24.1	54.0
1	2003	8	17	23.63	28.61	19.59	0.560	1.000	0.000	22.73	15	12.90	0.54	6.45	92.3	99.1	0.0	23.8	54.0
1	2003	8	18	23.75	30.91	19.09	0.527	1.000	0.000	21.33	13	0.00	0.00	0.00	88.9	99.9	0.0	23.6	53.0
1	2003	8	19	23.28	28.66	19.24	0.554	1.000	0.000	20.66	15	0.00	0.00	0.00	88.8	99.3	0.0	23.4	51.0
1	2003	8	20	23.57	30.12	18.07	0.573	1.000	0.000	20.98	15	0.00	0.00	0.00	89.0	100.2	0.0	23.3	48.0
1	2003	8	21	24.75	31.18	18.48	0.508	1.000	0.000	20.34	13	0.00	0.00	0.00	86.9	100.0	0.0	24.0	43.0
1	2003	8	22	25.85	30.74	22.31	0.578	1.000	0.000	20.65	14	0.00	0.00	0.00	88.7	99.6	0.0	25.0	36.0
1	2003	8	23	24.20	28.98	19.84	0.564	1.000	0.000	23.34	16	6.45	0.27	6.45	88.9	98.8	0.0	24.2	28.0
1	2003	8	24	21.06	28.10	15.36	0.560	1.000	0.000	22.20	14	0.00	0.00	0.00	82.4	99.7	0.0	22.1	19.0
1	2003	8	25	21.50	29.12	13.74	0.550	1.000	0.000	17.48	14	0.00	0.00	0.00	85.4	99.4	0.0	21.8	12.0
1	2003	8	26	25.71	32.53	20.61	0.552	1.000	0.000	16.98	14	0.00	0.00	0.00	86.8	99.4	0.0	24.3	7.0
1	2003	8	27	27.16	34.74	22.32	0.532	1.000	0.000	22.13	14	0.00	0.00	0.00	85.2	97.2	0.0	25.5	4.0
1	2003	8	28	27.55	33.65	22.80	0.431	1.000	0.000	23.57	11	0.00	0.00	0.00	87.3	98.4	0.0	26.2	2.0
1	2003	8	29	26.79	32.62	22.74	0.232	1.000	0.000	24.30	7	0.00	0.00	0.00	85.1	98.8	0.0	26.0	1.0
1	2003	8	30	26.83	32.30	22.99	0.123	1.000	0.000	23.21	3	0.00	0.00	0.00	78.9	98.5	0.0	26.0	1.0
1	2003	8	31	22.48	24.44	21.11	1.000	1.000	1.000	23.27	24	16.30	0.68	1.52	92.3	96.0	0.0	----	1.0
1	2003	9	1	24.87	31.98	18.97	0.397	1.000	0.000	21.15	10	6.45	0.27	6.45	91.8	139.5	0.0	24.5	54.0
1	2003	9	2	26.01	31.26	21.87	0.547	1.000	0.000	20.53	14	0.00	0.00	0.00	80.2	97.6	0.0	25.2	54.0
1	2003	9	3	26.24	31.05	22.28	0.538	1.000	0.000	23.21	14	58.10	2.42	51.60	88.7	98.9	0.0	25.3	53.0
1	2003	9	4	27.11	33.33	22.78	0.458	1.000	0.000	23.96	11	0.00	0.00	0.00	78.3	95.0	0.0	----	64.0
1	2003	9	5	22.35	29.48	16.93	0.494	1.000	0.000	21.30	13	6.45	0.27	6.45	89.9	102.0	0.0	23.1	79.0
1	2003	9	6	18.74	22.93	14.03	0.611	1.000	0.000	19.49	16	0.00	0.00	0.00	76.6	97.3	0.0	20.7	66.0
1	2003	9	7	19.93	25.40	14.38	0.410	1.000	0.000	16.69	10	0.00	0.00	0.00	79.9	96.5	0.0	20.6	54.0
