

ABSTRACT

NEUFELD, KATIE NICOLE. Epidemiology of Cucurbit Downy Mildew: Effects of Weather Variables on Infection Parameters and Disease Severity and Source Strength Relationships. (Under the direction of Dr. Peter S. Ojiambo).

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is one of most important diseases of cucurbitaceous crops worldwide. The pathogen is disseminated primarily through aerial transport of sporangia from either overwintering sources or infected cucurbit fields. The recent resurgence of the disease and unavailability of commercially resistant cultivars dictate that disease control now relies heavily on the use of fungicides. Thus, there is a need for continued search for improved methods to effectively use these fungicides for disease control. However, there are still critical gaps in the knowledge of the differential response of cucurbit host types to initial infection and how information on the aerobiology of *P. cubensis* can be incorporated in disease forecasting. Based on this premise, growth chamber experiments were conducted to determine the interactive effects of temperature and leaf wetness duration on infection parameters of *P. cubensis* for different cucurbit host types. Field experiments were also conducted in 2010 and 2011 to establish the effect of disease severity on concentration and escape of sporangia from a cucumber canopy.

In growth chamber experiments, host type, temperature, leaf wetness duration and their interactions affected sporangia germination and disease severity. Temperature and leaf wetness duration that supported a given level of germination or infection varied markedly between cucumber, cantaloupe and acorn squash. These results facilitate estimation of the potential risk of infection of cucurbit host types by *P. cubensis* based on prevailing or forecasted temperature and duration of leaf wetness. In field experiments, disease severity, hour of day and sampling height above the crop canopy affected aerial concentration of

sporangia and sporangia that escaped the canopy. Further, the increase of sporangia concentration and escaped sporangia with disease severity were well described by a log-normal model. These results on the aerobiology of cucurbit downy mildew will be incorporated in a large-scale spore transport model that is currently used to forecast sporangia transport and to predict the risk of cucurbit downy mildew spread and outbreak between and among cucurbit fields in eastern U.S.

Epidemiology of Cucurbit Downy Mildew: Effects of Weather Variables on Infection
Parameters and Disease Severity and Source Strength Relationships

by
Katie Nicole Neufeld

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Plant Pathology

Raleigh, North Carolina

2012

APPROVED BY:

Dr. Peter S. Ojiambo
Committee Chair

Dr. Barbara Shew

Dr. Gary Payne

BIOGRAPHY

Katie Nicole Neufeld was born January 30, 1986 in Hutchinson, Kansas. Katie had the exciting opportunity to live in many communities growing up, from Kansas to Nashville, Tennessee ending up in Raleigh, North Carolina. Katie graduated with honors from Cary Senior High School in 2004. She had a strong interest in science and a love for animals, which led her to pursue an undergraduate degree in Animal Science at North Carolina State University in the fall of 2004.

While working on her undergraduate studies, Katie worked as a veterinary technician and started her own horse boarding facility to help fund her education. She graduated in May of 2008 with a Bachelor of Science in Animal Science and a minor in Genetics. In an effort to gain research experience, Katie began working as a field technician under the direction of Dr. Gerald Holmes in the summer of 2008. Her work on assisting field and laboratory research eventually evolved into working on cucurbit downy mildew under Dr. Peter Ojiambo, a post-doctoral research associate, Dr. Loukas Kanetis, and a visiting scientist from University of Costa Rica, Dr. Luis Arauz, on projects designed to establish the physical variables that affect viability of *P. cubensis* sporangia and sensitivity of *P. cubensis* to various fungicides. After working with the pathogen for some time and having a passion for the field and laboratory work, Katie accepted a research assistantship in the fall of 2009 to work with Dr. Peter Ojiambo on improving aspects of the cucurbit downy mildew forecasting.

ACKNOWLEDGMENTS

I would like acknowledge my committee, Drs. Marc Cubeta and Barbara Shew in providing me with thoughtful insight and guidance during my time as a graduate student. I am most thankful to my advisor, Dr. Peter Ojiambo, for not only guidance, but for kindling the initial interest and providing the necessary support for me to achieve my goals. Sincere thanks are also extended to Dr. Gary Payne, who gracefully accepted to examine my thesis and replace Dr. Cubeta, who was leaving for a sabbatical in Sweden. To all members of the Ojiambo lab, both past and present that have put up with hours of planting and harvesting cucumbers, prepping for fieldwork and providing me with thoughtful insight. They include Mike Adams, Wendy Britton, Thomas Keever, Ashley Crook, Lucky Mehra, Rob Kautz, Charlie Shew and Rebecca Echerd.

A special thank you to my parents, who have continued to encourage and support me, especially during my graduate program, although, they may not fully understand what my research work was all about. My step-parents, for stepping up when you didn't count on having children and treating me like your own. To my little brothers and sisters, you inspire me to be a better person everyday. My friends, for providing invaluable advice, showing me the "ropes", impromptu lunches, or just listening to me rant.

I would also like to acknowledge funding from the following sources that support my graduate studies and research work: North Carolina Agriculture and Research Service, United States Department of Agriculture and Pest Information Platform for Extension and Education Program, and Regional Integrated Pest Management Program- Southern Region, without which my graduate research work would not have been possible.

TABLE OF CONTENTS

List of Tables	vii
List of Figures	ix
1. CHAPTER I: Literature Review	1
1.1. Cucurbitaceae	1
1.2. Pathogen Taxonomy	2
1.3. Pathogen Biology	4
1.4. Host Range and Pathogenicity	6
1.5. Epidemiology	7
1.6. Disease Management	10
1.7. Project Objectives	12
1.8. Literature cited	15
2. CHAPTER II: Interactive Effects of Temperature and Leaf Wetness Duration on Sporangia Germination and Infection of Cucurbit Host Types by <i>Pseudoperonospora cubensis</i>	22
2.1. Abstract	23
2.2. Introduction	24
2.3. Materials and Methods	27
2.3.1 Cucurbit host types and plant growth conditions	27

2.3.2 Pathogen isolate and inoculation of plants	27
2.3.3 Experimental design and data analysis	29
2.3.4 Risk chart models	31
2.4. Results	31
2.4.1 Sporangia germination and disease severity	31
2.4.2 Model evaluation and parameter estimates	33
2.4.3 Risk prediction charts	34
2.5. Discussion	35
2.6. Literature Cited	41
3. CHAPTER III: Quantifying the Relationship between Disease Severity and <i>P. cubensis</i> Sporangia Concentration and Escape from a Cucumber Canopy	54
3.1. Abstract	55
3.2. Introduction	56
3.3. Materials and Methods	59
3.3.1 Field sites, inoculum source and disease assessment	59
3.3.2 Standing crop of sporangia	61
3.3.3 Airborne concentration of sporangia	61
3.3.4 Meteorological measurements	63
3.3.5 Escape of sporangia from the canopy	64
3.3.6 Relationship between disease severity and sporangia concentration and escape	65

3.4. Results	66
3.4.1 Disease severity and standing crop of sporangia.....	66
3.4.2 Meteorological variables and dynamics of sporangia concentration	67
3.4.3 Escape of sporangia from the canopy	68
3.4.4. Relationship between sporangia concentration and escape and disease severity....	69
3.5. Discussion	70
3.6. Literature Cited	76
4. CHAPTER IV: Conclusions	91

LIST OF TABLES

CHAPTER II

TABLE 2.1	Sporangia germination and severity of cucurbit downy mildew on three cucurbit host types inoculated with <i>Pseudoperonospora cubensis</i> in two growth chamber experiments	44
TABLE 2.2	Analysis of variance for the effects of temperature (T), cucurbit host type (S), and leaf wetness duration (W) on germination of sporangia of <i>Pseudoperonospora cubensis</i> and severity of cucurbit downy mildew	45
TABLE 2.3	Parameter estimates for the model: $f(W, T) = f(T) \cdot (1 - \exp\{-[b \cdot W]^d\})$, characterizing the effects of temperature and leaf wetness duration on germination of <i>Pseudoperonospora cubensis</i> sporangia on three cucurbit host types	46
TABLE 2.4	Parameter estimates for the model: $f(W, T) = f(T) \cdot (1 - \exp\{-[b \cdot W]^d\})$, characterizing the effects of temperature and leaf wetness duration on infection by <i>Pseudoperonospora cubensis</i> for three cucurbit host types 5 days post-inoculation	47
TABLE 2.5	Parameter estimates for the model: $f(W, T) = f(T) \cdot (1 - \exp\{-[b \cdot W]^d\})$, characterizing the effects of temperature and duration of leaf wetness on infection by <i>Pseudoperonospora cubensis</i> for three cucurbit host types 7 days post-inoculation	48

CHAPTER III

TABLE 3.1	Standing crop of <i>Pseudoperonospora cubensis</i> sporangia from a cucumber field infected by cucurbit downy mildew at two field sites in North Carolina	79
TABLE 3.2	Concentration of <i>Pseudoperonospora cubensis</i> sporangia at different heights above a cucumber canopy at a source infected with different levels of cucurbit downy mildew severity at two field sites in North Carolina	80
TABLE 3.3	Pearson's coefficients for the correlation between <i>Pseudoperonospora cubensis</i> sporangia collected 0.5 m above a cucumber canopy (sporangia/m ³) and the prevailing relative humidity (%) monitored at a given level of disease severity and hour of day	81
TABLE 3.4	Escape of <i>Pseudoperonospora cubensis</i> from a source field naturally infected with different levels of severity of cucurbit downy mildew in Clayton and Clinton, North Carolina	82

TABLE 3.5 Parameter estimates from nonlinear regression analysis obtained using the log-normal model to describe the relationship between sporangia concentration (C), sporangia escape (F), and disease severity for combined data from two field sites in North Carolina83

LIST OF FIGURES

CHAPTER I

FIGURE 1.1	Total acreage of all cucurbits produced in the United States based on the 2007 USDA Agricultural Census.....	20
FIGURE 1.2	Symptoms of cucurbit downy mildew on cucumber (A), cantaloupe (B), squash (C) and watermelon (D)	21

CHAPTER II

FIGURE 2.1	Effects of temperature and leaf wetness duration on sporangia germination on leaves of three cucurbit host types inoculated with <i>Pseudoperonospora cubensis</i> . Observed values are for A , cantaloupe (cv. Kermit); C , cucumber (cv. Straight 8) and E , acorn squash (cv. Table Queen). Predicted values for B , cantaloupe; D , cucumber and F , acorn squash are from fitting observed values to the model: $f(W, T) = f(T) \cdot (1 - \exp\{-[b \cdot W]^d\})$. See main text for definition of the variables and parameters.....	49
FIGURE 2.2	Proportion of leaf area infected for three cucurbit host types 5 days after inoculation with <i>Pseudoperonospora cubensis</i> . Observed values are for A , cantaloupe (cv. Kermit); C , cucumber (cv. Straight 8) and E , acorn squash (cv. Table Queen). Predicted values for B , cantaloupe; D , cucumber and F , acorn squash are from fitting observed values to the model: $f(W, T) = f(T) \cdot (1 - \exp\{-[b \cdot W]^d\})$. See main text for definition of the variables and parameters.....	50
FIGURE 2.3	Proportion of leaf area infected for three cucurbit host types 7 days after inoculation with <i>Pseudoperonospora cubensis</i> . Observed values are for A , cantaloupe (cv. Kermit); C , cucumber (cv. Straight 8) and E , acorn squash (cv. Table Queen). Predicted values for B , cantaloupe; D , cucumber and F , acorn squash are from fitting observed values to the model: $f(W, T) = f(T) \cdot (1 - \exp\{-[b \cdot W]^d\})$. See main text for definition of the variables and parameters.....	51
FIGURE 2.4	Relationship between the asymptote, $f(T)$, and temperature based on model estimates for predicting sporangia germination and proportion of leaf area infected 7 days after inoculation of cucurbit host types with <i>Pseudoperonospora cubensis</i> . The varieties used for cantaloupe, cucumber and acorn squash were ‘Kermit’, ‘Straight 8’ and ‘Table Queen’, respectively.....	52
FIGURE 2.5	Charts for risk prediction for cucurbit downy mildew infection of three cucurbit host types. A , cantaloupe (cv. Kermit); B , cucumber	

(cv. Straight 8) and **C**, acorn squash (cv. Table Queen). Risk categories are based on the response surface models and isopaths separating the categories correspond to LGT = light (disease severity = 0.00 to 0.10); MOD = moderate (disease severity = 0.11 to 0.20) and SEV = severe (disease severity > 0.20). Isopaths are based on predicted response surfaces for leaf area infected 7 days after inoculating plants with *Pseudoperonospora cubensis*.....53

CHAPTER III

FIGURE 3.1 Temperature, relative humidity and wind speed monitored at Clayton and Clinton field sites in North Carolina in a study to determine the relationship between cucurbit downy mildew severity at the source and concentration and escape of *Pseudoperonospora cubensis* sporangia above the crop canopy. Data shown have been selected to show the range in the monitored weather variables. Wind speed data are based on measurements collected at 10 m above the ground.84

FIGURE 3.2 Diurnal pattern of concentrations of *Pseudoperonospora cubensis* sporangia at different heights above the canopy in a cucumber field naturally infected with different levels of cucurbit downy mildew severity at Clayton research station in Johnston County, North Carolina.....85

FIGURE 3.3 Diurnal pattern of concentrations of *Pseudoperonospora cubensis* sporangia at different heights above the canopy in a cucumber field naturally infected with different levels of cucurbit downy mildew severity at Clinton research station in Sampson County, North Carolina.....86

FIGURE 3.4 Vertical profiles of *Pseudoperonospora cubensis* sporangia concentrations measured above a source in a cucumber field from 0700 to 1400 h87

FIGURE 3.5 Escape of *Pseudoperonospora cubensis* sporangia from a source field naturally infected with different levels of cucurbit downy mildew in Clayton, North Carolina. On each assessment date, disease severity was assessed visually as percent of leaf area.....88

FIGURE 3.6 Escape of *Pseudoperonospora cubensis* sporangia from a source field naturally infected with different levels cucurbit downy mildew in Clinton, North Carolina. On each assessment date, disease severity was assessed visually as percent of leaf area.....89

FIGURE 3.7 Relationship between cucumber downy mildew severity and concentration of *Pseudoperonospora cubensis* sporangia (**A**) at the source or escape of sporangia (**B**) from a source in a cucumber

field at Clayton and Clinton, North Carolina. Solid circles (● or ○) are observed values, while curve is the predicted sporangia concentration or escaped sporangia obtained from fitting a log-normal model to combined data for sporangia concentration or escape from a canopy from the two sites90

1. CHAPTER I:

Literature Review

1.1. Cucurbitaceae

The family Cucurbitaceae is a large and heterogeneous group of plants, which includes over 118 genera with 825 species (Lebeda and Cohen, 2011). The family is distinct morphologically and biochemically from other plant families and is, therefore, considered monophyletic. Members of this family are grown in many different conditions and for many different purposes around the world (Zitter et al., 1998). The family includes economically important crops such as squashes (*Cucurbita* spp.), watermelon (*Citrullus lanatus*), melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus*). Fruits from cucurbit plants can be consumed raw or cooked, with squash and pumpkin having the most nutritional value. Seeds from many cucurbits are also used as an oil source for cooking and a protein source in several countries in Africa, Asia and Latin America. Other portions of the plant, such as leaves and flowers are also eaten and are rich sources of vitamins and minerals (Zitter et al., 1998).

Cucurbits are sensitive to frost and have differential reaction to heat and cold. Different cucurbit species are cultivated in tropical, temperate and desert regions (Zitter et al., 1998). Within the United States cucumbers are grown on more than 151,000 acres and the US ranks third in cucumber production worldwide with an average production of 2.3 billion pounds of cucumbers (Anon., 2011). Melons and squash (including pumpkins) are grown on over 243,000 and 147,000 acres, respectively. Cucurbits are typically grown in the southeastern states of Florida, Georgia, Alabama, South Carolina, North Carolina, Mississippi, and Tennessee and California on the west coast (Figure 1). California and Texas

are two of the largest growers of melons, while Michigan is a top producer of cucumbers and squash (Anon., 2011). North Carolina ranks third in cucumber production behind Michigan and Florida, respectively. Cucurbits grown in the southeast region are subject to warm humid weather that is conducive for the development of several foliar diseases. Of these diseases, cucurbit downy mildew caused by the obligate oomycete *Pseudoperonospora cubensis* is perhaps economically the most important (Lebeda and Cohen, 2011).

1.2. Pathogen Taxonomy

Pseudoperonospora cubensis is a biotrophic foliar pathogen that infects many species of the family Cucurbitaceae. The disease was first described by Berkeley and Curtis in herbarium plant material from Cuba in 1868 (Berkeley and Curtis, 1868). It was not until 1903 that the disease was described on live plant material in Russia (Skalický, 1961). In the past, *P. cubensis* has mistakenly been referred to as *Peronospora cubensis*, *Plasmopara cubensis*, or *Peronoplasmopara cubensis* (Dick, 2001). Based on recent taxonomic classification, *P. cubensis* belongs to kingdom Chromista, subdivision Peromosporomycotina, class Peronosporomycetes or Oomycetes under the order Peronosporales in the family Peronosporaceae. The difference between *Peronospora* and *Pseudoperonospora* is based on germination of sporangia. Members of *Peronospora* have sporangia that germinate directly via a germ tube whereas within *Pseudoperonospora*, sporangia germinate indirectly via cytoplasmic cleavage to produce zoospores (Palti and Cohen, 1980). Sporangia of *P. cubensis* typically are grey to purple in color, lemon-shaped with a papilla at the distal end and measure about 20 to 40 × 14 to 25 µm in diameter (Lebeda and Cohen, 2011). Sporangia are easily dislodged from sporangiophores and dispersed by

rain or air. Following germination of sporangia, the development of zoospores is dependent upon environmental conditions, especially temperature and moisture.

Sporangia form at the tips of sporangiophores and are of similar age at the ends of sterigmata (Choi et al., 2005). The shape and branching of sporangiophores have been used to characterize individual genera of *Pseudoperonospora* from *Plasmopara* and *Peronospora*. For example, in *Pseudoperonospora*, sporangiophores branch at acute angles irregularly for the first and second branches and then branches dichotomously with pointed tips (Waterhouse, 1973). In *Peronospora*, conidiophores branch acutely and resemble sporangiophores but they branch dichotomously (Waterhouse, 1973). *Plasmopara* sporangiophores branch at right angles, contain cross walls and have truncated tips (Waterhouse and Brothers, 1981). Although differences in sporangiophores can be used to identify genera, the cucurbit host type has a major impact on the morphology of *Pseudoperonospora* species. For example, *P. cubensis* from different host types have significant differences in the lengths of sporangia, sporangiophores and their branches as well as width of sporangia. As such, the sole use of morphological characteristics may not be adequate for species identification (Runge and Thines, 2010).

The genus *Pseudoperonospora* includes five species which are morphologically similar: *P. cubensis*, *P. humuli*, *P. cannabina*, *P. celtidis* and *P. urticae* (Choi et al., 2005; Constantinescu and Fatehi, 2002). It has been suggested that *P. cassiae* may be a sixth species of *Pseudoperonospora* (Waterhouse and Brothers, 1981). Although there are few morphological differences between *P. humuli* and *P. cubensis*, the use of single nucleotide polymorphisms (SNPs) has identified genetic differences between the two species (Mitchell

et al., 2009). These results strongly emphasize the role of genetic markers in identification of species and their relatedness within the genus *Pseudoperonospora* (Tian et al., 2011).

1.3. Pathogen Biology

Pseudoperonospora cubensis is an obligate foliar pathogen that causes chlorotic lesions on the adaxial leaf surface of infected hosts. In cucurbit species, lesions vary in size, shape and color (Figure 2). Lesions are typically angular in cucumbers due to the restriction by the leaf veins. In melon and watermelon, lesions are not always bound by leaf veins and are more circular and irregular. Among the plants parts, cotyledons are usually more susceptible than true leaves and symptoms on young, newly developing leaves are rare (Lebeda and Cohen, 2011). After initial infection, lesions expand in size and may become necrotic. Over the course of a few days, lesions expand and coalesce, killing leaves, a process that can result in death of the entire plant (Lebeda and Cohen, 2011). Under field conditions, incubation time ranges between 4 to 12 days depending on environmental conditions and the inoculum load (Cohen, 1977). Sporangiohores form within 5 to 7 days after initial infection and a new infection cycle can begin on susceptible hosts every 7 to 10 days (Lebeda and Cohen, 2011).

The main infective propagules of *Pseudoperonospora cubensis* are sporangia (asexual spores), which are light grey to deep purple in color (Thomas, 1996). Sporangia are attached to hyaline sporangiohores that form on the abaxial surface of leaves from stomata (Choi et al., 2005). Once sporangia are dislodged from the tips of sporangiohores, their lifespan is not usually longer than 48 h. Dispersal of sporangia onto susceptible host tissue is required during this time period for germination to occur. At higher temperatures (35°C and 40°C)

and humidity combinations (84 to 90%), infectivity of sporangia decreases. However, in presence of low humidity (5 to 28%), infectivity of sporangia is maintained even at higher temperatures (Cohen and Rotem, 1971). Contact with moisture is required for the germination and release of zoospores from sporangia (Cohen, 1981). Germination of sporangia will be disrupted when they are exposed to a dry period for 10 to 15 min (Cohen, 1977).

Sporangia germinate indirectly through cytoplasmic cleavage and release 5 to 15 biflagellate zoospores that are 10 to 13 μm in diameter (Thomas, 1996). Once zoospores form they settle, lose their flagella, and encyst near stomatal apertures (Cohen, 1981). Ambient temperature is important in cyst formation with high temperatures inducing cyst formation immediately with an optimum temperature of about 25°C (Cohen, 1981). A germ tube forms and produces an appressorium which then enters the host tissue through the stomatal opening. *P. cubensis* most frequently infects through the stomatal opening, however, direct penetration has also been reported (Lebeda and Cohen, 2011). Hyaline coenocytic hyphae form and grow through the mesophyll and palisade tissues. Within the mesophyll cells, clavate-branched haustoria colonize the plant cell membrane which provides the pathogen with nutrients. Sporangiohores then form in groups from the stomatal opening, on the lower leaf surface. The formation of the sporangiohores is dependent on high humidity and moisture on the leaf surfaces (Cohen, 1981).

Sexual reproduction of *P. cubensis* is rare and has not been reported in many locations in which *P. cubensis* is an important pathogen. Sexual reproduction takes place when compatible mating types fuse to form oospores when infected tissue becomes necrotic (Lebeda and Cohen, 2011). Recently, Cohen et al (2011) reported for the first time on

oospore formation in *P. cubensis* under controlled conditions in the laboratory. The study showed increased sensitivity of progenies to fungicides and altered host range relative to the parents. However, no oospores have been found or reported under field conditions. Thus, the role of sexual reproduction and oospores formation in the biology and epidemiology of cucurbit downy mildew is yet to be established.

1.4. Host range and pathogenicity

Pseudoperonospora cubensis infects a broad range of hosts, all within the Cucurbitaceae family. The pathogen infects nine cultivated hosts and some wild species of semi-cultivated plants. However, there are probably other cultivated species that have not yet been identified as hosts of *P. cubensis* (Lebeda and Cohen, 2011). It is estimated that 60 species and 20 genera are hosts of *P. cubensis* either through artificial inoculations or natural infection (Lebeda and Cohen, 2011). The host genus, *Cucumis*, has the largest number of susceptible hosts with 8 wild species and two cultivated species, *C. sativus* L. (cucumber) and *C. melo* L. (muskmelon). *Cucurbita* and *Citrullus* are also important genera of hosts that are infected by *P. cubensis*. Five species within *Cucurbita*, which is comprised of gourds and squashes, are widely grown in many parts of the world (Lebeda and Cohen, 2011).

