This is to certify that the

dissertation entitled

Epidemiology of Mildews of
Floricultural Crops

presented by

Janet M. Byrne

has been accepted towards fulfillment
of the requirements for

Ph.D degree in Plant Pathology

Mary K. Hausbeck
Major professor

Date 4-25-02

DATE DUE	DATE DUE	DATE DUE
APR 07 2011		

EPIDEMIOLOGY OF MILDEWS ON FLORICULTURAL CROPS

By

Janet M. Byrne

A DISSERTATION

Submitted to
Michigan State University
In partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Plant Pathology

2002

ABSTRACT

EPIDEMIOLOGY OF MILDEWS ON FLORICULTURAL CROPS

By

Janet M. Byrne

In the U.S., floriculture crops are grown in the greenhouse as potted plants or in the field as cut flowers. Due to their high value and the consumer's low tolerance for blemishes, foliar diseases are managed intensively. The mildews are especially troublesome because epidemics can develop rapidly and are difficult to control. In this study, the epidemiology of powdery mildew (*Oidium* sp.) on greenhouse poinsettias and downy mildew (*Peronospora antirrhini*) on field-grown snapdragons was investigated.

Atmospheric conidial concentrations (ACC) of *Oidium* sp. in research greenhouses containing infected poinsettias were monitored to investigate the role of environment in prompting conidial release and dissemination. The influence of temperature on disease development was studied by placing healthy poinsettias in each greenhouse for 7-day periods, removing them, and recording the days to the appearance of the first colony. When averaged over 5 December to 1 June, ACC were greatest during 1000 to 1800 h. Large numbers of conidia were sampled ($\geq 100/m^3$) within 1-h periods indicating conidial release events (CREs). Fluctuations in relative humidity (RH) prompted CREs. In both greenhouses, CREs (up to 23) occurred following RH fluctuations of 5 to 15%. When greenhouse temperatures exceeded 25C for 19 days in March and 21 days in May ACC were reduced by $\geq 75\%$ from the previous months.

Conidial germination and infection processes of *Oidium* sp. were quantified on

poinsettia foliage. Leaf disks were inoculated with conidia of *Oidium* sp. and incubated at 15, 20 and 25°C in chambers with glycerol/water solutions that provided 35, 50, 65, 80 and 92% (±2%) relative humidity (RH). Formation of appressoria and primary germ tubes were favored by 20°C. Haustorium formation appeared to be favored by 20°C and 35, 50, and 65% RH. The average length of germ tubes produced at 25°C (36.2μm) was longer than at 20°C (27.0μm) and 15°C (11.1μm). Effects of temperature on sporulation of *Oidium* sp. were quantified on inoculated leaf disks incubated for 14 days at 15 and 20°C under high relative humidity. Temperature had a significant effect on the number of conidia produced per conidiophore. At 15 and 20°C, a maximum of 4 and 7 conidia were produced per conidiophore, respectively.

Atmospheric concentrations of *Peronospora antirrhini* conidia in a commercial snapdragon production field were monitored over three growing seasons to investigate the influences environment has on the concentration of airborne conidia. The incidence of downy mildew among different cultivars was also determined. Atmospheric conidial concentrations followed a diurnal pattern and were greatest during 0500 to 1200 h. Minimum daily temperatures below 6.0°C and maximum daily temperatures above 30.0°C limited ACC. Long dew periods (≥6 hours) were associated with relatively large conidia releases. Consecutive days with short leaf wetness periods suppressed ACC. Snapdragon cultivar had a significant effect on area under the disease progress curve.

The information gained from this study will further the understanding of the relationship between the environment and disease development and may be incorporated into current disease management programs, enhancing their effectiveness.

ACKNOWLEDGEMENTS

I owe thanks to many people who have helped me accomplish this work. My major professor, Dr. Mary Hausbeck, has been a tremendous source of both personal and professional support. It is largely through her encouragement that I was able to complete my studies while initiating a career in plant pathology.

The assistance provided by undergraduate students who spent many hours at a microscope with the tedious task of counting spores is greatly appreciated. I also received a great deal of technical support from Brian Cortright, Sheila Linderman and Bill Quackenbush. My committee members Dr. Ray Hammerschmidt, Dr. Andrew Jarosz and Dr. Joseph Vargas are to be commended for their critical evaluations and flexibility.

Lastly, I would like to thank my family, both immediate and extended, who supported me in countless ways during this endeavor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I.

CHAPTER II.

CHAPTER III

LITERATURE REVIEW

POWDERY MILDEW OF POINSETTIA

Poinsettias (*Euphorbia pulcherrima* Willd ex Klotz) have a wholesale value in the United State of $237 million (14). Michigan growers produce 7% of the total U. S. crop (14). Poinsettias. have become a symbol of Christmas throughout the world, since being introduced to the United States from Mexico in 1825. In their native habitat poinsettias are a perennial. shrub-like plant. but are grown in the U.S. as a seasonal greenhouse-grown ornamental. Small flowers. or cyathia. are surrounded by brightly colored bracts ranging from red to variegated to white; flowering is initiated under short-day/long-night photoperiods.

Poinsettia cultivars are developed. maintained and patented by a few large primary propagators. Desirable cultivars are propagated vegetatively by taking tip or terminal cuttings from the branches of stock plants and placing them in rooting medium (12). Primary propagators maintain stock plants all year for cutting production. Secondary propagators purchase cuttings for stock plant production from primary propagators beginning in early spring. Cuttings from these stock plants are sold in midsummer to finishing growers who produce the flowering crop.

Historically, the most significant diseases of poinsettia have been leaf, stem, and bract blight caused by *Botrytis cinerea* Pers.:Fr., and root rot caused by *Pythium sp.* Pringsh, nom.cons. Management of both foliar and root rot diseases is a concern throughout all stages of poinsettia production. Most growers use regular calendar-based fungicide applications throughout the growing season to control these diseases. High fungicide costs. increasing energy, heating, and shipping costs and low retail prices set by large chain stores have greatly reduced the profitability of poinsettia production for

finishing producers. As a result, cost effective disease management strategies are of interest to many producers.

Powdery mildew occurred on poinsettias in Mexico in 1988 and 1989. The disease was first reported in the United States in 1990, and was epidemic in Michigan in 1992 (19). Growers in Puerto Rico have reported powdery mildew outbreaks, and the disease has also been observed on poinsettias in the landscape (9). Although the disease is not common, it occurs yearly and is an economically significant problem, especially for poinsettia growers in the midwestern and northern United States.

Powdery mildew fungi are characterized by the hyaline or white mycelium produced on external host surfaces. Taxonomically these fungi are grouped in the Erysiphaceae, order Erysiphales, class Ascomycetes, and are defined based on the sexual (perfect) stage. Although many genera relying on descriptions of conidia and conidiophores exist in the literature, classification based exclusively on the anamorph form is discouraged.

In the absence of an observed teleomorph, the causal agent of this powdery mildew is referred to simply as *Oidium* sp. and is recognized by its production of conidia in chains on upright conidiophores characterized by an arched basal cell. The conidia are oval to cylindrical, 15 to 20 x 25 to 50 µm, with a chain of up to nine conidia on a single conidiophore (26). The genus *Oidium* was established by Link in 1809 (51), and has been widely used for imperfect stages of Erysiphaceae. At least 89 species of *Oidium* have been named, and can infect over 1100 host species (51).

Disease signs.

Signs of powdery mildew appear as small, white, talcum-like colonies on bract or

leaf surfaces. Chlorotic spots appear opposite colonies on abaxial leaf surfaces. Under favorable conditions colonies enlarge and coalesce, blighting sections of the leaf. Stem and petiole tissue can also be infected. Severe infection results in defoliation.

In the initial stages of an epidemic initiated in the summer colonies form more frequently on the abaxial surface, where temperatures are cooler as a result of canopy shade and close proximity to the moist surface of the growing medium. If colonies first develop on the undersides of leaves, the disease may go undetected until late in production when bracts become infected, making plants unsaleable.

Temperature.

Optimum temperatures reported for powdery mildews (21.0°C) are generally lower than those for other plant pathogens (2). Kim et al. (26) observed that plants with active powdery mildew colonies, grown in a greenhouse throughout the summer and exposed to high temperatures and solar radiation, showed no signs of infection for a period of time. Controlled studies comparing effects of temperature on *Oidium* sp. on poinsettia found conidial germination was significantly reduced at 30°C versus 20.0°C (4). Temperature also influences conidial germination, haustorium formation, development of secondary germ tubes, and sporulation. At 30.0°C, formation of secondary germ tubes was significantly limited (<1%) compared with 20.0°C (≥53%). Less than 4% of germinated conidia formed haustoria at 30.0°C, compared with >53% of those at 20.0°C (4).

Relative humidity.

Unlike many other fungal plant pathogens, powdery mildew does not require leaf wetness for germination or penetration. The level of xerophytism differs depending on the

species of powdery mildew. Several researchers have observed germination of powdery mildew conidia at very low humidities (<25%) (10,50). This ability has been attributed to the moisture retained in large water storing vacuoles in the relatively large powdery mildew conidia (53). Others have suggested that the great water retaining ability of powdery mildew condia, created by a gelatinous sheath in the cell wall, is a more important factor (44). The ability of a conidium to germinate in low relative humidity conditions is a factor of temperature, tolerance to low relative humidity decreases with increasing temperatures. Conidia of even the most xerophytic species will shrivel and lose viability under low relative humidity and warm temperatures.

Infection process.

Powdery mildews are biotrophic pathogens, requiring live plant tissue to infect, grow, and reproduce. As a result, these pathogens have specialized infection processes, which minimize cell death or in some cases delay cell death until the pathogen has completed reproduction. Exceptions to this are some *Phyllactinia* species that have surface mycelia that infect through stomata to the mesophyll tissue where haustoria are formed, and fungi in the genus *Leveillula* that form well-developed internal mycelia.

The infection process begins with the production of germ tubes by a conidium on a host surface. Germ tubes are more sensitive to relative humidity than conidia. Conidia of *Erysiphe graminis* germinate at extremely low relative humidity (< 10%) but germ tube growth is poor below 98% relative humidity (32). Germ tube length of several *Erysiphe* and *Phyllactinia* species are affected by low relative humidity (2).

Once cued by the host surface, the germ tube lays down septation and differentiates into an appressorium. The host cuticle is penetrated with mechanical force

created by the appressorium and an infection peg. Cell wall degrading enzymes are involved in cell wall penetration by some *Erysiphe* species (8). In defense, the host deposits electron dense cytoplasm directly beneath the appressorium. Papilla are then formed which help prevent infection in incompatible host pathogen interactions.

Most powdery mildew fungi are restricted to the epidermal cell layer of the host. Powdery mildew are intracellular pathogens, penetrating the host cell wall but do not enter the cytoplasm of the host cell. The host plasmalemma invaginates allowing room for the formation of a haustorium. The haustorium suppresses further defense responses of the host and absorbs nutrients from the host which are supplied to the hyphae. The shape of the haustorium varies with the species and ranges from nearly spherical to an ellipsoid center with many finger-like projections or lobes. Successful formation of a haustorium is essential for further hyphal growth. Additional hyphae, develop from the appressorium and contribute to the expanding colony. Additional haustoria are produced in other epidermal cells.

Reproduction.

Asexual reproduction occurs on the host surface, where conidia are formed on upright conidiophores. Some species produce a single conidium every 24 hours, while others produce daily chains of conidia (2). Powdery mildew fungi, with the exception of *E. graminis* (18), produce conidia in a diurnal pattern. The periodicity of powdery mildew which form conidia in chains is determined by the final stage of sporulation, when the abstriction of the conidium from the germ cell of the conidiophore occurs (2). This maturation stage is photosensitive, proceeding faster in light than in dark; therefore, conidia produced at night remain immature and are released once exposed to light (6).

Condia dispersal.

Conidial release of *Oidium* sp. of poinsettias is associated with relative humidity fluctuations, but the mechanism of release is not known. Both passive and active liberation mechanisms have been proposed for powdery mildew species (28). Mechanical disruption (18), wind speed (16), and humidity (1) or temperature changes have been associated with large conidial releases of powdery mildews. In the field, wind plays a significant role in the release and dispersal of the conidia of many powdery mildew species, either directly or as a result of leaf movement dislodging conidia (13,17,49). An alternative method of dispersal is hygroscopic twisting in response to changing relative humidity. This twisting causes movement capable of dislodging spores of *Phytophthora* and *Peronospora* spp. (28). However, Jarvis (22) concluded that hygroscopic movements loosen spores of *B. cinerea* for subsequent rain-splash dispersal. Adams (1) proposed that a change from high to low relative humidity creates an electrical charge with sufficient voltage to remove powdery mildew conidia from conidiophores.

Disease management.

The ability of powdery mildew to become epidemic when poinsettias have colored bracts has made disease management difficult. Scouting the poinsettia crop for early signs of the disease can ensure timely fungicides applications prior to bract coloration. It is recommended that growers inspect eight fully expanded leaves of each newly received plant (19). Growers scout one out of 30 plants weekly thereafter. If powdery mildew is detected, the infected leaf tissue is removed carefully to deter further spread of conidia, an appropriate fungicide is applied, and scouting increases to one out of 10 plants weekly. Once the surrounding plants have been free of disease for three weeks, scouting drops to

one out of 30 plants weekly. Without carefully scouting for colonies that develop on the undersides of leaves, disease may go undetected until late in production when bracts become infected, making plants unsaleable. However, frequent scouting is time consuming and difficult for large greenhouse operations that may have more than an acre of closely-spaced poinsettias.

All popular poinsettia cultivars are susceptible to powdery mildew. Celio and Hausbeck (3) observed higher disease incidence among poinsettias with red bracts than those with pink, variegated, or white bracts. Kim et al., (25) found that 'Dark Red Hegg', was significantly less susceptible than 'Freedom Red', both are red bract varieties.

Several fungicides are labeled for use against this disease; Strike (triadimiefon) and Teraguard 50W (triflumizole) are both effective systemic products. Fungicide costs range from $0.48 to $2.44 per 1000 square feet of greenhouse area (19), and reduce growers' profits. Although applications of appropriate fungicides to poinsettia bracts prevent further colony development, the fungal mycelium remains visible and is commercially unacceptable. Additionally, residue and phytotoxicity resulting from fungicide applications to the bracts may result in an unmarketable crop.

DOWNY MILDEW OF SNAPDRAGON

Snapdragons (*Antirrhinum majus* L. (Huxley et al., 1992)) are produced for several markets, including cut flowers, bedding plants, and potted plants. The cut flower portion has a wholesale value in the U.S. of $18.5 million (14). Dwarf cultivars are a common greenhouse bedding plant crop. Cut flowers are produced in greenhouses or in fields. Taller cultivars (90 to 180 cm) are grown for cut flowers. The majority of the U. S. snapdragon cut flower production occurs in California and Florida(14). Snapdragons are increasing in popularity with floral designers and consumers and has become a profitable crop for growers looking to diversify their production with additional crops.

Snapdragons are seed propagated. Commercial producers buy finished plugs from specialty propagators and transplant them into production beds. The tall cultivars used for cut flower production require support, which is provided by mesh wiring or netting, to produce the tall-straight stalks (29). As the plant height increases the wiring is raised to provide continued support. In Florida, the second largest production state, planting occurs weekly from September to December. Planting is scheduled to provide a large volume of spikes for spring occasions such as Mother's and Valentine's days.

Snapdragons are a cool season crop, preferring temperatures between 7 to 10°C (7). Once flower initiation has occurred, night temperatures greatly influence flowering time and quality. When grown under cooler temperatures flowering spikes have thick stems and compact flowers, which are desirable characteristics that increase crop value. Spikes are harvested by hand when one-third of the florets are open.

Several commercial seed producers conduct plant breeding programs with tall snapdragons cultivars, most of the commonly grown cultivars are F_1 hybrids. Cut flower

cultivars are classified into flowering response groups, each with specific combinations of night temperature, light level and photoperiod duration (7,11). Cultivars are available in a wide variety of colors including, white, red, pink, rose, yellow, orange, and several bicolors. The coloration of the foliage varies with some cultivars, for example the orange and red varieties have purplish foliage.

Snapdragons are affected by several different foliar diseases including *Botrytis cinerea* (Pers.:Fr), rust (*Puccinia antirrhini)*, powdery mildew (*Oidium sp.)*, and downy mildew (*Peronospora antirrhini* Schroet., Hedwigia). *Peronospora antirrhini* infects snapdragon foliage both locally and systemically, causing economically significant damage (48). Local infections produce pale lesions that are visible on the upper leaf surface. Systemic infections result in downward curling of foliage, and shortened internodes. The disease can kill terminal buds of young transplants, causing undesirable branching, which decreases the crop value (48).

The downy mildews are obligate pathogens characterized by fungal morphology and their ability to rapidly reach epidemic proportions. The group contains economically significant pathogens of several important crops including tobacco, grapes, hops, sugar beets, sunflowers, soybeans and brassicas (47). Taxonomically these fungi are grouped in the *Peronosporaceae*, this group includes the genera *Peronospora*, *Pseudoperonospora*, *Plasmopora, Bremia*, and *Sclerospora.*

In general, crop losses caused by downy mildews can vary greatly between years and seasons, depending on climate and weather (52). Leaf wetness duration and temperatures are known to be critical environmental factors for sporulation, germination, and infection of downy mildew that affects tobacco (*Peronospora tabacina* (D.B. Adam))

(27,36) , grape (*Plasmopara viticola* (Berk. & Curt)) (30,31), and lettuce (*Bremia lactucae* (Regel)) (40-43).

History of occurrences of *Peronospora antirrhini*.