1	2003	9	8	21.76	26.83	19.04	0.093	1.000	0.000	15.68	4	25.80	1.12	19.40	78.2	92.7	0.0	21.7	54.0
1	2003	9	9	22.24	26.42	19.37	0.343	1.000	0.000	19.91	11	0.00	0.00	0.00	67.4	98.8	0.0	21.9	53.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C) Avg.	Air Temp. (C) Max.	Air Temp. (C) Min.	Leaf Wetness (LW)[b] Avg.	Leaf Wetness (LW)[b] Max.	Leaf Wetness (LW)[b] Min.	Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d] Total	Precipitation (mm)[d] Avg.	Precipitation (mm)[d] Max.	Relative Humidity Avg.	Relative Humidity Max.	Relative Humidity Min.	Soil Parameters Temp (C)	Soil Parameters Moist %
1	2003	9	10	19.97	23.55	14.27	0.149	1.000	0.000	20.01	4	0.00	0.00	0.00	62.7	91.7	0.0	20.6	51.0
1	2003	9	11	18.88	25.14	12.64	0.507	1.000	0.000	15.17	14	0.00	0.00	0.00	64.0	87.0	0.0	19.4	48.0
1	2003	9	12	21.37	25.56	15.00	0.000	0.000	0.000	15.29	0	0.00	0.00	0.00	56.0	79.0	0.0	----	100.0
1	2003	9	13	21.22	22.70	19.91	0.421	1.000	0.000	16.51	11	6.45	0.27	6.45	84.1	98.2	0.0	21.5	100.0
1	2003	9	14	22.64	29.97	16.29	0.529	1.000	0.000	20.81	14	0.00	0.00	0.00	80.1	101.3	0.0	21.9	89.0
1	2003	9	15	22.84	27.70	18.61	0.588	1.000	0.000	19.07	15	0.00	0.00	0.00	78.3	102.2	0.0	22.7	80.0
2	2003	7	29	26.32	29.44	25.00	0.500	1.000	0.000	25.68	12	1.78	0.07	1.78	83.5	95.0	0.0	28.6	48.0
2	2003	7	30	24.49	27.22	23.33	0.750	1.000	0.000	24.73	18	12.40	0.52	3.30	88.7	94.0	0.0	26.4	54.0
2	2003	7	31	24.81	28.89	22.22	0.875	1.000	0.000	23.97	21	0.51	0.02	0.51	86.5	95.0	0.0	26.1	54.0
2	2003	8	1	26.90	31.11	24.44	0.708	1.000	0.000	25.34	17	0.00	0.00	0.00	82.3	93.0	0.0	28.2	53.0
2	2003	8	2	27.50	31.11	24.44	0.292	1.000	0.000	26.21	7	3.56	0.15	3.56	79.4	93.0	0.0	28.6	51.0
2	2003	8	3	26.48	28.33	24.44	1.000	1.000	1.000	26.40	24	11.20	0.47	3.81	84.0	92.0	0.0	27.7	54.0
2	2003	8	4	25.79	28.89	23.89	0.875	1.000	0.000	25.33	21	11.40	0.48	6.60	86.3	94.0	0.0	27.1	54.0
2	2003	8	5	25.58	29.44	23.33	0.708	1.000	0.000	24.13	17	0.25	0.01	0.25	82.4	94.0	0.0	27.1	54.0
2	2003	8	6	25.39	28.33	22.22	0.750	1.000	0.000	24.86	18	14.50	0.60	5.84	83.7	93.0	0.0	26.2	54.0
2	2003	8	7	24.86	27.78	23.33	0.750	1.000	0.000	24.58	18	22.10	0.92	3.81	87.8	95.0	0.0	26.4	100.0
