EVALUATION OF PECAN SCAB DISEASE MANAGEMENT STRATEGIES TO IMPROVE ORCHARD PRODUCTION AND REDUCE PESTICIDE INPUTS

By

ANDREA F. PAYNE

Bachelor of Science in Microbiology
Oklahoma State University
Stillwater, OK
2007

Master of Public Health in Epidemiology
Tulane University
New Orleans, LA
2009

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
May, 2011

EVALUATION OF PECAN SCAB DISEASE MANAGEMENT STRATEGIES TO IMPROVE ORCHARD PRODUCTION AND REDUCE PESTICIDE INPUTS

Thesis Approved:

Dr. Damon L. Smith

Thesis Adviser

Dr. John Damicone

Dr. Michael Smith

Dr. Mark E. Payton

Dean of the Graduate College

ACKNOWLEDGMENTS

I would like to thank my advisor Dr. Damon L. Smith for not only accepting me as his first graduate student, but also being such a strong leader and role model over these past 2 years. He took a large leap of faith accepting a student from the medical field who had no background in horticulture and limited experience in agriculture and has turned her into a plant pathologist. For that faith and patience I will also be indebted. I would also like to thank the remaining members of my committee, Dr. John Damicone and Dr. Michael Smith. Their guidance has been incredibly helpful throughout my studies. With so many different ways of designing studies and analyzing their results having such a diverse committee was my greatest asset.

A special thanks to the Oklahoma Pecan Growers Association for partially funding this project and allowing me the opportunity to speak at their annual meetings and gain those experiences.

Finally, I would like to thank my family for all their support and words of encouragement during my graduate studies. It's been a long and arduous road that will continue, but with my family and friends behind me I know I'll make it.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

REVIEW OF LITERATURE

Definition of Epidemiology: The word epidemiology, is based on the Greek roots *epi* meaning 'on or upon' , *demos* typically understood as 'people' but also as 'populations', and *logos* which means the 'the study of'. Epidemiology, as defined by Leon Gordis (2000), is 'the study of how disease is distributed in populations and the factors that influence or determine this distribution'. Madden, Hughes, and van den Bosch (2007) define epidemiology as the 'study of epidemics'. For plant diseases, Agrios (1997) defines epidemiology as 'the study of epidemics and of the factors that influence them'. Considering these definitions we can further develop the following definition: epidemiology is the study of the distribution and determinants of disease within a population. For the purposes of the work presented here we will use the later definition to define plant epidemiology.

As a science, epidemiology was founded by John Snow after his study of the cholera outbreak in London in 1854 (Snow, 1855). Known as the 'Father of

Epidemiology', Snow showed many individuals with cholera were centrally located around a specific water pump. After the pump handle was removed the outbreak began to subside. Although other factors affected the cholera outbreak, the overall objectives and methodology of an epidemiologic study were developed for use in future studies on other populations.

The general objectives of epidemiology are to clarify etiology of disease, determine the extent of disease, study the natural history and progression of disease, and evaluate both existing and new control measures and modes of their delivery (Gordis, 2000). Although epidemiology was developed in the context of human diseases, plant disease epidemiology focuses on similar objectives. In studying the cause and effect of disease, a plant disease epidemiologist must be able to identify the disease and understand its role within a population. The ultimate goal of an epidemiologic study is to develop an understanding of how disease develops and progresses through a population in time and space. Based on this fundamental understanding, epidemiologists can begin to develop control strategies in order to maintain levels of disease under an economical threshold. As with any method of control, new technologies and sciences provide researchers with new tools to use in both the understanding of disease etiology, and in more efficient and cost effective methods of implementing disease control measures.

A conceptual framework often utilized by epidemiologists is the disease triangle (Agrios, 1997; Francl, 2001). This model incorporates the individual effect of host, pathogen, environment, and their interactive effects on disease development. When all components of this triangle are met (i.e. environmental conditions are favorable, a virulent pathogen is present, and the host is susceptible) disease development is likely.

Two parameters have been proposed for addition to the disease triangle, human involvement and time (Agrios, 1997; Francl, 2001). Human involvement has been proposed to explain the effect of certain conditions that are inherently man-made on disease progression. For example, certain cultural practices in agriculture have an effect on disease development and spread. However, human interventions are often viewed as changes to the environment making the addition of a human parameter to the plant disease triangle a redundancy. A second parameter proposed for addition to the plant disease triangle is the effect of time. Ultimately, host, environment, and pathogen factors must be favorable simultaneously in order to cause disease. The objective of most epidemiologic studies is to determine the interaction of the components of the triangle; it is unlikely other parameters will enter the triangle.

Background and Breeding of the Pecan Tree: The pecan tree, *Carya illinoinensis* (Wagenh.) K. Koch, is a deciduous tree native to North America and is found throughout the Mississippi River Valley and its tributaries from Louisiana, north to Illinois. The pecan tree is also found growing naturally in some areas of northeastern Mexico. Pecans can be grown outside their natural range extending into areas along the coast of the Gulf of Mexico, which account for the majority of the annual crop in the United States (Georgia, Louisiana, and Mississippi). Georgia typically produces the largest amount of pecans with 78 million, 100 million and 65 million pounds harvested during 2008, 2009 and 2010, respectively. With the use of irrigation, areas of western Oklahoma, western Texas, New Mexico, and Arizona are capable of producing pecans. Arizona and New Mexico's total production exceeds total production in Oklahoma. Arizona produced 17.5

million and 20 million pounds in 2008 and 2009, respectively. New Mexico produced 43 million pounds and 68 million pounds in 2008 and 2009, respectively. Oklahoma produced 5 million and 13.5 million pounds in 2008 and 2009, respectively (U.S. Department of Agriculture). Australia, portions of Latin America, Israel, and South Africa also have regions of pecan production (Johnson, 1997).

C. illinoinensis is a member of the Family *Juglandaceae* belonging to the hickory family of trees in section *Apocarya*. Other members of the *Apocarya* section are the Mockernut Hickory (*C. tomentosa*), Water Hickory (*C. aquaticus*), Bitternut Hickory (*C. cordiformis*), and Mexican Hickory (*C. palmeri*). Members of the *Apocarya* are diploid (n = 16), while members of the *Carya*, considered the true hickories, include both diploid and tetraploid species (n=32) (Thompson and Grauke, 1990). 'Native' trees are those trees occurring indigenously to an area. Improved cultivars have been selected from native and seedling populations, with a concentration on phenotypic traits such as larger fruit size, disease resistance, and overall quality of the fruit. Improved cultivars of pecan are typically planted in rows for crop production, similar to other orchard crops. Native pecan production systems are often found in low-lying areas near a source of water such as creeks and rivers. Where native pecan trees are densely populated, individuals can be thinned and managed for fruit production much like improved orchards.

Two pecan-breeding programs are located within the United States. These include the United States Department of Agriculture, Agricultural Research Services (USDA/ARS) stations in Somerville and Brownwood, Texas and at the University of Georgia College of Agricultural and Environmental Sciences. Both programs follow the same basic structure of controlled pollination to determine both the male and female

genetic contributors. Crosses are then grown, harvested, and fruit are examined for characteristics such as overall quality and scab disease resistance. Trees are also examined for vigor, cold hardiness, structural integrity and early fruit maturity. Crosses considered better than both parent trees undergo extensive evaluation across the U.S. pecan belt and may eventually be released as a cultivar.

Production from native trees typically does not return as much profit as improved cultivars. Average sale price per pound of nuts in Oklahoma was $1.15, $0.75, and $0.80 in 2006, 2007, and 2008, respectively. Average price per pound of nuts for improved cultivars was $1.70, $1.35, $1.60 and in 2006, 2007, and 2008, respectively (NASS 2009). Nuts which are more aesthetically pleasing to customers have a larger size and more desirable shell and kernel color. Due to consumer demand, kernels from cultivars exhibiting these traits command a higher price per pound as compared to those from native trees. Due to the different price between kernels from cultivars versus native trees, there is an increasing trend towards growing improved cultivars in Oklahoma. In 2007, total acreage of harvestable pecans increased to 141,675 acres of which 21,609 of those were cultivars and 120,066 were natives (NASS, 2009). This is an increase in total acreage as compared to 85,757 and 85,740 acres in 1997 and 2002, respectively.

Nutritional Requirements of the Pecan Tree: Considering the lower quality and profit obtained from native pecan production, many growers will typically rely on very few, or no inputs to manage native groves. Native pecan trees are often found on alluvial soils in flood plains that have better soil fertility levels, resulting in the need for fewer inputs as

compared to upland areas. In contrast, improved cultivar production often requires many inputs throughout the season to compensate for higher nutritional requirements. A nutrient that is commonly deficient is zinc (Zn). Symptoms of Zn deficiency are yellowing of new growth and undersized leaves, with short, clustered shoots. Clustering of shoots is also referred to as 'zinc rosette' (Alben and Boggs, 1936). A second nutrient that is commonly deficient is nitrogen (N). Trees that are deficient in nitrogen have been shown to exhibit increased tendencies to alternate bear, which is a naturally occurring phenomenon (Acuna-Maldonado et al., 2003). Alternate bearing is a cycling of high and low crop yields from season to season. Manganese (Mn) deficiency result in a decrease in photosynthetic capability because there is a decrease in chloroplast numbers in mesophyll cells (Henriques, 2004). Within the chloroplast, manganese is utilized in photosystem II during photosynthesis and deficiencies can disrupt the thylakoid membrane. Manganese is also used as a cofactor for many enzymes (Salisbury and Ross, 1992).

Common Diseases and Insect Pests of Pecan Trees: The most common diseases of pecan trees include pecan scab, anthracnose, vein spot, and leaf scortch. Pecan scab, caused by the fungus *Fusicladium effusum* (syn. *Cladosporium caryigenum*), is the most damaging disease in most pecan growing regions. High levels of scab severity on leaves can lead to an increased probability of shuck infection earlier in the season, resulting in early fruit drop and poor fruit quality (Gottwald and Bertrand, 1983; Hunter, 1983). Under extreme disease pressure, total crop loss can result from a scab epidemic. Anthracnose (caused by the fungus *Glomerella cingulata)* and vein spot (caused by the

fungus *Gnomonia nerviseda*) are typically controlled by routine fungicide application used to manage pecan scab (Rand, 1914). However, not all fungicides are effective and labeled for use against these fungal pathogens. A new emerging disease in pecan production is bacterial leaf scorch. Bacterial leaf scorch is caused by the xylem limited bacterium *Xylella fastidiosa* (Sanderlin and Heyderich-Alger, 2000).

Insect pests can severely reduce crop yield. Insects such as phylloxera (*Phylloxera sp.*), pecan nut casebearer (*Acrobasis nuxvorella)*, pecan weevil (*Curculio caryae)*, and hickory shuck worm (*Cydia caryana*) can cause serious damage to the fruit and foliage (Harris *et. al*, 1986). Gall formation from phylloxera can cause defoliation and weaken the tree resulting in reduced production in future years (Andersen and Mizell, 1987). Through multiple generations, pecan nut casebearer can destroy an entire crop within a given year.

Taxonomy of *Fusicladium effusum:* Pecan scab was first described by Winter in 1885 on leaves of the mockernut hickory from Cabden, Illinois. From this specimen Winter had identified the pathogen causing the disease as *Fusicladium effusum* (Winter, 1885). Forty years later, Demaree reclassified the fungus as a *Cladosporium* sp. based on the presence of chains of conidia (Demaree, 1928). However, the name *Cladosporium effusum* had already been given to a fungus that infects other members of the genus *Carya* by Ellis and Langlois (Ellis and Everhart, 1888). After consideration by Lentz, the causal agent of pecan scab was determined to be *Fusicladium effusum* based on the conidiophore structure and the lack of resemblance to *Cladosporium herbarum*, used as

the type *Cladosporium* specimen (Lentz, 1957). Later, Gottwald compared isolates from the National Fungus Collection and identified the fungus as *Cladosporium caryigenum* after finding conidial chains and ramoconidia. Ramoconidia are conidiogenous cells extending from the apical end of a conidiophore from which conidia form (Schubert et al, 2007). The presence of both characteristics is contrary to the Hughesian system of classification later amended by G.L. Barron. Using the Hughesian system, *Fusicladium* is classified with the family 'Venturiaceae', which never produce conidia in chains. The presence of conidia in long chains extending from ramoconidia is in direct contradiction to the family 'Venturiaceae'. *C. caryigenum* was adopted after the name *Cladosporium effusum* was found to be a synonym of *Fusicladium effusum* Wint. and was disregarded. Currently, the accepted taxonomic classification has reverted back to *F. effusum* Wint. The reversion of scientific name is due to the description of the conidiogeous loci as denticle-like with unthickened walls (Shubert, 2002). Another source of support for Shubert's assessment is the molecular study performed by Schnabel, Schnabel and Jones (1999) in which rDNA ITS regions were analyzed in both *Venturia* and *Cladosporium* species. In this study *C. caryigenum* clustered closely to species of *Venturia* with known *Fusicladium* anamorphs (Schnabel, Schnabel, and Jones, 1999). Seyran *et al*. (2010) gave further support for the taxonomic classification of the pecan scab fungus as *Fusicladium effusum*. Cytochrome b gene sequences where used to develop phylogenetic trees that clustered *F. effusum* with *Venturuia inaequalis* (causal agent of apple scab) with a 92% similarity (Seyran, Nischwitz, Lewis, Gitaitis, Brenneman, and Stevenson, 2010). At this time there is no known teleomorph of *F. effusum*.

Occurrence of Pecan Scab: Pecan scab is the most important and destructive fungal disease of pecans. Demaree performed the first epidemiologic studies of pecan scab in 1924. Severe disease was identified in pecan orchards within a 100 mile distance from the Gulf of Mexico in the southern pecan belt. Pecan scab had become economically significant at the time of this earliest assessment. After 100 miles inland, disease severity decreased and fewer cultivars were affected by pecan scab. States where scab had been a concern included Georgia, Florida, Alabama, Mississippi, Louisiana, and Arkansas. Texas had yet to experience serious epidemics of pecan scab during the period of Demaree's assessments.

The susceptibility of newly forming leaves and fruit were also noted in the initial observations. Disease progress began on leaves, where olive green lesions formed and appeared to reside in the vascular tissue (Demaree, 1924). Disease transmission was thought to be from an aphid vector; however, this was later demonstrated to be incorrect (Waite, 1911). Disease progress on the fruit begins as elongated spots running the long axis of the fruit, initially gray color and developing to a darker olive-brown, with gray borders.

Pecan Scab Disease Cycle: After conidia of *F. effusum* have reached susceptible plant parts and environmental conditions are favorable, conidia begin to germinate. The germ tube extends along the epidermis 3-24 hours after inoculation. Between 24 and 48 hours, germ tubes will begin to expand and at 36 hours the germ tube has typically penetrated the leaf epidermis. In a study by Latham and Rushing (1998), 82.2 % of germ

tubes typically penetrated into the tricomes. The remaining germ tubes penetrated the leaf epidermis near a vein. Hyphal growth does not occur on the leaf surface; however, it occurs in the sub-cuticular layer, rupturing epidermal cells as it continues to grow. Conidiophores were not observed expanding from stomata, but have been observed rupturing through the leaf cuticle. In infected areas, the epidermis and cuticle were found to be absolute by Latham and Rushing (1988).

Inoculum can arise from several generations in the same growing season, resulting in a polycyclic disease cycle. The primary inoculum comes from the previous season's lesions. Secondary inoculum is from the current season's epidemic. *F. effusum* overwinters in the lesions that result from the previous season's infection, most notably in the twigs, shucks, and petioles. Lesions appear as brown necrotic spots on the surface of the plant. When environmental conditions become favorable, around mid-April and coinciding with budbreak, stroma (hardened fungal mycelium) in the over-wintering lesions begin to produce conidia, which serve as primary inoculum. Dispersal of primary conidia occurs as early as March and is mediated by rainwater runoff and wind (Gottwald, 1985). Once conidia have landed on susceptible plant parts (e.g. newly budding leaves and twigs) and environmental conditions are favorable, a germ tube begins development.

A major concern to growers is the condition of the fruit during scab infections, as identified in Demaree's assessment. Like other parts of the tree, the newly developing fruit are more susceptible, and as they age, the level of susceptibility appears to decrease (Demaree, 1924). Pecan scab infection can lead to reduction in crop yield and quality of kernals. Scab can induce fruit drop and under sizing by cutting off the water and nutrient

flow while fruit are still developing (Gottwald, 1983). In addition, oil and moisture content are often reduced, leading to lower quality kernals (Gottwald, 1983). Latham (1982) reported that if the shuck is infected in June a crop loss is inevitable. However, if the shuck was not infected until August, there was only cosmetic damage. This was later refuted by Gottwald and Bertrand (1983) who found that early infection did not appear to affect fruit drop as much as quality.

Disease development can begin as early as 3 and as many as 24 days after infection, depending on the age of the leaf or fruit. Younger leaves and fruit are generally more susceptible to early disease development after infection compared to older ones (Turechek and Stevenson, 1998). This is due to the anatomical development of leaflet and shuck epidermis (Latham and Rushing, 1988). Latham and Rushing observed that the germ tube penetrated directly through the cuticle. They suggested that as leaves mature over time the cuticle becomes thicker and thus an infection is less likely because of this increasing barrier (Latham and Rushing, 1988). Conner and Stevenson (2004) suggested that resistance to infection by the fungus was also mediated by the speed at which plant cell walls thickened in resistant germplasm, thereby limiting growth of subcuticular fungal hyphae.

Environmental conditions that play an important role during fungal infection include temperature, moisture, wind, and rain. For *F. effusum,* temperature has been well studied in fungal populations from Georgia. Growth of *F. effusum* occurred from 15 to 30 °C. With 48 hours of continuous leaf wetness, growth was slow at 15 ° C and increased to maximal growth at 20°C. Growth gradually declined between 20°C to 30 °C with 48 hours of leaf wetness (Gottwald, 1985). Within 100-mile of the Gulf of Mexico, where

scab is severe, relative humidity tends to be high throughout the growing season with warmer temperatures and localized showers common. These showers tend to keep relative humidity higher throughout the day and southerly breezes from the Gulf of Mexico bring warmer, humid air from tropics. In the Gulf coast region, the environment is considered highly conducive to the growth of many fungal diseases including scab (Demaree, 1924; Gottwald, 1985).

Factors affecting Conidial Dispersal: Conidial dispersal occurs via wind and rainfall runoff. In Georgia, *F. effusum* conidial dispersal via heavy rainwater loads was minimal. However, maximum numbers of conidia were captured after periods of light rain showers. Similar numbers were captured after several consecutive days had 10-18 hour periods of relative humidity greater than 90% (Gottwald, 1982). Prolonged rainstorms resulted in the highest conidial dispersal occurring during the first few hours of a rain event; however, this number decreased sharply during the duration of rainstorms (Gottwald, 1982). Numerous studies have demonstrated that highest levels of conidial dispersal results after a drop in humidity, either after the morning hours when relative humidity is at its daily high, or following a rainstorm (Gottwald, 1982; Gottwald and Bertrand, 1982; Latham, 1982). The highest conidial dispersal in *Cladosporium carpophilum* (causal agent of peach scab) has been shown to occur during similar periods (Lan and Scherm, 2003). Minimal wind speeds required for spore dispersal were greater than 1.6 km/hr (Converse, 1960). Distance traveled in the wind is dependent on the wind speed.

Isolate Variability Within *F. effusum* Populations: Cultivar susceptibility is a dynamic continuum. Cultivars (*i.e.* 'Moneymaker', 'Stuart', 'Russel', 'Frotcher') of pecan once considered resistant to infection by *F. effusum* during the earliest pecan scab evaluations, are now either highly susceptible or moderately susceptible after decades of crop production (Demaree, 1924; Sutherland et al., 2005). During the earliest assessment in the Gulf Coast regions of the United States, 'Schley', 'Van Deman', and 'Pabst' were highly susceptible within a 100 miles of the Gulf Coast (Demaree, 1924).

Genetic variability has not been extensively explored in populations of *F. effusum* in Oklahoma. However, isolate variability has been identified in populations of *F. effusum* from other pecan growing areas. In greenhouse experiments performed in the late 1950s using isolates from the eastern portion of Oklahoma, Converse (1960) suggested that isolate specificity in *F. effusum* populations did exist based on host cultivar compatibility. Similar experiments have been performed in Georgia. When inoculations were performed using isolates from one cultivar on another, disease severity was lower compared to severity on the cultivar where the fungal isolate was recovered. When those individuals were re-isolated and used to inoculate the original cultivar, disease severity was similar to that which was originally observed (Conner and Stevenson, 2004).

Timing of Fungicide Applications to Manage Pecan Scab: Without effective management of pecan scab, susceptible cultivars can develop disease severe enough to cause complete crop loss in as little time as 3 years (Demaree, 1924). Preventative measures to manage pecan scab include pruning, tree thinning, sanitation, and fungicide

application. Pruning of trees allows better light penetration and air-flow through the canopy thereby reducing leaf wetness duration. In addition, infrared radiation has been implicated as an inhibitor to the growth of *F. effusum.* Conversely, infrared radiation has been shown to stimulate spore release (Gottwald, 1982). Several methods have been developed to determine when fungicide applications should be made including calendar dates, growth stage of the tree, and fundamentally and empirically derived disease prediction models. The purpose of predictive models is to provide growers a more efficient management program. Calendar-based schedules are effective, but can be expensive when numerous applications are recommended and not required to control pecan scab. Using an extension recommendation by Louisiana State University, a calendar-based program would include 2 pre-pollination sprays, starting in late March or early April, followed by 6 cover sprays, with the final spray occurring in August. Using this calendar-based schedule, a minimum of 8 sprays would be applied during the growing season. During periods when weather is not favorable for scab increase, unnecessary fungicide applications may be applied. This is not only inefficient, but the practice can result in populations of *F. effusum* that are resistant to fungicides, rendering those chemicals ineffective, which has occurred with propiconazole and fenbuconazole in Georgia (Reynolds, Brenneman et al. 1997). Other advantages of avoiding excessive or unnecessary application of fungicide include reduced human exposure and reduced chemical input to the environment (Sutton, 1996).