Peronospora antirrhini was described by Schroeter in 1874 on the wild *Antirrhinum orontium*, but was not described on cultivated snapdragons until 1936, when it caused severe damage to snapdragon seedlings (52). Some discrepancy exists in the literature as to whether both reports were the same pathogen (15,48). In 1940, *P. antirrhini* was found on snapdragons in California. From 1940 to1942, the disease was a problem throughout southern California, such that it was reportedly difficult to obtain healthy seedlings (52). At the same time, extensive losses were also occurring in areas of Europe. During the 1940's, Yarwood and a few other researchers published details of field observations and results of limited research projects on *P. antirrhini* (33,52). Since that time little has been published concerning this pathogen.

Reproduction.

Asexual reproduction in the Peronosporacea occurs through conidia or sporangia, which release zoospores. *Peronospora* species do not produce zoospores, their asexual reproductive structures are true conidia and germinate directly with a germ tube. Conidia of *P. antirrhini* are produced externally on the leaf surface and are therefore sensitive to environmental factors. Sporulation typically occurs on the abaxial leaf surface but with severe disease pressure and favorable environmental conditions sporulation can occur on the adaxial leaf surface (personal observation). Conidiophores are dicotomously branched, and the tips of the branches are narrowed. Conidia are 23-30 x 14-18μm, ellipsoid to ovoid with a small flat area of attachment (15).

The spore release of many downy mildews (*P. tabacina, P. manshurica, P. viciae, B. lactuca, Pseudoperonospora cubensis, P. humuli*, etc.) follows a diurnal pattern (38). Sporangia or conidia are produced at night, mature early in the morning and are released midmorning. The time at which spore release begins varies among species. Conidia of *P. tabacina* and *P. trifoliorum* are released in response to decreasing relative humidity. When exposed to dry air conidiophores twisted counter-clockwise instantaneously and spores were forcibly ejected (37). When the conidiophores were exposed to high relative humidity the twisting was reversed.

Sexual reproduction of downy mildew pathogens may be either homothallic or heterothallic depending on the species. *P. parasitica* is somewhat unique in that both homothallic and heterothallic isolates have been found (34). Oospore production has been associated with chlorotic and necrotic tissue. Therefore, oospore formation by several downy mildew pathogens has been suggested to be induced by senescence of the host tissue. However, others observed that the onset of sporulation occurs before chlorosis and is therefore the cause, rather than the result of sporulation (34). Oospore production is not as sensitive to the environment as conidial production.

Oospores of *P. antirrhini* are 24-28 μm in diameter and have a thick ridged wall. In Ireland, oospores were observed in the root cortex of infected nursery plants, and were found less frequently in above ground plant tissue (33,34).

Influence of leaf wetness.

Free moisture on the host surface is considered essential for infection by downy mildew pathogens (38). The duration of leaf wetness needed for infection varies with species, and is also dependent on the temperature during the leaf wetness period. In field

observations of both *Sclerospora* sp. and *Bremia lactuca*, a leaf wetness period between spore release and evaporation of leaf wetness in the morning was sufficient for infection to occur (38,42). Infection periods of *B. lactuca* occurred mainly on days on which leaf wetness ended late in the morning (0100 h Pacific standard time or later), thereby extending nightly leaf wetness durations (40,42).

The impact of leaf wetness on conidial production varies with species. Conidial production of *P. trifoliorum*, which is pathogenic on forage legumes, does not occur in free water on leaves (46). *Pseudoperonospora humuli* (pathogenic on hops) produces sporangia on dry leaf tissue, but is not inhibited by leaf wetness (45). Other downy mildews require leaf wetness for sporangium development. A minimum of 6 h of leaf wetness is required by *P. cubensis* (pathogenic to cucurbits) for sporangium formation (38).

Influence of relative humidity.

High relative humidity (>90%) is necessary for asexual reproduction of downy mildew fungi in general (34). Relative humidity level is most critical at night, when sporangium development occurs. A mean relative humidity below 64% between the hours of 2000 and 0600 curtailed sporulation of *P. humuli* (24), a relative humidity level between 95 - 100% is required for abundant sporulation (39). *P. destructor* (pathogenic on onions) also requires high relative humidity (≥95%) for sporulation, the critical time period for this pathogen appears to be between 0200 and 0600 hours (20). The disease predictive model proposed by Hildebrand (20) included the parameter of relative humidity above 95% beginning at 0200 hours or earlier and remaining until 0600 hours.

Influence of temperature.

Downy mildew pathogens are generally limited by temperatures over 25 - 30°C. *Peronospora antirrhini* sporulated on systemically infected snapdragons incubated at 13 and 19°C but not on those incubated at 7 and 22°C (52). Yarwood also observed that plants inoculated during the winter and early spring months were more successful than inoculations during the hotter summer months. Sporulation of *P. destructor* is inhibited when mean temperatures of the preceding day exceed 23-24°C (21). Limiting effects of high temperatures become more severe with increasing durations of warm temperatures (>30°C) (35).

Minimum temperatures below 5°C are limiting to downy mildew pathogens. *Pseudoperonospora humuli and P. cubensis* do not produce sporangia when the minimum night temperature is ≤5°C (5,24). An analysis of environmental factors over a 28 year period of hop production determined that minimum daily temperatures were generally higher in April and May in years with severe epidemics of *P. humuli*, than in years with mild or no epidemics (23). Average minimum daily temperatures were a better predictor of epidemics of *P. humuli* than the average daily temperature (23).

Management of *Peronospora antirrhini*.

It has not been determined whether this obligate pathogen survives on weeds and plant debris, or is annually introduced into the production area via seedling transplants (48,52). Currently, larger producers use chemical fumigants between the growing seasons to control soilborne pathogens. This option will likely be limited to ornamental producers in the long term.

Irrigation is required on a regular, almost daily, basis to maintain the crop.

Irrigation is done in late morning or early afternoon, after harvest has been completed. Timing irrigation to avoid prolonged durations of leaf wetness may help limit downy mildew as well as several other foliar diseases.

Tall snapdragon cultivars vary in their sensitivity to downy mildew (personal observation). Although thorough scouting for signs of infection is helpful for prompting fungicide sprays growers can target especially susceptible cultivars for scouting. Growers may also choose cultivars that are more resistant to downy mildew to ease disease pressure. Fungicides are applied at 1 - 3 week intervals throughout the growing season to control this disease.

LITERATURE CITED

1. Adams, G. . C., T. R. Gotwald, and C. M. Leach. 1986. Environmental factors initiating liberation of conidia of powdery mildews. Phytopathology 76:1239-1245.
2. Butt, D. J. 1978. Epidemiology of Powdery Mildews. In: The Powdery Mildews, edited by D. M. Spencer. New York: Academic Press.
3. Celio, G. J., and M. K. Hausbeck. 1997. Evaluation of poinsettia cultivars for susceptibility to powdery mildew. HortScience 32:259-261.
4. Celio, G. J., and M. K. Hausbeck. 1998. Conidial germination, infection structure formation, and early colony development of powdery mildew on poinsettia. Phytopathology 88:105-113.
5. Cohen, Y. 1981. Downy Mildew of Cucurbits. In: The Downy Mildews, edited by D. M. Spencer. London: Academic Press.
6. Cole, J.S. 1966. Powdery mildew of tobacco (*Erysiphe cichoracearum* DC) IV. Conidial content of the air within infected crops. Annals of Applied Biology 57:445-450.
7. Corr, B., and Laughner. L. Antirrhinum (Snapdragon). In: The Ball Red Book, edited by V. Ball. Batavia: Ball Publishing.
8. Coyier, D.L. 1985. Powdery mildews. In: Diseases of Floral Crops, edited by D. L. Strider. New York: Praeger Publishers.
9. Daughtrey, M.L., and J. Hall. 1992. Powdery mildew: A new threat to your poinsettia crop. Grower Talks, 23-31.
10. Delp, C. J. 1954. Effect of temperature and humidity on the grape powdery mildew fungus. Phytopathology 44:615-626.

11. Dole, J. M., and H. F. Wilkins. 1999. Antirrhinum. In: Floriculture Principles and Species. Upper Saddle River: Prentice Hall.

12. Ecke, P. , O.A. Matkin, and D.E. Hartley, eds. 1990. The Poinsettia Manual. Encinitas, CA: Paul Ecke Poinsettias.

13. Fernando, T.M. 1971. *Oidium* leaf disease; the effect of environment and control measures on incidence of disease and atmospheric spore concentration. [*Hevea brasiliensis*]. Quarterly Journal of Rubber Research 48:100-111.

14. Floriculture crops, 2000 Summary.2001., edited by N. A. S. Service. Washington, DC: United States Department of Agriculture.

15. Francis, S. M. 1981. *Peronospora antirrhini.* In: CMI Descriptions of Pathogenic Fungi and Bacteria No 685.

16. Frinking, H.D. 1977. Research on wind dispersion of rose-mildew spores (*Sphaerotheca pannosa*) in field, glasshouse and climate room. Grava 16:155-158.

17. Hammet, K.R.W. and Manners, J.G. 1971. Conidium liberation in *Erysiphe graminis* I. Visual and statistical analysis of spore trap records. Transactions of the British Mycological Society 56:387-401.

18. Hammett, K. R. W., and J. G. Manners. 1973. Conidium liberation in *Erysiphe graminis.* II. Conidial chain and pustule structure. Transactions of the British Mycological Society 61:121-133.

19. Hausbeck, M., J. Kalishek, M. Daughtrey, and L. Barnes. 1994. Keep the colonies at bay (Part 1). Greenhouse Grower:45-48.

20. Hildebrand, P. D., and J. C. Sutton. 1982. Weather variables in relation to an epidemic of onion downy mildew *Peronospora destructor*, near Bradford, Ontario.

Phytopathology 72:219-224.

21. Hildebrand. P. D., and J. C. Sutton. 1984. Interactive effects of the dark period, humid period, temperature, and light on sporulation of *Peronospora destructor*. Phytopathology 74:1444-1449.

22. Jarvis, W.R. 1962. The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. Transactions of the British Mycological Society 45:549-559.

23. Johnson, D. A., C.B. Skotland, and J. R. Alldredge. 1983. Weather factors affecting downy mildew epidemics of hops in the Yakima Valley of Washington. Phytopathology 73:490-493.

24. Johnson, D. A., and C.B. Skotland. 1985. Effects of temperature and relative humidity on sporangium production of *Pseudoperonospora humuli* on hop. Phytopathology 75:127-129.

25. Kim, S. H., and A. H. Micheal. 1995. Susceptibility of poinsettia cultivars to powdery mildew. Phytopathology (Abstr) 85:1171.

26. Kim, S. H. , and T. N. Olson. 1995. Recurrence of powdery mildew on over-summered asymptomatic poinsettias. Phytopathology (Abstract) 85:1171.

27. Kucharek, T. A. 1987. Rainfall, irrigation water, and temperatures associated with first occurrences of tobacco blue mold in leaf production area of north Florida from 1979 to 1984. Plant Disease 71:336-339.

28. Lacey, J. 1996. Spore dispersal-Its role in ecology and disease : The British contribution to fungal aerobiology. Mycological Research 100:641-660.

29. Langhans, ed. 1962. Snapdragons: a manual of the culture, insects and diseases and economics of snapdragon. Ithaca, NY.

30. Madden, L. V., G. Hughes, and M. A. Ellis. 1995. Spatial heterogeneity of the incidence of grape downy mildew. Phytopathology 85:269-275.

31. Madden, L. V., and M. A. Ellis. 2000. Evaluation of a disease warning system for downy mildew of grapes. Plant Disease 84:549-554.

32. Manners, J.G., and S.M.M. Hossain. 1963. Effects of temperature and humidity on conidial germination in *Erysiphe graminis*. Transactions of the British Mycological Society 46:225-234.

33. McKay, R. 1949. The production of oospores by the downy mildew of Antirrhinums. The Gardener's Chronicle:28.

34. Michelmore, R.W. 1981. Sexual and Asexual Sporulation in the Downy Mildews. In: The Downy Mildews, edited by D. M. Spencer. London: Academic Press.

35. Moss, M.A., and C.E. Main. 1988. The effect of temperature on sporulation and viability of isolates of *Peronospora tabacina* collected in the United States. Phytopathology 78:110-114.

36. Nesmith, W. C. 1984. The North American blue mold warning system [*Peronospora tabacina*, tobacco, Canada, USA]. Plant Disease 68:933-936.

37. Pinckard, J. A. 1942. The mechanism of spore dispersal in *Peronospora tabacina* and certain other downy mildew fungi. Phytopathology 32:505-511.

38. Populer, C. 1981. Epidemiology of Downy Mildews. In: The Downy Mildews, edited by D. M. Spencer. London: Academic Press.

39. Royle, D.J., and H. Kremheller. 1981. Downy Mildew of Hop. In: The Downy Mildews, edited by D. M. Spencer. London: Academic Press.

40. Scherm, H., and A. H. C. van Bruggen. 1994. Weather variables associated with

infection of lettuce by downy mildew (*Bremia lactucae*) in coastal California. Phytopathology 84:860-865.

41. Scherm, H., and A. H. C. van Bruggen . 1995. Concurrent spore release and infection of lettuce by *Bremia lactucae* during mornings with prolonged leaf wetness. Phytopathology 85:552-555.

42. Scherm, H., and A. H. C. Van Bruggen. 1995. Comparative study of microclimate and downy mildew development in subsurface drip- and furrow-irrigated lettuce fields in California. Plant disease 79:620-625.

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

44. Somers, and Horsfall. 1966. The water content of powdery mildew conidia. Phytopathology 56:1031-1035.

45. Sonoda, R.M., and J.M. Ogawa. 1972. Ecological factors limiting epidemics of hop downy mildew in arid climates. Hilgardia 41:457-474.

46. Stuteville, D.L. 1981. Downy mildew of forage legumes. In: The Downy Mildews, edited by D. M. Spencer. London: Academic Press.

47. Viennot-Bourgin, G. 1981. History and Importance of Downy Mildews. In: The Downy Mildews, edited by D. M. Spencer. San Francisco: Academic Press.

48. Wheeler, B.E.J. 1981. Downy Mildew of Ornamentals. In: The Downy Mildews, edited by D. M. Spencer. San Francisco: Academic Press.

49. Willocquet, L. , D. Colombet, M. Rougier, J. Fargues, and M. Clerjeau. 1996. Effect of radiation, especially ultraviolet B, on conidial germination and mycelial

growth of grape powdery mildew. European Journal of Plant Pathology 102:441-449.

50. Yarwood. C. E. 1936. The tolerance of *Erysiphe polygoni* and certain other powdery mildews to low humidity. Phytopathology 26:845-859.

51. Yarwood. C. E. 1957. Powdery Mildews. The Botanical Review XXIII:235-300.

52. Yarwood. C.E. 1947. Snapdragon Downy Mildew. Hilgardia 17:241-249.

53. Yarwood. C.E. 1950. Water content of fungus spores. American Journal of Botany 37:636-639.

CHAPTER I.

FACTORS AFFECTING CONCENTRATIONS OF AIRBORNE CONIDIA OF *OIDIUM SP.* AMONG POINSETTIAS IN A GREENHOUSE

ABSTRACT

Atmospheric concentrations of *Oidium* sp. conidia in two research greenhouses containing infected poinsettias were monitored to investigate the role of environment in prompting conidial release and dissemination. Hourly concentrations of conidia of *Oidium* sp. were estimated using a Burkard volumetric spore sampler. The influence of temperature on disease development was studied by placing healthy poinsettias in each greenhouse for 7-day periods, removing them, and recording the days to the appearance of the first colony. When averaged over 5 December to 1 June, atmospheric conidial concentrations in greenhouse 2 (GH 2) were greatest during 1000 to 1800 h with a peak (325 conidia/m^3/h) occurring at 1200 h. In greenhouse 11 (GH 11), peak concentrations occurred at in 1300 h (65 conidia/m^3/h) and 1600 h (75 conidia/m^3/h). Large numbers of conidia were sampled ($\geq$100/m^3) within 1-h periods indicating conidial release events (CREs). Fluctuations in relative humidity (RH) (either positive or negative) prompted CREs. In both greenhouses, the highest number of CREs (up to 23) occurred following RH fluctuations of 5 to 15%. Watering resulted in an immediate increase ($\leq$25%) followed by a rapid decrease in RH ($\leq$32%) beginning 1 to 2 h later. Eighty-nine percent (GH 2) and 48% (GH 11) of the CREs occurred within 3 h following greenhouse watering. When greenhouse temperatures exceeded 25C for 21 days in May (GH 2) and 19 days in March (GH 11) atmospheric conidial concentrations were reduced 80 and 75% from the previous months, respectively.

INTRODUCTION

Poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch) have a wholesale value in the United States of $212 million with California, Florida, Ohio, and Michigan leading production (19). Poinsettias are propagated by taking tip or terminal cuttings from the branches of vegetative stock plants and placing them in rooting medium (20). Primary propagators maintain stock plants all year for cutting production. Secondary propagators purchase cuttings for stock plant production from primary propagators beginning in early spring. Cuttings from these stock plants are sold in mid-summer to growers who produce the flowering crop. Management of foliar diseases is a concern throughout all stages of poinsettia production. The primary foliar disease of poinsettia historically has been leaf, stem, and bract blight caused by *Botrytis cinerea* Pers.:Fr. (16).

Powdery mildew on poinsettias in the United States was first reported in 1990 (9) and was epidemic in Michigan in 1992. While the disease is not common, it occurs yearly and is an economically significant problem especially for poinsettia growers in the midwestern and northern United States (M.K. Hausbeck, unpublished data). In absence of an observed teleomorph, the causal agent of this powdery mildew is referred to simply as *Oidium* sp. and is recognized by its production of conidia in chains on upright conidiophores characterized by an arched basal cell [Celio, 1998 #143].