2	2003	8	8	24.81	27.78	22.78	0.833	1.000	0.000	24.78	20	33.30	1.39	20.30	89.1	96.0	0.0	26.1	100.0
2	2003	8	9	25.28	27.22	24.44	1.000	1.000	1.000	25.17	24	13.20	0.55	2.29	88.7	94.0	0.0	26.7	100.0
2	2003	8	10	25.19	26.67	23.89	1.000	1.000	1.000	25.15	24	4.57	0.19	2.03	87.0	93.0	0.0	26.1	90.0
2	2003	8	11	25.30	28.89	23.33	0.833	1.000	0.000	24.43	20	16.50	0.69	5.59	87.1	96.0	0.0	26.5	100.0
2	2003	8	12	26.44	30.56	23.33	0.458	1.000	0.000	25.01	11	2.03	0.08	1.02	83.7	95.0	0.0	27.4	85.0
2	2003	8	13	26.44	30.56	23.33	0.792	1.000	0.000	26.55	19	0.76	0.03	0.25	84.0	96.0	0.0	27.7	71.0
2	2003	8	14	26.81	31.11	23.33	0.833	1.000	0.000	26.09	20	12.20	0.51	4.57	82.7	96.0	0.0	27.9	84.0
2	2003	8	15	27.57	32.78	22.78	0.458	1.000	0.000	25.01	11	0.25	0.01	0.25	79.6	96.0	0.0	28.3	67.0
2	2003	8	16	26.90	30.00	24.44	0.292	1.000	0.000	25.73	7	0.00	0.00	0.00	81.6	92.0	0.0	28.5	54.0
2	2003	8	17	25.39	29.44	23.33	0.625	1.000	0.000	24.53	15	12.70	0.53	5.59	85.0	95.0	0.0	27.3	70.0
2	2003	8	18	24.47	28.33	21.67	0.500	1.000	0.000	23.38	12	0.00	0.00	0.00	83.8	94.0	0.0	25.9	56.0
2	2003	8	19	24.98	28.33	22.22	0.583	1.000	0.000	23.85	14	0.00	0.00	0.00	83.7	95.0	0.0	26.1	54.0
2	2003	8	20	25.07	29.44	21.11	0.375	1.000	0.000	24.16	9	0.00	0.00	0.00	81.0	95.0	0.0	25.8	54.0
2	2003	8	21	26.39	31.11	21.67	0.458	1.000	0.000	24.19	11	0.00	0.00	0.00	79.3	95.0	0.0	26.9	53.0
2	2003	8	22	27.80	32.78	23.89	0.333	1.000	0.000	25.84	8	0.00	0.00	0.00	79.0	94.0	0.0	28.9	51.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
2	2003	8	23	26.32	29.44	22.78	0.083	1.000	0.000	24.61	2	0.00	0.00	0.00	78.6	95.0	0.0	27.4	48.0
2	2003	8	24	23.61	27.22	19.44	0.000	0.000	0.000	----	0	0.00	0.00	0.00	67.8	88.0	0.0	24.4	43.0
2	2003	8	25	23.10	30.00	16.67	0.000	0.000	0.000	----	0	0.00	0.00	0.00	72.4	90.0	0.0	23.6	36.0
2	2003	8	26	27.01	32.78	22.22	0.375	1.000	0.000	24.85	9	0.00	0.00	0.00	76.9	94.0	0.0	27.8	28.0
2	2003	8	27	28.98	35.00	24.44	0.000	0.000	0.000	----	0	0.00	0.00	0.00	73.2	89.0	0.0	30.2	19.0
2	2003	8	28	28.61	32.78	25.00	0.000	0.000	0.000	25.80	0	0.00	0.00	0.00	77.8	91.0	0.0	30.1	12.0
2	2003	8	29	28.82	33.89	25.00	0.000	0.000	0.000	25.32	0	0.00	0.00	0.00	76.9	91.0	0.0	30.1	7.0