Four states use prediction models to recommend fungicide application on pecans: Oklahoma, Georgia, Louisiana, and Alabama. AU-Pecan was developed at the University of Georgia and is currently in use in Georgia, Louisiana, and Alabama. This

advisory is a modification of the AUPnut program for controlling early and late leaf spot of peanut developed at Auburn University (Jacobi and Backman 1995; Jacobi, Backman, Davis, and Brannen, 1995). The advisory is based on the 5-day average percent chance of rain forecast and number of rain events after the fungicide protection period has ended. The protection period is determined by the grower based on observations made in his or her orchard; however, a 10-14 day period is recommended. A protection period is the time after a fungicide application when the tree is coated with an active residue of fungicide. A fungicide application using AU-Pecan is recommended if one of the following criteria is met:

1. No rain has occurred but the 5-day average chance of rain is 50% or greater.

OR

2. One rain event has been recorded and the 5-day average chance of rain is 40% or greater.

OR

3. Two rain events have been recorded and the 5-day average chance of rain is 20% or greater.

OR

4. Immediately after three rain events.

After fungicide has been applied the crop is considered protected. When the protection period has ended the grower must monitor the criteria previously mentioned to determine when the next application must be made.

An alternative system is the Oklahoma Agweather Pecan Scab Fungicide Advisory. The advisory was developed at Oklahoma State University during the mid-1990s. The advisory is currently available to Oklahoma growers via the Oklahoma Mesonet at www.agweather.mesonet.org (Driever 1998). This advisory assesses the need for fungicide application using cultivar susceptibility, relative humidity, and temperature as input variables. Timing of fungicide application is dependent on the accumulation of periods of weather favorable for scab development termed a 'scab hour'. A scab hour is defined as an hour in which the average relative humidity is greater than or equal to 90% and average temperature is greater than or equal to 21°C. A highly susceptible cultivar should be sprayed after ten scab hours, a moderately susceptible cultivar after twenty scab hours, and a resistant cultivar after thirty scab hours. Cultivar susceptibility is dependent upon previous observations (von Broembson, 2009). Weather data is collected from 120 weather stations situated throughout the state that are maintained by the Oklahoma Mesonet. Each county in Oklahoma has at least one weather station that measures weather variables every five minutes. Weather variables measured include but are not limited to temperature, relative humidity, solar radiation, rainfall, and soil temperature at several depths. Weather information is transmitted to a central server where the advisory can access the hourly average of relative humidity and temperature to calculate scab hours (von Broembson, 2009).

Recently growers have questioned the performance of the spray advisories resulting in speculation that the model may not be accurately identifying periods favorable for pecan scab epidemics. Inaccuracies in the advisory could be from two sources. First, weather stations are located in areas may not be representative of the

microclimate typically found in Oklahoma pecan groves. Pecan groves tend to be in low-lying areas and/or near bodies of surface water, which can dramatically change the microclimate. Another explanation for the poor accuracy of the advisory might be that pertinent weather variables are missing from the model. Finally, current temperature and/or relative humidity thresholds may be incorrect. The current thresholds were set to reflect levels of temperature and relative humidity known to be biologically significant for symptom development. The current thresholds were set to reflect levels known to cause an increase in disease in an orchard (Driever, 1998). The temperature threshold was based on research by Gottwald (1985) in Georgia and by Driever (1998) in Oklahoma that indicated temperatures ≥ 21.1 ˚C correlated with increases of pecan scab. Gottwald (1985) reported that isolates of *F. effusum* were capable of infecting leaves at temperatures of 15 to 25 ˚C. Turechek and Stevenson (1998) reported similar infection and disease development occurring at temperatures as low as 15 ˚C and no infection or disease development at 35 ˚C.

Relative humidity thresholds were determined by Driever (1998) and a value of 90% was selected to reflect favorable periods of scab increase. Relative humidity can be used to indirectly measure leaf wetness when leaf wetness sensors are not available and a value of 90% is a suitable indicator (Latham, 1982; Sentelhas et al., 2008). However, in some areas such as the upper Midwestern United States, leaf wetness can occur at lower levels of relative humidity (83-90%) (Sentelhas et al., 2008).

Fungicide Use in Pecan Production: Soon after *F. effusum* was identified as an economically important plant pathogen, control of pecan scab included cultural practices

and preventative fungicide applications. Grafting scion of resistant cultivars onto established susceptible cultivars and applications of Bordeaux mixture (composed of four pounds copper sulfate, four pounds of quicklime, and 50 gallons of water) were the earliest recommendations for the control of pecan scab (McMurran and Demaree, 1920). This integrated management program was successful during the growing season; however, applications made during dormancy were not successful. Cultural practices later recommended inclusion of pruning and thinning of trees to allow for better light penetration and air movement through the orchard. Increased flow of air reduces the duration of high humidity in the tree canopy creating a less conducive environment for disease initiation and progression (Pady, Kramer et al. 1969; Gottwald 1982; Gottwald and Bertrand 1982). Mowing the orchard floor also helps to improve airflow and reduce the duration of high humidity. A reduction in airflow can also occur as a result of tree overcrowding. In the 1920s and 30s, trees were planted approximately 18 m apart or less. More recently, trees have been planted 9 m apart or less. Both of these situations can result in a microclimate conducive for pecan scab.

Fungicide selection needs to be made based on mode of action, cost, and livestock grazing restrictions. Grazing restriction limits the type of fungicide that can be applied. A grazing restriction means livestock cannot graze in the treated orchards and grasses or other crops cannot be harvested as feed for livestock. Demethylation inhibitors (DMI), such as propiconazole, fenbuconazole, and tebuconazole, typically have grazing restrictions.

The development of fungicide resistance is a natural progression in many pathosystems where repeated applications of fungicides are made. While the process can

be very slow in nature, it can be accelerated by the misapplication of fungicides. Techniques such as repeated spraying using the same fungicide, using "off-label" rates, or improper spray volumes can all lead to premature development of fungicide resistance. Most of the fungicides labeled for use in pecans belong to mode of action (MOA) groups 3 and 11. The limited availability of several MOA groups coupled with grazing restrictions leave growers who do feed livestock in orchards or use hay from the orchard floor with a very limited availability of fungicides. Even with these limitations, fungicide resistance in *F. effusum* populations in Oklahoma has not been identified. However, resistance to fenbuconazole and propiconazole has been demonstrated in Georgia (Rushing and Latham 1991).

Statistical techniques - *Logistic regression*: In instances where datasets have a large number of missing data and the outcome can be easily transformed into a binomial outcome, logistic regression can be used to obtain probabilities of disease occurrence using one or more explanatory variables. Logistic regression is a generalized linear modeling (GLM) technique used when a non-Gaussian (binary [0, 1]) outcome of interest is modeled using a maximum likelihood estimation of the model parameters. For binary coding purposes a 0 traditionally constitutes no disease or disease below an economic threshold and 1 constitutes an occurrence of disease or disease above an economic threshold. The regression equation has the basic structure of $y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k$. By incorporating the logit link and fitting a sigmoidal curve (Figure 1), implying a curvilinear relationship, parameter estimation takes the following form as defined by Agresti (1990):

$$\pi(\alpha) = \frac{\exp(\alpha + \beta(x))}{1 + \exp(\alpha + \beta(x))}$$

Where α is the intercept β is a coefficiant to estimateand x is a parameter

(Agresti 1990)

In order to obtain probabilities of the occurrence of the outcome of interest, the value calculated by the regression equation requires a back-transformation using the following equation:

$$logit(\exp\big(\alpha + \beta(x)\big) = \frac{\exp(\alpha + \beta(x))}{1 - \exp(\alpha + \beta(x))}$$

When modeling the occurrence of disease, an increase in the logit value results in a higher probability of disease.

Within logistic regression, parameter selection can occur several ways; forward, reverse, or stepwise selection. Forward selection begins with a null model, intercept only, adding the next most significant parameter to the model one at a time. Backward selection occurs in a similar manner; however, the full model is the initial model and each step of the model build process deletes the least significant of the non-significant parameters from the model. When using both processes one can obtain different parameterizations of the models. Stepwise selection is a merger of forward and backward selection. Stepwise selection begins as forward selection with the null model. Stepwise selection then adds the most significant parameter to the model and checks the significance of all parameters in the model for loss of significance due to the addition of the new parameter. If a reduction in significance of the resulting model is detected, the non-significant

parameter is removed. This process is iterative and occurs until all statistically significant parameters have been added to the model and all non-statistically significant parameters have been removed from the model.

Determining Goodness-of-fit in Logistic Regression: *Akaike's Information Criteria*

The AIC is a tool to be used when comparing nested models and is calculated as follows: (Akaike, 1974)

$$AIC\left(\hat{\theta}\right) = (-2)L + 2k$$

where (-2)L is the maximum log-likelihood of the regression and k refers to the number of parameters in the model build.

A 'better' model is determined by the minimization of the AIC. As the AIC is not distributed across a particular distribution (e.g. χ^2 distribution) it cannot be associated with a p-value. The AIC calculation gets smaller based on the maximum likelihood score calculated for each model. The correction parameter, 2k, penalizes the model for each parameter in the model thus penalizing for over dispersion or over-parameterization. Each score of the AIC is merely a score; the values are only relative to those values from other models of the same model building process.

Statistical techniques - *Generalized Estimating Equations*: In situations where data is collected over time, or longitudinally, data has the possibility of being highly correlated or clustered. One of the assumptions of logistic regression is that observations are independent. To adjust GLM to fit correlated or clustered data, Liang and Zeger (1986) and Prentice (1988) developed an extension of generalized linear models (GLM),

generalized estimating equations (GEE), that is not dependent on independent observations. Assuming a non-Gaussian outcome (such as a binary outcome [0,1]), researchers are now capable of developing linear models within the context of a longitudinal study design. GEE is different from multivariate logistic regression in that GEE is testing within the same population, whereas multivariate logistic regression is testing two populations. GEE has been published in the journals of many disciplines including medical, political and veterinary journals (McDermott, Schukken et al. 1994; Barkema, Schukken et al. 1997; Zorn 2001; Liu and Suesse 2008).

GEE is a quasi-likelihood based approach to analyzing longitudinal data (Zeger and Liang 1986). This differs from traditional logistic regression in that logistic regression is a maximum likelihood approach. GEE takes the following form when calculating the score of β_i (Zeger and Liang 1986):

$$S_k(\beta) = \sum_{i=1}^{K} \left(\frac{\partial \mu_i^T}{\partial \beta}\right)' \upsilon_i^{-1}(y_i - \mu_i) = 0$$

Where the variance is as follows: $\upsilon_i = \frac{\boldsymbol{A_i^{\frac{1}{2}} R_i(\alpha) A_i^{\frac{1}{2}}}}{\Phi}$. $\boldsymbol{A_i^{\frac{1}{2}}}$is an *n* x *n* matrix where $g(\mu_i)$ is the *j*th diagonal element and $\boldsymbol{R_i}(\alpha)$ is the working correlation matrix, and ϕ is a scale parameter estimated using the following equation:

$$\hat{\phi} = \frac{1}{\sum_{i=1}^{n} n_i} \sum_{i=1}^{n} \sum_{t=1}^{n_i} \widehat{res}_{it}^2$$

By using a logit link, $\ln\left(\frac{p}{1+p}\right)$, in GEE, $S_k(\beta)$ is redefined within the logit transformation and requires the same back-transformation used in logistic regression.

Determining Goodness-of-fit in Generalized Estimating Equations: QIC

Few statistics are available to determine goodness-of-fit for GEE using SAS. The quasi-likelihood-under-the-independence-model information criteria (QICu) and the QIC adjusted for number of parameters in the model (QICuR) are association statistic used to determine goodness-of-fit and is defined as follows:

$$QICu = -2Q(\beta(R); I, D) + 2 * trace(\hat{\Omega}_I, \hat{V}_r) \qquad \text{(Pan 2001)}$$

An alternate form presented by Pan (2001) reduces the QICu to the QIC_u(R) by replacing $2 * trace(\hat{\Omega}_I, \hat{V}_r)$ with 2p where p represents the correction factor for the number of parameters in the model. However, as noted by the author, this form can only be used to determine the proper number of parameters and not selection of the correct correlation matrix.

As with the AIC in logistic regression, minimization of QICu demonstrates better model fit as compared to another QICu within a nested model building process. The QICu and AIC share a similarity in interpretation where the model is penalized for the addition of unnecessary parameters. Currently, the QICu is the only means of assessing model fit while using GEE without hand calculations or macro development if using SAS v.9.2. SAS v.9.1 does not give the QIC or QICu in the results, however it is capable of GEE.

Literature Cited

Acuna-Maldonado, L.E., Smith, M.W., Maness, N.O., Cheary, B.S., Carroll, B.L. and Johnson, G.V. 2003. Influence of nitrogen application time on nitrogen absorption, partitioning, and yield of pecan. Journal of American Society of Horticultural Sciences. 128: 155-162.

Agresti, A. 1990. Categorical data analysis. New York, Wiley.

Agrios, G. N. 1997. Plant Pathology. San Diego, Academic Press.

Akaike, H. 1974. New look at statistical-model identification. Ieee Transactions on Automatic Control 6: 716-723.

Alben, A.O. and Bogs, H.M. 1936. Zinc content of soils in relation to pecan rosette. Soil Science 41: 329-332.

Anderson, P.C. and Mizzell, R.F. 1987. Physiological effects of galls induced by *Phylloxera notabilis* (Homoptera: Phyllozeridae) on pecan foliage. Environmental Entomology 16: 264-268.

Barkema, H. W., Schukken Y. H., Lam, T.J.G.M., Galligan, D.T., Beiboer, M.L., and Brand, A. 1997. Estimation of interdependence among quarters of the bovine udder with subclinical mastitis and implications for analysis. Journal of Dairy Science. 80: 1592-1599.

Conner, P. J. and Stevenson, K. L. 2004. Pathogenic variation of *Cladosporium caryigenum* isolates and corresponding differential resistance in pecan. Hortscience 39: 553-557.

Converse, R. H. 1960. Physiologic specialization of *Fusicladium effusum* and its evaluation *in vitro*. Phytopathology 50: 527-531.

Demaree, J. B. 1924. Pecan scab with special reference to sources of the early spring infections. Journal of Agricultural Research 28: 321-330.

Demaree, J. B. 1928. Morphology and taxonomy of the pecan scab fungus, *Cladosporium effusum* (Wint.) comb. nov. Journal of Agricultural Research 37: 181-187.

Driever, G. F. 1998. Development of a threshold model for scheduling fungicide applications to control pecan scab**:** ix, 105 leaves.

Francl, L. J. 2001. The disease triangle: A plant pathological paradigm revisited. The Plant Health Instructor.

Gordis, L. 2000. Epidemiology. Philadelphia, W.B. Saunders.

Gottwald, T. R. 1982. Spore discharge by the pecan scab pathogen, *Cladosporium caryigenum*. Phytopathology 72: 1193-1197.

Gottwald, T. R. 1982. Taxonomy of the pecan scab fungus *Cladosporium caryigenum*. Mycologia 73: 382-390.

Gottwald, T. R. 1983. Factors affecting spore liberation by *Cladosporium carpophilum*. Phytopathology 73: 1500-1505.

Gottwald, T. R. 1985. Influence of temperature, leaf wetness period, leaf age, and spore concentration on infection of pecan leaves by conidia of *Cladosporium caryigenum*. Phytopathology 75: 190-194.

Gottwald, T. R. and Bertrand, P. F. 1982. Patterns of diurnal and seasonal airborne spore concentrations of *Fusicladium* effusum and its impact on a pecan scab epidemic. Phytopathology 72: 330-335.

Gottwald, T. R. and Bertrand P. F. 1983. Effect of time of inoculation with *Cladosporium caryigenum* on pecan scab development and nut quality. Phytopathology: 7144-7718.

Harris, M.K., Cutler, B.L., and Ring, D.R. 1986. Pecan nut loss from pollination to harvest. Journal of Economic Entomology 79: 1653-1657.

Hardin, J. W. and Hilbe J. M. 2003. Generalized estimating equations. Boca Raton, Chapman & Hall/CRC.

Henriques, F. S. 2004. Reduction in chloroplast number accounts for the decrease in the photosynthetic capacity of Mn-deficient pecan leaves. Plant Science 166: 1051-1055.

Hunter, R. E. 1983. Influence of scab on late season nut drop of pecans. Plant Disease: 806-807.

Jacobi, J. C. and Backman P. A. 1995. Au-Pnuts Advisory .2. modification of the rule-based leaf-spot advisory system for a partially resistant peanut cultivar. Plant Disease 79: 672-676.

Jacobi, J. C., Backman, P. A., Davis, D.P., and Brannen, P.M. 1995. Au-Pnuts Advisory .1. development of a Rule-based system for scheduling peanut leaf-spot fungicide applications. Plant Disease 79: 666-671.

Johnson, D. C. 1997. United States is world leader in tree nut production and trade. USDA-ERS Fruit and Tree Nuts Situation and Outlook (FTS-280).

Lan, Z. and Scherm, H. 2003. Moisture sources in relation to conidial dissemination and infection by *Cladosporium carpophilum* within peach canopies. Phytopathology 93: 1581-1586.

Latham, A. J. 1982. Effects of some weather factors and *Fusicladium effusum* conidium dispersal on pecan scab occurrence. Phytopathology 72: 1339-1345.

Latham, A. J. and Rushing, A. E. 1988. Development of *Cladosporium caryigenum* in Pecan Leaves. Phytopathology 78: 1104-1108.

Lentz, P. L. 1957. Taxonomy of the pecan scab fungus. Mycologia 49: 874-878.

Liang, K.Y. and Zeger, S. L. 1986. Longitudinal data analysis using generalized linear models. Biometrika 73: 13-22.

Liu, I. and Suesse, T. 2008. The analysis of stratified multiple responses. Biometrical Journal 50: 135-149.

McDermott, J. J., Schukken, Y. H., and Shoukri, M.M. 1994. Study design and analytic methods for data collected from clusters of animals. Preventive Veterinary Medicine 18: 175-191.

National Agricultural Statistical Survey (NASS) 2009. Noncitrus Fruits and Nuts 2008 Summary. A. S. Board, USDA.

Pady, S. M., Kramer, C. L., and Clary, R. 1969. Periodicity in Spore Release in *Cladosporium*. Mycologia 61: 87-98.

Pan, W. 2001. Akaike's information criterion in generalized estimating equations. Biometrics 57: 120-125.

Rand, F.V. 1914. Some disease of pecans. Journal of Agricultural Research. United States Department of Agriculture. 1: 303 - 338.

Reynolds, K. L., Brenneman, T. B., and Bertrand, P.F. 1997. Sensitivity of *Cladosporium caryigenum* to propiconazole and fenbuconazole. Plant Disease 81: 163-166.

Rushing, A. E. and Latham, A. J. 1991. Some ultrastructural observations of *Cladosporium caryigenum* growth in pecan leaves. Phytopathology 81: 1102-1108.

Sanderlin, R.S. and Heyderich-Alger, K.I. 2000. Evidence that *Xylella fastidiosa* can cause leaf scorch disease of pecan. Plant Disease 84: 1282-1286.

Salisbury, F. B. and Ross, C. W. 1992. Plant physiology. Belmont, Calif., Wadsworth Pub. Co.

Seyran, M., Nischwitz, C., Lewis, K.J., Gitaitis, R.D., Brenneman, T.B., and Stevenson, K.L. 2010. Phylogeny of the pecan scab fungus *Fusiclaidum effusum* G. Winter based on the cytochrome b gene sequence. Mycology Progress 9:305-308.

Schnabel, G., E. L. Schnabel, and Jones, A.L. 1999. Characterization of ribosomal DNA from Venturia inaequalis and its phylogenetic relationship to rDNA from other tree fruit *Venturia* species. Phytopathology 89: 100-108.

Schubert, K., Groenewald, J. Z., Braun, U., Dijksterhuis, J. Starink, M., Hill, C.F., Zalar, P., de Hoog, G.S. Crous, P.W. 2007. Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for Cladosprorium taxonomy and diagnostics. Studies in Mycology: 105-156.

Schubert, K. and Braun U. 2002. *Fusicladium effusum* [Descriptions of Fungi and Bacteria]. CMI descriptions of pathogenic fungi and bacteria 152: 1514.

Snow, J. 1855. On the mode of communication of cholera. London, J. Churchill.

Song, P. X. K. 2007. Correlated data analysis: modeling, analytics, and applications. New York City, Springer.

Szklo, M. and Nieto, F. J. 2007. Epidemiology : beyond the basics. Sudbury, Mass., Jones and Bartlett Publishers.

Thompson, T.E. and Grauke, L.J. 1990. Pecans and hickories (*Carya*). Acta Horticulturae 837-904.

Turechek, W. W. and Stevenson, K. L. 1998. Effects of host resistance, temperature, leaf wetness, and leaf age an infection and lesion development of pecan scab. Phytopathology 88: 1294-1301.

U.S. Department of Agriculture, National Agricultural Statistics Service. 2010. Crop Production. December 2010. Print.

Von Broembsen, S., Smith, D., Carlson, J.D., Sutherland, A., Hoey, M. 2009. Mesonet Agweather Pecan Scab Advior. Stillwater, Oklahoma State University: 1-2.

Zeger, S. L. and Liang, K. Y. 1986. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42: 121-130.

Zorn, C. J. W. 2001. Generalized estimating equation models for correlated data: a review with applications. American Journal of Political Science 45: 470-490.