Signs of disease appear as small, white, talcum-like colonies on bract and/or leaf surfaces; colonies can coalesce to cause blighting. If colonies first develop on the undersides of leaves, the disease may go undetected until late in production when bracts become infected, making plants unsaleable. While applications of appropriate fungicides to poinsettia bracts prevent further colony development, the fungal mycelium remains

visible and is commercially unacceptable. Additionally, residue and phytotoxicity resulting from fungicide applications to the bracts may result in an unmarketable crop.

Scouting the poinsettia crop for early signs of the disease can ensure timely fungicide applications prior to bract coloration. However, frequent scouting is time consuming and difficult for large greenhouse operations that may have more than an acre of closely-spaced poinsettias. Since popular poinsettia cultivars are susceptible to powdery mildew (6), growers have relied on preventive applications of systemic fungicides prior to bract coloration to manage the disease. An improved understanding of the epidemiology of this pathogen could contribute to development of a disease management program that reduces unnecessary fungicide applications without increased risk of disease. Recent research conducted under controlled environmental conditions indicates that temperature influences conidial germination, haustorium formation, development of secondary germ tubes, and sporulation (3,4). The objective of this study was to determine the role of environment in prompting atmospheric concentrations of *Oidium* sp. conidia in research greenhouses containing infected poinsettias. The influence of temperature on disease development was also of interest.

MATERIALS AND METHODS

Multi-stemmed, 8- to 10-week old poinsettias, with colored bracts, grown in 13.2-cm-diameter plastic pots in commercial soilless potting mix composed of 40% perlite and 60% sphagnum peat moss, were obtained from a commercial Michigan greenhouse in November 1994. A mixture of cultivars representing a range of disease susceptibility was used, cultivars included: Eckespoint Red Sails, Peace Jolly Red, Gross™ Supjibi Red, Gutbier™ V-14 Glory, Eckespoint Jingle Bells 3, Annette Hegg™ Hot Pink, Gutbier™

V-14 Pink, Eckespoint Pink Peppermint, Gutbier™ V-17 Angelika Marble, Annette Hegg™ Topwhite, Gutbier™ V-14 White and Gutbier™ V-17 Angelika White. A random assortment of cultivars was spaced eight to nine pots per m^2 on three benches in each of two glass research greenhouses located in the same complex, hereafter referred to as GH2 (9m x 8.5m) and GH11 (5.5m x 8.8m). Ideally, this study would have been conducted in adjacent greenhouses; however, greenhouse availability was a constraint. Plants were hand watered as needed, taking care to avoid wetting the foliage. Plants were fertilized during watering with 200 ppm 15-5-25 (N-P-K) poinsettia fertilizer (Grace-Sierra Horticultural Products Company, Milpitas, CA) at 2- to 3-day intervals. The irrigation water pH was maintained at 5.8. Glasshouse temperatures were set for 21.1 to 22.0C and vented when temperatures exceeded 26.0C. Temperature and relative humidity (RH) were monitored using a Neogen EnviroCaster (Neogen, Mason, MI) that recorded the environmental parameters every 15 min and calculated hourly averages. The number of days in each spore sampling week having one or more hourly average temperatures exceed 25.0 or 30.0C was calculated. Light levels were natural photoperiods. When entering the greenhouses, personnel documented the date, time of day, and greenhouse activity performed, such as irrigation, vent opening, equipment maintenance and cleaning of benches. Fungicides were not applied for the duration of the experiment.

In GH 2, inoculation occurred on 18 November by vigorously shaking mildew-infected plants with actively sporulating colonies above the healthy poinsettias. Colonies were observed on inoculated plants within 10 days. Although plants in GH 11 were not intentionally inoculated, colonies were first observed on 29 November. Both bracts and foliage were infected during the epidemic.

Concentrations of airborne conidia were monitored in each greenhouse using a 7-day volumetric spore sampler (Burkard Mfg. Co. Ltd., Rickmansworth, Herfordshire, England) placed in the center of a bench from 5 December to 1 June and from 5 December to 17 July in GH 11 and GH 2, respectively. Observations were not continued for an additional season due to the time-consuming nature of this type of study. The spore sampler was operated at a flow rate of 10 L/min and the orifice was set level with the top of the plant canopy and fixed in a direction perpendicular to the bench. Conidia were impacted onto tapes coated with an adhesive mixture of petroleum jelly and paraffin (9:1, w/w) dissolved in sufficient toluene to give a thick, liquid consistency. Tapes were removed weekly, cut into 48-mm lengths, marked at 2-mm intervals with a razor blade to indicate hourly intervals, stained with aniline blue in lactic acid (28 mg of aniline blue, 20 ml of distilled water, 10 mg of glycerol, and 10 ml of 85% lactic acid, diluted with 5 drops to 25 ml of distilled water), and mounted on glass slides beneath 22 x 50 mm coverslips. Under a compound microscope (x100), conidia were identified as *Oidium* sp. based on conidium size, shape, color, surface texture, and translucence. The number of conidia sampled during each 1-h period were recorded. When conidial concentrations were exceptionally large (>2,000/m^3/h), a portion of the 2-mm interval was counted and multiplied by the appropriate factor to provide an estimate of the concentrations for the 1-h period. Counts were converted to numbers of conidia per cubic meter air sampled per hour.

From 23 January to 20 March, nine healthy red-bracted poinsettias (cv. Freedom Red) grown as previously specified were placed in each greenhouse (three per each of three benches) for 7-day periods after which they were removed and incubated in a nearby

greenhouse under similar environmental conditions. The number of days to the appearance of the first visible colony following the exposure period was recorded for each plant. Once plants developed visible colonies they were immediately removed to minimize contamination of the greenhouse atmosphere and nearby poinsettias. Because of limited numbers of poinsettias available, three plants were used each week (one per bench) from 3 April to 3 July for GH 2.

RESULTS

Atmospheric conidial concentrations. When averaged over the observation period (5 December to 1 June), atmospheric conidial concentrations in GH 2 were greatest during 1000 to 1800 h with a peak (325 conidia/m^3/h) occurring at 1200 h (Fig. 1). Overall, atmospheric conidial concentrations were much lower in GH 11 compared to GH 2 with peak concentrations occurring at 1300 h (65 conidia/m^3/h) and 1600 h (75 conidia/m^3/h) (Fig. 1). During the course of this study, large numbers of conidia were sampled ($\geq$100/m^3) within 1-h periods indicating conidial release events (CREs). A total of 134 and 50 CREs occurred in GH 2 and GH 11, respectively, with maximum CREs occurring at 1100 and 1200 h (GH2) and 1300 h (GH 11) (data not shown). CREs were negligible ($\leq$1) from 2000 to 0800 h in both greenhouses.

Relative humidity fluctuations. Fluctuations in RH (either positive or negative) prompted CREs (Fig. 2). In both greenhouses, the highest number of CREs (up to 23) occurred following RH fluctuations (either positive or negative) of 5 to 15% (Table 1), although fluctuations in RH were as high as 32% (GH 2) and 27% (GH 11). One CRE occurred in GH 2 without a fluctuation in RH in the 3 h preceding the event.

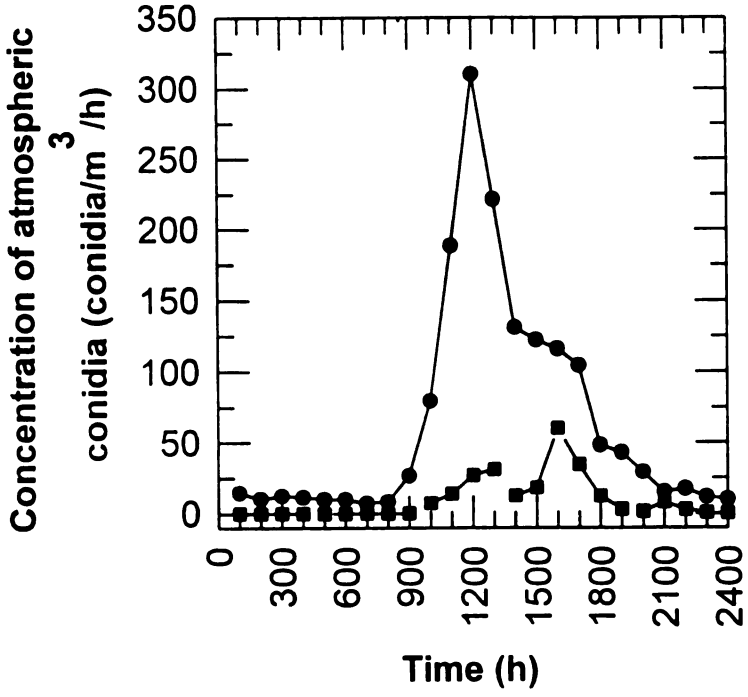


Figure 1. Hourly average concentration of airborne conidia of *Oidium* sp. in research greenhouses 2 (●) and 11(■), during 5 December to 1 June.

Figure 2. Association of greenhouse activity, including watering (▼) and venting (◆), and fluctuation of relative humidity with concentration of airborne conidia of *Oidium* sp. in research greenhouses 2 (A) and 11, (B) during 13 through 16 March.

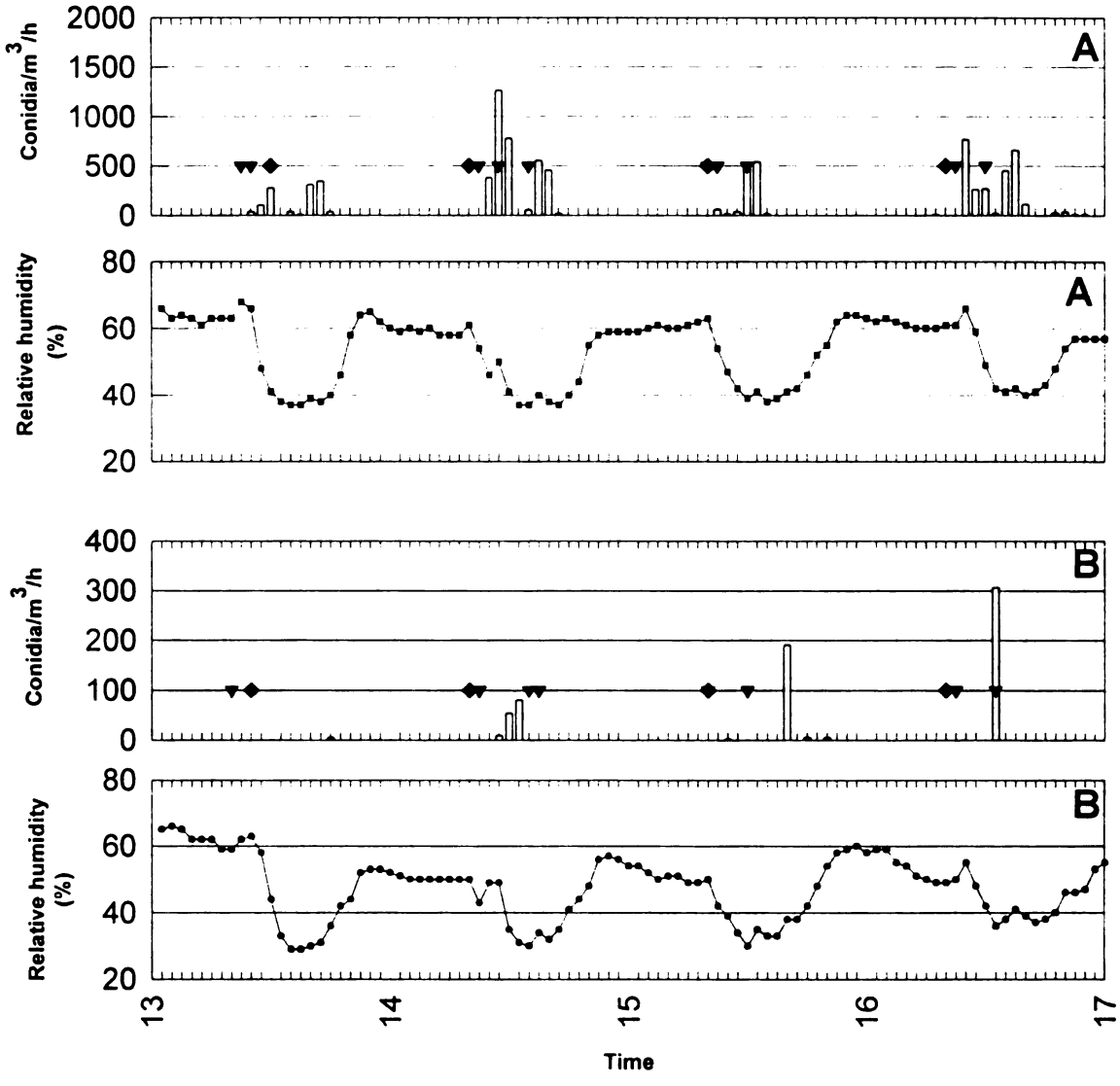

Conidia/m3/h
2000
1500
1000
500
0
A
Relative humidity (%)
80
60
40
20
A
Conidia/m3/h
400
300
200
100
0
B
Relative humidity (%)
80
60
40
20
B
13
14
15
16
17
Time

Table 1. Number of conidial release events associated with relative humidity fluctuations (positive or negative) occurring between 5 December to 1 June (greenhouse 11) and 5 December to 17 July (greenhouse 2).

Fluctuation in relative humidity (%)	Number of conidial release events			
	Greenhouse 2		Greenhouse 11	
	+	-	+	-
0	1	0	0	0
1-4	7	8	2	8
5-10	21	18	4	9
11-15	23	15	10	3
16-20	9	9	6	2
21-25	9	8	2	2
26-30	2	2	0	2
>30	0	2	0	0

Watering resulted in an immediate increase (≤25%) followed by a rapid decrease (≤32%) in RH beginning 1 to 2 h later. In GH 2, 89% of the 134 CREs occurred within 3 h following plant watering (data not shown). In GH 11, 48% of the 50 CREs were observed within 3 h of watering (data not shown). Some CREs were observed that were not associated with watering. In GH 2, 11 of these 15 CREs were associated with a fluctuation of 5 to 23% in RH within 3 h of the CRE onset (data not shown). In GH 11, 80% of the 25 CREs not associated with watering were preceded by a natural RH fluctuation ranging from 5 to 28% within 3 h of the CRE onset (data not shown).

The largest CRE in each greenhouse (estimated 8,500 conidia/m^3/h) was preceded by a 10% decrease in RH; in GH 11, a 10.0C decrease in temperature also occurred. In GH 2, CREs lasting 10 or more hours occurred eight times during the observation period. On 29 March, the CRE began at 1200 h, lasted 55 h, and released a total of 27,625 conidia/m^3 (Fig. 3). The onset of this event was associated with a watering event and an 8% increase in RH. Elevated peaks within this extended CRE period were associated with fluctuations in RH. Similar CREs did not occur in GH 11.

During the study, vents in GH 2 were opened on 50 days. On 34 of these days, plants were watered as the vents opened, prompting CREs within 3 h on 11 occasions. When venting occurred without watering, CREs were observed 14% of the time. Greenhouse 11 was vented 58 times during the course of this study. On three of these days, CREs occurred within 3 h of greenhouse venting or plant watering while venting.

Temperature. Although this study was conducted in greenhouses of similar size, construction, and orientation located in the same complex, the environmental conditions varied between greenhouses. In GH 2, temperatures did not routinely exceed 25.0C

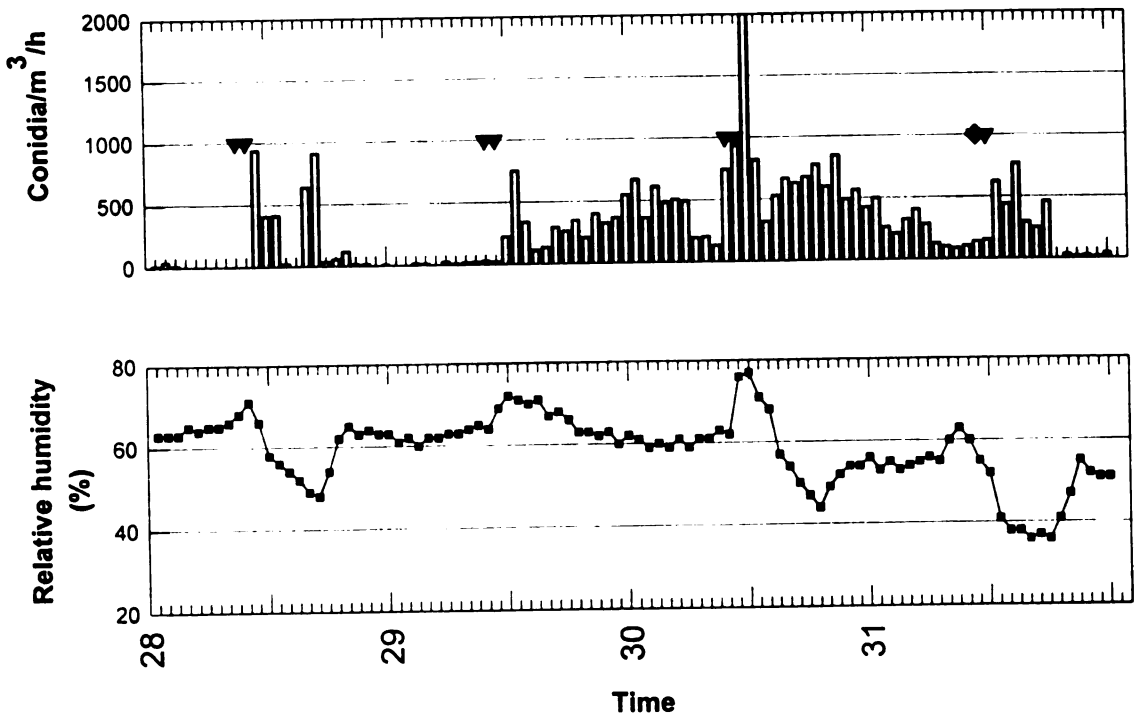


Figure 3. Extended conidial release event period and associated greenhouse activity, including watering (▼) and venting (◆), and fluctuations of relative humidity in greenhouse 2 during 28 through 31 March.

during the first six weeks (23 January to 6 March) of the study (Fig. 4). Thereafter, temperatures exceeded 25.0C three to six days during each week. However, during weeks 19 to 24 (29 May to 10 July), temperatures exceeded 30.0C one to seven days per week and corresponded to the lowest conidial concentrations occurring in this study (<1,200 m^3/week) (Fig. 4).

