BIOLOGY OF *Phakopsora pachyrhizi*, THE CAUSAL AGENT OF SOYBEAN RUST, IN FLORIDA

By

HEATHER MARIE YOUNG

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2012

To all those who have encouraged and supported me

ACKNOWLEDGMENTS

I would like to thank Dr. Jim Marois, my major professor, for his support, guidance, and help throughout my Doctoral degree program. I am thankful to Drs. David Wright, Ariena van Bruggen, and Phil Harmon, members of my advisory committee, for their useful counsel, critique and instruction. I am also very grateful to Drs. Dario Narváes, Daniele Liberti, Sheeja George, and Pratibha Srivastava, and Kelly O'Brien for their support and help with greenhouse and field experiments and laboratory analyses. I am also thankful for Maynard Douglas, Chuck Dickson, and Brian Kidd for their assistance with establishing and maintaining soybean plots.

I would also like to extend my admiration to all the faculty and staff of North Florida Research and Education Center – UF-IFAS in Quincy, Florida and to the Plant Pathology Department at the University of Florida in Gainesville, Florida for their camaraderie, inspiration, and support, which were very important in improving my scientific skills and knowledge of academics. I want to thank Dr. David Wright and North Central Soybean Research Program for financial and operational support – without their assistance this research would not have been possible. For those not mentioned, who had contributed to my success; I offer my sincere gratitude. Finally, I thank my boyfriend, Brett Kelly, and my parents, Dennis and Linda Young, for their love, encouragement, and support.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

BIOLOGY OF *Phakopsora pachyrhizi*, THE CAUSAL AGENT OF *SOYBEAN* RUST, IN FLORIDA

By

Heather Marie Young

May 2012

Chair: James J. Marois
Major: Plant Pathology

Soybean rust (SBR), caused by the biotrophic fungus *Phakopsora pachyrhizi*, is a potentially destructive disease to soybean (*Glycine max*) production in the United States. Winter temperatures and viable hosts limit the fungus to overwintering in the southeastern states, on the alternative host kudzu (*Pueraria montana*). The initial inoculum from kudzu and subsequent development of the disease within southern commercial soybean fields provide the inoculum for potential epidemics in the major soybean production areas in the U.S. An improved understanding of SBR epidemics and movement of initial inoculum from kudzu in the southeastern states is important in developing fungicide application decision models for managing epidemics and for improving the accuracy of current risk assessment models for long distance dispersal of SBR spores. This study examined the effects of environmental factors (i.e. temperature, relative humidity, leaf wetness, rainfall, and solar radiation) and soybean growth stage on SBR development and severity in soybean, examined genetic variation in kudzu populations with respect to possible resistant genes, and quantified resistance to SBR in kudzu and soybean through detached leaf assays. A disease and decision

model for fungicide application for SBR was developed and validated by the data obtained.

Disease and soybean data, from 2005 through 2008 collected in north Florida, indicated that the majority of SBR infections occur at reproductive growth stage R3 (beginning pod) or later, even though earlier maturing varieties are at reproductive stages earlier in the year. Precipitation was found to be the principal environmental factor affecting disease severity, where average maximum severity was 20.8 and 9.8% if precipitation was more and less than 300 mm, respectively, over a 1 month period after initial infection. Early or late infection in soybean fields was attributed in part to weather conditions occurring 1 month prior to disease detection and potential build up of inoculum over time. Data from shading treatments in the field and detached leaf assays suggest that solar radiation affects not only disease development in soybean canopies by affecting temperature and relative humidity and reducing spore germination, but also by increasing epicuticular wax on soybean leaves creating a thicker barrier that *P. pachyrhizi* has to penetrate through for infection.

Kudzu populations in Florida were screened for genetic resistance to SBR using primers designed in conserved nucleotide binding site (NBS) domain of the known soybean resistant gene *Rpp4* and restriction fragment length polymorphisms (RFLP). High genetic variation among kudzu populations and low variation within populations were found with respect to NBS region(s). No patterns of variation were observed in regard to geographic location of kudzu populations or infection history of SBR from 2005 through 2011. Results indicate that kudzu is a good source of resistance to SBR not only due to the genetic variation among kudzu populations but also due to an overall

reduction in severity of disease development on both susceptible and resistant kudzu as compared to soybean.

Detached leaf assays quantified different levels of resistance in susceptible, resistant, and immune kudzu with respect to susceptible and resistant soybean. From the results of the detached leaf assays the number of uredinia on susceptible kudzu was only 10% and on resistant kudzu was only 1% of the number of uredinia produced on susceptible soybean. Similarly, the number of spores produced per inoculation area, 20 days after infection on susceptible and resistant kudzu was only 23 and 2%, respectively, of that produced on susceptible soybean. Sporulation was delayed on resistant kudzu as compared to susceptible soybean and kudzu.

Sporulation, infection efficiency, and latent period were utilized from the detached leaf assay results in a spatial and temporal disease model estimating the number of infections occurring over time and amount of spores escaping into the atmosphere from soybean and kudzu areas in Florida. Utilizing the knowledge on environmental variables and potential inoculum from soybean and kudzu a decision model for fungicide application was developed and validated over the growing seasons of 2009 through 2011. Results from this dissertation identified environmental factors that contribute to SBR epidemics in the United States and have been used to better estimate potential spore loads available for long distance transport from southeastern states to the major soybean productions areas in the northern and mid-western states to improve risk assessment for the major soybean production areas.

CHAPTER 1
INTRODUCTION AND RESEARCH OBJECTIVES

Soybean

Physiology

Soybean [*Glycine max* (L.) Merrill] is an annual legume which typically grows 75 to 125 cm in height with 14-26 nodes (172). Leaves are simple compound trifoliates in an alternating pattern (172). Soybeans can either grow indeterminately, where the terminal bud continues to be active during most of the growing season, or determinately, where the terminal bud ceases growth at the onset of reproductive development (63,215). Soybean flowers have a typical papilionaceous flower with a high percentage of self-pollination (215). A soybean plant can produce 60-80 pods, each containing 3 pea-sized beans of 130-220 mg (172,184). Seed production is favored by warm mean temperatures of 20 °C to 30 °C. The seed content consists of approximately 38-56% protein and 8-27% oil, depending on variety (135). The root systems consist of a primary taproot and numerous lateral roots arranged in four longitudinal rows along the primary tap root; where most root dry weight (80-90%) occurs in the top 15 cm of the soil (172,188). Soybean above-ground vegetative structures consist of an erect, branched stem, attaining a height of 75-125 cm and possess 14-26 nodes (172).

Uses and Cultivation History

In 2009 soybeans represented 53% of the world's source of oilseed production and within the U.S. soybeans provide 69% of the edible consumption of fats and oils (184). The various uses of soybean include not only human and animal feed, but also many industrial uses from adhesives to biofuels. Linguistic, geographical, and historical evidence suggests domestication of soybean emerged around the eleventh century

B.C. in the eastern half of north China, most likely during the Shang dynasty (ca. 1700-1100 B.C.)(74). The movement of soybean within China and the peninsular Korea was most likely associated with the development, consolidation of territories, and degeneration of Chinese dynasties (74). Soybean seed was used primarily for human and livestock feed and for medicinal purposes. By the 15th and 16th century, soybeans were introduced in Japan, Indonesia, the Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal and north India.

In the early 1700's, soybeans were cultivated in Europe and in 1765 plantings of soybeans were recorded in the Colony of Georgia to manufacture soy sauce and vermicelli (74). USDA published its first bulletin about the use of soybeans as a forage crop in 1899, and by 1904 soybeans were evaluated for their use as an oil and protein source. By 1909, commercial soybean production had developed in many states from the eastern seaboard into the central states, with an estimated 800 ha of soybeans being planted that year (134). The top five U.S. states with the largest production areas of soybean in 2010 were Iowa, Illinois, Minnesota, Nebraska, and Indiana (134).

U.S. Cultivation

In the U.S. soybeans are generally planted in early May to mid June and harvested in mid September through mid November, but it is possible for soybeans to be planted as early as mid April and as late as early August in some areas (13,147,217). Soybean cultivars are classified into 13 maturity groups (MG) based on the time of flowering and maturity due to geographical adaptation (228). Specifically, a soybean cultivar is assigned a numeric number based on the days from planting to maturity at a defined latitude (or day length) and a specific planting date under optimum environmental conditions (228). The number of different varieties a farmer may plant in

one season varies based on the area being planted and other variables, and may range from 6 to 10 different varieties (201). In 1996 genetically modified soybeans were commercially released and by 2009 an estimated 91% of the soybean hectares planted in the U.S. were genetically modified soybean (134). Genetically modified soybean contains a gene derived from *Agrobacterium* sp. strain CP4, encoding a glyphosate-tolerant enzyme, called CP4 enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, which results in glyphosate-tolerant soybeans (53). Glyphosate is the main active ingredient in the herbicide RoundUp®, which can be used on the genetically modified, "RoundUp Ready" soybeans to control weeds.

Soybeans are grown in more than 30 states, making it the second largest crop in cash sales and the number one export crop in the U.S. (184). In 2009, the 31.4 million ha of soybeans planted in the U.S. was the largest soybean crop ever planted in the U.S and the 80.7 million metric tons harvested was also the largest in U.S. history, making the U.S. the world's leading soybean producer. Brazil had the second largest harvest of soybean with 57.0 million metric tons in 2009 (184). Soybeans accounted for 30% of the U.S. crop area planted in 2009, second only to corn. It accounted for 38% of the world's oilseed production and had a production value of $32.1 billion in 2009 (134).

Soybean Rust

Geographic Distribution

Soybean rust (SBR), caused by the fungal biotroph *Phakopsora pachyrhizi* Syd. & P. Syd., is present in all the major soybean producing areas of the world. The first report of the disease was from Japan in 1902 (20,118). By 1934, the pathogen had spread to several other Asian countries as well as Australia (21); and by 1951 SBR was reported in India (171). In 1996, the first confirmed report of the pathogen on the

African continent was published from Kenya, Rwanda, and Uganda (118). Since 1996, the pathogen has continued to spread across the African Continent with reports from Zambia and Zimbabwe in 1998, Nigeria in 1999, Mozambique in 2000, and South Africa in 2001 (3,118,152). The disease was found on soybeans on the Hawaii islands of Oahu, Kauai, and Hawaii in 1994, but dissemination to the continental U.S. from this location was never reported (86).

In February of 2001 the first detection of *P. pachyrhizi* in the New World was reported on soybeans in Paraguay. By 2002, the disease was widespread throughout Paraguay, limited areas of Brazil bordering Paraguay and northern Argentina (128,158,224). The pathogen spread north of the equator in Brazil and possibly into Venezuela, Guyana, and Suriname (79,224). In November 2004, the pathogen was found on soybeans in Baton Rouge, LA (167) and on soybeans and kudzu in Florida (65). It was hypothesized that Hurricane Ivan carried *P. pachyrhizi* spores from South America into the U.S. (79), thus making SBR present in every major soybean producing region in the world.

Since its introduction to the continental U.S. the disease has spread from the Southeast to as far west as Nebraska, to the north in the Canadian province of Ontario and to the south in Mexico (102,157,212). In the 2009 growing season, SBR was detected in 576 counties in 17 states (102).

Economic Impact and Yield Loss

SBR is one of the most detrimental diseases to soybeans worldwide causing devastating yield losses and increased production costs (66,102,118). The disease can reduce seed weight of soybeans by 40-80%, shoot weight by 20%, the overall number of pods per plant, and the photosynthetic area (222). Experiments conducted in Brazil

in the 2005-2006 and 2006-2007 growing season attributed SBR-induced yield loss to premature leaf loss, reduction in canopy green leaf area due to lesions, reduction in dry matter accumulation, and reduction in harvest index due to reduced seed set and seed mass (96).

In China, yield losses from SBR commonly range between 10 and 30%, and have been observed to be over 50% in severe disease years (61). In southern Japan, estimated yield losses have ranged up to 80% for individual fields, and total crop losses have been reported as high as 30% in Taiwan (222). Yield losses of 68% in susceptible varieties and 22% in tolerant cultivars have been reported in field studies in Korea (137). Research plots in Australia that had not received fungicide applications had yield losses of 60 to 70% (139).

In Africa, yield losses of 10 to 80% were observed in South Africa and from 60 to 80% in Zimbabwe (28). In recent years, yield losses of 100% have been reported in continuous mono-culture soybean production in Africa (28). Paraguay and Brazil have observed yield losses up to 60 and 63%, respectively (224).

Not only does yield loss due to SBR have an economic impact on soybean production, but also the cost of fungicides and their applications in response to SBR, increase production cost. In the 2003-2004 soybean production season in Brazil, economic losses due to the disease, including costs of control, were estimated at more than $2 billion US$ (72,180).

SBR poses a serious threat to the U.S. soybean production, as it does in other major soybean production areas in the world. In 2004, USDA estimated that in the first year the pathogen is established in the country the value of net economic losses would

range from $640 million to $1.3 billion, and annual losses in ensuing years could average anywhere between $240 million and $2.0 billion (104). Further estimates stated that yield losses could exceed 10% in the U.S. with up to 50% in the Mississippi delta and southeastern states (151,220,222).

Since its introduction in 2004, SBR has spread throughout the U.S. more gradually than was expected (45,169). The pathogen has not been detected in the major soybean producing regions of the Midwest until late in the growing seasons and the relatively late season arrival in the Southeast has resulted in only minor yield losses in commercial soybean fields. While the impact of yield loss in U.S. commercial fields has been minimal thus far, experimental plot losses have ranged from 19 to 35% in Alabama, Georgia, and Florida (37,130). Even though SBR has not yet caused major yield losses in the U.S., it has still had an economic impact through fungicide application on soybeans. According to data from the National Agriculture Statistics Service (NASS), very little fungicide application occurred on soybeans prior to 2006, but in 2006 over 362,000 pounds of fungicides were applied to soybeans across 7 different states, with that number continuing to increase in the following years (134). Since its introduction to the continental U.S. in 2004, researchers, producers, and industry representatives have been gaining valuable information from field trials about the biology and control of SBR in the U.S., despite its spread and severity continuing to be variable each year.

Pathogen Taxonomy and Morphology

P. pachyrhizi (synonym: *Malupa sojae*, *Uredo sojae*, *Phakopsora erythrinae*, and *Uredo erythrinae* (140)) is in the common rust fungi order Uredinales (73,118213). Fungi in Uredinales are generally biotrophs, and can have up to 5 different spore stages

on two plant hosts which are taxonomically unrelated. The 5 spore stages include urediniospores, teliospores (both on principal host), basidiospores, spermagonia (pycnia), and aeciospores (latter on alternate host) (213). In nature, only urediniospores and teliospores have been observed for *P. pachyrhizi* (66,118). Basidiospores have been produced in laboratory conditions; however no alternate host has been identified on which the fungus completes its life cycle. For this fungus, basidiospores are not considered a critical spore type (162). Hence, *P. pachyrhizi* is an autoecious, microcyclic rust fungus.

The urediniospores are produced in anamorphic fruiting structures called uredinia, which can be amphigenous (growth on both sides of leaf), but are mostly hypophyllous (growth on abaxial surface of leaf) (20,66). Uredinia are tan to reddish brown in color, range from 50-200 μm in diameter, and spatially sprinkled to clustered on yellowish lesions. A uredinium is surrounded by incurved paraphyses arising from a cellular basal peridium, which forms a volcano like structure, with urediniospores being borne on sporophores inside the structure. Paraphyses are cylindric to clavate, hyaline to yellowish-brown in color, range in size from 25 – 50 x 6 – 14 μm, and are slightly thickened at the apices. Urediniospores are released through an opening (ostiole) at the top of the volcano like structure. Urediniospores are sessile, obovoid to broadly ellipsoid, with a minute and dense echinulate surface (“spiny” spores). They are hyaline to yellowish brown in color and range in size from 18 – 38 μm long by 13 – 29 μm wide with walls that are approximately 1 μm thick (140).

Under appropriate environmental conditions the production of telia may be observed, often seen mixed on the abaxial leaf surface with uredinia. Telia are

chestnut-brown to dark brown in color, crustose and range in size from 150 – 250 μm. Teliospores are single-celled, with walls approximately 1 μm thick, oblate spheroid, and irregularly arranged in 2 to 7 spore layers within the telium. The spores range in size from 15 – 26 x 6 – 12 μm and are yellowish brown to colorless (21,140,223).

Host Range

P. pachyrhizi has been reported to infect, under controlled conditions, 158 species in 54 genera of the subfamily Papilionoideae (57,65,105,140,160,170,179). Unlike other rusts, *Phakopsora* spp. can directly penetrate host epidermal cells, which in *P. pachyrhizi,* might be, in part, the reason it can infect a wide range of legume species (91). Due to the early misidentification of the two rust species (*P. pachyrhizi* and *P. meiobmiae*) and the multiple responses of host species to different rust pathotypes, a complete host range has not been clearly defined (118). Current reported hosts in the U.S. are *Glycine max* (soybeans) (167), *Pueraria lobata* (kudzu) (65), *Desmodium tortuosum* (Florida beggarweed) (170), *Erythrina herbacea* (Coral bean) (57), *Phaseolus coccineus*, *P. lunatus*, and *P. vulgaris* (105). In greenhouse evaluations in 2008, 65 species were newly identified as hosts to *P. pachyrhizi*, of which 62 occur in Alabama, Florida, Louisiana, Mississippi, and Texas (179). The large host range of *P. pachyrhizi* reflects the diversity and complexity of its virulence patterns, as well as helps contribute to its survival and overwintering on 'green bridges,' such as kudzu in the southern U.S.

Disease Symptoms and Signs

P. pachyrhizi may infect soybean at any growth stage (47), but symptoms are most often observed at late vegetative to early reproductive stages (45,91,111). The most common visual symptoms of SBR are tan to dark brown to reddish brown angular

polygonal lesions (0.5 – 5 mm^2) (20,66,118). The color of the lesion is dependent upon its age and host/pathogen genotype interactions. Typically, symptoms are first observed on leaves in the lower canopy and mainly on leaves, but can be found on petioles, pods, and stems of soybean plants (176). Within lesions, multiple erumpent and globose uredinial may develop, on both abaxial and adaxial surfaces of soybean leaves (20,66). Telia are rare to observe, they are dark brown to black and are subepidermally distributed among uredinia. Production of multiple uredinia with a circular ostiole located at the top of each uredinia where spores emerge is a distinct sign of *P. pachyrhizi*. Severe or early infection can result in premature yellowing and defoliation, causing yield loss (197).

While *P. pachyrhizi* produces characteristic rust symptoms it may be confused with other diseases, such as brown spot (*Septoria glycines* Hemmi), downy mildew (*Peronospora manshurica* Naumov), frogeye leafspot (*Cercospora sojina* Hara), bacterial blight (*Pseudomonas syringae* van Hall), and bacterial pustules (*Xanthomonas axonopodis* pv. *Glycines* Nakano), especially at disease onset and very early stages of development (66,198).

Infection, Reproduction, and Dispersal

Urediniospores are predominately wind dispersed and serve as the only known infective spore type (118). Infection process begins with germination of urediniospores across host tissue, which can occur within 2 hours after spore deposition, at 20 °C when moisture is readily available (20). The germ tube can grow 5 to 400 μm long, with appressoria frequently forming when germ tube is less than 100 μm in length. Once the germ tube contacts an anticlinal wall or extends over epidermal cells, an appressorium

forms. Rarely will the appressorium develop over stomata (15,90,103,111). The appressorium will directly penetrate into epidermal cells, reaching the intercellular space of the mesophyll by way of the appressorial penetration peg, which works under turgor pressure to puncture the host epidermis (118). If an appressorium develops over a stomatum, it will penetrate one of the guard cells instead of entering through the stomatal opening. Direct penetration of host epidermis is a unique trait of *Phakopsora* rust species as compared to other rusts that require a natural opening or wound to enter the host.

Between 24 and 48 hours after penetration, growth and development of specialized feeding hyphae, called haustoria, occur in the intercellular space of the mesophyll (90,91). Uredinia have been observed as early as 5 days after infection with urediniospores being produced after 9 days (90,108). Sporulation can continue for 3 to 6 weeks for a single uredinium and up to 15 weeks in a single lesion while primary and secondary uredinia develop (90,118). On average, each uredinium can produce more than 12,000 urediniospres (21,45,90,108). Temperatures between 15 and 28°C (45,66,108) and a minimum of 6 hours of continuous leaf wetness (20,114) are required for infection to occur. As temperatures decrease below 18°C, the length of the moisture period needed for infection increases (109) and extended periods of leaf wetness in the field can increase the rate of spread of the pathogen in the upper canopy and overall disease severity (133). Temperatures near 20°C with 10 to 12 hours of free moisture promote maximum infection, which can result in high disease severity in less than 20 days after infection.

P. pachyrhizi spore dispersal can be illustrated in 5 stages: 1) urediniospore production on the host, 2) urediniospore escape from the canopy, 3) turbulent transport and dilution of urediniospore clouds through the atmosphere, 4) survival in transport, and 5) urediniospore deposition onto a host population (8,62).

Soybean Rust in the U.S.

In the U.S., the pathogen generally occurs on soybeans late in the growing season, when the majority of the soybeans are in the R3 or R4 reproductive growth stages or later (45,169). Based on observations recorded through the U.S. Department of Agriculture's (USDA) SBR surveillance and monitoring program, SBR overwinters in the Gulf Coast regions of the U.S. from Florida to Texas, and given suitable environmental conditions it spreads from a south to north direction in the U.S. (45,77,80,145,169). Being a biotroph, *P. pachyrhizi* requires living host tissue, as well as temperatures greater than 4 °C to survive (20,192), although urediniospores can survive short freeze/thaw cycles (83). Overwintering hosts may include volunteer soybeans or any of the numerous alternative hosts *P. pachyrhizi* can infect, but presently kudzu (*Pueraria montana* [Lour.] Merr. var. lobata [Willd.]) is the only known host that could provide large amounts of living tissue for overwintering in the southeastern U.S. in areas where it does not freeze (17,26,77,83,102140,160,178,192).

Soybean Rust Epidemiology and Risk Models

SBR models can be separated into two major groups: simulation and empirical models. Simulation models are process driven, based on concepts derived from a pathosystem and help improve understanding of structure and behavior of such a biological system (193). Empirical models are statistically driven, based on statistical relationship(s) of explanatory variables with experimental data. The reliability of model

predictions depends heavily on the quality of the dataset used for model development (93).

SBR simulation models can be further divided into epidemiological and aerobiological models. Epidemiological models aim to mimic biological processes in the disease cycle to estimate disease development considering local inoculum availability. Three such models are SOYRUST, specific to SBR (219,220), a general disease model for rusts (150), and CLIMEX (149) a climate computer model. SOYRUST is a computer model that simulates daily increase of disease severity by rate variables that estimate leaf area development and disease components, including infection, latent period, and uredinia senescence. Where infection rate is influenced by leaf wetness duration and temperature, latent period and uredinia senescence are a function of only temperature. SOYRUST was further refined by linking it to the soybean growth model, SOYGRO, to simulate disease effects on soybean yield and assess potential crop losses in some locations of the U.S. by generating disease progress curves and final yield in the presence or absence of SBR (219,220).

A general disease model using infection efficiency, latent period, infectious period, the number of spores produced per lesion, and proportion of spores landing on a potential infection site was adjusted to predict epidemic development for SBR and four other rust diseases (150). The general disease model was adjusted by the temperature effect on infection efficiency and latent period at the initial stages of an epidemic, in order to assess establishment potential by the estimation of daily increase of infection units and rate of disease increase. Results indicated that longer periods of time would

be required for SBR to increase from trace to visual detection levels in the field for specific locations within the U.S.

The computer model, CLIMEX (182), uses climatic suitability for biological processes and has been adjusted to determine stress-free zones for year-round survival of *P. pachyrhizi*. A stress free index for a single location was calculated using the stress indices of cold, warm, and moisture stresses as defined in the literature. Results identified Florida and other southeastern U.S. states boarding the Gulf of Mexico as potential regions where year-round inoculum survival would be most likely to occur. It also identified northern states in the U.S. as regions where SBR epidemics would depend on primary inoculum originating from long distances (149).

Aerobiological models aim to predict the transport and dissemination of airborne inoculum over long distances. SBR aerobiological models have been used to assess entry potential (HYSPLIT, 143, 88), entry and establishment potential (HYSPLIT, 132), and entry, establishment, and epidemic potential (SRAPS, 79). The atmospheric transport model NOAA ARL HYSPLIT_4 (HYSPLIT – Hybrid Single-Particle Lagrangian Integrated Trajectory) (136) simulates ingle trajectories of air parcels, dispersal, concentration and deposition of particles, originating from a source geographical location and time of year. The parameters for strength of inoculum sources, spore production, survival and deposition that were mechanistically and empirically estimated based on current knowledge on the system and physical principals of SBR were used in the HYSPLIT model (142). The climate prediction data from the Experimental Climate Prediction Center (ECPC) of the Scripps Institution of Oceanography was fed into the Pennsylvania State University/National Center for Atmospheric Research mesoscale

model (MM5) that estimates meteorological variables to input into the NOAA ARL HYSPLIT_4 model to predict spore dissemination months ahead. This SBR aerobiological model was used to hindcast likely dispersal of *P. pachyrhizi* spores from Africa into South America in 2002 and to forecast dispersal from central to northern soybean production regions in South America in 2004. Furthermore, spore dispersal maps generated by the model using a source of *P. pachyrhizi* in Colombia showed likely spread of spores to the southern U.S. which was confirmed by the findings of the disease in November 2004, in several locations in the gulf coastal states (142).

The HYSPLIT model was further adjusted to predict SBR risks in the U.S. during the 2005 growing season by combining biological and meteorological elements into a qualitative evaluation of the risk of disease development associated with one or a group of sources. This was done by including a description of the factors pertinent to the disease status, a general outlook that assessed the risk of disease development, and a map showing the spore-laden wind flowing away from an active source or source region using multiple, centrally-located trajectories (132). In such the model allowed the prediction of entry and establishment potential by estimating qualitative risks.

SBR empirical models can be separated into 3 kinds according to the epidemic component estimated: suitability or critical periods for infection, disease progress, and maximum or final disease severity (40). Empirical models that estimate suitability or critical periods for infection were developed and applied in Brazil (29,154). Reis et al (154) used a non-linear regression of interaction of leaf wetness duration and mean temperature during periods of wetness and infection (114) to develop daily values of probability for infection. Canteria et al. (29) also used a non-linear regression function

of the number of hours of leaf wetness and mean temperature (109) to calculate relative intensity of infection. Risk maps of infection efficiency were generated daily and results were summarized for periods of a week or a month to map regions where higher climatic favorability was predicted (29).

Management

There have been multiple approaches for controlling SBR including cultural practices, fungicide application, biological control, and breeding for resistance. Planting early or using an early maturing variety are cultural practices that may allow the crop to mature before conditions become favorable for disease development (20,118). In general, most cropping systems and cultural practices such as narrow-row or wide-row planting, double-crop soybean, and tillage system does not directly affect SBR incidence or severity; however, disease pressure may vary under some cropping systems and cultural practices due to extended time that the crop is exposed in the field and regulated crop free periods. In Brazil, mandatory 90-day soybean free periods, in which no soybeans are to be grown and all volunteer soybean plants are to be destroyed, were put into regulation to reduce inoculum accumulation and overall disease pressure (183). Other cultural practices for reducing SBR incidence and severity include mid-day and nighttime irrigation to avoid extended periods of leaf wetness (28).Furthermore, the destruction of alternative hosts will decrease the amount of potential initial inoculum; however, the eradication of kudzu and other wild alternative hosts of SBR are not practical.

Fungicide application has been most successful in controlling SBR epidemics. Historically, soybean diseases have not been managed by fungicide applications, but there are certain conditions that may necessitate the use of foliar fungicides. Other

soybean diseases that have been managed with fungicide application include frogeye leaf spot (*Cercospora sojina*) (1), Cercospora leaf blight (*C. kikuchii*) (168), Sclerotinia stem rot (*Sclerotinia sclerotiorum*) (129), brown spot (*Septoria glycines*) (106), and *Phomopsis* seed infection (216). Factors contributing to the effectiveness of fungicide applications for SBR control include the severity of the disease/disease pressure, the duration for which the chemical remains active on the host tissue, and the length of the reproductive stages of the plant (120). It has been observed when 20 to 30% of soybean leaves in the mid-canopy are infected fungicide applications are no longer effective in controlling the disease (45). Hence, early detection and prediction of SBR is critical for fungicide applications.

While one to two sprays from R1 through R6 is recommended for control of SBR in the U.S. (45), the timing and effectiveness of any given fungicide treatment is often dependent on when rust is first detected and the intensity of its development (130). In Africa, it is recommended to apply fungicides as a preventative or within a couple of weeks after first observations of the disease are confirmed since yields tend to decrease as the time between first detection and first application increase (101). Fungicide applications made prior to first detection of SBR tend to result in greater yields than those made after first detection. In general, occurrence of rust at any time during the first half of seed fill could justify protective fungicide applications (14,130). A forecasting system based on monitoring source regions for SBR and weather variables is established through the Integrated Pest Management – Pest Information Platform for Extension and Education (ipm-PIPE, sbr.ipmpipe.org). This system provides growers with information useful for determining timing and frequency of fungicide applications

and has and will continue to reduce the number of applications of fungicides applied to control SBR (80,130).

Another important factor for controlling disease with fungicides is the spray coverage on the host plant. Methods used to apply fungicides depend on the area that needs to be treated and the equipment available to make the application (120). Aerial applications of fungicides in Brazil provide adequate control of the disease over large soybean acreage (121). Alternatively, maximum disease control was achieved in Zimbabwe by penetrating the fungicide spray into the soybean canopy, by use of air assist and high-pressure lateral discharge equipment (100). In the U.S., effective application of fungicide have been attained using a conventional sprayer and flat-fan nozzle with high spray pressures to attain fine to medium droplet sizes (45,130,141,169).

The 2 chemical classes of fungicides predominantly used to manage SBR are strobilurins and triazoles. Strobilurins are within the Quinone Outside Inhibitors (QoI) fungicide group, blocking energy transfer at the site of quinol oxidation (Qo site) in the cytochrome bc 1 complex inhibiting fungal mitochondrial respiration resulting in the fungus death. This is a single site of inhibition; hence risk of the development of resistance is high. Resistance to QoI fungicides in plant pathogens has been detected and 3 amino acid substitutions in the cytochrome b gene were identified as mediating the resistance (87,173). The QoI fungicides have the ability to inhibit both spore germination and host penetration, but have little or no effect once the fungus has penetrated or colonized host tissue; hence they are most effective if applied prior to infection (45). Strobilurin fungicides have locally systemic, translaminar movement,

where some active ingredients can leak through the leaf and bind to the cuticle on other side, this aids in compensating for incomplete spray coverage (206). Most strobilurins have a residual period of approximately 14 to 21 days.

Triazoles are within the demethylation inhibitors (DMI) fungicide group. They target C14-demthylase in sterol biosynthesis in fungi, affecting membrane structure and function, resulting in abnormal growth and eventual death of the fungus (97). They have the ability to inhibit or stop development of infections that are already established at low levels, but have little or no effect on spore germination. Hence, DMIs are the fungicide of choice if SBR is established at low levels in a field. DMIs are locally systemic with a residual period of approximately 14 days. While DMIs also have a single site of inhibition, risk of development of resistance is medium due to resistance being mediated by the accumulation of several independent mutations (95).

Trials in India (162), Africa (100), and South America (121,122) have identified the triazole compounds of flusilazole, difenoconazole, triadimenol, tebuconazole, and tetraconazole, as well as strobilurins and strobilurin mixes of azoxystrobin, pycraclostrobin, pyrcalostrobin and boscalid, and trifloxystrobin and propiconazole as valuable fungicides for managing SBR. Trials conducted in the U.S. in 2005, indicate that Folicur® (tebuconazole), Headline® (pyraclostrobin), Headline SBR® (pyraclostrobin and tebuconazole), Laredo® (myclobutanil), and Stratego® (triflozystrobin and propiconazole) all provided acceptable control of SBR (169). However, Folicur® is no longer labeled for soybean and Headline SBR® is no longer on the market. Current U.S. and international research on the effectiveness of fungicides against SBR continues to support triazole and strobilurin chemistries in reducing

severity and yield loss in treated versus control soybean plots (45,123,130), although in the 2008-2009 growing season in South America, some *P. pachyrhizi* populations showed increasing tolerance to certain fungicides (59).

Limited options are available at present to manage SBR in organic soybean production, although recent research has reported a biological control agent that was effective in delaying disease development and reducing disease severity. Using some organic-approved copper fungicides against SBR have provided greater yields compared to untreated soybean in research trials and silicon amendments that delay disease onset and reduce final area under the disease progress curve have also been identified in research trials (45,99,211). Furthermore, application of saccharin to hydroponically grown soybeans can induce the systemic acquired resistance response of soybean to *P. pachyrhizi* (185). *Simplicillium lanosoniveum* was documented, through scanning electron microscopy, to wrap around urediniospores of *P. pachyrhizi* and colonize uredinia (210). Although *S. lanosoniveum* requires SBR to be present in some form on soybean leaves in order to colonize, it was effective in detached leaf assays and field trials in reducing disease severity of SBR (211). Additional research is still needed to evaluate the effects of sunlight on survival and colonization of leaf surface by *S. lanosoniveum* and the development of formulations with extended shelf life (211). Further research also is needed to investigate silicon application timings and combinations of silicon amendments with other fungicides or organic approved products, to manage SBR in organic soybean production (99).

Breeding for resistance is an important management strategy against any plant pathogen, and *P. pachyrhizi* is no exception. Planting resistant cultivars of soybean still

remains the most economical way to manage SBR, although due to the pathogens ability to move long distances by windborne urediniospores and evolve quickly, race shifts in regional *P. pachyrhizi* populations may occur rapidly (67,155). Such an example was observed in South America when resistance genes, *Rpp1* and *Rpp3*, in the Brazilian cultivar 'FT-2' were overcome within 2 years (175). Resistance to SBR in soybean is discussed in more detail in the following paragraphs. While using cultivar mixtures can reduce disease severity due to the epidemiological effect of host diversity, as Caldwell & Myer (27) observed mixtures of soybean varieties relative to pure stands restricted the spread of a pathogenic form of cyst nematode. There is no documented research on soybean cultivar mixtures to manage SBR.

Resistance to Soybean Rust in Soybean

Three lesion types have been observed on soybean, i) tan lesions (TAN) which have a shorter latent period, many uredinia, and abundant sporulation, ii) reddish-brown lesions (RB) which have a longer latent period, fewer uredinia and reduced sporulation, and iii) no visible uredinia; representing i) susceptible, ii) resistant, and iii) immune reactions (16,20,21). The formation of the visible reddish-brown lesions is suggestive of a hypersensitive-like response. Five genes mediating resistance to SBR have been identified at five independent loci, identified as "*Resistance to P. pachyrhizi*" (*Rpp*): *Rpp1* (112), *Rpp2* (21), *Rpp3* (22,69), *Rpp4* (68), and *Rpp5* (54). *Rpp1* – *Rpp4* were reported in the 1980s (21,22,68,69,112), while *Rpp5* was discovered in 2008 (54). *Rpp1* is the only *Rpp* gene that provides immunity to certain isolates of *P. pachyrhizi*, where no visible symptoms occur. The other *Rpp* genes provide resistance responses characterized by limited fungal growth and sporulation through the formation of RB

lesions when challenged with incompatible fungal isolates (21). None of the known *Rpp* genes provide resistance against all isolates of *P. pachyrhizi* (67,118).