Several studies have been conducted to determine the host range and differential disease severity on commonly cultivated cucurbit crops infected by *P. cubensis*. Doran (1932) conducted a host study using cucumbers, melons, gourds, squash and pumpkin and observed no infection on squash and pumpkin, while infection was moderate on muskmelon and severe on cucumber. Similarly, using cucumber, cantaloupe and watermelon and two isolates of *P. cubensis* derived from cucumber and watermelon, Hughes and Van Haltern

(1952) found that the cucumber isolate severely damaged cucumber and cantaloupe but caused few lesions on watermelon. The isolate from watermelon caused severe disease severity on watermelon but moderate disease severity on cucumber and cantaloupe. Thomas et al. (1987) conducted a host range study in order to develop data that could be used to characterize and identify *P. cubensis* isolates. The study was comprised of isolates and hosts from Japan, Israel and the United States in which 26 different cucurbit species were inoculated with their country's respective isolates. This study was able to distinguish five pathotypes of *P. cubensis*, all of which were highly compatible with cucumber and melon. The differential set used by Thomas et al. (1987) had several limitations (Lebeda and Widrlechner, 2003) and it did not include important host genera such as *Luffa*, *Lagenaria* and *Benincasa*. Lebeda and Widrlechner (2003) developed a new differential set consisting of 12 host genotypes which characterizes pathotypes by distinguishing among 12 'pathogenicity factors'. This differential set was to identify pathogenic variability among 22 isolates of *P. cubensis* originating from European countries (Lebeda and Gadasová, 2002). The results distinguished 13 pathotypes as compared to the five pathotypes described by Thomas et al. (1987). The pathotypes from Europe carried between 2 to 9 pathogenicity factors indicating that the European population of *P. cubensis* was highly diverse.

1.5. Epidemiology

Following infection and colonization of host tissues, sporangiophores form through stomatal openings when relative humidity is greater than 90%. Sporulation occurs on lesions of a certain physiological age and typically on chlorotic rather than necrotic lesions. Under the right conditions, sporulation can take place in as little as 4 to 5 days after infection

(Lebeda and Cohen, 2011). Heavy sporulation occurs after periods of darkness but this is strongly dependent on the ambient temperature (Cohen and Eyal, 1977). The pathogen requires at least 6 h of darkness in order to form sporangia (Cohen, 1977). Environmental conditions affect the timing and duration of sporulation. Lower temperatures delay the start of sporulation but increase the length of time a lesion will sporulate (Cohen, 1977).

The source of initial inoculum for infection by *P. cubensis* has been a major research focus for a number of workers. In the United States, the presumed lack of overwintering oospores for *P. cubensis* coupled with the sensitivity of cucurbits to frost implies that the pathogen can only survive as active mycelium in cultivated or wild species of cucurbits in frost-free areas (Bains and Jhooty, 1976). As such, *P. cubensis* can only survive within the continental United States (<30° latitude) in southern Florida and along the Gulf of Mexico (Thomas, 1996). Thus, in areas >30° latitude, onset of disease epidemics will depend on the aerial dispersal of *P. cubensis* sporangia from subtropical overwintering sources in southern Florida (Nusbaum, 1944) or protected greenhouse cultivation of cucurbit crops.

Although *P. cubensis* can be dispersed through both wind and water, aerial dispersal of sporangia is the primary method of dissemination and sporangia can be dispersed aurally over long distances on a regional scale (Thomas, 1996). A decrease in relative humidity results in the twisting of sporangiophores and the release of sporangia (Lange et al., 1989). Due to the change of humidity and leaf wetness in the morning to afternoon, concentrations of sporangia are higher in the morning compared to afternoon hours (Cohen and Rotem, 1971). The distance spores can be aurally transported depend on the release height within the canopy and the wind conditions during spore release (Aylor, 1990). Current empirical evidence indicates that *P. cubensis* sporangia at the source can be dispersed up to a distance

of 1,000 km (Ojiambo and Holmes, 2011). According to spore trapping studies conducted by Cohen and Rotem (1971) in Israel, spore release begins at sunrise and peaks about 2 h later. Dispersal ends about 10 h later with few spores being released afterwards. Short distance transport of sporangia is dependent on machinery or rain-splash dispersal within a field (Thomas, 1996). Survival of sporangia during transport is dependent on temperature, humidity and solar radiation (Kanetis et al., 2010; Thomas, 1996). Of these three weather variables, solar radiation is the most critical determinant of survival of sporangia (Kanetis et al., 2010). Depending on the prevailing weather, viability of sporangia during transport can range between 1 to 16 days (Cohen, 1981).

Due to the aerial nature of the spread of cucurbit downy mildew, forecasting is used to track the regional spread of the disease. The first indicators of spore dispersal are reports from southern Florida where infected cucurbit crops serve as the overwintering inoculum source for crops in the eastern regions of the United States and Canada (Lebeda and Cohen, 2011). Currently, forecasting uses reports of disease outbreaks and meteorological data to predict inoculum transport from overwintering sources to disease-free areas (Ojiambo et al., 2011). A network of over 40 collaborators has been established (Ojiambo et al., 2011) where collaborators routinely scout sentinel, commercial and research plots and report disease outbreaks to the forecasting website (<http://cdm.ipmpipe.org>). This information is also used to track the temporal and spatial movement of the disease from southern Florida northwards along the east coast in the United States as the season progresses (Ojiambo and Holmes, 2011).

Germination of sporangia of *P. cubensis* and subsequent infection of host plants by the pathogen are dependent on temperature and moisture or leaf wetness. Leaf wetness

allows for infection to occur, while temperature determines the extent of disease development (Arauz et al., 2010). In absence of leaf wetness, the development of cucurbit downy mildew is limited regardless to the ambient temperature (Palti and Cohen, 1980). The minimum duration of leaf wetness required for infection to occur is 2 h (Cohen, 1977) and the optimum temperature range is between 15 and 20°C (Cohen and Rotem, 1969). However, recent studies have suggested a broadening of the infection range of *P. cubensis* with infection occurring even at 30°C (Arauz et al., 2010). The interactive effects of these two variables also influence sporangia germination and the degree of disease severity (Arauz et al., 2010). For example, in the study by Arauz et al. (2010), a distinct optimum for infection was observed at 20°C for wetness periods of 4 to 8 h but broader optimum curves were observed for wetness periods > 8 h. Based on that work, risk threshold charts were constructed to estimate the potential risk for infection based on observed or forecasted temperature and leaf wetness duration (Arauz et al., 2010).

1.6. Disease Management

Breeding for resistance is dependent upon available sources for resistance and a standard method for evaluating genotypes for resistance to the disease of interest. Resistance among cucurbit species differs considerably with *Cucumis melo* and *Cucurbita pepo* having the most available sources for disease resistance (Lebeda, 1999). Resistance in cucumber was developed in early 1950s, but with a subsequent change in the population of the pathogen, a relatively rapid breakdown in host resistance has been observed (Lebeda and Cohen, 2011). Resistance was first identified in 1954 in the cucumber accession PI 197087 carrying the recessive gene *dm1* (Barnes and Epps, 1954). Plants with the recessive gene exhibited classic

hypersensitive response of sparse pathogen sporulation, small lesions and browning of tissue and rapid cell death. Within the United States, *dm1* has been used in commercial cultivars since 1950s to consistently control downy mildew until 2004 (Holmes and Thomas, 2006). A change in the pathogen population occurred in 2004 that resulted in breakdown of host resistance and subsequently widespread losses of cucumber crops in the US (Holmes and Thomas, 2006). Although high levels of resistance are no longer present in cucumber cultivars, the *dm1* gene still confers some level of resistance. In contrast, cultivars without *dm1* are infected early in the season and have high levels of disease (Holmes et al., 2004).

In the absence of cultivars with acceptable levels of resistance, management of cucurbit downy mildew now heavily relies on the use of fungicides. Sales of fungicides used against downy mildews were estimated to \$120 million in 1996 (Gisi, 2002). Of these sales, 10% is for fungicides used against *P. cubensis* on cucurbit crops (Urban and Lebeda, 2006). For adequate disease control, an aggressive spray program is recommended, with sprays every 5 to 7 days for cucumbers and 7 to 10 days for other cucurbits (Hausbeck and Cortright, 2009). In an effort to improve fungicide efficiency and apply fungicide only if and when it is necessary, a disease forecast system is now available to help growers apply the initial spray (Holmes et al., 2004; Ojiambo et al., 2011). In cucurbits, the initial spray is the key in the management of the disease during the season.

There are many different groups of chemistries (e.g., phenylamides, dithiocarbamates, cymoxanil, copper, chlorothalonil, fosetyl-A1, hymexazol, fentins, dimethomorph, propamocarb, fluazinam, phthalimides and strobilurins) that are used to control cucurbit downy mildew (Gisi, 2002). Contact fungicides such as dithiocarbamates and chlorothalonil have been used preventatively prior to disease onset. These contact fungicides prevent

zoospore release and cystospore germination and they are only effective if sprayed before infection or sporangia deposition (Urban and Lebeda, 2006). Systemic fungicides such as phenylamides, strobilurins, and carboxylic acid amides have curative effects by reducing disease development after infection but have been prone to loss of efficacy due to development of insensitivity by *P. cubensis*. Considered one of the top ten pathogens to develop resistance to fungicides by FRAC (2005), *P. cubensis* has developed resistance to several systemic fungicides. For example, resistance to phenylamide-based products developed shortly after release of the fungicides to the market (Lebeda and Schwinn, 1994). Similarly, resistance to mancozeb was reportedly shortly after the insensitivity to phenylamide-based products was documented (Reuveni et al., 1980; Thomas and Jourdain, 1992). Greenhouse studies have shown that resistance to strobilurins and mefenoxam containing products is widespread across the eastern United States (Colucci and Holmes, 2007). In a study to synthesize data from fungicide trials conducted over a period of ten years in the United States, the fungicide fluopicolide (Presidio) was found to be the most effective, followed by carbamates (e.g., propamocarb) and quinone inside inhibitors (e.g., cyazofamid) (Ojiambo et al., 2010). Fluopicolide is the first derivative of the new acylpicolide class of chemicals, whose mode of action is interference with the delocalization of spectrin-like proteins during mitosis (Tafforeau et al., 2005). This fungicide is also highly effective against other oomycetes such as *Phytophthora infestans* (Cooke and Little, 2006).

1.7. Project Objectives

Cucurbits respond differently to infection by *P. cubensis* under field conditions (Ojiambo et al. 2010). Models developed to describe the combined effects of temperature and

leaf wetness were developed using cantaloupe (Arauz et al., 2010) as the host type. However, it is not known how different host types will influence the predictive ability of these models. Since temperature and wetness duration are the key variables that influence sporangia germination and infection by *P. cubensis*, it is logical to expect that there will be a corresponding differential response of cucurbit host types to temperature and leaf wetness duration, either alone or in combination. The cucurbit downy mildew forecasting system issues forecasts for the initial infection based on the assumption that infection parameters for different cucurbits respond similarly to temperature and wetness duration (Ojiambo et al., 2011). The differential response of cucurbit host types to temperature and leaf wetness has not yet been determined for *P. cubensis* and such information, when developed, could be used to improve the performance and efficiency of the cucurbit downy mildew forecasting system. Such information will be derived here for different cucurbit host types in an effort to develop host-specific infection risks with the goal of improving the efficiency of the cucurbit downy mildew forecasting system.

P. cubensis is an aerially disseminated pathogen and the spread of downy mildew relies on the passive dispersal of sporangia between fields. The number of spores released from the source is integral to probability of infection (Aylor, 1986). However, cucurbit downy mildew forecasts are currently made with the assumption that all infected source fields have equal weights with respect to sporangia available for dispersal regardless of their corresponding levels of disease severity. Based on the biology of many obligate plant pathogens, one would expect a decrease in sporangia production at a certain critical threshold of disease severity. The relationship between source strength and sporangia available for transport has not yet been investigated and this will be the investigated here for cucurbit

downy mildew. When developed, such information can be used to parameterize a source area in available aerobiology models (Isard et al., 2007) to determine when and where deposition of sporangia will most likely to occur in the United States.

1.8. Literature Cited

1. Anonymous, 2011. National Agricultural Statistics Service, U. S. Department of Agriculture. 2011. Quick Stats - The National Agricultural Statistics Service Interactive, Online Statistical Database. www.quickstats.nass.usda.gov.
2. Arauz, L. F., Neufeld, K. N., Lloyd, A. L., and Ojiambo, P. S. 2010. Quantitative models for germination and infection of *Pseudoperonospora cubensis* in response to temperature and duration of leaf wetness. *Phytopathology* 100:959-967.
3. Aylor, D. E. 1986. A framework for examining inter-regional aerial transport of fungal spores. *Agric. For. Meteorol.* 38:263-288.
4. Aylor, D. E. 1990. The role of intermittent wind in the dispersal of fungal pathogens. *Annu. Rev. Phytopathol.* 28:73-92.
5. Bains, S. S., and Jhooty, J. S. 1976. Host-range and possibility of pathological races in *Pseudoperonospora cubensis*-cause of downy mildew of muskmelon. *Indian Phytopathol.* 29:214-216.
6. Barnes, W. C., and Epps, W. M. 1954. An unreported type of resistance to cucumber downy mildew. *Plant Dis. Rep.* 38: 620.
7. Berkeley, M. S., and Curtis, A. 1868. *Peronospora cubensis*. *J. Linn. Soc. Bot.* 10:363.
8. Choi, Y. J., Hong, S. B., and Shin, H.D. 2005. A re-consideration of *Pseudoperonospora cubensis* and *P. humuli* based on molecular and morphological data. *Mycol. Res.* 109:841-848.
9. Cohen, Y. 1977. The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Can. J. Bot.* 55:1478-1487.
10. Cohen, Y. 1981. Downy mildew of cucurbits. Pages 341-354 in: *The downy mildews*. D. M. Spencer, ed. Academic Press, Inc., London.
11. Cohen, Y., and Eyal, H. 1977. Growth and differentiation of sporangia and sporangiophores of *Pseudoperonospora cubensis* on cucumber cotyledons under various combinations of light and temperature. *Physiol. Plant. Pathol.* 10:93-103.
12. Cohen, Y., and Rotem, J. 1969. The effects of lesion development, air temperature, and duration of moist period on sporulation of *Pseudoperonospora cubensis* in cucumbers. *Israel J. Bot.* 18:135-140.

13. Cohen, Y., and Rotem, J. 1971. Dispersal and viability of sporangia of *Pseudoperonospora cubensis*. Trans. Br. Mycol. Soc. 57:67-74.
14. Cohen, Y., Rubin, A. E., and Galperin, M. 2011. Formation and infectivity of oospores of *Pseudoperonospora cubensis*, the causal agent of downy mildew in cucurbits. Plant Dis. 95:874.
15. Colucci, S. J., and Holmes, G. J. 2007. Fungicide insensitivity and pathotype determination of *Pseudoperonospora cubensis*, causal agent of cucurbit downy mildew. Phytopathology 97:S24.
16. Constantinescu, O., and Fatehi, J. 2002. Peronospora-like fungi (Chromista, Peronosporales) parasitic on Brassicaceae and related hosts. Nova Hedwigia 74:291-338
17. Cooke, L. R., and Little, G. 2006. Evaluation of fluopicolide-containing formulations for the control of potato late blight in Northern Ireland. Pflanzenschutz Nachr. Bayer (Ger. Ed.) 59:303-316.
18. Dick, M. W. 2001. Straminipilous fungi: Systematics of the Peronosporomycetes, including accounts of the marine Straminipilous Protists, the Plasmodiophorids, and similar organisms. Kluwer Academic Publishers, Dordrecht, The Netherlands.
19. Doran, W.L. 1932. Downy mildew of cucumbers. Mass. Agr. Exp. St. Re. B. 283:1-22.
20. Fungicide Resistance Action Committee (FRAC). 2005. Specialist Technical Group, Croplife International. Pathogen risk list- 2005. www.frac.info
21. Gisi, U. 2002. Chemical control of downy mildews. Pages 119-159 in: Advances in downy mildew research - Vol. 1). P. T. N. Spencer-Phillips, U. Gisi, and A. Lebeda, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
22. Hausbeck, M. K., and Cortright, B.D. 2009. Evaluation of fungicides for control of downy mildew of pickling cucumber, 2007. Plant. Dis. Manag. Rep. 3:V112.
23. Holmes, G. J., and Thomas, C. E. 2006. The history and re-emergence of cucurbit downy mildew (Abstr.). Phytopathology 99: S171.
24. Holmes, G. J., Main, C. E., and Zeever, Z. T. 2004. Cucurbit downy mildew: a unique pathosystem for disease forecasting. Pages 69-80 in: Advances in Downy Mildew Research – Vol. 1. P.T.N. Spencer-Phillips, and M. Jeger, eds. Kluwer Academic Publishers, Dordercht, The Netherlands.
25. Hughes, M. B., and van Halteren, F. 1952. Two biological forms of *Pseudoperonospora cubensis*. Plant Dis. Rep. 36:365-367.

26. Isard S. A., Russo, J. M., Ariatti, A. 2007. The integrated aerobiology modeling system applied to the spread of soybean rust into the Ohio River valley during September 2006. *Aerobiologia* 23:271-82.
27. Kanetis, L., Holmes, G. J., and Ojiambo, P. S. 2010. Survival of *Pseudoperonospora cubensis* sporangia exposed to solar radiation. *Plant Pathol.* 59:313-323.
28. Lange, L., Eden, U., and Olson, L. W. 1989. The zoospore of *Pseudoperonospora cubensis*. The causal agent of cucurbit downy mildew. *Nordic J. Bot.* 8:511-516.
29. Lebeda, A. 1999. *Pseudoperonospora cubensis* on *Cucumis* spp. and *Cucurbita* spp.– resistance breeding aspects. *Acta Hort.* 492:363-370.
30. Lebeda, A., and Schwinn, F. J. 1994. The downy mildews-an overview of recent research progress. *J. Plant Dis. Prot.* 101: 225–254.
31. Lebeda, A., and Cohen, Y. 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*)-biology, ecology, epidemiology, host-pathogen interaction and control. *Eur. J. Plant Pathol.* 129:157-192.
32. Lebeda, A., and Gadasova V. 2002. Pathogenic variation of *Pseudoperonospora cubensis* in the Czech Republic and some other European countries. *Acta Hort.* 588:137-141.
33. Lebeda, A., and Widrlechner, M. P. 2003. A set of cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Prot.* 110:337-349.
34. Mitchell, M. N., Ocamb, C., and Gent, D. 2009. Addressing the relationship between *Pseudoperonospora cubensis* and *P. humuli* by multigenic characterization and host specificity. *Phytopathology* 99:S87.
35. Nusbaum, C. J. 1944. The seasonal spread and development of cucurbit downy mildew in the Atlantic coastal states. *Plant Dis.* 28:82–85.
36. Ojiambo, P. S., Paul, P. A., and Holmes, G. J. 2010. A quantitative review of fungicide efficacy for managing downy mildew in cucurbits. *Phytopathology* 100:1066-1076.
37. Ojiambo, P. S., and Holmes, G. J. 2011. Spatiotemporal spread of cucurbit downy mildew in the eastern United States. *Phytopathology* 101:451-461.

38. Ojiambo, P. S., Holmes, G. J., Britton, W., Keever, T., Adams, M. L., Babadoost, M., Bost, S. C., Boyles, R., Brooks, M., Damicone, J., Draper, M. A., Egel, D. S., Everts, K. L., Ferrin, D. M., Gevens, A. J., Gugino, B. K., Hausbeck, M. K., Ingram, D. M., Isakeit, T., Keinath, A. P., Koike, S. T., Langston, D., McGrath, M. T., Miller, S. A., Mulrooney, R., Rideout, S., Roddy, E., Seebold, K.W., Sikora, E. J., Thornton, A., Wick, R. L., Wyenandt, C. A. and Zhang, S. 2011. Cucurbit downy mildew ipmPIPE: a next generation web-based interactive tool for disease management and extension outreach. Online. Plant Health Progress doi:10.1094/PHP-2011- 0411-01-RV.
39. Palti, J., and Cohen, Y. 1980. Downy mildew of cucurbits (*Pseudoperonospora cubensis*): The fungus and its hosts, distribution, epidemiology and control. *Phytoparasitica* 8:109-147.
40. Reuveni, M., Eyal, H., and Cohen, Y. 1980. Development of resistance to metalaxyl in *Pseudoperonospora cubensis*. *Plant Dis.* 64: 1108-1109.
41. Runge, F., and Thines, M. 2010. Host matrix has major impact on the morphology of *Pseudoperonospora cubensis*. *Eur. J. Plant Pathol.* 129:147-156.
42. Skalický, V. 1961. *Peronoplasmopara cubensis*. Pages 390-393 in: *Agricultural Phytopathology, Vol. III—Diseases of Vegetable Crops.* J. Benada, and J. Špaček, eds. Praha, Czechoslovakia: Státní zemědělské nakladatelství.
43. Tafforeau, S., Wegmann, T., Latorse, M. P., Gouot, J. M., Duvert, P., and Bardsley, E. 2005. Fluopicolide, a novel fungicide with a unique mode of action, setting a new standard for oomycete control. Pages 79-86 in: *Proc. BCPC Congress-Science & Technology, Volume 2.* Glasgow, UK.
44. Thomas, C. E. 1996. Downy mildew. Pages 25-27 in: *Compendium of Cucurbit Diseases.* T. A. Zitter, D. L. Hopkins, and C. E. Thomas, eds. American Phytopathological Society Press, St. Paul, MN.
45. Thomas, C.E., and Jourdain, E.L. 1992. Host effect on selection of virulence factors affecting sporulation by *Pseudoperonospora cubensis*. *Plant Dis.* 76:905-907.
46. Thomas, C. E., Inaba, T., and Cohen, Y. 1987. Physiological specialization in *Pseudoperonospora cubensis*. *Phytopathology* 77:1621-1624.
47. Tian, M., Win, J., Savory, E., Burkhardt, A., Held, M., Brandizzi, F., and Day, B. 2011. 454 Genome sequencing of *Pseudoperonospora cubensis* reveals effector proteins with a QXLR translocation motif. *Mol. Plant-Microbe Interact.* 24:543-553.
48. Urban, J., and Lebeda, A. 2006. Fungicide resistance in cucurbit downy mildew—methodological, biological and population aspects. *Ann. Appl. Bio.* 149:63-75.

49. Waterhouse, G. M. 1973. *Peronosporales*. Pages 165-183 in: *The Fungi An Advanced Treatise*. Vol. 4B. G. C. Ainsworth, F. K. Sparrow, and A. S. Sussman, eds. Academic Press, NY.
50. Waterhouse, G. M., and Brothers, M. P. 1981. The taxonomy of *Pseudoperonospora*. *Mycological Papers*. 148:1-28.
51. Zitter, T. L., Hopkins, D. L., and Thomas, C. E. 1998. *Compendium of Cucurbit Diseases*. American Phytopathological Society Press, St. Paul, MN.