2	2003	8	30	29.31	33.89	25.56	0.000	0.000	0.000	----	0	0.00	0.00	0.00	71.3	87.0	0.0	30.6	4.0
2	2003	8	31	26.55	29.44	23.89	0.292	1.000	0.000	25.72	7	0.00	0.00	0.00	82.5	95.0	0.0	28.2	2.0
2	2003	9	1	26.20	32.22	22.78	0.708	1.000	0.000	24.01	17	5.84	0.24	2.29	82.0	96.0	0.0	27.8	54.0
2	2003	9	2	27.82	32.22	23.89	0.208	1.000	0.000	25.56	5	0.00	0.00	0.00	74.8	94.0	0.0	28.9	54.0
2	2003	9	3	26.97	30.00	24.44	0.125	1.000	0.000	28.02	3	3.56	0.15	3.56	80.1	90.0	0.0	28.4	53.0
2	2003	9	4	26.71	31.67	23.33	0.792	1.000	0.000	25.58	19	25.10	1.05	7.11	81.4	92.0	0.0	28.0	69.0
2	2003	9	5	23.31	26.67	20.56	0.667	1.000	0.000	21.89	16	12.40	0.52	4.83	79.5	96.0	0.0	25.2	84.0
2	2003	9	6	20.83	23.89	17.22	0.000	0.000	0.000	----	0	0.00	0.00	0.00	76.1	89.0	0.0	21.7	70.0
2	2003	9	7	21.11	26.11	16.67	0.000	0.000	0.000	18.14	0	0.00	0.00	0.00	78.3	92.0	0.0	21.7	58.0
2	2003	9	8	22.48	25.56	20.00	0.000	0.000	0.000	----	0	0.00	0.00	0.00	76.1	84.0	0.0	23.6	54.0
2	2003	9	9	23.10	26.11	21.11	0.458	1.000	0.000	22.34	11	2.03	0.08	1.02	80.4	89.0	0.0	24.2	54.0
2	2003	9	10	21.50	23.33	20.00	0.000	0.000	0.000	----	0	0.00	0.00	0.00	75.2	88.0	0.0	22.8	53.0
2	2003	9	11	19.98	25.00	16.11	0.000	0.000	0.000	----	0	0.00	0.00	0.00	72.1	86.0	0.0	20.9	51.0
2	2003	9	12	22.06	23.89	20.56	0.750	1.000	0.000	22.34	18	43.20	1.80	5.84	87.5	93.0	0.0	23.1	100.0
2	2003	9	13	22.50	23.89	21.67	1.000	1.000	1.000	22.38	24	21.80	0.91	6.86	90.0	96.0	0.0	23.5	100.0
2	2003	9	14	22.96	27.22	18.89	0.750	1.000	0.000	21.50	18	0.00	0.00	0.00	84.8	96.0	0.0	23.2	89.0
2	2003	9	15	23.82	27.78	21.67	0.750	1.000	0.000	23.35	18	2.79	0.12	1.78	87.3	97.0	0.0	25.4	81.0
2	2003	9	16	23.13	26.67	20.00	0.542	1.000	0.000	21.23	13	0.00	0.00	0.00	72.3	93.0	0.0	24.2	65.0
2	2003	9	17	21.00	25.56	17.78	0.000	0.000	0.000	----	0	0.00	0.00	0.00	70.8	82.0	0.0	22.6	54.0
2	2003	9	18	22.31	22.78	21.11	0.875	1.000	0.000	22.33	21	80.00	3.33	7.87	89.2	96.0	0.0	23.1	100.0
2	2003	9	19	24.70	29.44	20.56	0.500	1.000	0.000	21.80	12	1.78	0.07	0.76	67.0	90.0	0.0	24.6	80.0
2	2003	9	20	23.08	28.89	17.22	0.000	0.000	0.000	----	0	0.00	0.00	0.00	71.8	90.0	0.0	23.9	67.0