In GH 11, temperatures exceeded 25.0C one to five days per week during the study (Fig. 5). Temperatures exceeding 30.0C were not observed until week 7 (6 to 13 March). On March 11 the temperature reached 39.1C and prompted several CREs (≤8,500 conidia/m^3/h) (data not shown). Total conidial concentrations for weeks one to seven (23 January to 13 March) were 31,653 conidia/m^3. Following the extreme high temperature occurring in week 7, CREs were reduced for the duration of the study. Total conidial concentrations for weeks 8 (13 to 20 March) to 18 (22 to 29 May) were 5,832/m^3 (Fig. 5). During week 15 (1 to 8 May), temperatures exceeded 30.0C each day of the week. Subsequently, atmospheric conidial concentrations were not detected for the remainder of the study.

Disease development. Disease developed on plants placed in GH 2 from week 1 to week 20 (23 January to 12 June). Time to colony development ranged between 0 (colonies developed during the exposure week) and 4.3 days after the exposure period (Fig. 4). Atmospheric conidial concentrations during this period ranged from 1,185 to 42,833 conidia/m^3/week. No colonies developed on plants placed in GH 2 during weeks 21 to 24 (12 June to 10 July). During this period, fewer than 600 conidia/m^3/week were released.

When plants were placed in GH 11 during weeks 1 to 6 (23 January to 6 March) and week 9 (20 to 27 March), colonies developed within an average of three to seven

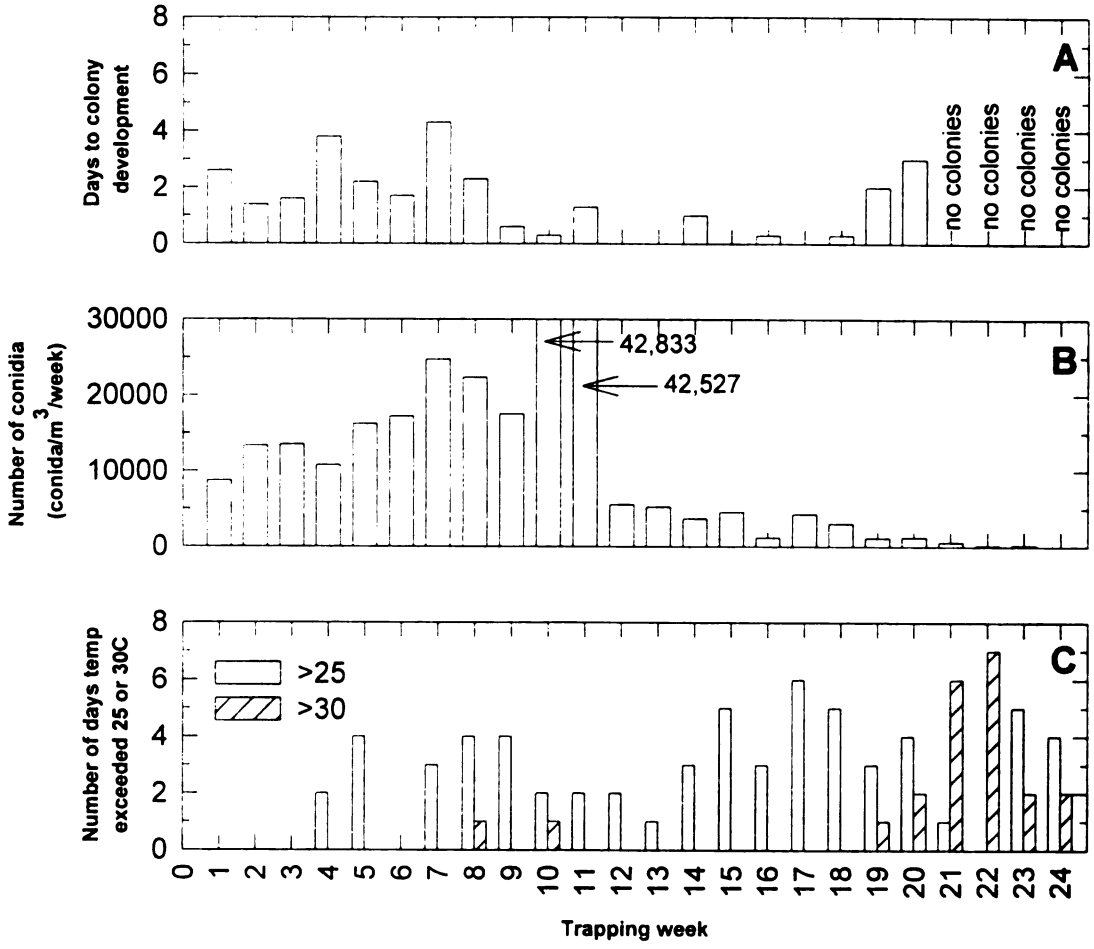


Figure 4. The A) days to colony development on poinsettias following exposure to atmospheric conidial concentrations, B) total number of conidia released during the trap week, and C) number of days the average hourly temperatures exceeded 25C and 30C in research greenhouse 2 during 23 January to 10 July.

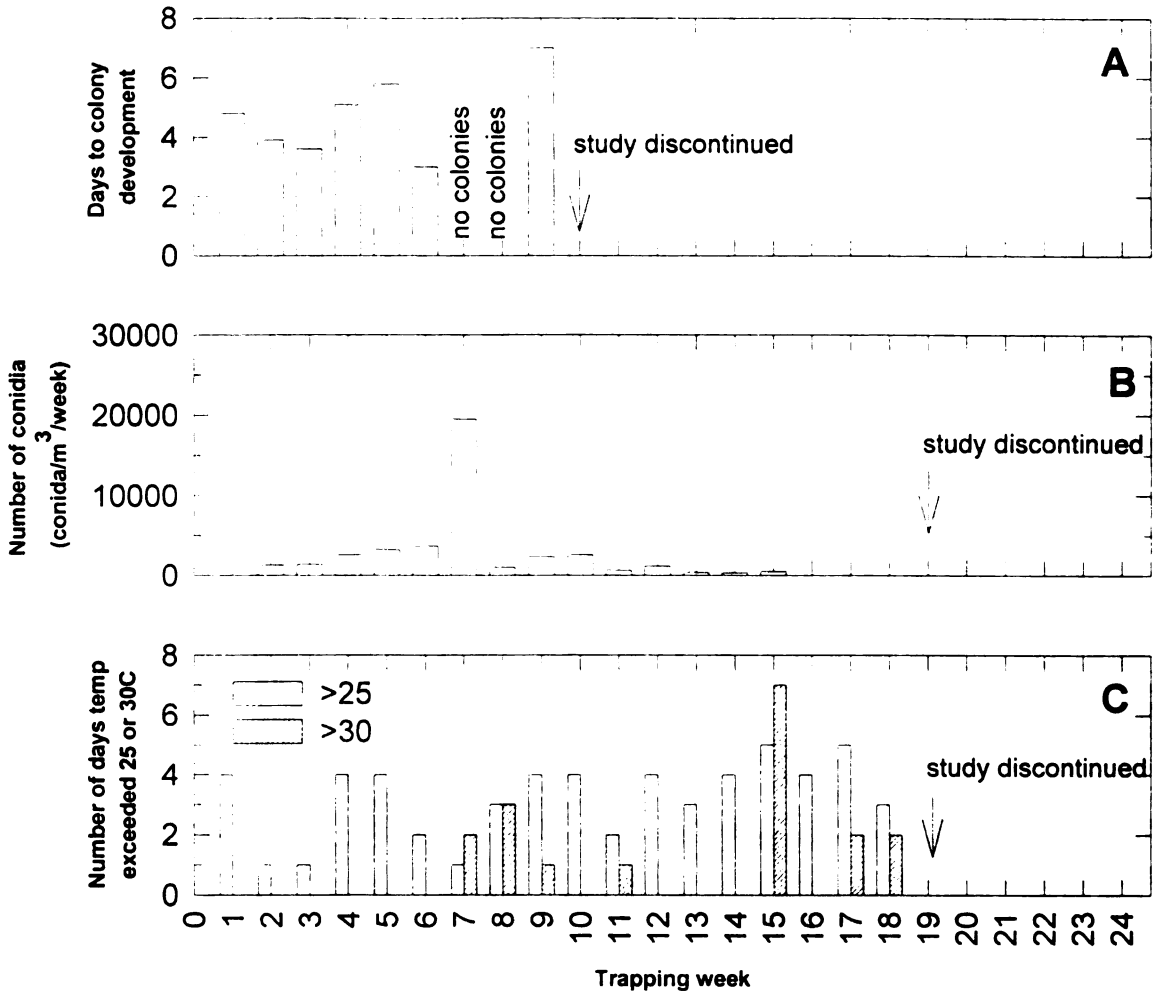


Figure 5. The A) days to colony development on poinsettias following exposure to atmospheric conidial concentrations, B) total number of conidia released during the trap week, and C) number of days the average hourly temperatures exceeded 25C and 30C in research greenhouse 11 during 23 January through 10 July.

days after the exposure period (Fig. 5). During week 7 (6 to 13 March) when temperatures reached 39.1C, 19,502 conidia/m^3 were released, although plants exposed during this time did not develop colonies. During week 9 (20 to 27 March), 2,322 conidia/m^3 were released and exposed plants developed colonies.

DISCUSSION

Concentrations of airborne conidia of *Oidium* sp. were present in the greenhouses throughout the monitored period. The production and release of conidia of many powdery mildew species is diurnal and we observed conidial release largely between 1000 and 1400 h. The periodicity of powdery mildews which form conidia in chains is determined by the final stage of sporulation, when the abstriction of the conidium from the germ cell of the conidiophore occurs (2). This maturation stage is photosensitive, proceeding faster in light than in dark, therefore conidia produced at night remain immature and are released once exposed to light (8). Observational field studies of powdery mildew on grapes (24), rubber (10), cherry (12), apple (22), barley (13) and tobacco (7) have also documented diurnal conidial release patterns. Release patterns are also influenced by wind speed, rainfall, fungicide applications, temperature, RH, and solar radiation, which also tend to follow diurnal patterns (23, 24).

In our study, a primary factor influencing the occurrence of CREs was fluctuation of relative humidity, often caused by watering. The association of conidial release in response to RH fluctuation has been similarly observed with other powdery mildew species including *Sphaerotheca pannosa*, *Erysiphe pisi* and *E. graminis* (1). Conidial release by *S. pannosa* occurs in response to abrupt decreases in RH while release by *E. graminis* occurs in response to increasing RH. Butt (2) proposed that the variation in

sensitivity to RH may be due to differences in the hygroscopic properties of the spore wall. *Eryisphe pisi* does not discharge conidia in response to RH fluctuation alone; conidial liberation occurs when RH change is accompanied by exposure to light and increasing temperature. In our study CREs occurred in response to both positive and negative changes in RH. Although many CREs occurred in conjunction with temperature changes, it was not required for conidial release.

Although we have found an association between RH fluctuations and conidial release in *Oidium* sp. of poinsettia, the mechanism of release is not known. Both passive and active liberation mechanisms have been proposed for powdery mildew species (18). Mechanical disruption (13), wind speed (11), and humidity (1) or temperature changes have been associated with high conidial releases of powdery mildews. In field situations, wind plays a significant role in the release and conidial dispersal of many powdery mildew species either directly or as a result of leaf movement dislodging conidia (10, 14, 23). In the greenhouse, significant air movement is limited to that caused by cooling fans or open vents. An alternative method of dispersal is hygroscopic twisting in response to changing RH. This twisting causes movement capable of dislodging spores of *Phytophthora* and *Peronospora* (18). However, Jarvis (15) concluded that hygroscopic movements loosen spores of *Botrytis cinerea* for subsequent or rainsplash dispersal. Adams (1) proposed that a change from high to low RH creates an electrical charge with sufficient voltage to remove powdery mildew conidia from conidiophores.

Optimum temperatures reported for powdery mildews (21.0C) are generally lower than those for other plant pathogens (2). Studies comparing effects of temperature on *Oidium* sp. on poinsettia found conidial germination was significantly reduced at 30C

(<64%) versus 20.0C (≥80%). At 30.0C formation of secondary germ tubes was significantly limited (<1%) compared with 20C (≥53%). Less than 4% of germinated conidia formed haustoria at 30.0C, compared to greater than 53% of those at 20.0C.

In our study, temperature exceeded 25.0C for 21 days in May in GH2 and the atmospheric conidial concentration was 20% of that occurring in April when only nine days exceeded 25.0C. Similarly in GH 11, when the number of days the temperature exceeded 25.0C increased from 9 in February to 19 in March, the atmospheric conidial concentration was reduced by 75%. As a result, the powdery mildew epidemics in the greenhouses naturally lost intensity in March (GH11) and June (GH2). For the last five weeks of the study, temperatures in GH2 exceeded 30.0C at least two days per week. At the conclusion of the study in GH2, colonies were no longer developing on exposed plants and atmospheric conidial concentrations were declining such that fewer than 100 conidia/m^3 were released in the final week of monitoring. In GH 11, following the high temperature of 39.1C in March (week 7), atmospheric conidial concentrations were reduced (≤833 conidia/m^3/h). Colonies did not develop on plants exposed during weeks 7 and 8, when temperatures exceeded 30.0C two to three days per week. Similar temperature effects have been observed with other powdery mildew species. The maximum temperature for germination and appressorium formation of *O. begoniae* is also 30.0C; colonies on mature leaves were eradicated by exposure to 32.0C for three days (21).

Kim et al. (17) observed that plants with active powdery mildew colonies, grown in a greenhouse throughout the summer and exposed to high temperatures and solar radiation showed no signs of infection for a period of time. In two commercial

greenhouses in Michigan where powdery mildew was detected in July or August, colonies were found only on the abaxial sides of the bottom leaves where temperatures would be cooler as a result of canopy shade and close proximity to the moist surface of the growing medium (Hausbeck, personal observation). Because of the colony formation on leaf undersurfaces, without thorough and frequent scouting, low levels of disease would thus escape undetected. However, as the season progresses and temperatures decrease, conditions will be more favorable for further colony development, with associated high and frequent CREs prompting rapid onset of an epidemic. At this point, colonies would likely be evident on the adaxial surface of upper leaves and/or bracts and widespread enough to be noticed by growers. Since Michigan conditions at this point would not naturally exceed 25.0C, the epidemic would proceed unhindered and result in entire poinsettia crops being unsaleable. This has occurred many times in Michigan in the last several years.

The ability of powdery mildew to become epidemic seemingly at a time when poinsettias have colored bracts and are especially vulnerable to phytotoxicity and residues from fungicides has made disease management difficult. While thorough scouting for signs of infection is optimum for prompting fungicide sprays, many growers do not have the necessary personnel to accomplish the task. Rather, some growers apply preventive fungicides during the entire production cycle until just prior to bract coloration. Results from this study may be helpful in the eventual development of a disease management strategy that utilizes environmental conditions to prompt fungicide sprays.

LITERATURE CITED

1. Adams, G. C., Jr., Gotwald, T. R., and Leach, C. M. 1986. Environmental factors initiating liberation of conidia of powdery mildews. Phytopathology 76:1239-1245.

2. Butt, D. J. 1978. Epidemiology of powdery mildews. Pages 51-81 in: The Powdery Mildews. D. M. Spencer, ed. Academic Press, New York.

3. Byrne, J. M., and Hausbeck, M. K. 1998. Influence of temperature and relative humidity on infection processes and sporulation on *Oidium* sp. on poinsettia foliage. (Abstr.) Phytopathology 88:S134.

4. Byrne, J. M., and Hausbeck, M. K. 1999. The effect of temperature on sporulation of *Oidum* sp. on poinsettia foliage. (Abstr.) Phytopathology 89:S10.

5. Celio, G. J., and Hausbeck, M. K. 1997. Evaluation of poinsettia cultivars for susceptibility to powdery mildew. HortScience 32:259-261.

6. Celio, G. J., and Hausbeck, M. K. 1998. Conidial germination, infection structure formation, and early colony development of powdery mildew on poinsettia. Phytopathology 88:105-113.

7. Cole, J. S. 1966. Powdery mildew of tobacco (*Erysiphe cichoracearum* DC) IV. Conidial content of the air within infected crops. Ann. Appl. Biol. 57:445-450.

8. Cole, J. S., and Fernandes, D. L. 1970. Effects of light, temperature and humidity on sporulation of *Erysiphe cichoracearum* on tobacco. Trans. Br. Mycol. Soc. 55:345-353.