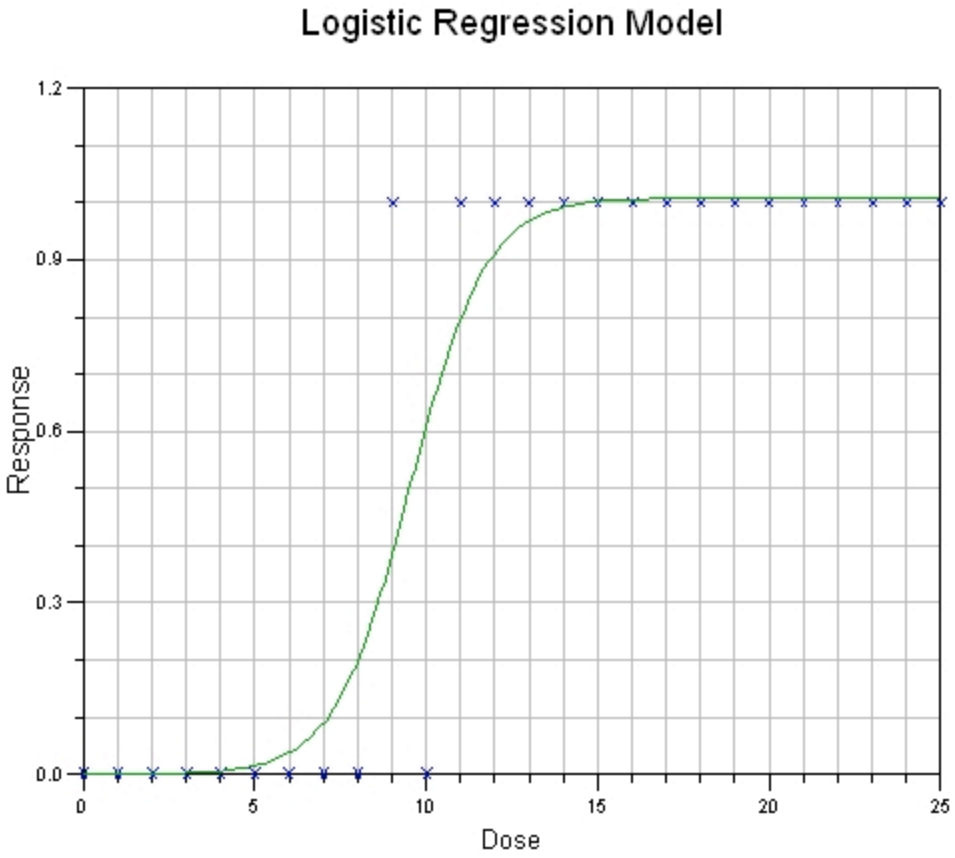


Figure 1: A sigmoidal curve demonstrating the probability of an event (response) on the y-axis in response to the independent parameter (Dose) on the x-axis.

CHAPTER II

INDUCING SPORULATION BY THE FUNGUS *FUSICLADIUM EFFUSUM IN VITRO*

Abstract

Fusicladium effusum, the causal agent of pecan scab, is characterized as a slow growing fungus with minimal spore production when mycelial plugs are inverted onto traditional media, such as potato dextrose agar (PDA) and malt extract agar (MEA). Sporulating growth and spore production were evaluated on five media. Mycelial plugs from six fungal isolates were macerated in 1 ml of sterile water with 3, 3-mm glass beads at 4600 rpm on a bead beating apparatus for 10 seconds. Media types tested were Sabouraud dextrose agar (SDA), PDA, MEA, potato carrot agar amended with 50% lactic acid (PCAL), and water agar (WA) as a negative control. Twenty µl of suspended hyphal material from each isolate and a water drop control were placed on each media type. Media were observed for growth and sporulation for 10 days at 12 hour intervals. Media were significantly different in sporulating area ($P < 0.001$) and spore production ($P < 0.001$). Sporulation on PCAL, PDA, and MALT were comparable while SDA was lower. No sporulation occurred on WA. Isolates were significantly different in sporulating area ($P < 0.0001$) and spore production ($P < 0.01$). A significant interaction

($P < 0.01$) of media and sporulation of isolate sporulating area was found due to high variability of isolates sporulating. Increased sporulation observed in this trial may be due using a maceration technique on hyphal tissue prior to plating.

Introduction

Pecan scab, caused by the fungus *Fusicladium effusum* (Wint.), is the most destructive fungal disease of pecans (*Carya illinoinensis* (Wangenh.) K. Koch). Severe epidemics of pecan scab can result in yield reduction and/or kernel quality if proper management techniques are disregarded (von Broembsen, 2009). The fungus overwinters in lesions produced during the previous season. Conidia produced from these lesions serve as the primary source of inoculum during the next season's epidemic. Infection events can occur as early as April, coinciding with budbreak of pecan trees.

In culture, *F. effusum* can be characterized as slow growing with limited spore production when mycelial plugs are inverted onto traditional media such as potato dextrose agar and malt extract agar. Carbohydrate utilization studies performed by Barnes and Adams (1963) and Hopp and Barnes (1967) demonstrated d-fructose, d+ mannose, and d+ raffinose were most suitable carbohydrates for the growth of *F. effusum*. Sucrose has been shown to be both suitable (Hopp and Barnes, 1967) and unsuitable (Barnes and

Adams, 1963) for the growth of *F. effusum.* Fructose ($C_6H_{12}O_6$) and mannose ($C_6H_{12}O_6$) are both monosaccharides commonly found in fruit. Raffinose ($C_{18}H_{32}0_{16}$) is a trisaccharide composed of galactose, fructose, and glucose. By being able to utilize these carbohydrates we can assume that *F. effusum* is able to make mannase, fructase, and an enzyme or group of enzymes capable of hydrolyzing raffinose into the monosaccharides galactose, fructose, and glucose. Since galactose and glucose are both poorly utilized by *F. effusum* (Barnes and Adams, 1963; Hopp and Barnes, 1967) it appears that the fructose component of raffinose is the primary monosaccharide component utilized for energy.

Currently potato dextrose agar is the medium most commonly used for the growth of *F. effusum*. Potato dextrose agar is a complex medium made by soaking unpeeled potatoes in distilled water and adding the monosaccharide dextrose ($C_6H_{12}O_6$) to the media producing potato dextrose broth. The result is a rich, slightly acidic media that is conducive to the growth of *F. effusum* (Hopp and Barnes, 1967). The effect of various nutrient rich media on sporulation by *F. effusum* has not been well studied. However, inducing sporulation in the laboratory can be difficult, thereby, limiting the ability to conduct certain epidemiological experiments *in vitro*. The objective of this study was to evaluate the effects of different media on mycelia growth and spore production by *F. effusum*.

Material and Methods:

Isolate Preparation. Six single-spore isolates (isolates 18, 21, 23, 25, 28, and 39) of *F. effusum* collected in Jeff Davis County, Georgia during the 1998 growing season were

maintained on potato dextrose agar at room temperature (19-20 °C) under 24 hours of continuous light provided by fluorescent light tubes. Once the mycelial colony grew to approximately 5 mm in diameter a #2 cork borer was used to harvest a mycelial plug for maceration. The plug was placed in a 1.5-ml tube with one ml of sterile deionized (DI) water and three sterilized 3-mm glass beads (Kimble-Chase). Tubes were then placed on a bead beating apparatus (BioSpec, Mini-Bead Beater) and cycled one time for 10 seconds at 4600 rpm. The macerated mycelium was then used in the experiments described below.

Media Evaluation. Media used included potato dextrose agar (PDA), potato carrot agar amended with 50% lactic acid (PCAL), malt extract agar (MEA), sabouraud dextrose agar (SDA), and water agar (control) (Table 1). Aliquots (20 µl) of the macerated mycelia suspension were spread onto Petri plates using a sterilized transfer loop. Plates were then wrapped in Parafilm then placed in a growth chamber (Conviron, Model No. PGR15) for 10 days at 21° C and 70% relative humidity. The experimental design was a randomized complete block with three replications. Experiments were repeated 7 times. Every 12 hours for the first 10 days, observations were made for the presence/absence of growth and sporulation. On day 11, images were taken of each plate with a Nikon D300 digital camera with AF-S VR micro-Nikkor 105mm f 12.8G lens over a blue background. Images were analyzed using Assess 2.0 (APS Press) for sporulating area, vegetative growth area, and total growth, measured as a percentage of the plate. Under green saturation, pigmentation between 0 and 56 was considered vegetative growth and pigmentation between 92 and 114 was considered sporulating area. Data on sporulating area data (percent of plate) were subjected to a square root transformation to normalize

the data. Sporulation was quantified by removing a mycelial plug from each plate using a #2 cork borer (5-mm diameter), which was selected arbitrarily from each plate. Each plug was placed in one ml of a 10% Tween 80 solution and spores were dislodged by vortex and physical manipulation. Ten µl of spore suspension was placed on a hemacytometer to quantify the number of spores on each plug. Using the percent sporulating area calculated by Assess 2.0 an estimation of the number of spores on each plate was calculated using the following formula:

$$(\Pi * r^2) * ((\%\ \text{sporulating area})/100) * (\#\ \text{of spores per 5 mm plug}/4)$$

Only mature conidia showing bud scars were counted.

Statistical Analysis. Analysis of variance (ANOVA) to determine the response of sporulating area and estimated spore production to media type, isolate, and media by isolate interaction was performed using PROC MIXED of SAS (v.9.2). Degrees of freedom were calculated using the Satterthwaite method to account for unequal variances.

Results:

Sporulating Area. Media, isolate, and the interaction of isolate by media were significant (P= 0.05) effects on percent sporulating area (Table 1). All isolates had similar sporulating area on PDA with the only differences observed between isolates 23 and 28 (Figure 2A). Isolates 39 and 25 had significantly higher sporulating area as compared to other isolates when grown on PCAL (Figure 2B). The highest individual sporulating area throughout this study was observed by isolate 39 on PCAL. Isolates 39, 25, and 18 were the only isolates significantly different from the water control when grown on PCAL. Isolates 25, 18, 28, and 21 were the only isolates significantly different

from the water control when grown on SDA (Figure 2C). The lowest sporulating area on SDA was isolate 23 (Figure 2C), which exhibited the highest sporulating area on PDA (Figure 3A). Isolates grew poorly on MEA, with only isolate 25 having significantly higher sporulating area than the water control (Figure 2D).

Spore Production. Media and isolate were significant ($P = 0.05$) effects on of spore production (Table 2). Significantly higher numbers of spores were produced on PDA as compared to other media types (Figure 3A). SDA was not significantly different from the water agar control (Figure 3A). The highest numbers of spores were produced by isolate 39 (Figure 3B) and lowest spore numbers were produced by isolate 21, which was not significantly different from the water control (Figure 3B).

Discussion:

PDA was the best medium for inducing spore production of *F. effusum* in this study. PCAL and MEA are suitable media for increasing production of mature spores. SDA is not a suitable media for inducing sporulation of *F. effusum*.

F. effusum was previously shown to grow best on a monosaccharide carbohydrate (Barnes and Adams, 1963; Hopp and Barnes, 1967). It is unclear why SDA, with an acidic pH and higher concentration of monosaccharide dextrose as compared to PDA, produced the least number of mature spores. PDA is an undefined media and might have an unknown concentration of other carbohydrate sources from the potato infusion that are favorable to growth and spore production by *F. effusum*. Infusion of potatoes primarily consist of carbohydrates starch, sucrose, glucose, and fructose. The unknown

concentration of these carbohydrate sources in PDA would explain the ability of potato carrot agar amended with 50% lactic acid to support growth and sporulation of *F. effusum* without further supplementation of dextrose to the medium. Alternatively, the enzymatic digest of casein and animal tissues could have inhibited sporulation of *F. effusum*. An inhibition of sporulation has been demonstrated in *Venutria inaequalis* and *Venturia pirina,* the causal agent of pear scab, on a media containing glucose and enzymatic digest of casein (Kirkman, 1957). Fatty acids have also been shown to inhibit sporulation of *F. effusum* (Gottwald and Wood, 1984).

The amount of variability in growth and sporulation among isolates in this trial was greater than expected given the limited area in both time and space isolates were gathered. A differential response of sporulating growth on media by isolates could be due to unknown genetic variability within the population of *F. effusum.* Conner and Stevenson (2004) demonstrated a high degree of variability with respect to pathogenicity within a collection of *F. effusum* isolates. This has also been demonstrated in related fungal species (Fothergill and Ashcroft, 1955). Conner and Stevenson's conclusions focused on the implications in pecan resistance genes, however with the finding of this study there may be additional factors that vary within the population of *F. effusum* than previously assumed.

Although not examined directly, using a maceration technique of hyphal tissue rather than inversion of mycelia plugs onto nutrient rich media appears to be more favorable to increased spore production. Streaking suspended, macerated hyphal material onto a media results in multiple foci from which vegetative growth can expand thereby increasing the area of sporulating growth and in turn the number of spores capable of

being produced. By using this maceration technique in conjunction with PDA, PCAL, or MEA, sporulation of *F. effusum* can be greatly increased.

Acknowledgments: This research was supported by the Oklahoma Pecan Growers Association. Special thanks to Dr. Katherine Stevenson, University of Georgia, for supplying isolates used during this study.

Literature Cited

Barnes, G. L. and Adams, I. N. 1963. A preliminary investigation on carbohydrate nutrition of the pecan scan fungus, *Fusicladium effusum* Wint. Proceeding of the Oklahoma academy of science. 44: 189-192.

Conner, P. J. and Stevenson, K. L.. 2004. Pathogenic variation of Cladosporium caryigenum isolates and corresponding differential resistance in pecan. Hortscience 39: 553-557.

Dhingra, O. D. and Sinclair, J. B. 1985. Basic plant pathology methods. Boca Raton, Fla., CRC Press.

Fothergill, P.G. and Ashcroft, R. 1955. The nutritional requirements of *Venturia inaequalis.* Journal of General Microbiology 12:387-395.

Fothergill, P.G. and Ashcroft, R. 1955. Further nutritional studies of *Venturia inaequalis*. Journal of General Microbiology 13: 399-407.

Gottwald, T. R. 1985. Influence of Temperature, Leaf Wetness Period, Leaf Age, and Spore Concentration on Infection of Pecan Leaves by Conidia of Cladosporium-Caryigenum. Phytopathology 75: 190-194.

Gottwald, T.R. and Wood, B.W. 1984. The effect of fatty acids on growth and sporulation of the pecan scab fungus Cladosporium caryigenum. Mycologia 76: 326-331.

Hopp, A.D. and Barnes, G.L. 1967. Utilization of carbohydrate compounds by the pecan scab fungus, fusicladium effusum Wint. Proceedings of the Oklahoma Academy of Science 48:210-215.

Kirkham, D.S. 1957. Studis of the significance of polyphenolic host metabolistes in the nutrition of *Venturia inaequalis* and *Venturia pirinia*. Journal of General Microbiology 17: 120-134.

Turechek, W. W. and Stevenson, K. L. 1998. Effects of host resistance, temperature, leaf wetness, and leaf age an infection and lesion development of pecan scab. Phytopathology 88: 1294-1301.

Von Broembsen, S., Smith, D., Carlson, J.D., Sutherland, A., Hoey, M. 2009. Mesonet Agweather Pecan Scab Advior. Stillwater, Oklahoma State University: 1-2.

Table 1: Composition of media used in this study.

Medium	Abbreviation	Composition (per liter of distilled water)	Distributor / Reference
Potato dextrose agar	PDA	200.0 g infusion from potatoes, 20.0 g dextrose, 15.0 g agar	HiMedia
Malt extract agar	MEA	30.0g malt extract, 5.0g peptone, 15.0 g agar	Difco
Potato and carrot agar amended with 50% lactic acid	PCAL	20.0g infusion from potatoes, 20.0g infusion from carrots, add 50% lactic acid to final pH of 7.0, 15.0g agar	Dhingra, O. D. and Sinclair, J. B. 1985
Sabouraud dextrose agar	SDA	5.0g enzymatic digest of casein, 5.0g enzymatic digest of animal tissue, 40.0g dextrose, 15.0g agar	Acumedia
Water agar	WA	15.0g agar	

Table 2: Analysis of variance (ANOVA) of main and interactive effects of media and isolate on percent sporulating area.

Source	df_n[a]	df_d[b]	F-value	*P*-value
Trial	4	48	3.46	0.0147
Repetition	2	48	0.45	0.6416
Media	4	48	10.82	<.0001
Trial*media	16	48	1.61	0.0909
Isolate	6	300	7.83	<.0001
Media*Isolate	24	300	1.89	.0080
Trial*Isolate	24	300	3.38	<.0001
Media*Trial*Isolate	96	300	1.56	0.0024

[a] numerator degrees of freedom

[b] denominator degrees of freedom

Table 3: Analysis of variance (ANOVA) of main and interactive effects of media and isolate on estimation of total spore production.

Source	df_n[a]	df_d[b]	F-value	*P*-value
Trial	4	48	1.65	0.1765
Repetition	2	48	0.16	.8494
Media	4	48	6.18	0.0004
Trial*Media	16	48	1.61	0.1026
Isolate	6	300	3.71	0.0014
Media*Isolate	24	300	1.02	0.4335
Trial*Isolate	24	300	1.79	0.0146
Media*Trial *Isolate	96	300	1.70	0.0004

[a] numerator degrees of freedom

[b] denominator degrees of freedom

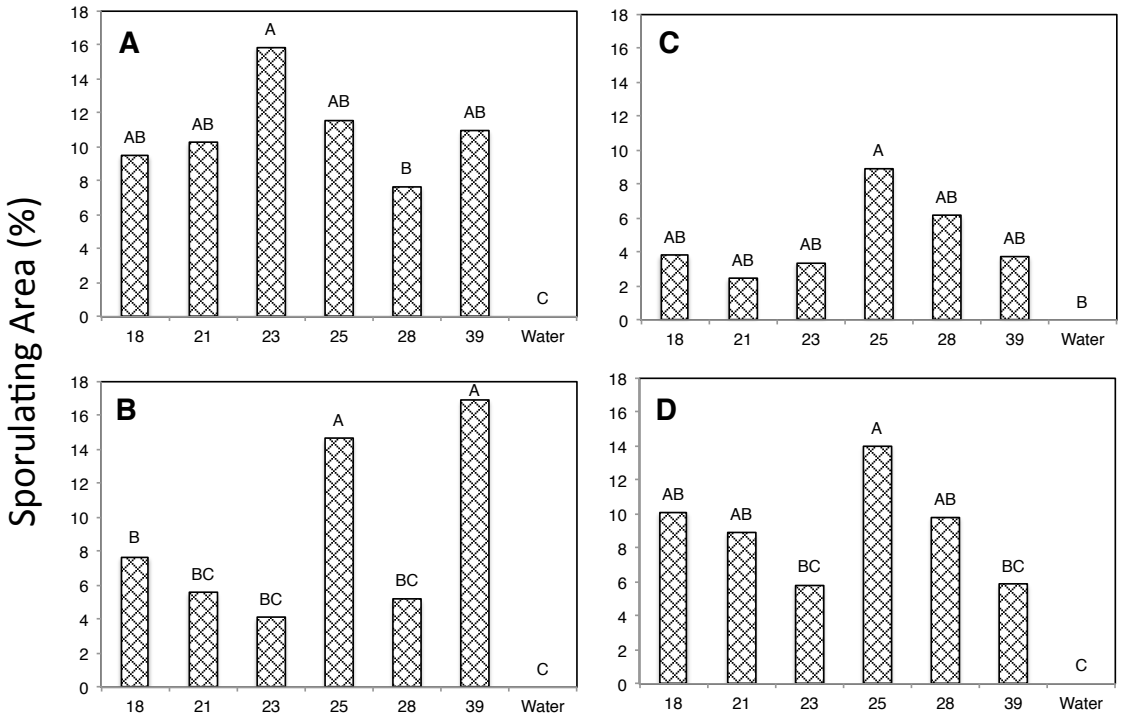


Figure 2: Means of percent sporulating area of isolates on **A,** Potato Dextrose Agar, **B,** Potato Carrot Agar amended with 50% Lactic Acid, **C,** Malt Extract Agar, and **D,** Sabouraud Dextrose Agar. The SLICE option was used in PROC MIXED of SAS to mean differences of isolate within media (LSD = 6.62). Means with a common letter within a study parameter signify isolates were not significantly different ($P > 0.05$).

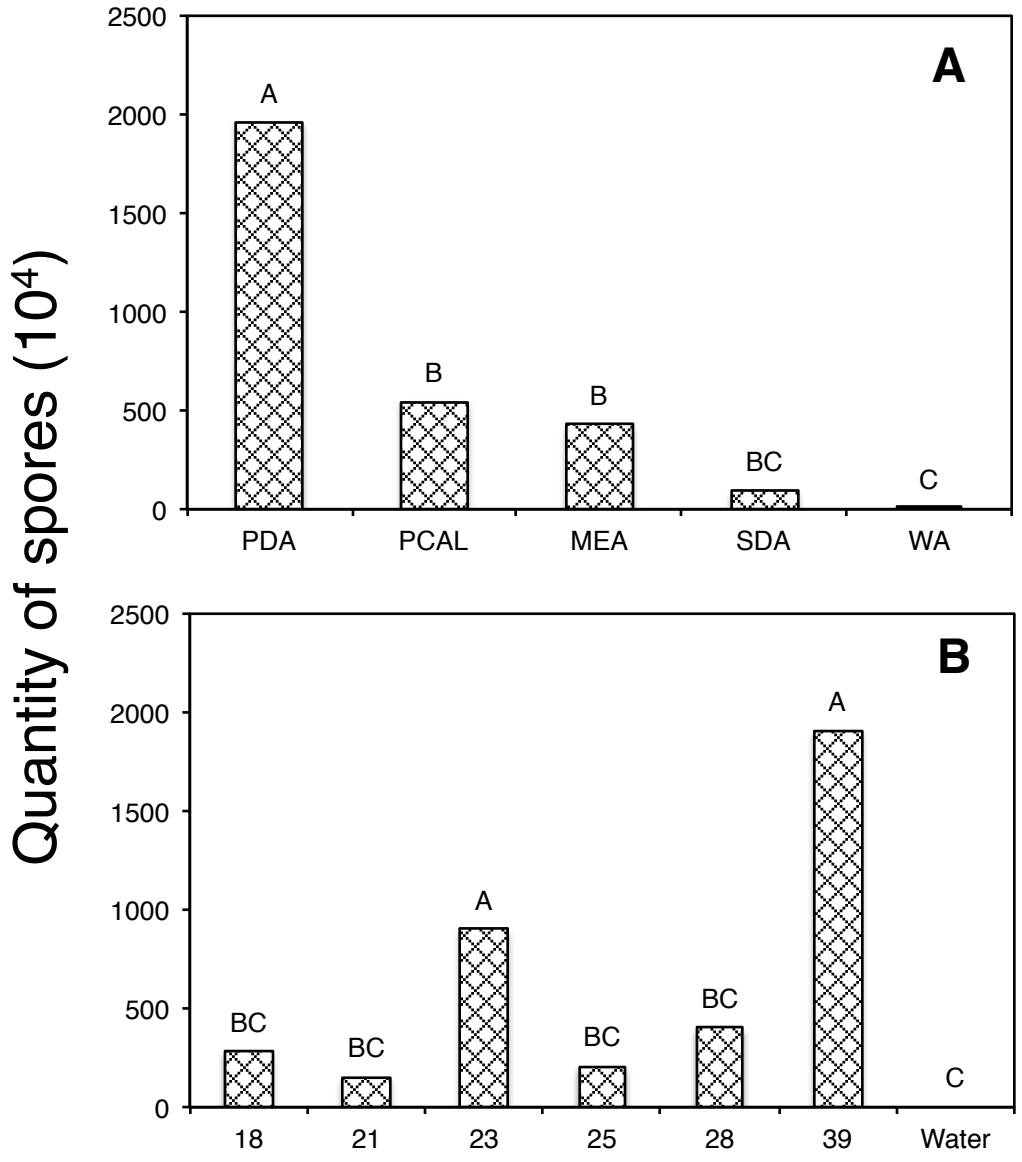


Figure 3: Main effects of **A,** media (LSD = 1899) and **B,** isolates (LSD = 2077) on spore production. Means with a common letter within a study parameter signify isolates were not significantly different ($P > 0.05$).