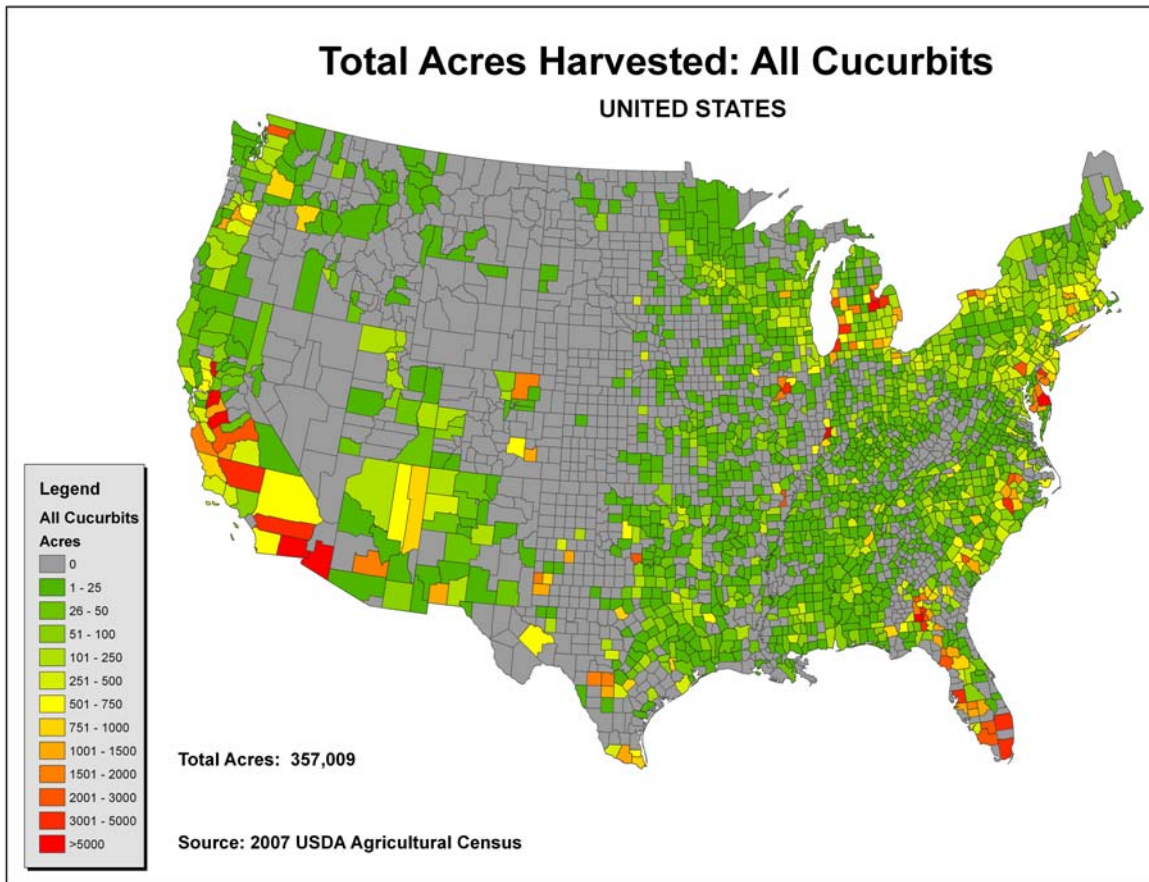


Figure 1.1 Total acreage of all cucurbits produced in the United States based on the 2007 USDA Agricultural Census.

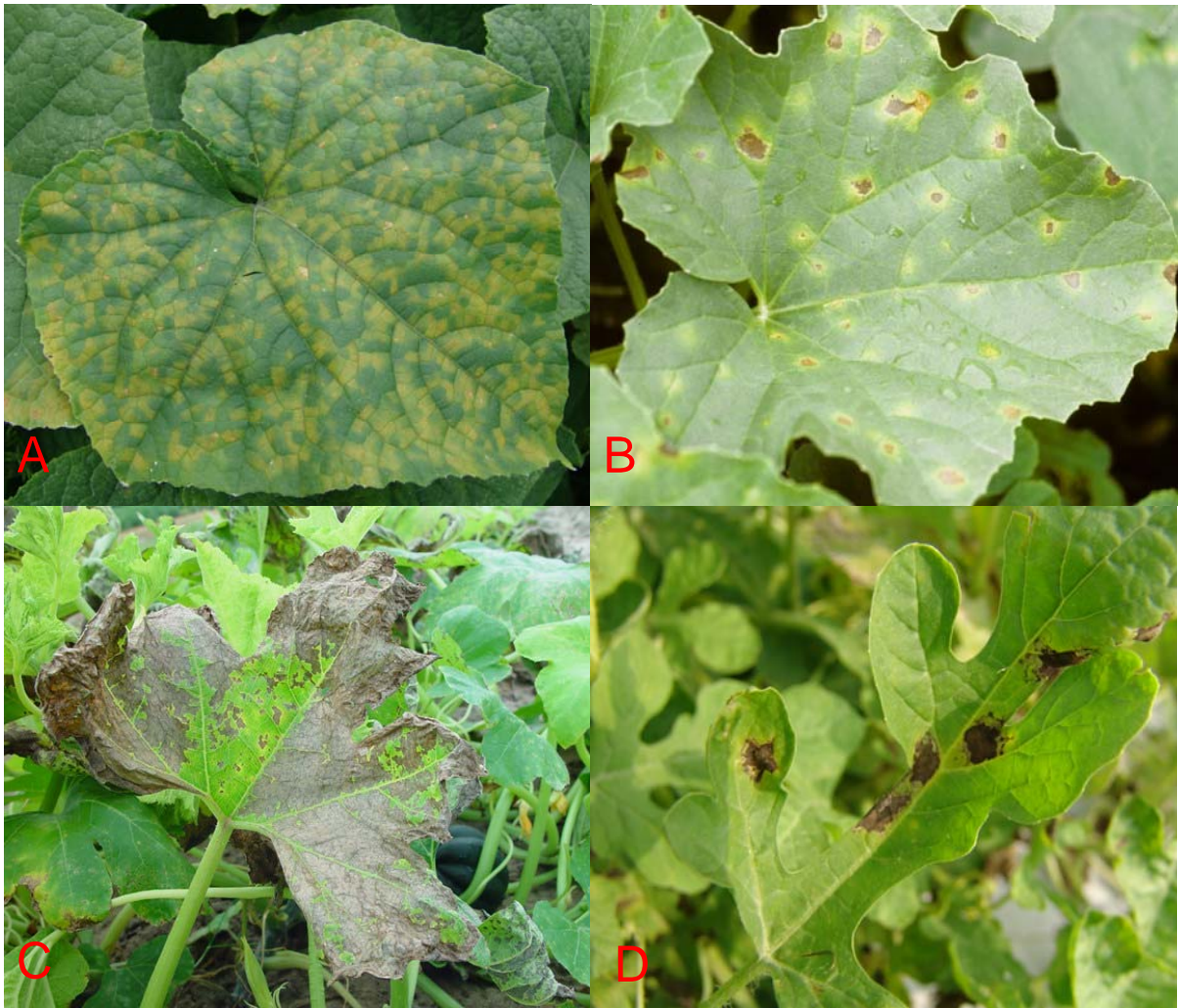


Figure 1.2 Symptoms of cucurbit downy mildew on cucumber (A), cantaloupe (B), squash (C) and watermelon (D). Lesions in cucumbers are typically more angular and vein bound. Lesions in squash and watermelon are typically more circular and irregular in size. Photos courtesy of Gerald J. Holmes, Valent USA Corporation.

2. CHAPTER II:

Interactive Effects of Temperature and Leaf Wetness Duration on Sporangia Germination and Infection of Cucurbit Host Types by *Pseudoperonospora cubensis*

Neufeld, K.N., and Ojiambo, P.S. 2012. *Plant Disease* 96: (345-353). Reprinted here with permission of publisher, 02/03/2012.

Katie N. Neufeld and Peter S. Ojiambo

K. N. Neufeld and **P. S. Ojiambo**, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695

2.1. ABSTRACT

Outbreaks of cucurbit downy mildew caused by *Pseudoperonopora cubensis* are dependent on the weather but effects of temperature and leaf wetness duration have not been studied for different cucurbits. To determine the effects of these two weather variables on sporangia germination and infection of cucurbit host types by *P. cubensis*, three host types; cucumber (cv. Straight 8), cantaloupe (cv. Kermit) and acorn squash (cv. Table Queen), were inoculated and exposed to leaf wetness durations of 2 to 24 h at six constant temperatures ranging from 5 to 30°C in growth chamber experiments. Sporangia germination was assessed after each wetness period, and leaf area infected was assessed 5 and 7 days after inoculation. Germination of sporangia was highest on cantaloupe (range 16.5 to 85.7%) and lowest on squash (range 10.7 to 68.9%), while disease severity was highest and lowest on cucumber and cantaloupe, respectively. Host type, temperature, wetness duration and their interactions significantly ($P < 0.0001$) affected germination and disease severity. Germination and disease data for each host type were separately fitted to a modified form of a Weibull function that characterizes a unimodal response and monotonic increase of germination or infection with temperature and wetness duration, respectively. The effect of host type on germination and infection was characterized primarily by differences in the upper limit parameter in response to temperature. Differences among host types based on other parameters were either small or inconsistent. Temperature and wetness duration that

supported a given level of germination or infection varied among host types. At 20°C, 15% leaf area infected was expected following 2, 4 and 8 h of wetness for cucumber, squash and cantaloupe, respectively. When temperature was increased to 25°C, 15% disease severity was expected following 3, 7 and 15 h of wetness for cucumber, squash and cantaloupe, respectively. Risk charts were constructed to estimation the potential risk of infection of cucurbit host types by *P. cubensis* based on prevailing or forecasted temperature and leaf wetness duration. These results will improve the timing and application of the initial fungicide spray in control of cucurbit downy mildew.

2.2. INTRODUCTION

Among the diseases that affect cucurbitaceous crops, downy mildew caused by the oomycete *Pseudoperonospora cubensis* is economically the most damaging (Lebeda and Cohen, 2011). Several cucurbit host types are infected by *P. cubensis*, of which cucumber (*Cucumis sativus*), squash, pumpkin and zucchini (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), and cantaloupe and muskmelon (*Cucumis melo*), are economically the most important crop plants (Lebeda and Widrlechner, 2003). The disease has a worldwide distribution and occurs wherever cucurbits are cultivated in the temperate and tropical areas and in the semi-arid areas in the Middle East. In these production regions, the disease is especially damaging in areas with warm and humid conditions which are conducive for disease development (Thomas, 1996). In the United States, these favorable environmental conditions commonly occur in the eastern half of the country where epidemics of the disease occur annually. Since 2007, cucurbit downy mildew now occurs annually in California (Ojiambo et al., 2011).

The degree of infection of cucurbits by *P. cubensis* is greatly influenced by temperature and leaf wetness. Leaf wetness allows for infection to occur, while temperature determines the degree of disease development (Arauz et al., 2010; Cohen, 1977). Thus, disease development is minimal in absence of leaf wetness irrespective of the prevailing temperature (Palti and Cohen, 1980). The minimum duration of leaf wetness required for infection is 2 h (Cohen, 1977) and the optimum temperature for infection is between 15 and 20°C (Cohen and Rotem, 1969). The interactive effects of temperature and leaf wetness duration also influence sporangia germination (Arauz et al., 2010) and the extent and degree of disease severity (Arauz et al., 2010; Cohen, 1977). Quantitative models based on modified forms of the Weibull function were recently developed to describe the combined effects of temperature and leaf wetness duration (Arauz et al., 2010). Using cantaloupe as the cucurbit host type, the models estimated 15 to 17 and 19 to 22°C as the optimum temperature range for germination and infection, respectively, with little germination or infection at 5 or 30°C (Arauz et al., 2010). For wetness periods of 4 to 8 h, a distinct optimum for infection was observed at 20°C but broader optimum curves resulted from wetness periods > 8 h. Based on these models, risk threshold charts were constructed for estimating the potential risk for infection based on observed or forecasted temperature and leaf wetness duration (Arauz et al., 2010). Studies on the influence of weather variables can generate useful information needed to improve management of plant diseases. For example, temperature and wetness duration are used together with information on known inoculum sources to forecast the risk of cucurbit downy mildew outbreak in the United States (Ojiambo et al., 2011).

Currently, there are no commercially available resistant cucurbit cultivars and control of cucurbit downy mildew relies heavily on the use fungicides such as fluopicolide

(Presidio), cyazofamid (Ranman) and propamocarb hydrochloride (Previcur Flex). In absence any resistance management strategies, heavy use of these fungicides can result in *P. cubensis* developing resistance to these chemistries. Field observations and empirical studies (Ojiambo et al., 2010; Urban and Lebeda, 2006) indicate that cucurbits respond differentially to infection by *P. cubensis*. For example, cucumber is generally more susceptible to *P. cubensis* than other cucurbitaceous host crops (Ojiambo et al., 2010; Urban and Lebeda, 2006). Since temperature and wetness duration are the key variables that influence sporangia germination and infection by *P. cubensis*, it is logical to expect that there will be a corresponding differential response of cucurbit host types to temperature and leaf wetness duration, either alone or in combination. However, the cucurbit downy mildew forecasting system (Ojiambo et al., 2011) issues forecasts for the initial infection based on the assumption that infection parameters for different cucurbits respond similarly to temperature and wetness duration. In a study on early leaf spot of peanut, Wu et al. (1999) observed that cultivars varied in their response to wetness and temperature and proposed improvement in the performance and efficiency of an early leaf spot advisory program through modification of wetness duration thresholds for cultivars that differ in their reaction to the disease. The differential response of cucurbit host types to temperature and leaf wetness has not yet been determined for *P. cubensis* and such information, when developed, could be used to improve the performance and efficiency of the cucurbit downy mildew forecasting system. Thus, this study was carried out with the following objectives: i) quantify the temperature and leaf wetness duration for sporangia germination and infection of cucurbit host types by *P. cubensis*, and ii) develop risk charts to predict infection of cucurbit host types by *P. cubensis* for a wide range of temperature and leaf wetness duration.

2.3. MATERIALS AND METHODS

2.3.1. Cucurbit host types and plant growth conditions. Three cucurbit host types were used in this study: cucumber (cv. Straight 8), cantaloupe (cv. Kermit) and acorn squash (cv. Table Queen). Cucumber and squash seeds were directly seeded and seedlings grown in circular 8 cm Styrofoam cups (one plant/cup) containing vermiculite. However, cantaloupe seeds were first germinated in Petri plates incubated in a growth chamber with no light at 30°C for 2 days, and then the seedlings were transplanted into circular 8.0 cm Styrofoam cups (one plant/cup) containing vermiculite. All plants were thereafter maintained in a greenhouse with a 12/12 h day/night natural light cycle and 32/26°C day/night temperature regime. Plants were watered daily with deionized water in the morning and half-strength Hoagland's solution (Hoagland and Arnon, 1950) in the afternoon during the experimental period.

2.3.2. Pathogen isolate and inoculation of plants. An isolate of *P. cubensis* (JCNC-09), which was isolated from the cucumber cultivar Straight 8 in Johnston County, North Carolina in 2009 was used for inoculation of test plants. This isolate (previously maintained on leaf tissue in a -80°C freezer) is capable of infecting different cucurbit host types and was used to produce adequate amounts of sporangia suspension for plant inoculation as described previously (Lebeda, 1986). At the two-true-leaf stage (approx. 21 days old), plants were randomly selected and first preconditioned in growth chambers at 5, 10, 15, 20, 25, and 30°C overnight. The first and second true leaves were then inoculated on the abaxial and adaxial sides of the leaves with a spore suspension containing 1×10^4 sporangia per ml in deionized water, until run-off using a Preval sprayer (Complete Unit 267, Precision Valve Corporation, Yonkers, NY). Following inoculation, plants were immediately covered with plastic bags

following inoculation to ensure that the required leaf wetness duration persisted during the test periods. Inoculated plants were then incubated in growth chambers with 24 h darkness at the above six temperatures to optimize infection of plants (Lebeda, 1986).

Leaf wetness durations evaluated in this study were 2, 4, 8, 12 and 24 h. To evaluate sporangia germination for each wetness period, inoculated plants incubated at each of the six temperatures were removed from growth chambers after a given leaf wetness duration. Leaf disks measuring 10-mm were removed from the first and second leaves using a cork borer and immediately placed in micro centrifuge tubes containing 12% CuSO₄ solution to inhibit germination of sporangia (Arauz and Sutton, 1989). The contents in the micro-centrifuge tubes were vortexed for 5 s and 25 µl of the solution was pipetted onto a slide and covered with a cover slip to examine sporangia for germination using a microscope. Sporangia of *P. cubensis* germinate indirectly by cytoplasmic cleavage to release zoospores and therefore, a sporangium was considered germinated when the cytoplasmic contents were emptied. A total of 50 sporangia were examined for each temperature and leaf wetness combination.

The effects of temperature and leaf wetness duration on infection of cucurbit host types by *P. cubensis* were evaluated in two steps. For each of the six temperature regimes, inoculated plants were first exposed to the five different leaf wetness durations by removing three plants from each growth chamber after 2, 4, 8, 12, and 24 h of leaf wetness. Plants were then dried with forced air for about 10 min and returned to their respective growth chambers where they were exposed to ambient humidity in the chamber for 24 h. In the second stage, all plants were removed from the growth chamber and exposed to standard incubation conditions (21/18°C and 12/12 h day and night temperature and light regimes, respectively; and 75-90% RH) in an incubator (Model I-36VL, Percival Scientific Inc., Perry, IA). Plants

were evaluated for disease severity (2 leaves per plant) at 5 and 7 days after inoculation (DAI) by visually assessing the percentage of leaf area affected with chlorotic and necrotic symptoms. The two disease assessment periods represent the range of the latent period of *P. cubensis* (Thomas, 1996). Both sporangia germination and infection experiments were conducted twice.

2.3.3. Experimental design and data analysis. Treatments were laid out in a split-split-plot design with temperature as the whole-plot treatment, host type as the split-plot treatment and leaf wetness duration as the split-split-plot treatment. Each combination of temperature and leaf wetness duration was comprised of two replications of two and three plants for germination and disease severity, respectively, with two inoculated leaves per plant.

A preliminary analysis of variance indicated no significant differences between the two experiments conducted for the germination and infection data. Thus, all subsequent analyses were performed on data averaged over the two experiments. Analysis of germination and infection data was conducted in two steps. First, the effects of temperature (t), host type (s) and leaf wetness duration (w) on sporangia germination and disease severity were compared by analysis of variance using PROC GLM of SAS (version 9.2; SAS Institute, Cary, NC).

In the second set of analysis, the response of individual host types to the combined effects of temperature and leaf wetness were modeled by fitting germination and infection data to the following nonlinear model (Arauz et al., 2010):

$$f(w,t) = f(t) \times (1 - \exp\{-[B \times w]^D\}) \quad (1.0)$$

where $f(t)$ which is the asymptote parameter, is defined as:

$$f(t) = E' \left\{ \exp[(t - F)G / (H + 1)] \right\} / \left\{ 1 + \exp[(t - F)G] \right\} \quad (1.1)$$

and where E' is defined as:

$$E' = E[(H + 1) / H] H^{1/(H+1)} \quad (1.2)$$

This model is a modified form of a Weibull function (Weibull, 1951) and was recently developed and validated to describe the response of sporangia germination and infection by *P. cubensis* to combined effects of temperature and leaf wetness using cantaloupe as the model system (Arauz et al., 2010). In the above equations, italicized lower case letters represent independent variables, while italicized upper case letters represent parameters. Briefly, the asymptote parameter, $f(t)$, characterizes the upper limit of the response variable as w is extended indefinitely. Parameter B refers to the intrinsic rate of increase of the response variable with respect to w , while parameter D is the intrinsic rate of acceleration. The parameter E characterizes the scale of the response to t . The parameters F and G characterize optimum temperature and the decline from maximum as temperature deviates from the optimum, respectively. The asymmetry in the response to t is characterized by parameter H . Sporangia germination and infection data averaged over replications were fitted to the nonlinear model using the PROC NLIN in SAS. Goodness-of-fit of the model was evaluated based on the significance of parameter estimates, magnitude of asymptotic standard errors, and simple correlation between observed and predicted germination or infection values.

2.3.4. Risk chart models. Curves derived from the final model parameter estimates for the range of temperature and leaf wetness duration required to attain thresholds values of disease severity evaluated at 7 DAI were used to generate isopaths for predicting risk of downy mildew development for each cucurbit host type. This was achieved by integrating the asymptote (equation 1.1), with the estimated values for parameters B , and D to generate predicted disease severity as a function of t and w . Based on the level of disease severity, risk categories were defined as follows: light, moderate and severe for 0 to 10%, 10 to 20% and >20% leaf area infected, respectively. These disease severity categories resulted in combinations of temperature and leaf wetness duration that were distinctly different among three risk categories.

2.4. RESULTS

2.4.1. Sporangia germination and disease severity. Sporangia germination averaged across all temperatures and leaf wetness combinations was highest for the cantaloupe and lowest for squash (Table 2.1) in both experiments, while sporangia germination was intermediate for cucumber. Disease severity evaluated at 5 and 7 DAI and averaged across all combinations of temperature and leaf wetness duration was highest for cucumber and lowest for cantaloupe in both experiments, while disease severity for squash was intermediate (Table 2.1). The extent of differences between host types was dependent on the response variable. For example, germination for cucumber and squash was 16% and 32%, respectively, lower than that for cantaloupe in both experiments. For disease severity, however, differences between host types were greater for disease severity evaluated at 5 DAI than for disease severity evaluated at 7 DAI. In experiment 1, for example, squash had 25%

less leaf area infected at 5 DAI than cucumber, while at 7 DAI, cantaloupe had 5% less leaf area infected than cucumber.

No significant ($P > 0.05$) differences in germination and disease severity were observed between the first and second experiments. In the analysis of variance, host type, temperature, and leaf wetness duration significantly ($P < 0.0001$) affected germination and disease severity at 5 and 7 DAI. Further, both sporangia germination and disease severity were also significantly ($P < 0.001$) affected by the interactions between host type, temperature, and leaf wetness duration. Variation in temperature response within experimental runs (split-plot error) was not significant ($P > 0.05$) for disease severity at 5 and 7 DAI but was marginally significant ($P = 0.045$) for sporangia germination. Similarly, variation in temperature response of germination and disease severity among host types within experimental runs was not significant ($P > 0.05$) except for disease severity evaluated at 7 DAI.

The range of temperature and wetness duration that supported high levels of germination varied among host types. For example, for cantaloupe, the highest proportion of germinated sporangia ($\geq 60\%$) was attained at $t = 10$ to 30°C for $w \geq 8$ h, while for squash, the same level of germination was attained at $t = 15$ to 20°C for $w \geq 12$ h (Fig. 2.1A, E). The corresponding level of germination for cucumber was attained for $t = 10$ to 25°C and $w \geq 8$ h (Fig. 2.1C). For all host types, the lowest levels of germination ($\leq 20\%$) were observed at $t = 5^\circ\text{C}$ and $w = 2$ or 4 h except for squash where this lowest level of germination was also observed for $t = 30^\circ\text{C}$ and $w \leq 4$ h. The optimum temperature for germination was 19.9 , 19.3 and 19.9°C for cantaloupe, cucumber and squash, respectively.

For all temperatures evaluated, an increase in wetness resulted in an increase in infection in all the host types except at 5°C where the increase infection was minimal. The optimum range of temperature and wetness duration for disease severity varied among host types. At 5 DAI, disease severity >15% was observed at $t = 15$ to 25°C for $w \geq 12$ h for cantaloupe, $t = 10$ to 30°C for $w \geq 4$ h for cucumber and $t = 20$ to 25°C for $w \geq 4$ h for squash (Fig. 2.2). A similar trend was observed for severity evaluated at 7 DAI except that the optimum range for infection was much broader for w than that observed at 5 DAI for all the host types (Fig. 2.3). For example, at $t = 15$ to 25°C, >15% leaf area infected on cantaloupe was observed for $w \geq 4$ h at 7 DAI compared to $w \geq 12$ h at 5 DAI. The optimum temperature for infection at 7 DAI was 20.5, 20.1 and 17.6°C for cantaloupe, cucumber and squash, respectively.