9. Daughtrey, M. L., and Hall, J. 1992. Powdery mildew: A new threat to your poinsettia crop. Grower Talks, Sept.:23-31.

10. Fernando, T. M. 1971. *Oidium* leaf disease: the effect of environment and control measures on incidence of disease and atmospheric spore concentration. *[Hevea brasiliensis]*. Quarterly J. Rubber Res. Inst. Ceylon 48:100-111.

11. Frinking, H. D. 1977. Research on wind dispersion of rose-mildew spores (*Sphaerotheca pannosa*) in field, glasshouse and climate room. Grava 16:155-158.

12. Grove, G. G. 1991. Powdery mildew of sweet cherry: influence of temperature and wetness duration on release and germination of ascospores of *Podosphaera clandestina*. Phytopathology 81:1271-1275.

13. Hammett, K. R. W., and Manners, J. G. 1971. Conidium liberation in *Erysiphe graminis*. I. Visual and statistical analysis of spore trap records. Trans. Br. Mycol. Soc. 56:387-401.

14. Hammett, K. R. W., and Manners, J. G. 1974. Conidium liberation in *Erysiphe graminis*. III. Wind tunnel studies. [Wheat]. Trans. Br. Mycol. Soc. 62:267-282.

15. Jarvis, W. R. 1962. The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. Trans. Br. Mycol. Soc. 45:549-559.

16. Jones, R. K., and Strider, D. L. 1985. Bedding plants. Pages 409-422 in: Diseases of Floral Crops, Vol. 1. D. L. Strider, ed. Praeger Publishers, New York.

17. Kim, S. H., and Olson, T. N. 1995. Recurrence of powdery mildew on over-summered asymptomatic poinsettias. (Abstr.) Phytopathology 85:1171.

18. Lacey, J. 1996. Spore dispersal–its role in ecology and disease: the British contribution to fungal aerobiology. Mycol. Res. 100:641-660.

19. National Agricultural Statistics Service. 1999. Floriculture crops, 1998 summary. Agricultural Statistics Board, U. S. Department of Agriculture, Washington, D. C.

20. Nau, J. 1998. *Euphorbia pulcherrima*. Pages 482-509 in: Ball Red Book, 16th ed. V. Ball, ed. Ball Publishing, Batavia, IL.

21. Quinn, J. A., and Powell, C. C., Jr. 1982. Effects of temperature, light, and relative humidity on powdery mildew of begonia *Oidium begoniae* on *Begonia x hiemalis*. Phytopathology 72:480-484.

22. Sutton, T. B., and Jones, A. L. 1979. Analysis of factors affecting dispersal of apple powdery mildew caused by *Podosphaera leucotricha* conidia. Phytopathology 69:380-383.

23. Willocquet, L., Berud, F., Raoux, L., and Clerjeau, M. 1998. Effects of wind, relative humidity, leaf movement and colony age on dispersal of conidia of *Uncinula necator*, causal agent of grape powdery mildew. Plant Pathol. 47:234-242.

24. Willocquet, L., and Clerjeau, M. 1998. An analysis of the effects of environmental factors on conidial dispersal of *Uncinula necator* (grape powdery mildew) in vineyards. Plant Pathol. 47:227-233.

CHAPTER II.

INFLUENCE OF ENVIRONMENT ON INFECTION AND SPORULATION OF *OIDIUM SP.* ON POINSETTIA FOLIAGE

ABSTRACT

The influence of temperature and relative humidity (RH) on conidial germination and infection processes of *Oidium* sp. were quantified on poinsettia foliage. Leaf disks were inoculation with conidia of *Oidium* sp. and incubated at 15, 20 and 25°C in chambers with glycerol/water solutions that provided 35, 50, 65, 80 and 92% (±2%) RH. Forty-eight hours after inoculation 100 conidia per leaf disk were counted and the presence of appressoria, germ tubes, haustoria, length of germ tubes and shriveled conidia quantified. Formation of appressoria and primary germ tubes were favored by 20°C. Haustorium formation appeared to be favored by 20°C and 35, 50, and 65% RH. The development of secondary germ tubes was favored by 25°C and was not consistently affected by RH. The average length of germ tubes produced at 25°C (36.2µm) was longer than at 20°C (27.0µm) and 15°C (11.1µm). Shriveling of conidia (18.8 to 23.8%) occurred across the range of temperatures and RH levels in this study. Effects of temperature on sporulation of *Oidium* sp. were quantified on inoculated leaf disks incubated for 14 days at 15 and 20°C under high relative humidity. The number of conidiophores produced per mm^2 of leaf tissue, 80 and 99 at 15 and 20°C, respectively, was not affected by temperature. Temperature had a significant effect on the number of conidia produced per conidiophore, at 15 and 20°C a maximum of 4 and 7 conidia were produced per conidiophore, respectively.

INTRODUCTION

Poinsettias (*Euphorbia pulcherrima* Willd. ex Klotzsch) have a wholesale value of $237 million in the United States with California, North Carolina, Texas, and Ohio leading production (6). Powdery mildew on poinsettias in the United States was first reported in 1990 and was epidemic in Michigan in 1992. While the disease is not common, it occurs yearly and is an economically significant problem especially for poinsettia growers in the midwestern and northern United States (M.K. Hausbeck, personal communication). In the absence of an observed teleomorph, the causal agent of this powdery mildew is referred to simply as *Oidium* sp. and is recognized by catenate conidia on upright conidiophores characterized by an arched basal cell (3).

Signs of powdery mildew appear as small, white, talcum-like colonies on bract, leaf, stem, or petiole surfaces that can coalesce to cause blighting. Chlorotic spots appear opposite colonies on abaxial leaf surfaces (7). If colonies first develop on the undersides of leaves, the disease may go undetected until later in production when bracts become infected, making plants unsaleable. While fungicide application to poinsettia bracts limit further colony development, the fungal mycelium remains visible and is commercially unacceptable. Additionally, residue and phytotoxicity resulting from fungicide applications to the bracts may result in an unmarketable crop. Growers who detect colonies early in production may eliminate the disease by removing infected leaves, and applying fungicides.

Initial epidemiological studies on this pathogen (*Oidium* sp.) detailed the temporal development of infection structures produced by conidia (3). Conidia placed on poinsettia leaf disks were found to germinate, form secondary germ tubes and haustoria within 24

hours at 20C. High temperatures (30C) reduce germination of conidia and the development of secondary germ tubes; haustorium development is also significantly limited. This coincides with observations made in both commercial and research greenhouses (10) where powdery mildew development is limited during growing periods where average hourly temperatures frequently exceed 30C (2). Powdery mildew vary in the ranges of relative humidity under which gemination can occur. The effect of relative humidity on germination and infection of *Oidium* sp. has not been established on poinsettia.

The objectives of this study were to examine the effects of relative humidity and temperature on the infection process. Earlier histological studies of foliar infection by *Oidium* sp. were conducted under a fixed RH level (85%) at 20 and 30°C (3). The effect of temperature on the pathogen's ability to sporulate was also examined and quantified.

MATERIALS AND METHODS

Plant growth. Multi-stemmed poinsettias (cv. Freedom Red) were grown in a research greenhouse in 13.2-cm-clay pots containing Baccto soilless potting mix (Michigan Peat Company, Houston, TX). The greenhouse was maintained at 23°C (day) and 18°C (night). Plants were watered daily or as needed and fertilized with 200 ppm 15-5-25 (N-P-K) poinsettia fertilizer (Grace-Sierra Horticultural Products Company, Milipitas, CA), twice weekly. Pesticides were not applied to the foliage of the plants during the experiment.

Inoculation. Leaf disks were excised from fully expanded leaves with a cork borer, surface-sterilized in a 0.525% sodium hypochlorite solution for 1 min, rinsed in sterile distilled water, and dried under a laminar flowhood. Inoculum of *Oidium* sp. (Michigan State University Herbarium, East Lansing, accession #361714, Mary Hausbeck 01) was

produced on infected poinsettias maintained in a growth chamber (Sherer-Gillett) at 20°C under high humidity (>70%) and 12 h photoperiod. To ensure uniform age and viability, conidia were dislodged 3 to 5 days before inoculations by shaking plants at the base of their stems to promote growth of new conidia. Leaf disks were inoculated by using a plastic-bristle paintbrush to transfer conidia from the infected leaves to each leaf disk.

Conidial germination and infection process. Inoculated leaf disks (1-cm-diam) were placed adaxial surface up on agar disks (1.4-cm-diam, 20 g agar/liter) suspended by a plastic mesh grid (1.7 squares/cm) in a glass humidity chamber (Mason jar, 476 ml) containing 120 ml of prepared glycerol/water solution. Glycerol/water solutions providing 35, 50, 65, 80 and 92% (±2%) relative humidity (RH) (Forney and Brandl, 1992) were made and measured with a hygrometer (VWR Scientific, McGaw Park, IL) which was accurate to ±0.5% RH. Glycerol solutions were allowed to equilibrate for at least 2 hours before RH measurements were taken. Leaf disks were incubated in tightly sealed humidity chambers at 15, 20 and 25C (±0.2) in a water bath (Precision Scientific, Inc.) under complete darkness for 48 hours.

Following incubation, leaf disks were fixed with an FAA solution (formalin, acetic acid, ethanol in 1:18:1 v/v/v) for 2 h and cleared in a saturated solution of chloral hydrate (250g/100ml) for 5 days. Disks were preserved in 1-dram vials of lactophenol solution (20 g phenol, 20 ml lactic acid, 40 g glycerin, 20 ml water) until microscopic observations were made. Cleared leaf disks were stained with cotton blue in lactophenol solution (100 ml lactophenol, 1 ml 1% aqueous cotton blue, 20 ml glacial acetic acid) and mounted in glycerol for observation. One hundred conidia on each disk were counted using light microscopy (400x) and observed for the presence of germ tubes, appressoria, and

haustoria. Conidia that were not germinated but were shriveled were also counted. Germination was defined as a conidium with either an appressorium or a primary germ tube equal in length to at least half the width of the conidium (Lacy, 1994). The length of up to five germ tubes on each disk were also determined. Each experiment included four leaf disks per humidity jar and two humidity jars per RH, and was conducted three times.

The experiment was analyzed as a split-split plot with RH being nested within experiment replicate and experiment replicate being nested within temperature. Experiment replicate, humidity jar and disk were classified as random effects while temperature and RH were fixed effects. Data was analyzed with the ANOVA procedure of the Statistical Analysis System (SAS Insititute, Cary, NC), germ tube length was analyzed with the proc mixed procedure, the remaining data was analyzed with a generalized mixed linear model (glimmix macro) and a binomial error distribution. Tukey's test was used to determine significant differences of least squares means for each temperature and RH combination. Iteration of the data on percentage of germinated conidia with germ tubes did not converge with the experimental design model used, therefore conclusions can not be made as to the significance of either temperature or relative humidity on this aspect of the infection process.

Sporulation. Inoculated leaf disks (1.7-cm-diam) were placed on agar disks (2.0-cm-diam) amended with 30 mg/l benzimidazole and were weighed down with sterile metal washers (1.3-cm-diam hole, 7/8 inch outer) and incubated in double petri dishes (4 leaf disks/dish). Double petri dishes were comprised of a lower petri dish that contained sterile distilled water and an upper compartment that housed the washer-leaf disk-agar stacks. The compartments were connected by an opening through which four filter paper wicks

were passed, to keep the agar disks hydrated throughout the experiment.

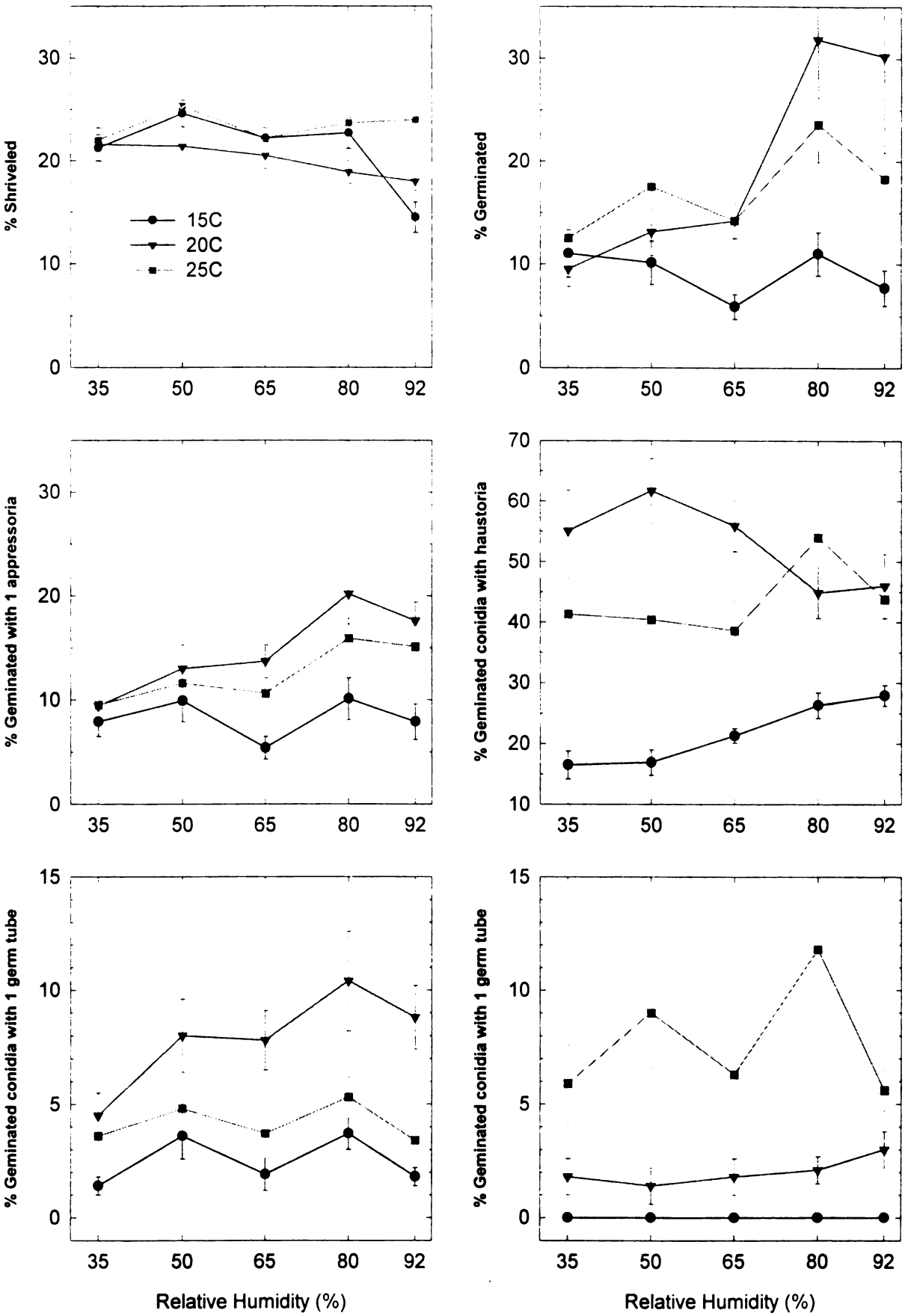
Leaf disks were incubated at 15 and 20C under 12-h photoperiods for 14 days. Initially, observations were made daily to determine the time to conidiophore and conidia development. Fourteen days after inoculation the number of conidiophores and conidia on two sites per disk were quantified with a compound dissecting microscope (100x). Each temperature was replicated two times, four disks per double petri dish and four double petri dish chambers per temperature replicate. The experiment was analyzed with the genmod procedure of the Statistical Analysis System with a multinomial distribution, experiment replicate was nested within temperature.

RESULTS

Germination and infection. There was a significant interaction between temperature and RH for conidial germination (P=0.0529), haustorium formation (P=0.0001), germ tube length (P=0.0028) and shriveling of conidia (P=0.0001). In general, the lowest temperature (15°C) was less conducive than the higher temperatures of 20 and 25°C for germination, appressorium and haustorium development, primary and secondary germ tube formation and germ tube elongation. (Fig. 1). Formation of appressoria and primary germ tubes were favored by 20°C.

Conidial germination was favored by warm temperatures (≥20°C) and high RH (≥80%). With one exception (35%RH), 15°C was not favorable for conidial germination. Appressorium formation was favored by 20°C and high RH (≥80%) with a minimum of 17.6 % of the germinated conidia with an appressorium. Fewer than 10.1% of the germinated conidia developed an appressorium at 15°C regardless of RH.

Figure 1. Effect of temperature and relative humidity on A) shrivelling, B) germination, C) appressorium formation, D) haustorium formation, E) primary germ tube formation, and F)secondary germ tube formation of *Oidium sp.* on poinsettia foliage.


% Shriveled
% Germinated
% Geminated with 1 appressoria
% Geminated conidia with haustoria
% Geminated conidia with 1 germ tube
% Geminated conidia with 1 germ tube
15C
20C
25C
0
5
10
15
20
30
40
50
60
70
35
50
65
80
92
Relative Humidity (%)
Relative Humidity (%)

Haustorium formation appeared to be favored by 20°C and RH levels of 35, 50, and 65%, where the percentage of germinated conidia with a haustorium ranged from 55.1 - 61.7. In contrast, haustorium development never exceeded 28% at 15C across all RH levels.

The 20°C treatment was conducive to the development of a primary germ tube, although 80% RH was more favorable than 35% RH. At 15 and 25°C there was little apparent impact of RH on the development of a single germ tube. The 15°C treatment was least favorable.