CHAPTER III

DEVELOPMENT AND EVALUATION OF PECAN SCAB PREDICTION MODELS USING REPEATED MEASURES LOGISTIC REGRESSION ANALYSIS

Abstract

Fusicladium effusum (Wint.) (syn. *Cladosporium caryigenum* (Wint.) Gottwald), causal agent of pecan scab, is the most economically important pathogen of pecans (*Carya illinoinensis)* (Wangenh.) K. Koch). Severe epidemics of pecan scab can reduce crop yield and quality. To manage pecan scab, fungicides sprays are routinely used. A weather-based advisory currently used to assess fungicide application in Oklahoma requires the accumulation of scab hours. A scab hour is defined as an hour of average temperature and relative humidity ≥ 21.1°C and 90%, respectively. To assess the validity of the thresholds in the advisory, repeated ratings of disease severity were taken on fruit - each year during the 1994-1996 and 2009-2010 growing seasons. Weather variables were also examined including temperature, relative humidity, dew point, dew point depression, total solar radiation, and total rainfall. Rain and disease severity were converted to binomial variables where a rain event (≥ 2.5mm) and disease severity (≥ 25%) were coded as 1 and all other events as 0. Logistic regression models adjusted for correlated data were developed using generalized estimating equations. Two models were

developed including a temperature/relative humidity model and a dew point/dew point depression model. For the temperature/relative humidity model, the best fitting model included all main effects. Using this model, validation exercises assuming no-rain and total solar radiation of 22.5 MJ m^{-2} resulted in a 0.62 probability of scab development when temperature was 21° C and relative humidity was 90%. Findings of this model were further validated during field studies that evaluated different combinations of temperature and relative humidity thresholds for operational use. These analyses indicate that the current thresholds of temperature and relative humidity are viable, but a modification of the temperature component should be considered. For the dew point/dew point depression model, a reduced model including dew point, dew point depression and a binomial rain variable was considered adequate for explaining scab events. This analysis suggests that future model building to describe pecan scab epidemics should include dew point, dew point depression, rain, and total solar radiation as independent variables.

Introduction

Fusicladium effusum (Wint.) (syn. *Cladosporium caryigenum* (Wint.) Gottwald), the causal agent of pecan scab, is the most economically important fungal pathogen of pecans (*Carya illinoinensis)* (Wangenh.) K. Koch). During severe epidemics of pecan scab, kernel quality can be reduced and crop losses can be high (Gottwald and Bertrand, 1983; Hunter, 1983). *F. effusum* overwinters as stroma on infected shoots and fruit shucks from the previous season. When springtime temperatures begin to warm, conidia

are liberated from the stroma and serve as primary inoculum. Symptoms of pecan scab can occur as early as budbreak in Oklahoma, beginning as small grey green lesions that develop into larger dark brown lesions (Demaree, 1924). Expanding leaves and twigs are the first plant parts infected. Fruit are infected via primary and secondary inoculum produced on leaves and twigs (Demaree, 1924; Hunter, 1983). In controlled environment studies using leaves, *F. effusum* grows optimally at 15 – 25°C with 48 hours of leaf wetness, while the greatest lesion numbers occurred at 20 °C with 48 hours of leaf wetness (Gottwald, 1985).

Leaf wetness events are periods when there is an accumulation of free moisture on leaf surfaces. Many fungal pathogens require leaf wetness to enable processes such as infection and spore discharge (Mwakutuya and Banniza, 2010; Gottwald and Bertrand, 1983). In previous research with *F. effusum*, infection can occur between 0 and 48 hours of continuous leaf wetness duration. However, optimal lesion development occurred during 48 hours of continuous leaf wetness (Gottwald, 1985). In the field, leaf wetness can be attributed to several sources including rainfall, irrigation, and dew events. There is no standardized protocol for the determination of leaf wetness although protocols have been suggested (Sentelhas *et. al,* 2004). Without a standardized protocol for preparation, placement, and programming of physical sensors, leaf wetness durations calculated cannot be compared between studies. Surrogate weather variables such as dew point and relative humidity are sometimes used to predict leaf-wetting events. According to the National Oceanic and Atmospheric Administration (NOAA), dew is 'moisture that has condensed on objects near the ground, whose temperatures have fallen below the dew point temperature' (National Weather Service Internet Services Team). Dew point is

further defined as 'a measure of atmospheric moisture. It is the temperature to which air must be cooled in order to reach saturation …' (National Weather Service Internet Services Team). Direct measurement of dew point can be accurately performed using a dew-point hygrometer and continues to be the standard for measurement. However, due to the sensitivity of the instrument to disruption by debris, dew-point hygrometers can be impractical for use in agricultural environments (Burch and Levetin, 2002). In meteorology, dew point is often estimated using the following formula (Lawrence, 2005):

$$t_d \approx t - \left(\frac{100 - RH}{5}\right)$$

where t_d and t are the dew point and ambient temperatures, respectively, and RH is relative humidity. The formula is capable of determining t_d when RH >50%.

Relative humidity as defined by NOAA is 'a ratio, expressed in percent, of the amount of atmospheric moisture present relative to the amount that would be present if the air were saturated at a specific temperature. Relative Humidity is derived from the associated ambient temperature and dew point…' (National Weather Service Internet Services Team). Both relative humidity and dew point are indicators of the amount of moisture in the air, however relative humidity is dependent upon the temperature at which it is was measured. As temperature begins to fluctuate, relative humidity can change independent of atmospheric moisture. Dew point is often favored over relative humidity because it is an indicator of absolute moisture content of the atmosphere.

When the ambient temperature reaches the dew point, the air is saturated and dew begins to condense. Dew deposition can be several nm to mm in depth and evaporation

of moisture is dependent upon canopy structure and weather conditions (Huber and Gillespie, 1992; Richards, 2004). Research in apples suggests a high degree of variability within the canopy during leaf-wetting and -drying events (Batzer *et al.,* 2008). This makes the definition of leaf wetness above, applicable only to individual leaves and not a plant or entire orchard. Because of the inability to define leaf wetness at the micro-meteorological scale or have a standardized means of determining leaf wetness (Sentelhas *et al.*, 2004), estimations of the formation of dew at the macro-meteorological scale aid in forecasting plant disease epidemics. Hence, disease prediction models often rely on relative humidity recorded on weather stations to estimate leaf wetness.

Current pecan scab management practices rely heavily on the use of fungicides to protect trees from the disease. In order to facilitate proper timing of fungicide applications the Oklahoma Agweather Pecan Scab Fungicide Advisory was developed in the mid-1990s. The advisory uses air temperature and relative humidity to determine 'scab hours' (Driever, 1998). A 'scab hour' is defined as an hour in which the average air temperature is greater than or equal to 21.1°C and the relative humidity is greater than or equal to 90%. Fungicide applications are not advised until 10 scab hours, 20 scab hours, or 30 scab hours have accumulated over the 14-day period for susceptible, moderately susceptible, and resistant cultivars, respectively (Sutherland, 2005). For the Oklahoma Agweather Pecan Scab Fungicide Advisory, temperature and relative humidity data are collected from the Oklahoma Mesonet weather station network (www.mesonet.org) at a standard height of 1.82 m above the ground. Currently there are 120 collection sites located throughout Oklahoma with at least one collection site in each county.

Recently growers have questioned the performance of the advisory program resulting in speculation that the model may not be accurately identifying periods favorable for pecan scab epidemics. Inaccuracies in the advisory could be from two sources. First, weather stations are located in areas that may not be representative of the microclimate typically found in Oklahoma pecan groves. Pecan groves tend to be in low-lying areas and/or near bodies of surface water, which can dramatically change the microclimate. Another explanation for the poor accuracy of the advisory might be that pertinent weather variables are missing from the model. Finally, current temperature and/or relative humidity thresholds may be incorrect. The current thresholds were set to reflect levels of temperature and relative humidity known to be biologically significant for symptom development. The temperature threshold was based on research in Georgia (Gottwald, 1985), and in separate research in Oklahoma (Driever, 1998), which indicated that temperatures ≥ 21.1 ˚C correlated with increases of pecan scab. Gottwald (1985) reported that isolates of *F. effusum* were capable of infecting leaves at temperatures of 15 to 25 ˚C. Turechek and Stevenson (1998) found similar trends with infection and disease development occurring at temperatures as low as 15 ˚C and no infection or disease development at 35 ˚C.

For the current Oklahoma advisory program, a relative humidity threshold for scab of 90% was selected to represent wetting events (Driever, 1998). As indicated previously, relative humidity can be used to predict wetting events and a value of ≥ 90% is a suitable indicator (Monteith, 1957). However, in some locations such as the upper Midwestern United States, leaf wetness has been reported to occur at lower thresholds of relative humidity from 83-85% (Sentelhas *et al.*, 2008). In Oklahoma, leaf-wetting events

have been observed at relative humidity values as low as 85% (*data not published*). Considering the wide variation in temperatures at which *F. effusum* can infect and cause disease, and the lower relative humidity thresholds that may correlate with leaf-wetting events, the current advisory may need modification to improve prediction accuracy. Also, other weather variables such as dew point or rain events should be considered as inputs. Therefore, the objectives of this study were to validate the thresholds required by the current fungicide advisory program and assess the inclusion of other weather variables for improving scab prediction.

Materials and Methods

2009 – 2010 Field Trials. Field trials were conducted on a commercial farm near Madill, OK. Trees were planted on a Madill fine sandy loam soil located on a lowland site in 2002. Bare root transplants consisted of 'Pawnee' scion grafted to 'Apache' rootstock. The cultivar 'Pawnee' is considered moderately susceptible to scab (Sutherland *et. al*, 2005). Trees were spaced 12.2 m apart with a between-row spacing of 12.2 m. Treatments consisted of applying fungicide according to the Oklahoma Agweather Pecan Scab Fungicide Advisory with various modifications to the air temperature and relative humidity combinations required to calculate a 'scab hour'. These included air temperature/relative humidity thresholds of 15.5°C/ 80%, 18.3°C / 80%, 15.5°C/ 85%, and the currently used thresholds of 21.1°C/ 90%. Treatments were compared to a non-spray control. At least two border trees separated each plot that received a treatment. The first fungicide applications for each treatment were applied according to tree phenology during the pre-pollination period, 19 May 2009 and 28 April

2010. Subsequent applications were applied according to the treatment thresholds after 20 scab hours accumulated. This is the recommended number of scab hours to be accumulated by a moderately resistant cultivar when using the advisory (Sutherland et al., 2005). In 2009, fungicides used were a rotation of fenbuconazole at 0.14 kg /ha (Enable; Dow AgroSciences), azoxystrobin at 0.25 kg /ha+ propiconazole at 0.16 kg /ha (Quilt; Syngenta Crop Protection), and azoxystrobin at 0.22 kg /ha. Trials were performed during the 2009 and 2010 growing seasons in a randomized complete block design with five replicates. During the 2010 growing season, fungicides used were a rotation of tebuconazole at 0.25 kg /ha (Folicur; Bayer CropScience), thiophanate-methyl 0.70 kg /ha (Topsin 4.5FL; United Phosphorus), and azoxystrobin at 0.22 kg /ha (Abound; Syngenta Crop Protection). Fungicides were applied with a tractor mounted air blast sprayer (Savage Equipment), calibrated to deliver 100 GPA. Disease severity was assessed visually by estimating the percentage of scab coverage on a single fruit at each cardinal direction, below and above the mid-line of the tree canopy (8 ratings/tree), every 14 or 28 days (depending on trial and location). Ratings for each tree were averaged to one value. Disease progress was analyzed using area under the disease progress curves (AUDPC) by season for all treatments (Shaner and Finney, 1977). Nuts were removed from each tree using a tractor-mounted tree shaker (Savage Equipment) and harvested using an orchard floor harvester (Savage Equipment). Yield data were collected after field cleaning and drying pecans to 6.5% moisture in 2009 and 4.5% moisture in 2010.

1994-1996 Fruit Severity Data. Fruit severity data were collected during the 1994-1996 growing seasons by Dr. G.F. Driever (1998). Rating sites included Sparks, OK (1995-1996), Burneyville, OK (1994-1995), Vinita,OK (1994-1995). For all sites

from 1994-1996, disease severity data were collected using a modified Horsfall-Barratt scale ranging from '1', disease free, to '8', 100% fruit severity (Driever, 1998). Fruit severity was rated per cluster across various locations of the tree canopy. Values as determined by the ordinal scale, were converted to percentages by using the midpoint indicated by each range of the rating scale. Multiple fruit were rated per tree and averaged to produce a single value for each rating per tree.

Weather Variables. Weather data for the 1994-1996 and 2009-2010 growing seasons were collected from the Oklahoma Mesonet weather stations in closest proximity to each rating site. These sites included Sparks, OK (1995-1996), Burneyville, OK (1994-1995), Vinita, OK (1994-1995), Perkins, OK (2009-2010), and Madill, OK (2009-2010). Moving averages over the time intervals between disease assessments were calculated from average daily weather data. Time intervals between disease ratings where 7,14, or 28 days depending on location and study. The weather variables evaluated included air temperature, relative humidity, dew point, dew point depression, total solar radiation, and daily rainfall total. Dew point depression was calculated as the absolute difference between air temperature and dew point. Rainfall was further converted to a binomial variable of rain events where 1 = ≥2.5 mm rain and 0 = <2.5mm. Both total daily rainfall and rain events were used in the model development process.

Model Development. For development of scab prediction models, disease severity data were converted to a binomial variable where ratings of 25% or above were coded as 1 and all other events as 0. Previous research demonstrated that an acceptable threshold for determining economically damaging levels of pecan scab on fruit was

approximately 25% severity (Hunter, 1983). Because multiple disease assessments were collected on the same tree throughout the season, all models were developed using the generalized estimating equations procedure, GEE (PROC GENMOD with the REPEATED statement) in SAS (version 9.2, SAS Institute, Cary, NC, USA) to account for the correlated nature of the data. The GEE procedure is a quasi-likelihood based approach to analyzing longitudinal data (Zeger and Liang, 1986). This differs from traditional logistic regression in that the latter is a maximum likelihood approach. When using the logit link function, GEE becomes a generalized linear model algorithm preferred to logistic regression where data have a repeated measure or clustered design, resulting in dependency between data points (Liang and Zeger, 1986). GEE takes the following form when calculating the score of $\boldsymbol{\beta_i}$ (Zeger and Liang, 1986):

$$S_k(\beta) = \sum_{i=1}^{k} \left(\frac{\partial \mu_i^T}{\partial \beta}\right)' v_i^{-1}(y_i - \mu_i) = 0 \quad \text{for k = 0 , 1, … , p}$$

Where:

$$\mu_i = h(x_i\beta)$$

$$v_i = \frac{A_i^{\frac{1}{2}} R_i(\alpha) A_i^{\frac{1}{2}}}{\Phi}$$

$\boldsymbol{A_i^{\frac{1}{2}} A_i^{\frac{1}{2}}}$ is an *n* x *n* matrix where $\boldsymbol{g(\mu_i)}$ is the *j*th diagonal element, $\boldsymbol{R_i(\alpha)}$ is the working correlation matrix, *h* is referred to as a link function, β is a *p* x 1 vector, and ϕ is a scale parameter estimated using the following equation:

$$\phi = \frac{1}{\sum_{i=1}^{n} n_i} \sum_{i=1}^{n} \sum_{t=1}^{n_i} \widehat{res}_{it}^2$$

By using the logit link, $\ln\left(\frac{p}{1-p}\right)$, in GEE, $S_k(\beta)$ is redefined within the logit transformation resulting in the following linearized transformation:

$$\ln\left(\frac{S_k(\beta)}{1-S_k(\beta)}\right) = \sum_{i=1}^{K} \frac{\partial \mu_i^T}{\partial \beta} v_i^{-1} (y_i - \mu_i)$$

The logit that is estimated by the resulting model can be back-transformed to obtain working probability estimates.

Goodness-of-fit for each model can be determined using the quasi-likelihood-under-the-independence-curve information criterion (QIC) where (Pan, 2001):

$$\text{QIC} = -2Q(\beta(R); I, D) + 2*\text{trace}\ (\Omega_I, V_r)$$

The QIC adjusted for the number of parameters in the model (QICu) is:

$$\text{QICu} = -2Q(\beta(R); I, D) + 2p,$$

Since the QICu can account for the number of parameters in the model (2p), the model is penalized for each parameter resulting in an increase in the QICu when a model is over-parameterized. Interpretation of QIC and QICu in GEE is similar to the AIC in traditional logistic regression (Pan, 2001). Smaller values of QIC or QICu indicate better model fit. The QIC and QICu are unitless and do not follow a distribution, as such a *P*-value cannot be calculated. For this analysis, QIC was used in determining overall fit and proper working correlation matrix. QICu was used to determine proper parameterization of the

models. Unadjusted effects were calculated for use in the model building process and significance was determined using a χ^2. All models were developed using a forward selection process beginning with the most significant unadjusted effect.

Results

2009 Field Trials: Following the pre-pollination fungicide application, fungicides were applied two times during the 2009 growing season for the 21.1°C / 90% treatment on 29 June and 31 July. Fungicide applications were applied four times (following the pre-pollination spray) for the remaining treatments on 8 June, 26 June, 23 July, and 14 August for 18.3 °C/ 80%; 12 June, 1 July, 31 July, and 25 August for 15.5 °C/ 85%; 10 June, 29 June, 23 July, and 14 August for 15.5 °C / 80%. Because of low levels of disease in the 2009 growing season, there were no significant differences in final disease severity or AUDPC among treatments (Table 4). However, numerically larger values of final disease severity and AUDPC were observed in the non-sprayed and 15.5°C/ 85% treatments compared to the 21.1°C / 90% treatment. Yield differences were not significantly different among treatments (Figure 4A).

2010 Field Trials. Following the pre-pollination application, fungicides were applied two times during the 2010 growing season according to the 21°C/ 90% treatment on 28 April, 8 June, and 28 June. Fungicide applications were applied four times for the remaining treatments on the following dates: 18 May, 4 June, 28 June, and 20 July for 15.5 °C/ 85%; 18 May, 4 June, 24 June and 16 July for 18.3 °C/ 80%; 18 May, 4 June, 24 June and 16 July for 15.5 °C / 80%. The highest levels of disease were observed in plots not treated with fungicide (Table 4). All plots that were sprayed with fungicides had

significantly less disease and lower AUDPC values than the non-sprayed control. Yield differences were not significantly different among all treatments. However, yield tended to be higher in plots treated with fungicide (Fig. 4B).

Model development. Analysis of the single-effects models demonstrated that temperature and total daily rain were not significant effects for predicting the probability of pecan scab, while relative humidity was the most significant unadjusted effect followed by dew point depression (Table 5). Because of previous research indicating the biological significance of temperature and total daily rain on pecan scab development, these variables where forced into one model during the build process. A second model was developed using dew point and dew point depression rather than temperature and relative humidity. Subsequent full and reduced model testing indicated that the full main effects model was the best fit in the temperature/ relative humidity model (Table 6), and a reduced dew point/dew point depression model was most appropriate (Table 7) to describe the probability of a significant (fruit severity ≥25%) pecan scab event.

To further evaluate both models and use the temperature/relative humidity model to test the validity of using 21.1°C temperature and 90% relative humidity thresholds in the current Oklahoma Agweather Pecan Scab Fungicide Advisory, temperature or relative humidity and dew point or dew point depression were held constant while adjusting the other variable within the back-transformed models. It was assumed that a 0.50 probability of significant pecan scab increase was a suitable action threshold (e.g. probabilities ≥ 0.50 indicated disease was likely). For the temperature/relative humidity model, temperature, relative humidity, and the binomial rain variable were positively

correlated to an increasing probability of observing significant levels of pecan scab on fruit. Increasing levels of total solar radiation were inversely correlated with the probability of economically damaging levels of pecan scab. The probability of significant scab increase never approached the 0.50 action threshold when relative humidity increased at a constant temperature of 18 °C, the minimum observed temperature (Figure 5A). At the mean observed temperature of 21.2 °C, the predicted probability of a significant increase in scab was above 0.50 when relative humidity was 87% or above (Figure 5B). At the maximum observed temperature of 30.6 °C the probability of a significant pecan scab event was predicted to be above 0.50 when relative humidity was ≥ 82% (Figure 5C). When temperature was adjusted at a constant relative humidity of 57% or 72%, significant levels of pecan scab were not predicted (Figure 5D and E). Only when relative humidity was fixed at 87% were significant increases in pecan scab predicted when temperature was ≥ 24°C (Figure 5F).