2.4.2. Model evaluation and parameter estimates. Combined effects of t and w on germination and infection of cucurbit host types by *P. cubensis* were well described by the model. For each host type, germination and disease severity increased sigmoidally when w was increased, whereas the response of the variables to temperature was unimodal with highest and lowest values being around the middle and the extreme range of temperatures, respectively. Plots of observed germination (Fig. 2.1) and infection (Figs. 2.2 and 2.3) were similar to those of predicted values for all host types. The correlation coefficients (r) between predicted and observed germination were = 0.962, 0.951 and 0.973 for cantaloupe, cucumber and squash, respectively. The r values for disease severity at 5 DAI were 0.919, 0.895 and 0.912, for cantaloupe, cucumber and squash, respectively, and the corresponding values for disease severity at 7 DAI were 0.893, 0.884 and 0.881, respectively. Parameters estimates were all significantly ($P < 0.001$) different from zero except for the parameter h . The

corresponding asymptotic standard errors (se) for germination (Table 2.3), disease severity at 5 DAI (Table 2.4) and disease severity at 7 DAI (Table 2.5) were not very large ($se < 4.0$) except for the parameter f where the standard error was fairly large for germination ($se = 10.38$) on cantaloupe. For all host types, G and F were the most- and least-precisely estimated parameters, respectively.

Differences among host types were mainly characterized by differences in the parameter E , the upper limit of germination or disease severity. For germination, E was highest for cucumber (90%), intermediate for cantaloupe (81%) and lowest for squash (72%) (Table 2.3). For disease severity at 5 DAI, the parameter E was highest for cucumber (32%) and lowest for cantaloupe (19%) (Table 2.4). Similarly, for disease severity at 7 DAI, parameter E was highest for cucumber and squash and lowest for cantaloupe (Table 2.5). At the optimum temperatures, germination and disease severity values approached their upper limits for all host types (Fig. 2.4). Although the rate of change with respect to t (parameter F) was the least-precisely estimated parameter, differences among host types in estimates of F were fairly consistent for disease severity (Table 2.4 and 2.5).

2.4.3. Risk prediction charts. Based on disease severity evaluated at 7 DAI, the risk threshold model indicated that 10% leaf area infected in cantaloupe would occur when $t = 6$ to 30°C and $w = 2$ to 10 h. The corresponding range for cucumber was $t = 5$ to 30°C and $w = 1$ to 5 h, while that for squash was $t = 5$ to 21°C and $w = 1$ to 14 (Figure 2.5). At any given temperature, the duration of leaf wetness required to cause disease was longer for cantaloupe than for cucumber (Figure 2.5). For example, at 18°C , 20% disease severity was expected on cantaloupe after 8 h of leaf wetness compared to only 2 h for cucumber.

2.5. DISCUSSION

In this study, we quantitatively defined the effects of temperature and duration of leaf wetness on sporangia germination and infection of three different cucurbit host types by *P. cubensis* in controlled conditions. Cucurbit host type, temperature and duration of leaf wetness significantly affected sporangia germination and disease severity. Further, sporangia germination and infection were significantly affected by the interaction between host type, and temperature and leaf wetness duration. Differences between the host types were mainly characterized by differences in the upper limit of sporangia germination or infection and the rate of change in these two response variables with respect to temperature. Prediction charts were developed from surface response models and they could be used to estimate the risk of cucurbit downy mildew based on prevailing or forecasted temperature and leaf wetness duration.

Previous studies (Cohen, 1977; Cohen and Rotem, 1969; Yang et al., 2007) investigated independently the effects of temperature and leaf wetness on the infection parameters of *P. cubensis*. Recently, quantitative models were developed that define combined effects of temperature and leaf wetness duration on sporangia germination and infection by *P. cubensis* (Arauz et al., 2010). Our results are generally in agreement with those reported in previous studies. For example, in the present study, the optimum temperature for infection ranged between 17 to 21°C which is similar to the range of 15 to 20°C reported by Cohen (1977). In the study by Arauz et al. (2010), the optimum temperature for sporangia germination ranged between 16 to 18°C which is similar to the 18 to 20°C range reported in this study. However, our results are different from previous reports on the maximal temperature for infection. Previous studies (Cohen, 1977; Cohen and Rotem,

1969) reported 25°C as the maximal temperature for infection with very minimal or no infection at 28°C. However, we observed infection at 30°C, a much higher temperature than previously reported. Our results on a high maximal temperature for infection are similar to those reported recently for *P. cubensis* (Arauz et al., 2010). It has been suggested that a change in the population structure of *P. cubensis* that has been linked to the resurgence of the disease in 2004 in Europe and the U.S., may have generated new strains of the pathogen (Lebeda and Cohen, 2011; Savory et al., 2011). This change in the population structure of *P. cubensis* may be due to the migration of virulent strains from Asia to Europe and the U.S. (Runge et al., 2011). Oospore formation and sexual reproduction in *P. cubensis* was reported for the first time under controlled conditions (Cohen et al., 2011) and although this process can also generate more aggressive strains of the pathogen, its impact on the population biology of *P. cubensis* in the field is yet to be determined. Nonetheless, these new strains of *P. cubensis* are more aggressive and adapted to much higher maximal temperatures for infection than older strains. Adaptation to high temperatures by a new strain of a pathogen has also been reported for *Puccinia striiformis* f. sp. *tritici* causing stripe rust of wheat (Milus et al., 2009) in the United States.

In earlier work on the infection parameters of *P. cubensis*, it was reported that the highest increase in infection occurred between 4 and 6 days after inoculation (Cohen, 1977), but the dynamics of this rapid increase in relation to temperature was not established. In this study, the range for the optimum temperature for infection became broader with increasing length of time between inoculation and disease assessment. The coalescing of individual lesions when lesions expand may explain this broadening of the optimum temperature. However, downy mildew lesions do not generally expand substantially after symptom

appearance (Populer, 1981). Thus, it's highly possible that a distributed delay in lesion development may be responsible for the broadening of optimum temperature for infection from 5 to 7 days after inoculation (Populer, 1981). This distributed delay in lesion development is probably more pronounced for infection that originated at the extremes than in the middle of the temperature range evaluated in this study. Given the similarity of the response surfaces and asymptotic parameters for germination and infection 7 days after inoculation, the 7-day data set probably depicts true terminal infection levels.

Advisory guidelines on the development of cucurbit downy mildew have been based on studies on the effects of temperature and leaf wetness duration involving a single cucurbit host type (Cohen, 1977; Cohen and Rotem, 1969; Yang et al., 2007). However, cucurbit host types differentially respond to the disease under field conditions (Ojiambo et al., 2010). Thus, it is expected that advisory guidelines based on studies conducted on a single host may be less accurate across host types. Thus, taking into consideration the differences among host types should increase the predictive capacity of disease advisory systems. In the present study, combined effects of temperature and leaf wetness duration on development of cucurbit downy mildew were investigated using three cucurbit host types. Our results clearly show that the interaction of host type, with temperature and wetness duration significantly influenced the magnitude and extent of either sporangia germination or infection. First, fewer hours of wetness were required to gain a specific level of germination or infection as temperature approached the optimum level. Secondly, at a given temperature, fewer hours of leaf wetness were required to result in a given level of disease severity on cucumber compared to squash or cantaloupe. The interaction of different host cultivars, with temperature and leaf wetness on infection parameters of plant pathogens has been reported in

other pathosystems. For example, Wu et al. (1999) characterized cultivar responses to temperature and wetness duration for components of infection by *Cercospora arachidicola* and observed that a longer wetness period was required to achieve a specific level of disease for a partially resistant compared with a susceptible cultivar. When all other factors that contribute to disease are held constant, cucumber is more susceptible to downy mildew than other cucurbitaceous host types (Ojiambo et al., 2010; Urban and Lebeda, 2006). Thus, the fewer hours of leaf wetness required to result in a given level of disease severity on cucumber compared to squash is most likely due to differences in host susceptibility to *P. cubensis*.

A modified version of a Weibull equation previously developed and validated to describe the response of the infection parameters of *P. cubensis* to combined effects of temperature and leaf wetness (Arauz et al., 2010) was used to model the effects on temperature and wetness duration on germination and infection for three cucurbit host types. This model resulted in a good description of our data as indicated by the relatively small asymptotic standard errors and high correlation coefficients between observed and predicted data. Further, there was a clear relationship between the asymptote and temperature for each host type. Differences among the host types were primarily characterized by the upper limit of germination or infection (parameter E). For both germination and infection, the parameter E was consistently larger for cucumber than either cantaloupe or squash. Differences in the upper limit of disease (Neher and Campbell, 1992; Park and Lim, 1985) or intrinsic rate of disease increase (Shaner and Finney, 1977; Wilcoxson et al., 1975) have been used to characterize host resistance. In this study, host type had a large effect on parameter E but had no effect on the optimum temperature. Further, unlike parameter E , intrinsic rate parameters

(parameters B or F) were more variable across host types when disease severity was assessed at 5 and 7 days after inoculation. Our results, thus, tend to support variation in the upper limit as the operating mechanism for cucurbit downy mildew. Nonetheless, cucurbit hosts are exposed to multiple cycles of disease in the field as opposed to a single cycle of infection used in this study. It is likely that a combination of other components of disease resistance determine the extent in the reduction of the disease on squash or cantaloupe versus cucumber over multiple infection cycles under field conditions.

A major goal of this study was to generate information that could be used to improve the cucurbit downy mildew forecasting system (Ojiambo et al., 2011). This advisory system issues a risk of initial infection based on the assumption that cucurbit host types respond similarly to infection by *P. cubensis*. The first spray is recommended if the risk of infection is moderate to high. In this study, we developed host based prediction charts that may have application in predicting the potential risk for disease development. Assuming that sporangia are available, these charts can be used in conjunction with the FLEXPART plume dispersal model (Stohl et al., 2005) present in the forecasting system, to identify locations of high risk for disease development based on forecasted temperature and duration of leaf wetness along projected trajectories of sporangia transport. For example, it's predicted that a moderate risk of the disease will be observed for the squash when the temperature is 20°C and the duration of leaf wetness is 5 h. Under the same environmental conditions, the predicted risk of disease development on the cantaloupe and cucumber will be light and severe, respectively. Given this scenario, application of the initial fungicide spray would only be recommended for cucumber and squash fields but not cantaloupe fields. Clearly, the inclusion of host based models describing the combined effects of temperature and leaf wetness on infection by *P.*

cubensis will greatly improve the predictive capacity of the cucurbit downy mildew advisory system. Applying fungicides with single modes of action only if and when it is necessary can also slow-down the development of resistance in *P. cubensis* (Skylakakis, 1981).

Under favorable conditions, downy mildew can spread rapidly and cucurbit growers are more concerned with whether infection will occur than in the actual level of infection. Thus, the timing of the initial fungicide spray is crucial in the management of the disease. It is important to note that presently, no advisory system is available to determine the risk of the disease development during the growing season. Such a system is useful to guide within-season fungicide application after initial infection and should improve fungicide efficiency compared to the current calendar-based application schedule. Models developed in this study can be extended to incorporate the effects of host growth, fungicides and interrupted leaf wetness to predict risk of disease development during the season. Thus, additional studies are needed to establish the impact of interrupted leaf wetness on the infection parameters of *P. cubensis* and the risk of disease development during the season. Further, although the Weibull model used to characterize the effects of cucurbit host types on infection parameters of *P. cubensis* has previously been validated (Arauz et al., 2010), the infection risks presented in this study will need to be verified under field conditions prior to implementation in commercial cucurbit production. Variation in some of the results present in study can be expected when using different cucurbit host types or if the susceptibility of a given host type changes due to changes in population of the pathogen.

2.6. LITERATURE CITED

1. Arauz, L. F., and Sutton, T. B. 1989. Temperature and wetness duration requirements for apple infection by *Botryosphaeria obtusa*. *Phytopathology* 79:440-444.
2. Arauz, L. F., Neufeld, K. N., Lloyd, A. L., and Ojiambo, P. S. 2010. Quantitative models for germination and infection of *Pseudoperonospora cubensis* in response to temperature and duration of leaf wetness. *Phytopathology* 100:959-967.
3. Cohen, Y. 1977. The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Can. J. Bot.* 55: 1478–1487.
4. Cohen, Y., and Rotem, J. 1969. The effects of lesion development, air temperature, and duration of moist period on sporulation of *Pseudoperonospora cubensis* in cucumbers. *Israel J. Bot.* 18:135-140.
5. Cohen, Y., Rubin, A. E., and Galperin, M. 2011. Formation and infectivity of oospores of *Pseudoperonospora cubensis*, the causal agent of downy mildew in cucurbits. *Plant Dis.* 95: 874.
6. Hoagland, D.R. and Arnon, D.I., 1950. The water culture method for growing plants without soil. *Calif. Agric. Exp. Stn. Circ.*, 347: 1-23.
7. Lebeda, A. 1986. *Pseudoperonospora cubensis*. Pages 81-85 in: *Methods of Testing Vegetable Crops for Resistance to Plant Pathogens*. A. Lebeda, ed. Sempra Research Institute of Vegetable Crops, Czech Republic.
8. Lebeda, A., and Cohen, Y. 2011. Cucurbit downy mildew (*Pseudoperonospora cubensis*) - biology, ecology, epidemiology, host-pathogen interaction and control. *Eur. J. Plant Pathol.* 129:157-192.
9. Lebeda, A., and Widrlechner, M. P. 2003. A set of cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Prot.* 110:337-349.
10. Milus, E. A., Kristensen, K., and Hovmøller, M. S. 2009. Evidence for increased aggressiveness in a recent widespread strain of *Puccinia striiformis* f. sp. *tritici* causing stripe rust of wheat. *Phytopathology* 99:89-94.
11. Neher, D. A., and Campbell, C. L. 1992. Underestimation of disease progress rates with the logistic, monomolecular, and Gompertz models when maximum disease intensity is less than 100 percent. *Phytopathology* 82:811-814.

12. Ojiambo, P. S., Holmes, G. J., Britton, W., Kever, T., Adams, M. L., Babadoost, M., Bost, S. C., Boyles, R., Brooks, M., Damicone, J., Draper, M. A., Egel, D. S., Everts, K. L., Ferrin, D. M., Gevens, A. J., Gugino, B. K., Hausbeck, M. K., Ingram, D. M., Isakeit, T., Keinath, A. P., Koike, S. T., Langston, D., McGrath, M. T., Miller, S. A., Mulrooney, R., Rideout, S., Roddy, E., Seebold, K.W., Sikora, E. J., Thornton, A., Wick, R. L., Wyenandt, C. A. and Zhang, S. 2011. Cucurbit downy mildew ipmPIPE: a next generation web-based interactive tool for disease management and extension outreach. Online. Plant Health Progress doi:10.1094/PHP-2011-0411-01-RV.
13. Ojiambo, P. S., Paul, P. A., and Holmes, G. J. 2010. A quantitative review of fungicide efficacy for managing downy mildew in cucurbits. *Phytopathology* 100:1066-1076.
14. Palti, J., and Cohen, Y. 1980. . Downy mildew of cucurbits: The fungus and its hosts, distribution, epidemiology and control. *Phytoparasitica* 8:109-147.
15. Park, E. W., and Lim, S. M. 1985. Empirical estimation of the asymptotes of disease progress curves and the use of the Richards generalized rate parameters for describing disease progress. *Phytopathology* 75:786-791.
16. Populer, C. 1981. Epidemiology of downy mildews. Pages 57-105 in: *The Downy Mildews* D. M. Spencer, ed. Academic Press, New York.
17. Runge, F., Choi, Y. -J., and Thines, M. 2011. Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. *Eur. J. Plant Pathol.* 129:135-146.
18. Savory, E. A., Granke, L. L., Quesada-Ocamop, L. M., Varbanova, M., Hausbeck, M. K., and Day, B. 2011. The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. *Mol. Plant Pathol.* 12:217-226.
19. Shaner, G., and Finney, R. E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* 67:1051-1056.
20. Skylakakis, G. 1981. Effects of alternating and mixing pesticides on the buildup of fungal resistance. *Phytopathology* 71:1119-1121.
21. Stohl, A., Forster, C., Frank, A., Seibert, P., and Wotawa, G. 2005. Technical note: The Lagrangian particle dispersion model FLEXPART version 6.2. *Atmos. Chem. Phys. Discuss.* 5:4739-4799.
22. Thomas, C. E. 1996. Downy mildew. Pages 25-27 in: *Compendium of Cucurbit Diseases*. T. A. Zitter, D. L. Hopkins, and C. E. Thomas, eds. APS Press, St. Paul, MN.

23. Urban, J., and Lebeda, A. 2006. Fungicide resistance in cucurbit downy mildew methodological, biological and population aspects. *Ann. Appl. Biol.* 149:63-75.
24. Weibull, W. 1951. A statistical distribution of wide applicability. *J. Appl. Mech.* 18:293-297.
25. Wilcoxson, R. D., Skovmand, B., and Atif, A. H. 1975. Evaluation of wheat cultivars for ability to retard development of stem rust. *Ann. Appl. Biol.* 80:275-281.
26. Wu, L., Damicone, J. P., Duthie, J. A., and Melouk, H. A. 1999. Effects of temperature and wetness duration on infection of peanut cultivars by *Cercospora arachidicola*. *Phytopathology* 89:653-659.
27. Yang, X., Li, M., Zhang, Z., and Hou, Y. 2007. Early warning model for cucumber downy mildew in unheated greenhouses. *New Zealand J. Agric. Res.* 50:1261-1268.

Table 2.1. Sporangia germination and severity of cucurbit downy mildew on three cucurbit host types inoculated with *Pseudoperonospora cubensis* in two growth chamber experiments

Experiment	Host type ^x	Sporangia Germination (%) ^y		Disease severity (%) (5 DAI) ^z		Disease severity (%) (7 DAI) ^z	
		Mean	<i>n</i>	Mean	<i>n</i>	Mean	<i>n</i>
1	Cantaloupe	53.3	60	8.2	112	13.4	127
	Cucumber	44.5	60	18.3	150	24.1	137
	Acorn Squash	36.1	60	13.6	120	22.7	108
	Mean	44.6	180	13.4	382	20.1	372
2	Cantaloupe	53.0	60	9.9	140	14.0	155
	Cucumber	44.3	60	16.6	164	23.4	147
	Acorn Squash	36.0	60	12.1	117	17.3	129
	Mean	44.4	180	12.9	421	18.2	431

^x The variety used was ‘Kermit’, ‘Straight 8’ and ‘Table Queen’ for cantaloupe, cucumber and acorn squash, respectively.

^y Sporangia germination values are means over six temperatures, five durations of leaf wetness, two plants, and two inoculated leaves per plant; *n* = sample size.

^z Disease severity values are means over six temperatures, five durations of leaf wetness, three plants, and two inoculated leaves per plant, assessed 5 and 7 days after inoculation (DAI); *n* = sample size.

Table 2.2. Analysis of variance for the effects of temperature (T), cucurbit host type (S), and leaf wetness duration (W) on germination of sporangia of *Pseudoperonospora cubensis* and severity of cucurbit downy mildew^x

Source	Sporangia germination (proportion)			Disease severity (5 DAI) ^y			Disease severity (7 DAI) ^y		
	df	SS	$P > F$	df	SS	$P > F$	df	SS	$P > F$
Replication (R)	3	0.00	0.7965	5	0.01	0.4450	5	0.01	0.0101
T	5	3.57	0.0001	5	1.22	0.0001	5	2.41	0.0001
$T(R)$ – error a	15	0.01	0.0364	25	0.03	0.4266	25	0.06	0.0449
Whole-plot total	23	35	35
S	2	1.75	0.0001	2	0.49	0.0001	2	0.87	0.0001
$S \times T$	10	0.27	0.0001	10	0.07	0.0001	10	0.30	0.0001
$S \times T(R)$ – error b	36	0.01	0.1558	60	0.07	0.4138	60	0.15	0.1016
Split-plot total	48	72	72
W	4	10.17	0.0001	4	0.74	0.0001	4	1.21	0.0001
$W \times T$	20	0.08	0.0001	20	0.10	0.0001	20	0.33	0.0001
$W \times S$	8	0.48	0.0001	8	0.03	0.0002	8	0.05	0.0002
$W \times S \times T$	40	0.14	0.0001	40	0.12	0.0001	40	0.27	0.0001
$W \times S \times T(R)$ – error c	216	0.08	...	317	0.34	...	318	0.47	...
Split-split-plot total	288	389	390
Total	359	496 ^z	497 ^z

^x Analysis of variance was conducted on mean values for two plants with two inoculated leaves per plant.

^y Disease severity (leaf area infected, expressed as a proportion) was determined 5 and 7 days after inoculation (DAI).

^z Forty-three and forty-two observations were missing when disease severity was assessed 5 and 7 days after inoculation, respectively, due to loss of plants.

Table 2.3. Parameter estimates for the model: $f(w,t) = f(t) \cdot (1 - \exp\{-[B \cdot w]^D\})$, characterizing the effects of temperature and leaf wetness duration on germination of *Pseudoperonospora cubensis* sporangia on three cucurbit host types ^a

Parameter	Cantaloupe (cv. Kermit)			Cucumber (cv. Straight 8)			Acorn Squash (cv. Table Queen)		
	Estimate	CI_L	CI_U	Estimate	CI_L	CI_U	Estimate	CI_L	CI_U
	e								
<i>B</i>	0.24	0.19	0.28	0.16	0.10	0.23	0.15	0.10	0.20
<i>D</i>	1.39	1.00	1.78	0.91	0.59	1.23	0.92	0.66	1.17
<i>E</i>	0.81	0.75	0.88	0.90	0.75	1.05	0.72	0.61	0.82
<i>F</i>	22.65	1.21	44.08	18.99	11.09	26.89	22.54	14.75	30.33
<i>G</i>	0.14	0.07	0.19	0.21	0.17	0.24	0.19	0.41	0.24
<i>H</i>	1.48	-2.46	5.42	0.94	-0.24	2.12	1.66	-0.44	3.77

^a In the model, $f(t) = E' \{ \exp[(t - f)G / (H + 1)] \} / \{ 1 + \exp[(t - f)G] \}$ in which $E' = E[(H + 1) / H]H^{1/(H+1)}$. The parameters *B*, *D*, *E*, *F*, *G* and *H* are as defined in the main text; *t* = temperature and *w* = leaf wetness duration.

^b CI_L and CI_U = asymptotic lower and upper limits of the 95% confidence interval, respectively, around the parameter estimate.

Table 2.4. Parameter estimates for the model: $f(w,t) = f(t) \cdot (1 - \exp\{-[B \cdot w]^D\})$, characterizing the effects of temperature and leaf wetness duration on infection by *Pseudoperonospora cubensis* for three cucurbit host types 5 days post-inoculation^a

Parameter	Cantaloupe (cv. Kermit)			Cucumber (cv. Straight 8)			Acorn Squash (cv. Table Queen)		
	Estimate	CI_L	CI_U	Estimate	CI_L	CI_U	Estimate	CI_L	CI_U
	e								
<i>B</i>	0.19	0.06	0.33	0.27	0.13	0.42	0.23	0.09	0.35
<i>D</i>	0.78	0.34	1.21	0.84	0.30	1.39	0.86	0.34	1.37
<i>E</i>	0.19	0.15	0.25	0.32	0.26	0.38	0.26	0.20	0.31
<i>F</i>	19.13	12.50	25.77	17.25	8.94	25.56	15.34	9.13	21.56
<i>G</i>	0.24	0.20	0.29	0.23	0.18	0.29	0.26	0.19	0.34
<i>H</i>	0.81	-0.18	1.80	0.61	-0.30	1.51	0.45	-0.13	1.04

^a In the model, $f(t) = E' \{ \exp[(t - f)G / (H + 1)] \} / \{ 1 + \exp[(t - f)G] \}$ in which $E' = E[(H + 1) / H]H^{1/(H+1)}$. The parameters *B*, *D*, *E*, *F*, *G* and *H* are as defined in the main text; *t* = temperature and *w* = leaf wetness duration.