Rpp1 and *Rpp4* have been mapped to two different loci on chromosome 18 (75,175), *Rpp2* to chromosome 16 (175), *Rpp3* to chromosome 6 (76), and *Rpp5* to chromosome 3 (54). Other resistant alleles have been mapped to the vicinities of the previously mentioned loci, including *Rpp?*(Hyuuga) to *Rpp3* locus (127), and *Rpp1-b* to the *Rpp1* locus (37). With *Rpp5*, resistance was dominant in plant introduction (PI) 200487 and PI 200526, incompletely dominant in PI 471904, and recessive in PI 200456 (54). Furthermore, a recessive resistance allele at or near the *Rpp2* locus in PI 224270 was reported also (54). Mapping of *Rpp* genes offers breeders the opportunity to pyramid two or more *Rpp* genes to obtain broader and/or more durable SBR resistance that may delay and/or reduce SBR growth and sporulation (148).

Of the five *Rpp* genes identified to date, extensive studies have been conducted on *Rpp2* and *Rpp4*. In microarray analyses of *Rpp2*, it was observed that expression of basal defense pathway genes increased during the first 12 hours after inoculation, returned to mock levels before a second phase of differential gene expression occurred in both susceptible and resistant (*Rpp2*) plants. The second phase of differential expression was stronger and detected 1 to 2 days earlier in the resistant (*Rpp2*) plants than in the susceptible plants (204). *Rpp2* through *Rpp5* display similar resistant phenotypes (RB lesions) suggesting that *Rpp3* through *Rpp5* mediate responses similar to *Rpp2* and are likely governed by disease resistance genes that mediate gene-for-gene recognition (117).

In 2009, the *Rpp4* gene was identified as a member of the coiled-coiled (CC) - nucleotide binding site (NBS) - Leucine rich repeat (LRR) family of disease resistance genes based on significant sequence similarity to other CC-NBS-LRR genes, in particular the lettuce *Resistant Gene Candidate2 (RGC2)* (117). The NBS-LRR gene family accounts for the largest number of known disease resistance genes, and is one of the largest gene families in plant genomes. NBS-LRR are identified by their unique sequence motifs. In plants, LRR motifs have been found in a large number of proteins involved in both disease resistance and development and provides a potential binding surface for protein-protein interactions (89,98). The probable function of NBS regions is to interact with ATP or GTP and act as a signaling molecule (196). In general, sources of resistance to SBR in soybean are relatively rare possibly due to the cost of maintaining resistance genes when no pathogen is present overriding the benefits of resistance during pathogen attack (117). Also, the loss of resistance genes by selection may explain their rarity in soybean. The multitude of alternative leguminous hosts may be potential sources with an abundance of resistance mechanisms to SBR.

Kudzu

Physiology

Kudzu (*Pueraria* spp.) has been identified as the only known host that could provide large amounts of living tissue for overwintering of SBR in the southeastern U.S. (83). With economic loss caused by SBR in the U.S. depending largely on whether or not *P. pachyrhizi* can survive winters in the absence of soybean, kudzu is of great importance in understanding and predicting SBR epidemics in the U.S. In addition to being important as an initial inoculum source of urediniospores, kudzu could also be a source of selection pressure on the pathogen and/or a source of resistance to the

pathogen. Kudzu is a perennial semi-woody, climbing leguminous vine, belonging to the tribe Phaseoleae Benth., subtribe Glycininae Benth. (203). There are approximately 17 species in the genus *Pueraria* recognized globally (203) and different scientific names are often used in literature to refer to kudzu in the U.S., including *Pueraria montana* (Lour.) Merr., *Pueraria lobata* (Willd.) Ohwi., *Pueraria thomsoni* (Benth) Maesen, or *Pueraria montana* var. *lobata* (Willd.) Maesen and Almeida (209). Lobed leaflets and the size of wing and keel petals have been used to differentiate *P. lobata* from *P. montana* and *P. thomsoni*, but all these morphological characteristics can be quite variable; furthermore, possible hybrids exist between these related *Pueraria* species (203).Based on investigation by Ward (209), the widespread taxon, if treated at varietal rank, must bear the name *Pueraria montana* (Lour.) Merr. var. lobata (Willd.) Maesen & Almeida.

Geographic Distribution

Kudzu is considered native to Indo-China, Korea, Japan, Malaysia, Oceania, and the Indian subcontinent (189,203). Successful introductions of kudzu have occurred in South America, Switzerland, Queensland, New South Wales, Australia, and United States. It is only considered a serious pest in the southeastern U.S. (23). It was first introduced into the U.S. in 1876 at the Centennial Exposition as an ornamental (189). Estimates of kudzu infestation in the southeastern U.S. vary from 2 million acres (~ 800,000 hectares) to 7 million acres (~ 3,000,000) (34,48). Its closest relative in the continental U.S. is soybean (*Glycine max*) (23).

History in U.S.

In 1907, kudzu was promoted as a drought-resistant, high-nitrogen forage crop exhibition at Jamestown, Virginia. One farmer in Chipley, Florida was so enthralled with

the growth potential of kudzu that he grew 35 acres of it to sell as a fodder crop and sold rooted cuttings through the mail (23). By the 1920-30s, kudzu was propagated and promoted by the Soil Conservation Service as a means of holding soil on eroding gullies of the deforested southern landscape, predominantly in the southern states of Alabama, Georgia, and Mississippi (23). More than 1.2 million acres (485 thousand hectares) were planted under the Soil Erosion Service, which paid farmers, a onetime payment of $8.00 per acre (~$20 per hectare) planted in kudzu. Kudzu seedling and nurseries produced and distributed more than 73 million seedlings between 1936 and 1941 (23). Kudzu is now estimated to cover between 2 and 7 million acres (0.81 and 2.8 million hectares) of the U.S. primarily in the southeast (23). Present spread of kudzu is slow through local movement of infested soil.

Eventually, farmers found drawbacks to kudzu including difficulty baling the kudzu hay and the tendency for it to grow so rapidly and extensively that it covered all other vegetation. In the 1950s, kudzu was recognized as a weed and removed from the list of species acceptable for use under the Agricultural Conservation Program. Finally, in 1998 Congress placed kudzu on the federal list of noxious weeds. Economic impact of kudzu is estimated at \$48 $acre^{-1}$ $year^{-1}$ (\$118 ha^{-1} $year^{-1}$) for infested forest land which has lost productivity and control costs by power companies alone at $1.5 million per year (23).

Reproduction

Kudzu produces purple flowers with a sweet aroma in relative abundance. Its corolla is papilionaceous and 14-20 mm long. Kudzu can reproduce sexually through insect pollination and asexually through rhizome spread. It has been estimated that seed production by kudzu in the U.S. varies from 0 to 1,800 seeds per m^2 soil surface,

with higher values occurring where vines are climbing on structures (195). However, kudzu seeds have extremely low viability demonstrated to be the result of arthropod damage, mostly due to feeding by native Hemiptera (195). Kudzu seedlings develop woody root crown, with multiple runners and extensive tuberous roots, which contain carbohydrate reserves that enable the plant to survive repeated mowing and herbicide application.

Management

While no cultural or chemical controls exist for kudzu, some biological control agents native to the U.S. have been identified, but have limited success in controlling the weed. The bacterium *Pseudomonas syringae* pv. *phaseolicola* has been reported to kill eight to ten week old kudzu seedlings, but produced few, if any, secondary infections under dry conditions in the field (230). The fungus *Myrothecium verrucaria* provided 95 to 100% control of kudzu in field tests within 14 days of inoculation by girdling runner stems with the fungus. Although, it can affect a number of important crops, ornamentals, and weeds, representing 6 different families, a patent for kudzu control has been applied for using this fungus (19). Also, an isolate of the fungus *Colletrotrichum gloeosporioides* was collected from kudzu in Houston County, Georgia and grown on Czapek Cox medium amended with kudzu extract to increase virulence on kudzu. Field inoculations showed a synergistic effect was achieved by a mixture of spores with 20% of the organochlorine compound dicamba. The fungus attacks both leaves and vines (51). Despite these studies, an effective and efficient control measure for kudzu is yet to be discovered.

Species Variation

In 2005, inter-simple sequence repeat (ISSR) analysis was used to investigate the genetic variation of *Pueraria lobata* and closely related species *P. montana*, *P. thomsoni*, *P. edulis*, and *P. phaseoloides* (189). A total of 260 kudzu samples were collected both from China and 19 states of the U.S. The Shannon diversity indices of *P. lobata* for Chinese and U.S. samples were 0.208 and 0.221, respectively (189). Such similar diversity indices is contradictory to most cases of plant colonization, because when invasive species colonize a new area, genetic variation is often lower than in the source population due to frequent founder effects (161,199). Furthermore, *P. lobata* samples from the U.S. had high genetic diversity and low population differentiation (189). This supports the theory that in the U.S. multiple introductions from multiple sources, with subsequent gene exchange among different regions or sources of kudzu occurred, resulting in high genetic diversity and low genetic differentiation.

Reactions to Soybean Rust

Previous studies have reported kudzu exhibiting the same reactions to *P. pachyrhizi* as soybean: TAN, RB, and no visible symptoms (17,82). Contrary to soybean reactions, RB lesions were more common (50% frequency) when evaluating 125 kudzu plants with 3 different *P. pachyrhizi* isolates (17). Furthermore, in 64% of instances where multiple plants from a site were tested, each reacted the same to the individual pathogen isolate, suggesting a tendency for plants at specific sites to be genetically identical with respect to rust reaction (17). Only 15% of individual plants produced a different reaction to one isolate than to the other two isolates suggesting that susceptibility or resistance to *P. pachyrhizi* in individual kudzu sites is often broad, extending over multiple isolates of *P. pachyrhizi*.

Further characterization of kudzu resistance to *P. pachyrhizi* was conducted in Florida in 2008 (82). An evaluation conducted on 139 kudzu sites in north Florida resulted in 25 sites free of SBR infection and 32 sites with reduced sporulation (82). Ten accessions of kudzu from north-central Florida were examined in detached and attached leaf assays with a single isolate of *P. pachyrhizi* under controlled laboratory conditions. Of the 10 accessions examined, 6 were susceptible, 3 were immune, and 1 was resistant. Resistant interactions had early onset of a multicell hypersensitive response (HR), while immune interactions were the result of a cell wall deposition that blocked penetration in combination with early onset of HR (82). Furthermore, using quantitative real-time polymerase chain reaction 15 days after inoculation, resistant kudzu had 10-fold less *P. pachyrhizi* DNA as compared to susceptible kudzu and *P. pachyrhizi* DNA was below detection level in immune kudzu (82). Susceptible kudzu had approximately half the amount of *P. pachyrhizi* DNA present when compared with susceptible soybean cultivar (82).

Summary

With the introduction of SBR to the continental U.S., environmental factors previously identified to affect SBR in other areas of the world need to be examined in the soybean fields of the U.S. to determine major environmental factors that would drive the epidemic in the U.S. Furthermore, the unique relationship of SBR on the overwintering, alternative host, kudzu needs further investigation to examine possible resistance genes in the alternative host which would indicate selection pressure on the pathogen due to resistant populations. Potential initial inoculum produced on the alternative host is also of great importance in understanding SBR epidemics in the U.S. and needs further quantification and modeling. Lastly, knowledge of the environmental

factors influencing SBR epidemics in north Florida and potential initial inoculum can be utilized to develop a disease and decision model for fungicide application for SBR. Based on the previous listed needs the following objectives were created.

Research Objectives

The objectives of the present research are: 1) to determine environmental and other site specific variables that influence SBR epidemics in soybean in north Florida, 2) to determine molecular attributes in resistant kudzu accessions, 3) to estimate potential initial inoculum from kudzu populations in Florida to better characterize SBR epidemics in Florida, and 4) to evaluate a spray forecasting model for SBR in Florida. To achieve objective 1, data from soybean sentinel plots and corresponding environmental data were analyzed to identify climatic and environmental factors important to SBR epidemics in soybean in north Florida (Chapter 2). Furthermore, field experiments were conducted where shading was manipulated in soybean fields and disease development and environmental factors were recorded and analyzed; and detached leaf assays analyzed disease development without the influence of environmental factors on disease development (Chapter 3). The identification of candidate resistant genes to *P. pachyrhizi* in the alternative host kudzu (Chapter 3) was performed to achieve objective 2. To achieve objective 3, historical infection data of kudzu populations were utilized and restriction fragment length polymorphisms (RFLPs) and detached leaf assays were carried out to identify and quantify potential inoculum sources from kudzu populations in Florida (Chapter 4). Examining the use of different parameters for a spray forecasting model based on previous studies and potential initial inoculum (Chapter 5) was conducted to achieve objective 4.

CHAPTER 2
EPIDEMIOLOGY OF SOYBEAN RUST IN SOYBEAN SENTINEL PLOTS IN FLORIDA

Introduction

Soybean rust (SBR), caused by the fungus *Phakopsora pachyrhizi*, is one of the most destructive diseases to soybean production and is monitored in the eastern United States by a series of coordinated sentinel plots. Since SBR was discovered in the southeast United States in 2004 (167), its distribution and severity has been variable from year to year. This study examined data from sentinel plots in north Florida and corresponding weather conditions to help understand this variability.

Del Ponte et al. (40) highlighted the importance of precipitation influencing SBR epidemics, especially for regions where temperature is not a limiting factor for disease development, such as Florida. Examples of the importance of precipitation on SBR development include: severe epidemics in high-rainfall areas of the lower Richmond Valley in Australia (187), yield losses of up to 100% during rainy seasons and only 10 to 15% losses during a dry season in Thailand (163), and suppressed disease development in Brazil and Paraguay during moderate to severe droughts in mid to late growing season (224). The objective of this study was to examine environmental and climatic factors that affected SBR development within the soybean sentinel plots of north Florida. It was hypothesized that disease onset and development would be highly correlated with moisture values calculated from the Florida Automated Weather Network. Results from this study may lead to a better understanding of the impact of weather on the epidemiology of this disease.

Materials and Methods

Sentinel Plot Data

Beginning in 2005, a national soybean sentinel plot program was established through the Integrated Pest Management – Pest Information Platform for Extension and Education (ipm-PIPE, sbr.ipmpipe.org) (80), to which the data for this study are reported. Data were utilized from forty-six soybean sentinel plots (SSP) from 2005 through 2008 from 12 different counties in north Florida. Location of the SSPs was dependent upon agronomic land availability in the counties and when possible were planted in the same location each year. SSPs that did not get planted within 4.8 km of the previous year's planting were from the following counties and years: Escambia 2007, Santa Rosa 2006, Okaloosa 2007 & 2008; Walton 2006 & 2008; Holmes 2006; Jackson 2008; Jefferson 2006; Madison 2006; Suwannee 2008; Columbia 2008 (Figure 2-1). Seven to 11 different SSPs became infected with SBR each year and were used for analysis. On average, SSPs were planted in a 225 m^2 area with 25 cm row spacing. All locations across the years were planted in March or April (Julian date 83-119) to detect SBR at the earliest date it developed in the county. Two different maturity group 3 (MG3) varieties, 2 different MG5 varieties, and 3 different MG7 varieties were used in SSPs, from 2005 through 2008, (Table 2-1). All varieties used are susceptible to SBR. The SSPs were managed using standard production practices for Florida (217) and were not irrigated.

Data Collection

Each SSP was scouted bi-weekly and then scouted weekly for SBR after one of the following conditions occurred: first bloom of soybean (growth stage R1) (113), presence of SBR in the surrounding counties, forecast or spore deposition data

suggested increased risk, or environmental conditions became conducive to SBR development. Scouting consisted of arbitrarily collecting 100 leaflets from each maturity group, so each site had a total of 300 leaflets collected per sampling date. Leaflets collected from each maturity group were placed in 3 separate plastic bags, stored at room temperature (~ 25 C) and evaluated within 1 to 7 days. Maturity group data were recorded individually each year, except in 2005 and SSP data from Santa Rosa County in 2006 where maturity group data were compiled together and reported. As such 2005 data and Santa Rosa County data from 2006 were omitted when analyzing maturity group data.

Disease severity was determined using a dissecting microscope for symptomatic leaf area on individual leaflets. An arbitrary rating scale was used with the following values and corresponding severity ranges based on a visual key developed by Bayer Crop Science (Kansas City, Kansas), 0 = no disease, 1 = up to 2.5%, 2 = 2.5-5%, 3 = 5-10%, 4 = 10-15%, 5 = 15-25%, 6 = 25-35%, 7 = 35-67.5%, and 8 = 67.5-100% of the leaf area affected by SBR. The following mid-point rating was used to create severity values, i.e., 0=0%, 1=1.25%, 2=3.75%, 3=7.5%, 4=12.5%, 5=20%, 6=30%, 7=51.25%, and 8=83.75%. Incidence and severity data for each individual MG was averaged for each sampling date, and this average was used to calculate weekly and yearly averages. Maximum incidence and severity values for each SSP were averaged to determine yearly maximum incidence and severity, regardless of date (Table 2-1). Incidence and severity values were aligned chronologically and used to obtain the area under the disease progress curve (AUDPC) (Table 2-1, Figure 2-2). The previous year's maximum incidence and severity data used was in reference to the year being

analyzed (i.e. when analyzing Jefferson SSP 2006 maximum incidence data, the previous year's maximum incidence – Jefferson SSP 2005 maximum incidence data was used). SSP 2005 data did not have any previous year's maximum incidence and maximum severity to use in analysis (i.e. no data was recorded in 2004). In the circumstance where a SSP did not become infected, 0 was used for that year's maximum incidence and severity. In such a case if a SSP did not become infected until 2007 there would only be previous year's maximum incidence and severity data for 2008. In the event the SSP was lost due to poor germination or deer damage these data were omitted.

The duration of the epidemic was calculated from the week of first detection to the week of maximum severity (Table 2-2). Average incidence and severity were plotted over time (Figure 2-2) and the AUDPC was calculated using Equation 2-1(205).

$$AUDPC = \sum_{i=1}^{n} \frac{(x_{i+1} + x_i)}{2}(t_{t+1} - t_i) \qquad (2\text{-}1)$$

where x_i = proportion of tissue affected (disease severity) at the *i*th observation, t = time (days), and n = total number of observations. $\sum$ is the sum of areas of all of the individual trapezoids or areas from i to $n - 1$. i and $i+1$ represent observations from 1 to n.

Julian days were used for date calculations, where January 1st is Julian day 1. Yearly disease incidence and severity averages, discussed previously, were used to calculate the AUDPC for each year. AUDPC was calculated through the second week in September each year based on natural senescence of plots (Figure 2-2).

The apparent infection rate per week for each year was calculated from weekly severity averages from the week of detection to the week of maximum severity, these

were also averaged together to get average infection rate per year for the duration of the epidemic (Table 2-1). Apparent infection rate was calculated using Equation 2-2 (146).

$$r = \left(\frac{1}{t_1 - t_2}\right) \ln\left(\frac{x_2(1-x_1)}{x_1(1-x_2)}\right) \quad (2\text{-}2)$$

Where t is time during which infection has occurred, x is disease severity and "ln" is the natural logarithm. Disease severity at time $t_1=x_1$ (disease severity at first disease detection) and disease severity at time $t_2=x_2$ (disease severity when maximum severity reached).

Six climatic time periods were used for analysis: 2, 3, and 4 weeks before disease detection (including first week of detection) and 4, 6, and 8 weeks before maximum incidence and severity. These time periods were used to correlate preceding environmental conditions with first disease detection, maximum incidence, and maximum severity. The Florida Automatic Weather Network stations (FAWN) were utilized to obtain environmental data collected from five counties in the Florida Panhandle (Escambia, Jackson, Gadsden, Jefferson, and Suwannee) (Figure 2-1).Weekly average temperatures were calculated from the daily average air temperatures from FAWN stations, recorded at 60 cm above the soil. Similarly, the weekly average relative humidity (RH %), solar radiation (watts per square meter), and precipitation (mm) was calculated from FAWN stations at 2 m above soil level, and soil temperature taken at 10 cm below soil surface. These weekly averages were used to create the yearly averages presented in Figure 2-2. The dew point was calculated from the daily average air temperature and RH using Equation 2-3 (10).

$$T_d = \frac{b * \alpha(T, RH)}{a * \alpha(T, RH)} \quad (2\text{-}3)$$

Where *α (T, RH) = (a*T)/(b+T) +ln (RH),* where T is the temperatures in degrees Celsius, RH is the measured relative humidity, T_d is the calculated dew point temperature (°C) and "ln" refers to the natural logarithm. The constants are: a = 17.27 and b = 237.7 (°C). The uncertainty in the measured dew point temperature is a function of the measured temperature and relative humidity and the uncertainties associated with those measurements. The uncertainty in the calculated dew point temperature is ±0.4° C. This expression is based on the "Magnus" (or "Magnus-Tetens") approximation for the saturation vapor pressure of water in air as a function of temperature (10). It is considered valid for 0 °C < T < 60 °C; 1% < RH < 100%; 0 °C < Td < 50 °C.

Dew point temperatures were then compared with the hourly average air temperature and hours where the difference between the two variables was 0 to 2 were counted as dew leaf wetness hours for daily calculations (55). Daily leaf wetness hours were averaged over the different time periods. The number of days with >0 mm, >1 mm, and >5 mm of precipitation and cumulative precipitation were recorded for each time period in question using FAWN data. All variables utilized in this study are listed in Table 2-2.

Data Analysis

Statistical analyses were performed using SAS version 9.1 (SAS Institute, Inc., Cary, NC). The PROC CORR procedure of SAS was used to conduct Pearson's correlation test. Correlations were examined between first detection date of SBR and weekly average air temperature, soil temperature, relative humidity, solar radiation,

precipitation, daily leaf wetness hours, number of days with precipitation >0mm, >1mm, and >5mm, the number of years a site was previously infected, and sites' previous maximum incidence and severity. Each site's first disease detection date, in each year, was correlated with climatic variables averaged over 2, 3, and 4 weeks prior to detection date (including week of detection). Pearson's correlation test was also used to examine the correlations between SBR maximum incidence and severity (percentage), first detection date, weekly average air temperature, soil temperature, relative humidity, solar radiation, precipitation, daily leaf wetness hours, number of days with precipitation >0mm, >1mm, and >5mm, the number of years a site was previously infected, and site's previous maximum incidence and severity. Maximum incidence and severity for each site and year was correlated with climatic variables averaged over 4, 6, and 8 weeks prior to a site's maximum incidence and severity.

Results

All correlations were significant with a *p*-value less than 0.05. Significant correlations between climatic variables and first detection date of SBR, maximum incidence and maximum severity are listed in Tables 2-3, 2-4, and 2-5, variables not listed were nonsignficant. Heavy rainfall (> 80 mm) occurred during 2005 and 2008 with earlier disease detection and higher maximum incidences and severities. In contrast, dry to drought conditions (< 80 mm of total rain) occurred during 2006 and 2007, with later disease detection, and lower maximum incidences and severities (Figure 2-2). Disease duration (time period from first detection to maximum severity) increased from 2005 through 2008, while area under the disease progress curve (AUDPC), first disease detection, maximum incidence and severity varied between years (Table 2-1). Five sentinel plots in 2005 and one in 2007 and 2008 were lost due to poor germination or

deer damage before infection could occur, and in 2006 three sentinel plots never became infected. The majority of SSPs (33 plots) did not become infected with SBR until growth stage R4 or later; however, 2 SSPs were infected by growth stage R2 and 1 SSP was infected by growth stage R3 (113) (Figure 2-3). In SSPs from 2006 through 2008, SBR infections were first discovered in MG3, MG5, MG7 a total of 4, 17, and 14 times, respectively (Table 2-6).

First disease detection was significantly correlated with the number of years a site had been infected (-0.35), previous year's maximum incidence (-0.49), planting date (-0.34), and maximum incidence and severity (-0.66 and -0.44, respectively) (Table 2-3). Environmental variables to which first disease detection were negatively correlated with included the number of days with greater than 0 mm and 1 mm precipitation (-0.39 and -0.49, respectively), cumulative solar radiation (-0.54), and average leaf wetness (-0.53) during the 4-week time period prior to first disease detection (Table 2-3). Temperature and relative humidity did not have a significant correlation with first disease detection.

Maximum disease incidence significantly decreased 23%, 17%, and increased 59% in 2006, 2007, and 2008, respectively, in comparison with maximum incidence of the previous year (Table 2-2). Maximum incidence had a significant positive correlation with average weekly precipitation (0.39) for the 8 week period prior to maximum incidence date (Table 2-4). Maximum severities increased 22%, decreased 64%, and increased 275% in 2006, 2007, and 2008, respectively, in comparison with the maximum severity of the previous year (Table 2-2). Climatic variables that significantly correlated with maximum severity included average weekly precipitation (0.38) and average leaf wetness (0.37) 4 weeks prior to maximum severity date. Cumulative solar

radiation 4 weeks prior to maximum severity date was weakly correlated to maximum severity (p-value 0.0417 and correlation coefficient -0.35) (Table 2-5). Plotting cumulative precipitation (mm) recorded 4 weeks prior to maximum severity date and the maximum severity (%) from 2005 through 2008 revealed a threshold of 300 mm cumulative precipitation (Figure 2-4).

Discussion

This study examined the epidemic development of SBR from 2005 through 2008. Epidemic development was correlated to multiple environmental variables with important trends apparent between the first detection of SBR and the plant descriptor variables of growth stage and maturity group. While SBR can infect soybean at any growth stage, it has been indicated that earlier SBR infections within a growing season increases the chances of yield loss due to higher disease incidence and severity (222). Rarely did SBR infection occur on MG3 soybeans before the other varieties or before the MG3 soybeans reached growth stage R8 (full maturity) (Table 2-6, Figure 2-3). Most often, MG5 and MG7 soybean varieties were first detected with SBR, and at a range of growth stages including R2 (full bloom) through R8 (full maturity) (Table 2-6, Figure 2-3). The majority of SSPs were in growth stage R4 (full pod) or later when SBR was first detected (Figure 2-3), even though MG3 varieties were at reproductive stages earlier in the year. Hence, soybeans did not become infected at earlier growth stages, most likely due to lack of inoculum and/or conducive environmental conditions.

From 2006 through 2008, fluctuating first disease detection was correlated with the previous number of years a site had been infected and the previous year's maximum incidence, suggesting that accumulated inoculum could contribute to first disease detection. While first disease detection was negatively correlated with planting date,

this might be attributed to other factors over time, such as accumulated inoculum, in that the planting dates were slightly later each year (Table 2-2). First disease detection data also were correlated strongly with maximum incidence and severity, which can be expected, because with earlier disease onset greater maximum incidence and severity can be achieved and at an earlier date.

The environmental variables most strongly correlated to SBR first detection were precipitation of greater than 1 mm, cumulative solar radiation intensity, and average leaf wetness calculated over the 4 week period prior to SBR detection (Table 2-3). Although temperature and relative humidity were not directly correlated with first disease detection, the significant correlations of calculated leaf wetness provide an indirect correlation of these variables. The observed correlations with first disease detection imply days with greater than 1 mm of precipitation, increased leaf wetness hours, and decreased solar radiation promote earlier disease onset. This concurs with reports by Marchetti et al. (109), Melching et al. (114), and Narvaez et al. (133) that increased moisture, increased SBR disease intensity. However, the lack of a correlation between first disease detection and the number of days with greater than 5 mm of precipitation may be explained by the observation that subsequent rainfall for a 30 minute period can remove up to 91% of urediniospores from soybean leaves (46). Furthermore, the correlation between first disease detection and solar radiation coincides with Isard et al. (78) report of the proportion of *P. pachyrhizi* spores that germinate decrease with increasing exposure to solar radiation.

Environmental conditions, such as periods of high moisture and low solar radiation, can occur throughout the soybean growing season in Florida during

hurricanes and tropical storms. For example, on July 10th of 2005 Hurricane Dennis made landfall on Santa Rosa Island in north Florida, and resulted in substantial amounts of precipitation (max = 118 mm) across the north Florida area. The environmental conditions created by Hurricane Dennis could account for the 2005 average disease detection date of August 19 (Julian day 231), being earlier than the following years of 2006 and 2007, which had dry to drought conditions. Notably, precipitation is a readily available variable throughout many regions across the U.S. and the correlation of first disease detection with precipitation can be used to determine areas that will become infected after possible spore deposition events.

Del Ponte et al. (40) demonstrated that accumulated precipitation from the following month that SBR was initially detected could be used as a predictor for final disease severity. Similarly, in this study we found maximum incidence and severity correlated with average precipitation prior to maximum incidence and severity dates. In particular, maximum incidence correlated with average precipitation over 8 weeks prior to maximum incidence date (Table 2-4), whereas maximum severity was most significantly correlated to the average precipitation over 4 weeks prior to maximum severity date (Table 2-5). The positive correlation of maximum incidence with precipitation over an 8-week time period suggests that incidence is influenced more than maximum severity by precipitation events occurring prior to disease onset. Hurricane Dennis's precipitation may account for not only the early disease onset, but also a greater average maximum incidence in 2005 as compared to 2006 and 2007. In addition, the greatest maximum incidence and severity averages in 2008 may be attributed to the environmental conditions resulting from Tropical Storm Fay, which

traveled across north Florida August 17 – 24 and accounted for the peak of average precipitation during the third week in August (Julian day 230) (Figure 2-2). The greatest maximum severity and precipitation occurred in 2008, indicating that rain, after initial infection, can contribute to the severity of a SBR epidemic in north Florida and that relatively dry to drought conditions, observed in 2006 and 2007, can reduce the maximum severity reached during a growing season (Figure 2-2).

Accumulated precipitation 4 weeks prior to maximum severity displays a threshold of 300 mm cumulative precipitation (Figure 2-4); where average maximum severity was 20.8% and 9.8% if precipitation was more and less than 300 mm, respectively. Similarly, Del Ponte et al. (40) found thresholds in rainfall correlating to SBR severity in Brazil during the 1-month period after disease detection, where severity was >70% and <30% if rainfall ranged between 250-459 mm and 10-125 mm, respectively.

Compounding factors contributed to fluctuating first disease detection, maximum incidences and severities from 2005 to 2008 in north Florida soybean sentinel plots. These factors include, but are not limited to, varying levels of initial inoculum, leaf wetness hours, and amount of precipitation before and after infection. Correlations between climatic variables and maximum incidence and severity imply certain environmental conditions are favorable for SBR and may allow the pathosystem to reach a maximum impact earlier in the season. Furthermore, correlation between early disease detection and early maximum incidence and severity reached may lead to earlier available inoculum contributing to rust epidemics in the major soybean regions in the U.S. This study indicates that improved prediction of SBR epidemic severity and understanding of the variability of epidemics from year to year in the United States can

be gained from continued research and monitoring of SBR in the southeastern United States as we have yet to see the maximum potential of a SBR epidemic.

Table 2-1. Data summary of north Florida soybean sentinel plots (SSP) and the soybean rust epidemic data within those plots from 2005 through 2008

	2005	2006	2007	2008
Variety Planted*	MG3-DP 3861RR MG5-DP 5915RR MG7-DP 7870RR	MG3-HS 3456RR MG5-DP 5915RR MG7-DP 7220RR	MG3-HS 3861RR MG5-DP 5915RR MG7-DP 7220RR	MG3-DP 3861RR MG5-AG 5905RR MG7-DP 7330RR
Total number of (SSP)	12	10	12	12
Number SSP infected	7	7	11	11
AUDPC[a] (SEV[b])	146	39	20	204
AUDPC[a] (INC[c])	19	4	4	21
Plant date in Julian day	83	88	94	99
First disease detection in Julian day	231	258	243	216
Days after planting disease was detected in Julian day	148	170	148	115
Duration (days)	24	33	34	58
Avg. max. SEV[b] (%)	9	11	4	15
Avg. max. INC[c] (%)	82	64	52	83
Avg. apparent Infection rate	0.22	0.11	0.17	0.24

[a] AUDPC = area under disease progress curve.
[b] SEV = disease severity.
[c] INC = disease incidence.
* Abbreviations for varieties planted include, MG = maturity group, RR = roundup ready, DP = Delta and Pine Land seed, HS = Hyland Seed, AG = Asgrow seed.

Table 2-2. A description of variables. Variables were used to examine the relationship between soybean rust first disease detection, maximum disease incidence, severity, and environmental conditions in north Florida soybean sentinel plots from 2005 through 2008.

Variable	Description
Onset	First disease detection date
MaxIncid	Maximum disease incidence (%) reached at a particular site and year
MaxSev	Maximum disease severity (%) reached at a particular site and year
Planting date	Date planting occurred
PrevYRMaxSev	Previous year's maximum disease severity (%)
PrevYRMaxIncid	Previous year's maximum disease incidence (%)
NumyrInf	Number of years site was previously infected
InfectRate	Infection rate calculated using initial and maximum disease severity
AvgRain*	Average precipitation (mm)
RD>0mm*	Number of days with precipitation >0mm
RD>1mm*	Number of days with precipitation >1mm
RD>5mm*	Number of days with precipitation >5mm
SolRad*	Cumulative solar radiation (W/m^2)
AvgTemp*	Average air temperature at 60 cm height
AvgSoilTemp*	Average soil temperature at 10cm depth
RH*	Average relative humidity
LeafWetAvg*	Average of leaf wetness hours

*Each variable was determined for each of the six time periods of climate examined: 2, 3, and 4 weeks before first disease detection (including first week of detection) and 4, 6, and 8 weeks before maximum incidence and severity was achieved.

Table 2-3. Variables correlated with first disease detection date. Variables correlated, using Pearson's correlation coefficient, with first disease detection of soybean rust during the growing seasons of 2005 through 2008 in soybean sentinel plots in north Florida.

	Onset[a]	
Variable	P-value	Correlation coefficient
MaxIncid[b]	<0.0001	-0.66
MaxSev[c]	0.0080	-0.44
Planting date	0.0434	-0.34
PrevYRMaxIncid[d]	0.0048	-0.49
NumyrInf[e]	0.0381	-0.35
RD>0mm4wks[f]	0.0193	-0.39
RD>1mm4wks[g*]	0.0026	-0.49
SolRad 2 wks[h]	0.0025	-0.49
SolRad 3 wks[i]	0.0007	-0.54
SolRad 4 wks[j*]	0.0006	-0.54
LeafWetAvg 4wks[k*]	0.0009	-0.53

[a] Onset = First disease detection date.
[b] MaxIncid = Maximum disease severity (%) reached at a particular site and year.
[c] MaxSev = Maximum disease severity (%) reached at a particular site and year.
[d] PrevYRMaxIncid = Previous year's maximum disease incidence (%).
[e] NumyrInf = Number of years site was previously infected.
[f] RD>0mm4wks = Number of days with precipitation >0mm, over 4 weeks prior to first disease detection date.
[g] RD>1mm4wks = Number of days with precipitation >1mm, over 4 weeks prior to first disease detection date.
[h] SolRad 2 wks = Cumulative solar radiation (W/m^2), over 2 weeks prior to first disease detection date.
[i] SolRad 3 wks = Cumulative solar radiation (W/m^2), over 3 weeks prior to first disease detection date.
[j] SolRad 4 wks = Cumulative solar radiation (W/m^2), over 4 weeks prior to first disease detection date.
[k] LeafWetAvg 4wks = Average of leaf wetness hours, over 4 weeks prior to first disease detection date.
[*] Most significant climatic variables correlated.