Appendix I (continued)

Site[a]	Season	Month	Day	Air Temp. (C)			Leaf Wetness (LW)[b]			Temp. During Leaf Wetness (C)[c]	Leaf Wetness Hours	Precipitation (mm)[d]			Relative Humidity			Soil Parameters	
				Avg.	Max.	Min.	Avg.	Max.	Min.			Total	Avg.	Max.	Avg.	Max.	Min.	Temp (C)	Moist %
2	2003	9	21	22.73	28.33	18.33	0.333	1.000	0.000	20.89	8	0.00	0.00	0.00	77.4	92.0	0.0	24.2	54.0
2	2003	9	22	23.40	28.33	20.00	0.375	1.000	0.000	22.00	9	0.00	0.00	0.00	80.0	92.0	0.0	24.9	54.0
2	2003	9	23	24.12	26.11	21.67	0.667	1.000	0.000	23.60	16	21.10	0.88	5.33	78.5	91.0	0.0	25.0	93.0
2	2003	9	24	20.88	26.11	16.67	0.292	1.000	0.000	17.51	7	0.00	0.00	0.00	74.3	91.0	0.0	22.1	80.0
2	2003	9	25	21.67	27.78	16.11	0.292	1.000	0.000	19.21	7	0.00	0.00	0.00	73.8	92.0	0.0	22.4	67.0
2	2003	9	26	22.18	27.78	16.67	0.208	1.000	0.000	19.32	5	0.00	0.00	0.00	75.0	92.0	0.0	22.9	54.0
2	2003	9	27	23.24	28.33	20.00	0.667	1.000	0.000	23.90	16	3.05	0.13	2.29	81.0	93.0	0.0	24.8	54.0
2	2003	9	28	22.99	25.56	21.11	0.875	1.000	0.000	22.55	21	19.80	0.83	3.30	84.1	93.0	0.0	24.6	93.0
2	2003	9	29	18.06	22.22	13.33	0.583	1.000	0.000	17.28	14	0.00	0.00	0.00	66.9	86.0	0.0	18.9	80.0
2	2003	9	30	15.81	20.56	11.67	0.000	0.000	0.000	----	0	0.00	0.00	0.00	61.3	80.0	0.0	17.0	68.0
2	2003	10	1	15.09	21.11	10.00	0.000	0.000	0.000	----	0	0.00	0.00	0.00	69.3	88.0	0.0	16.1	59.0
2	2003	10	2	15.97	19.44	12.78	0.000	0.000	0.000	----	0	0.00	0.00	0.00	64.1	85.0	0.0	17.0	54.0
2	2003	10	3	12.57	17.78	6.67	0.000	0.000	0.000	----	0	0.00	0.00	0.00	51.1	71.0	0.0	13.1	54.0
2	2003	10	4	16.06	24.44	10.56	0.000	0.000	0.000	----	0	0.00	0.00	0.00	64.8	84.0	0.0	17.0	53.0
2	2003	10	5	17.13	21.67	12.78	0.000	0.000	0.000	----	0	0.00	0.00	0.00	65.3	87.0	0.0	18.3	51.0
2	2003	10	6	17.20	24.44	12.22	0.000	0.000	0.000	----	0	0.00	0.00	0.00	71.0	89.0	0.0	18.5	48.0
2	2003	10	7	18.70	25.00	13.89	0.000	0.000	0.000	----	0	0.00	0.00	0.00	74.7	90.0	0.0	20.0	43.0
2	2003	10	8	19.24	25.00	14.44	0.333	1.000	0.000	16.87	8	0.00	0.00	0.00	78.8	92.0	0.0	20.4	36.0
2	2003	10	9	21.62	25.00	19.44	0.208	1.000	0.000	22.69	5	1.78	0.07	1.27	80.2	91.0	0.0	22.9	28.0
2	2003	10	10	21.11	21.11	21.11	0.000	0.000	0.000	----	0	0.00	0.00	0.00	80.0	80.0	0.0	21.9	54.0