^b CI_L and CI_U = asymptotic lower and upper limits of the 95% confidence interval, respectively, around the parameter estimate.

Table 2.5. Parameter estimates for the model: $f(w,t) = f(t) \cdot (1 - \exp\{-[B \cdot w]^D\})$, characterizing the effects of temperature and duration of leaf wetness on infection by *Pseudoperonospora cubensis* for three cucurbit host types 7 days post-inoculation^a

Parameter	Cantaloupe (cv. Kermit)			Cucumber (cv. Straight 8)			Acorn Squash (cv. Table Queen)		
	Estimate	CI_L	CI_U	Estimate	CI_L	CI_U	Estimate	CI_L	CI_U
	e								
<i>B</i>	0.27	0.13	0.39	0.34	0.16	0.52	0.21	0.01	0.39
<i>D</i>	0.87	0.34	1.41	0.84	0.19	1.49	0.76	0.16	1.36
<i>E</i>	0.25	0.20	0.29	0.44	0.32	0.51	0.42	0.31	0.59
<i>F</i>	21.65	13.33	29.96	17.65	7.90	27.41	16.17	10.67	21.67
<i>G</i>	0.23	0.16	0.30	0.22	0.17	0.27	0.30	0.23	0.37
<i>H</i>	1.29	-0.63	3.21	0.59	-0.42	1.59	0.65	-0.11	1.41

^a In the model, $f(t) = E' \{ \exp[(t - f)G / (H + 1)] \} / \{ 1 + \exp[(t - f)G] \}$ in which $E' = E[(H + 1) / H]H^{1/(H+1)}$. The parameters *B*, *D*, *E*, *F*, *G* and *H* are as defined in the main text; *t* = temperature and *w* = leaf wetness duration.

^b CI_L and CI_U = asymptotic lower and upper limits of the 95% confidence interval, respectively, around the parameter estimate.

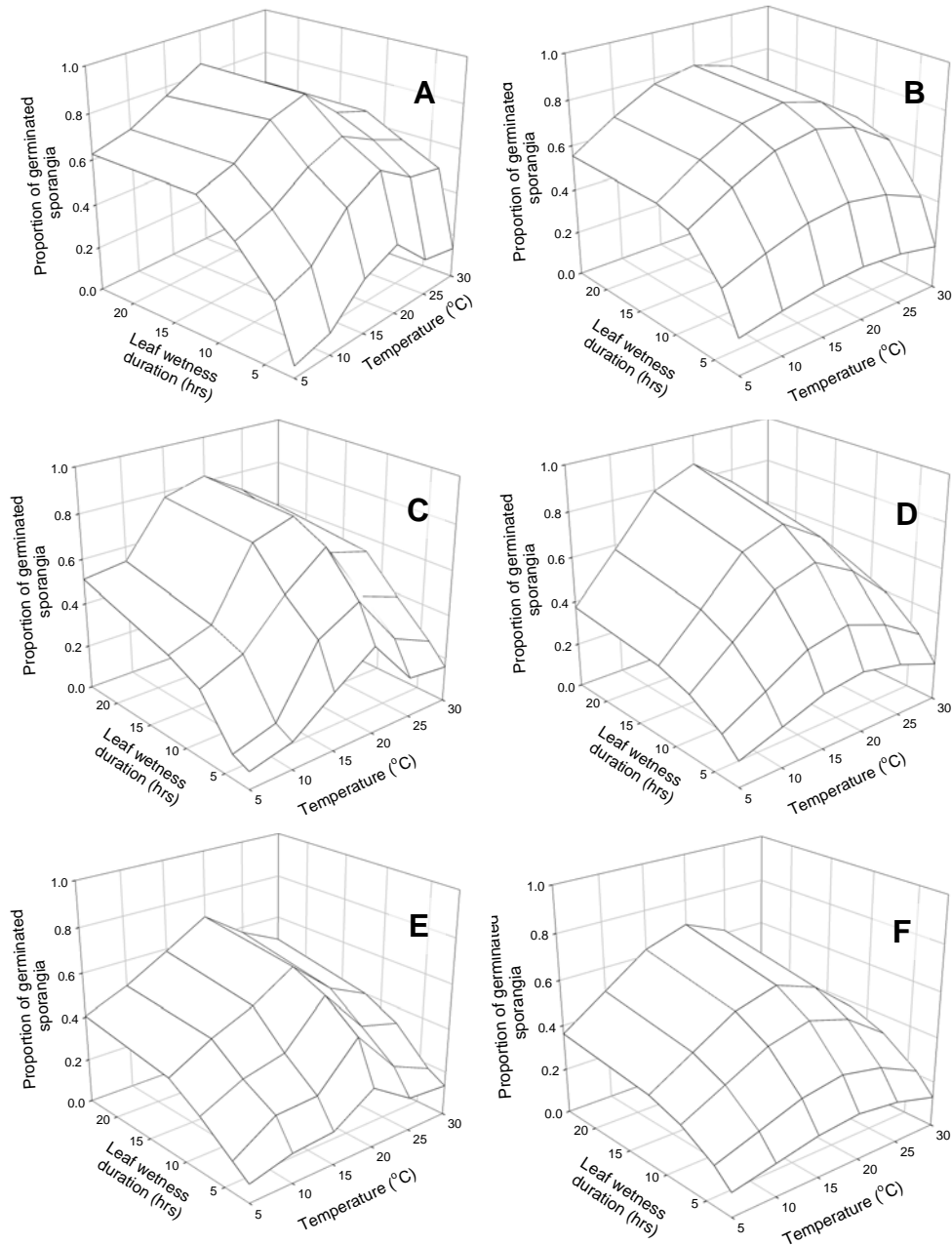


Figure 2.1. Effects of temperature and leaf wetness duration on sporangia germination on leaves of three cucurbit host types inoculated with *Pseudoperonospora cubensis*. Observed values are for **A**, cantaloupe (cv. Kermit); **C**, cucumber (cv. Straight 8) and **E**, acorn squash (cv. Table Queen). Predicted values for **B**, cantaloupe; **D**, cucumber and **F**, acorn squash are from fitting observed values to the model: $f(w, t) = f(t) \cdot (1 - \exp\{-[B \cdot w]^D\})$. See main text for definition of the variables and parameters.

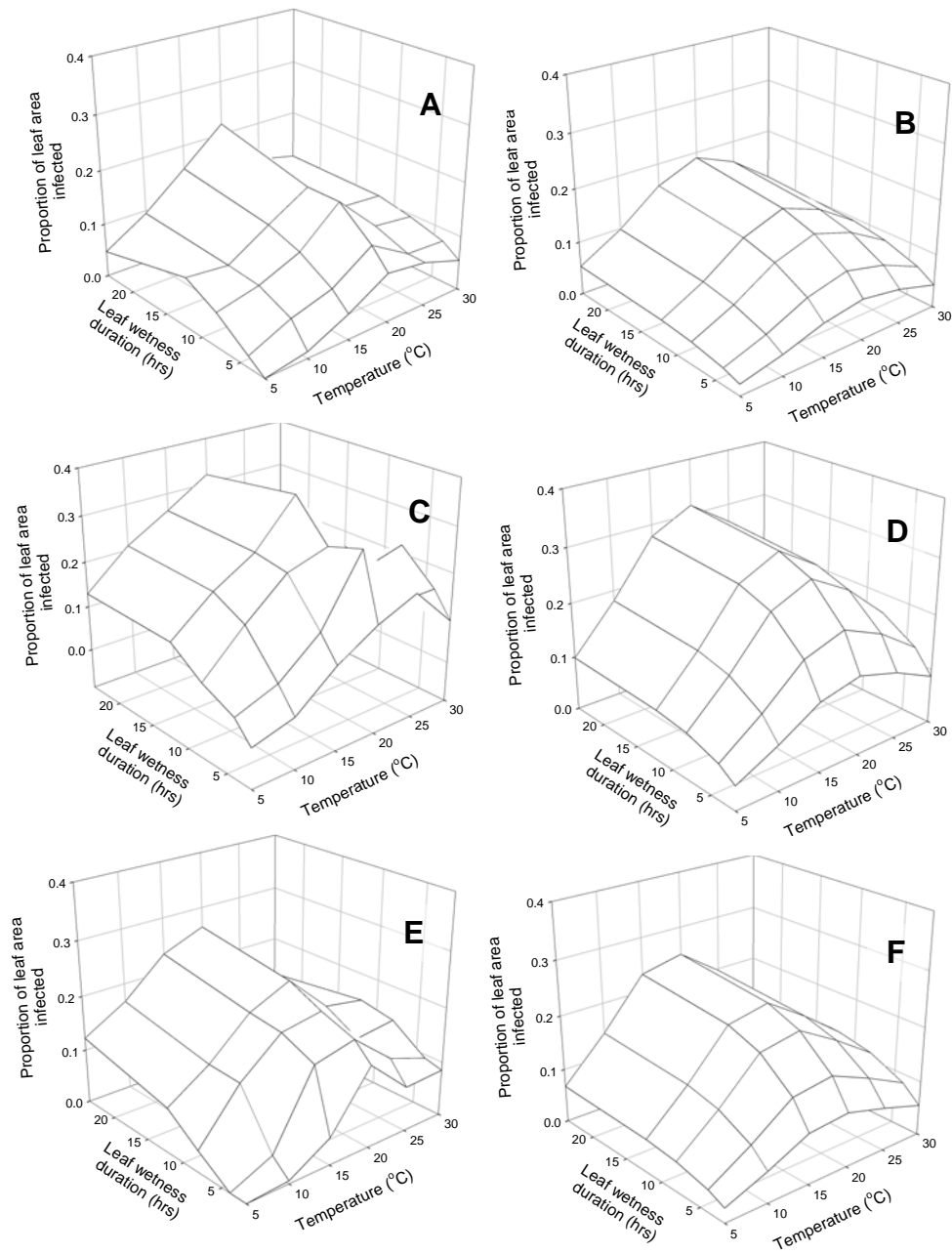


Figure 2.2. Proportion of leaf area infected for three cucurbit host types 5 days after inoculation with *Pseudoperonospora cubensis*. Observed values are for **A**, cantaloupe (cv. Kermit); **C**, cucumber (cv. Straight 8) and **E**, acorn squash (cv. Table Queen). Predicted values for **B**, cantaloupe; **D**, cucumber and **F**, acorn squash are from fitting observed values to the model: $f(w,t) = f(t) \cdot (1 - \exp\{-[B \cdot w]^D\})$. See main text for definition of the variables and parameters.

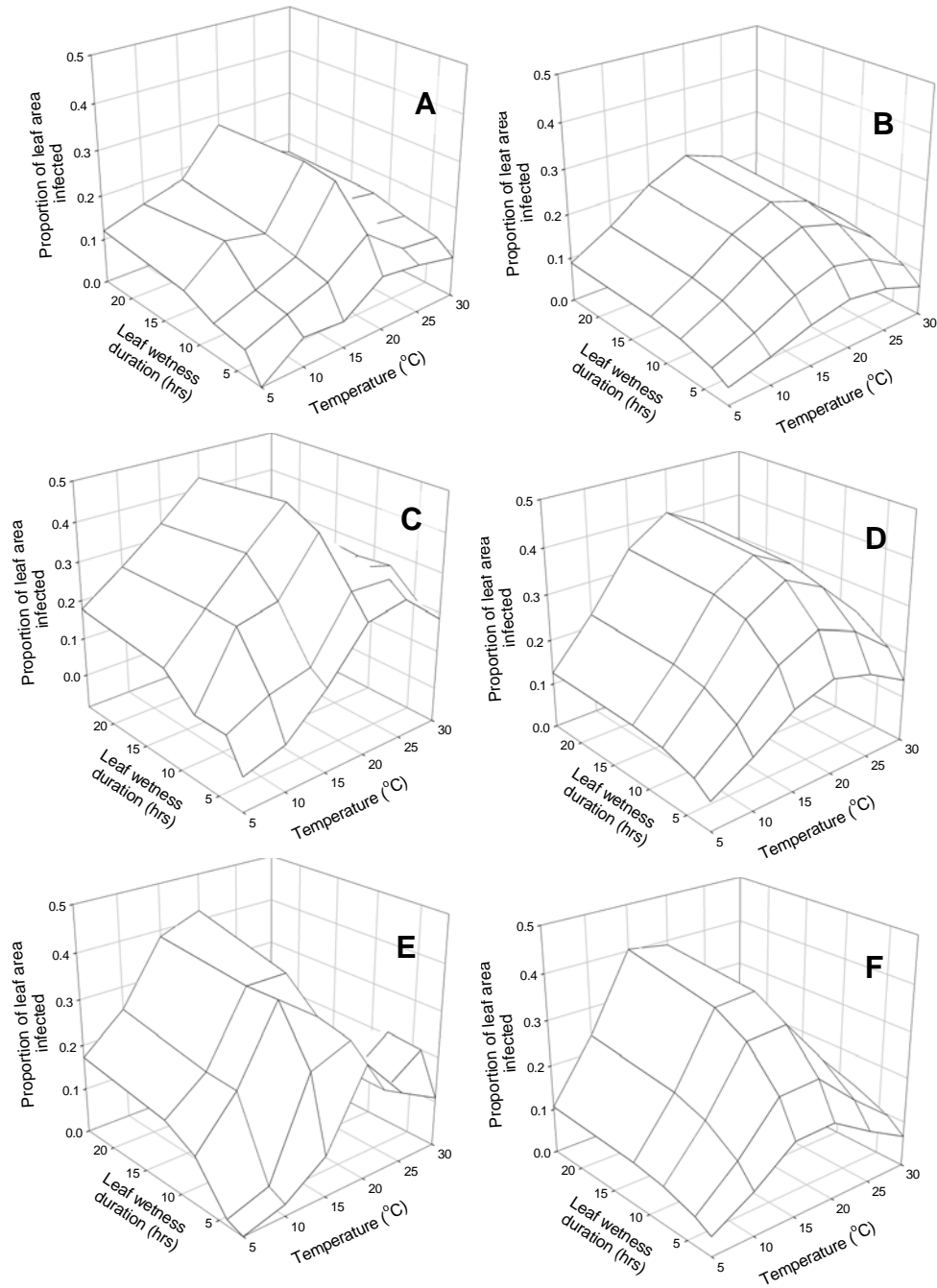


Figure 2.3. Proportion of leaf area infected for three cucurbit host types 7 days after inoculation with *Pseudoperonospora cubensis*. Observed values are for **A**, cantaloupe (cv. Kermit); **C**, cucumber (cv. Straight 8) and **E**, acorn squash (cv. Table Queen). Predicted values for **B**, cantaloupe; **D**, cucumber and **F**, acorn squash are from fitting observed values to the model: $f(w,t) = f(t) \cdot (1 - \exp\{-[B \cdot w]^D\})$. See main text for definition of the variables and parameters

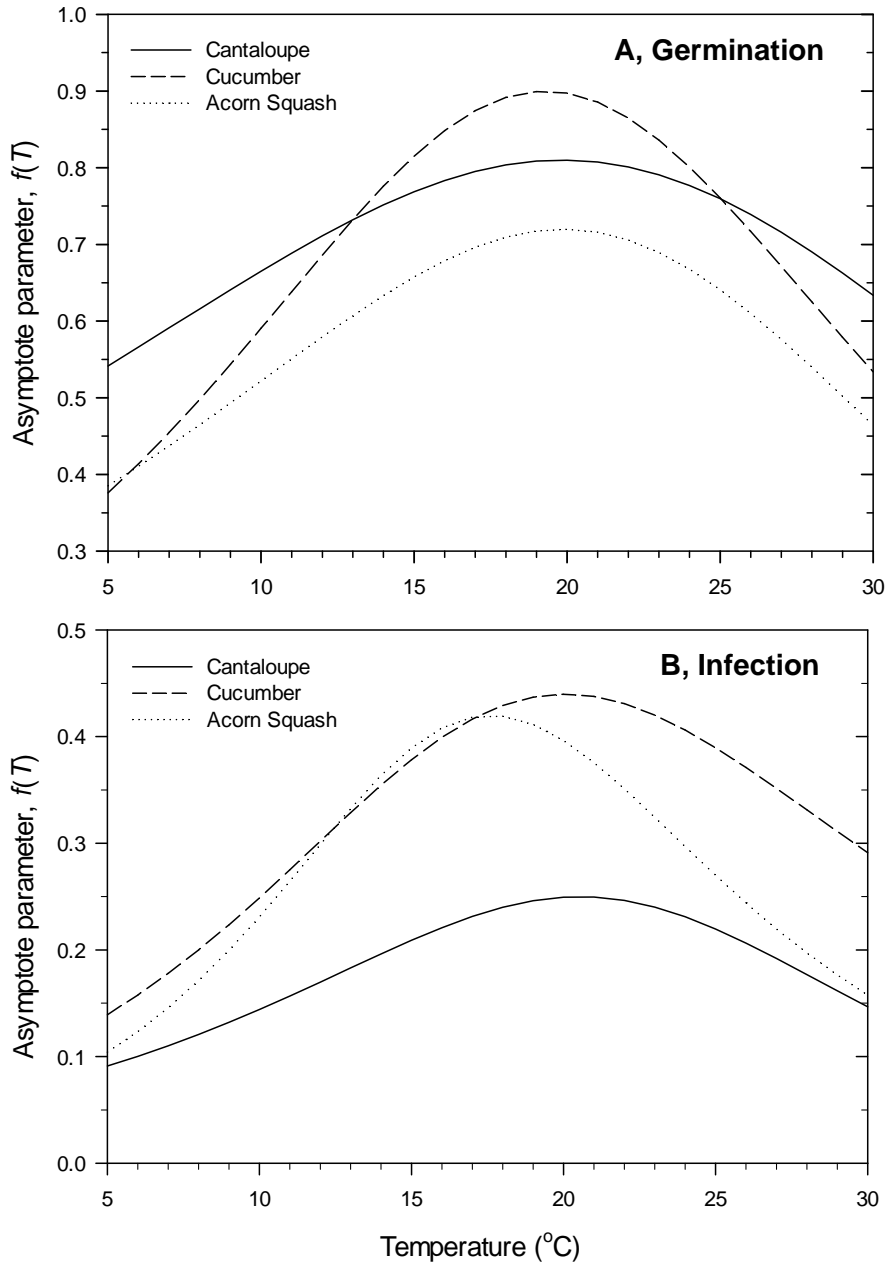


Figure 2.4. Relationship between the asymptote, $f(T)$, and temperature based on model estimates for predicting sporangia germination and proportion of leaf area infected 7 days after inoculation of cucurbit host types with *Pseudoperonospora cubensis*. The varieties used for cantaloupe, cucumber and acorn squash were ‘Kermit’, ‘Straight 8’ and ‘Table Queen’, respectively.

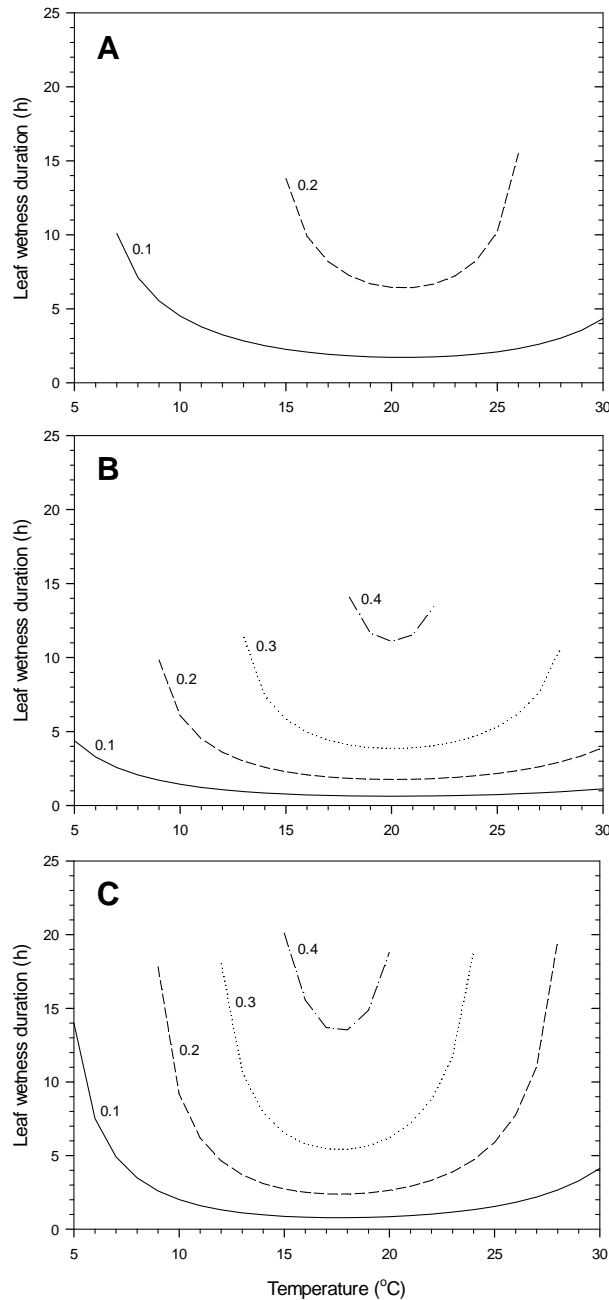


Figure 2.5. Charts for risk prediction for cucurbit downy mildew infection of three cucurbit host types. **A**, cantaloupe (cv. Kermit); **B**, cucumber (cv. Straight 8) and **C**, acorn squash (cv. Table Queen). Risk categories are based on the response surface models and isopaths separating the categories correspond to LGT = light (disease severity = 0.00 to 0.10); MOD = moderate (disease severity = 0.11 to 0.20) and SEV = severe (disease severity > 0.20). Isopaths are based on predicted response surfaces for leaf area infected 7 days after inoculating plants with *Pseudoperonospora cubensis*.

3. CHAPTER III:

Quantifying the Relationship between Disease Severity and Concentration and Escape of *Pseudoperonospora cubensis* Sporangia from a Cucumber Canopy

Neufeld, K.N., Isard, S.A., and Ojiambo, P.S. 2012. *Agricultural and Forest Meteorology* 157: (in review).

Katie N. Neufeld, Scott A. Isard and Peter S. Ojiambo

K. N. Neufeld, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695; **S. A. Isard**, Departments of Plant Pathology and Meteorology, Pennsylvania State University, University Park, PA 16802; and **P. S. Ojiambo**, Department of Plant Pathology, North Carolina State University, Raleigh, NC 27695

3.1. ABSTRACT

Fundamental to the development of models to predict aerial spread of cucurbit downy mildew is the ability to determine the airborne concentrations and escape of *Pseudoperonospora cubensis* sporangia at a source. Aerial concentrations of sporangia, C (sporangia m^{-3}), were monitored using Rotorod samplers deployed at 0.5 to 3.0 m above a cucumber canopy at two sites where disease severity ranged from about 1 to 40%. Escape of sporangia, F_s (sporangia $\text{m}^{-2} \text{s}^{-1}$), was estimated using the diffusivity of sporangia and the vertical gradient of C as measured at the various sampling heights. Disease severity, hour of day and sampling height above the crop canopy significantly ($P < 0.0001$) affected C . Sporangia concentration was higher at moderate levels of disease than at low or high levels of disease severity. Values of C decreased rapidly with height above the canopy and values of C at a height of 2.0 m were only 7% of values measured at 0.5 m when disease severity was moderate. No sporangia were collected at a height of 3.0 m above the canopy. F_s varied with the hour of the day and level of disease severity and the maximum F_s was about 930 sporangia $\text{m}^{-2} \text{s}^{-1}$. The increase of C and F_s with increasing disease severity was well

described ($P < 0.0001$) by a log-normal model and 15% disease severity was estimated a threshold above which C and F_s will decrease as disease severity increases. These results will be incorporated in a large-scale spore transport model that is currently used to forecast sporangia transport and to predict the risk of cucurbit downy mildew spread and outbreak between and among cucurbit fields in eastern U.S.