Table 2-4. Variables correlated with maximum incidence. Variables correlated, using Pearson's correlation coefficient, with maximum incidence of soybean rust during the growing seasons of 2005 through 2008 in soybean sentinel plots in north Florida.

	Maximum Incidence	
Variables	P-value	Correlation coefficient
MaxSev[a]	<0.0001	0.70
AvgRain 8wks[b]	0.0196	0.39

[a] MaxSev = Maximum disease severity (%) reached at a particular site and year.
[b] AvgRain8wks = Average precipitation (mm), over 8 weeks prior to maximum incidence date.

Table 2-5. Variables correlated with maximum severity. Variables correlated, using Pearson's correlation coefficient, with maximum severity of soybean rust during the growing seasons of 2005 through 2008 in soybean sentinel plots in north Florida.

	Maximum Severity	
Variables	P-value	Correlation coefficient
MaxIncid[a]	<0.0001	0.70
AvgRain 4 wks[b]	0.0256	0.38
SolRad 4 wks[c]	0.0417	-0.35
LeafWetAvg 4 wks[d]	0.0266	0.37

[a] MaxIncid = Maximum disease incidence (%) reached at a particular site and year
[b] AvgRain 4 wks = Average precipitation (mm), over 4 weeks prior to maximum severity date.
[c] SolRad 4 wks = Cumulative solar radiation (W/m^2), over 4 weeks prior to maximum severity date.
[d] LeafWetAvg 4 wks = Average of leaf wetness hours, over 4 weeks prior to maximum severity date.

Table 2-6. The number of soybean sentinel plots (SSP) in which a maturity group was first detected with soybean rust (SBR). Data from 2006, 2007, and 2008 SSP.

	Maturity group first detected with SBR			
	MG3	MG5	MG7	Total Number of diseased SSP/year
2006	1	4[a]	4[a]	6[d]
2007	2[b]	4[b]	7[b]	11
2008	1	9[c]	3[c]	11
Total	4	17	14	

[a] At 3 SSP, both MG5 and MG7 were detected with SBR at the same date.
[b] Only 6 of the 7 SSPs in 2006 had maturity group data reported and used in this study.
[c] At 1 SSP all 3 maturity groups were detected with SBR at the same date.
[d] At 2 SSPs both MG5 and MG7 were detected with SBR at the same date.

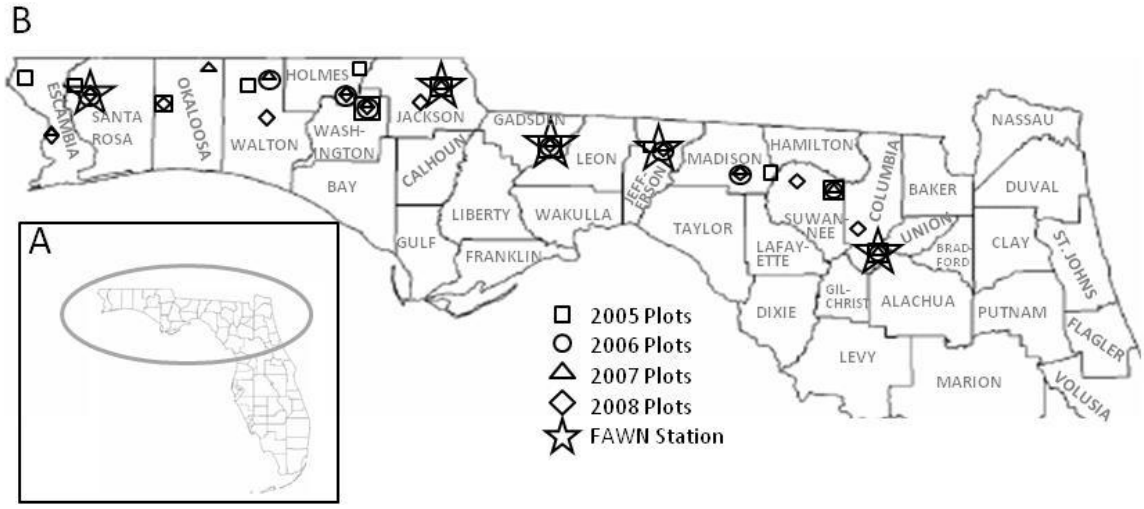

Figure 2-1. Florida map of soybean sentinel plots and Florida Automated Weather Network stations. A, Florida map indicating, with the circle, the study area of north Florida. B, Enlarged area of north Florida with approximate locations of soybean sentinel plots from 2005 – 2008 used to monitor soybean rust and Florida Automated Weather Network (FAWN) stations that were used to obtain environmental data utilized in this study.

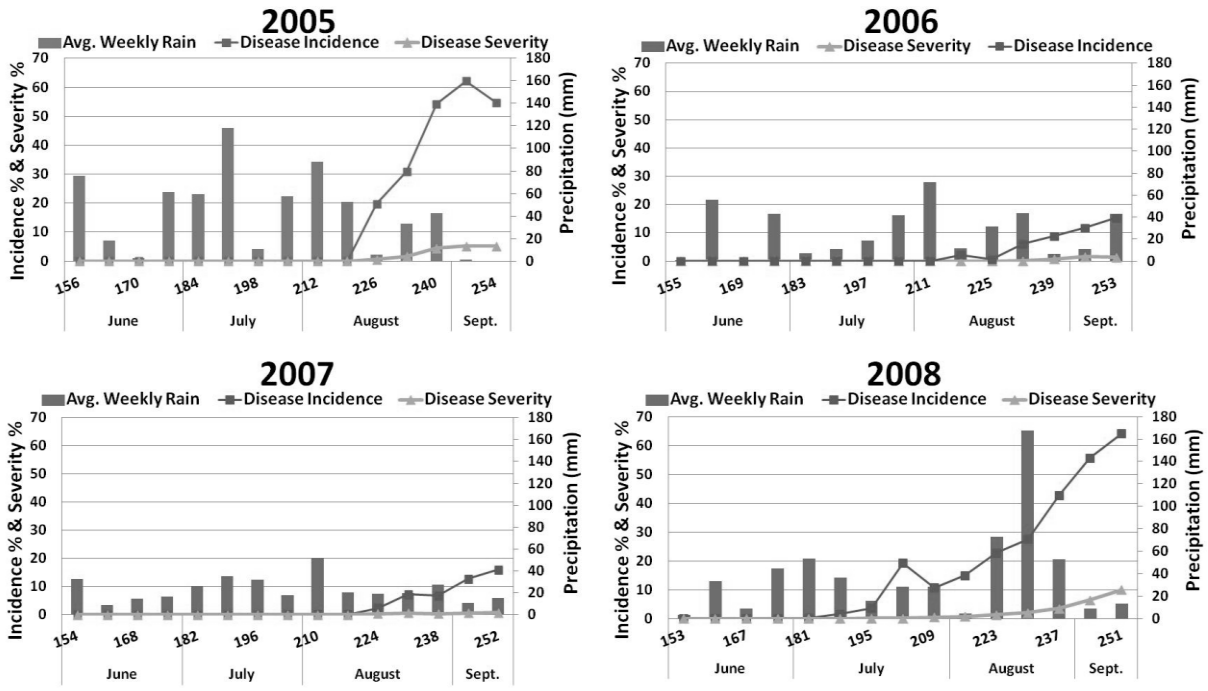

Figure 2-2. Weekly cumulative rain and disease incidence and severity over time. The disease incidence (square symbol, primary y-axis) and severity (triangle symbol, primary y-axis) of soybean rust, and weekly cumulative rain (bars, secondary y-axis) were averaged across soybean sentinel plot locations in north Florida and plotted over time (in Julian days, x-axis) during the seasons of 2005 through 2008.

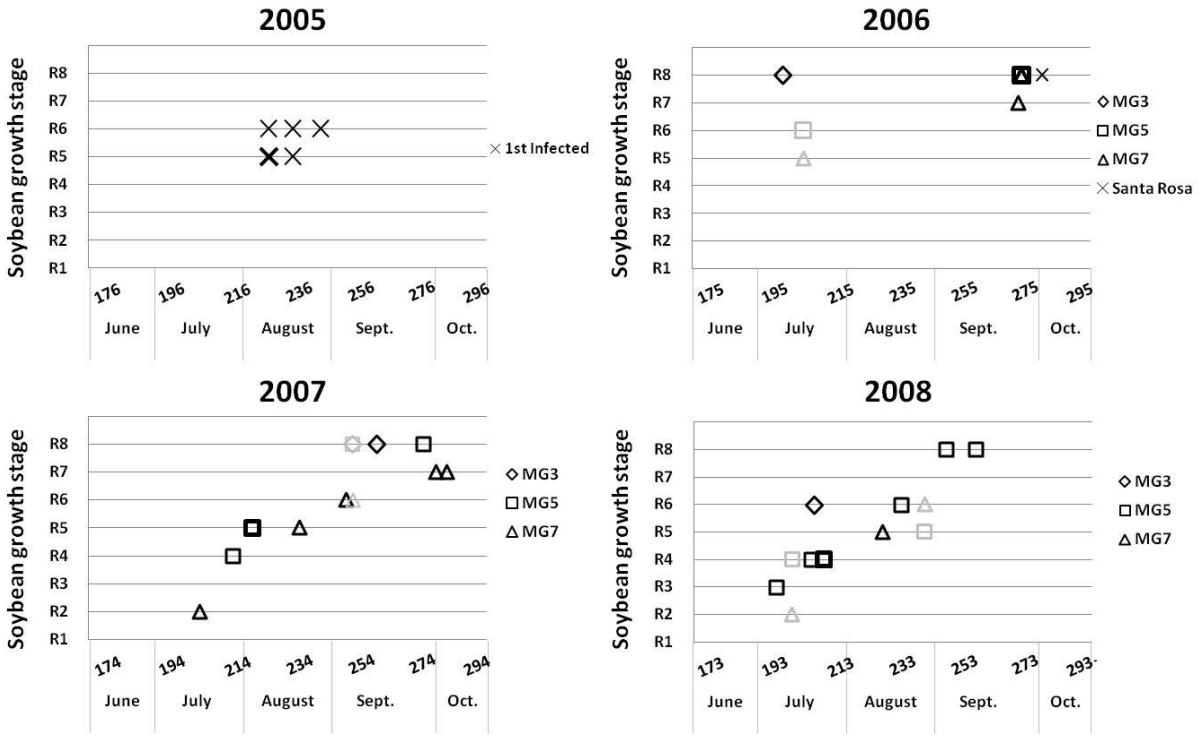

Figure 2-3. Soybean growth stage and maturity group (MG) data over time. Soybean growth stage and maturity group (MG) data are plotted over time (in Julian days) for each soybean sentinel plot's (SSP) first detection of soybean rust (SBR) during the seasons of 2005 through 2008. Soybean MG was not recorded for 2005 SSPs or Santa Rosa SSP in 2006, only soybean growth stage is reported in this figure for these data. In 2005, 3 of the 7 SSPs were first detected with SBR at growth stage R5 on 15 August (Julian day 227). The bolded "x" on the 2005 graph represents those 3 SSP data. In 2006, 1 out of 7 SSPs were first detected with SBR on MG5 and MG7 on 27 July (Julian date 208) at growth stages R6 and R5, respectively. The grayed "square" and "triangle" on the 2006 graph represents that 1 SSP datum. Two other SSP in 2006 were first detected with SBR on MG5 and MG7 on 28 September (Julian date 271) all at growth stage R8; and 1 other SSP was first detected with SBR on 28 September (Julian ate 271) at growth stage R8. The bolded "square" and the "triangle" within that square on the 2006 graph represents these 3 SSP data. In 2007, 2 out of 11 SSPs were first detected with SBR on MG5 on 8 August (Julian date 220), both at growth stage R5. The bolded "square" on the 2007 graph represents those 2 SSP data. One other SSP in 2007 was first detected with SBR on MG3, MG5, and MG7 on 7 September (Julian date 250) at growth stages R8, R8, and R6, respectively. The grayed "square" with the grayed "diamond" within it and the grayed "triangle" directly below them on the 2007 graph represents that SSP datum. In 2008, 1 out of 11 SSPs were first detected with SBR on MG5 and MG7 on 8 July (Julian date 190) at growth stages R4 and R2, respectively. Another SSP in 2008 was first detected with SBR on MG5 and MG7 on 27 August

(Julian date 240) at growth stages R5 and R6, respectively. The grayed squares and grayed triangles aligned vertically on the 2008 graph represent these SSP data. Two other SSP in 2008 were first detected with SBR on MG5 on 20 July (Julian date 202) at growth stage R4. The bolded square on the 2008 graph represents those 2 SSP data. Figure 2-3. Continued.

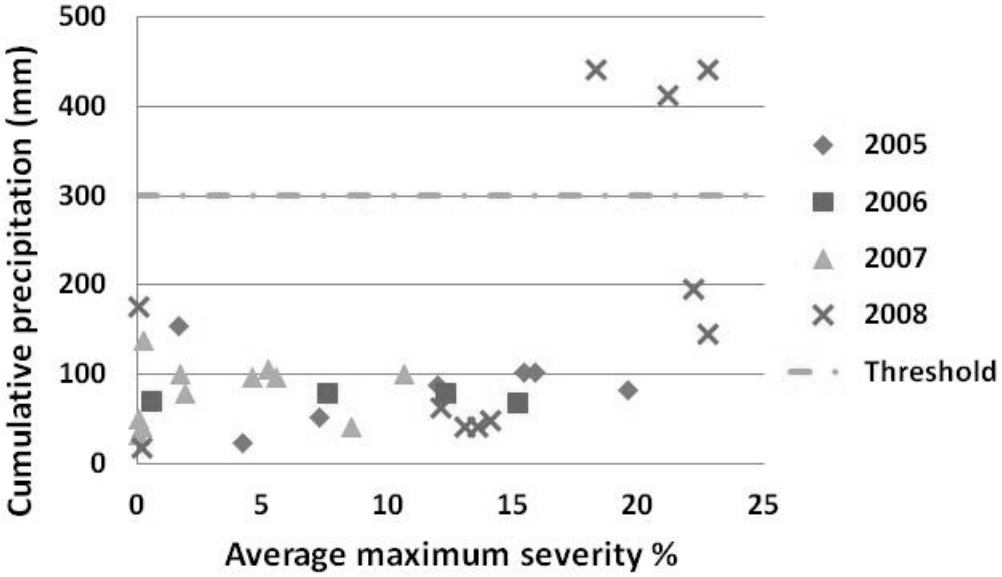

Figure 2-4. Precipitation threshold for average maximum disease severity of soybean rust in soybean sentinel plots (SSP) recorded from 2005 through 2008. Symbols represent each SSP average maximum disease severity, as a percentage, reached each year and cumulative precipitation (mm) over the 4 weeks prior to each site reaching maximum disease severity each year (different symbols representing each year); and the line is the threshold at 300 mm of precipitation.

CHAPTER 3
EFFECT OF SOLAR RADIATION ON SEVERITY OF SOYBEAN RUST

Introduction

Soybean rust (SBR), caused by Phakopsora pachyrhizi Syd. and P. Syd., has the potential to be a damaging fungal disease of soybean (Glycine max (L.) Merr). SBR often is first detected in low light environments and regularly progresses from the lower to upper soybean canopy. Favorable meteorological and environmental conditions such as temperature, relative humidity and rainy days with prolonged cloudiness are critical components that affect the progression of disease development (38).

Identification and quantification of the key factors that drive a SBR epidemic are critical components for assessing and managing SBR risks. Such studies provide a rational basis to improve disease management and subsequently prevent yield loss. There is very little quantitative information on the effect of environmental and meteorological conditions in the field when compared to the body of literature from growth chamber and greenhouse studies.

Since long-distance dispersal of SBR urediniospores has been confirmed (94), there has been a surge in new epidemiological studies for SBR to accurately predict and manage the disease across a large geographical area. As a consequence, several disease models have been developed that integrate meteorological data such as wind speed, wind direction, and solar radiation as predictors to assess the risk of the disease at various geographical scales (40,79,142).

While airborne, the survival of fungal spores is affected by temperature, solar radiation, ultraviolet radiation, and relative humidity (79). Pathogenicity of fungal spores, in the course of spores long-distance aerial spread, is dependent on spore survival

during variation in temperature, relative humidity, and solar radiation over the time that they are suspended in the atmosphere and after landing on a susceptible host. Solar radiation has been demonstrated to have the potential to increase canopy temperature, reduce relative humidity (RH), and reduce SBR urediniospore survival (79), but limited field information is available on how solar radiation triggers physiological changes in the structure of the soybean plant canopy, which might also influence SBR development. This information could be another important factor for accurate predictions using aerobiological models for SBR forecasting (79,142).

Components of solar radiation (UVB radiation; 280 to 320 nm) can affect crop growth directly through photosynthesis and photomorphogenic systems, up regulation of pathways producing defense compounds (e.g., flavonoids and related phenolic compounds or waxes), decreased vegetative growth, and decreased developmental times. In contrast, the UVB (280-320 nm) component of solar radiation also has a range of effects on invading pathogens that include damage to DNA and proteins thus affecting metabolic and physiologic responses of the organism (7). Ghajar et al. (58) and Rotem and Aust (159) reported that UV radiations in sunlight, and not temperature, are the major factor affecting germination of fungal propagules. The extent of damage caused by biotic factors (pest, pathogens and weeds) on growth and development of crop plants can be modified by abiotic factors such as solar radiation, possibly making the plants more or less susceptible to pest pressure (84).

Soybean leaves from canopies exposed to solar UVB showed significantly higher levels of soluble phenolics and lower levels of lignin than leaves that developed in canopies covered by polyester films (227). Canopy shading can reduce solar radiation

and impact the microclimate affecting plant physiology including cuticular wax. Experimental and observational data reports show that high radiant energy, low humidity, and higher temperatures (23 to 30 °C) are favorable conditions for maximum wax production (9,33,177). Once the canopy closure is 100%, creating a completely shaded area between rows, the entire exposed upper canopy begins to act as a more uniform evaporative surface diminishing the differences in shading of the soil surface and variation in heat from soil between rows due to reflection of radiation (181).

Recently, the effects of sunlight (both total solar and ultraviolet radiation) on urediniospore survival have been used to predict P. pachyrhizi urediniospore germination (78). The recommendations, based on the findings of Isard et al. (78), have been included in USDA's aerobiological model for SBR epidemiology. While field experiments on influence of total solar radiation on P. pachyrhizi urediniospore germination have been conducted by Isard et al. (78), these factors have not been studied under laboratory conditions in the absence of other environmental parameters that may otherwise confound the correlation between them and urediniospore survival and viability. The objectives of this study were: to investigate the effect of solar radiation on progress of SBR in the field and to evaluate the effect of solar radiation on epicuticular wax content of soybean and if it is correlated to disease susceptibility.

Material and Methods

Canopy Manipulation

To investigate the effect of solar radiation on the progress of SBR, four soybean plantings were established at the North Florida Research and Education Center (NFREC) in Quincy, Florida. Early plantings occurred on 11 April 2008 and 24 April 2009 and late plantings on 4 August 2008 and 23 July 2009; henceforth designated as

early or *late* plantings, respectively. All plantings were in the same field. Asgrow cv. 5905 Roundup Ready, maturity group 5 soybeans were planted on 76 cm rows, over a total area of 15,600 square meters, on average. Three 3 m x 3 m square structures of black knitted shade cloth (Rimol Greenhouse Systems, Inc. Hooksett, NH) were constructed at least 4.6 m apart from each other and had a minimum of 4.6 m soybean boundary around the perimeter. Control plots without shade cloth with the same area and parameters were also established. The shade cloth attenuated 30, 40, or 60% sunlight as stated by the manufacturer and determined by Optronic OL754 spectroradiometer (Optronic Inc., Orlando, FL) measurements of energy values (W m^{-2}) under each shade cloth in field plots (Table 3-1). All plantings had 3 replicates (total of 12 plots). The shade cloth structures were constructed over the soybeans at vegetative growth stage V3-V4 (113) in a complete randomized block design. Subsequently, leaves were marked on 3 individual plants in each plot, starting at growth stage V3, to be utilized for leaf assays.

Plant height was measured at reproductive growth stage R3 in the early and late planting of 2008 in all treatments. At reproductive growth stage R3 there is little to no further increase in height in determinant soybeans and hence this stage was chosen to measure the overall effect the treatments had on plant height. Data log tags (MicroDAQ.com, Ltd. Contoocook, NH) were used to record temperature and relative humidity every 20 minutes in the middle of each plot at approximately 36 cm above the soil for the duration of the experiment. The weeks during reproductive growth stage R3-R4 (pod growth) of each planting were selected as typical examples of temperature and relative humidity observed throughout the growing season and critical time periods for

disease development. Leaf area indices (LAI) were obtained for early and late plantings of soybeans in 2008 on 9 July and 3 October at reproductive growth stage R3and for the late planting in 2009 on 11 October at reproductive growth stage R6 by measuring photosynthetically active radiation (PAR; 400 to 700 nm) approximately 10 to 20 cm above the top of the canopy and 25-30 cm below the top of the canopy using a LI-COR 191 quantum sensor and LI-1400 Data Logger (LI-COR Biosciences; Lincoln, NE). All measurements were taken between 11:00 AM and 2:00 PM (EDT) (UTC 16:00 – 19:00). The following equation was used to calculate LAI from the PAR values: $LAI = -(1/k) \ln(Q_b/Q_a)$ (214), where Q_a is the above canopy PAR, and Q_b is the below canopy PAR. The extinction coefficient, k, of 0.4 was used based on Flenet et al. (52) study on row spacing effects on extinction coefficients of soybean. Average light transmissions at the upper soybean canopies in plots covered by the 30, 40, and 60% shade material were measured using an Optronic OL754 spectroradiometer (Optronic Inc., Orlando, FL) measuring energy values (Wm^{-2}) between 270 and 775 nm (Table 3-1). Similar measurements were also made in the lower, middle, and upper canopies of control plots (Table 3-2). All light transmission measurements were taken on 28 September, 2009.

Field Inoculations and Evaluations

Urediniospores used for inoculation were collected the previous week from soybeans (Asgrow cv. 5905 Roundup Ready, maturity group 5) infected with a population of urediniospores collected from naturally infected soybean at the North Florida Research and Education Center in Quincy, Florida the previous year. Diseased plants were maintained in a greenhouse and urediniospores were collected using a cyclone spore collector (G-R manufacturing Co., Manhattan, KS) from the underside of infected leaves and stored in a 10 ml glass vial at -20 °C until spore suspension was

made. The early plantings of 2008 and 2009 were inoculated at growth stage R1 with a suspension of approximately 75,000 urediniospores per ml of water and 10 μl of Tween 20 between 6 and 8 p.m. Eastern Standard Time. Inoculation occurred in the evening to ensure all plots were exposed to similar environmental conditions for inoculation and to enhance probability of infection. Late plantings were naturally infected with SBR with no addition of inoculum. Germination of urediniospore was assessed by plating 100 μl of urediniospore suspension on 1% water agar and recording the number of germinated urediniospores out of 100. A spore was considered germinated if the germ tube was twice as long as the spore's length. Approximately 0.5 liters of the spore suspension was applied to the lower canopy in each plot using a hand-held spray bottle. Weekly evaluations were made, starting 10 to 14 days after inoculation for early plantings or for late plantings at growth stage R1 (flowering) because natural SBR infection usually occurs at the beginning of soybean reproductive stages. Ten randomly pre-assigned areas in each plot were evaluated at the lower, middle, and upper canopies for severity of SBR based on visual assessment of symptomatic leaf area using the scale, 0= no disease, 1 = up to 2.5%, 2 = 2.5-5%, 3 = 5-10%, 4 = 10-15%, 5 = 15-25%, 6 = 25-35%, 7 = 35-67.5%, and 8 = 67.5-100% of the leaf area affected by SBR. Average severity rating was determined by converting each rating to the midpoint of its range, i.e., 0=0%, 1=1.25%, 2=3.75%, 3=7.5%, 4=12.5%, 5=20%, 6=30%, 7=51.25%, and 8=83.75% (225). The lower, middle, and upper canopy included plant area from nodes 1 to 3, 3 to 6, and 6 to 10, respectively. In the early plantings, the lower, middle, and upper canopy were designated between 10 and 20 cm, 20 to 40 cm, and 40 to 70 cm from the ground, respectively. In the late plantings, the lower, middle, and upper canopy were

designated between 10 to 30 cm, 30 to 60 cm, and 60 to 100 cm from the ground, respectively.

Detached Leaf Assays

At growth stage R1 (flowering)(113), two pre-marked leaflets each from the lower, middle, and upper canopies, as previously described, were collected for leaf assays to assess the amount of epicuticular wax and susceptibility of leaves to SBR. An additional leaflet was collected from the lower canopy of each plot to verify SBR was not present in the field at that time. The detached leaflets were rinsed with sterile distilled water and allowed to dry. One set of leaflets was immediately used to assess susceptibility and the other set was placed in resalable plastic bags and placed in a freezer at -20 °C for later analysis of epicuticular wax. Leaflets collected from both early plantings and the late planting in 2008 were used in susceptibility assays and leaflets from the early planting in 2009 and both late plantings were used in epicuticular wax assays.

For the susceptibility assay the detached leaflets were inoculated with urediniospores of *P. pachyrhizi* which were collected from diseased soybeans in a greenhouse the previous day and kept in frozen storage (approximately -20 °C) until use. A suspension of 15,000 urediniospores per mL of water with 10 μl of Tween 20 was used to inoculate the upper and lower surface of each leaflet using approximately 3 mL of suspension per leaflet by using a KC-566CG air sprayer (Lowes; North Wilkesboro, NC). The suspension was also sprayed on a plate of 1 % water agar and germination was assessed as previously described. After inoculation, the petiole of each leaflet was cut diagonally with a scalpel to produce a clean, fresh surface. Each leaflet was then placed in an individual Petri plate (150 x 15 mm) half-filled with 1% water agar

with the petiole inserted into the agar. Petri plates were kept at room temperature (approximately 24°C) and exposed to approximately 30% of natural sunlight for 8 hours per day. Leaflets were evaluated 10 to 14 days after inoculation using the rating scale previously given. Non inoculated control leaflets from the lower canopy were also maintained in Petri plates half-filled with 1% water agar and evaluated along with inoculated leaflets.

The other set of leaflets were removed from the freezer and allowed to thaw 2 to 3 months later. Epicuticular wax was then measured using the following modified procedures from Beattie and Marcell (12). Individual leaflets were submerged in 10 mL of chloroform in pre-weighed aluminum foil boats for 30 s. The waxes were dried by placing the foil boats on a hotplate (approximately 150 °C) until chloroform had evaporated. After an hour of incubation at room temperature the foil boats were weighed. The area of each leaflet was obtained using the LI-COR 3000 portable area meter with LI-COR 3000 transparent conveyer belt accessory (LI-COR Biosciences; Lincoln, NE). The amount of wax extracted from a leaflet was expressed in mg of wax per cm^2 of leaf area.

Data Analysis

Statistical analyses were performed using SAS 9.1 (Statistical Analysis Software version 9.1, SAS Institute Inc., Cary, NC). Temperature and relative humidity were averaged across repetitions and 7 days soybean was in reproductive growth stage R3-R4 (pod development) each planting, resulting with data in 20 minute intervals over a 24 hour period typical of temperature and relative humidity observed throughout the growing season. These data were analyzed by the general linear model procedure and

Fisher's least significant difference test at a significant level of 0.05. Disease severity recorded at growth stage R5 was averaged across each planting date at each canopy level and treatment. Disease severity recorded in field experiments at growth stage R5, severity of detached leaves, and epicuticular wax from detached leaves were analyzed by two-factor analysis of variance (ANOVA) with interaction. Factors used as main effects were shade treatment, canopy height, and plant date. Fisher's least significant difference test at a significant level of 0.05 was used for multiple comparisons among treatments and canopy height by means of the general linear model procedure of SAS when significant differences were found in the analysis of variance table. Significant correlations were determined using Pearson's correlation test.

Results

At growth stage R3, in the early planting (11 April) of 2008, plant height under 40 and 60% shade were significantly greater than those under 0 and 30% shade, whereas in the late planting (4 August) of 2008 there was no significant difference in plant height among treatments (Figure 3-1). Furthermore, plant height from the early planting of 2008 was significantly greater than plant height from the late planting of 2008. Leaf area indices from the early planting of 2008, with the exception of LAI from 60% shaded soybean, were significantly greater than LAI from the late plantings in 2008 and 2009 (Figure 3-2). There were no significant differences in LAI across treatments from the 2008 plantings, however LAI from the 2009 planting non-shaded and 30% shaded treatments had significantly greater LAI than that from 40 and 60% shaded treatments. The two-way ANOVA of the disease severity at reproductive stage R5 showed a significant effect of the shade treatments (F = 10.78, P = 0.0001) and canopy heights (F

= 67.97, $P < 0.0001$), but not of their interaction ($F = 8.06$, $P = 0.339$). Plant date, as expected, also had a significant effect ($F = 8.06$, $P = 0.002$).

Temperature was significantly affected by shade treatments ($F = 8.80$, $P < 0.0001$), although only temperature in the non-shaded treatment was significantly greater than the shaded treatments (Figure 3-3). Temperatures ranged from 20 to 30 °C throughout typical days of the growing season, where morning and evening temperatures were within 10 to 28.5 °C. Relative humidity was not significantly affected by shade treatments ($F = 2.00$, $P = 0.11$) and ranged from 76 to 100% throughout typical days of the growing season and had an average of 93% across all treatments. UVB, UVA, and PAR values, when converted to a percentage of the upper canopy energy values, were affected approximately in proportion to the percent shade (Figure 3-4 and Table 3-1).

In both inoculated and naturally infected plots, disease was first detected in the lower canopy and progressed upwards (Figure 3-5). Final disease severity, recorded at growth stage R5, was significantly greater in the lower canopy of all treatments and in the middle canopy of 40 and 60% shade treatments (Figure 3-6). In the middle canopy, the final disease severity in the non-shaded and 30% shade treatments were significantly lower from the 40 and 60% shade treatments. In the upper canopy, the final disease severity in the 40 and 60% shade treatments were significantly greater than the non-shaded treatments.

Similar to severity results from the field, the greatest disease severity was observed on leaves collected from the lower canopy of all treatments (Figure 3-7). Disease severity on leaves collected from the lower canopy was significantly greater

then severities observed on leaves from the upper canopy within the same treatment. Amount of epicuticular wax from leaves taken from the upper canopy in non-shaded treatments was significantly greater than all other shaded treatments at all canopy heights, with the exception of wax from the upper canopy in 30% shaded treatments (Figure 3-8). Amount of epicuticular wax was not significantly different across all treatments at the lower and middle canopies and from 40 and 60% shaded treatments at the upper canopy. Only the epicuticular wax and disease severity in the upper canopy, regardless of treatment, had significant negative correlation at $P = 0.009$, $R = 0.84$.

Discussion

The present study examined the effects of solar radiation on the progress of SBR under field and detached leaf conditions and susceptibility of detached leaves developed under different solar radiation intensities. The greater difference of disease severities over time across treatments in the early planting of 2008 as compared to the late plantings (Figure 3-5) may be attributed to increased plant height (Figure 3-1) and LAI (Figure 3-2) across treatments in the early planting. The increase in plant height, LAI and greater differences among treatments in the early planting are most likely due to longer day length allowing solar radiation to have a greater effect on plant physiology and disease development. Furthermore, the early planting of 2008 may have had a more uniform inoculation in the lower canopy, allowing the effect of the shade treatments to be enhanced over time and across the treatments.

Temperatures recorded throughout typical days of the growing season are within the range for SBR development. Morning and evening temperatures are within the range for *P. pachyrhizi* germination and infection of 10 to 28.5 °C (109), and never

reached above 30 °C which can retard disease development (30), suggesting temperature was not a limiting factor in this experiment. Temperature may have caused minor differences in disease development between non-shaded and shaded treatments, but not to differences between shaded treatments where temperature was not significantly different. The high relative humidity recorded throughout typical days of the growing season was within the optimum range for SBR development and was not significantly affected by shade treatments, suggesting relative humidity was not a limiting factor for disease development. Similarly, Batchelor et al. (11) suggested moisture was a nonlimiting factor when adjusting SBR neural network models by the inclusion of a variable for cumulative days when relative humidity exceeded 90% did not improve the accuracy of the model.

Intensity of solar radiation is more likely influencing disease development and final disease severity in the field, especially with the 60% shade treatment where the amount of UVB, UVA, and PAR was reduced to 36, 34, and 32%, respectively, of the initial intensity in the non-shaded treatment (Table 3-1). These reduced intensities are comparable to those in the non-shaded middle canopy (Table 3-2), which might be contributing to the lack of significant difference between the final disease severity of the upper canopy of the 60% shade treatment and the middle canopy of the non-shaded treatment (Figure 3-6). Our data suggests the greater final disease severities in the lower canopy and shaded treatments are most likely due to difference in solar radiation and its direct and indirect effects on disease development. Similarly, Dias et al. (41) concluded that sunlight likely affects post-germination or post-infection processes in SBR contributing to final severity levels.

In addition to the direct effect of solar radiation on disease development in the field, its influence on soybean physiology may have been a contributing factor in the difference in disease severities observed in the detached leaf assays. The amount of epicuticular wax on soybean leaves could affect the pathogen's ability to infect, especially if the pathogen penetrates directly, as *P. pachyrhizi* does. In rice cultivars, infection rates by *Rhizoctonia solani* were negatively correlated with the amount of cuticular wax deposits on the outer sheath surface (107). It was found that the wax deposits interfered with the formation of appressorial and infection cushions of *Rhizoctonia solani* and when wax deposits were removed with chloroform, infection of previously resistant cultivars occurred (107). Previously reported, UVB radiation can affect leaf ultrastructure and anatomy, photosynthetic pigments, UVB absorbing compounds, and growth and development (84). In particular, UVB radiation could affect development of the epicuticular wax layer, which acts as the first line of defense against biotic and abiotic factors.

While there have been reports of environmental stresses affecting the epicuticular wax layer (84), only a few studies (60,186) have reported UVB effects on epicuticular waxes of crop plants. Steinmuller and Tevini (186) reported that enhanced UVB radiation produced 23 and 28% increase in wax content on leaf area in barley and bean, respectively. Another study with six pea genotypes differing in amount of leaf surface wax, showed an increase in wax content when the genotypes were exposed to UVB radiation (60). Additionally, UVB radiation can also alter the chemical composition of leaf surface waxes (186). Production of UV-absorbing compounds such as flavonoids and other phenolics that function as phytoalexins or antifungal compounds have also

been attributed to UVB radiation (85,125). Marta et al. (110) reported that grapevine leaves maintained at 100% sun light had high polyphenolic content and significantly lower downy mildew severity compared to leaves under shading nets. While the upper canopy epicuticular wax amounts were significantly correlated to the disease severity in the detached leaf assays more than just the amount of epicuticular wax could be contributing to disease reduction. Other components of the wax, such as chemical composition, need to be analyzed to further investigate the effect of solar radiation on the epicuticular wax layer and its interaction with *P. pachyrhizi* urediniospores.