In the dew point/dew point depression model, the reduced model that included dew point, solar radiation, and rain variables was the best fitting model (Table 7). However, because of the lack of dew point depression in this model a reduced model that included dew point, dew point depression, and rain variables was selected as the most appropriate model. In order for dew point to be an appropriate parameter for addition to the model, a reference to the temperature must be included in the model build. Dew point depression is the difference between ambient and dew point temperatures. Although dew point is the point at which atmospheric saturation occurs, the proximity of the ambient temperature to this point of saturation must be included. As the dew point depression gets

smaller, the more likely it is that a dew event will occur. Resulting in conditions that are conducive for scab epidemics.

In the reduced model that included dew point, dew point depression, and the binomial rain variable, dew point was positively correlated with an increasing probability of observing significant increases in pecan scab. Rain events greater than 2.5 mm and dew point depression were inversely correlated to an increasing probability of observing significant pecan scab. When dew point was fixed at a constant value of 14.4 °C, the probability of a significant scab event was above the 0.50 action threshold at dew point depression values greater than 4 °C (Fig. 6A). At a fixed dew point of 20.6 °C, the predicted probability of a significant increase in scab was above 0.50 when dew point depression was greater than 9 °C (Figure 6B). At the maximum observed dew point of 24.4 °C the probability of a significant pecan scab event was predicted to be above 0.50 at a dew point depression greater than 12 °C (Figure 6C). When dew point was adjusted at a constant dew point depression of 4 °C, significant levels of pecan scab were predicted when dew point was greater than 14 °C (Figure 6D). When dew point depression was fixed at 10 °C, significant levels of pecan scab were predicted at dew points greater than 22 °C (Figure 6E). When dew point depression was fixed at 18 °C, significant levels of pecan scab were not predicted across all observed dew points (Figure 6F).

Discussion

The models presented here are the first attempt to use regression analysis to determine timing of fungicide application in pecan scab management by using weather

variables as inputs. These models offer insight into how the currently established Oklahoma Agweather Pecan Scab Fungicide Advisory can be improved in the future by suggesting that other variables, in addition to temperature and relative humidity, might improve pecan scab prediction. Furthermore, the temperature and relative humidity model and separate field trials used to test other temperature and relative humidity thresholds in the advisory confirm that the current temperature and relative humidity thresholds of 21.1°C/90% RH are reasonable for use in predicting pecan scab epidemics.

The negative influence of solar radiation in the temperature/relative humidity and dew point/dew point depression models is biologically significant. Solar radiation has been shown to inhibit the ability of conidia to germinate in *Venturia inaequalis* in field and laboratory experiments (Aylor and Sanogo, 1997). Solar radiation doses of 0.176 MJ m^{-2} reduced germination of *V. inaequalis*, the causal agent of apple scab, in field experiments and UV_{254} doses of 10.8 kJ m^{-2} reduced germination by 95% in laboratory experiments. Similar findings have been identified in other fungal genera (*Cladosporium* spp., *Alternaria* spp., *Arthrinium* spp., and *Aspergillus* spp.) (Ulevicius et al., 2004).

The effect of rain has been shown to have a dualistic effect on the capture of spores from the atmosphere (Hjelmroos, 1993). In research examining common aerial fungal spores, spore liberation has been shown to greatly increase immediately prior to a rain event. During the rain event spore captures decreased; however, after the rain event, the number of spores captured increased again (Aylor and Sutton, 1992; Burch and Levetin, 2002; Hjelmroos, 1993; Kurkela, 1997; Venables et al., 1997).

The positive influence of rain in the temperature and relative humidity model are supported by a long held belief that rain events are highly conducive to the development of epidemics. These events are known to be an indicator of leaf wetness onset and increase relative humidity resulting in conditions conducive for spore liberation and infection events (Huber and Gillespie, 1992). The approach used by the AU-Pecan advisory is based on the occurrence of rain and has been used successfully since its adaptation. Light misting rain has also been attributed in deposition of conidia onto leaf surfaces without enough force to coalesce droplets and wash spores off leaf surfaces (Rich and Waggoner, 1962).

Contradictory to the idea of increased disease during rain events, the dew point model shows a decrease in the probability of economically damaging levels of disease during rain events. This relationship is logical if one considers washing events that take place during significant rain events. Rain events measuring 23 mm and 33 mm resulted in a decrease in concentrations of *Cladosporium* spp. captured from the atmosphere (Troutt and Levetin, 2001). These findings were amplified if rain events occurred during the late night hours. During prolonged heavy rain events, spores can also be washed from the leaf surface to the grassy understory of the orchard. Studies have shown in *V. inaequalis* and other fungal species that escape of spores from grassy understories was insignificant when disease pressure was considered low (Aylor, 1998; Aylor and Qiu, 1996; Nicholson, 1998).

This study demonstrates that current thresholds used by the Agweather Pecan Scab Advisory appear to closely estimate scab epidemics in Oklahoma orchards. Field

experiments indicated that the 21.1°C / 90% RH thresholds were typically suitable for controlling scab to levels that did not influence yield, while providing two-spray savings over the other spray regimes. However, there is evidence that there is a slight underestimation of pecan scab when using the current advisory T/RH thresholds. An adjustment of the relative humidity threshold to 85% or reduction of the temperature threshold to 19 °C may reduce type-II error if assuming an action threshold of 0.50. More sustainable predictions may be observed by reducing the temperature threshold alone. Findings by Sentelhas *et al.* (2008) demonstrated that constant relative humidity could be used to predict leaf wetness when the relative humidity threshold was calibrated to each site. This could be cumbersome in some situations. However, lower relative humidity thresholds have also been shown to accurately predict disease in a field situation and maintain a high degree of sensitivity and specificity (Mwakutuya and Banniza, 2010).

The dew point/dew point depression model developed during the course of this study has provided insight into predicting pecan scab epidemics while relying on a more appropriate meteorological phenomenon to determine leaf-wetting events via dew formation. Few studies have looked at the importance of dew point depression when predicting leaf wetness for use in a fungicide advisory although the importance of dew on epidemics has been well documented (Wallin, 1967). Continued work on the validation of this technique in needed in order to better understand the ability of dew point depression to predict epidemics. Dew point depressions less than 1.8 °C have been proposed as an estimator of leaf wetness onset and dew point depression greater than 2.2 °C as leaf wetness dry-off (Rao et al., 1998). These values were compared to an RH

threshold of 90% and an extended relative humidity threshold model using 87% as the base in another study (Sentelhas et al., 2004). Those findings demonstrated a locally calibrated dew point depression and relative humidity threshold approach was suitable and capable of predicting leaf wetness duration with an accuracy of less than 2 hours, which made this approach suitable for operational use.

The temperature/relative humidity model presented here not only offers insight into the performance of the Oklahoma Agweather Pecan Scab Advisor, but also may have an advantage over the current threshold approach if it was used directly to predict pecan scab increases. This model has been adjusted for the effects of solar radiation and the accumulation of rain in a dynamic fashion. By doing so, it is evident that the thresholds used by the current advisory closely estimated periods of scab increase, but accuracy might be improved by adding the effects of solar radiation and rain. Furthermore, a new model using dew point and dew point depression was developed. Further validation studies using these models should be performed to identify any improvement in the ability to predict fungicide application.

Acknowledgements

Published with the approval of the Director, Oklahoma Agricultural Experiment Stations, Stillwater, OK. This project was supported in part by the Oklahoma Agricultural Experiment Station Project 2667. The authors would like to thank the Oklahoma Pecan Growers Association for partially funding this project. We would also like to thank Dr.

George Driever for access to historical data and the Hauani Creek Ranch for field support.

Literature cited

Aylor, D. E. 1998. The aerobiology of apple scab. Plant Disease 82: 838-849.

Aylor, D. E. and Sanogo, S. 1997. Germinability of *Venturia inaequalis* conidia exposed to sunlight. Phytopathology 87: 628-633.

Aylor, D. E. and Sutton, T. B. 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: relationship to spore rransport and deposition. Phytopathology 82: 532-540.

Aylor, D. E. and Qiu, J. 1996. Micrometeorological determination of release rate of *Venturia inaequalis* ascospores from a ground-level source during rain. Agricultural and Forest Meteorology 81: 157-178.

Batzer, J.C., Gleason, M.L., Taylor, S.E., Koehler, K.J., and Monteiro, J.E. 2008. Spatial heteogeneity of leaf wetness duration in apple trees and its influence on performance of a warning system for sooty blotch and flyspeck. Plant Disease 92: 164-170.

Burch, M. and Levetin, E. 2002. Effects of meteorological conditions on spore plumes. International Journal of Biometeorology 46: 107-117.

Demaree, J.B. 1924. Pecan scab with special reference to sources of the early spring infection. Journal of Agricultural Research 28: 321-330.

Driever, G. F. 1998. Development of a threshold model for scheduling fungicide applications to control pecan scab. Ph.D. dissertation, Oklahoma State University. pp 105.

Gottwald, T. R. 1985. Influence of temperature, leaf wetness period, leaf age, and spore concentration on infection of pecan leaves by conidia of *Cladosporium caryigenum*. Phytopathology 75: 190-194.

Gottwald, T. R. and Bertrand, P. F. 1983. Effect of time of inoculation with *Cladosporium caryigenum* on pecan scab development and nut quality. Phytopathology 73: 714-718.

Hjelmroos, M. 1993. Relationship between airborne fungal spore presence and weather variables - Cladosporium and Alternaria. Grana 32: 40-47.

Huber, L. and Gillespie, T.J. 1992. Modeling leaf wetness in relation to plant disease epidemiology. Annual Review of Phytopathology. 30: 553-577.

Hunter, R. E. 1983. Influence of scab on late season nut drop of pecans. Plant Disease 67: 806-807.

Kurkela, T. 1997. The number of Cladosporium conidia in the air in different weather conditions. Grana 36: 54-61.

Lawrence, M.G. 2005. The relationship between relative humidity and the dewpoint temperature in moist air. Bulletin of the American Meteorological Society. 225-233.

Liang, K.Y. and Zeger, S. L.1986. Longitudinal data analysis using generalized linear models. Biometrika 73: 13-22.

Monteith, J.L., 1957. Dew. J. R. Meteorol. Soc. 83: 322–341.

Mwakutuya, E. and Banniza, S. 2010. Influence of temperature and wetness periods on the development of stemphylium blight on lentil. Plant Disease 94: 1219-1224.

National Weather Service Internet Services Team. National Weather Service Glossary. National Oceanic and Atmospheric Administration. Retrieved 4 February 2011 from http://www.weather.gov/glossary/.

Nicholson, K. W. 1988. A review of particle resuspension. Atmospheric Environment 22: 2639-2651.

Pan, W. 2001. Akaike's information criterion in generalized estimating equations. Biometrics 57: 120-125.

Rao, P.S., Gillespie, T.J. and Schaafsma, A.W. 1998. Estimating wetness on maize ears from meteorological observations. Canadian Journal of Soil Sciences 78: 149-154.

Rich, S. and Waggoner, P. E. 1962. Atmospheric concentration of *Cladosporium* spores. Science 137: 962-965.

Richards, K. 2004. Observation and simulation of dew in rural and urban environments. Progress in Physical Geography 28: 76-94.

Sentelhas, P. C., Dalla Marta, A., Orlandini, S., Santos, E.A., Gillespie, T.J. and Gleason, M.L. 2008. Suitability of relative humidity as an estimator of leaf wetness duration. Agricultural and Forest Meteorology 148: 392-400.

Sentelhas, P. C., Gillespie, T. J., Gleason, M.L., Monteiro, J.E.B.A. and Helland, S.T. 2004. Operational exposure of leaf wetness sensors. Agricultural and Forest Meterology 126: 59-72.

Shaner, G., and Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056.

Sutherland, A., von Broembsen S., Smith, M., and Carlson, J.D. 2005. Pecan Crop and Scab Disease Overview. Stillwater, Oklahoma State University**:** 1-4.

Troutt, C. and Levetin, E. 2001. Correlation of spring spore concentrations and meteorological conditions in Tulsa, Oklahoma. International Journal of Biometeorology 45: 64-74.

Turechek, W. W. and Stevenson, K. L. 1998. Effects of host resistance, temperature, leaf wetness, and leaf age an infection and lesion development of pecan scab. Phytopathology 88: 1294-1301.

Ulevicius, V., Peciulyte D., Lugauskas, A., and Andriejauskiene, J. 2004. Field study on changes in viability of airborne fungal propagules exposed to UV radiation. Environmental Toxicology 19: 437-441.

Venables, K. M., Allitt, U., Collier, C.G., Emberlin, J., Greig, J.B., Hardaker, P.J., Highham, J.H., Latng-Morton, T., Maynard, R.L., Murray, V., Strachan, D., and Tee, R.D. 1997. Thunderstorm-related asthma - The epidemic of 24/25 June 1994. Clinical and Experimental Allergy 27: 725-736.

Wallin, J.R. 1967. Agrometeorological aspects of dew. Agricultural Meteorology. 4:85-102.

Zeger, S. L. and Liang, K.Y. 1986. Longitudinal data analysis for discrete and continuous outcomes. Biometrics 42: 121-130.

Table 4: Effects of weather-based advisory programs on levels of pecan scab during 2009 and 2010 growing seasons in Madill, OK.

Treatment [u]	2009		2010	
	Severity[v] (%)	AUDPC [w] (%-days)	Severity[x] (%)	AUDPC [w] (%-days)
Non-treated	30.7	963.0	57.2 a [y]	1589.2 a [y]
21.1°C/ 90%	12.8	387.0	26.1 b	671.2 b
15.5°C/ 85%	39.3	925.3	12.7 b	658.9 b
18.3°C / 80%	0.0	0.0	13.2 b	333.9 b
15.5°C/ 80%	1.5	38.5	11.1 b	266.6 b
LSD (*P*=0.05)	NS [z]	NS [z]	13.5	582.2

[u] Treatments included scab hour thresholds (Temperature/Relative humidity) of 15.5°C/ 80%, 18.3°C / 80%, 15.5°C/ 85%, 21.1°C/90%; and a non-treated control. A fungicide was applied after 20 scab hours accumulated (see text).

[v] Mean observed fruit severity taken on the final disease rating, 9/18/2009.

[w] Area under the disease progress curve

[x] Mean observed fruit severity taken on the final disease rating, 8/16/2010.

[y] Mean values within a column followed by the same letter, are not significantly different according to Fisher's Protected Least Significant Difference test ($P > 0.05$).

[z] Not Significantly different ($P = 0.05$)

Table 5: Single-effects models describing the probability of a scab event greater than 25% fruit severity using either dew point (DP), air temperature (T), relative humidity (RH), total solar radiation (SR), rain events (R where 1=rain event ≥ 2.5mm; 0=0 to < 2.5mm), and total rainfall (TR) as independent variables. Lower values of QIC and QICu indicate better model fit.

Model	**QIC** [a]	**QICu** [b]	X^2	$P > X^2$
-12.72 + .1566 RH	411.39	408.81	29.30	<0.0001
2.11 - 0.3219 DPD	419.39	416.36	28.30	<0.0001
-17.91 + 0.2405 DP	438.98	433.02	13.40	0.0003
-0.31 - 1.47 R	442.6	437.65	11.98	0.0005
0.91 - 0.093 SR	476.89	471.53	8.84	0.0029
-1.13 - 0.2809 TR	481.50	476.60	0.46	0.4958
-.7197 - 0.0058 T	482.76	477.25	0.070	0.7906

[a] Quasi-likelihood under the independence model information criterion

[b] Quasi-likelihood under the independence model information criterion adjusted for the number of parameters in the model

Table 6: Evaluation of models predicting the probability of a scab event greater than 25% fruit severity using air temperature (T), relative humidity (RH), total solar radiation (SR), rain events (R where 1=rain ≥ 2.5mm; 0= < 2.5 mm), and total rainfall (TR) as independent variables. Models were developed using a forward selection approach starting with the most significant unadjusted effect as chosen by the lowest QICu.

Model	**QICu**[a]
-12.72 + 0.1566 RH	408.81
-30.12 + 0.2099 RH + .1687 T	389.66
-13.57 + 0.1799 RH – 1.639 R	370.40
-18.63 - 0.2450 RH – 4.342 TR	478.50
-15.30 + 0.1713 RH + 0.0661 SR	408.66
-29.40 + 0.2286 RH – 1.630 R+ 0.1538 T	353.95
-9.540 + 0.1585 RH – 1.932 R- 0.1021 SR	369.11
-33.40 + 0.1803 RH + 0.3806 T - 0.5770 SR +3.39 R	313.31

[a] Quasi-likelihood-under-the-independence-curve information criterion adjusted for the number of parameters in the model

Table 7: Best fitting multivariable models describing the probability of a scab event using, dew point depression (DPD), dew point (DP), binomial output of rain events (R where 1=rain event ≥ 2.5mm and 0 < 2.5 mm), rain accumulation (TR), and total solar radiaiton (SR) as independent variables. Models were developed using a forward selection approach starting with the most significant unadjusted effect as chosen by the lowest QICu.

Model	**QICu**[a]
2.11 – 0.3219 DPD	416.36
-17.91 + 0.2405 DP	433.02
-9.6818 - .2735 DPD + 0.1624 DP	397.85
-7.567 - 0.3252 DPD + 0.1517 DP – 1.542 TR	364.59
-11.36 - .2061 DPD + .2122 DP – 0.1093 SR	396.27
-5.715 - 0.4233 DPD + 0.1341 DP - 3.220 R	381.27
-12.53 + .3771 DP - 3.340 R - 0.5809 SR	317.77
-12.91 + 0.0231 DPD + .3859 DP – 3.394 R - .6005 SR	319.70

[a] Quasi-likelihood-under-the-independence-curve information criterion adjusted for number of parameters in the model

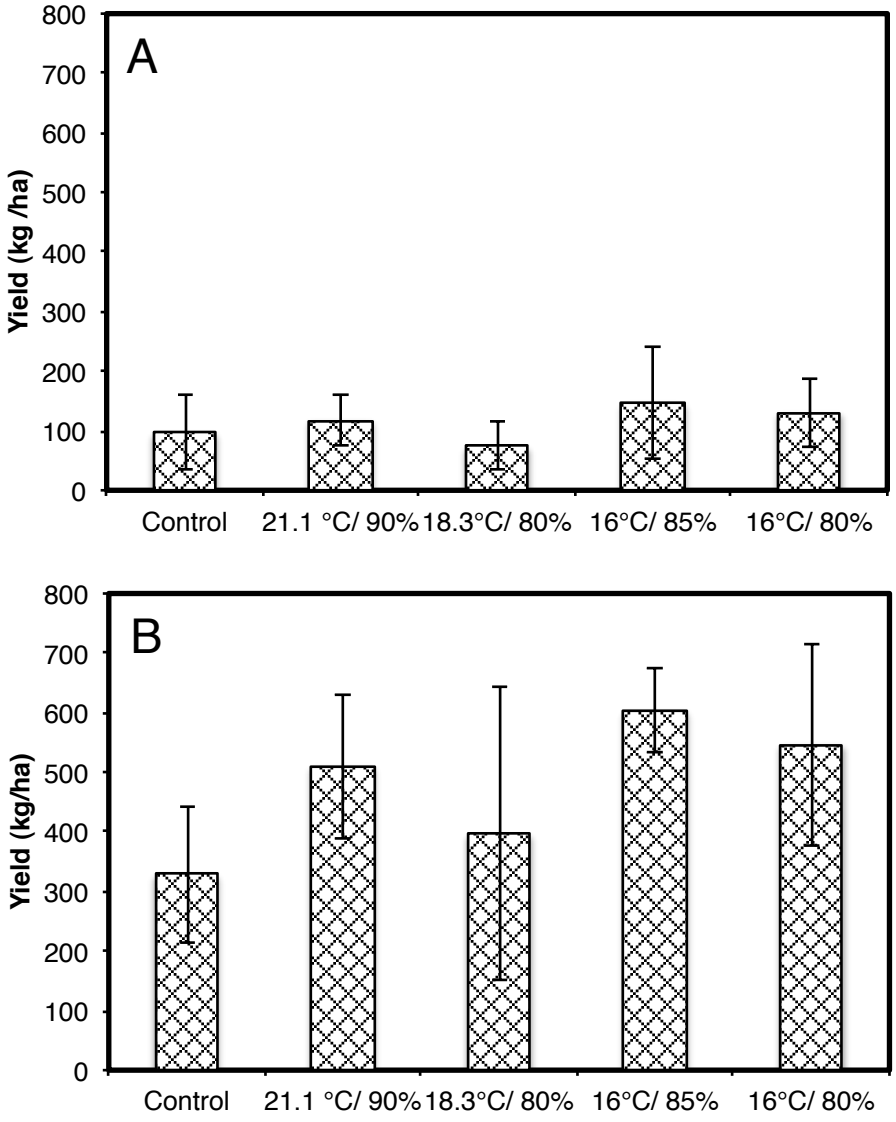


Figure 4: Pecan yield during the **A,** 2009 and **B,** 2010 growing seasons for plots sprayed with fungicide according to the Oklahoma Agweather Pecan Scab Fungicide Advisory - using five combinations of air temperature and relative humidity thresholds to calculate 'scab hours.' Treatments included air temperature/relative humidity thresholds of 15.5°C/ 80%, 18.3°C / 80%, 15.5°C/ 85%, 21.1°C/90%, and a non-treated. Error bars represent a 95% confidence interval about the treatment mean.