3.2 INTRODUCTION

Cucurbit downy mildew, caused by *Pseudoperonospora cubensis*, is economically the most damaging disease of cucurbitaceous crops worldwide. The disease is favored by warm and humid weather (Thomas, 1996) that prevail in many parts of the eastern U.S. Since the 1960s, cucurbit downy mildew was controlled in cucumber by planting resistant cultivars (Holmes et al., 2006). However, there was a resurgence of the disease in 2004 which resulted in heavy losses of the cucumber crop in the eastern region of the U.S. (Holmes et al., 2006) and host resistance alone is no longer sufficient to control the disease (Lebeda and Widrlechner, 2003). A change in the pathogen population structure due to the migration of virulent strains of *P. cubensis* from Asia to Europe and the U.S. has been suggested as the cause of the resurgence of the disease in Europe and the U.S. (Runge et al., 2011; Savory et al., 2011). The resurgence of the cucurbit downy mildew necessitates renewed and improved disease management guidelines. Currently, disease control relies heavily on application of fungicides, thus there is a need for continued search for the most efficient methods to apply these fungicides that will result in more effective control of the disease.

Pseudoperonospora cubensis reproduces primarily through asexual production of sporangia and thus, the primary mechanism by which the pathogen can be introduced in a disease-free field is through aerial transport of sporangia from outside inoculum sources. Sporangia can survive in the atmosphere for several hours depending on the cumulative exposure to varying degrees of solar radiation (Kanetis et al., 2010). Viable sporangia that survive during transport get deposited on plant surfaces and germinate and penetrate host tissues. Depending on environmental conditions, new sporangia are produced 4 to 12 days after infection (Cohen, 1977) and the cycle of aerial dispersal and infection is repeated. Aerial transport of *P. cubensis* sporangia from infected or overwintering sources to disease-free fields was earlier demonstrated using anecdotal evidence based on disease records collected along the Atlantic coast states in the U.S. (Nusbaum, 1944). A recent study suggests that *P. cubensis* sporangia can be transported up to a distance of 1,000 km (Ojiambo and Holmes, 2011). Thus, aerial transport of *P. cubensis* sporangia to disease-free cucurbit fields from infected or overwintering sources present a risk of crop loss due to the disease. Based on this knowledge of aerial transport and the biology of *P. cubensis*, a disease forecasting system has been developed as part of the Cucurbit ipmPIPE program to facilitate decision-making on the application of the initial fungicide spray (Ojiambo et al., 2011).

Several factors determine the infection risk for plant pathogens such as *P. cubensis* whose infectious propagules are dispersed aurally (Aylor, 1986; Skelsey et al., 2008); i) number of spores available for transport, ii) proportion of available spores that escape the canopy, iii) dilution of spores by the wind and their removal from the air, iv) survival of spores during transport; v) efficiency of spore deposition on susceptible host tissue; and vi)

amount of susceptible host tissue per unit ground area. Currently, the Cucurbit ipmPIPE forecasting system (Ojiambo et al., 2006) provides a general framework for predicting aerial transport of *P. cubensis* sporangia. However, the factors that determine the infection risk due to aerial transport of *P. cubensis* sporangia have not been quantified for incorporation into the system. For example, there is no available information on number of *P. cubensis* sporangia available for dispersal at any given time or the proportion of sporangia that will escape the canopy and become airborne. Quantification of spores available for transport and escape from a source area are the key factors to successful modeling of spore transport over any distance from a source (Aylor, 1990). If the number of *P. cubensis* sporangia escaping from a canopy is known, then the number of sporangia reaching nearby cucurbit fields can be calculated using meteorological methods (Aylor, 1978; Gifford, 1968).

The number of source fields that provide inoculum for transport to disease-free field is an integral component in determining the disease risk of aerially dispersed plant pathogens. In most aerobiology studies involving plant diseases, spore production and escape are usually investigated in source fields where disease severity is assumed to be constant or not defined (e.g., Andrade et al., 2009; Aylor et al., 1983; Aylor et al., 2001; Aylor et al., 2011). In most of these studies, disease severity in the experimental plots is usually very severe (e.g., Aylor et al., 1983) and there is little attempt to relate measured spore fluxes to different levels of disease severity. Currently, the Cucurbit ipmPIPE forecasting system (Ojiambo et al., 2011) determines the risk of infection by *P. cubensis* based on the assumption that all source fields of equal size have the same source strength irrespective of their levels of disease severity. When all factors are held constant, spore production for obligate plant pathogens such as *P.*

cubensis is dependent on the amount of available living host tissue. As disease severity increases, sporangia production and thus, escape of sporangia, will increase up to a certain severity threshold and then decrease thereafter as disease severity increases. Establishing the relationship between disease severity and spore production and escape at the source can generate information that can be used to parameterize a source area in an aerobiology model such as IAMS (Isard et al., 2007) to accurately determine the risk of sporangia being transported to disease-free fields and where and when sporangia deposition will likely to occur. Thus, the objectives of this study were: i) examine the temporal dynamics of concentration of *P. cubensis* sporangia and quantify the escape of *P. cubensis* sporangia at different levels of disease severity, and ii) establish the relationship between disease severity and concentration and escape of *P. cubensis* from a cucumber canopy.

3.3 MATERIALS AND METHODS

3.3.1. Field sites, inoculum source and disease assessment. Field experiments were conducted at two research sites during the summer of 2011. The first site was located at the Central Crops Research Station located in Clayton, while the second site was located at the Horticultural Crops Research Station located in Clinton, North Carolina. The Central Crops Research Station in Clayton is located in Johnston County in the central North Carolina, while the Horticultural Crops Research Station is located in Sampson County in eastern North Carolina. Seed of cucumber cultivar ‘Poinsett 76’ were planted in experimental plots at Clayton and Clinton on 20 June and 5 July, 2011, respectively. The cultivar ‘Poinsett 76’ is moderately susceptible to cucurbit downy mildew. Cucumber plots at Clayton consisted of

11 rows that were 17 m long and a spacing of 0.5 m between rows. Plots at Clinton consisted of 10 rows, with each row measuring 20 m and a spacing of 0.6 m between rows. Black plastic mulch with drip irrigation was used at the two sites. Within each row, holes were punched with a bulb planter every 0.6 m and two seeds were planted for each hill for a total of 28 to 33 plants per row. When necessary, plots were reseeded 1 week after initial seeding to replace non-germinated seeds. Two weeks after initial seeding, plots were thinned to one plant per hill at the two sites. Beds covered in black mulch were raised about 0.11 m above ground and the canopy of fully grown plants were 0.15 m above the plastic mulch.

Plants were regularly monitored for growth and development when the plots were fully established. Once plants reached ‘tip-over’ or about 30 days old, ten plants were randomly selected and tagged in each plot. Plants were randomly chosen by using a random number generator by row and by number of plant (within the row). The sixth, seventh, eighth leaf of every randomly chosen plant was tagged using zip-ties to allow for consistent disease assessment throughout the experiment. At both sites, disease was initiated from natural infection and tagged plants were regularly monitored for disease symptoms during the study period. Disease severity was visually assessed by estimating the percentage of leaf area with chlorotic and necrotic symptoms on all the tagged leaves. Thus, the estimated disease severity of a plot was the mean leaf area infected across all the tagged leaves in the whole plot. Disease assessment was initiated when initial symptoms (about 1% leaf area infected) were observed. Disease severity was subsequently assessed and recorded in roughly 5% increments until disease severity in a plot was about 40%. Disease severity was assessed

from 16 August to 29 August, 2011 at Clayton and 12 August to 01 September, 2011 at Clinton.

3.3.2. Standing crop of sporangia. The number of sporangia produced at the source was estimated each morning (around 7:00 am) for each level of disease severity (~1 to 40%) in experimental plots at Clayton and Clinton as described by Aylor et al. (2001). Assessments of the standing crop of sporangia were made in sampling units measuring $0.25 \times 0.25 \text{ m}^2$ within the plot. New locations of the sampling units in the plot were selected for each level of disease severity. All the lesions in the $0.25 \times 0.25 \text{ m}^2$ grids were counted. For each sampling grid, three lesions were destructively sampled and separately placed in a tube containing 5 ml of 12% CuSO_4 solution to inhibit any sporangia germination (Arauz et al., 1989). Each lesion was vortexed for a few seconds to dislodge sporangia into solution, and then removed from the tube and discarded. The concentration of sporangia in the resultant spore suspension from each lesion was then sampled twice (subsamples) and determined using a hemacytometer to estimate the number of sporangia per lesion. The standing spore crop (sporangia m^{-2}), was then determined from by first multiplying the mean number of sporangia per lesion by the number of lesions per plot and then dividing this resulting number by the area of the sampling unit.

3.3.3. Airborne concentration of sporangia. At each level of disease severity (~1 to 40%), the aerial concentration of *P. cubensis* sporangia, C (sporangia m^{-3}), was measured above the cucumber canopy by Rotorod spore samplers with retracting type heads (Model 82; Sampling Technologies, Inc., Los Altos Hills, CA). A sampling array of Rotorods was supported vertically by three separate poles (each pole mounted with four Rotorods) located

in the field plot. The three poles were placed in the center of the field in such a way that they formed a triangular shape. The spore collection rods were mounted on the poles at heights of 0.5, 1.5, 2.0 and 3.0 m above the canopy. Rotorods were operated every hour from 0700 to 1400 h. The sampling period within any given hour was 10 min and at the end of each sampling period, the sampling rods were replaced and a new sampling period commenced. A total of six consecutive sampling periods of 10 min were carried out within each hour.

A thin layer of high vacuum silicon grease (Dow Corning Corp. Midland, MI) was applied on the sampling surfaces of the Rotorods to facilitate capturing of sporangia. Sporangia captured on the greased surface were counted with the aid of a microscope at 100× magnification. Counts of sporangia on the sampling Rotorods were converted to sporangia concentrations by accounting for the proportion of sample surface that was counted, sampling rate (38 l min^{-1}), duration of sampling period and the efficiency of the Rotorod sampler. In addition to the Rotorods, a Burkard 7-day volumetric spore sampler (Burkard Scientific Sales, Ltd., Hertfordshire, England) was operated during the experiment and overnight. The Burkard sampler was attached to a platform that was above ground level and the orifice was approximately 0.5 m above the canopy. The surface of the spore trapping tape was made sticky first with a treatment of gelvatol and coated with adhesive mixture when it was dry. The start and stop period of spore collection was denoted by a knife inserted through the orifice. Sporangia were counted at 100× magnification in 2 mm strips for each hour during the sampling period. These counts gave a daily record of changes in C in the air at the source.

For each level of disease severity, values of C from the three vertical poles were averaged by sampling height and summarized by the hour of day from 0700 to 1400. The LOESS procedure of SAS (version 9.2; SAS Institute, Cary, NC) was used to perform loess nonparametric regression analyses (Cleveland and Grosse, 1991) to model daily temporal trends for hourly values of C at Clayton and Clinton for each level of disease severity. The smoothing parameter used in the loess regression analysis was chosen to minimize a bias-corrected Akaike Information Criterion (Hurvich et al., 1998). To qualitatively describe the relationship between disease severity and sporangia concentration at the various sampling heights, three classes of disease severity were defined: low (0 to 5%), moderate (5 to 20%) and high (20 to 40%). Differences in mean sporangia concentration at three sampling heights and between disease severity classes were determined using ANOVA.

3.3.4. Meteorological measurements. Temperature and relative humidity (RH) were monitored onsite at Clayton and Clinton during the experiment using Watchdog data loggers (Model 450, Spectrum Technologies, Inc., Plainfield, IL) located in the center of the triangulated poles within the field. Readings of temperature and RH were taken every 5 min and averaged every hour for subsequent analysis. Wind speed data were obtained from the weather stations located onsite at the research stations. Wind speed and wind direction were measured using a helicoid propeller and wind vane sensor suite (Model 05103, R.M. Young, Traverse City, MI) located at 10 m above ground level. These measurements were recorded every 5 sec and averaged for 1 h. Correlation analyses were performed to determine if any association existed between sporangia concentrations, temperature and RH within each hour

of the day. The CORR procedure of SAS was used on combined data from the two sites for this analysis.

Assuming, among other factors, that the wind speed profile above the crop canopy is approximately logarithmic, the wind speed (u , cm s^{-1}) at 0.5, 1.5, 2.0 and 3.0 m above the ground was calculated using the following equation (Ro and Grunt, 2007):

$$u = (u_* / k) \times \ln[(z - D) / z_0] \quad (1)$$

where, u_* is the friction velocity, k is the von Karman's constant (approximately = 0.4), z is the vertical direction or height above the canopy(cm), D is the zero-plane displacement height (cm) and z_0 is the roughness length (cm). D and z_0 are set to 0.7 and 0.2, respectively, of the height of the typical roughness elements. The frictional velocity u_* was first calculated using the wind speed measured at 10 m above the ground and then subsequently applied to calculate u at the four sampling heights above the canopy.

3.3.5. Escape of sporangia from the canopy. Calculations of sporangia that escaped the canopy were based on the assumption that the vertical diffusion of sporangia is determined by the vertical diffusivity of the air (Smith and Hay, 1961). Thus, the diffusivity of the sporangia (K_s) was equated to the diffusivity of the momentum (K_m). The friction velocity and the rate of change of the average wind speed were used to calculate K_s using the following equation (Sutton, 1953):

$$K_s = K_m = u_*^2 / (\partial u / \partial z) \quad (2)$$

where u_* , u and z are as defined above, and $(\partial u / \partial z)$ is the vertical speed gradient.

For each level of disease severity, the vertical flux of sporangia F_s (spores per square meter per second) was calculated using the following equation:

$$F_s = -K_s \times (\partial C / \partial z) = -u_*^2 \times (\partial C / \partial u) \quad (3)$$

in which, $\partial C / \partial z$ is the vertical gradient of sporangia concentration as determined from the sporangia concentration at the various heights of the Rotorods. Calculation of F_s above the canopy using equation 3 ignores sedimentation under gravity that reduces F_s above the canopy by 10% at a height of 1 to 2 m (Aylor, 1983).

3.3.6. Relationship between disease severity and sporangia concentration and escape. For each level of disease severity, sporangia concentrations summarized per hour as described above were averaged across hours (from 0700 to 1400) to generate mean sporangia concentrations (\bar{C}). Similarly, data on sporangia escape were summarized per hour and summed across hours from 0700 to 1400 to generate a daily flux (DF_s) for each level of disease severity.

The relationship between disease severity and \bar{C} or DF_s was first examined graphically for separate data collected at Clayton and Clinton. Preliminary analysis did not indicate substantial differences in the shape parameters for plots of disease severity and \bar{C} and DF_s from Clayton and Clinton. Thus, combined data from the two sites was used in the final analysis to establish the quantitative relationship between disease severity and \bar{C} and DF_s . Based on the visual inspection of the plots of disease severity versus \bar{C} and DF_s , data were fitted to a three parameter log-normal model of the form:

$$y = \frac{a}{x} \exp \left[-0.5 \left[\frac{\ln(x/x_0)}{b} \right]^2 \right] \quad (4)$$

where, a is the vertical scale parameter, b is the log standard deviation and x_0 is the log mean. Data was fitted to this nonlinear model in SAS using NLIN procedure. Goodness-of-fit of the model was evaluated based on the significance of parameter estimates, magnitude of asymptotic standard errors, and simple correlation between observed and predicted values of sporangia concentration and escape.

3.4 RESULTS

3.4.1. Disease severity and standing crop of sporangia. Symptoms of cucurbit downy mildew were first observed in the field plot at Clinton on 12 August with a disease severity of 1.6%. Initial symptoms of the disease at the field plots in Clayton were observed on 16 August with a field plot severity of 1.1% (Table 3.1). At both sites, the disease increased rapidly and final disease assessment was recorded approximately 2 weeks after initial symptoms were observed in the cucumber plots. Disease severity in the plots at the final assessment date at Clayton and Clinton was 37.2% and 35.1% leaf area infected, respectively.

Across the two sites, the standing crop of sporangia ranged from 320 sporangia m⁻² at Clayton to 16,170 sporangia m⁻² at Clinton (Table 3.1). At both sites, the standing crop of sporangia was dependent on the level of disease in the field and there was a clear threshold relationship between the two variables. The standing crop of sporangia increased with increasing disease severity until a disease severity level of 12.2% and 16.1% at Clayton and

Clinton, respectively, and then decreased thereafter. Yields of sporangia m^{-2} at disease severity levels $> 20\%$ were 67 to 88% lower than the corresponding yields of sporangia m^{-2} at disease severity levels of 12.2% or 16.1%. Variations in the standing crop of sporangia were much more pronounced at moderate levels of disease severity (10 to 20%) than at lower (1 to 5%) or higher ($>30\%$) levels of disease severity.

3.4.2. Meteorological variables and dynamics of sporangia concentration.

Temperature, RH and wind speed at any given date of disease and sporangia assessment followed a similar pattern during the day at both Clayton and Clinton sites (Figure 3.1). Generally, temperature increased while RH decreased with time of the day, except on 12 August at Clinton where temperature increased from 0700 h until 1100 h and decreased thereafter, while RH decreased until 1100 h and slightly increased thereafter (Figure 3.1). Wind speed measured at 10 m ranged from 0.04 to 4.65 m s^{-1} across the two sites. Generally, wind speeds remained constant or increased slightly with the hour of the day except on 16 August at Clayton and 12 August at Clinton where wind speed fluctuated during day (Figure 3.1).

Aerial concentration of sporangia, C , was dependent on the height above the canopy and level of disease severity (Table 3.2). The highest concentration of sporangia was recorded at the 0.5 m above the canopy irrespective of the level of disease severity at both Clayton (Figure 3.2) and Clinton (Figure 3.3). The shapes of the vertical profiles of concentrations of sporangia at the two sites were similar although the absolute values were different (Figure 3.4). At both sites, sporangia decreased rapidly with increasing height above the canopy and were lowest at 2.0 m, while no sporangia were captured at 3.0 m (Figure 3.4).

At any given height above the canopy, C increased with increasing level of disease severity until a threshold of 16 to 18% disease severity and then decreased thereafter (Figures 3.2 and 3.3). Across both sites, values of C at 0.5 m above the canopy ranged from 15 sporangia m^{-3} at 1.1% disease severity to 21,122 sporangia m^{-3} at a disease severity of 18.3%.

There was a marked daily trend in C with the highest hourly concentration between 0800 and 1100 h and a peak concentration between 0900 and 1000 h (Figures 3.2 and 3.3). Generally, sporangia concentrations measured in the 1-h periods were not significantly ($P > 0.05$) correlated with temperature or relative humidity recorded in the same 1-h periods at both Clayton and Clinton. However, when measurements were grouped according the level of disease severity, sporangia concentrations were significant ($P < 0.05$) negatively correlated with RH ($-0.97 < r < -0.99$) for hourly measurements recorded at 0900 and 1000 for a disease severity of 20 and 40% (Table 3.3).

3.4.3. Escape of sporangia from the canopy. Estimates of sporangia that escaped the canopy (F_s) were dependent on the disease severity and hour of day. Escape of sporangia during the day followed a typical diurnal pattern with maximum escape rates at 0900 and 1000 h irrespective of the level of disease severity (Figures 3.5 and 3.6). Further, F_s increased with increasing disease severity until a specific disease severity threshold and then decreased thereafter. For example, F_s at Clayton was lowest at 1.1 % disease severity with maximum of 7.98 sporangia $m^{-2} s^{-1}$ and increased with increasing severity until 18.3% disease severity where the maximum value was 926.43 sporangia $m^{-2} s^{-1}$ and then decreased thereafter (Figure 3.5). The corresponding release rates at 12.2 and 37.2% disease severity

were 824.16 and 69.24 sporangia $\text{m}^{-2} \text{s}^{-1}$. Release rates followed a similar pattern at Clinton with sporangia $\text{m}^{-2} \text{s}^{-1}$ being lowest at the lowest level of disease severity (maximum = 2.75 sporangia $\text{m}^{-2} \text{s}^{-1}$) and highest at 9.4% leaf area infected (maximum = 378.48 sporangia $\text{m}^{-2} \text{s}^{-1}$). Release rate reduced dramatically with increasing levels of disease and at the highest level of disease severity the maximum F_s was 17.6 sporangia $\text{m}^{-2} \text{s}^{-1}$ (Figure 3.6).

Extrapolating total F_s values recorded on a given day to estimate the number of sporangia that escaped from a hectare of source area showed that the number of sporangia that would escape from 1 ha of source area ranged from 0.09 to 8.07×10^{10} sporangia ha^{-1} at Clayton and from 0.02 to 2.9×10^{10} sporangia ha^{-1} at Clinton (Table 3.4). Further, the number of escaped sporangia were highest at moderate levels of disease severity (10 to 18%) than at lower (< 5%) or higher (>20%) levels of disease severity.

3.4.4. Relationship between sporangia concentration and escape and disease severity. Sporangia concentration (C) and escape (F_s) both increased with increasing level of disease severity until a specific threshold and then decreased sharply thereafter with a thin tail as disease severity increased (Figure 3.7). This relationship was well described by the log-normal model ($P < 0.0001$). All the three parameters in model were significant ($P < 0.001$) for both C and F_s (Table 3.5) and plots of residual versus observed values did not reveal any systematic pattern in the residuals (Data not shown). Simple correlations between observed and predicted as values were high for both C ($r = 0.82$) and F_s ($r = 0.75$). The log-mean and standard deviation from the model were similar for both sporangia concentration

and escape and thus, the disease severity threshold above which there is decrease in either C or F_s as disease severity increases is was estimated to be approximately 15%.

3.5. DISCUSSION

Quantifying the release and escape of sporangia from a given source is the vital in successful modeling of *P. cubensis* sporangia transport over any distance. Such knowledge on sporangia release and escape is fundamental to the development of decision support systems for rational and effective application of fungicides. Data on spore release and escape can also assist in tracking the movement and introduction of the new and aggressive strains of *P. cubensis* in to the U.S. or Europe from other parts of the world (Runge et al., 2011; Savory et al., 2011). In this study, we quantified the aerial concentration and escape of *P. cubensis* sporangia from cucumber fields and related these variables to different severity levels of cucurbit downy mildew. Values of C and F_s were highly dependent on the amount of disease and exhibited a log-normal distribution with respect to disease severity. A disease severity of 15% was estimated as the threshold above which both C and F_s will drastically reduce with increasing level of disease. The maximum value F_s at this disease threshold was approximately 930 sporangia $m^{-2} s^{-1}$.