The development of secondary germ tubes was favored by 25°C. Limited development of secondary germ tubes occurred at 20°C and secondary germ tubes were not formed at 15°C. There was no consistent trend in the effect of RH on development of secondary germ tubes at either 20 or 25°C. The average length of germ tubes produced at 25°C (36.2 μm) was longer than at 20°C (27.0μm) and 15°C (11.1μm) (Fig. 2). There was no consistent trend in RH effects on germ tube length. Shriveling of conidia (18.8 to 23.8%) occurred across the range of temperature and RH levels included in this study.

Sporulation.

Sporulation was initiated 9 days after inoculation regardless of temperature. Chains of 3 - 6 conidia developed 11 days after inoculation. Fourteen days after inoculation the maximum chain length was 7 conidia per conidiophore.

The number of conidiophores produced per mm^2 of leaf tissue, 80 and 99 at 15 and 20°C, respectively, was not affected by temperature (Fig. 3). Temperature did have a significant effect on the number of conidia produced per conidiophore ($P<0.0001$) (Fig. 4). The effect of the experiment replicate was also significant. At 15°C, a maximum of

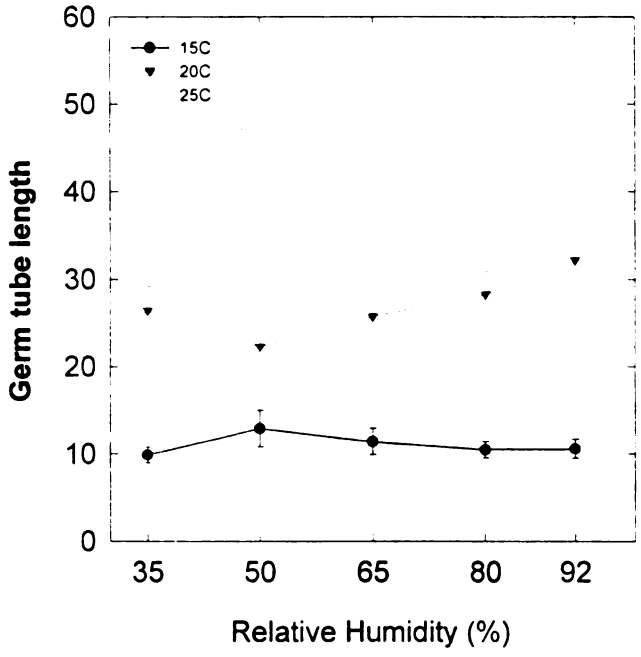


Figure 2. Effects of temperature and relative humidity on the length of germ tubes produced by conidia of *Oidium* sp. on poinsettia foliage.

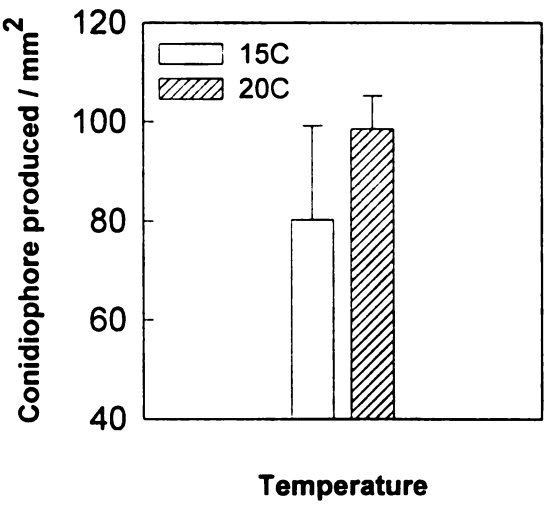


Figure 3. The effect of temperature (15 and 20°C) on conidiophores produced per mm2 of leaf disk, 14 days after inoculation.

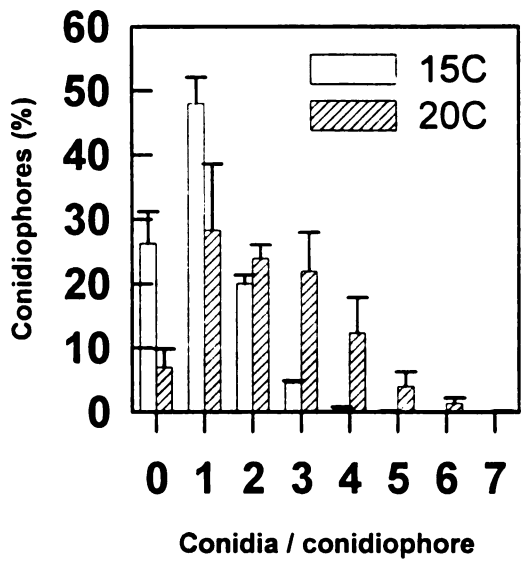


Figure 4. Sporulation of *Oidium* sp. on poinsettia leaves, incubated at 15 and 20°C, 14 days after inoculation.

4 conidia were produced per conidiophore. At 20°C up to 7 conidia were produced per conidiophore.

DISCUSSION

Effects of temperature and relative humidity on the conidial germination and infection processes of *Oidium* sp. were quantified on poinsettia foliage. Optimum temperatures reported for powdery mildews (21.0°C) are generally lower than those for other plant pathogens (1). Celio found that many of the infection processes were inhibited at 30°C. The lowest temperature used in our study (15C) was generally less conducive to aspects of the infection process than the higher temperatures of 20 and 25°C. This observation coincides with results of similar studies with other powdery mildews (1). With an extended incubation period some powdery mildews are able to germinate at lower temperatures. At 4°C, both *O. begoniae* and *Podosphaera leucotricha* conidia germinate on host tissue (5,12). Low temperatures lengthen the period between infection and symptom development (1).

Haustorium development appeared to be severely limited at 15°C. Additionally, secondary germ tubes did not develop at 15°C. The formation of a haustorium is considered a visible measure of successful infection. The main function of a haustorium is to absorb nutrients from the host which are then supplied to the hyphae. Since haustoria are considered essential for additional hyphal growth it is not surprising that development of secondary germ tubes did not occur at 15C (9).

The formation of secondary germ tubes and their elongation were favored by the warmest temperature (25°C). (11). Manners and Hussain (11) found that the optimum temperature for germ tube development of *Erysiphe graminis* was higher than the

temperature that favored optimum germination. In this study, germ tubes of *Oidium* sp. elongated regardless of RH and differs from *E. graminis* where germ tubes did not elongate below 98% RH (11).

Powdery mildews in general are able to germinate and infect under conditions of low RH. High amounts of moisture within the relatively large conidia provide a resource, allowing germination and infection under low RH.. Powdery mildews are actually inhibited by free moisture on host surfaces, conditions that favor germination of many other fungal pathogens. Conidia of some powdery mildew species are susceptible to bursting in the presence of free water due to the additional water that spores contain (13). Even under controlled environmental conditions it is difficult to maintain a level of very high RH (>90%) without small fluctuations that promote condensation and subsequent leaf wetness. The reduced efficiency of the infection processes at the 92% RH level may be due to temporary formation of condensation on the leaf disk surface. The moderate temperature (20°C) and lower RH levels of 35, 50, and 65 were most favorable to the formation of haustoria. This stage of the infection process appears less dependent on high RH than germination and appressorium formation.

The density of conidiophores on the leaf tissue was not affected by the incubation temperature; however, the number of conidia produced on a conidiophore was affected. Sporulation of *Oidium* sp. began nine days after inoculation of the poinsettia foliage. Conidiophores of *E. polygonia* differentiate from mycelium five days after infection, and the conidium at the tip of the conidiophore subsequently matures with each additional 24 hour period (4). The lower temperature (15°C) used in this study apparently slowed the development and maturation of conidia. The optimum temperature for sporulation of *E.*

graminis is 20°C. conidial production was significantly decreased at 25°C (14). Relative humidity was not controlled in the chambers used for these sporulation studies, but high RH is also considered an important influence of sporulation of powdery mildews.

Viability of the conidia produced in this study was not assessed in this study but it is reportedly affected by the environmental conditions during their production (1). Hossain (8) found that humid conditions were necessary for the production of conidia with high germinability. Ward and Manners (14) concluded that the development of epidemics is more likely to be affected by the environmental conditions that impact sporulation, than by those that influence conidial germination and infection.

Temperature manipulation may be a useful tool in managing powdery mildew on poinsettia. Based on results of high temperature studies Celio (3) and Quinn (12) suggested warm temperatures could be used to help manage powdery mildews on greenhouse grown poinsettias and begonias. Depending on the time of year and the climate it may be more feasible and cost effective to reduce greenhouse temperatures. Reducing greenhouse temperatures to 15°C would create an environment less favorable for sporulation and infection of poinsettia foliage by *Oidium* sp. Low temperatures could be helpful when trying to suppress disease development in the time period between diagnosis and fungicide application. For horticultural reasons this low temperature could not be maintained for extended periods of time, and therefore could not be used alone in a disease management program. There may be the potential to incorporate temperature manipulations with scouting and fungicide applications to enhance powdery mildew management.

LITERATURE CITED

1. Butt, D. J. 1978. Epidemiology of Powdery Mildews. In: The Powdery Mildews, edited by D. M. Spencer. New York: Academic Press.

2. Byrne, J.M., M. K. Hausbeck, and B.D. Shaw. 2000. Factors affecting concentrations of airborne conidia of *Oidium sp.* among poinsettias in a greenhouse. Plant disease 84:1089-1095.

3. Celio, G. J., and M. K. Hausbeck. 1998. Conidial germination, infection structure formation, and early colony development of powdery mildew on poinsettia. Phytopathology 88:105-113.

4. Cole, J. S. 1976. The formation and dispersal of *Erysiphe conidia.* In: Microbiology of Aerial Plant Surfaces, edited by C. H. Dickinson and T. H. Preece. London: Academic Press, Inc.

5. Coyier, D.L. 1968. Effect of temperature on germination of *Podosphaera leucotricha* conidia. Phytopathology 58:1047-1048.

6. Floriculture crops, 2000 Summary.2001., edited by N. A. S. Service. Washington, DC: United States Department of Agriculture.

7. Hausbeck, M., J. Kalishek, M. Daughtrey, and L. Barnes. 1994. Keep the colonies at bay (Part 1). Greenhouse Grower:45-48.

8. Husain, S.I., and M. Akram. 1995. Effect of temperature and relative humidity on conidial germination and germ tube elongation of *Sphaerotheca fuliginea* (Schlecht ex Fr.) Poll. on sunflower. Journal of Plant Diseases and Protection 102:509-513.

9. Jenkyn, J. F., and A. Bainbridge. 1978. Biology and pathology of cereal powdery

mildews. In: The Powdery Mildews, edited by D. M. Spencer. London Press: Academic Press.

10. Kim, S. H. , and T. N. Olson. 1995. Recurrence of powdery mildew on over-summered asymptomatic poinsettias. Phytopathology (Abstract) 85:1171.

11. Manners, J.G., and S.M.M. Hossain. 1963. Effects of temperature and humidity on conidial germination in *Erysiphe graminis*. Transactions of the British Mycological Society 46:225-234.

12. Quinn, J. A., and C. C. Powell, Jr. 1982. Effects of temperature, light, and relative humidity on powdery mildew of begonia *Oidium begoniae* on *Begonia X hiemalis*. Phytopathology 72:480-484.

13. Smith, R. F., K. Subbarao, S. T. Koike, F. Laemmien, and M. Davis. 1999. Several fungicides control powdery mildew in peppers. California Agriculture, 40-43.

14. Ward, S. V., and J. G. Manners. 1974. Environmental effects on the quantity and viability of conidia produced by *Erysiphe graminis*. Transactions of the British Mycological Society 62:119-128.

CHAPTER III.
INFLUENCE OF ENVIRONMENT ON ATMOSPHERIC CONCENTRATIONS OF *PERONOSPORA ANTIRRHINI* CONIDIA IN FIELD GROWN SNAPDRAGONS

ABSTRACT

Atmospheric concentrations of *Peronospora antirrhini* conidia in a commercial snapdragon production field were monitored over three growing seasons to investigate the influences environment has on the concentration of airborne conidia. Hourly concentrations of conidia of *P. antirrhini* were estimated using a Burkhard volumetric spore sampler. The incidence of downy mildew among different cultivars was also of interest. Atmospheric conidial concentrations followed a diurnal pattern and were greatest during 0500 to 1200 hours. Peak conidial concentrations occurred between 0700 and 0900 hours. Minimum daily temperatures below 10.0°C appeared to have a moderate limiting effect on atmospheric conidia concentrations, while temperatures below 6.0°C had more severe limiting effects. Maximum daily temperatures higher than 30.0°C limited concentrations of atmospheric conidia. Long dew periods (≥6 hours) were associated with relatively large conidia releases. On 69 days (1999-2001), the daily total of conidia trapped was >100 and the average length of leaf wetness duration prior to these releases was 11 hours. Consecutive days with short leaf wetness periods suppressed atmospheric conidial concentrations. Snapdragon cultivar had a statistically significant effect on area under the disease progress curve.

INTRODUCTION

Snapdragons (*Antirrhinum majus* L. (Huxley et al., 1992) are grown commercially as a cut flower crop with production in the United States located mainly in Florida and California. Snapdragons are an annual crop established in the field from transplants that are propagated by seed. Flowers are cut by hand one to two times during the growing season with the longer stemmed and therefore more valuable blooms harvested first. Snapdragons have a high value and strict requirements for floret size and stem length, and no tolerance for blemishes.

Downy mildew incited by *Peronospora antirrhini* (Schroet., Hedwigia) infects snapdragon foliage both locally and systemically, causing economically significant damage (16). Local infections produce pale lesions that are visible on the upper leaf surface. Systemic infections result in downward curling of foliage, and shortened internodes. The disease can kill terminal buds of young transplants, causing undesirable branching, which decreases the crop value (16). Fungicides are applied every 1 to 3 weeks throughout the growing season to control this disease.

Depending on climate and weather crop losses caused by downy mildews can vary greatly between years and seasons (11). Leaf wetness duration and temperatures are known to be critical environmental factors for sporulation, germination and infection of downy mildew that affect tobacco (*Peronospora nicotianae* (Speg).) (5,10) , grape (*Plasmopara viticola* (Berk. & Curt)) (6,7), and lettuce (*Bremia lactucae* (Regel)) (12-15).

It has not been determined whether this obligate pathogen survives on weeds and plant debris, or if it is annually introduced into the production area via seedling transplants

(16,18). Research on *P. antirrhini* is limited to symptomology, pathogen morphology, preliminary studies of temperature effects on sporulation, and observations of oospores within plant tissue (8,18). Incident inoculum is an important consideration in control strategies, however, airborne concentrations of conidia of *P. antirrhini* have not, to our knowledge, been studied. Therefore, hourly concentrations of airborne conidia of *P. antirrhini* among snapdragons in a commercial production field were estimated to determined: 1)if conidia are present throughout the growing season and 2)what influences environment has on the concentration of airborne conidia. The incidences of downy mildew among different cultivars was also of interest.

MATERIALS AND METHODS

This study was conducted in 1999 (2 December 1998 to 13 April 1999), 2000 (22 November 1999 to 5 May 2000), and 2001 (14 December 2000 to 18 April 2001) on a 74.1 hectare commercial cut flower farm in Palm Beach county, Florida.. Research sites were established within the snapdragon production areas of the farm. In 1999 and 2001 there was only one research site each year. In 2000, there were two research sites (designated 1 and 2) located in different areas of the farm, located 106.7m apart. Plants were grown on raised (17.8 cm) plant beds (104 cm x 30.5 m) established on 1.5m centers. In 1999 there were 176 beds in the research site. In 2000 there were 176 and 88 beds in sites 1 and 2, respectively. In 2001 there were 124 beds in the research site.

Snapdragon seedlings (grown in 288-cell flats) were planted from 20 Sept. to 30 Nov. 1998, 11 Oct. to 6 Dec 1999 and 9 Oct. to 26 Oct. 2000. All seedlings within a bed were planted on the same date. For the 1999 and 2000 growing seasons seventeen cultivars were grown and included the following: Allure Pink, Allure Red, Attraction

Pink, Attraction Rose, Attraction White, Potomac Red, Potomac Apple Blossom, Potomac Dark Orange, Potomac Early Pink, Potomac Ivory, Potomac Pink, Potomac Red, Potomac Rose, Potomac Royal, Potomac Soft Yellow, Rocket Lemon, and Rocket White. Each plant bed was established with one cultivar and cultivars were arbitrarily arranged within each section.

Plant beds were prepared and maintained according to standard commercial production practices including annual fumigation with methyl bromide, and amendment with a granular application of metalaxyl (Subdue 2E, Novartis, Greensboro, NC) prior to planting. Plantings were maintained with foliar insecticide and fungicide applications. The fungicides copper sulphate pentahydrate (Phyton 27, Source Technology, Edna, MN) fosety-al (Alliette, Rhone-Poulenc, Research Triangle Park, NC) chlorothalonil (Daconil, Zeneca, Inc., Wilmington, DE), azoxystrobin (Heritage, Zeneca Inc.), EBDC (Manzate, Griffen, Valdosta, GA), mancozeb (Dithane, Rohm and Haas, Philadelphia, PA), iprodione (Rhone-Poulenc), fenhexamid (Decree, SePRO Corp., Carmel, IN) were applied during the growing season. Plots were watered frequently (3 to 6 times/week) with overhead irrigation, heads were set 1.32m above the ground. Research sites remained in commercial production throughout the experiment and were harvested regularly.

Temperature, rainfall, relative humidity, and leaf wetness were recorded every 15 min with averages calculated hourly using a Neogen EnviroCaster (Neogen, Mason, MI). A leaf wetness sensor was placed within a plant bed and was set at a 45° angle. The sensor was kept within the plant canopy and raised as the plants grew and the canopy thickened.