[a] Site 1 was in Perquimans County, NC; located on the Hollowell Brother's Farm; different fields were utilized in 2002 and 2003. Site 2 was in Chowan County, NC; located on the C.I. Ward Farm, 2003.

[b] Leaf wetness is measured as 0 representing absolute dryness, and 1 representing total surface wetness.

[c] Temperature during leaf wetness was calculated by averaging the temperature for each hour that the leaves were wet each day

[d] Total Precipitation is the total rain accumulated for every hour of that day. Minimum precipitation is the hour that accumulated the least amount of rain. Maximum precipitation is the hour that accumulated the high amount of rain.

Appendix II. United States Department of Agriculture Market Grades for all plots at all sites, 2002 and 2003.

Site[a]	Season	Cultivar	Fungicide[b]	Rate (kg ai/ha)	Schedule[c]	Total SMK (%)	Other Kernels (%)	Damaged Splits (%)	Total Kernels (%)	ELK (%)	Fancy (%)
1	2002	NC12C	Fluazinam	0.43	Calendar	64	3	4	71	47	80
1	2002	NC12C	Fluazinam	0.87	Calendar	66	3	3	72	51	86
1	2002	NC12C	Fluazinam	NA	NA	68	2	1	71	49	---
1	2002	Perry	Fluazinam	NA	NA	59	6	3	68	34	84
1	2002	Perry	Fluazinam	0.87	Calendar	60	6	2	68	34	79
1	2002	Perry	Fluazinam	0.43	Calendar	58	6	3	67	35	75
1	2002	NC12C	Fluazinam	0.87	Calendar	64	3	4	73	52	87
1	2002	NC12C	Fluazinam	0.43	Calendar	64	4	5	73	48	82
1	2002	NC12C	Fluazinam	NA	NA	63	4	3	70	40	76
1	2002	Perry	Fluazinam	NA	NA	55	7	3	65	29	71
1	2002	Perry	Fluazinam	0.43	Calendar	63	3	5	71	44	75
1	2002	Perry	Fluazinam	0.87	Calendar	58	6	3	67	36	77
1	2002	NC12C	Fluazinam	0.43	Calendar	64	3	5	72	41	87
1	2002	NC12C	Fluazinam	0.87	Calendar	66	3	4	73	52	89
1	2002	NC12C	Fluazinam	NA	NA	64	3	3	70	47	88
1	2002	Perry	Fluazinam	0.43	Calendar	64	4	3	71	42	88
1	2002	Perry	Fluazinam	0.87	Calendar	58	7	3	68	35	78
1	2002	Perry	Fluazinam	NA	NA	59	5	4	68	36	83
1	2002	Perry	Fluazinam	NA	NA	62	6	2	70	37	82
1	2002	Perry	Fluazinam	0.43	Calendar	63	4	3	70	37	80
1	2002	Perry	Fluazinam	0.87	Calendar	63	3	3	69	45	84
1	2002	NC12C	Fluazinam	NA	NA	61	4	2	67	39	86
1	2002	NC12C	Fluazinam	0.87	Calendar	63	3	4	70	45	86
1	2002	NC12C	Fluazinam	0.43	Calendar	61	4	3	68	40	81
1	2002	NC12C	Fluazinam	NA	NA	58	5	4	67	38	82
1	2002	NC12C	Fluazinam	0.87	Calendar	61	4	3	68	42	83
1	2002	NC12C	Fluazinam	0.43	Calendar	59	4	4	67	---	85
1	2002	Perry	Fluazinam	NA	NA	57	8	2	67	31	75
1	2002	Perry	Fluazinam	0.43	Calendar	58	6	3	67	29	71
1	2002	Perry	Fluazinam	0.87	Calendar	55	7	3	65	31	81
1	2002	NC12C	Fluazinam	0.87	Calendar	60	4	3	67	42	82
1	2002	NC12C	Fluazinam	0.43	Calendar	62	4	3	67	44	87
1	2002	NC12C	Fluazinam	NA	NA	61	4	3	68	44	89

Appendix II (continued)