Aerial concentration of sporangia showed a strong diurnal periodicity, irrespective of the level of disease severity, with peak C occurring between 0900 and 1000 h. Our results are largely similar to those reported for other downy mildew pathogens (Aylor and Taylor, 1983; Aylor et al., 2001; Carrise and Philion, 2002). Sporangia release episodes of downy mildew

pathogens have been associated with a decrease in RH, an increase in temperature and evaporation of moisture from leaf surfaces (Populer, 1981; Sutton and Hildebrand, 1985). For example, sporangia of *B. lactucae* have been reported to be airborne in the morning concomitant with increases in temperature and a decrease in RH (Carrise and Phillion, 2002). In the present study, values of *C* were significantly negatively correlated with RH at 0900 and 1000 h when disease severity was high. The decrease in RH causes hygroscopic twisting of sporangiophores as they dry, which in turn actively releases sporangia into the air (Lange et al., 1989). Unlike in a previous study (Granke and Hausbeck, 2011) on the dynamics of *C*, we did not observe a significant correlation between temperature and *C*. This can partly be explained by differences in temperatures. In the study by Granke and Hausbeck (2011), temperatures ranged between 14 to 30°C, while in the present study temperatures were much warmer and ranged from 23 to 40°C except for one assessment date at Clinton. Sporulation of *P. cubensis* is optimum within a temperature range of 15 to 20°C (Thomas, 1996).

As expected for spores that are released from a ground level source (Csanady, 1973), aerial concentrations of *P. cubensis* sporangia were highest closer to the cucumber canopy and decreased with increasing height above the canopy. On average, the values of *C* at a height of 2.0 m were only 7% of values measured at 0.5 m above the canopy when disease severity was moderate or severe. This rapid decrease in *C* with height which has also been reported for other oomycetes (Aylor and Taylor, 1983; Aylor et al., 2001) is mainly due to a rapid increase in wind speed and eddy diffusivity with height above the ground (Aylor, 1995). In the present study, wind speeds ranged from 0.01 to 3.1 m s⁻¹ at 0.5 to 2.0 m above the canopy and increased by more than 64% as the height above the canopy increased from

0.5 to 2.0 m. Based on the model developed by Aylor (1995), the rate of release rate of spores and the physical transport dilution of airborne spores by the wind were the main determinants of the vertical variation of the concentration of *V. inaequalis* ascospores released from a ground level source. Further, although the absolute values of C were different at Clayton and Clinton, the shapes of the vertical profiles of C were similar at the two sites and also similar to those reported for other *P. infestans* (Aylor, 2001). This indicates that a common underlying atmospheric diffusion and transport process is responsible for the dispersal of *P. cubensis* sporangia from a source. Knowledge of the actual value of C is important for estimating the potential risk of infection from a given sporangia releases event. Thus, development of models to predict C based on u and K would be useful in predicting the spread of cucurbit downy mildew. Ratios of C at 1.5 m: 2.0 m at Clayton (~1.62) and Clinton (~1.75) were not significantly different which suggests that our field plots were the main source of most of the sporangia captured in this study.

Escape of sporangia varied with the hour of the day and the level of disease severity within the plot. Our results indicate that peak F_s occurred between 0800 and 1000 h which coincides with peak period for C . Values of F_s reported in this study (maximum = 930 sporangia $\text{m}^{-2} \text{s}^{-1}$) were similar to those reported for *P. infestans* that ranged between 0 to 700 sporangia $\text{m}^{-2} \text{s}^{-1}$ (Aylor, 2011). Canopy structure and the height of spore release inside the canopy are some of the factors that can influence C , and thus indirectly affect estimates of F_s . Given that the present study was conducted within a 2 week window, it is unlikely that the canopy structure changed substantially enough to influence F_s . Nonetheless, it has been

shown that height of spore release within the canopy has relatively a larger effect on C and thus, F_s , compared to the canopy structure (Aylor, 2001). The higher the inoculum source is located in the canopy and the stronger the wind velocity, the greater the number of spores that will escape from the canopy. Fewer sporangia will escape the canopy at lower wind speed since turbulence is less (i.e., low u_*) and spores are expected to settle out quickly in low turbulence. These results demonstrate that wind speeds recorded in this study (up to 3.1 m s^{-1}) were sufficient to result in a fairly high amount of sporangia that escaped the canopy. A wind speed of 3 m s^{-1} can transport sporangia for 20 km in about 2 h. Transmission of *P. cubensis* over such distances will depend strongly on viability of sporangia. Solar radiation is the most important physical variable that affects survival of *P. cubensis* sporangia (Kanetis et al., 2010). Thus, the extent of the spatial spread of cucurbit downy mildew will depend on the interaction between inoculum source strength, sporangia escape and viability of *P. cubensis* sporangia during transport.

In most aerobiology studies involving plant diseases, disease severity at the source has been assumed to be relatively constant (Andrade et al., 2009; Aylor et al., 1983; Aylor et al., 2001; Aylor et al., 2011) or the experiments have been conducted in severely infected fields (Aylor and Taylor, 1983). Our results clearly indicate a predictable relationship between disease severity and either C or F_s . The effects of disease on C and F_s were well described by a log-normal model and the estimates of the log-mean and standard deviation were similar for both C and F_s . This model predicts an increase in C and F_s as disease increases up to a severity threshold of 15% followed by a decrease in both variables with

increasing disease severity. This threshold relationship was also evident with the standing crop at the source that was used as a measure of source strength. The source strength was higher at moderate levels of disease severity (10 to 20%) and proportionately lower at low (< 5%) or high (> 20%) levels of disease severity. Our results tend to support corollary observations reported by Aylor (2001), where results of a model developed to quantify the escape of *P. infestans* sporangia suggested that that F_s will be low when the source of inoculum is from plants with a low level of disease severity and located close to the ground in a vigorously growing canopy. F_s will increase when the crop tissue becomes more affected by disease and the canopy begins to disintegrate. Obligate pathogens such as *P. cubensis* and *P. infestans* reproduce only on living host tissues. Thus, as disease severity increases, pathogen sporulation (and hence C and F_s) will increase up to a given disease threshold and decrease thereafter as the available host tissue becomes predominantly necrotic and less favorable for reproduction.

A major goal of this study was to generate information that can be incorporated in the cucurbit downy mildew forecasting system (Ojiambo et al., 2011) to improve its efficiency as a decision support tool for disease management. The cucurbit downy mildew forecasting utilizes the FLEXPART (Stohl et al., 2005) particle dispersion model to simulate the long-range and mesoscale transport and deposition of *P. cubensis* sporangia. These simulations are coupled with weather data along the projected pathway of sporangia transport and deposition to predict the risk of disease outbreak. In the current implementation of the FLEXPART, there are still large uncertainties in several model parameters. For example, the

total number of spores released (> 1) assumes a linear relationship between disease severity and source strength. Our results indicate that the total number of spores released needs to be adjusted appropriately depending on the disease severity at the source. It should be possible to scale down the variables using the model developed in this study where by a source with a moderate severity ($\sim 15\%$ leaf area infected) is assigned a value of 0.5, while sources with higher or lower levels of disease are assigned correspondingly lower emission rates that follows a log-normal distribution. Dry deposition in FLEXPART is described by a deposition velocity $v(z) = -F_s / C(z)$. For a given source area, this equation can also be parameterized based on the relationship between C , F_s and disease severity that was obtained from this study. Clearly, the use of the biology of *P. cubensis* to parameterization key components within the FLEXPART will result in better representation of the model simulations and substantially improve forecasts of cucurbit downy mildew.

3.6. LITERATURE CITED

1. Andrade, D., Pan, Z., Dannevik, W., and Zidek, J. 2009. Modeling Soybean Rust Spore Escape from Infected Canopies: Model Description and Preliminary Results. *J. Appl. Meteor. Climatol.* 48:789–803.
2. Arauz, L.F., Sutton, T.B., 1989. Temperature and wetness duration requirements for apple infection by *Botryosphaeria obtusa*. *Phytopathology.* 79:440-444.
3. Aylor, D.E., 1993. Relative collection efficiency of Rotorod and Burkard spore samplers for airborne *Venturia inaequalis* ascospores. *Phytopathology* 83, 1116-1119.
4. Aylor, D.E., 1995. Vertical variation of aerial concentration of *Venturia inaequalis* ascospores in an apple orchard. *Phytopathology* 85, 175-181.
5. Aylor, D. E. 1999. Biophysical scaling and the passive dispersal of fungus spores: Relationship to integrated pest management strategies. *Agric. For. Meteorol.* 97:275-292.
6. Aylor, D.E. and Taylor, G.S. 1983. Escape of *Peronospora tabacina* spores from a field of disease tobacco plants. *Phytopath.* 73:525-529.
7. Aylor, D.E., Fry, W.E., Mayton, H., and Andrade-Piedra, J.L. 2001. Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopath.* 91:1189-1196.
8. Aylor, D.E., Schmale III, D.G., Shields, E.J., Newcomb, M., Nappo, C.J., 2011. Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. *Agric. For. Meteorol.* 151, 215-260.
9. Carrisse, O., Philion, V., 2002. Meteorological factors affecting periodicity and concentration of airborne spores of *Bremia lactucae*. *Can. J. Plant Pathol.* 24, 184-193.
10. Cleveland, W.S., Grosse, E., 1991. Computational methods for local regression. *Stat. Comput.* 1, 47-62.
11. Cohen, Y. 1977. The combined effects of temperature, leaf wetness, and inoculum concentration on infection of cucumbers with *Pseudoperonospora cubensis*. *Can. J. Bot.* 55:1478–1487.
12. Csanady, G.T., 1973. *Turbulent Diffusion in The Environment*. Reidel, Dordrecht, 248 pp.

13. Granke, L.L., Hausbeck, M.L., 2011. Dynamics of *Pseudoperonospora cubensis* sporangia in commercial cucurbit fields in Michigan. *Plant Dis.* 95, 1392-1400.
14. Holmes, G.J., Wehner, T., Thornton, A., 2006. An old enemy re-emerges: downy mildew rears its ugly head on cucumber, impacting growers up and down the Eastern U.S. *Amer. Veg. Grow. February*: 14-5.
15. Hurvich, C.M., Simonoff, J.S., Tsai, C.L., 1998. Smoothing parameter selection in nonparametric regression using an improved Akaike Information Criterion. *J. R. Stat. Soc. Ser. B Stat. Methodol.* 60, 271-293.
16. Isard, S.A., Russo, J.M., Ariatti, A., 2007. The integrated aerobiology modeling system applied to the spread of soybean rust into the Ohio River valley during September 2006. *Aerobiologia* 23, 271-282.
17. Kanetis, L., Holmes, G.J., Ojiambo, P.S., 2010. Survival of *Pseudoperonospora cubensis* sporangia exposed to solar radiation. *Plant Pathol.* 59, 313-323.
18. Lange, L., Eden, U., Olson, L.W., 1989. Zoosporogenesis in *Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew. *Nord. J. Bot.* 8, 497-504.
19. Lebeda, A., and Widrlechner, M. P. 2003. A set of cucurbitaceae taxa for differentiation of *Pseudoperonospora cubensis* pathotypes. *J. Plant Dis. Prot.* 110:337-349.
20. Lowry, W. P., Lowry II, P.P., 1989. *Fundamentals of Biometeorology: Interactions of Organisms and the Atmosphere, Volume I: The Physical Environment.* Peavine Publications, McMinnville, Oregon. 310 pp.
21. Nusbaum, C. J. 1944. The seasonal spread and development of cucurbit downy mildew in the Atlantic coastal states. *Plant Dis. Rep.* 28:82-85.
22. Ojiambo, P.S., Holmes, G. J., 2011. Spatiotemporal spread of cucurbit downy mildew in the eastern United States. *Phytopathology* 101, 451-461.
23. Ojiambo, P. S., Holmes, G. J., Britton, W., Keever, T., Adams, M. L., Babadoost, M., Bost, S. C., Boyles, R., Brooks, M., Damicone, J., Draper, M. A., Egel, D. S., Everts, K. L., Ferrin, D. M., Gevens, A. J., Gugino, B. K., Hausbeck, M. K., Ingram, D. M., Isakeit, T., Keinath, A. P., Koike, S. T., Langston, D., McGrath, M. T., Miller, S. A., Mulrooney, R., Rideout, S., Roddy, E., Seebold, K.W., Sikora, E. J., Thornton, A., Wick, R. L., Wyenandt, C. A. and Zhang, S. 2011. Cucurbit downy mildew ipmPIPE: a next generation web-based interactive tool for disease management and extension outreach. Online. *Plant Health Progress* doi:10.1094/PHP-2011-0411-01-RV.

24. Populer, C., 1981. Epidemiology of downy mildews. Pages 57-105 in: The Downy Mildews. D.M. Spencer, ed. Academic Press, New York.
25. Ro, K.S., Hunt, P.G., 2007. Characteristic wind speed distributions and reliability of the logarithmic wind profile. J. Environ. Eng. 133, 313-318.
26. Runge, F., Choi, Y. -J., Thines, M., 2011. Phylogenetic investigations in the genus *Pseudoperonospora* reveal overlooked species and cryptic diversity in the *P. cubensis* species cluster. Eur. J. Plant Pathol. 129, 135-146.
27. Savory, E. A., Granke, L. L., Quesada-Ocampo, L. M., Varbanova, M., Hausbeck, M. K., Day, B., 2011. The cucurbit downy mildew pathogen *Pseudoperonospora cubensis*. Mol. Plant Pathol. 12, 217-226.
28. Skelsey, P., Holtslag, A. A. M., Moene, A. F., and van der Werf, W. 2008. Development and validation of a quasi-Gaussian plume model for the transport of botanical spores. Agric. For. Meteorol. 148:1383-1394.
29. Sutton, O.G., 1953. Micrometeorology, McGraw-Hill, New York.
30. Sutton, J.C., Hildebrand, P.D., 1985. Environmental water in relation to *Peronospora destructor* and related pathogens. Can. J. Plant Pathol. 7, 323-330.
31. Stohl, A., Forster, C., Frank, A., Seibert, P., and Wotawa, G. 2005. Technical note: The Lagrangian particle dispersion model FLEXPART version 6.2. Atmos. Chem. Phys. Discuss. 5:4739-4799.
32. Thomas, C. E. 1996. Downy mildew. Pages 25-27 in: Compendium of Cucurbit Diseases. T. A. Zitter, D. L. Hopkins, and C. E. Thomas, eds. APS Press, St. Paul, MN.

Table 3.1. Standing crop of *Pseudoperonospora cubensis* sporangia from a cucumber field infected by cucurbit downy mildew at two field sites in North Carolina

Site	Date	Disease severity (%) ^a	Sporangia [$\times 10^2$] m ⁻² plot area ^b	
			Mean	Std deviation
Clayton	16-Aug	1.1	3.20	1.39
	17-Aug	1.6	11.45	0.79
	24-Aug	7.9	73.73	9.12
	25-Aug	12.2	131.31	9.89
	26-Aug	18.3	110.93	24.01
	29-Aug	37.2	17.97	21.12
Clinton	12-Aug	1.6	5.33	4.62
	23-Aug	9.4	42.46	33.55
	25-Aug	16.1	161.73	36.14
	28-Aug	22.6	54.86	47.51
	30-Aug	27.9	54.20	46.93
	01-Sept	35.1	24.73	22.84

^a Disease severity was assessed visually as the percentage of leaf area infected on each date when sporangia were collected.

^b Standing crop of sporangia was estimated by counting number of lesions (x) in a 0.25×0.25 m plot area and the average number of sporangia washed from 3 sampled lesions (y) as: $(x \times y)/0.0625$.

Table 3.2. Concentration of *Pseudoperonospora cubensis* sporangia at different heights above a cucumber canopy at a source infected with different levels of cucurbit downy mildew severity at two field sites in North Carolina^x

Site	Height above canopy (m)	Sporangia m ⁻³ air					
		Low severity (0 to 5%) ^y		Moderate severity (5 to 20%) ^y		High severity (20 to 40%) ^y	
		Mean	SE	Mean	SE	Mean	SE
Clayton	0.5	114.7	14.9	8711.9	1462.6	748.4	106.6
	1.5	39.5	6.2	1233.7	221.7	95.4	16.2
	2.0	31.4	5.4	630.1	121.1	57.3	10.4
Clinton	0.5	102.8	10.5	3498.7	577.2	796.9	142.9
	1.5	90.4	9.2	319.8	44.5	119.7	19.1
	2.0	56.3	8.4	173.2	19.3	67.1	10.6

^x Sporangia concentrations are averages of spores m⁻³ air recorded from 0700 to 1400 for each level of disease severity and height above the canopy of a cucumber crop.

^y SE denotes the standard error of the mean for a sample size of $n = 42$ for low and high disease severity and $n = 84$ for moderate disease severity at both locations.

Table 3.3. Pearson's coefficients^a for the correlation between *Pseudoperonospora cubensis* sporangia collected 0.5 m above a cucumber canopy (sporangia/m³) and the prevailing relative humidity (%) monitored at a given level of disease severity and hour of day

Hour of day	Correlation between sporangia concentration and relative humidity ^a					
	Low severity (0 to 5%) ^b		Moderate severity (5 to 20%) ^b		High severity (20 to 40%) ^b	
	<i>r</i>	<i>P</i> > <i>F</i>	<i>r</i>	<i>P</i> > <i>F</i>	<i>r</i>	<i>P</i> > <i>F</i>
0700	0.918	0.2559	-0.154	0.8048	-0.445	0.5550
0800	0.941	0.1731	-0.501	0.3900	-0.807	0.1932
0900	0.987	0.1029	-0.430	0.4698	-0.973	0.0267
1000	0.547	0.6314	-0.041	0.9484	-0.993	0.0073
1100	0.933	0.2332	-0.276	0.6530	0.279	0.7206
1200	0.719	0.4888	-0.471	0.4234	-0.129	0.8713
1300	-0.721	0.4877	-0.266	0.6651	-0.434	0.5664

^a *P*-values are for the test to determine if the correlation coefficient (*r*) is significantly different from zero.

^b Disease severity was assessed visually as the percentage of leaf area infected on each date when sporangia were collected.

Table 3.4. Escape of *Pseudoperonospora cubensis* from a source field naturally infected with different levels of severity of cucurbit downy mildew in Clayton and Clinton, North Carolina

Site	Date	Disease severity (%) ^a	Sporangia escape [$\times 10^{10}$ sporangia per ha] ^b
Clayton	16-Aug	1.1	0.09
	17-Aug	1.6	0.94
	24-Aug	7.9	2.57
	25-Aug	12.2	8.07
	26-Aug	18.3	5.73
	29-Aug	37.2	0.57
Clinton	12-Aug	1.6	0.02
	23-Aug	9.4	2.90
	25-Aug	16.1	2.14
	28-Aug	22.6	0.44
	30-Aug	27.9	0.43
	01-Sept	35.1	0.25

^a Disease severity was assessed visually as percent leaf area infected on each sporangia monitoring date.

^b Based on the total hourly vertical fluxes (0700 to 1400 h) during the disease severity and sporangia assessment date over a canopy area of 1 ha.

Table 3.5. Parameter estimates from nonlinear regression analysis obtained using the log-normal model to describe the relationship between sporangia concentration (C), sporangia escape (F), and disease severity for combined data from two field sites in North Carolina^x

Respon e	Parameter ^y	Estimate	Asymptotic ^z SE	Asymptotic ^z CI_L	Asymptotic ^z CI_U	$P > F$
C	a	66994.53	9358.95	45823.10	88165.90	0.0001
	b	0.39	0.07	0.24	0.55	0.0003
	x_0	15.13	1.11	12.60	17.65	0.0001
F	a	24645.13	4824.88	13732.40	35557.93	0.0005
	b	.30	0.07	0.14	0.46	0.0009
	x_0	13.87	0.98	11.65	16.08	0.0001

^x Sporangia concentrations are averages of sporangia m^{-3} recorded from 0700 to 1400 at each level of disease severity at 0.5 m above the canopy of a cucumber crop.

^y Parameters a , b and c are the vertical scale parameter, log-std and log-mean, respectively.

^z SE is the standard error and CI_L and CI_U = lower and upper limits of the 95% confidence interval around the parameter estimates.

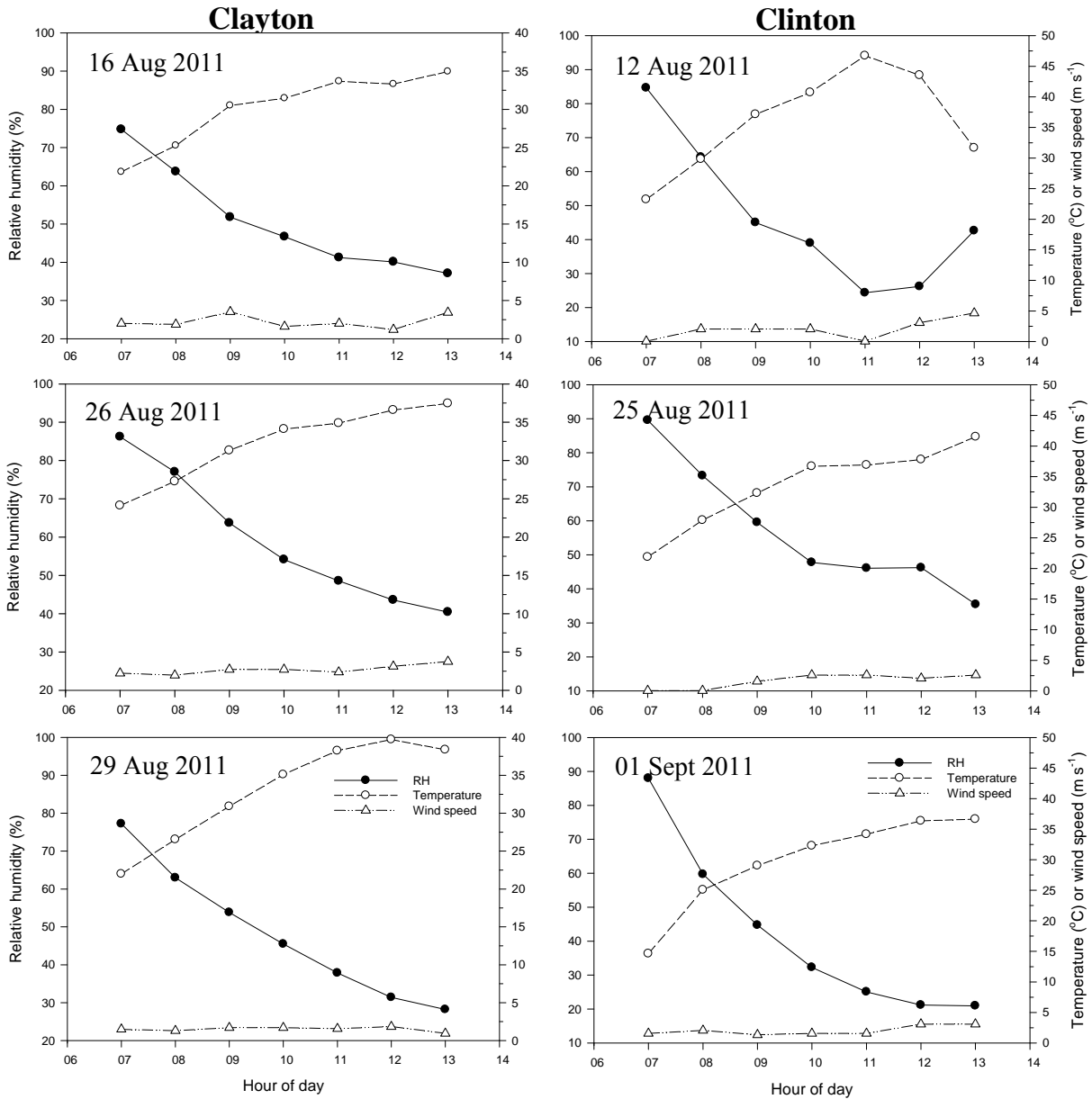


Figure 3.1. Temperature, relative humidity and wind speed monitored at Clayton and Clinton field sites in North Carolina in a study to determine the relationship between cucurbit downy mildew severity at the source and concentration and escape of *Pseudoperonospora cubensis* sporangia above the crop canopy. Data shown have been selected to show the range in the monitored weather variables. Wind speed data are based on measurements collected at 10 m above the ground.