Solar radiation could be affecting many aspects of soybean physiology with the amount of epicuticular wax being just one aspect. Similar results where SBR was consistently more prevalent and more severe under shaded conditions (36) and soybean plants kept in the dark up to 16 h following inoculation had greater amount of lesions even though penetration rates remained constant after 8 h of darkness (15) imply sunlight affects SBR development after infection. Solar radiation may elicit defense mechanisms affecting post-infection processes. One possible defense mechanism induced by light is the production of the phytoalexin glyceollin. Greater resistance to *Phytophthora megasperma* f. sp. *glycinea* was reported in soybean seedlings incubated under light for more than 12 h and had a 30 times greater concentration of glyceollin as compared to seedlings incubated in the dark (208). More research needs to be conducted to further identify physiological and biochemical factors affected by solar radiation which might contribute to disease development.

Results from field trials and detached leaf assays indicate that solar radiation influences progress of SBR caused by *P. pachyrhizi* by affecting disease development

across canopy heights and epicuticular wax content. Significant differences in disease severity in upper canopy heights in detached leaf assays correlated well with epicuticular wax values, with wax values decreasing and disease severity increasing as canopy height decreased. Solar radiation has a direct influence on SBR severity, as observed across canopy heights and shade treatments. This study suggests that in order to make useful epidemiological predictions in forecasting models by factoring in the influence of solar radiation on disease progress and severity of SBR, effect of its UV component at given environmental conditions (temperature, relative humidity etc.) cannot be undermined.

Table 3-1. Energy values (W m^{-2}) recorded in 0, 30, 40, and 60% shaded soybean plots. Data recorded on 28 September 2009 at the North Florida Research and Education Center in Quincy, Florida.

	UVB[ab]		UVA[ac]		PAR[ad]	
Shade Treatment (%)	W m^{-2}	Percent of non-shaded treatment	W m^{-2}	Percent of non-shaded treatment	W m^{-2}	Percent of non-shaded treatment
0	3.37	100	47.53	100	349.2	100
30	2.32	69	31.57	66	226.0	65
40	1.84	55	24.66	52	173.7	50
60	1.23	36	16.22	34	111.9	32

[a]First columns for each UV band report raw data (W m^{-2}) recorded using an Optronic OL754 spectroradiometer (Optronic Inc., Orlando, FL) measuring energy values between 270 and 775 nm. Second columns of each UV band represent the UV flux relative to the upper canopy in the full sun (non- shaded) soybeans.
[b]UVB – ultraviolet radiation at 300 to 320 nm
[c]UVA – ultraviolet radiation at 320 to 400 nm
[d]PAR – photosyntetically active radiation at 400 to 700 nm

Table 3-2. Energy values (W m^{-2}) recorded in non-shaded control soybean plots. Data recorded on 28 September 2009 at the North Florida Research and Education Center in Quincy, Florida.

	UVB[ab]		UVA[ac]		PAR[ad]	
Canopy Level	W m^{-2}	Percent of upper canopy	W m^{-2}	Percent of upper canopy	W m^{-2}	Percent of upper canopy
Upper	3.00	100	43.80	100	328.3	100
Middle	1.19	40	10.50	23	78.7	23
Lower	0.34	11	3.26	7	14.9	4

[a] First columns for each UV band report raw data (W m^{-2}) recorded using an Optronic OL754 spectroradiometer (Optronic Inc., Orlando, FL) measuring energy values between 270 to 775 nm. Second columns of each UV band represent the UV flux relative to the upper canopy.
[b]UVB – ultraviolet radiation at 300 to 320 nm
[c]UVA – ultraviolet radiation at 320 to 400 nm
[d]PAR – photosyntetically active radiation at 400 to 700 nm

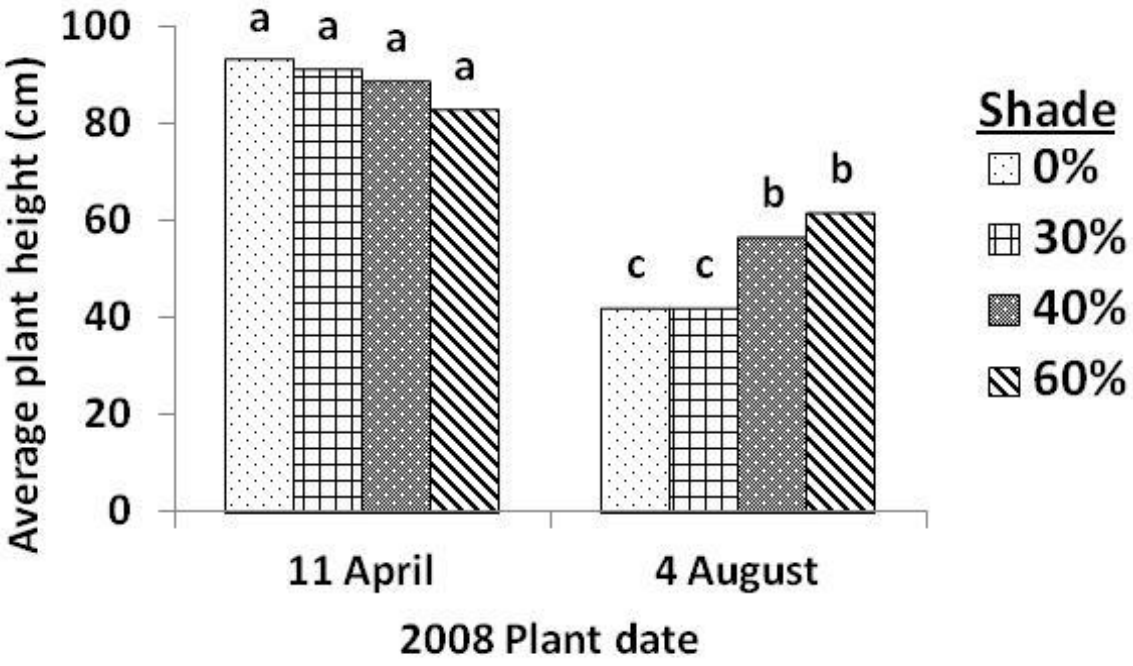

Figure 3-1. Average plant height. Average plant height (cm) was recorded at growth stage R3 for early (11 April) and late (4 August) planted soybeans in 2008, under 0, 30, 40, and 60% shade treatment installed at growth stage V3-V4. Different letters on the bars indicate significant differences between early and late planting dates and among treatments based on averages analyzed by least significant differences at $P \leq 0.05$.

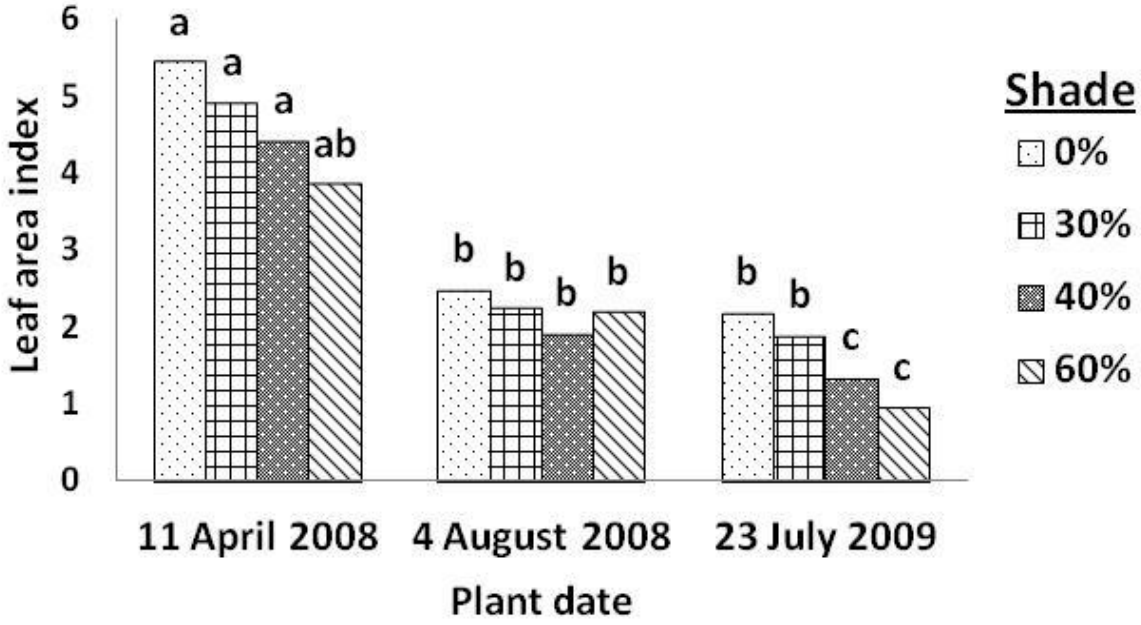

Figure 3-2. Average leaf area indices. Average leaf area indices were measured at reproductive growth stage R3 for early (11 April 2008) and late (4 August 2008) planted soybeans on 9 July 2008 and 3 October 2008, respectively; and at reproductive growth stage R6 for late (23 July 2009) planted soybeans on 11 October 2009 under 0, 30, 40, or 60% shade installed at growth stage V3-V4. Leaf area indices were measured using LI-COR 191 quantum sensor and LI-1400 Data Logger (LI-COR Biosciences; Lincoln, NE). Different letters on the bars indicate significant differences between planting dates and among shade treatments based on averages analyzed by least significant differences at P≤0.05.

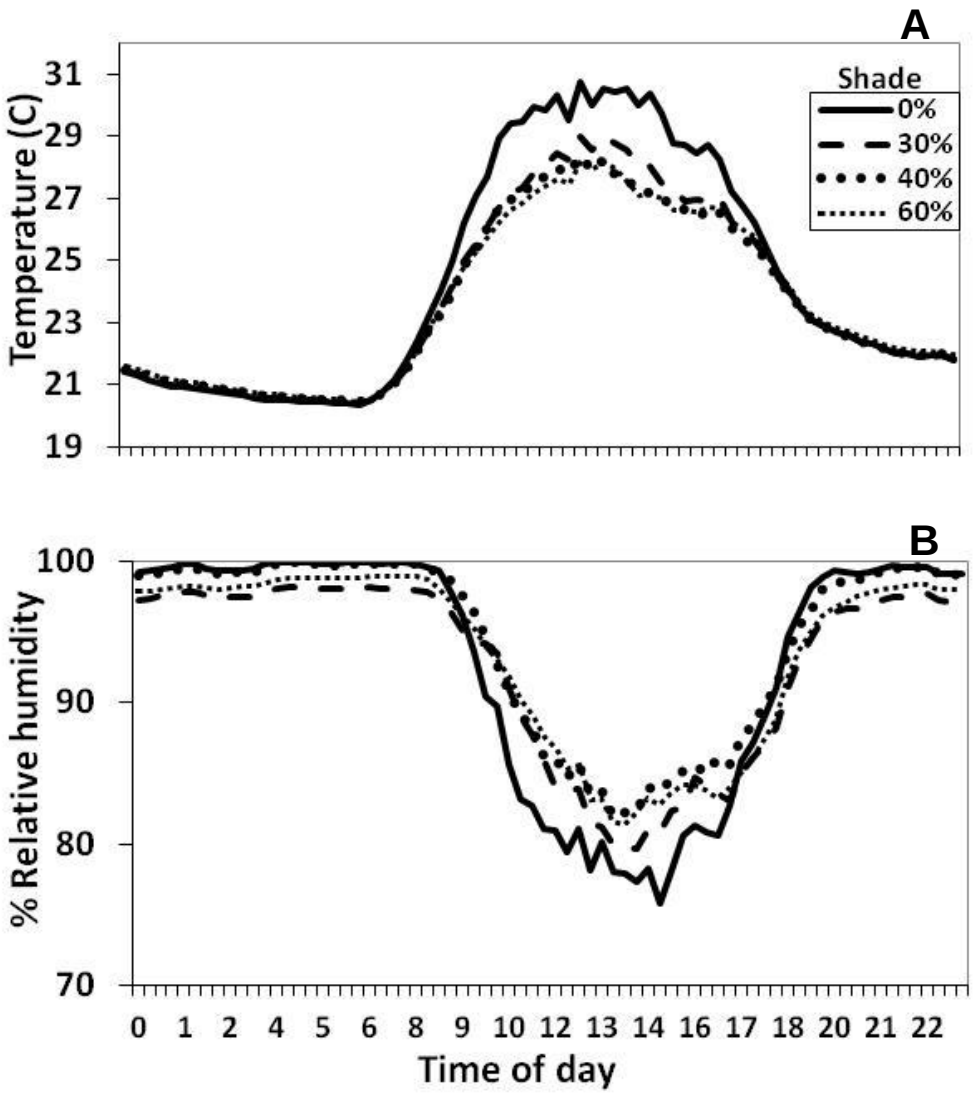

Figure 3-3. Ambient temperature and relative humidity recorded in shade treatments. Ambient temperature (A) and relative humidity (B) were recorded approximately 36 cm above the soil (mid canopy) within soybean rows every 20 minutes in soybeans grown under 0, 30, 40, and 60% shade installed at vegetative growth stage (V3-V4).

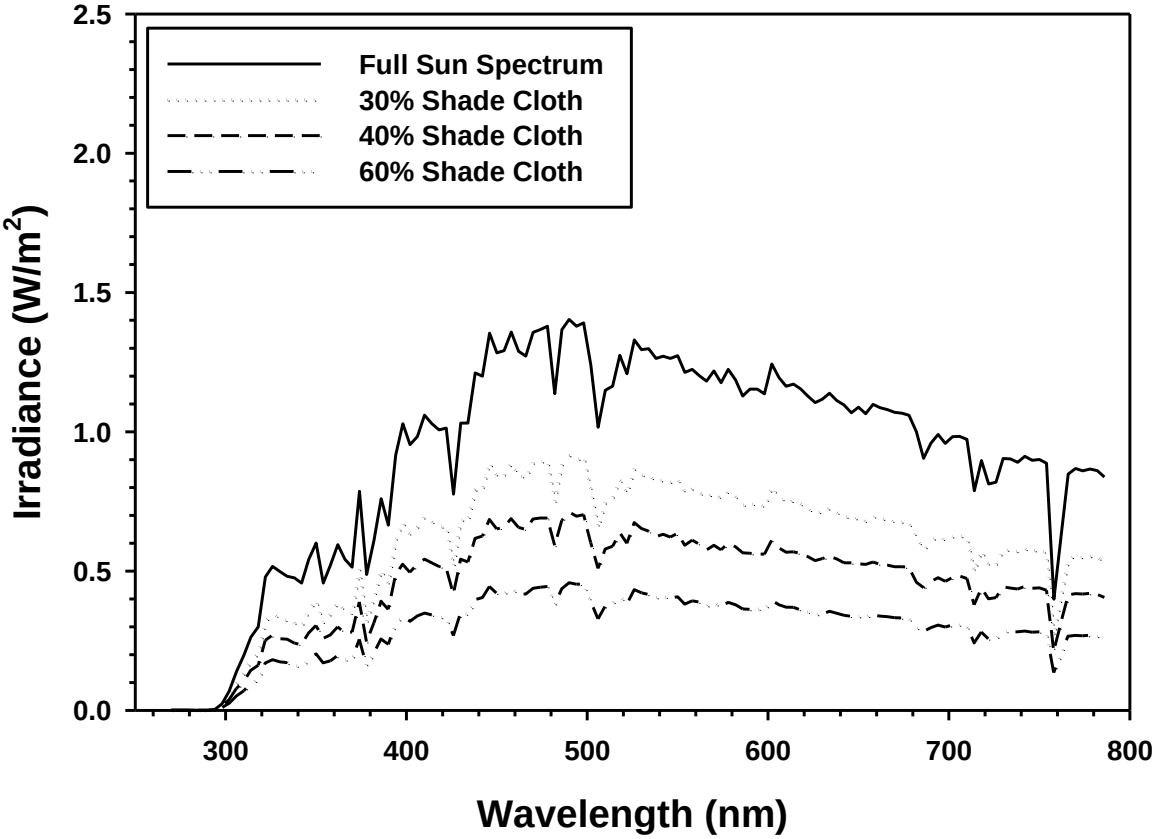

Figure 3-4. Comparative ultraviolet-visible solar spectrum recorded under shade treatments. Comparative ultraviolet-visible solar spectrum (270 to775 nm) of overhead sun at 2:00 PM EDT was measured under 0, 30, 40, and 60% shade plot treatments on 28 September 2009. The spectrum was obtained using an Optronic OL754 spectroradiometer (Optronic Inc., Orlando, FL)

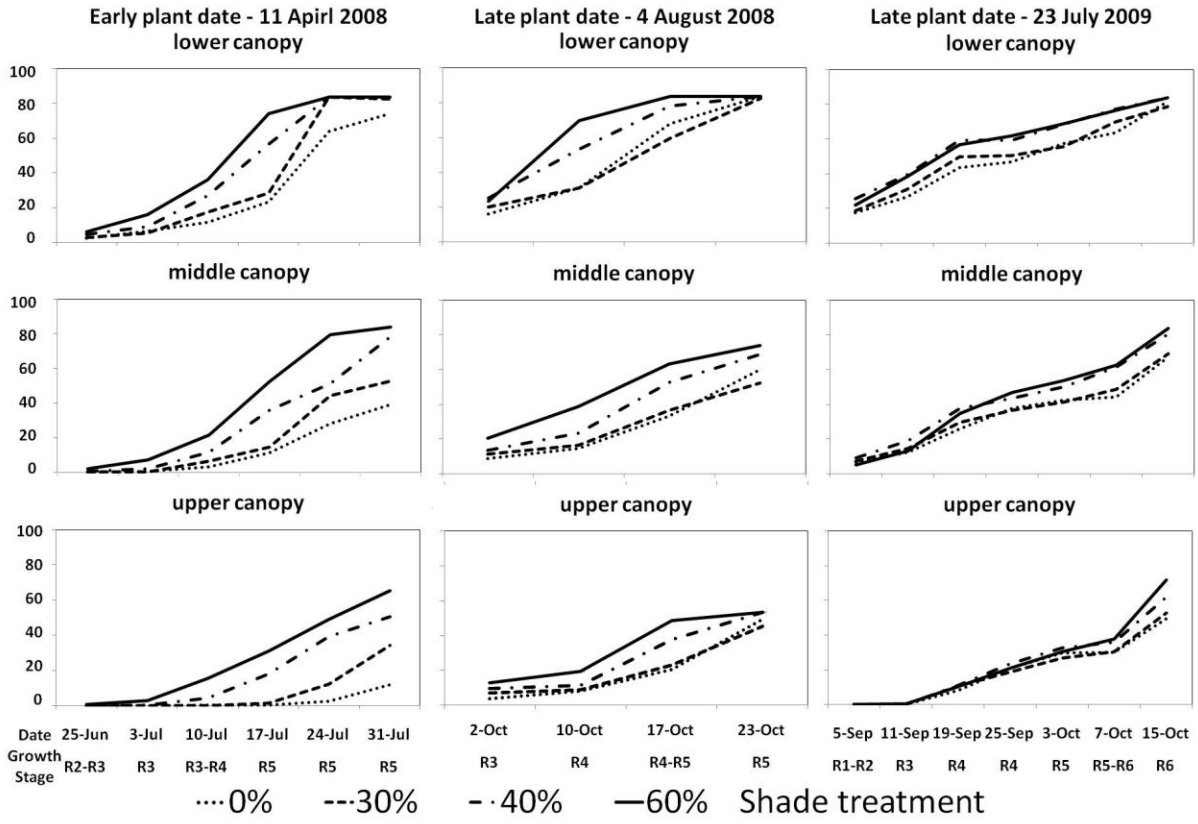

Figure 3-5. Severity of soybean rust (SBR) from field plots over time. SBR was rated weekly under different shade treatments (0, 30, 40, and 60% shade), canopy heights (lower, middle, and upper), and planting dates. X-axis displays date and growth stage of soybean at each evaluation. Shade cloth structures were installed at vegetative growth stage V3-V4. Severity of SBR was based on visual assessment of symptomatic leaf area using the scale, 0= no disease, 1 = up to 2.5%, 2 = 2.5-5%, 3 = 5-10%, 4 = 10-15%, 5 = 15-25%, 6 = 25-35%, 7 = 35-67.5%, and 8 = 67.5-100% of the leaf area affected by SBR and averages were calculated by converting each rating to the midpoint of its range.

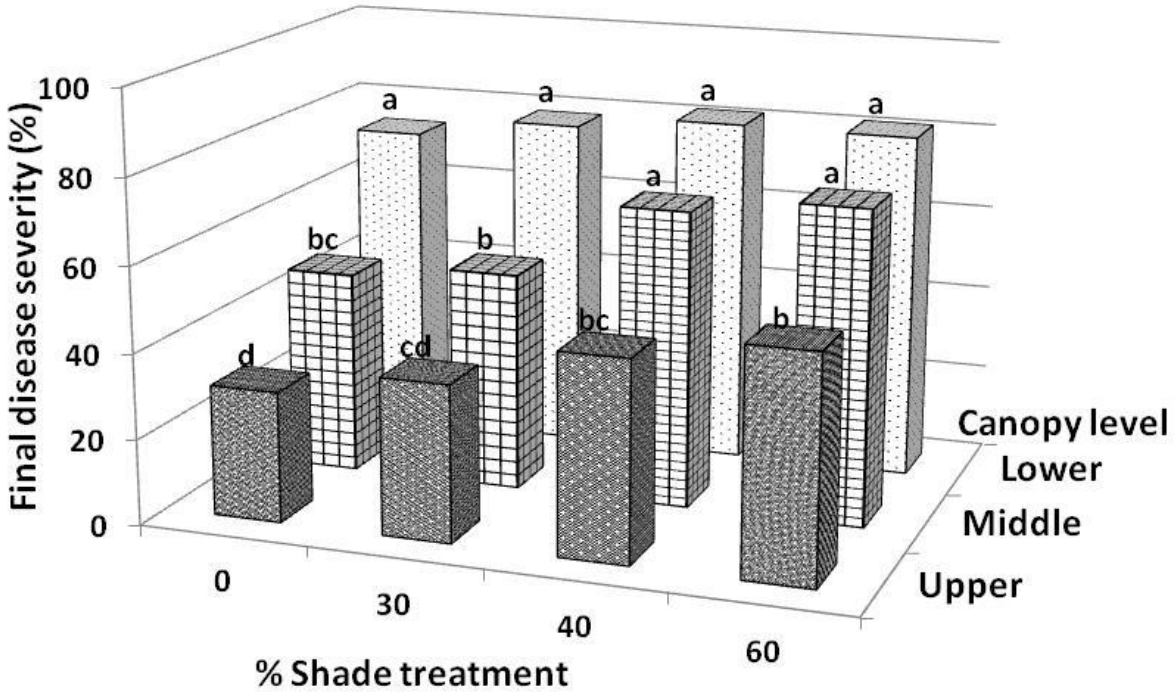

Figure 3-6. Final soybean rust (SBR) disease severity among shade treatments. Average SBR severity at reproductive growth stage R5 (seed development) at lower, middle, and upper soybean canopies under 0, 30, 40, and 60% shade treatments in the field were calculated to determine final SBR severity. Shade cloth structures were installed at vegetative growth stage V3-V4. Disease severity was assessed at ten randomly pre-assigned areas in each plot by visual assessment of symptomatic leaf area using the scale, 0= no disease, 1 = up to 2.5%, 2 = 2.5-5%, 3 = 5-10%, 4 = 10-15%, 5 = 15-25%, 6 = 25-35%, 7 = 35-67.5%, and 8 = 67.5-100% of the leaf area affected by SBR and averages were calculated by converting each rating to the midpoint of its range. Different letters on the bars indicate significant differences among soybean canopy heights and shade treatments based on averages analyzed by least significant differences at $P \leq 0.05$, across treatments and canopy heights.

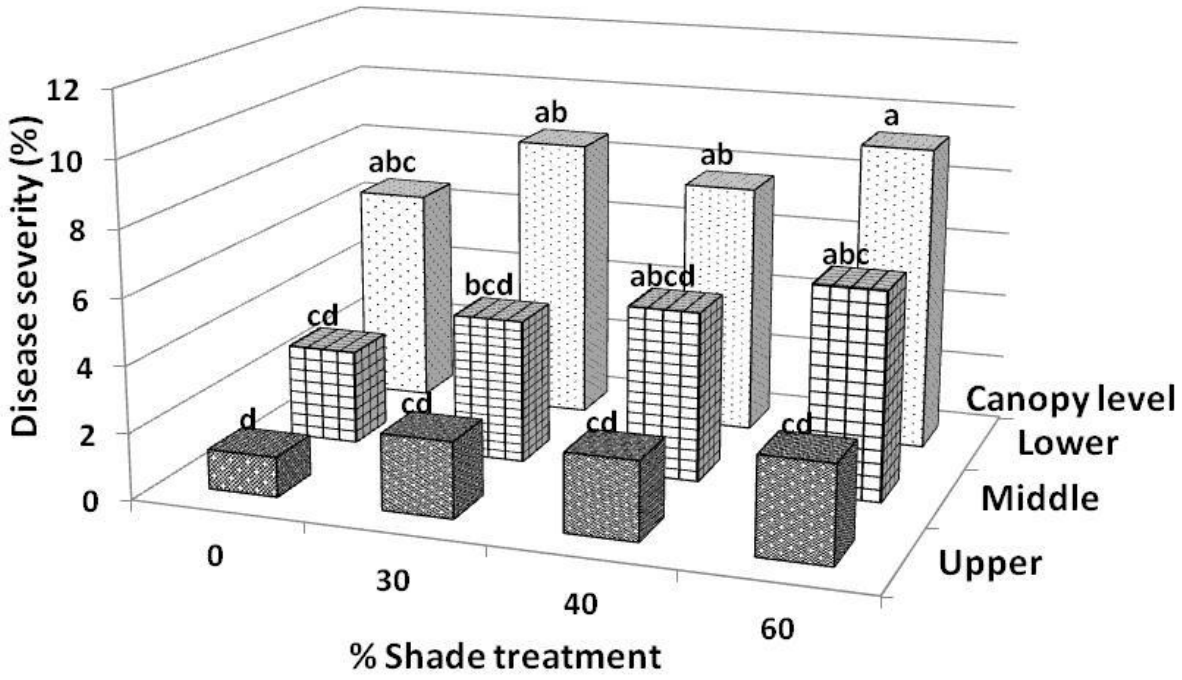

Figure 3-7. Average soybean rust (SBR) severity on detached soybean leaves. The average SBR severity on detached soybean leaves taken from lower, middle, and upper canopies under 0, 30, 40, and 60% shade treatments, which were installed at vegetative growth stage (V3-V4). Leaves were detached at reproductive growth stage R1, and inoculated with *Phakopsora pachyrhizi* urediniospore suspension containing approximately 15,000 spores/ml. Disease severity was determined based on visual assessment of symptomatic leaf area using the scale, 0= no disease, 1 = up to 2.5%, 2 = 2.5-5%, 3 = 5-10%, 4 = 10-15%, 5 = 15-25%, 6 = 25-35%, 7 = 35-67.5%, and 8 = 67.5-100% of the leaf area with soybean rust. Averages severity was calculated by converting each rating to the midpoint of its range. Different letters on the bars indicate significant differences among soybean canopy heights and shade treatments based on averages analyzed by least significant differences at $P \leq 0.05$, across treatments and canopy heights.

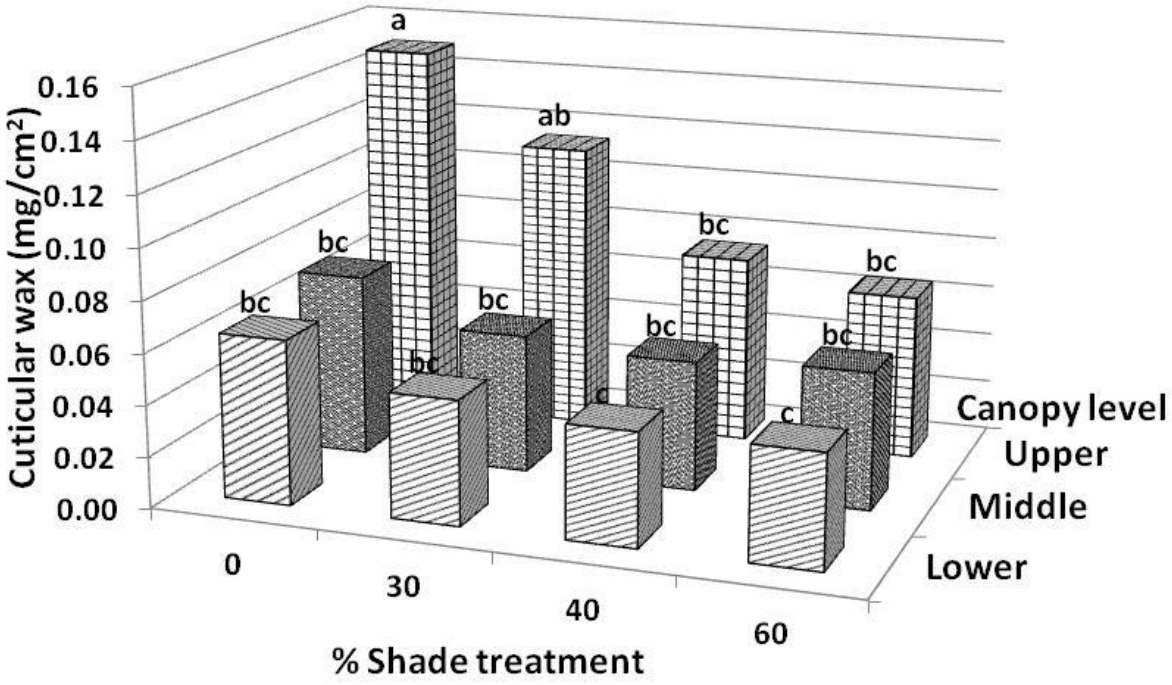

Figure 3-8. Average epicuticular wax (mg/cm^2) extracted from soybean leaves. Average epicuticular wax (mg/cm^2) was extracted from soybean leaves at reproductive growth stage, R1, from lower, middle, and upper canopies under 0, 30, 40, and 60% shade treatments. Different letters on the bars indicate significant differences among soybean canopy heights and shade treatments based on averages analyzed by least significant differences at $P \leq 0.05$, across treatments and canopy heights.

CHAPTER 4
VARIATION IN THE NUCLEOTIDE BINDING SITE (NBS) REGIONS OF KUDZU (*PUERARIA MONTANA*): POSSIBLE SOURCE OF RESISTANCE TO PHAKOPSORA PACHYRHIZI, THE CAUSAL AGENT OF SOBYEAN RUST

Introduction

Soybean rust (SBR), caused by the biotrophic fungus *Phakopsora pachyrhizi*, is potentially destructive to soybean (*Glycine max*) production worldwide. In the U.S., the fungus over-winters on exotic, invasive kudzu species, predominantly on the species *Pueraria montana* (Lour.) Merr. var. lobata (Willd.) Maesen & Almeida. (83,209) in the southeastern states. Urediniospores originating from infection on kudzu provide the initial inoculum during every soybean growing season and has the potential to disperse to northern soybean producing areas (79,80,83).

Kudzu was first promoted in the U.S. in 1907 as a drought-resistant, high-nitrogen forage crop. By the 1920-30s, kudzu was propagated and promoted by the Soil Conservation Service as a means of holding soil on eroding gullies of the deforested southern landscape. Kudzu seedlings and nurseries produced and distributed more than 73 million seedlings between 1936 and 1941 (23). Kudzu is now estimated to cover between 0.81 and 2.8 million hectares of the U.S., primarily in the southeast. Eventually, farmers found drawbacks to kudzu including difficulty baling kudzu hay and the tendency for it to grow so rapidly and extensively that it covered all other vegetation. In 1998 Congress placed kudzu on the federal list of noxious weeds.

Kudzu exhibits similar susceptible, resistant, and immune response reactions to *P. pachyrhizi* as soybean, with susceptible responses characterized by tan lesions with profuse sporulation, resistant responses characterized by reddish-brown lesions with delayed, reduced sporulation, and immune responses characterized by no lesion

development (82). Of 10 kudzu accessions obtained from north central Florida and tested by Jordan et al. (82), 6 were identified as susceptible, 3 immune, and 1 resistant to *P. pachyrhizi*. The resistant kudzu had 10-fold less *P. pachyrhizi* DNA present 15 days after inoculation compared with susceptible kudzu quantified by quantitative real-time polymerase chain reaction (82).

In the U.S, a resistant reaction, characterized by reddish-brown lesions, was common on kudzu plants collected from areas of Mississippi, Louisiana, Kentucky, and North Carolina when challenged with three isolates of *P. pachyrhizi*, one each from the U.S states of Alabama (AL) and Louisiana (LA), and one from Brazil (16). It was further reported that plants at specific sites were genetically identical with respect to their reaction to urediniospores from a wide range of *P. pachyrhizi* isolates when tested with 8 additional isolates from several areas of the world. Of the kudzu populations tested from the southeastern U.S., 46% produced a resistant response to the three *P. pachyrhizi* isolates from AL, LA and Brazil (16). In kudzu populations from north Florida, 32 out of 139 tested had reduced sporulation and red brown lesions characteristic of resistant reactions to *P. pachyrhizi* (82). Data from the Integrated Pest Management – Pest Information Platform for Extension and Education (ipm-PIPE) collected from 2005 through 2010 provide evidence of further resistance to *P. pachyrhizi* in kudzu populations throughout Florida (80).

Current SBR prediction models for the U.S. are based upon initial inoculum from kudzu and assume all kudzu populations to be susceptible (79,80). A more precise understanding of resistance in kudzu may provide better estimates of initial inoculum for forecasting models, shed light on probable evolutionary mechanisms of host-parasite

interactions of SBR, and lead to identifying a potential source of resistance that could be utilized against SBR in soybean.

To date, five major sources of SBR resistance, termed resistant to *Phakopsora pachyrhizi* (*Rpp*), (Rpp1, *Rpp2*, *Rpp3*, *Rpp4*, and *Rpp5*) have been identified in soybean (21,22,54,68,69,112). A resistance candidate gene, *Rpp4C*, belonging to the coiled coiled-nucleotide binding site- leucine rich repeat (CC-NBS-LRR) gene family has been associated with resistance conferred by *Rpp4* (117).

Resistant genes with regions encoding products that contain nucleotide-binding site (NBS) and a series of leucine rich repeats (LRRs) have been shown to mediate a hypersensitive reaction (HR) to defend against pathogen attack (35,42,43,126,226). The NBS region is responsible for the binding and hydrolysis of ATP and GTP and acts as a signaling molecule (190), whereas the LRR encodes for a potential binding surface for protein-protein interactions (89). It has been confirmed that the majority of 149 NBS-LRR-encoding genes in Arabidopsis (*Arabidopsis thaliana*) are clustered physically in the genome, although a cluster can contain highly dissimilar NBS-LRR encoding genes (115,116,156). Similarly, NBS-LRR encoding gene clusters have been identified in many other species including rice (*Oryza sativa*; 535 NBS-LRR encoding genes; (229)) and common bean (*Medicago truncatula*; 333 NBS-LRR encoding genes; (5)). Yet, a BLASTX comparison of the soybean genome scaffold with *Rpp4* candidate sequences did not identify any additional NBS-LRR encoding genes in soybean (4,117). Likewise, an evaluation of the entire U.S. germplasm collection (16,000 accessions) against a mixture of five *P. pachyrhizi* isolates identified less than 5% (805 accessions) with partial tolerance or resistance reactions (119). The rarity of resistance to SBR in

soybean suggests other sources should be investigated for resistance to *P. pachyrhizi* such as wild legumes, including kudzu.