Site[a]	Season	Cultivar	Fungicide[b]	Rate (kg ai/ha)	Schedule[c]	Total SMK (%)	Other Kernels (%)	Damaged Splits (%)	Total Kernels (%)	ELK (%)	Fancy (%)
1	2002	Perry	Fluazinam	NA	NA	64	4	2	70	41	87
1	2002	Perry	Fluazinam	0.43	Calendar	62	4	4	70	40	84
1	2002	Perry	Fluazinam	0.87	Calendar	59	6	2	67	35	78
1	2003	Perry	Fluazinam	NA	NA	75	1	0	76	43	80
1	2003	Perry	Fluazinam	0.87	Calendar	76	1	0	77	50	80
1	2003	Perry	Fluazinam	0.43	Calendar	76	1	0	77	45	76
1	2003	NC12C	Fluazinam	0.43	Calendar	74	1	0	75	51	91
1	2003	NC12C	Fluazinam	0.87	Calendar	74	1	0	75	---	90
1	2003	NC12C	Fluazinam	NA	NA	77	1	0	78	49	88
1	2003	NC12C	Fluazinam	0.87	Calendar	75	1	0	76	54	91
1	2003	NC12C	Fluazinam	NA	NA	74	1	0	75	51	---
1	2003	NC12C	Fluazinam	0.43	Calendar	75	1	0	76	44	85
1	2003	Perry	Fluazinam	0.87	Calendar	76	1	0	77	46	79
1	2003	Perry	Fluazinam	0.43	Calendar	76	1	0	77	40	82
1	2003	Perry	Fluazinam	NA	NA	75	1	0	76	37	80
1	2003	Perry	Fluazinam	0.87	Calendar	73	1	0	74	43	75
1	2003	Perry	Fluazinam	NA	NA	74	1	0	75	42	98
1	2003	Perry	Fluazinam	0.43	Calendar	74	2	1	77	40	78
1	2003	NC12C	Fluazinam	0.43	Calendar	74	1	1	76	49	83
1	2003	NC12C	Fluazinam	NA	NA	75	0	0	75	52	90
1	2003	NC12C	Fluazinam	0.87	Calendar	74	1	1	76	53	88
1	2003	NC12C	Fluazinam	0.87	Calendar	72	1	1	74	49	86
1	2003	NC12C	Fluazinam	NA	NA	73	1	0	74	46	88
1	2003	NC12C	Fluazinam	0.43	Calendar	72	1	1	74	51	89
1	2003	Perry	Fluazinam	0.87	Calendar	75	1	0	76	48	73
1	2003	Perry	Fluazinam	0.43	Calendar	74	1	0	75	42	81
1	2003	Perry	Fluazinam	NA	NA	74	1	0	75	39	81
1	2003	NC12C	Fluazinam	NA	NA	72	1	1	74	49	96
1	2003	NC12C	Fluazinam	0.87	Calendar	73	1	0	74	50	92
1	2003	NC12C	Fluazinam	0.43	Calendar	74	1	0	75	57	90
1	2003	Perry	Fluazinam	NA	NA	74	1	0	75	46	87
1	2003	Perry	Fluazinam	0.43	Calendar	75	1	0	76	67	88
1	2003	Perry	Fluazinam	0.87	Calendar	76	1	0	77	50	84
1	2003	NC12C	Fluazinam	NA	NA	74	1	0	75	54	91

Appendix II (continued)