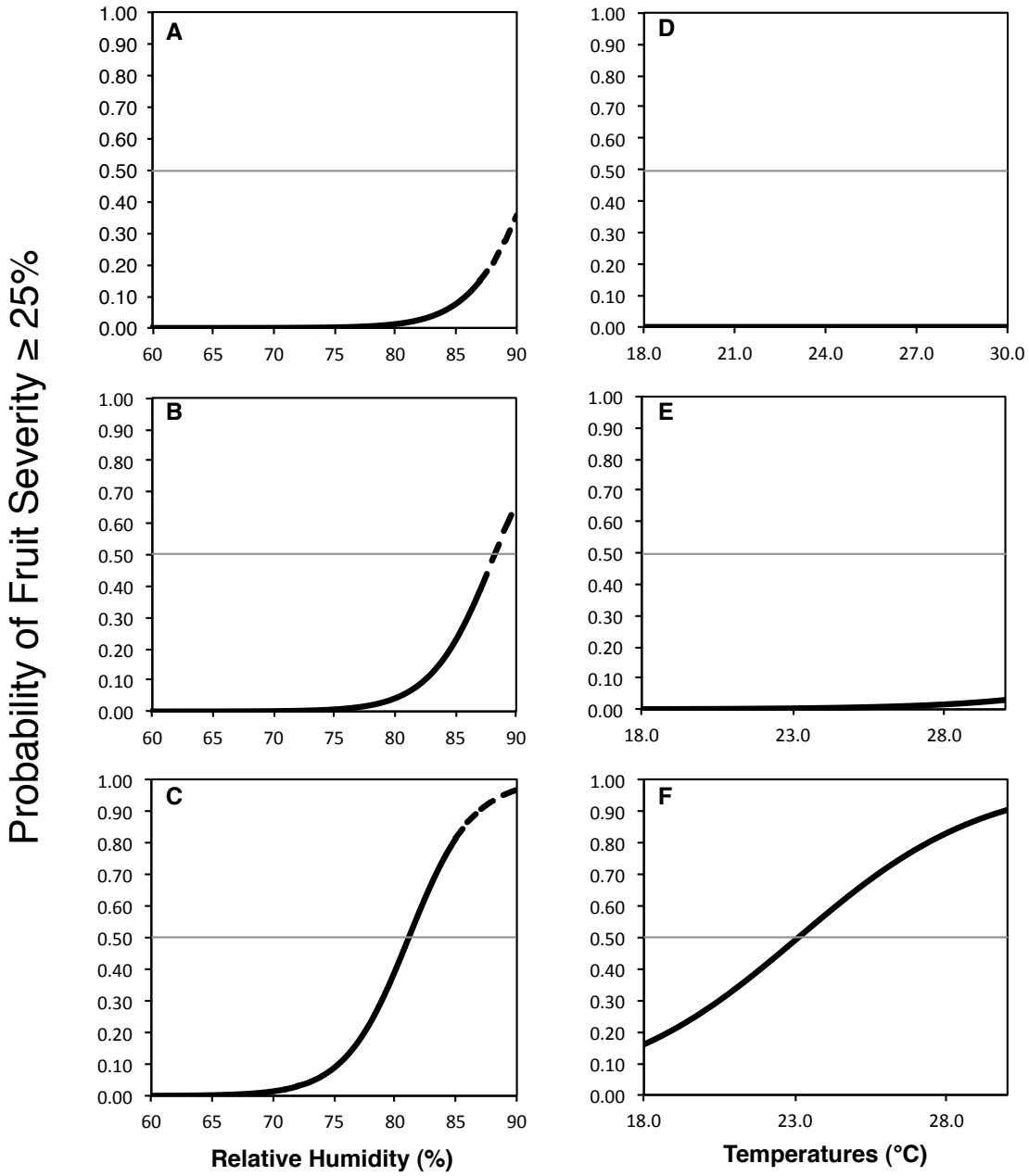


Figure 5: Evaluation of best fitting T/RH based model that included temperature (T), relative humidity (RH), total solar radiation (SR), and a binomial rain variable (R) where 1 = ≥ 2.5mm of rain and 0 = < 2.5 mm rain. Probability of a significant fruit severity

event (fruit severity ≥ 25%) as predicted by the model: fruit severity ≥ 25% = -exp (-33.40 + 0.1803 RH + 0.3806 T - 0.5770 SR +3.39 R)/[1+exp (-33.40 + 0.1803 RH + 0.3806 T - 0.5770 SR +3.39 R)]; when T was fixed at the observed **A,** minimum (18.0°C), **B,** mean (21.2°C), and **C,** maximum (30.6°C) values and RH was adjusted or when RH was fixed at the observed **D,** minimum (57%), **E,** mean (72%), and **F,** maximum (87%) values and T was adjusted. All predictions assumed no rain (e.g. R=0) and total solar radiation held constant at the mean observed level of 22.5 MJ/m^2. Dotted lines represent probabilities extrapolated outside the context of the observed data. Horizontal line occurs at the action threshold of 0.50.

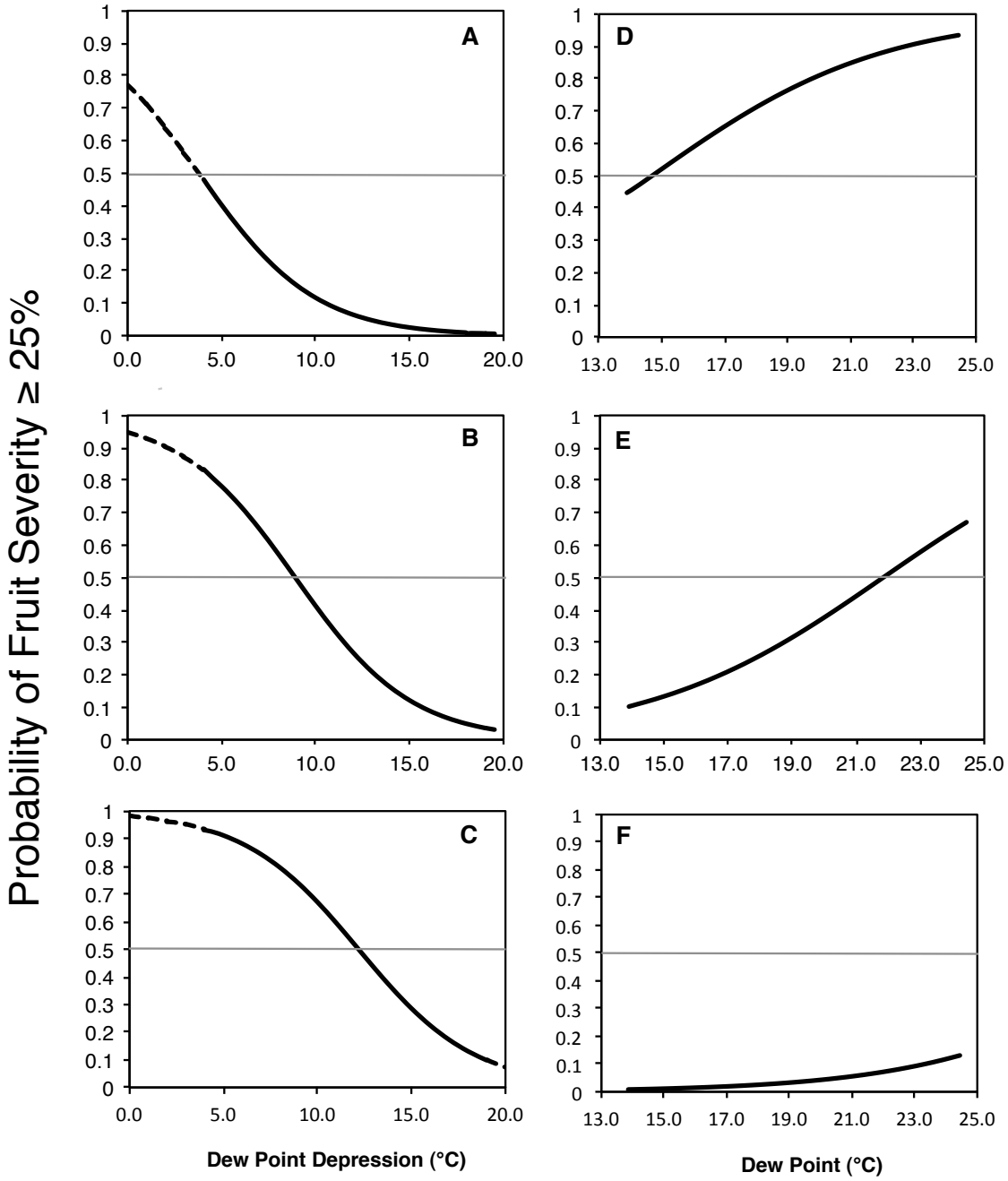


Figure 6: Evaluation of most appropriate DP/DPD based model that included dew point (DP), dew point depression (DPD), and a binomial rain variable (R) where 1 = ≥ 2.5mm of rain and 0 = < 2.5 mm rain. Probability of a significant fruit severity event as predicted by the model: fruit severity ≥ 25% = -exp (-5.715 - 0.4233 DPD + 0.1341 DP - 3.220

R)/[1+exp (-5.715 - 0.4233 DPD + 0.1341 DP - 3.220 R)]; when DP was fixed at the observed **A,** minimum (14.4°C), **B,** mean (20.6°C), and **C,** maximum (24.4°C) values and DPD was adjusted or when DPD was fixed at the observed **D,** minimum (4°C), **E,** mean (10 °C), and **F,** maximum (18 °C) values and DP was adjusted. All predictions assumed no rain (e.g. R=0). Dashed lines represent probabilities extrapolated outside the context of the observed data. Horizontal line occurs at the action threshold of 0.50.

CHAPTER IV

FIELD EVALUATION OF REDUCED FUNGICIDE PROGRAMS FOR CONTROL OF PECAN SCAB

Abstract

Fusicladium effusum (Wint.) (syn. *Cladosporium caryigenum* (Wint.) Gottwald), causal agent of pecan scab, is the most economically important pathogen of pecans (*Carya illinoinensis)* (Wangenh.) K. Koch). Severe epidemics of pecan scab can reduce crop yield and quality. To manage pecan scab, fungicides sprays are routinely used. Oklahoma growers currently use a weather-based advisory to assess fungicide application that requires the accumulation of scab hours. A scab hour is defined as an hour of average temperature and relative humidity ≥ 21.1°C and 90%, respectively. This advisory has been used with success for nearly 20 years, however does not have defined rules for when the first fungicide application should be made. The influence of early fungicide applications is largely not understood. To assess the influence of these early applications repeated ratings of disease severity were taken on fruit each year during the 2009-2010 growing seasons. Fungicides were applied every other week beginning with a pre-pollination application. Treatments included 2, 4, 6, and 9-sprays during the growing

season and a non-spray control. After the treatment was met no further applications were made. Monthly scab hours were calculated using temperature and relative humidity data collected from the Oklahoma Mesonet. Months most conducive for scab development were from June through August in 2009 and 2010. Final fruit severity and area under the disease progress curve (AUDPC) were significantly different during the 2009 and 2010 growing season ($P < 0.05$). The 9-spray treatment had significantly higher disease severity and AUDPC as compared to other treatments in 2009. In 2010 only the 9-spray treatment had significantly lower fruit severity compared to the non-spray check and only the 2-spray AUDPC was not dissimilar from the non-spray control. From this study, early fungicide applications can reduce fruit severity later in the growing season. By beginning fungicide applications at pre-pollination it be hypothesized that amounts of secondary inoculum were reduced. The lack of influence of fungicide during the 2009 season could have been due to low disease pressure.

Introduction

Fusicladium effusum (Wint.) (syn. *Cladosporium caryigenum* (Wint.) Gottwald), causal agent of pecan scab, is the most economically important pathogen of pecans (*Carya illinoinensis* (Wangenh.) K. Koch). Without effective management of pecan scab, susceptible cultivars can develop epidemics so severe that significant losses in yield and kernel quality can result in as little as 3 years if left uncontrolled (Demaree, 1924). Preventative measures to manage pecan scab include pruning, tree thinning, sanitation, and fungicide application. Several methods have been developed to determine when

fungicide applications should be performed including schedules based on the calendar, tree phenology, and disease prediction models.

Effectively managing pecan scab using preventative fungicide programs can be complicated due to the disease incubation period, which can be highly variable and/or extended. Disease symptoms can appear 3 to 24 days after infection, depending on the age of the leaf or shuck. Younger leaves and shucks showed a general trend of earlierdisease development as compared to older ones (Turechek and Sevenson, 1998). This is due to the anatomical development of leaflet and shuck epidermis (Latham and Rushing, 1988). Latham and Rushing observed that the germ tube penetrated directly through the cuticle. They suggested that as leaves mature over time, the cuticle becomes thicker and serves as a physical barrier to infection (Latham and Rushing, 1988). Conner and Stevenson (2004) suggested that resistance to infection by the fungus was also mediated by the speed at which plant cell walls thickened in resistant germplasm, thereby limiting growth of subcuticular fungal hyphae.

To assist growers in application of fungicides, agricultural scientists have developed disease prediction models to be used in disease advisories for recommending fungicide applications. The purpose of predictive models is to provide growers a more efficient management program as compared to a calendar-based program for fungicide application. States that currently offer prediction models for the control of pecan scab include Oklahoma, Georgia, Louisiana, and Alabama. AU-Pecan was developed at the University of Georgia and is currently in use in Georgia, Louisiana, and Alabama. This model is a modification of the AUPnut program developed at Auburn University for controlling early and late leaf spot of peanut (Jacobi and Backman, 1995; Jacobi,

Backman, Davis, and Brennan, 1995). Oklahoma pecan producers use the Agweather Pecan Scab Advisory developed at Oklahoma State University during the mid-1990s (Driever, 1998).

Calendar-based schedules are effective, but can result in over-spraying for pecan scab. Using an extension recommendation from Louisiana State University, a calendar-based program would include two pre-pollination sprays, starting in late March or early April, followed by six cover sprays, with the final spray occurring in August resulting in eight fungicide applications per season. During periods when weather is not favorable for scab increase, unnecessary fungicide applications may be applied using this approach. This is not only inefficient, but the practice can result in populations of *F. effusum* that are resistant to fungicides. Resistance to propiconazole and fenbuconazole has been identified in populations of *F. effusum* in Georgia (Reynolds, Brenneman, and Bertrand, 1997). Other advantages of avoiding excessive or unnecessary application of fungicide include reduced chemical exposure to growers, decreased input to the environment, and reduced costs in production.

Regardless of the schedule for fungicide application, determining when to initiate fungicide programs can be difficult. Considering the increased susceptibility of pecans to damage by pecan scab in early growth stages (leaves and fruit), the critical time for controlling the disease using fungicides should be early in the growing season and mid-season during initial fruit development. Determining timing of the first fungicide application is largely left to the growers when using disease advisories or calendar-based programs. If following the Oklahoma Pecan Scab Advisory, a grower could choose to make his/her first application when the advisory indicates a spray is required, using a

calendar and past disease history, or based on tree phenology. All three approaches can result in different application dates and levels of disease. The objectives of this study were to determine the most critical time(s) to apply fungicides during the pecan-growing season to maximize pecan scab control and determine the efficacy of a reduced fungicide program on control of pecan scab.

Materials and Methods

Field trials were conducted at the Cimarron Valley Research Station in Perkins, OK during the 2009 and 2010 growing seasons. Trees consisted of 'Pawnee' scion grafted to 'Peruque' rootstock which were planted as bare-root transplants in 1994 and 1997. 'Pawnee' is considered a moderately susceptible cultivar to pecan scab (16). Trees were planted on a Konawa fine sandy loam and Dougherty loamy fine sand with a10.6 m between-tree spacing and 10.6 m between-row spacing. Treatments were various numbers of fungicides applied under different timing sequences. The 2-spray treatment involved the application of fungicide at pre-pollination and an additional application 14-d later. The 4-spray treatment was initiated at pre-pollination with three subsequent sprays on a 14-d interval. 6-spray treatment received five applications of fungicide on a 2-week interval after the pre-pollination spray. The nine-spray treatment involved fungicide protection throughout the season initiated at pre-pollination. Treatments were compared to a non-treated control. Fungicides used included rotations of azoxystrobin at 0.25 kg/ha+ propiconazole at 0.16 kg/ha (Quilt; Syngenta Crop Protection), Thiophanate-methyl 0.70 kg/ha (Topsin 4.5FL; United Phosphorus), Tebuconazole at 0.25 kg/ha (Folicur; Bayer CropScience), and fenbuconazole at 0.14 kg/ha (Enable; Dow

AgroSciences) during the 2009 and 2010 seasons. Fungicides were applied with a tractor mounted air blast sprayer (Savage Equipment), calibrated to deliver 100 GPA. Following recommended management practices for Oklahoma, thinning to a 50% crop-load was performed prior to nut fill during the 2009 growing season to reduce the severity of alternate bearing (McCraw, Smith, and Reid, 2007). This practice was not needed during the 2010 growing season. The experimental design was a randomized complete block with four replicates.

Fruit severity was assessed by visually estimating percent foliar and single-fruit scab coverage at each cardinal direction, below and above the mid-line of the tree canopy (8 ratings/tree), every 14 days beginning with the first symptoms of foliar disease. Ratings for each tree were averaged to one value per tree for analysis. Disease progression was analyzed using area under the disease progress curves (AUDPC) (Shaner and Finney, 1977). Nuts were removed from each tree using a tractor-mounted tree shaker (Savage Equipment) and harvested using an orchard floor harvester (Savage Equipment). Yield data were collected after mechanical shaking, field cleaning, and drying pecans to 6.5% moisture in 2009 and 5.5% moisture in 2010.

Scab hours were calculated for each month during the growing season using temperature and relative humidity data from the Oklahoma Mesonet station located on the Cimarron Valley Research Station (www.mesonet.org; 35N 59’ 55”, 97W 2’ 53”).

Results

Accumulated Scab Hour Data. April and May were not conducive for scab outbreaks with less than 10 hours being accumulated during the 2009 and 2010 growing seasons

(Table 8). June was the first month growers with susceptible and moderately susceptible cultivars would have made fungicide applications as per the advisory. June, July, and August were months conducive for scab development during the 2009 and 2010 growing seasons (Table 8). The most conducive month for scab development was August in 2009 and July in 2010 (Table 8).

2009 Field Trial. Fungicide applications were initiated on 4 May 2009. Subsequent fungicides were applied on 18 May, 1 June, 15 June, 29 June, 13 July, 27 July, 10 August, and 24 August according to treatment. The 9-spray treatment had significantly higher levels of disease as compared to the other treatments including the non-sprayed control (Table 9). Fruit severity rose between 13 August and 23 August across all treatments and fell slightly between 23 August and 25 September (Figure 7A). No significant differences were found in yield in the 2009-growing season (Figure 8A).

2010 Field Trial. Fungicide applications were initiated on 27 April 2010. Subsequent fungicides were applied on 10 May, 25 May, 8 June, 22 June, 6 July, 20 July, 3 August and 17 August according to treatment. Fruit severity for the non-sprayed and 2-spray treatments rose sharply between 21 July and 4 August. Fruit severity for the 4-spray, 6-spray, and 9-spray treatments increased slightly between 2 July and 4 August and remained static during the next assessment on 19 August (Figure 7A). All treatments significantly reduced AUDPC compared to the non-sprayed control treatment except the 2-spray treatment. Only the 9-spray treatment reduced final disease severity compared to the non-spray control (Table 9). No significant differences were found in yields during the 2010-growing season (Figure 8B).

Discussion

Previous studies examining reduced fungicide management programs for pecan scab control successfully demonstrated that they were a viable option (Gottwald and Bertrand, 1983). However, these studies failed to identify when the abbreviated fungicide programs should be initiated, thus leaving the decision up to interpretation. The consequences of this technique could result in severe disease if sprays were not initiated early enough in the growing season to protect rapidly expanding plant parts from initial inoculum. By focusing fungicide applications during the early part of the season, fewer initial infections should result leading to lower levels of secondary inoculum later in the season. Therefore, an overall reduction in the level of final disease can result as demonstrated in this research even with just two early season applications of fungicide without significantly reducing yields.

In Oklahoma, reduced fungicide programs may be even more appropriate because weather during late fruit maturation can be unfavorable for disease development. If weather is not favorable for disease development during these periods, then fungicide applications are not warranted. This may explain why disease progress did not occur during mid-to-late stages of fruit development. Weather was still favorable for scab develop during shell hardening when fruit is considered least susceptible to infection by *F. effusum* (Gottwald and Bertrand, 1983).

The trend found in fruit severity during the 2010-growing season demonstrates the importance of controlling early season scab development. Without fungicide

applications in June, highly susceptible expanding leaves and fruit are left unprotected and subjected to infection and colonization by *F. effusum*. During favorable weather for disease development, this can also contribute to a substantial increase in secondary inoculum levels leading to high levels of disease at the end of the season. The similarity between the non-spray and 2-spray treatments demonstrated the impact of allowing epidemics to progress during periods of the growing season when fruit are highly susceptible and levels of secondary inoculum can dramatically increase. The 4-spray, 6-spray, and 9-spray treatments protected the fruit during the most susceptible periods of development resulting in low levels of disease at the end of the season.

Using a 4-spray fungicide application program initiated at pre-pollination, fruit remain protected during the most susceptible stages while end-of-season fruit severity remained below the economic threshold of 25% (Hunter, 1983). Fruit severity greater than the economic threshold has the potential of decreasing crop yield and quality. By using a 4-spray approach a grower would reduce fungicide output by five applications if using a season-long calendar approach.

These data suggest that in Oklahoma growers can use an abbreviated fungicide program, which provides scab control during the early stages of the season and is relaxed during months of July and August. Considering the increased susceptibility of pecans to damage by pecan scab during early stages of development, this study has demonstrated that fungicide protection be used when conditions are favorable for disease during the early growing season.

Literature Cited

Conner, P. J. and Stevenson, K. L.. 2004. Pathogenic variation of *Cladosporium caryigenum* isolates and corresponding differential resistance in pecan. Hortscience 39: 553-557.

Demaree, J. B. 1924. Pecan scab with special reference to sources of the early spring infections. Journal of Agricultural Research 28: 321-330.

Driever, G. F. 1998. Development of a threshold model for scheduling fungicide applications to control pecan scab. Ph.D. dissertation, Oklahoma State University. pp 105.

Gottwald, T. R. 1985. Influence of temperature, leaf wetness period, leaf age, and spore concentration on infection of pecan leaves by conidia of *Cladosporium caryigenum*. Phytopathology 75: 190-194.