Previously identified SBR resistance in kudzu (82) may be associated with the NBS-LRR gene family, which exhibits a resistance reaction similar to *Rpp4* mediated resistance in soybean when challenged with a compatible *P. pachyrhizi* isolate. There is a need for improved understanding of the degree of resistance in kudzu to *P. pachyrhizi* as well as the underlying variables to which such resistance can be attributed. The objectives of this study were to identify and characterize probable resistance conferring NBS regions and examine the genetic diversity of NBS regions of kudzu populations in Florida by using restriction fragment length polymorphisms (RFLP).

Materials and Methods

Kudzu Populations for Initial Screening of NBS Region

Individual kudzu plants representing 5 kudzu populations, FLAL15, FLAL13, FLMA01, FLAL11, located in north-central Florida and FLMD12 located in north Florida were collected in the spring of 2007 and in the summer of 2010, respectively. Kudzu plants were collected by digging up a healthy, asymptomatic root and vine from each location, planted in 5-gal plastic pots containing Metro Mix 300 potting soil (Sun Gro Horticultural Distributors, Inc., Bellevue, WA) and maintained in a rust-free greenhouse without shade until spring of 2011, at the University of Florida campus located in Gainesville, Florida. In the spring of 2011 the potted kudzu plants were relocated to rust-free greenhouse at the North Florida Research and Education Center campus located in Quincy, Florida. Kudzu population FLAL15 was previously reported as resistant, FLAL13 and FLMA01 as susceptible, and FLAL11 as immune to the *P.*

pachyrhizi isolate (FL05) collected from infected soybean in the summer of 2005 from the Plant Science Research and Education Center near Citra, FL (82). Kudzu population FLMD12 had not previously been characterized with respect to its reaction to *P. pachyrhizi*.

P. pachyrhizi Inoculum

The isolate of *P. pachyrhizi* used in this study was collected from infected kudzu (population FLGA09) in the summer of 2010 from the North Florida Research and Education Center in Quincy, Florida. This isolate was maintained on susceptible maturity group 5 soybean (Pioneer 95Y20RR) in a greenhouse on the North Florida Research and Education Center campus in Quincy, Florida. Newly produced urediniospores were collected by vacuum cyclone separation (G-R manufacturing Co., Manhattan, KS, USA) from the abaxial side of infected soybean leaves that were washed the previous day to remove old urediniospores. Urediniospores were suspended in a solution of 0.2% (vol/vol) Tween 20 in sterile distilled water and gently mixed for 20 to 30 s using a vortexer. Final urediniospore concentration was adjusted to 10^5 spores per ml using a hemacytometer.

DNA extraction, polymerase chain reaction (PCR), and partial sequencing of NBS region

Immature leaflets approximately 0.6 to 2.6 cm in length and 0.25 to 2.0 cm in width were collected from individuals from each kudzu population and pulverized in mortars with liquid nitrogen. Total genomic DNA was extracted using the DNeasy Plant Mini kit (QIAGEN, Valencia, CA, USA). Primers designed in the conserved region of the nucleotide binding site (NBS) domain of the resistant gene, *Rpp4* of soybean (117) were used to amplify NBS domain(s) in kudzu populations (Table 1). The PCR mixture

contained 12.5 μl of Extract-N-Amp™ (Sigma-Aldrich, Inc., St. Louis, MO, USA), 1.25 μl each of the forward and reverse primers , 5 μl of deionized (DI) water, and 5 μl of template DNA (approximately 250 ng) . The PCR program used for amplification was as follows: initial denaturation at 94 °C for 1 min; 34 cycles of 94 °C for 30 sec, annealing at 55 °C for 30 sec, elongation at 72 °C for 1.4 min, and final extension at 72 °C for 7 min. PCR products were purified using the MiniElute PCR Purification kit (QIAGEN, Valencia, CA), and cloned into *Escherichia coli* JM109® High Efficiency Competent cells (Promega, Fitchburg, WI, USA) using pGEM®-T Easy vector system II Kit (Promega, Fitchburg, WI, USA). A total of 19 clones were sequenced (ICBR, University of Florida, Gainesville, FL). Sequences were aligned using Clustal W (194) and phylogenetic trees were generated using the software Mega5 (191).

Sampling of Kudzu Populations across Florida

Kudzu populations in Florida were identified and monitored for the presence of *P. pachyrhizi* beginning in 2005, with additional populations added in subsequent years. The latitude and longitude coordinates and the approximate area of each kudzu population were recorded. Kudzu populations were separated geographically by at least 500 m or by physical barriers, such as a paved roadway. Multiple samples were taken from 4 kudzu populations (FLAL15, FLJef12, FLMA01, and FLMD12), at least 3 m apart and screened separately to examine variation within a kudzu population. For RFLP analysis, kudzu populations were sampled from 31 counties, across the 6 regions of Florida (Figure 4-1). Kudzu samples were collected from as far south as Miami-Dade county (15.50805, -80.30267 decimal degrees (DD)), as far northeast as Duval county

(30.29280, -81.61590 DD), and as far northwest as Escambia county (30.99080, -87.57790 DD).

Using data collected from 2005 through 2011, 179 kudzu populations out of 266 were infected 3 of the years scouted or less and were identified as possibly resistant to *P. pachyrhizi*. Of the 179 possibly resistant populations identified 105 populations were sampled with an additional 8 susceptible kudzu populations that were infected 4 years or more. Immature leaflets of kudzu, as described previously, were collected from the 113 populations during 2011.

Restriction Fragment Length Polymorphism (RFLP) Analysis

DNA extraction and PCR amplification of the NBS region from all sampled kudzu populations was performed as described previously. Using aligned sequences and NEBcutter V 2.0 (http://tools.neb.com/NEBcutter2/) the enzyme *Taq*$^{\alpha}$*I* was identified and utilized to further differentiate between kudzu populations. A RFLP analysis of the amplified NBS region of the kudzu populations was performed. The restriction enzyme, *Taq*$^{\alpha}$*I* (New England Biolabs, Inc.,Ipswich, MA; 0.3 μl containing 30 units) was incubated with 10 μl of purified PCR product for approximately 2 h at 65°C following the manufacturer's recommendations. Electrophoresis was performed on a 2.0% agarose gel (2% agarose in 1X TBE buffer containing 0.002% ethidium bromide) for approximately 1.5 h at 50 V. Gels were photographed on a UV transilluminator. RFLP band profiles were analyzed using the software package GelCompar II (version 6.5; Applied Maths, Kortrijk, Belgium). Molecular weights were assigned to each fragment using a 100 bp ladder. A fragment that was present was scored as "1" and a fragment that was absent was scored as "0". A cluster analysis was performed and an

unweighted pair group method with arithmetic means (UPGMA) phylogenetic tree was generated using DICE similarity coefficients to determine genetic similarities among the kudzu populations sampled.

To infer population genetic structure and differentiation an analysis of molecular variance (AMOVA; (50)) was done by using the program ARLEQUIN (version 3.1; (49)). Populations were assigned to 6 groups on the basis of the region they are located (Northwest, Northeast, Central, South Central, and South) (Figure 4-1 and Table 4-2) to assess the effect of geography on genetic variation. Another grouping based on number of years of previous SBR infections (obtained from historical records) grouped the sampled populations into 7 groups: 47 populations never observed to be infected, 37 populations infected 1 year, 21 populations infected 2 years, 3 populations infected 3 years, 2 populations infected 4 years, 1 population infected 5 years, and 2 populations infected 6 years.

In the AMOVA, banding patterns are treated as molecular haplotypes of NBS regions. A distance matrix based on pairwise squared Euclidean distances between the haplotypes was generated, based on which, the total observed variance was partitioned into covariance components at different levels of genetic structure: among populations, among groups of populations, and among populations within groups, to generate the *F* statistics (F_{ST}, F_{CT}, or F_{SC}). The measure of F_{ST} is the proportion of the total observed variance that resides among all sampled populations, whereas F_{CT} represents the proportion of the total variance that resides among the groups of populations. The statistic F_{SC} estimates the proportion of total variance in a group that resides among populations in that group (49,50,56). The *F* values can range from 0 to 1. The *P* values

obtained are the proportion of 1000 permutations that gave an *F* value larger than the observed value and represent the probability of the *F* statistic being measured.

Detached Leaf Assay

Detached leaf assays were conducted using kudzu plants, representative of populations FLAL15, FLMA01, FLAL11, and FLMD12, to verify resistance in FLMD12 as identified by RFLP. Susceptible soybean variety (Pioneer 95Y20RR) and resistant soybean, PI 459025B the original source of the *Rpp4* gene, were also used in the detached leaf assay. Two or four leaflets were excised from each kudzu plant and soybean variety. Fully expanded leaflets were collected from the third to sixth node of kudzu and fully expanded leaflets from the upper most nodes of soybean at vegetative growth stage V8 (last vegetative stage before flowering) to reproductive growth stage R3 (pod development) and used in the assays. The detached leaflets were rinsed with sterile distilled water and allowed to air dry in the laboratory. The petiole of each leaflet was cut diagonally with a scalpel to produce a clean, fresh surface. Each leaflet was placed in an individual Petri plate (150 x 15 mm) half-filled with 1% water agar with the petiole inserted into the agar. Within each Petri plate a sterile, moist paper towel (8 x 8 cm) was placed to increase moisture. Each leaflet was inoculated on its adaxial side in 9 to 10 defined areas with 5 μl of spore suspension (10^5 spores/ml) (prepared as described previously). After inoculation Petri plates were placed in the dark in a moist chamber for approximately 48 h. After removing Petri plates from the moist chamber they were parafilmed and maintained at room temperature (approximately 24°C) and exposed to approximately 30% intensity of natural sunlight for 8 hours per day for the entire duration of the assay.

At 10, 15, and 20 days post inoculation (DPI), leaf discs, (approximately 8 mm in diameter) were removed from the detached leaflets at the inoculated sites using a cork borer, and inspected for number of sporulating uredinia. The leaf discs were then placed individually in 1.5 ml microcentrifuge tubes and 100 μl of 0.2% (vol/vol) Tween 20 in sterile distilled water was added to each tube, followed by mixing for 30 s using a vortexer. Spore concentration was determined for each leaf disc using a hemacytometer. The number of spores produced at each inoculated site was determined using the spore concentration and collection volume. At each evaluation date (10, 15, and 20 DPI), 3 inoculated sites were harvested from each leaflet, giving a total of 6 or 12 sites for each plant. The assay was carried out three times (once with 2 leaflets of each plant and twice with 4 leaflets of each plant) for a total of 30 inoculation sites for each plant at each time point of evaluation. Statistics were applied to the data using the general linear model procedure and Fisher's least significant difference test at a significance level of 0.05 in SAS 9.1 (Statistical Analysis Software version 9.1, SAS Institute Inc., Cary, NC).

Results

Primers targeting the conserved NBS domain of the soybean *Rpp4C* resistance gene were used to amplify similar regions from kudzu populations. Amplicons from kudzu populations were the same in size as that from soybean (1.2 kb using *Rpp4*_NB_F/R, Table 4-1). Sequencing of 19 clones of the NBS domain from kudzu populations FLMA01, FLAL13, FLAL11, and FLAL15 revealed motifs identifying the regions as NBS domains. Clones from the different kudzu populations had high sequence similarity (81 to 100%) with each other and a neighbor joining phylogenetic

tree delineated multiple clusters (Figure 4-2). However, clones representing a given kudzu population did not always cluster together implying the primers were amplifying multiple NBS regions from kudzu. A GenBank search, performed with the BLASTX algorithm showed homology of the kudzu sequences with *Rpp4* candidate genes from soybean and *Phaseolus vulgaris*, respectively sharing from 80 to 86% and 73 to 77% protein identity.

Individuals that were at least 3 m apart within a kudzu population, did not display variation in the RFLP banding pattern, indicating that individuals within populations examined in this study are clonal . Compared to the known susceptible kudzu populations, FLMA01 and FLAL13, the known resistant kudzu population, FLAL15, had a distinct banding pattern when digested with the restriction enzyme, *Taq*$^{\alpha}$*I* (Figure 4-3). Out of the 113 kudzu populations screened, 1 population (FLMD12) had the same banding pattern as the known resistant kudzu population FLAL15. Results from the detached leaf assays further indicated kudzu population FLMD12 is resistant to *P. pachyrhizi*. The number of uredinia and urediniospores produced per inoculated area on FLAL15 and FLMD12 in the detached leaf assay were not significantly different (Figure 4-4). Whereas, the number of uredinia and urediniospores produced per inoculated area were significantly lower on susceptible and resistant kudzu compared to susceptible and resistant soybean. Furthermore, uredinia did not develop on resistant kudzu populations (FLAL15 and FLMD12) until 15 DPI and majority of sporulation did not occur until 20 DPI, and no uredinia developed on the immune kudzu (FLAL11) (data not shown).

A UPGMA dendrogram that was generated based on RFLP of the NBS domain identified grouped the sampled kudzu populations into 28 distinct clusters (Figure 4-5 and Table 4-3). Although kudzu populations did not always cluster based on infection history, some clusters with high cophenetic correlation and average percent similarity may indicate those populations have a genetic pre-disposition of resistance or susceptibility to *P. pachyrhizi*. Specifically, the identified resistant kudzu populations FLAL15 and FLMD12 clustered together with population FLMD13, which has never been found to be infected, with a cophenetic correlation coefficient of 95 and average similarity of 81.4% among those populations (cluster 18 in Table 4-4 and Figure 4-5). Cluster 22 and 24 groups kudzu populations that have never been observed to be infected with cophenetic correlation coefficients of 82 and 64, respectively; and average similarity of, respectively, 66.5 and 85.8%, among those populations (Table 4-4; Figure 4-5). Whereas, cluster 11 and 19 groups kudzu populations that have been observed to be infected every year that they were scouted along with populations that have never been observed to be infected or infected 1 to 3 years, with cophenetic correlation coefficients of 92 and 88, respectively, and average similarity of, respectively, 92 and 88%, among those populations. Similarly, only 6 clusters group kudzu populations that are in the same geographic region in Florida and of those 6 clusters only 1 (cluster 18) has a high cophenetic correlation coefficient and average similarity among the populations.

Population structure determined by AMOVA showed almost identical results when grouped by geographic region and by the number of years a population was observed to be infected with SBR (Table 4-4). All the variation was attributed to variation among

populations and among populations within groups. The proportion of total variance residing among populations within groups (F_{SC}) was 1.000 (P = 0.000) and 1.000 (P = 0.000) when populations were grouped by geographic location or number of years of previous infection, respectively.

Discussion

Based on the results, primers designed in conserved regions of the NBS domain from soybean can be used to amplify similar regions from kudzu populations in Florida. While sequencing of the NBS region from 4 kudzu populations had 80 to 86% sequence identity to *Rpp4* candidate genes in soybean multiple polymorphisms, evidenced by multiple clusters on the phylogenetic tree, indicate that the conserved primers were amplifying multiple NBS regions within a single kudzu population. The RFLP results further provide evidence of great variation in the NBS region in kudzu which may be due to variability within a single NBS region and/or variability among NBS regions in kudzu populations. This is expected, as other studies have identified multiple NBS genes within an organism using primers designed in conserved regions of the NBS (124). The sequence analysis and phylogenetic tree imply greater genetic diversity within kudzu compared to soybean, with respect to the NBS regions. Bonde et al. (18) reported that kudzu plants previously shown to produce resistant reaction to 3 *P. pachyrhizi* isolates also produced resistant reactions to an additional 8 isolates from around the world. This broad resistance displayed by kudzu against multiple isolates of *P. pachyrhizi* may be due to multiple resistance genes, indicated by multiple NBS regions identified in this study. Furthermore, the high variation of the NBS region(s) in both susceptible and resistant kudzu populations may explain the reduction in uredinia and urediniospore production on all kudzu populations examined compared to soybean.

Geographic location and infection history of the sampled kudzu populations does not appear to influence the genetic structure of these populations. The low F_{CT} values from an AMOVA indicate that populations within the same region of Florida and those with similar infection history from 2005 through 2011 are genetically distant from each other as they are from populations from different regions of Florida or from populations that differ in the number of years they have been observe to be infected with SBR.

Most of the sampled kudzu populations did not cluster according to their infection history, although clusters 18, 22, and 24 were clusters grouping only those kudzu populations that were never observed to be infected with SBR. These clusters may represent NBS region(s) that contribute to resistance to *P. pachyrhizi* with reduction in uredinia and reduction and delay in sporulation. Other clusters that grouped kudzu populations that had a range in infection history from never being observed to be infected to being infected every year may be representing NBS region(s) that likely do not contribute to resistance. Furthermore, the lack of any pattern based on infection history in these clusters could be due to lack of inoculum or conducive environmental conditions for SBR infection and disease development. Further investigation and exhaustive sampling of susceptible kudzu populations may better resolve polymorphisms in the NBS region contributing to resistance to *P. pachyrhizi*.

Consistent with the findings from this research, Pappert et al. (144) found southeastern U.S. kudzu populations to have high genetic diversity with no regional patterns of variation and relatively little overlap of genotypes between populations when examining 14 allozyme loci. Furthermore, kudzu populations from the southeastern U.S. have been reported to have similar diversity index to the native Chinese kudzu

populations from examination of inter-simple sequence repeat (ISSR) variations (189). Such high levels of genetic diversity among populations and low diversity within kudzu populations can be explained by limitation of gene flow among populations and high genotypic diversity. With growth in kudzu populations being primarily clonal by rhizomes, low levels of seed set and seed viability and restriction of gene flow via seed dispersal could lead to genetic differentiation among populations (1,138). When kudzu populations were initially becoming established, pollination and seedling establishment may have contributed to genotypic variation within a population. But once a population became established with a dense structure of growth and rhizomes, only a very small proportion of seedlings may become established. This was reported by Abramovitz (1), where out of 245 naturally occurring seedlings observed at the periphery of populations only one became established. The high level of genetic variability among kudzu populations in the southeastern U.S. can be primarily attributed to establishment by introductions from different sources and gene exchange and recombination during initial establishment.

Variability in the NBS region(s) in kudzu may partially explain why kudzu, both susceptible and resistant populations, display greater resistance to *P. pahcyrhizi* with reduction in uredinia and reduction and delay in sporulation as compared to soybean. Meyers et al. (117) found that amino acid differences among the *Rpp4* candidate genes may play a key role in resistance. Resistance to rice blast and flax rust, conferred by LRR-NBS gene(s), has been reversed by changing as few as 1 and 6 amino acids, respectively (25,44). This shows that even small variations in the NBS region could significantly alter susceptibility or resistance in a kudzu population which perhaps is the

basis of a lack of correlation between apparent high similarity among RFLP of the NBS region(s) and resistance or susceptibility to SBR observed in this study.

While the NBS marker used in this study did have high variation among kudzu populations a more robust marker may better differentiate between susceptible and resistant kudzu populations or possibly a more extensive digest of the NBS region(s) using a combination of enzymes may identify more polymorphisms contributing to resistance. Furthermore, there may be other resistance genes, non-LRR-NBS genes, contributing to the observed reduction in uredinia and urediniospore production in kudzu populations and multiple defense mechanisms. Different defense mechanisms in kudzu populations were reported by Jordan et al. (82). Where *P. pachyrhizi* infection of susceptible kudzu only differed from susceptible soybean 120 h after infection by limiting colonization of the mesophyll, infection of resistant kudzu displayed a hypersensitive response (HR) between 24 and 48 h after inoculation, and infection of immune kudzu displayed an early onset of an HR with a cell wall deposition around the penetration hyphae (82). These defense mechanisms may be mediated by different genes hence other markers than the NBS region(s) used in this study should be used to further investigate potential sources of resistance to *P. pachyrhizi* in kudzu.

Based on the results, using the number of previous years a kudzu population has been observed to be infected may be a poor parameter to estimate resistance in kudzu populations, as this may only be a result of low disease pressure or unfavorable conditions in the area for SBR development. However, the reduction in disease development on susceptible, resistant, and immune kudzu observed in this study and patterns in infection history could be used to better estimate initial inoculum from kudzu

populations which begin SBR epidemics each year in the U.S. Furthermore, as SBR severity has been variable since its introduction into the continental U.S., continued observation and research on kudzu populations will provide valuable information for improved forecasting of SBR movement and development, and estimating the potential risk SBR poses to U.S. soybean production. This research indicates that kudzu is a good source of resistance to SBR not only due to the genetic variation among kudzu populations but also due to an overall reduction in severity of disease development on both susceptible and resistant kudzu as compared to soybean.

Table 4-1. Primers used to amplify the nucleotide binding site (NBS) domain in kudzu populations. The primers were designed in the conserved NBS domain of the resistant gene, *Rpp4* of soybean (117).

Primer Name	Sequence
*Rpp4*_NB_F	CCTTATAATCCTAGATGATC
*Rpp4*_NB_R	CGTAATTTCTTTAACTCTCC

Table 4-2. Distribution of kudzu populations in Florida.

Region	Kudzu Populations	Possible Resistant Populations[a]	Sampled Kudzu Popluations[b]
Northwest	160	88	63 (67%)
Northeast	52	41	30 (63%)
Central	24	20	11 (55%)
South Central	11	11	7 (64%)
South	19	19	2 (11%)

[a] Kudzu populations were identified as possibly resistant to *Phakopsora pachyrhizi* if from observational data from 2005 through 2011 a population was infected 3 years or less

[b] Number and percent (in parentheses) of kudzu populations sampled out of the kudzu populations identified as possible resistant population

Table 4-3. Average percent similarity among clusters of kudzu populations in regard to the nucleotide binding site (NBS) region(s). Average percent similarity with standard deviation calculated based on restriction fragment length polymorphisms (RFLP) of NBS region(s), among kudzu populations within a cluster in an unweighted pair group method with arithmetic means (UPGMA) phylogenetic tree (Figure 4-4), generated using DICE similarity coefficients.

Cluster[a]	Average percent similarity with Std. Dev.[b]	Cophenetic correlation coefficient[c]
1	58.0 +/- 0.0	66
2	64.3 +/- 5.8	67
3	74.6 +/- 5.6	73
4	79.1 +/- 2.2	81
5	73.2 +/- 1.9	84
6	-	72
7	69.8 +/- 2.8	62
8	76.9 +/- 1.8	89
9	85.0 +/- 0.0	75
10	84.1 +/- 2.5	78
11	84.9 +/- 1.5	92
12	79.2 +/- 3.4	76
13	77.0 +/- 3.3	78
14	78.0 +/- 1.7	74
15	75.2 +/- 0.0	62
16	68.2 +/- 0.76	97
17	77.1 +/- 1.6	85
18	81.4 +/- 1.2	95
19	84.9 +/- 0.0	88
20	76.3 +/- 0.0	79
21	-	81
22	66.5 +/- 0.0	82
23	-	82
24	85.8 +/- 0.0	64
25	61.7 +/- 6.1	60
26	68.4 +/- 1.7	87
27	58.4 +/- 5.3	86
28	-	70

[a] Kudzu population clusters generated by UPGMA tree using RFLP of the NBS region(s)

[b] Average percent similarity and standard deviation for clusters with 2 or more populations.

[c] Cophenetic correlation coefficient computed by Gel Compar II (version 6.5; Applied Maths, Kortrijk, Belgium)

Table 4-4. Analysis of molecular variance (AMOVA) of restriction fragment length polymorphism (RFLP) data. ARLEQUIN 3.1 was used to determine the genetic structure among populations of kudzu (*Pueraria montana*) sampled in 2011 by AMOVA of RFLP data. The populations were grouped by geographic region: Northwest (NW), Northeast (NE), Central (C), South Central (SC), and South (S); and by the number of years a population was observed to be infected with *Phakopsora pachyrhizi* from 2005 through 2011 (0, 1, 2, 3, 4, 5, or 6). Computations for AMOVA are based on a matrix of pairwise squared Euclidean distances between populations. Percent variation is the distribution of variation at a given level of hierarchy (among groups/among populations within groups/among populations). The *F* statistics are a measure of genetic structure at different levels of hierarchy with values ranging from 0 to 1. The *P* value is the proportion of 1000 permutations that gave an *F* statistic value ≥ the observed value.

Grouping of populations	Source of variation	Variation (%)	*F* statistic[a]	*P*
By geographic region				
Five groups: NW, NE, C, SC, and C	Among groups	1.60	F_{CT} = 0.016	0.060
	Among populations within groups	98.40	F_{SC} = 1.000	0.000
	Among populations	100.00	F_{ST} = 1.000	0.000
By number of years infected				
Seven groups: 0, 1, 2, 3, 4, 5, and 6	Among groups	0.00	F_{CT} = 0.000	0.690
	Among populations within groups	100.00	F_{SC} = 1.000	0.000
	Among populations	100.00	F_{ST} = 1.000	0.000

[a] F_{CT} = proportion of total variance residing in groups; F_{SC} = proportion of total variance in a group residing among populations in that group; F_{ST} = proportion of total variance residing among populations

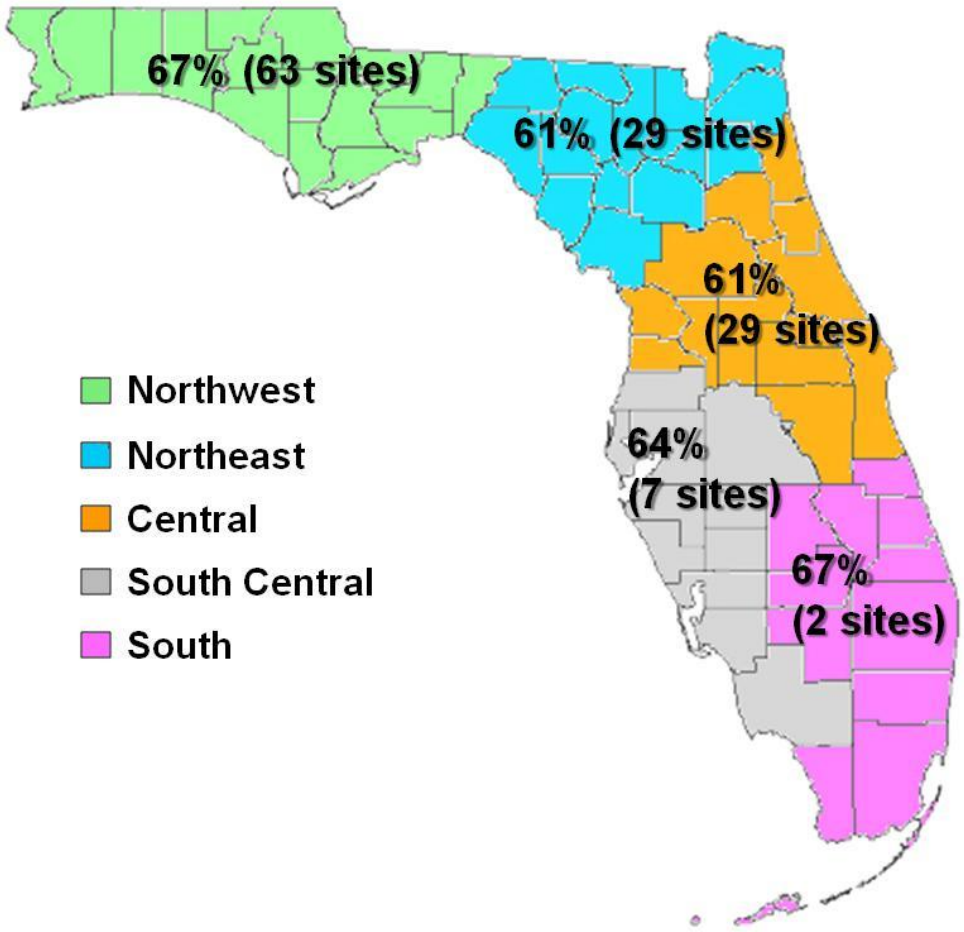

Figure 4-1. Distribution of kudzu populations in Florida. The percentage (and number) of possibly resistant kudzu populations that were sampled from each region is listed. Populations were identified as possibly resistant based on being infected with soybean rust 3 years or less from 2005 through 2011.

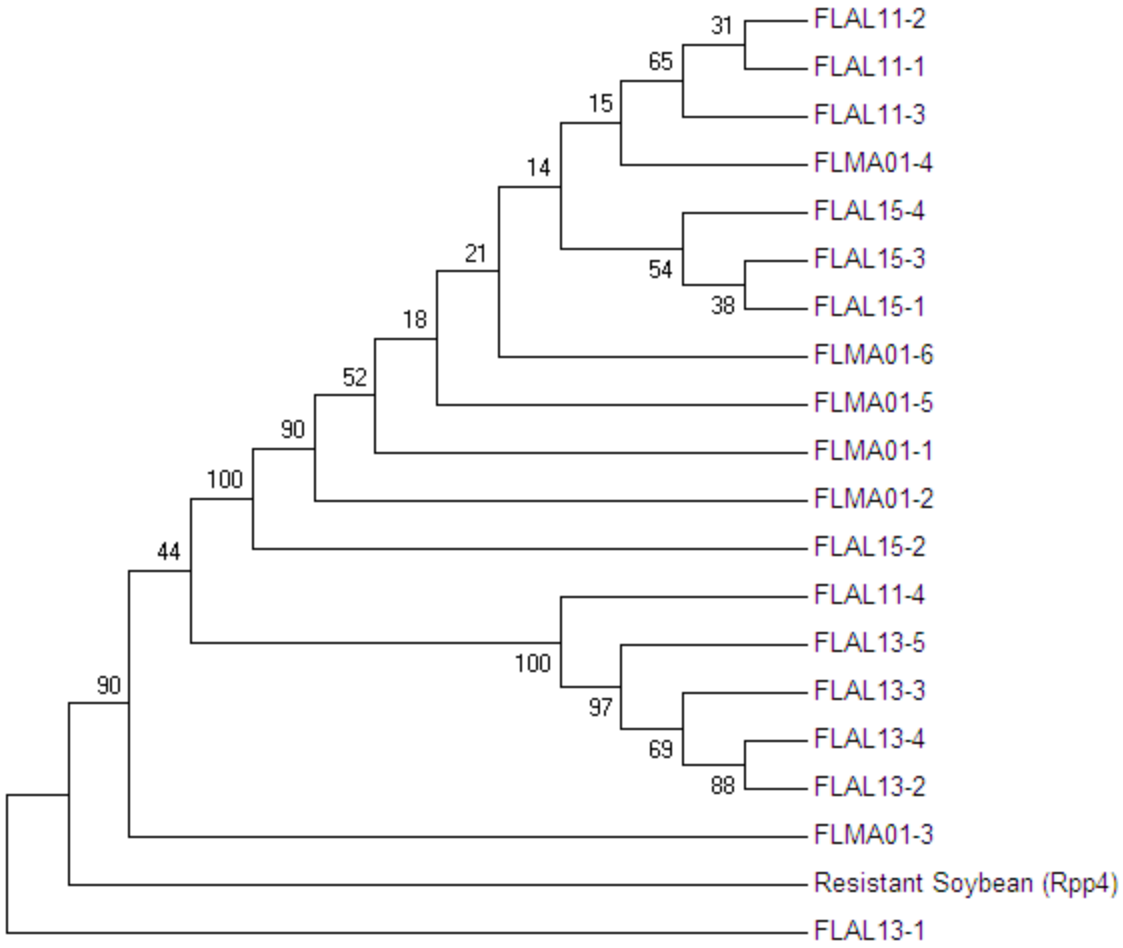

Figure 4-2.Neighbor joining phylogenetic tree. The tree and bootstrap values are generated by the maximum composite likelihood method using the nucleotide binding site (NBS) sequences of 19 clones from 4 kudzu populations (FLAL11,FL AL13,FL MA01, and FLAL15) and rooted with the sequence of soybean PI 459025B, the original source of *Rpp4*.

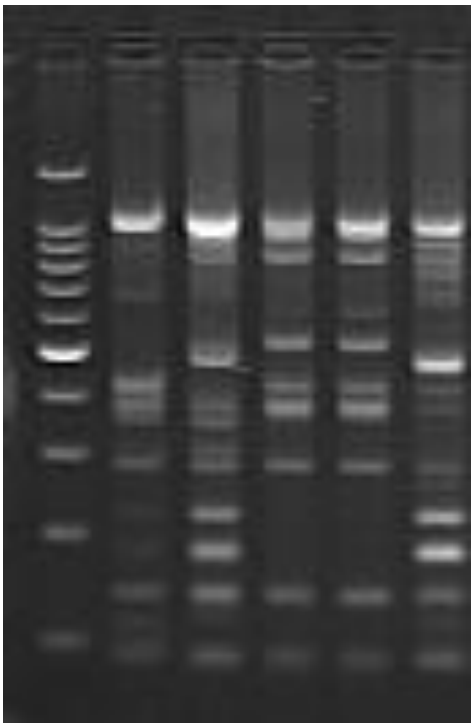

Figure 4-3. Restriction fragment length polymorphisms (RFLP) of kudzu populations susceptible, resistant, and immune to *Phakopsora pachyrhizi*. Lane 1: 100 bp ladder, lane 2: FLAL13 known susceptible, lane 3: FLMA01 known susceptible, lane 4: FLAL15 known resistant, lane 5: FLMD12 not previously identified as susceptible, resistant, or immune, and lane 6: FLAL11 known immune.