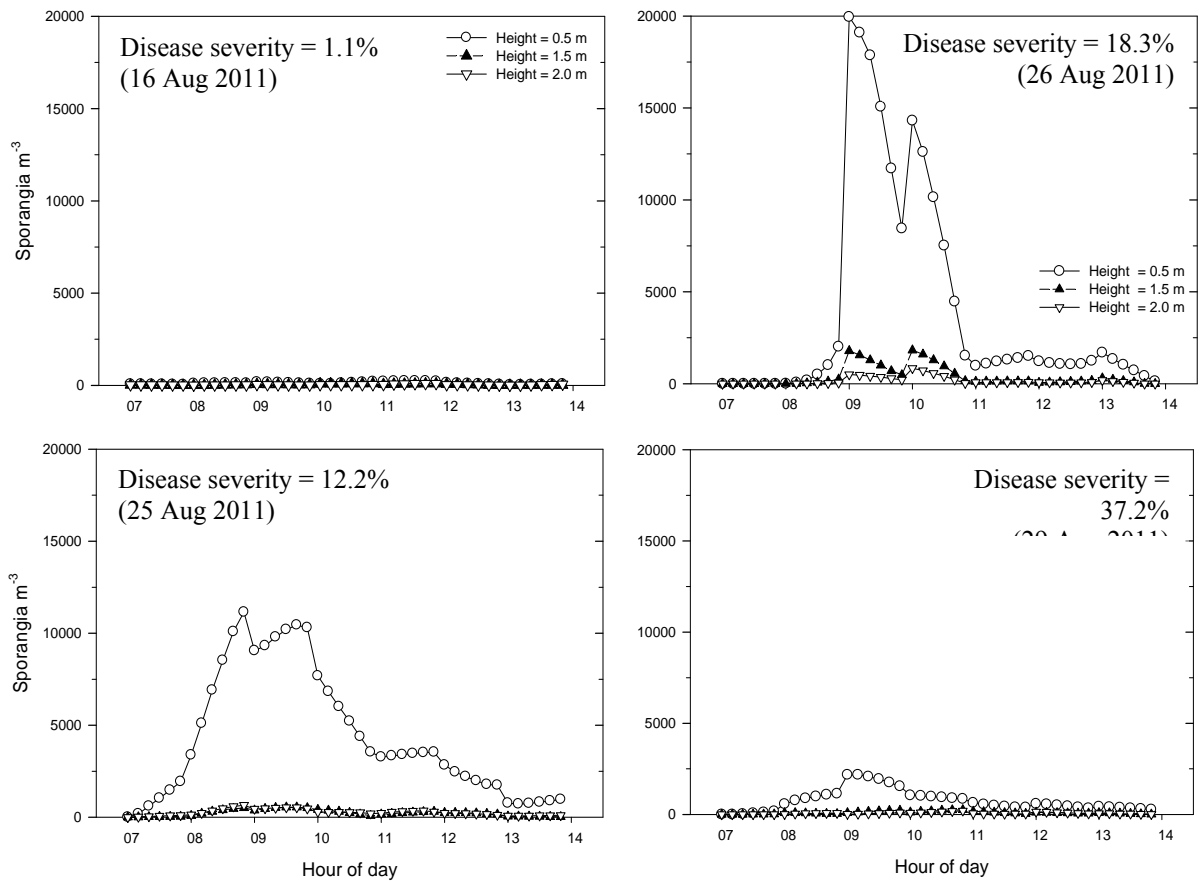


Figure 3.2. Diurnal pattern of concentrations of *Pseudoperonospora cubensis* sporangia at different heights above the canopy in a cucumber field naturally infected with different levels of cucurbit downy mildew severity at Clayton research station in Johnston County, North Carolina.

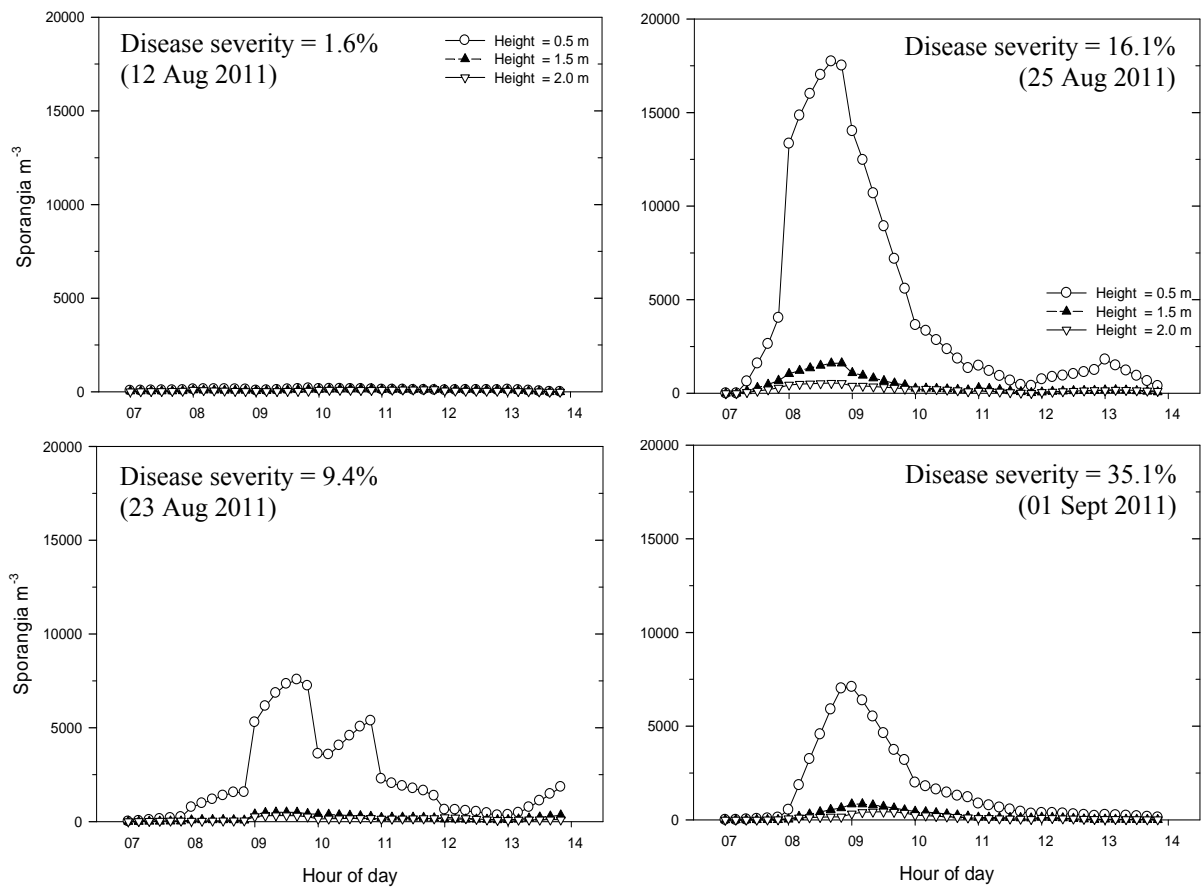


Figure 3.3. Diurnal pattern of concentrations of *Pseudoperonospora cubensis* sporangia at different heights above the canopy in a cucumber field naturally infected with different levels of cucurbit downy mildew severity at Clinton research station in Sampson County, North Carolina.

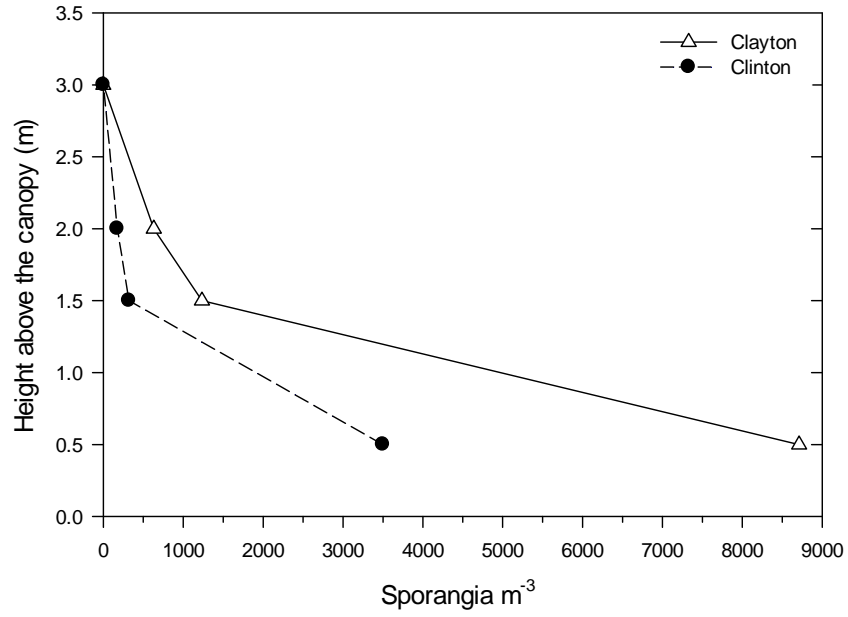


Figure 3.4. Vertical profiles of *Pseudoperonospora cubensis* sporangia concentrations measured above a source in a cucumber field from 0700 to 1400 h. The severity of cucurbit downy mildew at the source at Clayton and Clinton sites was 16.1 and 18.3%, respectively.

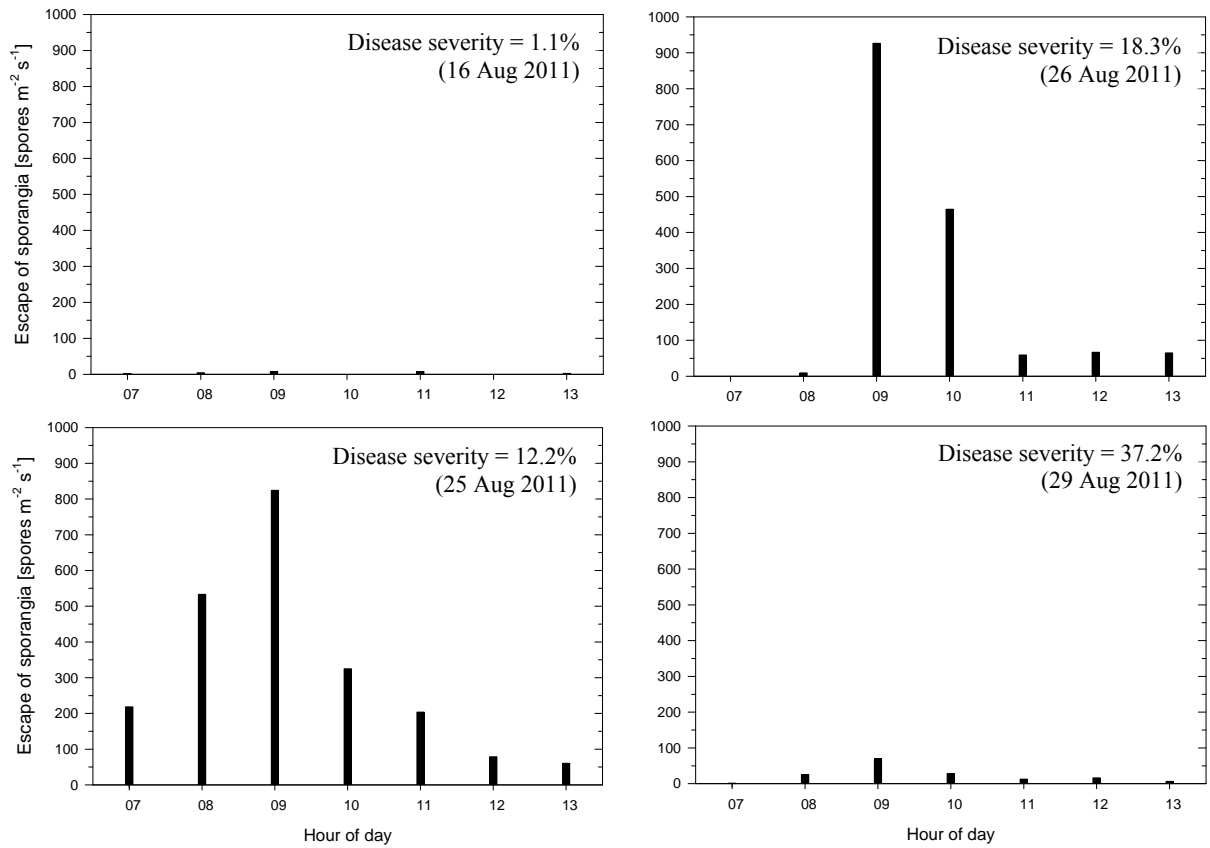


Figure 3.5. Escape of *Pseudoperonospora cubensis* sporangia from a source field naturally infected with different levels of cucurbit downy mildew in Clayton, North Carolina. On each assessment date, disease severity was assessed visually as percent of leaf area infected on the cucumber cultivar Poinsett76.

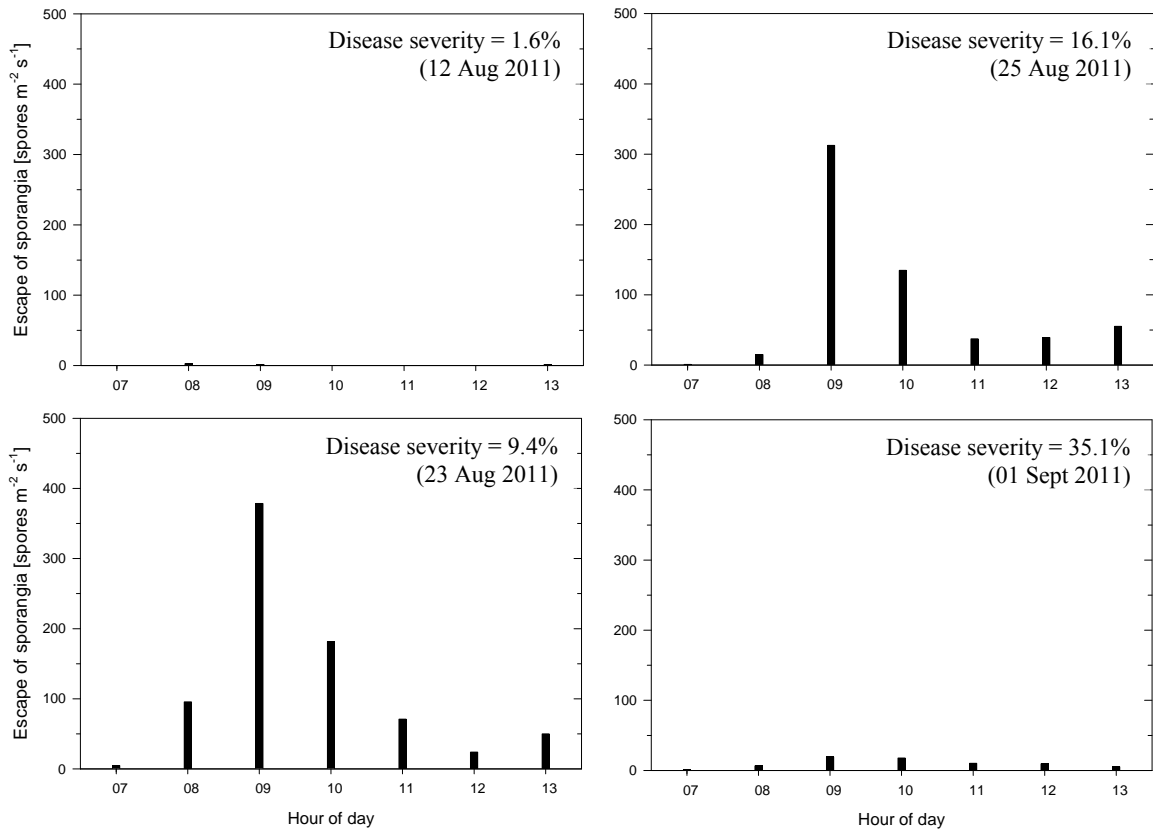


Figure 3.6. Escape of *Pseudoperonospora cubensis* sporangia from a source field naturally infected with different levels cucurbit downy mildew in Clinton, North Carolina. On each assessment date, disease severity was assessed visually as percent of leaf area infected on the cucumber cultivar Poinsett76.

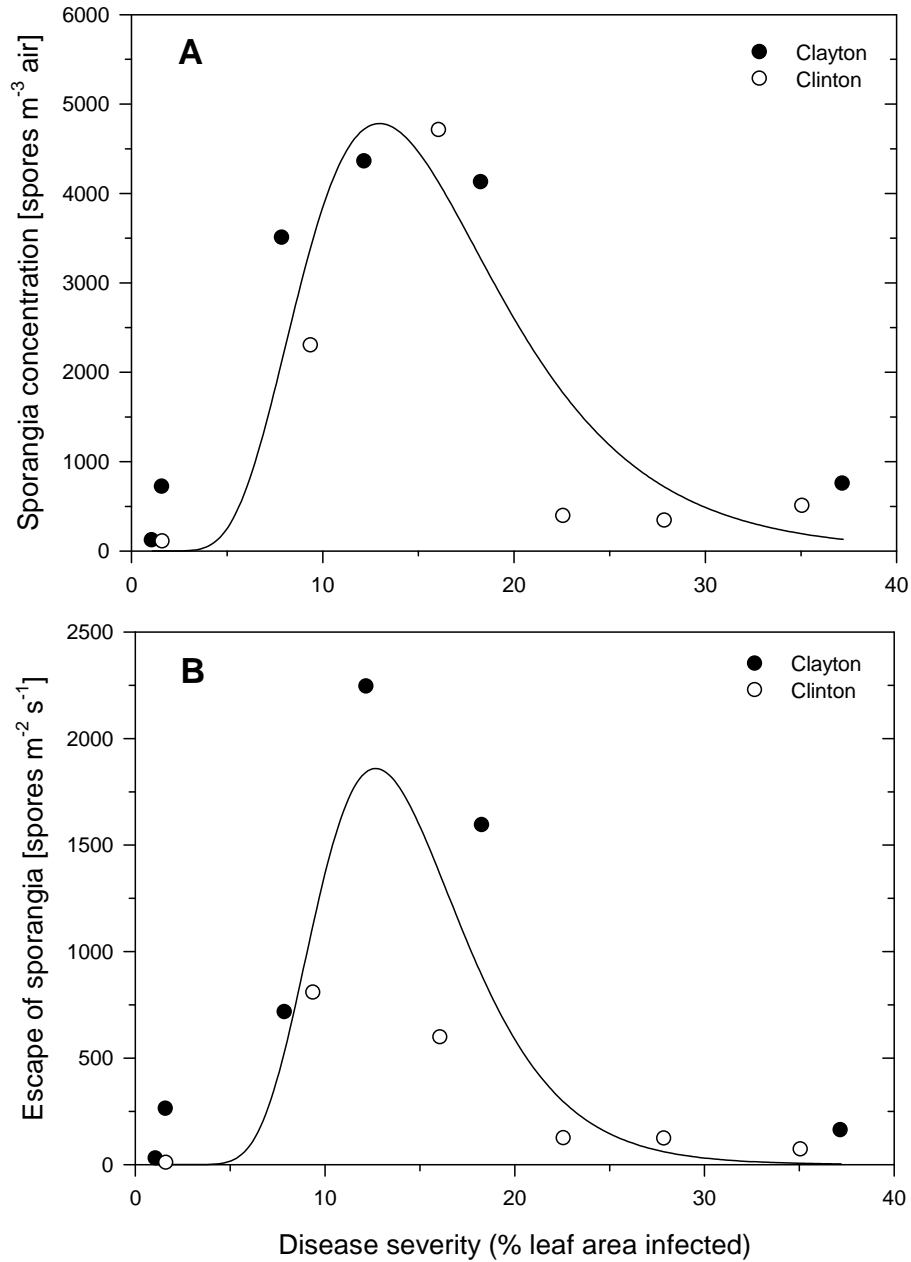


Figure 3.7. Relationship between cucumber downy mildew severity and concentration of *Pseudoperonospora cubensis* sporangia (**A**) at the source or escape of sporangia (**B**) from a source in a cucumber field at Clayton and Clinton, North Carolina. Solid circles (● or ○) are observed values, while curve is the predicted sporangia concentration or escaped sporangia obtained from fitting a log-normal model to combined data for sporangia concentration or escape from a canopy from the two sites.

4. CHAPTER IV:

Conclusions

The research outlined in this thesis was carried out to fill critical knowledge gaps, such as differential host type response to infection and the effect of source strength in the epidemiology and aerobiology of cucurbit downy mildew. This knowledge is needed to efficiently control the disease using disease forecasting to guide when and where to apply fungicides. Timing of the first fungicide spray is the key in the effective control of cucurbit downy mildew. The difficulty in controlling cucurbit downy mildew has been due partly to the uncertainties related to prediction of initial infection and thus, predicting when the first spray is needed. This study generated specific information that will improve our ability to predict infection of various cucurbit host types based on forecasted temperature and leaf wetness durations. Data on the aerobiology of *P. cubensis* will lead to improved performance of the current forecasting system, and improve forecasting of initial infection and the timing of the first fungicide spray.

Cucurbit host types differentially responded to infection in response to weather variables. Previous advisory guidelines based on studies conducted on a single host may, thus, be less accurate across host types. Host based charts were derived to predict initial infection based on forecasted temperature and leaf wetness durations. Thus, the predictive capacity of current disease advisory systems will increase significantly if they take into account differences among host types. These host-based findings, however, present a challenge on how they can be implemented at the field level. For example, if predicted weather conditions indicate that the risk of initial infection for cantaloupe and cucumber and

is low and high, respectively, it is unlikely that growers will spray only the cucumber crop if both crops are planted in the same or adjacent plots. It is well known that many growers view calendar-based spraying as insurance, i.e., better to have it and not need it than the other way around. Thus, predictive systems for cucurbit downy mildew and other plant diseases need to be extremely accurate to reduce average management costs compared with calendar-based fungicide applications because the cost of a false negative is much greater than a false positive. Incorporation of knowledge on the aerobiology of *P. cubensis* in the cucurbit downy mildew forecasting will greatly enhance the ability of this decision support tool to accurately predict the infection of cucurbit host types. Prior to this study, parameters within the FLEXPART Lagrangian plume dispersal model were primarily based on properties of gas particles in the air rather than biologically relevant properties of *P. cubensis* sporangia.

Based on the studies of the aerobiology of *P. cubensis*, wind speeds of up to 3.1 m s^{-1} were sufficient to result in a fairly high amount of sporangia that escaped the cucumber canopy. A wind speed of 3 m s^{-1} can transport sporangia for 20 km in about 2 h. Thus, everything else being constant, sporangia of *P. cubensis* are expected to travel a distance of about 240 km within a period of 24 h. However, the dissemination of sporangia over such distances will depend strongly on viability of sporangia and solar radiation is the most important meteorological variable that affects survival of sporangia. The model used to describe the survival of *P. cubensis* sporangia in response to cumulative solar radiation can be incorporated in the FLEXPART to accurately determine the amount of sporangia that will be available for deposition. Clearly, the more information on the biology of cucurbit downy mildew that is incorporated within the FLEXPART dispersion model, the more accurate the

forecasts will be. Additional studies are needed to generate biologically relevant information for cucurbit downy mildew. For example, knowledge of the actual concentration of sporangia is important for estimating the potential risk of infection from a given sporangia release event. Thus, the development of models to predict sporangia concentration in the air based on wind speed and eddy diffusivity will be useful in predicting the spread of cucurbit downy mildew.