Disease was assessed on 5 Jan., 2 and 25 Feb., 18 Mar., 14 April, and 5 May 2000

and on 7 and 22 Mar., 3 and 17 Apr., and 8 May 2001. Ratings of disease incidence were based on estimates of the percentage of infected plants within each plant bed. Each plant bed was divided into ten 3.04m plots. Disease ratings were taken from alternating plots within each bed and were not taken from the end plots. Disease ratings were used to calculate area under the disease progress curves (AUDPC). In 2000 there were 5 (site1) and 4 (site 2) replicates. In 2001, there were 4 replicates. A replicate consisted of four 3.04 m plots within a single 30.5 m bed. Area under the disease progress curve data from sites 1 and 2 in 2000 were pooled and analyzed using a split plot design after meeting the assumptions of Bartlett's test for homogeneity of variances. The significance of cultivar effects in each year were determined through analysis of variance (ANOVA). Cultivar means were compared using Fisher's least significant difference (LSD) test with significance at $P < 0.05$.

Concentrations of airborne conidia were monitored in each field using a 7-day volumetric spore sampler (Burkard Mfg. Co. Ltd., Rickmansworth, Herfordshire, England) placed between the two sections of each site from 2 Dec. 1998 to 13 Apr. 1999, and 22 November 1999 to 5 May 2000, 14 Dec. 2000 to 18 Apr. 2001. The spore samplers were operated at a flow rate of 10 liters/min and the orifice was free to move with changing wind direction. Conidia were impacted onto tapes coated with an adhesive mixture of petroleum jelly and paraffin (9:1, wt/wt) dissolved in sufficient toluene to give a thick, liquid consistency. Tapes were removed weekly, cut into 48-mm lengths, marked at 2-mm intervals with a razor blade to indicate hourly intervals, stained with aniline blue in lactic acid (28 mg of aniline blue, 20 ml of distilled water, 10 mg of glycerol, and 10 ml of 85% lactic acid, diluted with 5 drops to 25 ml of distilled water), and mounted on glass

slides beneath 22-by-50-mm coverslips. Conidia were identified as *P. antirrhini* based on size (23-30 x 14-18μm) and the ellipsoid to ovoid shape using a compound microscope (x200) (1). The number of conidia sampled during each 1-h period were recorded. When conidial concentrations were exceptionally large (>2,000/m^3/h), a portion of the 2-mm interval was counted and multiplied by the appropriate factor to provide an estimate of the concentrations for the 1-h period. Counts were converted to numbers of conidia per cubic meter of air sampled per hour.

RESULTS

Atmospheric conidial concentrations. Over the three years, atmospheric conidial concentrations were greatest during 500 to 1200 hours (Fig. 1). Overall, atmospheric conidial concentrations were much lower in 1999 and 2001 compared with 2000. Within each year peak concentrations occurred at 0700 in 2000 (94 conidia/m^3/hr) and 2001 (7 conidia/m^3/hr), and at 0900 (13 conidia/m^3/hr) in 1999 (Fig. 1). Between 1500 and 0300 hours fewer than 4 conidia/m^3 were trapped each hour, irrespective of the year.

Temperature effects. Minimum daily temperatures (MinDT) below 10.0°C appeared to have a moderate effect of limiting atmospheric conidial concentrations, while temperatures below 6.0° had more severe limiting effects (Tables 1-3). Temperatures were 6.0°C or lower 6, 9, and 22 days in the 1999, 2000, and 2001 growing seasons respectively. In the 1999 growing season when MinDTs below 6.0°C occurred in January (weeks 5-6), the total conidia trapped on these days was <25. Minimum daily temperatures (MinDT) below 6.0°C also occurred in February 1999 (weeks 12 -14), and a decrease in atmospheric conidia occurred in weeks 12 and 13. In 2001, a MinDT

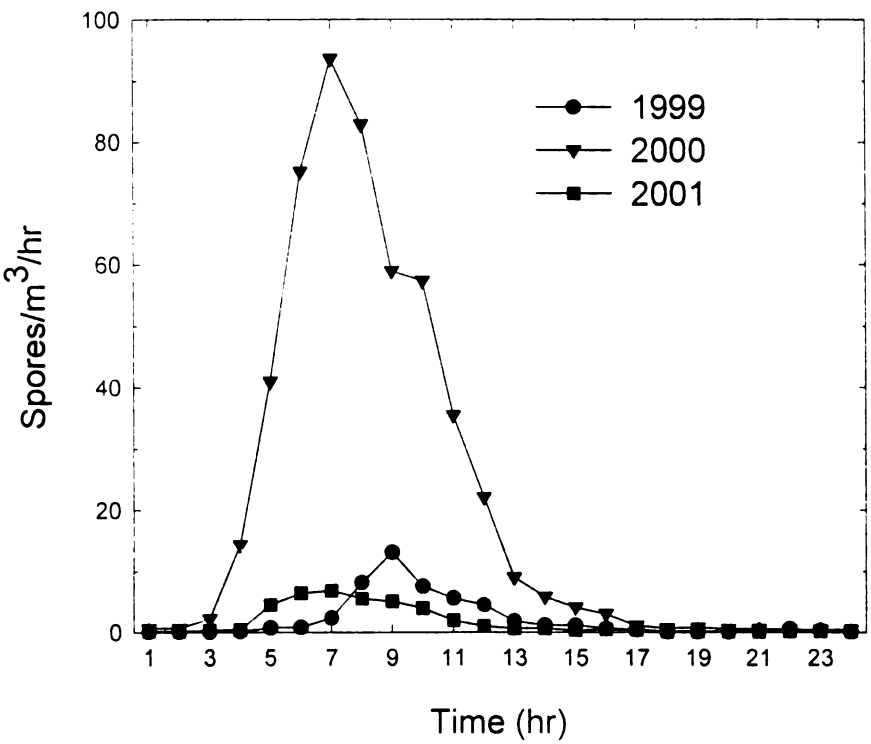


Figure 1. Hourly average concentration of airborne conidia of *Peronospora antirrhini* in research plots from (●) 2 Dec. 1998 to 15 April, 1999, from (▼) 23 Nov., 1999 to 5 May 2000, and from (■) 14 Dec. 2000 to 18 April, 2001.

Table 1. Association of atmospheric conidial concentration of *Peronospora antirrhini* with rainfall, leaf wetness and cold temperatures from 2 December 1998 to 13 April 1999.

Week number	Julian days	Total rainfall	Leaf wetness	Avg. Min temp.	Days <10°C	Days <6°C	Hours <10°C	Hours <6°C	Total spores
1	336-342	9.9	72	13.6	0	0	0	0	0
2	343-349	13.0	69	16.3	0	0	0	0	3
3	350-356	11.2	62	13.1	3	0	28	0	1
4	357-363	38.6	74	16.7	0	0	0	0	244
5	364-05	48.7	45	11.1	4	1	32	9	292
6	06-12	5.3	47	8.7	5	1	37	8	413
7	13-19	10.4	83	14.1	0	0	0	0	53
8	20-26	20.3	88	14.9	0	0	0	0	82
9	27-33	18.3	83	14.2	0	0	0	0	30
10	34-40	11.9	83	14.1	0	0	0	0	284
11	41-47	23.9	74	11.1	4	0	23	0	2280
12	48-54	7.1	64	10.0	3	1	29	2	1502
13	55-61	12.2	77	7.3	5	1	34	1	60
14	62-68	18.3	52	9.9	3	1	21	8	295
15	69-75	19.1	54	11.2	3	1	14	1	188
16	76-82	11.9	80	11.4	1	0	2	0	143
17	83-89	–	–	–	–	–	–	–	–
18	90-96	18.3	46	16.8	0	0	0	0	238
19	97-102	11.4	43	13.9	0	0	0	0	0

Table 2. Association of atmospheric conidial concentration of *Peronospora antirrhini* in Sites 1 and 2 with rainfall, leaf wetness and cold temperatures from 22 November 1999 to 5 May 2000.

Week number	Julian days	Total rainfall	Leaf wetness	Avg. Min temp.	Days <10°C	Days <6°C	Hours <10°C	Hours <6°C	Total spores	Total spores
1	336-342	10.7	81	13.5	1	0	10	0	0	0
2	343-349	12.4	82	16.5	0	0	0	0	0	0
3	350-356	45.5	71	17.7	0	0	0	0	48	0
4	357-363	10.2	64	8.8	4	2	47	7	206	0
5	364-05	13.0	80	12.2	2	0	15	0	131	4
6	06-12	11.0	95	14.4	1	0	5	0	45	26
7	13-19	13.7	82	11.2	1	0	8	0	45	32
8	20-26	34.0	58	7.0	5	3	54	25	10	153
9	27-33	12.2	18	10.5	2	1	30	12	10	47
10	34-40	34.5	27	9.4	3	2	30	4	5	13
11	41-47	13.2	7	9.8	3	1	29	4	21	50
12	48-54	10.9	12	13.6	0	0	0	0	9	60
13	55-61	14.5	57	13.8	0	0	0	0	312	360
14	62-68	14.5	82	13.1	0	0	0	0	2528	4169
15	69-75	50.3	74	13.7	0	0	0	0	6450	4218
16	76-82	42.9	74	18.0	0	0	0	0	24323	7549
17	83-89	38.5	77	14.5	0	0	0	0	56232	3708
18	90-96	31.0	68	17.1	0	0	0	0	2897	646
19	97-102	11.7	37	9.68	1	0	7	0	1045	1003

Table 3. Association of atmospheric conidial concentration of *Peronospora antirrhini* with rainfall, leaf wetness and cold temperatures from 14 December 2000 to 18 April 2001.

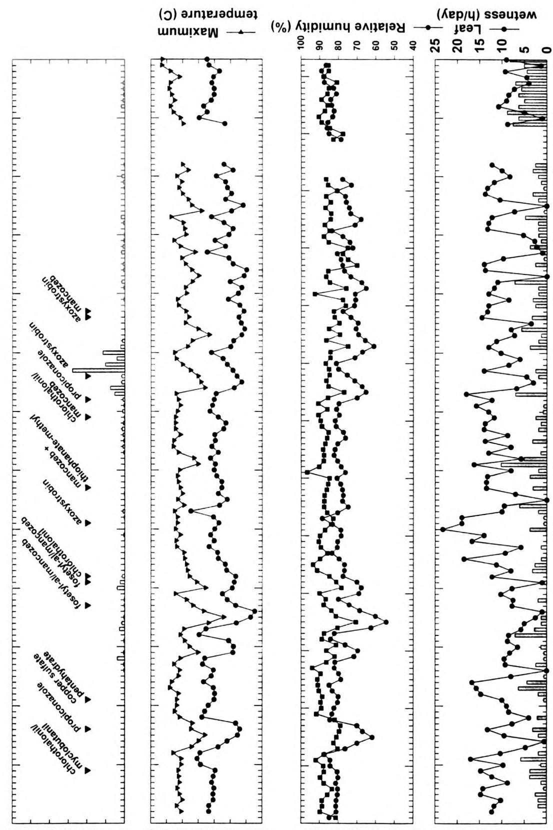
Week number	Julian days	Total rainfall	Leaf wetness	Avg. Min temp.	Days <6°C	Days <6°C	Hours <10°C	Hours <6°C	Total spores
1	336-342	–	–		–	–	–	–	–
2	343-349	–	–		–	–	–	–	–
3	350-356	6.4	118	7.5	4	3	44	22	15
4	357-363	14.2	100	13.0	0	0	0	0	36
5	364-05	16.5	126	.9	7	7	107	71	11
6	06-12	19.8	123	4.4	6	5	69	31	30
7	13-19	8.6	122	11.2	1	0	6	0	73
8	20-26	9.7	80	4.3	6	4	65	32	20
9	27-33	3.6	120	11.2	3	2	34	12	33
10	34-40	54.1	80	14.3	1	0	10	0	12
11	41-47	7.1	162	17.9	0	0	0	0	73
12	48-54	5.1	39	17.2	0	0	0	0	56
13	55-61	14.0	55	18.7	0	0	0	0	389
14	62-68	13.0	52	110	4	1	28	3	119
15	69-75	5.3	82	19.3	0	0	0	0	506
16	76-82	18.5	50	14.7	2	0	4	0	400
17	83-89	77.5	50	15.5	0	0	0	0	119
18	90-96	6.4	93	14.8	0	0	0	0	1188
19	97-102	1.8	93	15.4	0	0	0	0	203

below 10.0°C occurred in each month of the observation period for a total of 39 days, temperatures were as low as -1.7°C in January. With only one exception, atmospheric conidia in the 2001 growing season did not exceed 400 conidia per day, significantly less than daily totals in the 1999 and 2000 growing seasons (Figs 2-4). When temperatures below 6.0°C occurred for 12 consecutive days in January 2001 (weeks 5 - 6), total conidia trapped per day did not exceed 10. Four days of MinDTs below 10.0°C in March 2001 (week 14) were accompanied by daily atmospheric conidia totals of less than 10.

Maximum daily temperatures (MaxDT) higher than 30.0°C limited concentrations of atmospheric conidia. The durations of leaf wetness that occurred on these warm days were adequate (>8 hours) for sporangium development. Temperatures were 30.0°C or higher 4, 5, and 16 days in 1999, 2000, and 2001, respectively. In 1999 high temperatures occurred in the last 4 days of monitoring and no conidia were trapped on these days. Maximum daily temperatures of 30.7 and 30.5°C and average daily temperatures of 23.5 and 25.3°C occurred on March 30 and 31, 2000 (JD 90-91) (week 18). For the six days prior to these high temperatures daily atmospheric conidia totals were greater than 250, conidia totals did not reach this level again for five days, despite moderate rainfall (3.05 - 5.58 mm) and adequate leaf wetness (6 - 13 hours). In 2001, MaxDTs above 30.0°C occurred sporadically throughout the growing season (Feb, March, April) and on the last eight days of monitoring. Atmospheric conidial concentrations were reduced on days immediately following 14 of the 16 days with MaxDT above 30.0°C.

Leaf wetness and rain. Leaf wetness was often detected beginning at 1900 hours, persisted throughout the night, and usually dried by 0700 hours. Long dew periods (≥6

Figure 2. Number of conidia of *Peronospora antirrhini* trapped per day and associated minimum (●) and maximum (▼) and average temperatures (●), relative humidity (■), rainfall and leaf wetness (●) during 14 Dec 1998 to 13 April 1999.

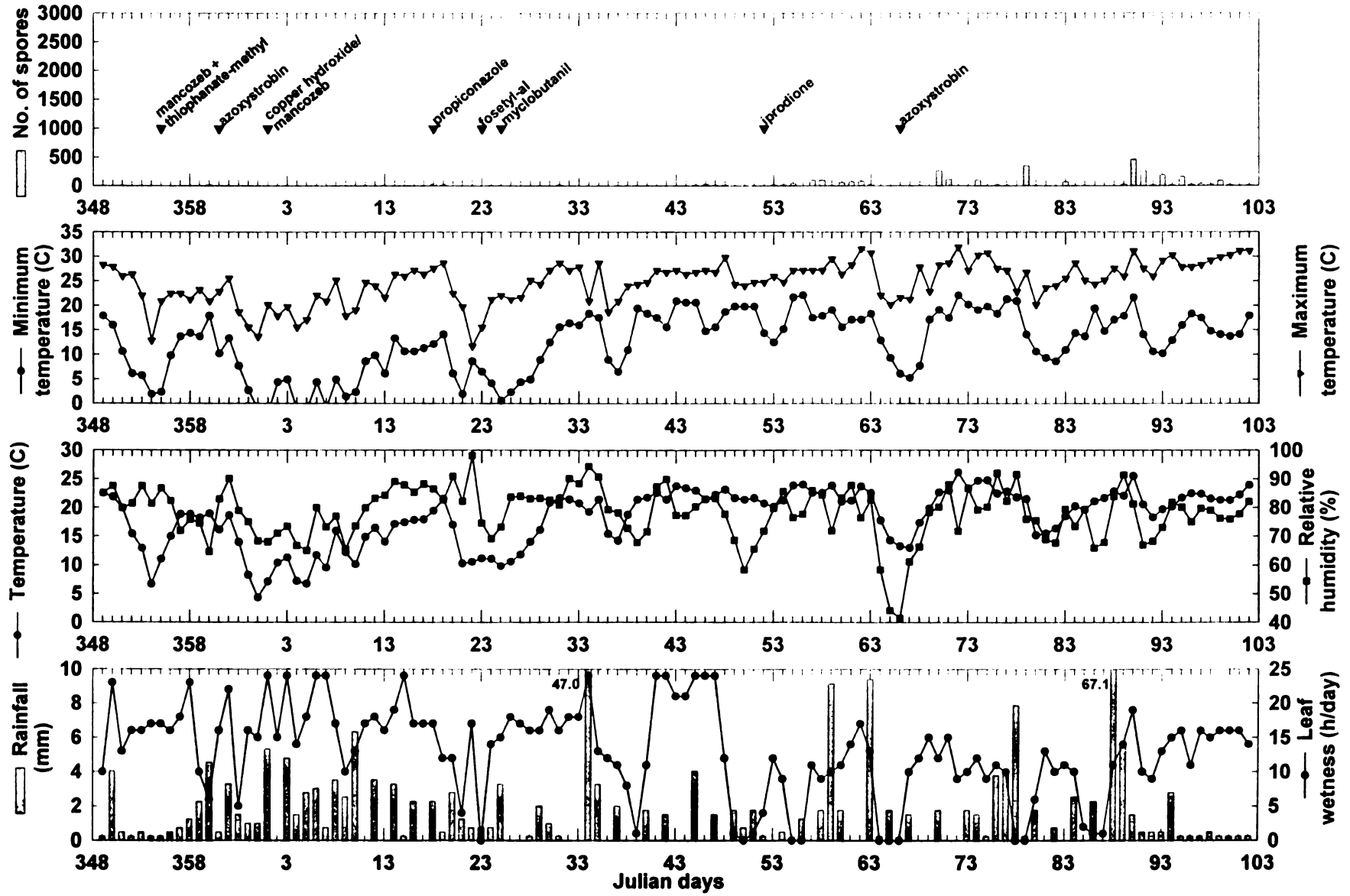


No. of spores
Minimum temperature (C)
Maximum temperature (C)
Temperature (C)
Relative humidity (%)
Rainfall (mm)
Leaf wetness (h/day)
Julian days
chlorothalonil/ myclobutanil
propiconazole
copper sulfate pentahydrate
fosetyl-al/mancozeb
fosetyl-al/mancozeb chlorothalonil
azoxystrobin
mancozeb + thiophanate-methyl
chlorothalonil/ mancozeb
propiconazole
azoxystrobin
azoxystrobin mancozeb

Figure 3. Number of conidia of *Peronospora antirrhini* trapped per day and associated minimum (●) and maximum (▼) and average temperatures (●), relative humidity (■), rainfall and leaf wetness (●) in section1 during14 Dec. 1999 to 13 April 2000.