Site[a]	Season	Cultivar	Fungicide[b]	Rate (kg ai/ha)	Schedule[c]	Total SMK (%)	Other Kernels (%)	Damaged Splits (%)	Total Kernels (%)	ELK (%)	Fancy (%)
1	2003	NC12C	Fluazinam	0.87	Calendar	76	1	0	77	54	92
1	2003	NC12C	Fluazinam	0.43	Calendar	76	1	0	77	50	90
1	2003	Perry	Fluazinam	0.87	Calendar	75	1	0	76	46	84
1	2003	Perry	Fluazinam	NA	NA	75	1	0	76	47	85
1	2003	Perry	Fluazinam	0.43	Calendar	76	0	0	76	53	92
2	2003	NC V11	Boscalid	0.45	Calendar	67	3	4	74	35	75
2	2003	NC V11	Fluazinam	0.87	Calendar	71	2	2	75	31	69
2	2003	NC V11	Fluazinam	0.87	Advisory	70	2	2	74	34	68
2	2003	NC V11	Check	NA	NA	69	2	0	71	37	82
2	2003	NC V11	Boscalid	0.45	Advisory	68	2	4	74	32	73
2	2003	NC V11	Boscalid	0.32	Advisory	73	2	0	75	35	74
2	2003	NC V11	Boscalid	0.32	Advisory	70	2	2	74	40	69
2	2003	NC V11	Boscalid	0.45	Advisory	72	2	1	75	41	70
2	2003	NC V11	Fluazinam	0.87	Advisory	70	3	0	73	40	63
2	2003	NC V11	Fluazinam	0.87	Calendar	73	2	0	75	44	74
2	2003	NC V11	Boscalid	0.45	Calendar	69	2	4	75	42	76
2	2003	NC V11	Check	NA	NA	68	3	2	73	41	78
2	2003	NC V11	Boscalid	0.32	Advisory	70	2	2	74	36	76
2	2003	NC V11	Fluazinam	0.87	Advisory	72	2	1	75	31	70
2	2003	NC V11	Boscalid	0.45	Calendar	73	2	0	75	35	81
2	2003	NC V11	Check	NA	NA	70	2	2	74	35	76
2	2003	NC V11	Fluazinam	0.87	Calendar	74	1	0	75	42	77
2	2003	NC V11	Boscalid	0.45	Advisory	68	2	3	73	35	77
2	2003	NC V11	Boscalid	0.45	Calendar	72	2	1	75	45	76
2	2003	NC V11	Boscalid	0.45	Advisory	70	3	0	73	---	73
2	2003	NC V11	Boscalid	0.32	Advisory	68	3	3	74	39	74
2	2003	NC V11	Fluazinam	0.87	Advisory	72	2	1	75	42	77
2	2003	NC V11	Fluazinam	0.87	Calendar	70	3	1	74	41	76
2	2003	NC V11	Check	NA	NA	70	3	1	74	43	74
2	2003	NC V11	Boscalid	0.32	Advisory	70	2	0	72	42	81
2	2003	NC V11	Fluazinam	0.87	Advisory	72	1	1	74	32	79
2	2003	NC V11	Boscalid	0.45	Advisory	67	3	3	73	32	74
2	2003	NC V11	Check	NA	NA	65	4	3	72	33	81
2	2003	NC V11	Boscalid	0.45	Calendar	69	2	2	73	38	75

Appendix II (continued)

Site[a]	Season	Cultivar	Fungicide[b]	Rate (kg ai/ha)	Schedule[c]	Total SMK (%)	Other Kernels (%)	Damaged Splits (%)	Total Kernels (%)	ELK (%)	Fancy (%)
2	2003	NC V11	Fluazinam	0.87	Calendar	71	2	1	74	38	73
2	2003	NC V11	Boscalid	0.45	Advisory	60	4	0	64	39	76
2	2003	NC V11	Boscalid	0.45	Calendar	71	2	1	74	38	66
2	2003	NC V11	Boscalid	0.32	Advisory	73	2	1	76	45	75
2	2003	NC V11	Fluazinam	0.87	Calendar	72	2	0	74	33	80
2	2003	NC V11	Fluazinam	0.87	Advisory	72	2	0	74	46	73
2	2003	NC V11	Check	NA	NA	70	2	1	73	---	73

[a] Site one was in Perquimans County, NC; located on the Hollowell Brother's Farm; different fields were utilized in 2002 and 2003. Site two was in Chowan, County, NC; located on the C.I. Ward Farm, 2003.
[b] Fluazinam was applied as Omega 500 F and boscalid was applied as Endura 70% WG.
[c] Calendar fungicide applications were made at site 1 on 15 Jul and 6 Aug 2002 and 21 Jul and 18 AUG 2003. In 2003, fluazinam calendar applications were made at site 2 on 21 Jul and 19 Aug. Boscalid calendar applications were made at site 2 on 21 Jul, 8 Aug, 2 Sep. All advisory treatments were made on 8 Aug and 2 Sep 2003.