Gottwald, T. R. and Bertrand, P.F. 1983. Effect of time of inoculation with *Cladosporium caryigenum* on pecan scab development and nut quality. Phytopathology: 73: 714-718.

Gottwald, T.R. and Bertrand, P.F. 1988. Effects of an abbreviated pecan disease control program on pecan scab disease increase and crop yield. Plant Disease 72: 27-32.

Huber, L. and Gillespie, T.J. 1992. Modeling leaf wetness in relation to plant disease epidemiology. Annual Review of Phytopathology. 30: 553-577.

Hunter, R. E. 1983. Influence of scab on late season nut drop of pecans. Plant Disease 67: 806-807.

Jacobi, J. C. and Backman, P. A. 1995. Au-Pnuts advisory .2. modification of the rule-based leaf-spot advisory system for a partially resistant peanut cultivar. Plant Disease 79: 672-676.

Jacobi, J. C., Backman, P. A., Davis, D.P., and Brannen, P.M. 1995. Au-Pnuts advisory .1. development of a rule-based system for scheduling peanut leaf-spot fungicide applications. Plant Disease 79: 666-671.

Latham, A. J. and Rushing, A. E. 1988. Development of *Cladosporium caryigenum* in pecan leaves. Phytopathology 78: 1104-1108.

McCraw, D., Smith, M.W., and Reid, W. 2007. Pecan crop load management. OK coop ext. HLA-6251.

Reynolds, K. L., Brenneman, T. B., and Bertrand, P.F. 1997. Sensitivity of *Cladosporium caryigenum* to propiconazole and fenbuconazole. Plant Disease 81:163-166.

Rich, S. and Waggoner, P. E. 1962. Atmospheric Concentration of *Cladosporium* spores. Science 137: 962-965.

Shaner, G. and Finney, R.E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67: 1051-1056.

Sutherland, A., von Broembsen S., Smith, M., and Carlson, J.D. 2005. Pecan Crop and Scab Disease Overview. Stillwater, Oklahoma State University**:** 1-4.

Turechek, W. W. and Stevenson, K. L. 1998. Effects of host resistance, temperature, leaf wetness, and leaf age an infection and lesion development of pecan scab. Phytopathology 88: 1294-1301.

Table 8: Monthly accumulated scab hours[a] for Perkins, Oklahoma during the 2009 and 2010 growing seasons.

	2009	2010
April	0	0
May	5	7
June	23	32
July	24	89
August	34	23

[a] A scab hour is an hour in which the average temperature is ≥ 21.1 °C and the relative humidity is ≥ 90% as collected by the Mesonet weather collection site.

Table 9: Effects of fungicide programs on levels of pecan scab during 2009 and 2010 growing seasons in Perkins, OK.

Treatment [v]	2009		2010	
	Severity (%)[w]	AUDPC (%-days) [x]	Severity (%) [y]	AUDPC (%-days) [x]
Non-treated	4.0 b [z]	363.9 b [z]	11.5 a [z]	272.8 a [z]
2-spray	2.3 b	323.6 b	6.8 ab	188.8 ab
4-spray	4.0 b	341.3 b	3.4 ab	78.5 b
6-spray	2.6 b	377.0 b	2.7 ab	51.6 b
9-spray (Season-long)	7.6 a	492.4 a	1.9 b	50.1 b
LSD (*P*=0.05)	3.5	105.6	6.6	111.8

[v] Fungicides were applied every two weeks following a pre-pollination application. Treatments included a 2, 4, 6, and 9-sprays during the growing season; and a non-spray control. After the treatment was met no further applications were made.

[w] Mean observed fruit severity taken on the final disease rating, 9/25/2009.

[x] Area under disease progress curve

[y] Mean observed fruit severity taken on the final disease rating, 8/19/2010.

[z] Mean values within a column followed by the same letter, are not significantly different according to Fisher's Protected Least Significant Difference test ($P = 0.05$).

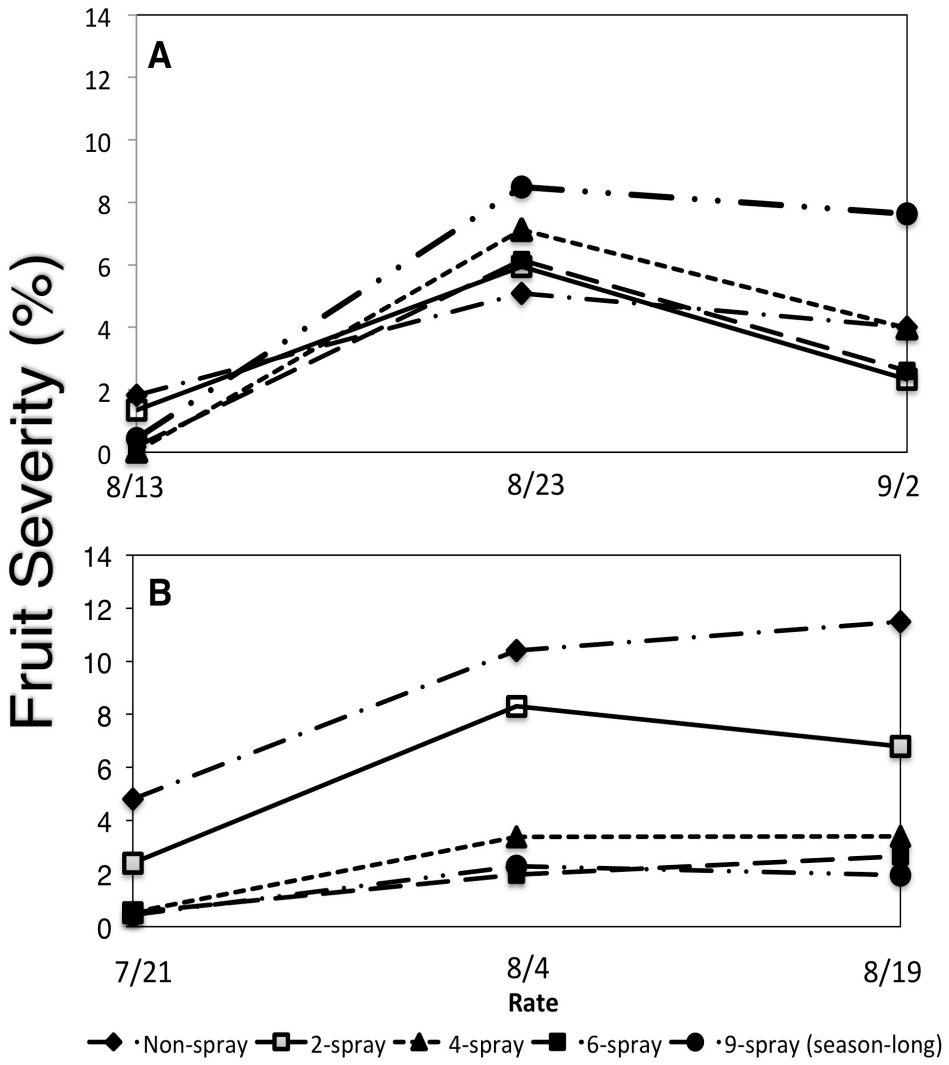


Figure 7: Disease progress in response to fungicide programs during the **A,** 2009 and **B,** 2010 growing seasons. Treatments included different numbers of fungicide applications on 14-d intervals beginning with pre-pollination sprays on 4 May 2009 and 27 April 2010. Error bars represent a 95% confidence interval about the treatment mean.

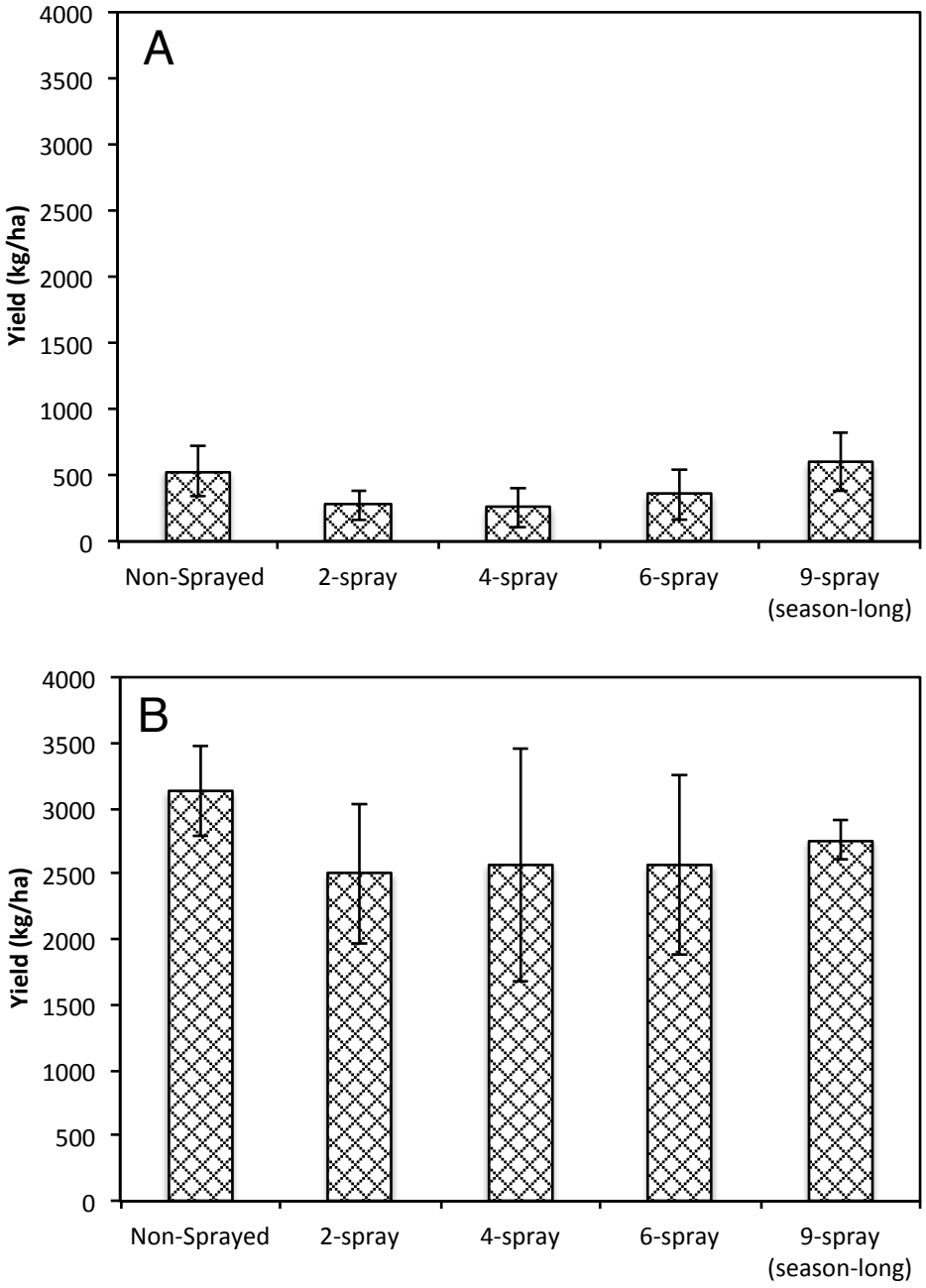
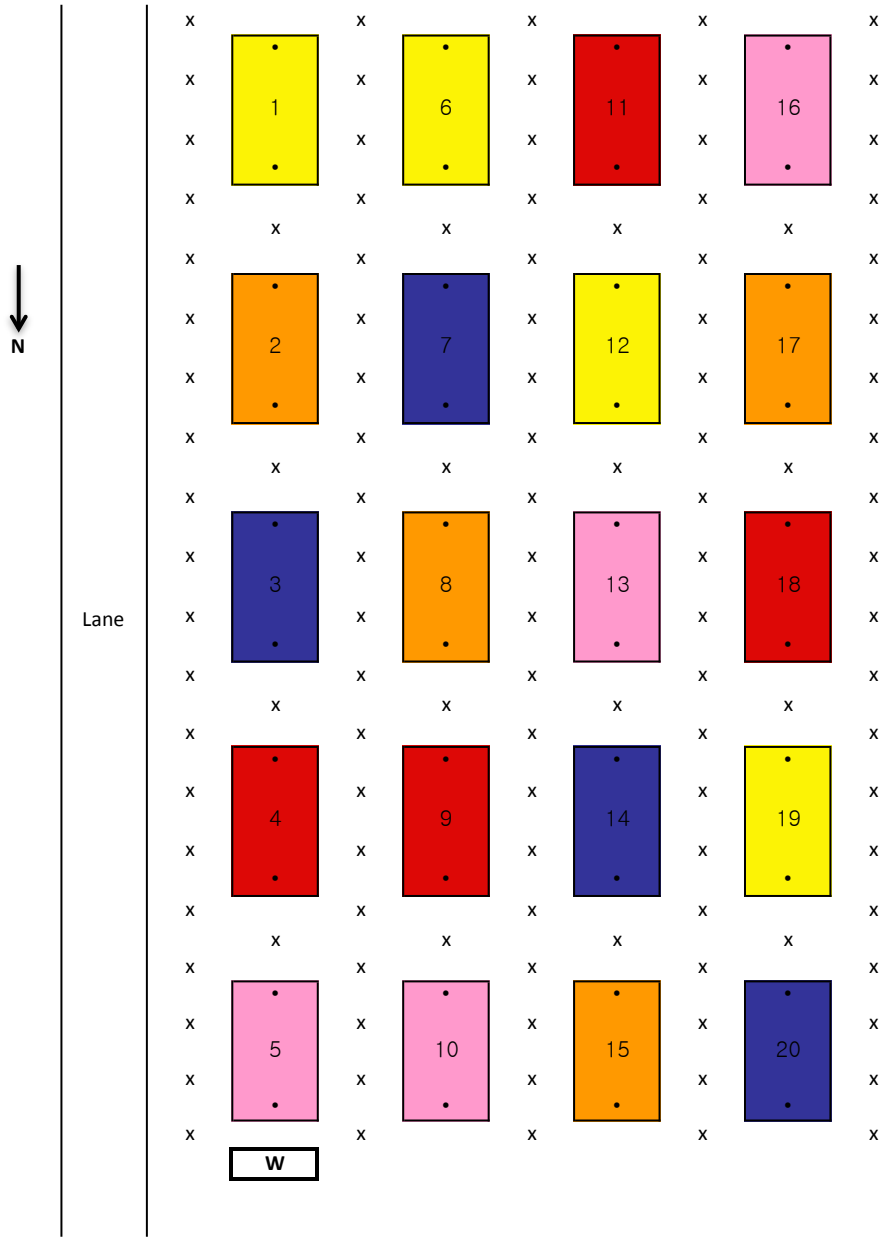


Figure 8: Effect of fungicide programs on pecan yield during the **A,** 2009 and **B,** 2010 growing seasons for plots sprayed with fungicide every two weeks. Treatments included different numbers of fungicide applications on 14-d intervals beginning with pre-

pollination sprays on 4 May 2009 and 27 April 2010. Error bars represent a 95% confidence interval about the treatment mean.

APPPENDICES

2009 BIXBY FIELD TRIAL PLOT PLAN



Appendix 1: Fruit and foliar disease incidence and severity during the 2009-growing season at a private commercial orchard located near Bixby, OK.

Date	Tree	Leaf_Inc	Leaf_Sev	Nut_inc	Nut_Sev	Cultivar
06/10/09	1	70	4	.	.	Pawnee
06/10/09	1	90	5	.	.	Pawnee
06/10/09	1	50	3	.	.	Pawnee
06/10/09	1	20	3	.	.	Pawnee
06/10/09	1	50	2	.	.	Pawnee
06/10/09	1	50	3	.	.	Pawnee
06/10/09	1	20	3	.	.	Pawnee
06/10/09	1	60	4	.	.	Pawnee
06/10/09	2	45	6	.	.	Pawnee
06/10/09	2	90	8	.	.	Pawnee
06/10/09	2	25	4	.	.	Pawnee
06/10/09	2	85	4	.	.	Pawnee
06/10/09	2	10	4	.	.	Pawnee
06/10/09	2	50	4	.	.	Pawnee
06/10/09	2	100	5	.	.	Pawnee
06/10/09	2	50	8	.	.	Pawnee
06/10/09	3	100	10	.	.	Pawnee
06/10/09	3	100	4	.	.	Pawnee
06/10/09	3	100	9	.	.	Pawnee
06/10/09	3	100	12	.	.	Pawnee
06/10/09	3	90	15	.	.	Pawnee
06/10/09	3	80	9	.	.	Pawnee
06/10/09	3	90	12	.	.	Pawnee
06/10/09	3	90	5	.	.	Pawnee
06/10/09	4	50	12	.	.	Pawnee
06/10/09	4	50	27	.	.	Pawnee
06/10/09	4	100	11	.	.	Pawnee
06/10/09	4	60	16	.	.	Pawnee
06/10/09	4	50	13	.	.	Pawnee
06/10/09	4	40	3	.	.	Pawnee
06/10/09	4	50	8	.	.	Pawnee
06/10/09	4	70	9	.	.	Pawnee
06/10/09	5	50	4	.	.	Pawnee
06/10/09	5	60	2	.	.	Pawnee
06/10/09	5	40	8	.	.	Pawnee
06/10/09	5	60	12	.	.	Pawnee
06/10/09	5	80	6	.	.	Pawnee

06/10/09	5	20	5	.	.	Pawnee
06/10/09	5	100	9	.	.	Pawnee
06/10/09	5	100	14	.	.	Pawnee
06/10/09	6	70	6	.	.	Pawnee
06/10/09	6	25	6	.	.	Pawnee
06/10/09	6	60	7	.	.	Pawnee
06/10/09	6	100	15	.	.	Pawnee
06/10/09	6	30	7	.	.	Pawnee
06/10/09	6	40	11	.	.	Pawnee
06/10/09	6	75	2	.	.	Pawnee
06/10/09	6	80	9	.	.	Pawnee
06/10/09	7	60	7	.	.	Pawnee
06/10/09	7	50	5	.	.	Pawnee
06/10/09	7	75	6	.	.	Pawnee
06/10/09	7	50	14	.	.	Pawnee
06/10/09	7	100	9	.	.	Pawnee
06/10/09	7	20	7	.	.	Pawnee
06/10/09	7	30	33	.	.	Pawnee
06/10/09	7	60	2	.	.	Pawnee
06/10/09	8	80	4	.	.	Pawnee
06/10/09	8	55	16	.	.	Pawnee
06/10/09	8	80	13	.	.	Pawnee
06/10/09	8	90	14	.	.	Pawnee
06/10/09	8	40	6	.	.	Pawnee
06/10/09	8	50	11	.	.	Pawnee
06/10/09	8	55	11	.	.	Pawnee
06/10/09	8	50	16	.	.	Pawnee
06/10/09	9	50	8	.	.	Pawnee
06/10/09	9	70	12	.	.	Pawnee
06/10/09	9	45	3	.	.	Pawnee
06/10/09	9	60	8	.	.	Pawnee
06/10/09	9	60	16	.	.	Pawnee
06/10/09	9	65	12	.	.	Pawnee
06/10/09	9	90	14	.	.	Pawnee
06/10/09	9	90	10	.	.	Pawnee
06/10/09	10	80	2	.	.	Pawnee
06/10/09	10	50	3	.	.	Pawnee
06/10/09	10	75	12	.	.	Pawnee
06/10/09	10	90	9	.	.	Pawnee
06/10/09	10	80	4	.	.	Pawnee
06/10/09	10	100	7	.	.	Pawnee
06/10/09	10	70	11	.	.	Pawnee
06/10/09	10	75	9	.	.	Pawnee

06/10/09	11	100	13	.	.	Pawnee
06/10/09	11	100	14	.	.	Pawnee
06/10/09	11	100	7	.	.	Pawnee
06/10/09	11	90	6	.	.	Pawnee
06/10/09	11	90	5	.	.	Pawnee
06/10/09	11	75	5	.	.	Pawnee
06/10/09	11	100	17	.	.	Pawnee
06/10/09	11	100	10	.	.	Pawnee
06/10/09	12	100	7	.	.	Pawnee
06/10/09	12	50	13	.	.	Pawnee
06/10/09	12	100	6	.	.	Pawnee
06/10/09	12	100	9	.	.	Pawnee
06/10/09	12	40	8	.	.	Pawnee
06/10/09	12	75	5	.	.	Pawnee
06/10/09	12	60	12	.	.	Pawnee
06/10/09	12	80	12	.	.	Pawnee
06/10/09	13	70	6	.	.	Pawnee
06/10/09	13	70	12	.	.	Pawnee
06/10/09	13	40	7	.	.	Pawnee
06/10/09	13	80	15	.	.	Pawnee
06/10/09	13	50	4	.	.	Pawnee
06/10/09	13	60	10	.	.	Pawnee
06/10/09	13	100	13	.	.	Pawnee
06/10/09	13	90	8	.	.	Pawnee
06/10/09	14	50	10	.	.	Pawnee
06/10/09	14	60	8	.	.	Pawnee
06/10/09	14	50	3	.	.	Pawnee
06/10/09	14	30	6	.	.	Pawnee
06/10/09	14	50	3	.	.	Pawnee
06/10/09	14	50	7	.	.	Pawnee
06/10/09	14	40	6	.	.	Pawnee
06/10/09	14	20	3	.	.	Pawnee
06/10/09	15	70	5	.	.	Pawnee
06/10/09	15	50	9	.	.	Pawnee
06/10/09	15	35	2	.	.	Pawnee
06/10/09	15	40	9	.	.	Pawnee
06/10/09	15	90	3	.	.	Pawnee
06/10/09	15	50	2	.	.	Pawnee
06/10/09	15	75	4	.	.	Pawnee
06/10/09	15	95	7	.	.	Pawnee
06/10/09	16	50	4	.	.	Pawnee
06/10/09	16	50	7	.	.	Pawnee
06/10/09	16	50	6	.	.	Pawnee