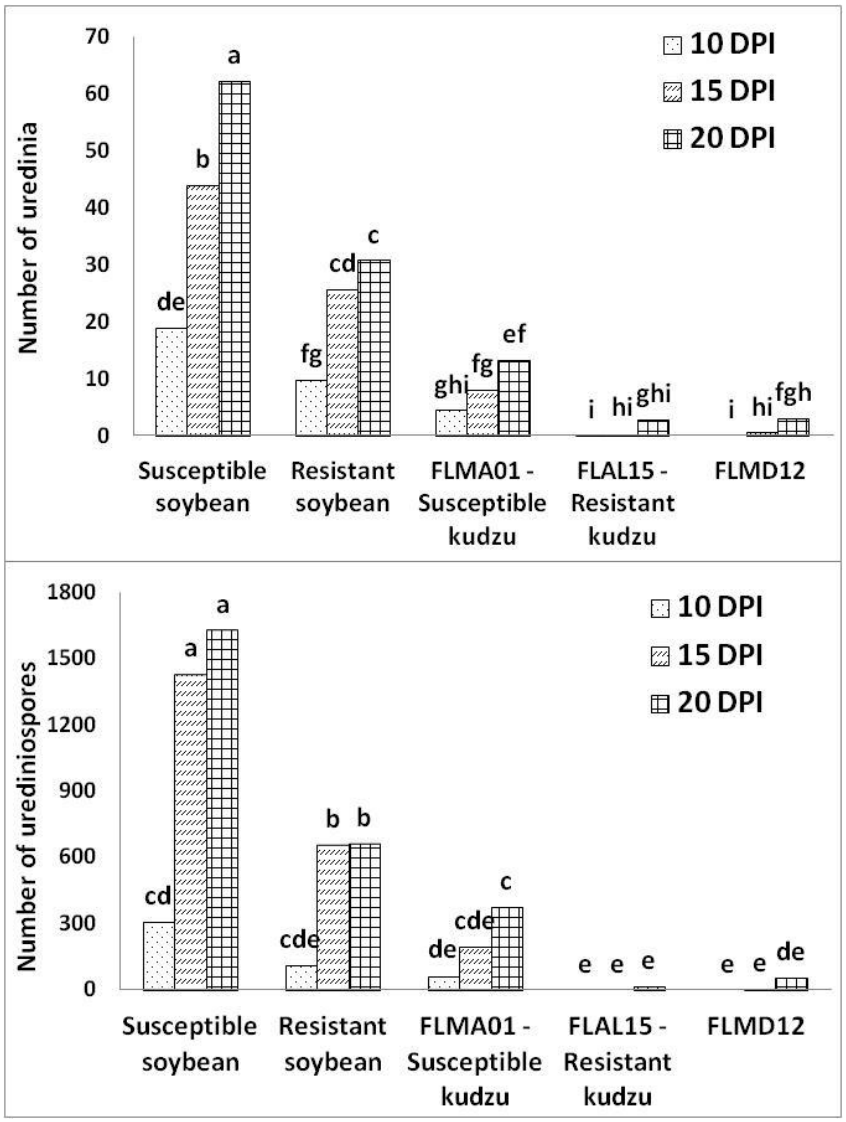

Figure 4-4. Average number of uredinia and urediniospores produced per inoculation site on detached leaflets from different hosts. Hosts used included: susceptible soybean (Pioneer 95Y20RR), resistant soybean (PI 459025B), susceptible kudzu population (FLMA01), resistant kudzu population (FLAL15), and kudzu population (FLMD12) identified as possibly resistant. Fully expanded leaflets were detached from the third to sixth node of kudzu and from the upper most nodes of soybean at vegetative growth stage V8 to reproductive growth stage R3. Leaflets were inoculated at 9 to 10 defined areas on the adaxial side with approximately 500 spores at each area. Three inoculated areas were evaluated 10, 15, and 20 days post inoculation (DPI) for number of uredinia and urediniospores produced per inoculated area. Different letters on the bars indicate significant differences among DPI and hosts based on averages analyzed by least significant differences at $P \leq 0.05$, across DPI and hosts.

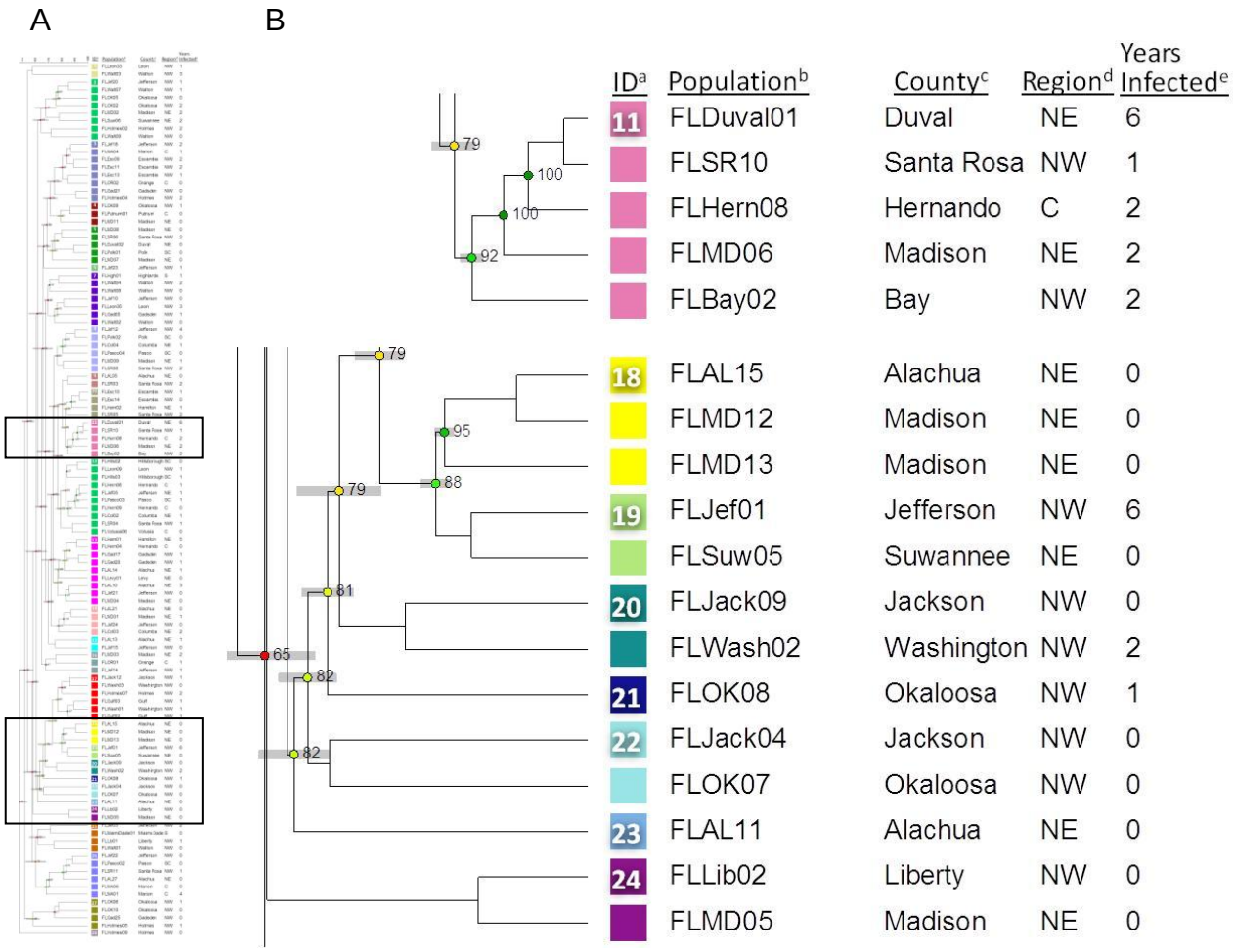

Figure 4-5. Unweighted pair-group method using arithmetic mean (UPGMA) dendrogram. A, UPGMA dendrogram generated with Gel Compar II (version 6.5; Applied Maths, Kortrijk, Belgium), based on RFLPs of NBS region(s) in the sampled kudzu populations in Florida. Boxes indicate areas enlarged in B. B, clusters with high cophenetic correlation coefficients and average percent similarities. [a] ID-color and number identification for clusters, [b] Kudzu population name, [c] County kudzu population is located. [d] Region of Florida kudzu population is located: Northwest (NW), Northeast (NE), Central (C), South Central (SC), and South (S), [e] The number of years the kudzu population has been observed to be infected with *Phakopsora pachyrhizi* from 2005 through 2011.

CHAPTER 5
EPIDEMIOLOGY OF SOYBEAN RUST ON SOYBEAN AND SUSCEPTIBLE AND RESISTANT KUDZU AND DECISION MODEL FOR FUNGICIDE APPLICATIONS FOR SOYBEAN RUST IN FLORIDA

Introduction

Soybean rust (SBR), caused by the obligate fungal parasite *Phakopsora pachyrhizi*, is a potentially devastating disease of soybean in the U.S. Each year initial inoculum is produced on the alternative host kudzu (*Pueraria lobata*) in the southeastern U.S. Kudzu has been estimated to cover 800,000 to 3,000,000 hectares in the southeastern U.S. with most severe infestations occurring in the piedmont regions of Mississippi, Alabama, and Georgia (48,34,23). Based on surveys of kudzu in Florida from 2007 through 2011, it is estimated Florida has approximately 201,000 hectares of kudzu, predominately in the north central to northeast and northwest regions (K. O'Brien, *personal communication*). Epidemiological models for SBR have been developed that estimate disease development considering biological processes in the disease cycle and local inoculum availability (39). For example, Pivonia and Yang (150) calculated the number of infections and rate of disease increase amongst five rust pathogens, including *P. pachyrhizi*, by adjusting a general disease model (64) and climatology data for specific locations within the U.S. The models for the U.S. are based upon the initial inoculum originating in kudzu and assume all kudzu populations to be susceptible to SBR (79). While multiple risk assessment and risk prediction models have been developed for SBR, uncertainties in inoculum availability weaken their accuracy (150).

Management of SBR epidemics in soybean in the southeast is necessary not only to avoid yield loss, but to also reduce inoculum that can potentially infect other soybean

producing areas of the country. Fungicides can be applied, but are costly. To be efficient and effective at managing disease, growers need to know when applications will be most beneficial. The effectiveness of different fungicides and timing of applications for SBR have often depended on when SBR was first detected, the intensity of its development, recent weather conditions, and proximity to sources of SBR (131,174). To assess proximity to sources of SBR the Integrated Pest Management – Pest Information Platform for Extension and Education (Ipm–PIPE, www.sbrusa.net) can be used to view reports of SBR in the U.S. on soybean and alternative hosts. SBR development has been predicted using simulation and empirical models based on the effects of environmental variables on epidemiological parameters such as infection rate, latent period, and senescence of disease lesions. Data from Marchetti et al (109) on temperature and dew duration for maximal infection on soybean have been utilized in models to assess the potential impact of SBR on U.S. agriculture prior to its arrival in 2004 (220,222). Similarly, daily weather variables, such as rainfall events, have been used to predict epidemic potential in field epidemics (40).

To better understand and manage SBR epidemics in north Florida, a simple decision model was developed based on presence of *P. pachyrhizi* and leaf wetness duration or rainfall events, and tested against traditional timing of fungicide applications. The results were validated and further interpreted based on the estimated SBR potential inoculum, calculated using the differences in SBR epidemiology on soybean and susceptible and resistant kudzu and the area of these hosts in Florida (Chapter 4). The objectives of this study were to use the differences in epidemiology of SBR on soybean and susceptible and resistant kudzu and the area of these hosts in Florida to 1)

estimate the potential maximum inoculum load, 2) the time of year the maximum inoculum load can occur in Florida and 3) use the inoculum estimates to validate simple decision application models.

Material and Methods

Estimation of Potential Inoculum

Spore production per area per day on susceptible and resistant soybean and kudzu was obtained from detached leaf assays with a *P. pachyrhizi* isolate collected from naturally infected kudzu growing on the University of Florida, North Florida Research and Education Center in Quincy, Florida in the fall of 2010 and maintained on susceptible soybean in the greenhouse (Chapter 4). The difference between the amount of spores produced per inoculation area (50 mm^2) 10 and 15 days post inoculation for each host was divided by 5 days to calculate the sporulation per 50 mm^2 per day for each host and is considered to be representative of the exponential phase. The total area of soybean planted in Florida in 2010 (134) and the estimated area of kudzu in Florida (K. O'Brien, *personal communication*) and the corresponding leaf area indices were used to estimate the total available host tissue in Florida. Based on previous research on the characterization of resistance to SBR in kudzu (17,82) and screening of genetic resistance in kudzu populations (Chapter 4) the proportion of immune and resistant kudzu to the total area was estimated at 18 and 32%, respectively.

Furthermore, the total available host tissue was estimated at half of the actual area due to the observation of leaf senescence after approximately 50% of the total area exhibits SBR symptoms and it is assumed to no longer contribute to the inoculum escaping the canopy. The spore escape rate from Andrade et al. (6) based on a simple

turbulence closure method and a parameterization of the canopy porosity in soybean was used to calculate the amount of spores escaping the canopy from the total amount produced. The spore production per 50 mm^2 (SP_a), 50% of the total area of host in Florida (A_{50}), host leaf area index (LAI), and spore escape rate (E) parameters (Table 5-1) were used to estimate the potential maximum amount of inoculum escaping from hosts in Florida using Equation 5-1(6).

$$M_i = \left[\frac{(SP_a * A_{50})}{LAI} \right] * E \qquad (5\text{-}1)$$

Where M_i is the maximum amount of potential inoculum escaping host canopies; SP_a is the spore production per 50 mm^2 per day, A_{50} is 50% of the total area (ha) of host; LAI is leaf area index; and E is the spore escape rate from the host canopy.

From detached leaf assay data (Chapter 4) the amount of spores applied to each inoculation area (500 spores) and the average germination rate (79.5%) were used to calculate the total number of viable spores applied to each inoculation area (398 spores per 50 mm^2). The average number of uredinia produced 20 days post inoculation on each host was divided by the number of viable spores applied to calculate the infection efficiency (IE). The number of uredinia produced per day was calculated using the difference between the uredinia produced 10 and 15 days post inoculation for each host and dividing by 5 days. The spore production per 50 mm^2 per day, previously discussed was divided by the number of uredinia produced per day per host to calculate the spore production per uredinium per day (SP_u). Previously Twizeyimana *et al.* (200) proved the utility of detached-leaf assay for screening soybean for rust resistance. Furthermore spore production per uredinia on susceptible soybean used in this study concurrs with results from Twizeyimana *et al.* (200). From detached leaf assay data

and other research (Chapter 4,92) the latent period (*LP*), the number of days from inoculation until uredinium eruption, was calculated for each host. The proportion of spores deposited within the canopy of the spore origin (*D*) used was set at the upper limit of 0.1 (64) and the infectious period (*IP*) was obtained from the literature (150,224). These parameters (Table 5-2) were assumed optimal and used to calculate the number of new uredinia per day over time using Equation 5-2 (150).

$$Y(t) - Y(t-1) = IE * D * SP_u\left(Y\left(t - LP\right) - Y\left(t - LP - IP\right)\right) \quad (5\text{-}2)$$

With the initial conditions, $Y(t) = 0$ at $-(LP + IP) < t < 0$ and $Y(t) = IE \times I$ at $t = 0$, where $Y(t)$ is the number of new uredinia at day t; IE is infection efficiency; D is the proportion of spores deposited within the canopy of the spore origin; SP_u is the spore production per uredinium per day; LP is latent period; IP is the infectious period in days as previously described; and I is the initial influx number of spores at the beginning of the year. In the model, 100 viable spores were assumed successfully infecting host plants ($Y(t) = IE \times I = 100$ at $t = 0$). The measure $\rho = Y(t)/Y(t\text{-}1)$ represents the rate of disease increase per day. While Equation 5-2 had no spatial component, the maximum amount of new uredinia was set by the number of uredinia required to produce the maximum amount of spores on 50% of the total area of each host as calculated by Equation 5-1 and the number of spores produced per uredinium. The presence of SBR in Gadsden County in north Florida on kudzu and soybean was recorded from 2005 thru 2011 and the first observation of the pathogen on each host was used as the initial infection date ($t = 0$).

To increase the accuracy of Equation 5-2, infection efficiency (*IE*) and latent period (*LP*) parameters were adjusted based on the effect of temperature. Monthly average

temperatures were calculated from daily observations recorded from Florida Automatic Weather Network (FAWN) stations in five counties in north Florida (Escambia, Jackson, Gadsden, Jefferson, and Suwannee, accessed from http://fawn.ifas.ufl.edu/) from 2005 through 2008 (Table 5-3). The effect of temperature on the infection efficiency of SBR was assumed to follow a classical response curve for biological systems with three cardinal temperatures: optimum (T_{opt} = 22.5 °C), maximum (T_{max} = 27.5 °C), and minimum (T_{min} = 10 °C) (109) and was calculated using Equation 5-3 (24,153).

$$Y = \alpha(T - T_{\min})(T_{\max} - T)^{\beta} \quad (5\text{-}3)$$

Where $\alpha = Y_{max}/[(T_{opt} - T_{min})(T_{max} - T_{opt})^{\beta}] = 0.042$ and $\beta = (T_{max} - T_{opt})/(T_{opt} - T_{min}) = 0.40$. Y is the infection index and Y_{max} = 1 when wetness conditions are optimal. Chances for infections in a field are usually higher during the night and early morning than day time because relative humidity is higher and leaf wetness duration is longer in the night than in daytime (150). Therefore, the average infection index for each month was determined using mean nighttime temperatures. Mean night time temperature was estimated by averaging the mean and the minimum monthly temperatures (Table 5-3). Multiplying infection efficiency at optimal conditions (Y_{max}) and infection index (Y) gave the infection efficiency value for each temperature.

To calculate the average latent period for a given temperature latent period was defined as a function of physiological days (PD) of SBR. PD_i can be a linear function of temperature with Equation 5-4 (70,221):

$PD_i = (T_i - T_{min})/(T_{opt} - T_{min})$, if $T_{min} \le T_i < T_{opt}$

$PD_i = (T_{max} - T_i)/(T_{max} - T_{opt})$, if $T_{max} \ge T_i > T_{opt}$ (5-4)

Optimal temperature (T_{opt} = 22 °C) is where latent period was found to be the shortest and maximum temperature (T_{max} = 42 °C) and minimum temperature (T_{min} = 11°C) were from the literature (92). T_i is the mean temperature calculated from 2005 through 2008 data. The average latent period during a given month was calculated with Equation 5-5 (150):

$$LP \text{ (at } T_i) = LP \text{ (at } T_{opt})/PD_i \quad (5\text{-}5)$$

After disease initiation, development continues according to the average temperature conditions of the month examined.

Data from the 2008 and 2009 National Wind-Vane Spore Trap Program for SBR was used to validate the estimate of spores escaping host canopies in Florida. In a wind vane spore trap a white petrolatum-coated slide was placed at approximately a 45 degree angle and exchanged every 2 to 7 days from June to October. Wind vane spore traps were aligned approximately 165 cm above the ground and placed approximately 95 cm apart. Three wind vanes with diameters of approximately 15 cm were used in 2008 and one in 2009. Microscopic evaluation was performed on each slide and DNA was extracted from whatever residue was present on the slide and assayed by quantitative polymerase chain reaction (qPCR) for *P. pachyrhizi* (71). In 2008 slides that contained greater than 50 visual spores on a slide were only recorded as having 50 visual spores. To better adjust these values time periods in 2008 where 2 to 3 slides were collected the number of visual spores was totaled over the time period. In 2009, all visual spores were recorded.

Monthly temperatures for Gadsden County from 2009 to 2011 were calculated, as previously described, from the FAWN station located on the North Florida Research and

Education Center in Quincy, Florida (http://fawn.ifas.ufl.edu/). The temperatures were used in Equations 5-3 and 5-4 to calculate the infection efficiency and latent period and used in Equation 5-2 to estimate the rate of disease increase and number of sporulating uredina. This was used to better understand disease severity and yield results from the decision application model in 2009 to 2011.

Decision Application Model

Decision application model for SBR was develop and tested in 2009 to 2011, with one soybean planting in 2009 and 2010 and two soybean plantings in 2011 at the North Florida Research and Education Center in Quincy, FL. Pioneer 97M50 RR soybean was planted 5 May 2009, 17 May 2010, 25 June 2011, and 18 July 2011 in a 700 m^2 area with 51-cm row spacing and 6 to 7 seeds per 30 cm. Each treatment plot was approximately 4 x 6 m with approximately 2 to 4 m between each plot. Approximately 2.5 m border of soybean was planted around the entire area. All plantings had 4 replicates arranged in a complete randomized block design. Disease severity was evaluated at four corners (approximately one row and 2 m in from the border rows) and one central point within each plot. Severities were averaged to calculate overall disease severity in each plot. Approximately 9 m^2 area in the center of each plot was harvested at the end of each season and seed moisture was taken and seed weight and yield were adjusted to 13% moisture to calculate final yield.

Applications of the triazole fungicide Folicur® 3.6 (tebuconazole) were determined based upon the presence of SBR, on soybean or kudzu, in Gadsden County and environmental variables determined by data from Marchetti *et al.* (109) on maximal infection or by rainfall events determined by data from Del Ponte *et al.* (40) on maximum severity. Marchetti *et al.* (109) reported maximal infection occurred on soybean at 20 to

25 °C with 10 to 12 hours of dew. Correlation analysis in Del Ponte *et al.* (40) indicated that rainfall variables were more strongly correlated with final disease severity than temperature variables over a 1-month period from the time of first disease detection. Specifically, severity was >30% in southern Brazil locations with rainfall events greater than 12.5 cm, distributed over 5 to 10 rainy days during the 1-month period. In accordance with the previous mentioned environmental variables the following parameters were set up for application treatments if SBR was present in the Gadsden County and: if 3 consecutive days with 10 hours or more of leaf wetness and night time temperatures (from 2200 hours to 0500 hours) between 20 to 25 °C occurred fungicide was applied to the temperature/dew duration treatments; if over 5 days within the past 30 days, cumulative rain was equal or greater than 13 cm fungicide was applied to the rainfall event treatments. These treatments were compared to a control (no fungicide application) and a traditional fungicide application schedule with one application at soybean growth stage R1 (flowering) and one application at growth stage R3 (pod development). Presence of SBR in the county, on soybean or kudzu was obtained from the Ipm-PIPE website (www.sbrusa.net). Fungicide was applied using a platform sprayer with a 7-nozzle 11002 Turbo teejet at 35 psi and 25.7 L per ha.

Weather data were obtained from a FAWN station located on the North Florida Research and Education Center campus in Quincy, FL, which records observations every 15 min at 60 cm above the soil. Hourly averages of temperature and relative humidity were calculated and used to calculate the dew point using Equation 5-6 (10).

$$T_d = \frac{[b * \alpha(T, RH)]}{[a * \alpha(T, RH)]} \quad (5\text{-}6)$$

Where $\alpha (T, RH) = (a*T)/(b+T) + \ln (RH)$, where T is the temperatures in degrees Celsius, RH is the measured relative humidity, T_d is the calculated dew point temperature (°C) and "ln" refers to the natural logarithm. The constants are: a = 17.27 and b = 237.7 (°C). The uncertainty in the measured dew point temperature is a function of the measured temperature and relative humidity and the uncertainties associated with those measurements. The uncertainty in the calculated dew point temperature is ±0.4° C. This expression is based on the "Magnus" (or "Magnus-Tetens") approximation for the saturation vapor pressure of water in air as a function of temperature (10). It is considered valid for 0 °C < T < 60 °C; 1% < RH < 100%; 0 °C < Td < 50 °C. Dew point temperatures were then compared with the hourly average air temperature and hours where the difference between the two variables was 0 to 2 were counted as dew leaf wetness hours for daily calculations (55). Weather data, from the previously mentioned FAWN station, was also used to calculate the number of hours below 0 °C from 30 November to 17 April prior to each planting and total rainfall from March through September.

Results

Based on infection of 50% of the total area of susceptible soybean, susceptible kudzu, and resistant kudzu in Florida the calculated maximum potential amount of spores escaping from the host canopy is 8.1×10^{12}, 5.3×10^{12}, and 8.5×10^{11}, respectively. The number of uredinia required to produce the maximum amount of inoculum from susceptible soybean, susceptible kudzu, and resistant kudzu is 1.5×10^{12}, 1.2×10^{12}, and 5.4×10^{11}, respectively. The average date that infection has been first observed on kudzu and soybean in Florida from 2005 to 2010 was used as the

initial infection date, which on average was in mid March for kudzu and mid June for soybean. Using the average initial infection date and optimum parameters (Table 5-2) the maximum amount of inoculum produced on soybean occurred 93 days after initial infection (DAI) in mid September and on susceptible kudzu 196 DAI at the end of September (Figure 5-1). The point in time that the maximum amount of inoculum would be produced on resistant kudzu was not predicted (Figure 5-1) due to the epidemic staying in the early lag phase (Figure 5-2).

Based on the calculated rates of disease increase (ρ) of SBR on different hosts during different months of a growing season in north Florida (Table 5-4), temperature conditions from April through October are suitable for the development of SBR on susceptible soybean and susceptible kudzu, but not on resistant kudzu (Figure 5-2). SBR epidemics on resistant kudzu never reached the logistic phase and hence never attain a rate of disease increase (ρ) greater than 1.00. All temperature conditions examined are more suitable for SBR development on soybean than kudzu. May through October have the highest rates of disease increase for soybean and susceptible kudzu, with May and September temperature conditions near optimal promoting the shortest latent period (*LP* = 6 to 7 on soybean and *LP* = 9 to 10 on susceptible kudzu). The first increase in spores collected by the wind-vane spore traps occurred in August, followed by the highest amount of spores collected in mid September in both 2008 and 2009 (Figure 5-3). This agrees with the high calculated rate of disease increase, ρ and the shorten latent period calculated by the disease model (Table 5-4, Figure 5-2).

Disease pressure varied over the 3 years the decision model was tested in the field, as indicated by the first observation of SBR on kudzu and soybean (Table 5-5).

Furthermore, visual disease symptoms in plots were only observed in 2009 (Figure 5-4), although soybean yield was only significantly lower in control plots regardless of treatment in 2009 (Figure 5-5). In 2009 the rainfall event and leaf wetness-based decision applications were effective for controlling SBR (Figure 5-4), although the yields from these treatments were not significantly greater than a traditional application at growth stages R1 (flowering) and R3 (pod development) (Figure 5-5). In 2010 a traditional application and leaf wetness-based application resulted in the highest yield, although they were not significantly greater than the control yield. Furthermore, in 2010 the yields from the control and rain based application were not significantly different, which was expected because of the lack of significant rain events no applications were applied in the rain based treatments. In 2011 yields across treatments and controls were not significantly different, due to the lack of disease pressure.

The calculated rates of disease increase, ρ, of SBR on soybean and susceptible kudzu during the growing seasons in 2009, 2010 and 2011 in Quincy, FL (Table 5-6) indicate that sufficient temperature conditions occurred May through September each year; although greater and more consistent disease increase occurred in 2009. The lower ρ values in 2010 and 2011 season indicate later disease onset and slower disease development. Similar to the calculated ρ of SBR on resistant kudzu from the average temperature conditions (Table 5-4), the calculated ρ of SBR on resistant kudzu using temperature conditions in 2009 to 2011 was not greater than 1.00, because epidemics on resistant kudzu never reach the logistic phase (data not shown). Based on first detection of SBR on kudzu and soybean in 2009 through 2011 and monthly temperature conditions high disease severity was estimated in 2009, but not in 2010

and 2011 (Figure 5-6). Similarly, high disease severity estimated in 2008 can be contributed to early disease onset and previously reported rain events (225). Infections and potential initial inoculum survival through winter temperatures most likely affected disease onset each year. The winter of 2008 to 2009 in Quincy, Florida had approximately 180 hr below 0 °C, whereas winters of 2009 to 2010 and 2010 to 2011 had 265 and 306 hr below 0 °C, respectively. In both 2010 and 2011 growing seasons there were drought conditions. Total rainfall from March through September in 2009 was 1107 mm in Quincy, FL, whereas in 2010 and 2011 total rainfall was only 719 and 739 mm, respectively.

Discussion

Under optimal conditions, the available area of hosts in Florida, and the average disease onset of SBR, soybean and susceptible kudzu have the potential to contribute a significant number of spores for long distance transport, while resistant kudzu has very low to no potential to contribute spores for long distance transport. The lack of severe SBR epidemics on resistant kudzu is due to the reduced infection efficiency and spore production. This further reduces the total area of kudzu available to contribute spores for long distance transport and initial inoculum for infection of soybean. Temperature conditions in May, June, and September were near optimal for SBR development (Table 5-4, Figure 5-2) on soybean and susceptible kudzu. Similarly, Pivonia and Yang (150), reported the optimal month for SBR reproduction in Baton Rouge, LA based on temperature conditions was in May and September.

Comparing SBR incipience times in Florida, the results indicate conditions for disease development are suitable for infection of soybean 2 months prior to the actual

appearance on soybean using the average of mid June. Although comparing SBR development on susceptible kudzu in Florida the results indicate less suitable conditions until May. This may explain why SBR epidemics on kudzu have been observed to develop slowly during the spring and early summer despite early detection (32). Furthermore, a lower disease increase rate ρ implies a longer time for disease to develop to a detectable level (150). Although kudzu canopies may have a more conducive environment for SBR establishment and development than portrayed by the temperature conditions used in this study.

The estimates of the potential maximum inoculum load occurring in September in Florida correspond to the maximum amount of spores collected in September from wind-vane spore traps in Quincy, FL in 2008 and 2009 (Figure 5-1 and 5-3). Furthermore, the greater number of sporulating uredinia on susceptible kudzu in 2009 (Figure 5-6), due to earlier disease onset and more conducive temperature conditions, may account for the higher number of spores collected in 2009 than in 2008. These data indicate the parameters used in this study can accurately predict time periods of potential inoculum based on disease onset and temperature conditions.

This study evaluated the effect of host and temperature to address suitability for disease development and estimation of potential inoculum for long distance transport. The effect of temperature conditions on infection efficiency and latent period taken from the literature were from studies conducted at constant temperatures, while effects on infection efficiency and latent period may differ under naturally fluctuating temperature conditions. Comparing the temperature effect on the latent period of lettuce downy mildew using average temperatures from naturally and theoretically fluctuating

temperatures to previous work conducted at constant temperature conditions revealed that under fluctuating temperatures there was a smaller temperature effect on the length of the latent period than reported under constant temperature conditions (165,166). Hence, effect of temperature on latent period and infection efficiency may be improved by the inclusion of daily temperature oscillations. Moreover, calculated dew durations are affected by the actual temperature and relative humidity at a particular time of day, the estimates being generally least accurate early in the morning (164,218). Future studies which include more factors into a disease simulation model might further increase the accuracy of time period of SBR onset and development.

In 2009 all soybean plants that received fungicide treatments had significantly greater yields than untreated soybean plants. The rain and leaf wetness based applications were significantly greater at controlling SBR in 2009, although the yields from these treatments were not significantly greater than the traditional application at growth stages R1 (beginning bloom) and R3 (pod development) (Figure 5-4 and 5-5). While disease severity reached approximately 75% in traditional application plots the yield was not significantly different from those that did not develop disease symptoms, most likely because disease did not develop until reproductive stage R7 (beginning maturity) after seed fill. Fungicide spray programs for SBR tested in 2006 and 2007 in Alabama, found fungicide applications at soybean reproductive growth stages R2 (full bloom) and R5 (beginning seed) resulted in significantly higher yields ($P \leq 0.1$) than untreated soybean when SBR was first detected at growth stages R4-R5 (pod fill and beginning seed) (174). Hence, soybean that has fungicide applied at early soybean reproductive growth stages (R1 and R3 or R2 and R5) can provide significantly greater

yield than untreated soybean when SBR is present in the surrounding area and conducive conditions for disease development occur.

Depending on the soybean growth stage when symptoms of SBR are first detected and environmental conditions additional fungicide applications may or may not result in greater yields. In this study, additional fungicide applications (more than the two applications at soybean growth stages R1 and R3) did not result in significantly greater yield due to the late appearance of SBR symptoms at soybean growth stage R7 (Table 5-5 and Figure 5-5). However, three applications of tebuconazole at R1, R3, and R5 did result in significantly greater yields than two applications at R1 and R3 when SBR was first detected at R4 in fungicide trials conducted in Quincy, FL in 2006 (131). Therefore, as first disease detection occurs earlier in the growing season (i.e. on earlier soybean growth stages) additional fungicide applications may provide greater protection from yield loss due to SBR.

In 2010 and 2011 fungicide applications did not significantly increase yield compared to non-treated soybean due to the lack of substantial SBR inoculum and conducive environmental conditions for disease development. While SBR was detected in other soybean fields in Quincy, FL in 2010 and 2011 when soybeans were at growth stages R6 and R1-R2, respectively, SBR symptoms were never observed in the experimental plots. This can be explained by the low number of estimated sporulating uredinia based on first detection and temperature conditions (Figure 5-6). Hence, a model should use both soybean growth stage when SBR is first detected as well as environmental conditions to guide fungicide applications to reduce impact on yield loss.

Furthermore, increased hours of winter temperatures below 0 °C prior to the 2010 and 2011 seasons, compared to 2009, reduced initial inoculum and contributed to later disease onset. Similarly, drought conditions in 2010 and 2011 reduced inoculum dispersal and disease development. Therefore, while temperature conditions were conducive for SBR development in 2010 and 2011, the lack of initial inoculum due to winter temperatures delayed disease onset and lack of substantial rain events retarded disease development. First detection of SBR within the county of planted soybean can be used as a risk indicator, but would be best utilized in combination with environmental data to assess potential dispersal of inoculum and rate of disease increase to aid in fungicide application decisions.

Based on temperature conditions in north Florida from 2005 through 2011, there is a narrow window of suitable conditions for SBR establishment and development in May and June and again in September, which is similar to that found for Baton Rouge, LA (150). This may hinder early SBR establishment in the south and consequently its northward spread, but may still potentially impact yields of late-planted soybean in the south. This study provides fundamental data on inoculum potential from infected kudzu in Florida which can improve the accuracy of SBR forecasting models, but further incorporation of other variables affecting inoculum dispersal are needed to analyze potential risk in northern soybean production areas. The potential impact on yield due to low levels of SBR severity and the lack of resistant varieties commercially available affirms the destructive potential of SBR. The destructive potential of SBR and milder winters and significant changes in rainfall events due to climate change emphasizes the

need for further research and forecasting of SBR to better assess and predict SBR risk to U.S. soybean production.

Table 5-1. Variables used to estimate the potential maximum amount of soybean rust inoculum escaping from different host canopies in Florida

Variables	Susceptible Soybean	Susceptible Kudzu	Resistant Kudzu
Spore production per 50 mm^2 per day (SP_a)	224	26	7
Half of total area (ha) of host in FL (A_{50})	4500	50500	32500
Leaf area index (LAI)	3	6	6
Spore escape rate (E)	0.12	0.12	0.12

Table 5-2. Variables, at optimal conditions, used to calculate the number of new uredinia per day over time

Variables	Susceptible Soybean	Susceptible Kudzu	Resistant Kudzu
Infection efficiency (*IE*)	0.19	0.09	0.009
Spore production per uredinium (SP_u)	45	36	13
Proportion of spores deposited (*D*)	0.1	0.1	0.1
Latent period (*LP*)	6	9	12
Infection period (*IP*)	21	21	21

Table 5-3. Monthly temperature for 2005 to 2008 across 5 different locations in north Florida

	March	April	May	June	July	August	September	October
Mean	15.3	18.4	22.9	26.0	26.6	26.8	24.9	21.1
Minimum	8.0	10.7	15.7	20.0	21.6	22.0	19.5	15.2
Maximum	22.8	26.2	30.4	33.3	33.8	33.9	31.9	28.5
Mean night[a]	11.6	14.5	19.3	23.0	24.1	24.4	22.2	18.2

[a] Mean night temperature is estimated as the average of the mean and minimum temperatures.

Table 5-4. Rates of disease increase. Rates of disease increase (ρ)[a] calculated for different hosts and months as affected by temperatures, during the exponential phase of soybean rust, where higher ρ values represent more suitable conditions for rust reproduction

Host	Optimal conditions	March	April	May	June	July	August	September	October
Susceptible soybean	1.30	1.06	1.16	1.29	1.25	1.21	1.21	1.25	1.24
Susceptible kudzu	1.13	1.02	1.07	1.12	1.12	1.11	1.11	1.12	1.11
Resistant kudzu[b]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] Rate of disease increase per day [ρ=$Y(t)/Y(t\text{-}1)$] was calculated using $Y(t) = IE \text{x} D \text{x} SP_u[Y(t\text{-}LP) – Y(t\text{-}LP\text{-}IP)] + Y(t\text{-}1)$, using the initial conditions, $Y(t) = 0$ at $–(LP + IP) < t < 0$ and $Y(t) = IE \text{ x } I$ at $t = 0$, where $Y(t)$ is the number of new sporulating uredinia at day t; IE is infection efficiency; D is the proportion of spores deposited within the canopy of the spore origin; SP_u is the spore production per uredinium per day; LP is latent period; IP is the infectious period in days; and I is the initial influx number of spores at the beginning of the year. In the model, 100 viable spores were assumed successfully infecting host plants ($Y(t) = IE \text{ x } I = 100$ at $t = 0$).