No. of spores
3000
2500
2000
1500
1000
500
0
azoxystrobin
12729
4721
16543
13107
7757
6278
8080
boscalid
difenoconazole
azoxystrobin
boscalid-difenoconazole
azoxystrobin
Minimum temperature (C)
35
30
25
20
15
10
5
0
Minimum relative humidity (%)
100
90
80
70
60
50
40
Rainfall (mm)
25
20
15
10
5
0
24.1
13.5
27.2
16.5
36.1
16.0
11.4
10.2
Leaf wetness (h/day)
25
20
15
10
5
0
Maximum relative humidity (%)
Maximum temperature (C)
335
345
355
365
10
20
30
40
50
60
70
80
90
100
Julian days

Figure 4. Number of conidia of *Peronospora antirrhini* trapped per day and associated minimum (●) and maximum (▼) and average temperatures (●), relative humidity (■), rainfall and leaf wetness (●) during 14 Dec 2000 to 13 April 2001.


No. of spores
mancozeb + thiophanate-methyl
azoxystrobin
copper hydroxide/ mancozeb
propiconazole
fosetyl-al
myclobutanil
iprodione
azoxystrobin
Minimum temperature (C)
Maximum temperature (C)
Temperature (C)
Relative humidity (%)
Rainfall (mm)
Leaf wetness (h/day)
47.0
67.1
Julian days

hours) were associated with relatively large (>25 conidia/day) conidia releases at the end of the leaf wetness period (Figs. 5-6). Occasionally, conidia releases were observed after a brief (≤4 hours) leaf wetness period. When leaf wetness duration was short (average 2.7 hours) from 26 Jan. to 23 Feb., 2000 (weeks 8 - 12), the daily totals of conidia were low (≤ 42). Atmospheric conidial concentrations were also suppressed immediately following consecutive days (2-3) with short leaf wetness periods. Short, mid-day leaf wetness periods, frequently caused by irrigation, did not impact subsequent conidia releases.

Irrigation, which was applied almost daily or daytime rainfall did not appear to have an affect on atmospheric conidia concentrations. Rainfall occurring between 0200 hours and 0600 hours also did not appear to have an effect on subsequent conidial concentrations

Disease incidence and severity. In 2000 the average disease incidence was less than 10% at the first four disease ratings. Disease incidence was highest at the April 14 (JD 105) rating when the average disease incidence on the most susceptible cultivar, was 44.4%. Disease severity (data not shown) was also highest on this rating. Both the older foliage that was enclosed within the canopy as well as foliage higher on the stalks were infected on the more susceptible cultivars. Sporulation was very heavy on both the abaxial and adaxial sides of the foliage.

Between Julian days 78 and 109, the 4th and 5th disease ratings in the 2000 growing season, the duration of leaf wetness consistently averaged 12 hours each night. These extended leaf wetness periods were also associated with a significant increase in disease incidence that occurred over this same time period. In 2000, the most significant

Figure 5. Association of atmospheric conidial concentration of *Peronospora antirrhini* in snapdragon fields with temperature (▼), relative humidity (●), leaf wetness (■) and rainfall during 12 through 18 February 1999.

No. of spores
500
Temp. (C)
Relative Humidity (%)
Leaf Wetness (min/hr)
Rainfall (mm)

Figure 6. Association of atmospheric conidial concentration of *Peronospora antirrhini* in snapdragon fields with temperature (▼), relative humidity (●), leaf wetness (■) and rainfall in sites 1 and 2 during 7 through 13 March 2000.

Site 1
Site 2
No. of spores
No. of spores
Temp. (C)
Relative Humidity (%)
Leaf Wetness
Rainfall (mm)
648
511
67
68
69
70
71
72
73

increase in disease incidence occurred in the midst of the highest concentrations of conidia during the season. Disease incidence decreased after the fifth rating.

Cultivar susceptibility. The snapdragon cultivar had a statistically significant ($P < 0.0001$) effect on AUDPC in both growing seasons. 'Rocket White' had significantly higher AUDPC values (Table 4) than the other six cultivars grown in both the 2000 and 2001 growing seasons. In both growing seasons 'Potomac Ivory' had the second highest AUDPC values. Cultivars Potomac Rose, Potomac Apple, and Potomac Dark Orange had significantly lower AUDPC values than the remaining cultivars grown in each of the two seasons.

Table 4. AUDPC values of disease ratings of *Peronospora anthirrini* on field grown snapdragon cultivars.

Cultivar	2000	2001
Rocket White	95.5 a	187.75 a
Potomac Ivory	55.93 b	140.16 b
Potomac Royal	50.6 bc	131.03 bc
Potomac Pink	34.4 cd	87.47 cd
Potomac Rose	29.4 d	17.13 e
Potomac Apple	27.5 d	67.66 d
Potomac Dark Orange	27.8 d	52.72 de

DISCUSSION

In this study, concentrations of atmospheric conidia of *P. antirrhini* followed a diurnal cycle. Sporulation of many downy mildews is dependent on cycling of light and darkness; continuous light or darkness prevents the sporulation of *Bremia lactucae*, *P. destructor*, *Plasmopara viticola* and *Pseudoperonospora humuli* (17). The timing of

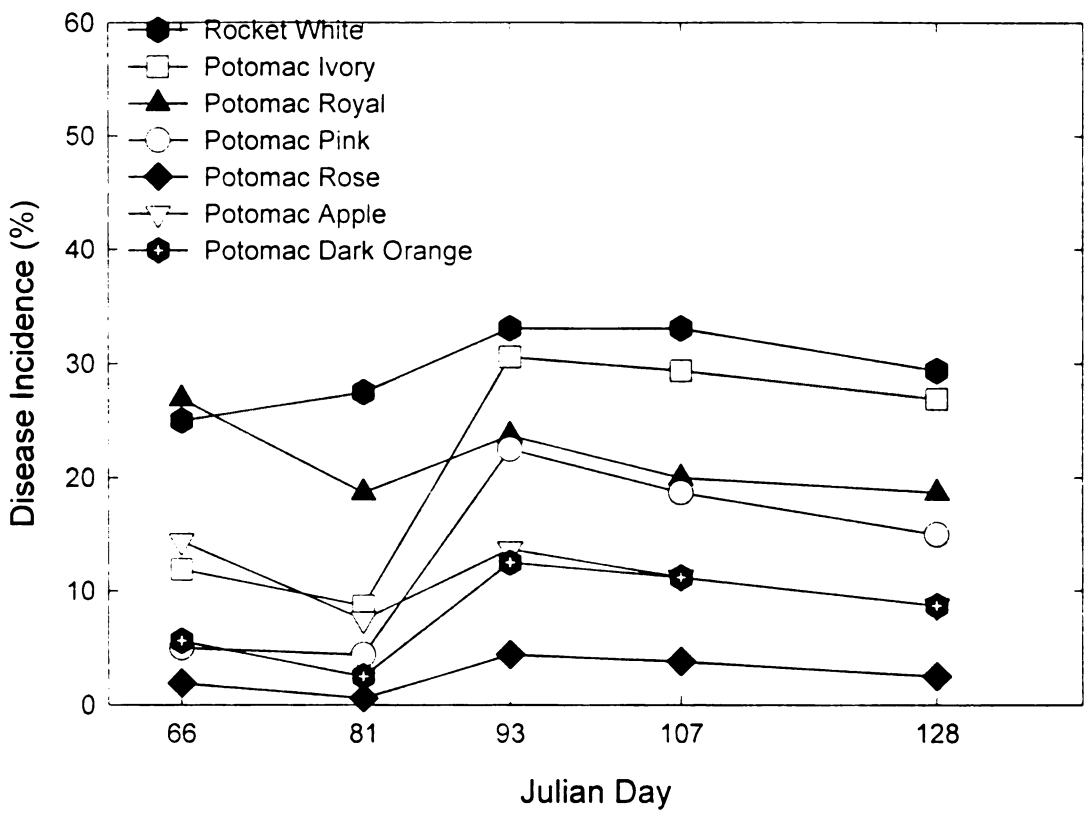


Figure 7. Disease progress curve of *Peronospora antirrhini* for 7 snapdragon cultivars from 7 March 2001 (Julian day 66) to 8 May 2001 (Julian day 128).

the sporulation process (sporangiophore emergence, differentiation, conidial formation, maturation and liberation) including spore liberation varies with species. The sporulation process of *P. antirrhini* appeared to be completed earlier and perhaps more quickly than other downy mildew pathogens. Conidial release in our study began at 0500 h and in 1999 and 2001 peak conidial concentrations occurred at 0700 hours. Field observations of *P. humuli* determined that sporangiophores emerged from stomata by midnight, differentiation was complete and small conidia formed by 0300 h, conidia were full-sized at 0600 h and were mature and being liberated at 0900 h (17).

Several mechanisms may be involved in the liberation of conidia, however the timing of peak daily releases in our study appears to be associated with decreasing relative humidity and increasing temperature. These factors tend to follow diurnal patterns and cannot be evaluated independently in this field study. Hildebrand and Sutton (2) found peak releases of *P. destructor* occurred as relative humidity dropped, leaf wetness evaporated, and wind speeds increased. However, in field studies of *Bremia lactuca* low numbers of spores were released on mornings with prolonged leaf wetness and high relative humidity (13). Red-infrared radiation and solar radiation have been implicated as triggers of spore release under such conditions (11,13).

Maximum daily temperatures above 30.0°C limited atmospheric conidial concentrations. This coincides with Yarwood's (18) earlier observations that sporulation of *P. antirrhini* occurred on systemically infected plants incubated at 13°C and 19°C but not on those incubated at 7°C or 22°C. Additionally, Yarwood observed that inoculations during the winter and early spring months were more successful than inoculations during the hotter summer months (18). The average daily temperatures (20 - 25°C) on days that

MaxDT exceeded 30.0°C were tempered by cooler night temperatures. Sporulation was affected by high day-time temperatures, although sporangiophores are likely not initiated until evening.

In this study, the greatest number of consecutive days with high temperatures occurred at the end of each monitoring period and atmospheric conidial concentrations decreased progressively at these times. The limiting effects of high temperatures were more severe with increasing durations of warm temperatures. The same trend has been reported with *P. tabacina,* which has a higher temperature tolerance (9). Increasing temperature, maturing plant tissue, and less available tissue (due to harvesting) are all thought to be involved in the decrease in disease incidence that occurred at the end of the growing seasons.

Minimum daily temperatures below 6.0 °C occurred periodically in all three growing seasons, temporarily reducing atmospheric conidial concentrations. Average MinDT is an important component in disease prediction of other downy mildew pathogens. An analysis of environmental factors over a 28 year period of hop production determined that MinDTs were generally higher in April and May of years with severe epidemics of *P. humuli,* than in years with mild or no epidemics (3).

Effects of rainfall or irrigation did not have a notable effect on the atmospheric conidial concentrations. Yarwood also concluded that rain did not play an important role in the disease progress of *P. antirrhini* based on observations of more significant epidemics in the greenhouse than on outdoor plantings (18). Rainy weather is unfavorable for the sporulation of *P. destructor;* sporulation failed to occur on 8 of 10 nights that were otherwise considered favorable based on the relative humidity and temperature (2).

The duration of leaf wetness at night affected the number of atmospheric conidia trapped on the subsequent day. Among the three growing seasons there were 69 days where the daily total of conidia was ≥100. The average length of leaf wetness durations on the evenings prior to these large releases was 11 hours. There were several occasions where limited leaf wetness (0-3 h) directly preceded sizable (20-100 conidia/day) releases. However, it was noted that these days followed an extended leaf wetness period (11-16 h) that occurred on the prior day. A dry night in the middle of a series of days with high concentrations of atmospheric conidia resulted in a decreased spore release on the following day. This has also been observed with epidemics caused by *P. tabacina* (11).

When leaf wetness is prolonged and overlaps with conidia release, infection is likely favored. Field studies of *B. lactucae* found nightly leaf wetness durations were consistent predictors of infection days (12). Infection periods occurred mainly on days on which leaf wetness ended late in the morning (0100 h Pacific standard time or later) (14).

Disease incidence differed significantly with cultivar. Although thorough scouting for signs of infection is optimum for prompting fungicide sprays growers can use 'Rocket White' and other especially susceptible cultivars for targeted and more time efficient scouting. Other especially susceptible cultivars are no longer being grown by this grower cooperator based on the high disease incidence ratings obtained in the first year of this cultivar susceptibility study (data not shown). The variability in disease susceptibility complicates development of a disease predictive model that prompts fungicide application and has been a problem with other downy mildew predictive models. For example, in grape production the relationship between temperature, wetness duration, and relative humidity, and the resulting infection and sporulation depends on the grape species or

hybrid (7).

While MinDT, relative humidity and leaf wetness are important factors in downy mildew development, a favorable level of one may compensate for a marginal level in the other (4). This compensatory ability is one of the factors that has prevented the development of accurate and effective disease prediction models for other downy mildew pathogens. Differing climates in various production areas of the world also prevent wide scale adoption of existing disease prediction systems. For example monitoring systems used in European grape production are too conservative for use in the mid-western U.S. (7), and models for hop downy mildew used in Europe are not used in the western U.S. because the environmental factors that restrict disease development differ in the two locations. U.S. cut flower snapdragon production is primarily located in Florida and southern California. It is our intent that information gained from this study will help growers understand the relationship between environment and disease development and encourage the implementation of disease resistant cultivars. These tools may be incorporated into current disease management programs, enhancing their effectiveness.

LITERATURE CITED

1. Francis, S. M. 1981. *Peronospora antirrhini*. In: CMI Descriptions of Pathogenic Fungi and Bacteria No 685.

2. Hildebrand, P. D., and J. C. Sutton. 1982. Weather variables in relation to an epidemic of onion downy mildew *Peronospora destructor*, near Bradford, Ontario. Phytopathology 72:219-224.

3. Johnson, D. A., C.B. Skotland, and J. R. Alldredge. 1983. Weather factors affecting downy mildew epidemics of hops in the Yakima Valley of Washington. Phytopathology 73:490-493.

4. Johnson, D. A., J. R. Alldredge, and J. R. Allen. 1994. Weather and downy mildew epidemics of hop in Washington state. Phytopathology 84:524-527.

5. Kucharek, T. A. 1987. Rainfall, irrigation water, and temperatures associated with first occurrences of tobacco blue mold in leaf production area of north Florida from 1979 to 1984. Plant Disease 71:336-339.

6. Madden, L. V., G. Hughes, and M. A. Ellis. 1995. Spatial heterogeneity of the incidence of grape downy mildew. Phytopathology 85:269-275.

7. Madden, L. V., and M. A. Ellis. 2000. Evaluation of a disease warning system for downy mildew of grapes. Plant Disease 84:549-554.

8. McKay, R. 1949. The production of oospores by the downy mildew of Antirrhinums. The Gardener's Chronicle:28.

9. Moss, M.A., and C.E. Main. 1988. The effect of temperature on sporulation and viability of isolates of *Peronospora tabacina* collected in the United States. Phytopathology 78:110-114.

10. Nesmith, W. C. 1984. The North American blue mold warning system [*Peronospora tabacina*, tobacco, Canada, USA]. Plant disease 68:933-936.

11. Populer, C. 1981. Epidemiology of Downy Mildews. In: The Downy Mildews, edited by D. M. Spencer. London: Academic Press.

12. Scherm, H., and A. H. C. van Bruggen. 1994. Weather variables associated with infection of lettuce by downy mildew (*Bremia lactucae*) in coastal California. Phytopathology 84:860-865.

13. Scherm, H., and A. H. C. van Bruggen . 1995. Concurrent spore release and infection of lettuce by *Bremia lactucae* during mornings with prolonged leaf wetness. Phytopathology 85:552-555.

14. Scherm, H., and A. H. C. Van Bruggen. 1995. Comparative study of microclimate and downy mildew development in subsurface drip- and furrow-irrigated lettuce fields in California. Plant Disease 79:620-625.

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

16. Wheeler, B.E.J. 1981. Downy Mildew of Ornamentals. In: The Downy Mildews, edited by D. M. Spencer. San Francisco: Academic Press.

17. Yarwood, C.E. 1937. Relation of light to the diurnal cycle of sporulation of ceratin downy mildew. Journal of Agricultural Research 54:365-373.

18. Yarwood, C.E. 1947. Snapdragon Downy Mildew. Hilgardia 17:241-249.