06/10/09	16	60	5	.	.	Pawnee
06/10/09	16	60	3	.	.	Pawnee
06/10/09	16	50	4	.	.	Pawnee
06/10/09	16	70	6	.	.	Pawnee
06/10/09	16	60	10	.	.	Pawnee
06/10/09	17	50	12	.	.	Pawnee
06/10/09	17	50	14	.	.	Pawnee
06/10/09	17	50	6	.	.	Pawnee
06/10/09	17	40	5	.	.	Pawnee
06/10/09	17	60	7	.	.	Pawnee
06/10/09	17	50	5	.	.	Pawnee
06/10/09	17	60	6	.	.	Pawnee
06/10/09	17	40	12	.	.	Pawnee
06/10/09	18	30	8	.	.	Pawnee
06/10/09	18	50	11	.	.	Pawnee
06/10/09	18	50	4	.	.	Pawnee
06/10/09	18	70	3	.	.	Pawnee
06/10/09	18	90	16	.	.	Pawnee
06/10/09	18	60	7	.	.	Pawnee
06/10/09	18	50	10	.	.	Pawnee
06/10/09	18	90	12	.	.	Pawnee
06/10/09	19	40	8	.	.	Pawnee
06/10/09	19	50	6	.	.	Pawnee
06/10/09	19	50	4	.	.	Pawnee
06/10/09	19	70	12	.	.	Pawnee
06/10/09	19	80	9	.	.	Pawnee
06/10/09	19	70	10	.	.	Pawnee
06/10/09	19	70	5	.	.	Pawnee
06/10/09	19	50	9	.	.	Pawnee
06/10/09	20	60	14	.	.	Pawnee
06/10/09	20	50	12	.	.	Pawnee
06/10/09	20	60	5	.	.	Pawnee
06/10/09	20	50	10	.	.	Pawnee
06/10/09	20	75	4	.	.	Pawnee
06/10/09	20	50	13	.	.	Pawnee
06/10/09	20	50	6	.	.	Pawnee
06/10/09	20	70	10	.	.	Pawnee
07/22/09	1	80	3	.	.	Pawnee
07/22/09	1	80	5	.	.	Pawnee
07/22/09	1	90	4	.	.	Pawnee
07/22/09	1	90	7	.	.	Pawnee
07/22/09	1	30	3	.	.	Pawnee
07/22/09	1	50	2	.	.	Pawnee

07/22/09	1	70	6	.	.	Pawnee
07/22/09	1	100	12	.	.	Pawnee
07/22/09	2	60	5	.	.	Pawnee
07/22/09	2	60	4	.	.	Pawnee
07/22/09	2	80	9	.	.	Pawnee
07/22/09	2	60	8	.	.	Pawnee
07/22/09	2	80	5	.	.	Pawnee
07/22/09	2	50	4	.	.	Pawnee
07/22/09	2	50	6	.	.	Pawnee
07/22/09	2	100	7	.	.	Pawnee
07/22/09	3	100	12	.	.	Pawnee
07/22/09	3	90	15	.	.	Pawnee
07/22/09	3	100	10	.	.	Pawnee
07/22/09	3	100	8	.	.	Pawnee
07/22/09	3	100	6	.	.	Pawnee
07/22/09	3	100	5	.	.	Pawnee
07/22/09	3	100	9	.	.	Pawnee
07/22/09	3	100	5	.	.	Pawnee
07/22/09	4	100	18	.	.	Pawnee
07/22/09	4	100	15	.	.	Pawnee
07/22/09	4	100	5	.	.	Pawnee
07/22/09	4	50	3	.	.	Pawnee
07/22/09	4	100	8	.	.	Pawnee
07/22/09	4	100	8	.	.	Pawnee
07/22/09	4	80	10	.	.	Pawnee
07/22/09	4	100	12	.	.	Pawnee
07/22/09	5	100	8	.	.	Pawnee
07/22/09	5	100	6	.	.	Pawnee
07/22/09	5	60	8	.	.	Pawnee
07/22/09	5	30	4	.	.	Pawnee
07/22/09	5	90	10	.	.	Pawnee
07/22/09	5	40	5	.	.	Pawnee
07/22/09	5	100	9	.	.	Pawnee
07/22/09	5	100	8	.	.	Pawnee
07/22/09	6	100	10	.	.	Pawnee
07/22/09	6	50	8	.	.	Pawnee
07/22/09	6	50	6	.	.	Pawnee
07/22/09	6	100	15	.	.	Pawnee
07/22/09	6	20	4	.	.	Pawnee
07/22/09	6	90	12	.	.	Pawnee
07/22/09	6	90	6	.	.	Pawnee
07/22/09	6	100	10	.	.	Pawnee
07/22/09	7	100	2	.	.	Pawnee

07/22/09	7	90	6	.	.	Pawnee
07/22/09	7	60	4	.	.	Pawnee
07/22/09	7	70	10	.	.	Pawnee
07/22/09	7	100	15	.	.	Pawnee
07/22/09	7	90	2	.	.	Pawnee
07/22/09	7	100	12	.	.	Pawnee
07/22/09	7	90	11	.	.	Pawnee
07/22/09	8	100	3	.	.	Pawnee
07/22/09	8	70	6	.	.	Pawnee
07/22/09	8	100	2	.	.	Pawnee
07/22/09	8	50	5	.	.	Pawnee
07/22/09	8	80	7	.	.	Pawnee
07/22/09	8	100	6	.	.	Pawnee
07/22/09	8	100	3	.	.	Pawnee
07/22/09	8	80	6	.	.	Pawnee
07/22/09	9	80	3	.	.	Pawnee
07/22/09	9	100	12	.	.	Pawnee
07/22/09	9	90	6	.	.	Pawnee
07/22/09	9	60	4	.	.	Pawnee
07/22/09	9	90	9	.	.	Pawnee
07/22/09	9	100	12	.	.	Pawnee
07/22/09	9	100	13	.	.	Pawnee
07/22/09	9	90	15	.	.	Pawnee
07/22/09	10	80	9	.	.	Pawnee
07/22/09	10	50	4	.	.	Pawnee
07/22/09	10	100	12	.	.	Pawnee
07/22/09	10	90	8	.	.	Pawnee
07/22/09	10	70	6	.	.	Pawnee
07/22/09	10	60	10	.	.	Pawnee
07/22/09	10	60	5	.	.	Pawnee
07/22/09	10	100	8	.	.	Pawnee
07/22/09	11	90	13	.	.	Pawnee
07/22/09	11	100	6	.	.	Pawnee
07/22/09	11	100	10	.	.	Pawnee
07/22/09	11	80	11	.	.	Pawnee
07/22/09	11	80	3	.	.	Pawnee
07/22/09	11	90	6	.	.	Pawnee
07/22/09	11	100	8	.	.	Pawnee
07/22/09	11	100	12	.	.	Pawnee
07/22/09	12	20	4	.	.	Pawnee
07/22/09	12	90	15	.	.	Pawnee
07/22/09	12	100	6	.	.	Pawnee
07/22/09	12	90	8	.	.	Pawnee

07/22/09	12	70	6	.	.	Pawnee
07/22/09	12	50	8	.	.	Pawnee
07/22/09	12	100	5	.	.	Pawnee
07/22/09	12	90	9	.	.	Pawnee
07/22/09	13	100	8	.	.	Pawnee
07/22/09	13	80	13	.	.	Pawnee
07/22/09	13	80	5	.	.	Pawnee
07/22/09	13	70	12	.	.	Pawnee
07/22/09	13	100	9	.	.	Pawnee
07/22/09	13	100	3	.	.	Pawnee
07/22/09	13	100	17	.	.	Pawnee
07/22/09	13	100	6	.	.	Pawnee
07/22/09	14	80	4	.	.	Pawnee
07/22/09	14	100	6	.	.	Pawnee
07/22/09	14	60	5	.	.	Pawnee
07/22/09	14	70	2	.	.	Pawnee
07/22/09	14	70	2	.	.	Pawnee
07/22/09	14	40	2	.	.	Pawnee
07/22/09	14	100	6	.	.	Pawnee
07/22/09	14	80	7	.	.	Pawnee
07/22/09	15	100	4	.	.	Pawnee
07/22/09	15	100	5	.	.	Pawnee
07/22/09	15	100	9	.	.	Pawnee
07/22/09	15	60	5	.	.	Pawnee
07/22/09	15	100	6	.	.	Pawnee
07/22/09	15	80	7	.	.	Pawnee
07/22/09	15	100	9	.	.	Pawnee
07/22/09	15	100	8	.	.	Pawnee
07/22/09	16	80	5	.	.	Pawnee
07/22/09	16	100	7	.	.	Pawnee
07/22/09	16	100	11	.	.	Pawnee
07/22/09	16	50	6	.	.	Pawnee
07/22/09	16	90	5	.	.	Pawnee
07/22/09	16	100	4	.	.	Pawnee
07/22/09	16	65	12	.	.	Pawnee
07/22/09	16	100	7	.	.	Pawnee
07/22/09	17	60	12	.	.	Pawnee
07/22/09	17	50	6	.	.	Pawnee
07/22/09	17	60	4	.	.	Pawnee
07/22/09	17	100	8	.	.	Pawnee
07/22/09	17	60	5	.	.	Pawnee
07/22/09	17	70	5	.	.	Pawnee
07/22/09	17	100	5	.	.	Pawnee

07/22/09	17	90	3	.	.	Pawnee
07/22/09	18	100	7	.	.	Pawnee
07/22/09	18	90	7	.	.	Pawnee
07/22/09	18	90	6	.	.	Pawnee
07/22/09	18	100	8	.	.	Pawnee
07/22/09	18	100	3	.	.	Pawnee
07/22/09	18	90	8	.	.	Pawnee
07/22/09	18	100	10	.	.	Pawnee
07/22/09	18	100	4	.	.	Pawnee
07/22/09	19	95	15	.	.	Pawnee
07/22/09	19	100	5	.	.	Pawnee
07/22/09	19	90	5	.	.	Pawnee
07/22/09	19	100	4	.	.	Pawnee
07/22/09	19	90	3	.	.	Pawnee
07/22/09	19	100	6	.	.	Pawnee
07/22/09	19	100	10	.	.	Pawnee
07/22/09	19	100	6	.	.	Pawnee
07/22/09	20	90	10	.	.	Pawnee
07/22/09	20	100	6	.	.	Pawnee
07/22/09	20	100	4	.	.	Pawnee
07/22/09	20	100	3	.	.	Pawnee
07/22/09	20	100	11	.	.	Pawnee
07/22/09	20	100	9	.	.	Pawnee
07/22/09	20	100	8	.	.	Pawnee
07/22/09	20	100	8	.	.	Pawnee
08/28/09	1	100	7	.	.	Pawnee
08/28/09	1	100	9	.	.	Pawnee
08/28/09	1	100	8	.	.	Pawnee
08/28/09	1	100	10	.	.	Pawnee
08/28/09	1	100	8	.	.	Pawnee
08/28/09	1	100	6	.	.	Pawnee
08/28/09	1	100	13	.	.	Pawnee
08/28/09	1	100	10	.	.	Pawnee
08/28/09	2	100	30	.	.	Pawnee
08/28/09	2	100	21	.	.	Pawnee
08/28/09	2	100	15	.	.	Pawnee
08/28/09	2	100	10	.	.	Pawnee
08/28/09	2	100	10	.	.	Pawnee
08/28/09	2	100	15	.	.	Pawnee
08/28/09	2	100	28	.	.	Pawnee
08/28/09	2	100	32	.	.	Pawnee
08/28/09	3	100	5	.	.	Pawnee
08/28/09	3	100	6	.	.	Pawnee

08/28/09	3	100	4	.	.	Pawnee
08/28/09	3	100	4	.	.	Pawnee
08/28/09	3	100	10	.	.	Pawnee
08/28/09	3	90	8	.	.	Pawnee
08/28/09	3	100	7	.	.	Pawnee
08/28/09	3	100	4	0	0	Pawnee
08/28/09	4	100	8	75	10	Pawnee
08/28/09	4	100	7	25	15	Pawnee
08/28/09	4	100	8	100	6	Pawnee
08/28/09	4	100	8	100	10	Pawnee
08/28/09	4	100	10	100	6	Pawnee
08/28/09	4	80	7	.	.	Pawnee
08/28/09	4	100	4	100	10	Pawnee
08/28/09	4	100	5	100	10	Pawnee
08/28/09	5	100	7	.	.	Pawnee
08/28/09	5	100	5	.	.	Pawnee
08/28/09	5	100	6	.	.	Pawnee
08/28/09	5	100	8	.	.	Pawnee
08/28/09	5	100	8	.	.	Pawnee
08/28/09	5	90	6	.	.	Pawnee
08/28/09	5	100	4	.	.	Pawnee
08/28/09	5	100	6	.	.	Pawnee
08/28/09	6	100	20	.	.	Pawnee
08/28/09	6	100	10	.	.	Pawnee
08/28/09	6	100	20	.	.	Pawnee
08/28/09	6	90	9	.	.	Pawnee
08/28/09	6	100	8	.	.	Pawnee
08/28/09	6	100	12	.	.	Pawnee
08/28/09	6	100	5	.	.	Pawnee
08/28/09	6	100	6	.	.	Pawnee
08/28/09	7	100	10	.	.	Pawnee
08/28/09	7	100	10	.	.	Pawnee
08/28/09	7	90	6	.	.	Pawnee
08/28/09	7	100	10	.	.	Pawnee
08/28/09	7	100	15	.	.	Pawnee
08/28/09	7	100	10	.	.	Pawnee
08/28/09	7	100	8	.	.	Pawnee
08/28/09	7	100	10	.	.	Pawnee
08/28/09	8	100	5	.	.	Pawnee
08/28/09	8	100	15	.	.	Pawnee
08/28/09	8	100	8	.	.	Pawnee
08/28/09	8	90	6	.	.	Pawnee
08/28/09	8	100	5	.	.	Pawnee

08/28/09	8	90	4	.	.	Pawnee
08/28/09	8	100	10	.	.	Pawnee
08/28/09	8	100	5	.	.	Pawnee
08/28/09	9	100	16	.	.	Pawnee
08/28/09	9	100	13	.	.	Pawnee
08/28/09	9	100	3	.	.	Pawnee
08/28/09	9	100	6	.	.	Pawnee
08/28/09	9	90	7	.	.	Pawnee
08/28/09	9	100	6	.	.	Pawnee
08/28/09	9	100	15	.	.	Pawnee
08/28/09	9	100	8	.	.	Pawnee
08/28/09	10	100	5	.	.	Pawnee
08/28/09	10	60	4	.	.	Pawnee
08/28/09	10	100	5	.	.	Pawnee
08/28/09	10	100	4	.	.	Pawnee
08/28/09	10	100	7	.	.	Pawnee
08/28/09	10	100	8	.	.	Pawnee
08/28/09	10	100	6	.	.	Pawnee
08/28/09	10	100	5	.	.	Pawnee
08/28/09	11	100	5	.	.	Pawnee
08/28/09	11	100	8	.	.	Pawnee
08/28/09	11	100	7	.	.	Pawnee
08/28/09	11	100	6	.	.	Pawnee
08/28/09	11	100	4	.	.	Pawnee
08/28/09	11	80	5	.	.	Pawnee
08/28/09	11	100	4	.	.	Pawnee
08/28/09	11	100	5	.	.	Pawnee
08/28/09	12	100	7	.	.	Pawnee
08/28/09	12	100	12	.	.	Pawnee
08/28/09	12	100	5	.	.	Pawnee
08/28/09	12	100	10	.	.	Pawnee
08/28/09	12	100	6	.	.	Pawnee
08/28/09	12	100	5	.	.	Pawnee
08/28/09	12	100	4	.	.	Pawnee
08/28/09	12	100	6	.	.	Pawnee
08/28/09	13	100	10	.	.	Pawnee
08/28/09	13	100	8	.	.	Pawnee
08/28/09	13	100	8	.	.	Pawnee
08/28/09	13	100	6	.	.	Pawnee
08/28/09	13	100	4	.	.	Pawnee
08/28/09	13	100	7	.	.	Pawnee
08/28/09	13	100	9	.	.	Pawnee
08/28/09	13	100	8	.	.	Pawnee

08/28/09	14	100	11	.	.	Pawnee
08/28/09	14	100	12	.	.	Pawnee
08/28/09	14	100	10	.	.	Pawnee
08/28/09	14	100	9	.	.	Pawnee
08/28/09	14	100	10	.	.	Pawnee
08/28/09	14	100	12	.	.	Pawnee
08/28/09	14	100	6	.	.	Pawnee
08/28/09	14	100	13	.	.	Pawnee
08/28/09	15	100	7	.	.	Pawnee
08/28/09	15	100	5	.	.	Pawnee
08/28/09	15	90	6	.	.	Pawnee
08/28/09	15	100	4	.	.	Pawnee
08/28/09	15	100	5	.	.	Pawnee
08/28/09	15	90	4	.	.	Pawnee
08/28/09	15	100	6	.	.	Pawnee
08/28/09	15	100	8	.	.	Pawnee
08/28/09	16	100	8	.	.	Pawnee
08/28/09	16	100	6	.	.	Pawnee
08/28/09	16	100	4	.	.	Pawnee
08/28/09	16	100	6	.	.	Pawnee
08/28/09	16	90	6	.	.	Pawnee
08/28/09	16	100	5	.	.	Pawnee
08/28/09	16	100	8	.	.	Pawnee
08/28/09	16	100	6	.	.	Pawnee
08/28/09	17	90	5	.	.	Pawnee
08/28/09	17	100	4	.	.	Pawnee
08/28/09	17	100	3	.	.	Pawnee
08/28/09	17	.	.	.	.	Pawnee
08/28/09	17	100	5	.	.	Pawnee
08/28/09	17	100	4	.	.	Pawnee
08/28/09	17	100	3	.	.	Pawnee
08/28/09	17	100	5	.	.	Pawnee
08/28/09	18	100	10	.	.	Pawnee
08/28/09	18	100	6	.	.	Pawnee
08/28/09	18	100	2	.	.	Pawnee
08/28/09	18	100	13	.	.	Pawnee
08/28/09	18	100	8	.	.	Pawnee
08/28/09	18	100	7	.	.	Pawnee
08/28/09	18	100	5	.	.	Pawnee
08/28/09	18	100	8	.	.	Pawnee
08/28/09	19	100	4	.	.	Pawnee
08/28/09	19	100	6	.	.	Pawnee
08/28/09	19	100	4	.	.	Pawnee

08/28/09	19	100	6	.	.	Pawnee
08/28/09	19	90	5	.	.	Pawnee
08/28/09	19	80	4	.	.	Pawnee
08/28/09	19	100	5	.	.	Pawnee
08/28/09	19	100	3	.	.	Pawnee
08/28/09	20	100	5	.	.	Pawnee
08/28/09	20	100	4	.	.	Pawnee
08/28/09	20	100	8	.	.	Pawnee
08/28/09	20	100	5	.	.	Pawnee
08/28/09	20	100	6	.	.	Pawnee
08/28/09	20	100	5	.	.	Pawnee
08/28/09	20	100	10	.	.	Pawnee
08/28/09	20	100	8	.	.	Pawnee

VITA

Andrea Payne

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF PECAN SCAB DISEASE MANAGEMENT STRATEGIES TO IMPROVE ORCHARD PRODUCTION AND REDUCE PESTICIDE INPUTS

Major Field: Entomology and Plant Pathology

Biographical:

Education:

Completed the requirements for the Master of Science in Entomology and Plant Pathology at Oklahoma State University, Stillwater, Oklahoma in May 2011.

Completed the requirements for the Master of Public Health in Epidemiology at Tulane University, New Orleans, Louisiana in January 2009.

Completed the requirements for the Bachelor of Science in Microbiology at Oklahoma State University, Stillwater, Oklahoma in 2007.

Professional Memberships:

Entomology and Plant Pathology Graduate Student Association
American Phytopathological Society
Epidemiology Committee
Graduate Student Committee
Southern Division of the American Phytopathological Society

Awards:

The Richard L. Gabrielson Award and The Raymond G. Grogan Award
Women's Faculty Council Excellence in Research
2nd Place Southern Division of American Phytopathological Society student paper competition
Finalist of the President's Outstanding Leadership for Masters Student Award
Winner Phoenix Award for Outstanding Masters Student

Name: Andrea F. Payne Date of Degree: May, 2011

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATION OF PECAN SCAB DISEASE MANAGEMENT STRATEGIES TO IMPROVE ORCHARD PRODUCTION AND REDUCE PESTICIDE INPUTS

Pages in Study: 109

Candidate for the Degree of Master of Science

Major Field: Entomology and Plant Pathology

Scope and Method of Study: To facilitate the subsequent studies, initial experiments evaluated growth media best suited for maximizing sporulation of *F. effusum in vitro*. Additional studies were aimed at improving our understanding of weather conditions that are favorable for pecan scab epidemic initiation and progression in Oklahoma. In addition, influential relative humidity and temperature thresholds in pecan orchards were reevaluated for use in models to predict epidemics of pecan scab. Other biologically significant weather variables were also evaluated for inclusion in an updated advisory. Finally, experiments were conducted to understand the influence of early fungicide treatments in Oklahoma pecan orchards.

Findings and Conclusions: Potato dextrose agar was found to be the best medium for the growth and sporulation of *F. effusum.* Malt extract agar and potato carrot agar amended with 50% lactic acid were also favorable for the growth and sporulation of *F. effusum in vitro*. The disease prediction model developed during this study was adjusted for the effects of solar radiation and the accumulation of rain in a dynamic fashion. By doing so, it was evident that the thresholds used by the current advisory closely estimated periods of scab increase, but accuracy might be improved by adding the effects of solar radiation and rain. Furthermore, a new model using dew point and dew point depression was developed. Further validation studies using these models should be performed to identify any improvement in the ability to predict fungicide application. Data from field trials suggest that in Oklahoma growers can use an abbreviated fungicide program, which provides scab control during the early stages of the season and can be relaxed during months of July and August. Considering the increased susceptibility of pecans to damage by pecan scab during early stages of development, this study has demonstrated that fungicide protection be used when conditions are favorable for disease during the early growing season.

ADVISER'S APPROVAL: Dr. Damon L. Smith