[b] Soybean rust epidemics on resistant kudzu never reach the logistic phase and hence never attain a rate of disease increase (ρ) greater than 1.00

Table 5-5. Application decision model results and first observations of soybean rust (SBR) on kudzu and soybean each year

Plant date	SBR detection on kudzu[a]	SBR detection on soybean[b]	Treatment[c]	Applications[d]	Yield (Kg/H)
5 May 2009	24 April 2009	7 July 2009	Control	0	1181
			Tradational	2	2193
			Rain event	4	2632
			Leaf wetness	6	2569
17 May 2009	15 July 2010	10 Sept. 2010	Control	0	3350
			Tradational	2	4011
			Rain event	0	3912
			Leaf wetness	4	3231
24 June 2011	12 July 2011	25 Aug. 2011	Control	0	3149
			Tradational	2	3123
			Rain event	1	3215
			Leaf wetness	3	3465
18 July 2011	12 July 2011	25 Aug. 2011	Control	0	2810
			Tradational	2	2648
			Rain event	1	2627
			Leaf wetness	2	2696

[a] First report of SBR establishment on kudzu after winter conditions

[b] First report of SBR establishment on soybean after winter conditions

[c] Treatments included: Control - no application of fungicide; Traditional - fungicide application at soybean growth stage R1 (flowering) and R3 (pod development); Rain event - fungicide applied if SBR present in county and if over 5 days within the past 30 days cumulative rain was equal or greater than 13 cm; Leaf wetness - fungicide applied if SBR present in county and if 3 consecutive days with 10 hours or more of leaf wetness and night time temperatures (from 2200 to 0500 hours) between 20 to 25 °C occurred

[d] The number of application of the triazole fungicide Folicur ® 3.6 (tebuconazole)

Table 5-6. Rates of disease increase. Rates of disease increase (ρ)[a] calculated for soybean and susceptible kudzu and months of different years as affected by temperatures, during the exponential phase of soybean rust, where higher ρ values represent more suitable conditions for rust reproduction

Year	Host	March	April	May	June	July	August	September	October
2009	Susceptible soybean	-	1.00	1.26	1.27	1.22	1.22	1.22	1.24
	Susceptible kudzu	-	1.00	1.11	1.11	1.10	1.11	1.10	1.12
2010	Susceptible soybean	-	1.00	1.27	1.27	1.23	1.21	1.24	1.24
	Susceptible kudzu	-	1.00	1.11	1.11	1.10	1.11	1.12	1.12
2011	Susceptible soybean	-	1.08	1.20	1.27	1.23	1.22	1.23	1.24
	Susceptible kudzu	-	1.02	1.08	1.11	1.10	1.11	1.11	1.12

[a] Rate of disease increase per day [ρ=$Y(t)/Y(t\text{-}1)$] was calculated using $Y(t) = IE \times D \times SP_u[Y(t\text{-}LP) - Y(t\text{-}LP\text{-}IP)] + Y(t\text{-}1)$, using the initial conditions, $Y(t) = 0$ at $-(LP + IP) < t < 0$ and $Y(t) = IE \times I$ at $t = 0$, where $Y(t)$ is the number of new sporulating uredinia at day t; IE is infection efficiency; D is the proportion of spores deposited within the canopy of the spore origin; SP_u is the spore production per uredinium per day; LP is latent period; IP is the infectious period in days; and I is the initial influx number of spores at the beginning of the year. In the model, 100 viable spores were assumed successfully infecting host plants ($Y(t) = IE \times I = 100$ at $t = 0$).

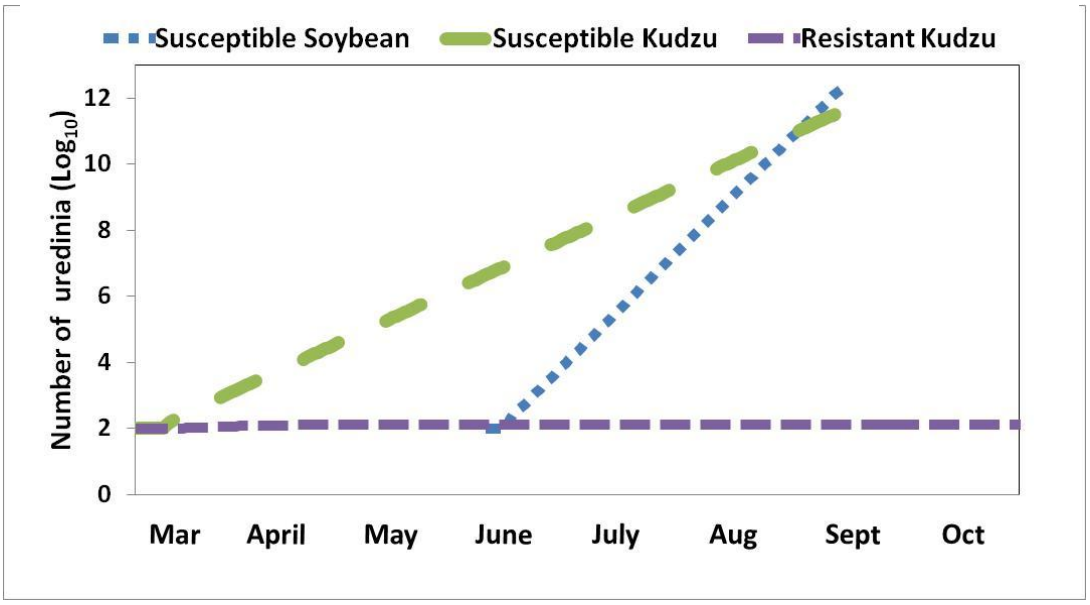

Figure 5-1. The number of uredinia developing on different hosts in Florida over time at optimal condition for disease development.

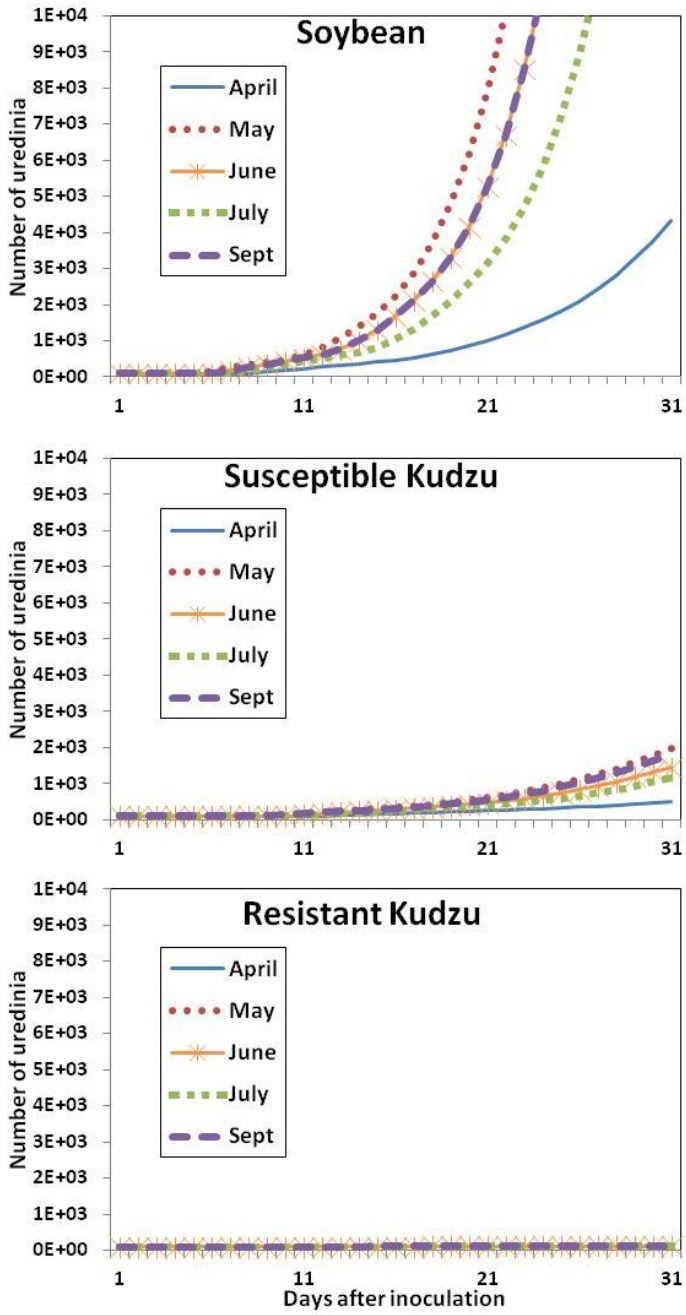

Figure 5-2. Soybean rust infection progress curves. Soybean rust infection progress curves were calculated for hypothetical epidemics, staying in the exponential phase, on 3 different hosts in Florida, under temperature conditions of several months during the soybean growing season.

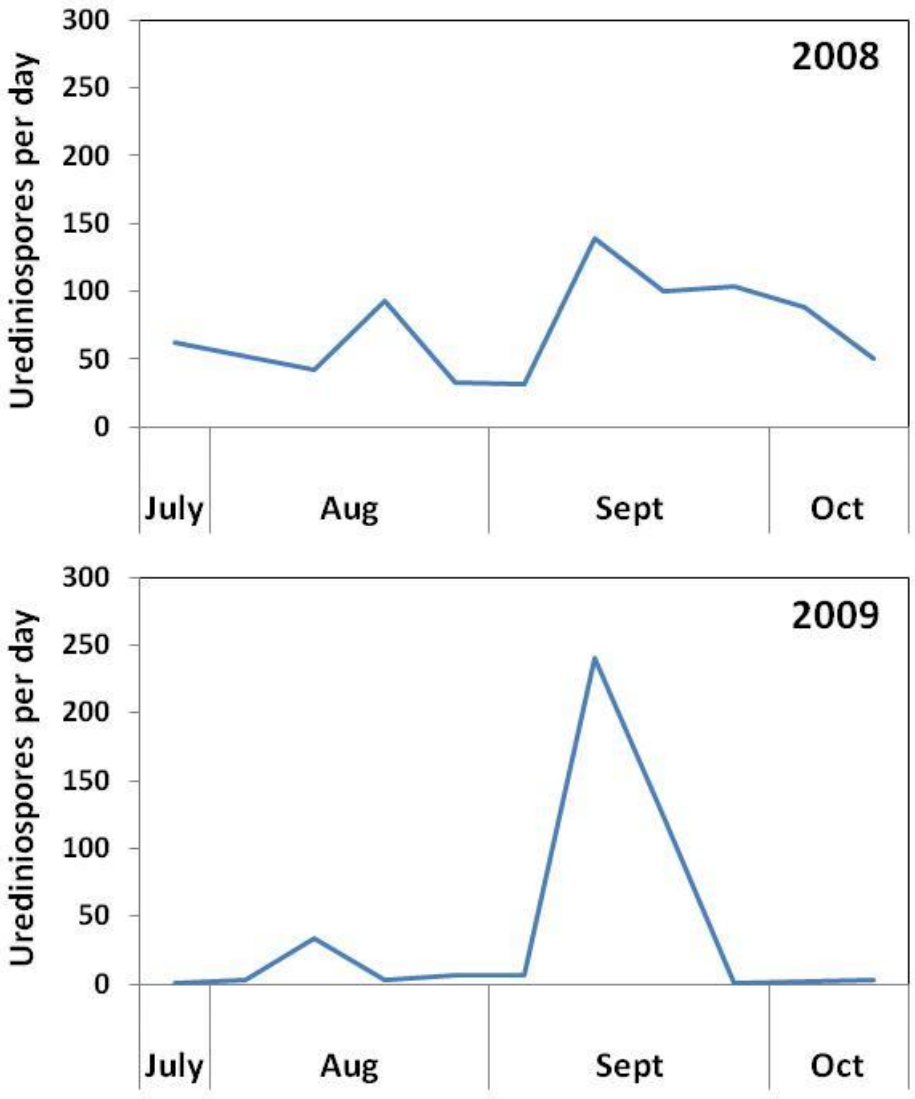

Figure 5-3. Enumeration of visual urediniospores from passive wind-vane spore traps. Wind-vane spore traps utilized white petrolatum coated slides to capture spores, which were then analyzed by microscopic evaluation. Wind-vane spore traps were location at North Florida Research and Education Center, Quincy, FL. from June to October.

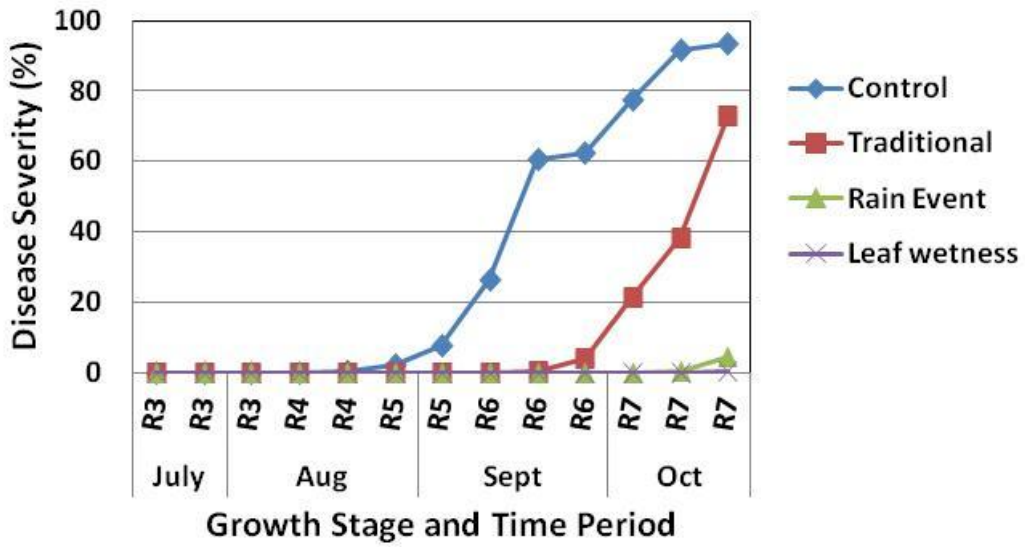

Figure 5-4. Disease severity of soybean rust (SBR) within fungicide treatment plots. Severity of SBR recorded in plots in 2009 located at the North Florida Research Education Center in Quincy, FL. Treatments included Control - no application of fungicide; Traditional - fungicide application at soybean growth stage R1 (flowering) and R3 (pod development); Rain event - fungicide applied if SBR present in county and if over 5 days within the past 30 days cumulative rain was equal or greater than 13 cm; Leaf wetness - fungicide applied if SBR present in county and if 3 consecutive days with 10 hours or more of leaf wetness and night time temperatures (from 2200 to 0500 hours) between 20 to 25 °C occurred.

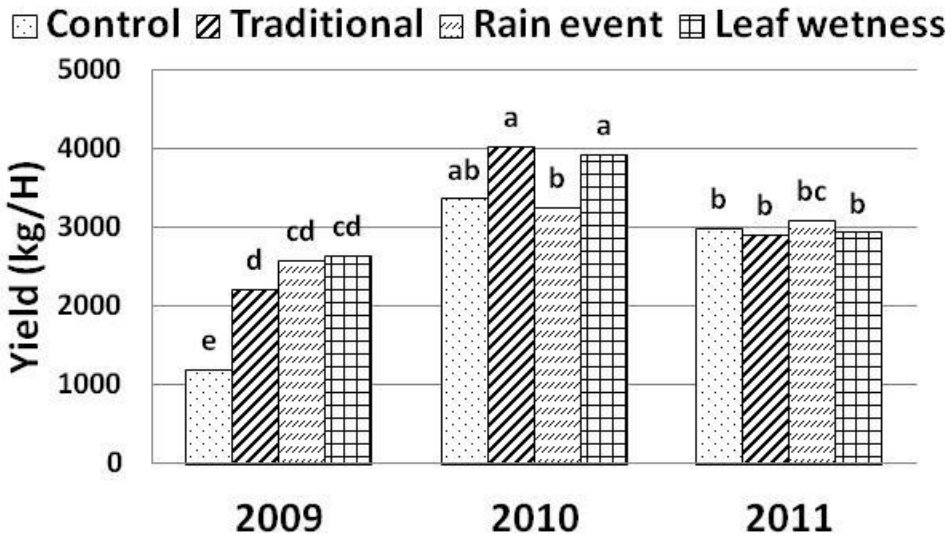

Figure 5-5. Average soybean yields (kg/H) from fungicide application treatment plots. Plots were planted at the North Florida Research and Education Center, in Quincy, FL in 2009, 2010, and 2011. Treatments included: Control - no application of fungicide; Traditional - fungicide application at soybean growth stage R1 (flowering) and R3 (pod development); Rain event - fungicide applied if soybean rust (SBR) present in county and if over 5 days within the past 30 days cumulative rain was equal or greater than 13 cm; Leaf wetness - fungicide applied if SBR present in county and if 3 consecutive days with 10 hours or more of leaf wetness and night time temperatures (from 2200 to 0500 hours) between 20 to 25 °C occurred.

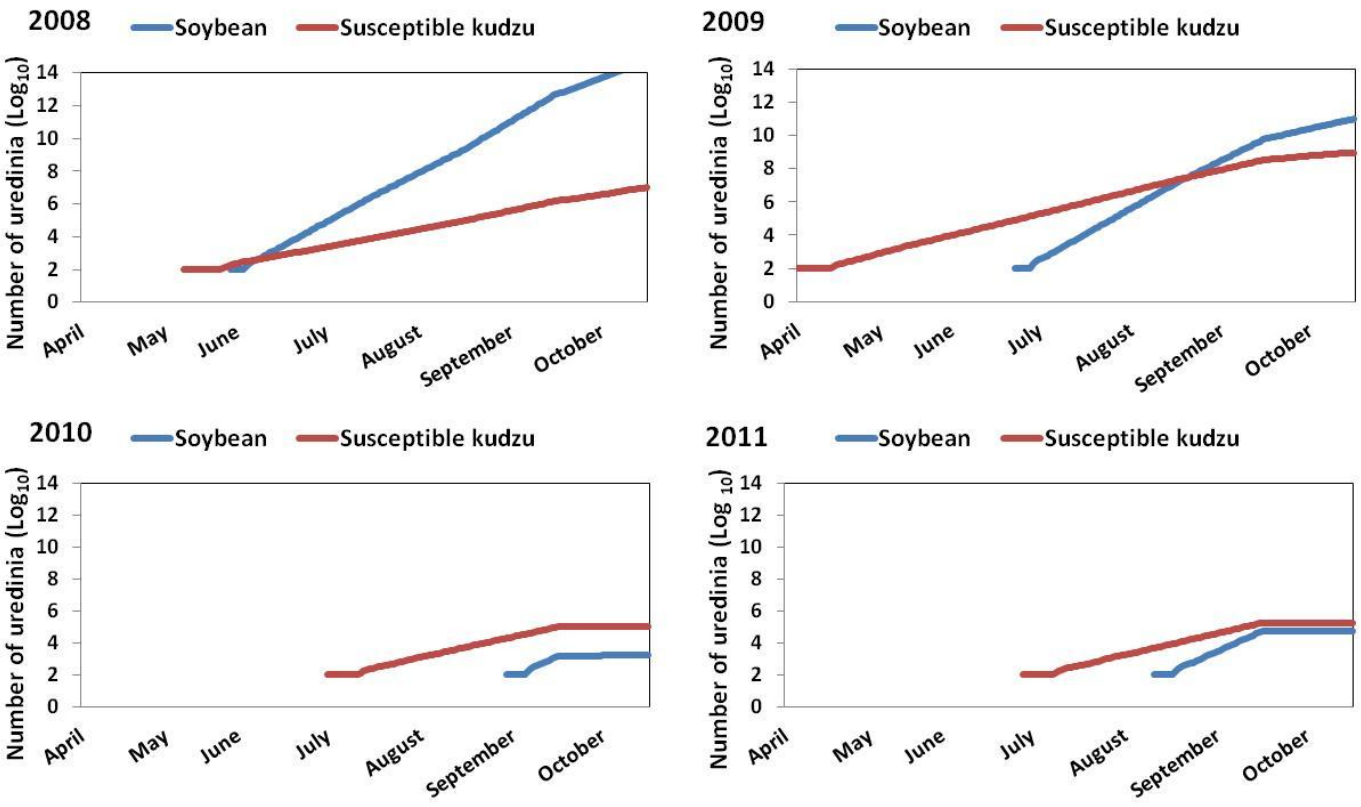

Figure 5-6. Estimated number of sporulating uredinia over time on soybean and susceptible kudzu. Estimation based on temperature conditions effect on infection efficiency and latent period; using first detection data and temperature data from Gadsden County in north Florida in 2008 through 2011.

LIST OF REFERENCES

1. Abramovitz, J. N. 1983. *Pueraria lobata* Willd. (Ohwi) kudzu: limitations to sexual reproduction. MS thesis, University of Maryland, College Park, MD, USA.

2. Akem, C. N. 1995. The effect of timing of fungicide applications on the control of frogeye leaf spot and grain yield on soybeans. European J. of Plant Path. 101:183- 187.

3. Akinsanmi, O. A., Ladipo, J. L., and Oyekan, P. O. 2001. First report of soybean rust (*Phakopsora pachyrhizi*) in Nigeria. Plant Dis. 85: 97.

4. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

5. Ameline-Torregrosa, C., Wang, B. B., O'Bleness, M. S., Deshpande, S., Zhu, H., Roe, B., Young, N. D., and Cannon, S. B. 2007. Identification and characterization of nucleotide-binding site-leucine-rich repeat genes in the model plant *Medicago truncatula*. Plant Phys. 146:5-21.

6. Andrade, D., Pan, Z., Dannevik, W., and Zidek, J. 2009. Modeling soybean rust spore escape from infected canopies: model description and preliminary results. Journal of Applied Meteorology and Climatology. 48:789-803.

7. Asthana, A., and Tuveson, R. W. 1992. Effects of UV and phototoxins on selected fungal pathogens of citrus. Int. J. Plant Sci. 153:442-452.

8. Aylor, D. E. 1986. A framework for examining inter-regional aerial transport of fungal spores. Ag. and For. Meteorol. 38:263-288.

9. Baker, E. A. 1974. The influence of environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. New Phytol. 73:955-966.

10. Barenbrug, A. W. T. 1974. Psychrometry and Psychrometric Charts, 3rd Edition, Cape and Transvaal Printers Ltd.,Cape Town, S.A.

11. Batchelor, W. D., Yang, X. B., and Tschanz, A. T. 1997. Development of a neural network for soybean rust epidemics. Tans. ASAE 40:247-252.

12. Beattie, G. A., and Marcell, L. M. 2002. Effect of alterations in cuticular wax biosynthesis on the physicochemical properties and topography of maize leaf surfaces. Plant, Cell Environ. 25:1-16.

13. Beuerlein, J. and Dorrance, A. E. 2005. Chapter 5: Soybean production. Ohio Agronomy Guide, 14th Edition.

14. Board, J. E., and Harville, B. G. 1998. Late-planted soybean yield response to reproductive source/sink stress. Crop Sci. 38:763-771.

15. Bonde, M. R., Melching, J. S., and Bromfield, K. R. 1976. Histology of the suscept-pathogen relationship between *Glycine max* and *Phakopsora pachyrhizi*, the cause of soybean rust. Phytopathology 66:1290-1294.

16. Bonde, M. R., Nester, S. E., Austin, C. N., Stone, C. L., Frederick, R. D., Hartman, G. L., and Miles, M. R. 2006. Evaluation of virulence of *Phakopsora pachyrhizi* and *P. meibomiae* isolates. Plant Dis. 90:708-716.

17. Bonde, M. R., Nester, S. E., Berner, D. K., and Frederick, R. D. 2008. Comparative susceptibility of legume species to infection by *Phakopsora pachyrhizi*. Plant Dis. 92:30-36.

18. Bonde, M. R., Nester, S. E., Moore, W. F., and Allen, T. W. 2009. Comparative susceptibility of kudzu accessions from the southeastern United States to infection by *Phakopsora pachyrhizi*. Plant Dis. 93:593-598.

19. Boyette, C. D., Abbas, H. K., and Walker, H. L. 2000. Bioherbicidal control of kudzu with *Myrothecium verrucaria*. Phytopathology 90:S9.

20. Bromfield, K. R. 1984. Soybean rust. Monograph. The American Phytopathological Society: St. Paul, MN. pg. 1-58.

21. Bromfield, K. R., and Hartwig, E. E. 1980. Resistance to soybean rust and mode of inheritance. Crop Sci. 20:254-255.

22. Bromfi eld, K. R., and J. S. Melching. 1982. (Abstract) Sources of specific resistance to soybean rust. Phytopathology 72:706.

23. Britton, O. K., Orr, D., and Sun, J. H. 2002. Biological control of Kudzu, *Pueraria montana* var*. lobata*. In: Biological Control of Invasive Plants in the Eastern United States (eds R Van Driesche, B Blossey, M Hoddle, S Lyon & R Reardon), 323– 325, USDA, Forest Service FHTET-2002–04, Morgantown, WV, USA.

24. Butler, D. R., and Jadhav, D. R. 1991. Requirements of leaf wetness and temperature for infection of groundnut by rust. Plant Pathol. 40:395-400.

25. Bryan, G. T., Wu, K. S., Farrall, L., Jia, Y., Hershey, H. P., McAdams, S. A., Faulk, K. N., Donaldson, G. K., Tarchini, R., and Valent, B. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell 12:2033-2046.

26. CAB International. 2008. Crop Protection Compendium. CABI. Online publication.

27. Caldwell, S. P. and Myers, O. J. 1980. Seasonal field mapping of soybean cyst nematode populations as influenced by cultivars and blends. In World Soybean Research Conference II, 1979, ed. F. T. Corbin. Boulder, CO: Westview (Abstr.).

28. Caldwell, P. and Laing, M. 2002. Soybean rust - A disease on the move. Southern African Society for Plant Pathology Monthly Feature. March 2002. Online publication.

29. Canteri, M. G., Caramori, P., Tsukahara, R., Silva, O. C., Faria, R., and Godoy, C. V. 2005. A system to map risk of infection by *Phakopsora pachyrhizi* for Parana State, Brazil. Phytopathology 95:S16.

30. Casey, P. S. 1979. The epidemiology of soybean rust – *Phakopsora pachyrhizi* Syd. Ph.D. thesis, University of Sydney, 203 pp.

31. Chakraborty, N., J. Curley, R. D. Frederick, D. L. Hyten, R. L. Nelson, G. L. Hartman, and Diers, B. W. 2009. Mapping and confirmation of a new allele at *Rpp1* from soybean PI 594538A conferring RB lesion-type resistance to soybean rust. Crop Sci. 49:783–790.

32. Christiano, R. S. C. and Scherm, H. 2007. Quantitative aspects of the spread of Asian soybean rust in the southeastern United States, 2005 to 2006. Phytopathology 97:1428-1433.

33. Clark, J. A., and Levitt, J. 1956.The basis of drought resistance in the soybean plant. Physiol. Plant. 9:598-606.

34. Corley, R. N. Woldeghebriel, W., and Murphy, M. R. 1997. Evaluation of the nutritive value of kudzu (*Pueraria lobata*) as a feed for rumints. Animal Feed Science and Technology 68:183-188.

35. Dangl, J. L. and Jones, J. D. G. 2001. Plant pathogens and integrated defense responses to infection. Nature 411:826-833.

36. Dias, A. P. S., Li, X., Harmon, P. F., Harmon, C. L., and Yang, X. B. 2011. Effects of shade intensity and duration on Asian soybean rust caused by *Phakopsora pachyrhizi*. Plant Dis. 95:485-489.

37. Delaney, D. P., Lawrence, K. S., Sikora, E. J., Lawence, G. W., Delaney, M. A., and Pegues, M. D. 2007. Efficacy of foliar fungicides for Asian soybean rust disease management and yield enhancement in Alabama. Proc. 2007 National Soybean Rust Symposium.

38. Del Ponte, E. M., and Esker, P. D. 2008. Meteorological factors and Asian soybean rust epidemics-A systems approach and implications for risk assessment. Sci. Agric. (Piracicaba, Braz.) 65:88-97.

39. Del Ponte, E. M., Godoy, C. V., Canteri, M. G., Reis, E. M., and Yang, X. B. 2006. Models and applications for risk assessment and prediction of Asian soybean rust epidemics. Fitopatol. Bras. 31:533-544.

40. Del Ponte, E. M., Godoy, C. V., Li, X., and Yang, X. B. 2006. Predicting severity of Asian soybean rust epidemics with empirical rainfall models. Phytopathology. 96:797-803.

41. Dias, A. P. S., Li, X., Harmon, P. F., Harmon, C. L., and Yang, X. B. 2011. Effects of shade intensity and duration on Asian soybean rust caused by *Phakopsora pachyrhizi*. Plant Dis. 95:485-489.

42. Dixon, M. S., Hatzixanthis, K. Jones, D. A. Harrison, K., and Jones, J. D. G. 1998. The tomato Cf 5 disease resistance gene and six homologs show pronounced allelic variation in leucine-rich copy number. Plant Cell 10:1915-1926.

43. Dixon, M. ,Jones, D. A., Keddie, J. S. Thomas, C. M., Harrison, K. and Jones, J. D. 1996. The tomato Cf 5 disease resistance locus comprises two functional genes encoding leucine-rich repeat proteins. Cell 84:451-459.

44. Dodds, P. N., Lawrence, G. J., and Ellis, J. G. 2001. Six amino acid changes confined to the leucine-rich repeat β-strand/β-turn motif determine the difference between the *P* an *P2* rust resistance specificities in flax. Plant Cell 13:163-178.

45. Dorrance, A. E., Draper, M. A., and Hershman, D. E. eds. 2008. Using foliar fungicides to manage soybean rust. Land-Grand Universities Cooperation NCERA-208 and OMAF, Bulletin SR-2008.

46. Dufault, N. S., Isard, S. A., Marois, J. J., and Wright, D. L. 2010. Removal of wet deposited *Phakopsora pachyrhizi* urediniospores from soybean leaves by subsequent rainfall. Plant Dis. 94:1336-1340.

47. Dufresne, L. and Bean, G. 1987. Effects of temperature and light intensity on telia development by Puerto Rico and Taiwan isolates of *Phakopsora pachyrhizi*, the soybean rust fungus. Plant Dis. 71:629-631.

48. Everest, J. W., Miller, J. H., Ball, D. M., and Patterson, M. G. 1991. Kudzu in Alabama. Alabama Cooperative Extension Service Circular ANR-65, Auburn University, Auburn, Alabama, USA.

49. Excoffier, L., Laval, G., Schneider, S. 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. Evol. Bioinform.1:47–50.

50. Excoffier, L., Smouse, P. E., and Quattro, J. M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

51. Farris, J. and Reilly, C. C. 2000. The biological control of kudzu (*Pueraria lobata*). Proceeding of the American Association for the Advancement of Science, Washington, D.C. 116: AB2.

52. Flenet, F. Kiniry, J. R., Board, J. E., Westgate, M. E., and Reicosky, D. C. 1996. Row spacing effects on light extinction coefficients of corn, sorghum, soybean, and sunflower. Agron. J. 88:185-190.

53. Funke, T. Han, H., Healy-Fried, M. L., Fischer, M., and Schönbrunn, E. 2006. Molecular basis for the herbicide resistance of Roundup Ready crops. PNAS. 103. 13010-13015.

54. Garcia, A., Calvo, E. S., de Souza Kiihl, R.A., Harada, A., Hiromoto, D.M., and Vieira, L.G.E. 2008. Molecular mapping of soybean rust (*Phakopsora pachyrhizi*) resistance genes: Discovery of a novel locus and alleles. Theor. Appl. Genet. 117:545–553.

55. Gates, D. M. 1968. Transpiration and Leaf Temperature. Annl Rev Plant Physiol. 19:211-238.

56. George, S., Sharma, J., and Yadon, V. L. 2009. Genetic diversity of the endangered and narrow endemic *Piperia yadonii* (Orchidaceae) assessed with ISSR polymorphisms. Am. J. Bot. 96:2022-2030.

57. Gevens, A. J., Nequi, N., Vitoreli, A., Marois, J. J., Wright, D. L., Harmon, C. L., and Harmon, P. F. 2008. First Report of Soybean Rust Caused by Phakopsora pachyrhizi on *Erythrina herbacea* (Coral Bean). Plant Dis. 92:1472.

58. Ghajar, F., Holford, O., Cother, E., and Beattie, A. 2006. Effects of ultraviolet radiation, simulated or as natural sunlight, on conidium germination and appressorium formation by fungi with potential as mycoherbistats. Biocontrol Science and Technology 16:451-469.

59. Godoy, C. 2009. Changes in performance of soybean rust fungicides over years and new management strategies adopted in Brazil. 2009 Natl. Soybean Rust Symp., New Orleans, LA. 9-11 Dec. 2009.

60. Gonzalez, R., Paul, N. D., Percy, L., Ambrose, M. McLaughlin, C. K., Barnes, J. D., Aresea, M., and Wellburn, A. R. 1996. Responses to ultraviolet-B radiation

(280-315 nm) of pea (*Pisum sativum*) lines differing in leaf surface wax. Physiol. Plant. 98: 852-860.

61. Green, A. 1984. Soybean Rust. Pest Not Known to Occur in the United States or of Limited Distribution No. 56. USDA-APHIS-PPQ.

62. Gregory, P. H. 1973. Microbiology of the Atmosphere, ed. N. Polunin. Leonard Hill Books. Aylesbury. 377.

63. Guard, A. T. 1931. Development of floral organs of the soybean. Bot. Gaz. 91:97-102.

64. Gumpert, F. M., Geiger, H. H. and Stahle, U. 1987. A mathematical model of the epidemics in homogeneous and heterogeneous host stands. Zeitschrift Pflanzendrankheiten Pflanzenschutz 94:206-215.

65. Harmon, P. F., Momol, M. T., Marois, J. J., Dankers, H., and Harmon, C. L. 2005. Asian soybean rust caused by *Phakopsora pachyrhizi* on soybean and kudzu in Florida. Plant Health Progress. doi:10.1094/PHP-2005-0613-01-RS.

66. Hartman, G. L., Sinclair, J. B., and Rupe, J. C. 1999. Compendium of Soybean Disease 4th Edition. APS Press.

67. Hartman, G. L, Miles, M. R., and Frederick, R. D. 2005. Breeding for resistance to soybean rust. Plant Dis. 89:664-666.

68. Hartwig, E. E. 1986. Identification of a fourth major gene conferring resistance to soybean rust. Crop Sci. 26:1135–1136.

69. Hartwig, E. E., and K. R. Bromfield. 1983. Relationships among three genes conferring resistance to rust in soybeans. Crop Sci. 23:237–239.

70. Hau, B., Eisensmith, S. P., and Dranz, J. 1985. Construction of temporal model: II. Simulation of aerial epidemics. Pages 31-66 in: Advances in Plant Pathology, Vol 3. C. A. Gilligan, ed. Academic Press, Orlando, FL.

71. Haudenshield, J. S., Chaudhary, P., and Hartman, G. L. 2010. Recovery of *Phakopsora pachyrhizi* urediniospores from passive spore trap slides and extraction of their DNA for quantitative PCR. Phytopathology 100:S185.

72. Henning, A. A. and Godoy, C. V., 2006. Situaça˜o da ferrugem da soja no Brasil e no mundo. In: Zambolim, L. (Ed.), Ferrugem Asia´ tica da Soja. UFV. Viçosa, Brazil, pp. 1–14.

73. Holliday, P. 1998. A Dictionary of Plant Pathology, 2 ed. Cambridge University Press. Cambridge.

74. Hymowitz, T. 1990. Soybeans: The Success Story. Advances in New Crops. Pages 159-163.

75. Hyten, D. L., Hartman, G. L., Nelson, R. L., Frederick, R. D., Concibido, V. C., Narvel, J. M., and Cregan P. B. 2007. Map location of the *Rpp1* locus that confers resistance to soybean rust in soybean. Crop Sci. 47:837–840.

76. Hyten, D. L., Smith, J. R., Frederick, R. D., Tucker, M. L., Song, Q., and Cregan, P. B. 2009. Bulk segregate analysis using the Golden Gate assay to locate the *Rpp3* locus that confers resistance to *Phakopsora pachyrhizi* (soybean rust) in soybean. Crop Sci. 49:265–271.

77. Isakeit, T., Miller, M. E., Saldana, R., Barnes, L. W., McKemy, J. M., Palm, M. E., Zeller, K. A., DeVries-Paterson, R., and Levy, L. 2006. First Report of rust 23 caused by *Phakopsora pachyrhizi* on soybean and kudzu in Texas. Plant Dis. 90:971.

78. Isard, S. A., Dufault, N. S., Miles, M. R., Hartman, G. L., Russo, J. M., de Wolf, E. D., & Morel, W. (2006). The effect of solar irradiance on the mortality of *Phakopsora pachyrhizi* urediniospores. Plant Disease. 90:941-945.

79. Isard, S. A., Gage, S. H., Comtois, P., and Russo, J. M. 2005. Principles of the atmospheric pathway for invasive species applied to soybean rust. BioScience 55:851-861.

80. Isard, S. A., Russo, J. M., and De Wolf, E. D. 2006. The establishment of a national Pest Information Platform for Extension and Education. Published Online at Plant Heath Progress. doi: 10.1094/PHP-2006-0915-01-RV.

81. Ishii, H., Fraaije, B. A., Sugiyama, T., Noguchi, K., Nishimura, K., Takeda, T., Amano, T., and Hollomon, D. W. 2001. Occurrence and Molecular Characterization of Strobilurin Resistance in Cucumber Powdery Mildew and Downy Mildew. Phytopathology 91:1166-1171.

82. Jordan, S. A., Mailhot, D. J., Gevens, A. J., Marois, J. J., Wright, D. L., Harmon, C. L., and Harmon, P. F. 2010. Characterization of kudzu (*Pueraria* spp.) resistance to *Phakopsora pachyrhizi*, the causal agent of soybean rust. Phytopathology 100:941-948.

83. Jurick, W. M., Narváez, D. F., Brennan, M. M., Harmon, C. L., Marois, J. J., Wright, D. L., and Harmon, P. F. 2008. Winter survival of the soybean rust pathogen, *Phakopsora pachyrhizi*, in Florida. Plant Dis. 92:1551-1558.

84. Kakani, V. G., Reddy, K. R., Zhao, D., and Sailaja, K. 2003. Field crop responses to ultraviolet-B radiation: a review. J. Agric. Forest. Meteorol. 120:191-218.

85. Keller, M., Rogiers, S. Y., and Schultz, H. R. 2003. Nitrogen and ultraviolet radiation modify grapevines' susceptibility to powdery mildew. Vitis 42:87-94.

86. Killgore, E., and Heu, R. 1994. First report of soybean rust in Hawaii. Plant Dis. 78:1216.

87. Kim, Y. S., Dixon, P., Vincelli, P., and Farman, M. L. 2003. Field resistance to strobilurin (QoI) fungicides in *Pyricular grisea* caused by mutations in the mitochondrial cytochrome b gene. Phytopathology 93:891-900.

88. Kim, K. S., Wang, T. C., and Yang, X. B. 2005. Simulation of apparent infection rate to predict severity of soybean rust using a fuzzy logic system. Phytopathology 95:1122-1131.

89. Kobe, B. and Deisenhofer, J. 1995. A structural basis of the interactions between Leucine-rich repeats and protein ligands. Nature 374:183-186.

90. Koch, E., Ebrahim Nesbat, F., and Hoppe, H. H. 1983. Light and electron microscopic studies on the development of soybean rust (*Phakopsora pachyrhizi* Syd.) in susceptible soybean leaves. Phytopathol. Z. 106:302-320.

91. Koch, E. and Hoppe, H. H. 1988. Development of infection structures by the direct penetrating soybean rust fungus (*Phakopsora pachyrhizi* Syd.) on artificial membranes. J. Phytopathol. 122:232-244.

92. Kochman, J. K. 1979. The effect of temperature on development of soybean rust. Aust. J. Agric. Res. 30:273-277.

93. Krause, R. A. and Massie, L. B. 1979. Predictive systems: modern approaches to disease control. Annual Review of Phytopathology 13:31-47.

94. Krupa, S., Bowersox, V., Claybrooke, R., Barnes, C. W., Szabo, L., Harlin, K., and Kurle, J. 2006. Introduction of Asian soybean rust urediniospores into the Midwestern United States - A case study. Plant Dis. 90:1254-1259.

95. Kuck, K. H. and Mehl, A. 2004. Prothiconazole: Sensitivity profile and anti-resistance strategy. Pflanzenschutz-Nachrichten Bayer 57:225-236.

96. Kumudini, S., Godoy, C. V., Board, J. E., Omielan, J., Tollenaar, M. 2008. Mechanisms Involved in Soybean Rust-Induced Yield Reduction. Crop Sci. 48:2334-2342.

97. Kwok, I. M. Y. and Loeffler, R. T. 1993. The biochemical mode of action of some newer azole fungicides. Pestic. Sci., 39:1-11.

98. Leister, R. T. and Katagiri, F. 2000. A resistance gene product of the nucleotide binding site – Leucine rich repeats class can form a complex with bacterial avirulence proteins in vivo. Plant J. 22:345-354.

99. Lemes, E. M., Mackowiak, C. L., Blount, A., Marois, J. J., Wright, D. L., Coelho, L., and Datnoff, L. E. 2011. Effects of silicon application on soybean rust development under greenhouse and field conditions. Plant Dis. 95:317-324.

100. Levy, C. 2003. A field evaluation of eight fungicides in the control of soybean rust in Zimbabwe during 2002-03. Commercial Oilseeds Producer Association, Harare, Zimbabwe.

101. Levy, C. 2005. Epidemiology and chemical control of soybean rust in southern Africa. Plant Dis. 89: 699-647.

102. Li, X., Esker, P. D., Pan, Z., Dias, A. P., Xue, L., and Yang, X. B. 2010. The Uniqueness of the Soybean Rust Pathosystem: An Improved Understanding of the Risk in Different Regions of the World. Plant Dis. 94:796-806.

103. Lin, S. Y. 1966. Studies on the physiologic races of soybean rust fungus, Phakopsora pachyrhizi Syd. J. Agric. Res. (Taiwan) 15:24-28.

104. Livingston, M., Johansson, R., Daberkow, S., Roberts, M., Ash, M., and Breneman, V. 2004. Economic and policy implications of wind-borne entry of asian soybean rust into the United States. U. ERS. Editor. Online publication.

105. Lynch, T. N., Marois, J. J., Wright, D. W., Harmon, P. F., Harmon, C. L., Miles, M. R., and Hartman, G. L. 2006. First report of soybean rust caused by Phakopsora pachyrhizi on Phaseolus species in the United States. Plant Dis. 90:970.

106. Mantecon, J. 2001. Timing of fungicide applications for the control of soybean brown spot. F & N Tests 60:FC126.

107. Marchall, D. S., and Rush, M. C. 1980. Infection cushion formation on rich sheaths by *Rhizoctonia solani*. Phytopathology 70:947-950.

108. Marchetti, M. A., Urecker, F. A., and Bromfield, K. R. 1975. Uredial development of *Phakopsora pachyrhizi* in soybeans. Phytopathology 65:822-823.

109. Marchetti, M. A., Melching, J. S., and Bromfield, K. R. 1976. The effect of temperature and dew period on germination and infection by urediniospores of *Phakopsora pachyrhizi*. Phytopathology 66:461-463.

110. Marta, A. D., Di Stefano, V., Cerovic, Z. G., Agati, G., and Orlandini, S. 2008. Solar radiation affects grapevine susceptibility to *Plasmopara viticola*. Sci. Agric. 65:65-70.

111. McLean, R. J. 1979. Histological studies of resistance to soybean rust, Phakopsora pachyrhizi Syd. Aust. J. Agr. Res. 30:77-84.

112. McLean, R. J., and D. E. Byth. 1980. Inheritance of resistance to rust (*Phakopsora pachyrhizi*) in soybeans. Aust. J. Agric. Res. 31:951–956.

113. McWilliams, D. A., Berglund, D. R., and Endres, G. J. 2004. Soybean Growth and Management Quick Guide. Online Publication: NDSU Agriculture and University Extension. A-1174.

114. Melching, J. S., Dowler, W. M., Koogle, D. L., and Royer, M. H. 1989. Effects of duration, frequency, and temperature of leaf wetness periods on soybean rust. Plant Dis. 73:117-122.

115. Meyers, B. C., Dickerman, A. W., Michelmore, R. W., Sivaramakrishnan, S., Sobral, B. W., and Young, N. D. 1999. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding superfamily. Plant J. 20:317-332.

116. Meyers, B. C., Kozik, A., Griego, A., Kuang, H., and Michelmore, R. W. 2003. Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. Plant Cell 15:809–834.

117. Meyer, J. D. F., Silva, D. C. G., Yang, C., Pedley, K. F., Zhang, C., van de Mortel, M., Hill, J. H. Shoemaker, R. C., Abdelnoor, R. V., Whitham, S. A., and Graham, M. A. 2009. Identification and analyses of candidate genes for *Rpp4*-mediated resistance to Asian soybean rust in soybean. Plant Physiol. 150:295-307.

118. Miles, M. R., Frederick, R. D. and Hartman, G. L. 2003. Soybean Rust: Is the U.S. Soybean Crop At Risk? APSnet Features. Online. doi: 10.1094/APSnetFeature-2003-0603.

119. Miles, M., Frederick, R., and Hartman, G. 2006. Evaluation of soybean germplasm for resistance to Phakopsora pachyrhizi. Plant Health Prog. 10: doi/ 10.1094/PHP-2006-0104-01-RS.

120. Miles, M. R., Hartman, G. L., Levy, C., and Morel, W. 2003. Current status of soybean rust control by fungicides. Pesticide Outlook:197-200.

121. Miles, M. R., Morel, W., and Hartman, G. 2003. Summary of the USDA fungicide efficacy trials to control soybean rust in Paraguay 2002-2003. p. 1 - 8.

122. Miles, M. R., Morel, W., Steinlage, T., and Hartman, G. L. 2004. Summary of the USDA fungicide efficacy trials to control soybean rust in Paraguay 2003-2004. p. 1 -11.

123. Miles, M. R., Levy, C., Morel, W., Meuller, T., Steinlage, T., van Rij, N., Frederick, R. D., and Hartman, G. L. 2007. International fungicide efficacy trials for the management of soybean rust. Plant Dis. 91:1450-1458.

124. Miller, R. N. G. Bertioli, D. J., Baurens, F. C., Santos, C. M. R., Alves, P. C., Martins, N. F., Togawa, R. C., Souza Junior, M. T., and Pappas Junior, G. J. 2008. Analysis of non-TIR NBS-LRR resistance gene analogs in *Musa acuminate* Colla: isolation, RFLP marker development, and physical mapping. BMC Plant Biol. 8:15 doi:10.1186/1471-2229-8-15.

125. Modolo, L. V., Cunha, F. Q., Braga, M. R., and Salgado, I. 2002. Nitric oxide synthase-mediated phytoalexin accumulation in soybean cotyledons in response to the *Diaporthe phaseolorum* f. sp. meridionalis elicitor. Plant Physiol. 130:1288-1297.

126. Moffett, P., Farnham, G., Peart, J., and Baulaombe, D. C. 2002. Interaction between domains of a plant NBS-LRR protein in disease resistance-related cell death. EMBO Journal 21:4511-4519.

127. Monteros, M. J., A. M. Missaoui, D. V. Phillips, D. R. Walker, and Boerma, H. R. 2007. Mapping and confirmation of the 'Hyuuga' red-brown lesion resistance gene for Asian soybean rust. Crop Sci. 47:829–836.

128. Morel, W. and Yorinori, J. 2002. Ministerio de Agricultura y Ganaderia, Centro Regional de Investigacion Agricola, Capitan Miranda, Paraguay. Bol. Divulg. No. 44.

129. Mueller, D. S., Dorrance, A. E., Derksen, R. C., Ozkan, E., Kurle, J. E., Grau, C. R., Gaska, J. M., Hartman, G. L., Bradley, C. A., and Pedersen, W. L. 2002. Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of Sclerotinia stem rot on soybean. Plant Dis. 86:26-31.

130. Mueller, T. A., Miles, M. R., Hartman, G. L., O'Brien, G. K., Marois, J. J., and Wright, D. L. 2008. Evaluation of fungicides and fungicide timing for the control of soybean rust in northwest Florida, 2006. Plant Disease Management Reports (online). Report 2:FC086. doi:10.1094/PDMR02. American Phytopathological Society, St. Paul, MN.

131. Mueller, T. A., Miles, M. R., Morel, W., Marois, J. J., Wright, D. L., Kemerait, R. C., Levy, C., and Hartman, G. L. 2009. Effect of fungicide and timing of application on soybean rust severity and yield. Plant Dis. 93:243-248.

132. NAPDFC. 2006. North American Plant Disease Forecast Center. Soybean Rust Forecast Homepage. Online publication.

133. Narváez, D. F., Jurick, W. M., Marois, J. J., and Wright, D. L. 2010. Effects of surface wetness periods on development of soybean rust under field conditions. Plant Dis. 94:258-264.

134. National Statistics for Soybeans – National Agricultural Statistics Service (NASS). Agricultural Statistics Board USDA. 2010. Online publication.

135. Nguyen, H. T. 2011. Seed Composition. Molecular Genetics & Soybean Genomics Laboratory, University of Missouri. Online publication.

136. NOAA. 2006. National Oceanic and Atmospheric Administration. Atmospheric Research Laboratory. NOAA ARL HYSPLIT Model. Online publication.

137. NPAG 2002. NPAG Data: *Phakopsora pachyrhizi* Australasian soybean rust. Exotic Fungus Not in the Continental United States. p. 1-14.

138. Nybom, H. and Bartish, IV. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Perspectives in Plant Ecology, Evolution and Systematics 3:93-114.

139. Ogle, H., Byth, D., and Mclean, R. 1979. Effect of rust (*Phakopsora pachyrhizi*) on soybean yield and quality in Southeastern Queensland. . Australian J. of Ag. Res. 30:883-893.

140. Ono, Y., Buriticá, P., and Hennen, J. F. 1992. Delimitation of *Phakopsora*, *Physopella* and *Cerotelium* and their species on *Leguminosae*. Mycol. Res. 96:825-850.

141. Ozkan, H. E., Zhu, H., Derksen, R. C., Guler, H., and Krause, C. R. 2006. Evaluation of spraying equipment for effective application of fungicides to control Asian soybean rust. Aspects of App. Bio. 77:423-431.

142. Pan, Z., Yang, X. B., Pivonia, S., Xue, L., Pasken, R., and Roads, J. 2006. Long-term prediction of soybean rust entry into the continental United States. Plant Dis.90:840-846.

143. Pan, Z., Xue, L., Del Ponte, E. M., Andrade, D., Li, X., Pasken, R. and Yand, X. B. 2005. Soybean rust dispersal prediction and analysis in the U.S. for 2005 growing season. Proceedings, National Soybean Rust Symposium, Nashville, TN, USA. Online publication.

144. Pappert, R. A., Hamrick, J. L., and Donovan, L. A. 2000. Genetic variation in *Pueraria lobata* (Fabaceae), an introduced, clonal, invasive plant of the southeastern United States. Am. J. Bot. 87:1240-1245.

145. Park, S., Chen, Z. Y., Chanda, A. K., Schneider, R. W., & Hollier, C. A. 2008. Viability of *Phakopsora pachyrhizi* urediniospores under simulated southern Louisiana winter temperature conditions. Plant Dis. 92: 1456-1462.

146. Parry, D. W. (1990). Plant pathology in agriculture. New York City, NY: Cambridge University Press.

147. Pedersen, P. 2007. Soybean planting date. Soybean Extension and Research Program, Department of Agronomy, Iowa State University. Online publication.

148. Pedersen, W. L. and Leath, S. 1988. Pyramiding major genes for resistance to maintain residual eff ects. Annu. Rev. Phytopathol. 26:369–378.

149. Pivonia, S. and Yang, X. B. 2004. Assessment of potential year round establishment of soybean rust throughout the world. Plant Dis. 88:523-529.

150. Pivonia, S. and Yang, X. B. 2006. Relating epidemic progress from a general disease model to seasonal appearance time of rusts in the United States: implications for soybean rust. Phytopathology 96:400-407.

151. Pivonia, S., Yang, X. B., and Pan, Z. 2005. Assessment of epidemic potential of soybean rust in the United States. Plant Dis. 89:678-682.

152. Pretorius, Z. A., Kloppers, F. J., and Frederick, R. D. 2001. First Report of Soybean Rust in South Africa. Plant Dis. 85: 1288.

153. Reed, K. L., Hamerly, E. R., Dinger, R. F., and Jarvis, P. G. 1976. An analytical model for field measurements of photosynthesis. J. Appl. Ecol. 13:925-942.

154. Reis, E. M., Sartori, A. F., and Camara, R. K. 2004. Modelo climatic para a previsão da ferrugem da soja. Summa Phytopathologica 30:290-292.

155. Ribeiro, A. S., Moreira, J. U. V., Pierozzi, P. H. B., Rachid, B. F., de Toledo, J. F. F., Arias, C. A. A., Soares, F. M., and Godoy, C. V. 2007. Genetic control of Asian rust in soybean. Euphytica 157:15-25.

156. Richly, E., Kurth, J., Leister, D. 2002. Mode of amplification and reorganization of resistance genes during recent Arabidopsis thaliana evolution. Mol. Biol. Evol. 19:76–84.

157. Rodríguez, A. C., Rios, J. A., and Hernández, J. R. 2006. First Report of Asian Soybean Rust Caused by *Phakopsora pachyrhizi* from Mexico. Plant Dis. 90: 1260.

158. Rossi, R. L. 2003. First Report of *Phakopsora pachyrhizi*, the Causal Organism of Soybean Rust in the Province of Misiones, Argentina. Plant Dis.87: 102.

159. Rotem, J., Wooding, B., and Aulor, D. E. 1985. The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. Phytopathology 75: 510-514.

160. Rytter, J. L., Dowler, W. M., and Bromfield, K. R. 1984. Additional alternative hosts of *Phakopsora pachyrhizi*, causal agent of soybean rust. Plant Dis. 68:818-819.

161. Sakai, A. K., Allendorf, F. W., Holt, J. S. et al. 2001. The population biology of invasive species. Ann. Rev. Ecol. Syst. 32, 305–332.

162. Saksirirat, W. and Hoppe, H. H. 1991. Teliospore germination of soybean rust fungus (Phakopsora pachyrhizi Syd.). J. Phytopathol. 32:339-342.

163. Sangawongse, P., Kittisin, S., and Nunthapun, M. 1977. Chemical control of soybean rust in Thailand. Thai. J. Agric. Sci. 10:1-8.

164. Scherm, H. and van Bruggen, A. H. C. 1993. Sensitivity of simulated dew duration to meteorological variations in different climatic regions of California. Agric. For. Meteorol. 66:229-245.

165. Scherm, H. and van Bruggen, A. H. C. 1994. Effects of fluctuating temperatures on the latent period of lettuce downy mildew (*Bremia lactucae*). Phytopathology 84:853-859.

166. Scherm, H. and van Bruggan, A. H. C. 1994. Global warming and nonlinear growth: how important are changes in average temperature? Phytopathology 84:1380-1384.

167. Schneider R. W., Hollier, C. A., and Whitam, H.K. 2005. First report of soybean rust caused by *Phakopsora pachyrhizi* in the continental United States. Plant Dis.89:774.

168. Schneider, R. W., Robertson, C. A., Giles, C. G., and Mumma, E. P. 2005. Evaluation of fungicides, registered to control Asian soybean rust, for the control of Cercospora leaf blight. F & N Tests 61:FC015.

169. Sconyers, L. E., Kermerait, R. C., Brock, J., Phillips, D. V., Jost, P. H., Sikora, E. J., Gutierrez-Estrada, A., Mueller, J. D., Marois, J. J., Wright, D. L., and Harmon,

C. L. 2006. Asian soybean rust development in 2005: A perspective from the Southeastern United States. APSnet Feature. Online publication.

170. Sconyers, L. E., Kemerait, Jr. R. C., Brock, J. H., Gitaitis, R. D., Sanders, F. H., Phillips, D. V., and Jost, P. H. 2006. First report of Phakopsora pachyrhizi, the causal agent of Asian soybean rust, on Florida beggar weed in the United States. Plant Dis. 90:972.

171. Sharma, N. D., and Mehta, S. K. 1996. Soybean rust in Madhya Pradesh. Acta Botanica Indica 24:1150116.

172. Shibles, R., Anderson, I. C., and Gibson, A. H. 1975. Soybean. Pages 151-189 in: Crop physiology: Some case histories, L. T. Evans, ed. Cambridge University Press, NY.

173. Sierotzki, H., Wullschleger, J., and Gisi, U. 2000. Point-mutation in cytochrome b gene conferring resistance to strobilurin fungicides in *Erysiphe graminis* f. sp. *tritici* field isolates. Pesticide Biochemistry and Physiology 68:107-112.

174. Sikora, E. J., Delaney, D. P., Delaney, M. A., Lawrence, K. S., and Pegues, M. 2009. Evaluation of sequential fungicide spray programs for control of soybean rust. Online. Plant Health Progress doi: 10.1094/PHP-2009-0402-01-RS.

175. Silva, D. C. G, Yamanaka, N., Brogin, R. L., Arias, C. A. A., Nepomuceno, A. L., Mauro, A. D., Pereira, S. S., Noguiera, L. M., Passianotto, A. L. L., and Abdelnoor, R. V. 2008. Molecular mapping of two loci that confer resistance to Asian rust in soybean. Theor. Appl. Genet. 117:57-63.

176. Sinclair, J. B. and Hartman, G. L. eds. 1996. Soybean rust workshop. College of Agricultural, Consumer, and Environmental Sciences. National Soybean Research Laboratory Pub. n. 1.

177. Skoss, J. D. 1955. Structure and composition of the plant cuticle in relation to environmental factors and permeability. Bot. Gaz .117:55-72.

178. Slaminko, T. L., Miles, M. R., Frederick, R. D., Bonde, M. R., and Hartman, G. L. 2008. New legume hosts of *Phakopsora pachyrhizi* based on greenhouse evaluations. Plant Dis. 92:767-771.

179. Smits, M. M., Slaminko, T. L., Miles, M. R., Frederick, R. D., Bonde, M. R., and Hartman, G. L. 2008. New legume hosts of Phakopsora pachyrhizi based on greenhouse evaluations. Plant Dis. 92:767-771.

180. Soares, R. M. 2007. Balanço da ferrugem asia´ tica na safra 2006/07 com base nos nu´ meros do Sistema de Alerta. In: Anais do Simpo´ sio Brasileiro de

Ferrugem Asia´ tica da Soja. Se´ rie Documentos 281, Embrapa. Londrina, Brazil, pp. 9–13.

181. Sojka, R. E., and Parsons, J. E. 1983. Soybean water and canopy microclimate relationships of four row spacings. Agron. J. 75:961-968.

182. Southerst, R. W. and Maywald, G. F. 1985. A computerized system for matching climates in ecology. Agriculture, Ecosystems and Environment 13:281-299.

183. Soybean and Corn Advisor. 2011. Soybean free period in Brazil Starts. Online publication.

184. Soy Stats. 2010. Soy Statistics and U.S. Soy Crop Statistics. Online publication.

185. Srivastava, P., George, S., Marois, J. J., Wright, D. L., and Walker, D. R. 2011. Saccharin-induced systemic acquired resistance against rust (*Phakopsora pachyrhizi*) infection in soybean: Effects on growth and development. Crop Prot. 30:726-732.

186. Steinmuller, D., and Tevini, M., 1985. Action of ultraviolet radiation (UV-B) upon cuticular waxes in some crop plants. Planta 164, 557-564.

187. Stovold, G. E., and Smith, H. J. P. 1991. The prevalence and severity of diseases in the coastal soybean crop of New South Wales. Aust. J. Exp. Agric. 31:545-550.

188. Sun, C. N. 1955. Growth and development of primary tissues in aerated and non-aerated roots of soybean. Bull. Torr. Bot. Club 82:491-502.

189. Sun, J. H., Li, Z.-C., Jewett, D. K., Britton, K. O., Ye, W. H., and Ge, X.-J. 2005. Genetic diversity of *Pueraria lobata* (kudzu) and closely related taxa as revealed by inter-simple sequence repeat analysis. Eur. Weed Res. Soc. Weed Res. 45:255-260.

190. Tameling, W. I. Elzinga, S. D. Darmin, P. S., Vossen, J. H. Takken, F. L., Haring, M. A., and Cornelissen, B. J. 2002. The tomato R gene products I-2 and MI-1 are functional ATP binding proteins with ATPase activity. Plant Cell 14:2929-2939.

191. Tamural, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28:2731-2739.

192. Tan, Y. J., Yu, Z. L., and Yang, C. Y. 1996. Soybean Rust. China Agriculture Press, Beijing, China.

193. Teng, P. S. 1985. A comparison of simulation approaches to epidemic modeling. Annual Review of Phytopathology 23:351-379.

194. Thompson, J. D., Higgins, D. G., and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22:4673-4680.

195. Thornton, M. R. 2001. Arthropod fauna associated with kudzu (*Pueraria lobata* Willd.) in North Carolina. M.S. thesis, North Carolina State University, Raleigh, North Carolina, USA.

196. Traut, T. W. 1994. The functions and consensus motifs ofnine types of peptide3 segments that form different types of nucleotide-binding sites. Eur J Biochem. 222:9-19.

197. Tschanz, A. T. and Tsai, B. 1982. Effect of maturity on soybean rust development. Soybean Rust Newsletter. 5:38-41.

198. Tschanz, A. T. and Shanmugasundaram, S. 1985. Soybean rust. Pages 562-567 in: III Proceedings of World Soybean Research Conference, R. Shibles ed. Westview Press. Boulder, CO.

199. Tsutsui N. D., Suarez A. V., Holway D. A., and Case T. J. 2000. Reduced genetic variation and the success of an invasive species. Proceedings of the National Academy of Sciences of the USA 97, 5948–5953.

200. Twizeyimana, M. Ojiambo, P. S., Ikotun, T., Paul, C., Hartman, G. L., and Bandyopadhyay, R. 2007. Comparison of field, greenhouse, and detached-leaf evaluations of soybean germplasm for resistance to *Phakopsora pachyrhizi*. Plant Dis. 91:1161-1169.

201. U.S. Soy Production. American Soybean Association – World Initiate for Soy in Human Health. 2010. Online publication.

202. USDA. 2010. United State Soybean Industry: Background Statistics and Information. Online publication.

203. van der Maesen, L. J. G. 1985. Revision of the genus Pueraria DC. With some notes on Teyleria Backer. Agricultural University of Wagenigen Paper 85–1.

204. van de Mortel, M., Recknor, J. C., Graham, M. A., Nettleton, D., Dittman, J.D., Nelson, R.T., Godoy, C.V., Abdelnoor, R. V., Almeida, A. M. R., and Baum, T. J. 2007. Distinct biphasic mRNA changes in response to Asian soybean rust infection. Mol Plant Microbe In. 20: 887–899.

205. Vidhyasekaran, P. 2004. Concise encyclopedia of plant pathology, Food Products Press and The Haworth Reference Press, Binghamton, N.Y.

206. Vincelli, P. 2002. QoI (Strobilurin) fungicides: benefits and risks. The Plant Heath Instructor DOI: 10.1094/PHI-I-2002-0809-02.

207. Walker, D. R., Boerma, H. R., Phillips, D. V., Schneider, R. W., Buckly, J. B., Shipe, E. R., Mueller, J. D., Weaver, D. B., Sikora, E. J., Moore, S. H., Hartman, G. L., Miles, M. R., Harris, D. K., Wright, D. L., Marois, J. J., and Nelson, R. L. 2011. Evaluation of USDA soybean germplasm accessions for resistance to soybean rust in the southern United States. Crop Sci. 51:678-693.

208. Ward, E. W. B., and Buzzell, R. I. 1983. Influence of light, temperature and wounding on the expression of soybean genes for resistance to *Phytophthora megasperma* f. sp. *glycinea*. Physiol. Plant Pathol. 23:401-409.

209. Ward, D. B. 1998. Pueraria montana: the correct scientific name of the kudzu. Castanea 63: 76–77.

210. Ward, N. A., Schneider, R. W., and Aime, M. C. 2011. Colonization of soybean rust sori by *Simplicillium lanosoniveum*. Fungal Eco. 4:303-308.

211. Ward, N. A. 2011. *Simplicillium lanosoniveum*, a mycoparasite of *Phakopsora pachyrhizi* and its use as a biological control agent. Dissertation. Louisiana State University.

212. Watson, S. R., Giesler, L. J., Ziems, A. D. and Brovont, T. E. 2008. Report of Soybean Rust Caused by *Phakopsora pachyrhizi* in Nebraska. Plant Dis.92: 1588.

213. Webster, J. and Weber, R. Introduction to Fungi. 3rd Ed. Cambridge, UK: Cambridge University Press, 2007. 609-635.

214. Welles, J. M., and Cohen, S. 1996. Canopy structure measuring by gap fraction analysis using commercial instrumentation. J. Exp. Bot. 47:1335-1342.

215. Williams, L. F. 1950. Structure and genetic characteristics of the soybean. Pages 11-134 in: Soybeans and soybean products, K. S. Markley, ed. Interscience Publishers, NY.

216. Wrather, J. A., Shannon, J. G., Stevens, W. E., Sleper, D. A., and Arelli, A. P. 2004. Soybean cultivar and foliar fungicide effects on *Phomopsis* sp. seed infections. Plant Dis. 88:721-723.

217. Wright, D. L., Rich, J. R., Marois, J. J., Sprenkel, R. K., and Ferrell, J. A. 2002. Soybean Production in Florida. Online Publication: Electronic Data Information Source of UF/IFAS Extension (EDIS). SS-AGR-182.

218. Wu, B. M., van Bruggen, A. H. C., Subbarao, K. V., and Pennings, G. G. H. 2001. Validation of weather and leaf wetness forecasts for a lettuce downy mildew warning system. Can. J. Plant Pathol. 23:371-383.

219. Yang, X. B., Dowler, W. M., and Tschanz, A. T. 1991. A simulation model for assessing soybean rust epidemics. Journal of Phytopathology 133:187-200.

220. Yang, X. B., Dowler, W. M., and Royer, M. H. 1991. Assessing the risk and potential impact of an exotic plant disease. Plant Dis. 75:976-982.

221. Yang, X. B., Royer, M. H., Tschanz, A. T., and Tsai, B. Y. 1990. Analysis and quantification of soybean rust epidemics from seventy-three sequential planting experiments. Phytopathology 80:1421-1427.

222. Yang, X. B., Tschanz, A. T., Dowler, W. M., and Wang, T. C. 1991. Development of yield loss models in relation to reductions of components of soybean infected with *Phakopsora pachyrhizi*. Phytopathology. 81:1420-1426.

223. Yeh, C. C., Tschanz, A. T., and Sinclair, J. B. 1981. Induced teliospore formation by Phakopsora pachyrhizi on soybeans and other hosts. Phytopathology 71:1111-1112.

224. Yorinori, J. T., Paiva, W. M., Frederick, R. D., Costamilan, L. M., Bertagnolli, P. F., Hartman, G. L., Godoy, C. V., and Nunes Jr, J. 2005. Epidemics of soybean rust (*Phakopsora pachyrhizi*) in Brazil and Paraguay from 2001 to 2003. Plant Dis.89:675-677.

225. Young, H. M., Marois, J. J, Wright, D. L., Narváez, D. F, and O'Brien, K. 2011. Epidemiology of Soybean Rust in Soybean Sentinel Plots in Florida. Plant Dis.95:744-750.

226. Youssef, B., Subramaniam, R., and Dangl, J. L. 2004. Plant disease resistance protein signaling: NBS-LRR proteins and their partners. Current Opinion in Plant Biology 7:391-399.

227. Zavala, J. A, Scopel, A. L., and Ballaré, C. L. 2001. Effects of ambient UV-B radiation on soybean crops: Impact on leaf herbivory by *Anticarsia gemmatalis*. Plant Ecology 156:121-130.

228. Zhang, L. X., Kyei-Boahen, S., Zhang, J., Zhang, M. H., Freeland, T. B., Watson, C. E., Jr., and Liu, X. M. 2007. Modifications of optimum adaptation zones for soybean maturity groups in the USA. Online. Crop Management. Sept. 2. doi:10.1094/CM-2007-0927-01-RS.

229. Zhou, T., Wang, Y., Chen, J.-Q., Araki, H., Jing, Z., Jiang, K., Shen, J., and Tian, D. 2004. Genome-wide identification of NBS genes in *japonica* rice reveals significant expansion of divergent non-TIR NBS-LRR genes. Mol. Gen. Genomics. 271:402-415.

230. Zidak, N. K. and Backman, P. A. 1996. Biological control of kudzu (*Pueraria lobata*) with the plant pathogen *Pseudomonas syringae* pv. *phaseolicola*. Weed Sci. 44:645-649.

BIOGRAPHICAL SKETCH

Heather Marie Young, daughter of Dennis and Linda Young, was born in Orlando, Florida in 1984. Heather attended Auburndale high school and graduated valedictorian in 2003. While still in high school she attended Polk Community College and in 2004 was awarded and Associate of Arts in Business. She attended Florida State University where she majored in biological sciences and was awarded a Bachelor of Science degree in April 2007 and a Master of Science in science teaching in April 2008. In May 2008, Heather began working at University of Florida as a biological scientist at the North Florida Research and Education Center. Within a year of working in agricultural research she decided to obtain her doctorate and began her doctorate of philosophy in Plant Pathology in the fall of 2009. Under the guidance of Dr. James Marois and Dr. David Wright she conducted research on the biology of the obligate fungal parasite, *Phakopsora pachyrhizi*, the casual agent of soybean rust. Heather was awarded multiple travel grants to present her research at the American Phytopathological Society Annual Meeting in Oahu, Hawaii in summer 2011, at the CropWorld Global International Meeting in London, United Kingdom in fall 2011, and at the American Phytopathological Society Southern Division Meeting in Birmingham, Alabama in spring 2012. She was also awarded first place for her paper and oral presentation at the Southern Soybean Disease Workers conference in Pensacola, Florida in spring 2012. After her PhD, Heather plans on continuing her agricultural research career in academics.