EPIDEMIOLOGY AND MANAGEMENT OF COLEUS AND IMPATIENS DOWNY MILDEW; NEW AND EMERGING PATHOGENS IN THE UNITED STATES

By

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ABSTRACT

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Coleus (*Solenostemon scutellarioides*) and impatiens (*Impatiens walleriana*) are popular annuals used extensively in shaded landscape beds in the U.S. Coleus plants exhibiting disease symptoms were observed in New York and Louisiana in 2005 and throughout the country by 2006. This pathogen is an unclassified *Peronospora* species. Downy mildew (DM) on impatiens, caused by the oomycete *Plasmopara obducens*, rapidly defoliates impatiens and epidemics have been observed throughout the U.S. Epidemiological studies on both pathogens were conducted to determine the optimal environmental conditions for infection and sporangial production and release in the greenhouse atmosphere. A volumetric spore sampler was used in a greenhouse containing DM-infected coleus for two extended periods of time and environmental parameters recorded. A rapid reduction in recorded relative humidity prompted the release of high concentration of sporangia into the atmosphere. *P. obducens* sporulation was observed on inoculated impatiens when incubated at 20°C and 25°C while no pathogen sporulation was observed at 30°C. A leaf wetness period of ≥6 hours yielded a greater proportion of inoculated leaves developing *P. obducens* sporangia than a reduced leaf wetness period of 3 hours. Twenty-one coleus cultivars were screened for DM susceptibility. None of the tested cultivars showed complete disease resistance. Reduced-risk fungicides were tested for efficacy against DM in coleus and impatiens. Defining the environmental conditions that favor DM sporulation, and identifying cultivars that are less susceptible to DM along with reduced-risk fungicides with proven efficacy provide an integrated management program.

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INTRODUCTION

Coleus (*Solenostemon scutellariodes* L. (Codd)) is a popular foliage plant grown in gardens, containers, and interiorscapes throughout the United States. In recent years, coleus has become increasing popular with gardeners who are drawn to its vibrant colored foliage and variegation patterns. In 2014, wholesale value of coleus produced in Michigan was over $1 million, while the value for the entire United States was $14 million (USDA, 2014). Impatiens (*Impatiens walleriana* L. (Hooker)) is an annual shade plant of the Balsaminaceae family known for its ability to flower continuously for the entire summer season. The wholesale value of impatiens in the United States in 2014 was $114 million with Michigan producing $14 million (USDA, 2014). Michigan ranks first in the U.S. in bedding plant production with a wholesale value of over $200 million in 2015 (USDA, 2014). At retail greenhouses, coleus and impatiens can be purchased as flats, hanging baskets, or individual potted plants.

SOLENOSTEMON SCUTELLARIODES AND *IMPATIENS WALLERIANA*

Coleus was first classified as *Majana aurea* by George Everhard Rumphius is his work titled *Herbarium amboinensis* published in 1747. The original illustrations were made before 1673, when he lost his sight. In 1687, a fire destroyed most of his work, but still determined, he was able to finish his work with the aid of his son until his death in 1702 (Pedley and Pedley, 1974). In 1826, the botanist Blume classified the plant as *Coleus blumei*, and it was under this name that that it was introduced into Amsterdam in 1851. Throughout the latter half of the 19th century, coleus became a favorite among gardeners and plant breeders alike. One advertisement from the period stated that a single breeder, Mr. Bull, had raised one hundred and fifty new varieties himself (Pedley and Pedley, 1974). The *Gardeners' Chronicle* of May 1 1869 stated,

"Few groups of plants have so rapidly emerged from comparative obscurity into prominence and notoriety as that of the coleus . . ." One auction of twelve plants realized an amazing grand total of £ 390, approximately $50,000 in present U.S. dollars. Also during this time, new varieties were being transported from the South Pacific, which added to the almost endless number of cultivars. By the beginning of the 20th century, the popularity of coleus began to wane. From 1904 to 1934, coleus is mentioned only four times in the index of the *The Gardener's Chronicle* (Pedley and Pedley, 1974). In recent years, coleus has seen a resurgence in popularity with new and heirloom varieties available for sale.

Accounts of impatiens species date back to the 17th and 18th century; George Bowles discovered *Impatiens noli-tangere* in 1632, and Carolus Linnaeus classified seven different species in the mid 1700s (Cumo, 2013). British botanist J.D. Hooker wrote the first monograph on impatiens in 1859. He went on to describe over 120 species in several publications before his death in 1911. The *walleriana* species was named in tribute to Horace Waller - an English botanist who collected impatiens on an expedition to Zanzibar, Africa during the late 19th century (Cumo, 2013). In the 1950s, a breeder from the PanAmerican Seed Company, Claude Hope, began improving impatiens, and they quickly became popular with several varieties available by the mid-1960s (Klingaman, 2002). Over 1,000 species of impatiens have been identified, the most popular being *Impatiens walleriana*.

CULTIVATION TECHNIQUES

Coleus are tropical to subtropical plants, preferring temperatures ranging between 16°C and 24°C (Pedley and Pedley, 1974). Temperatures higher than 24°C will not negatively affect growth, although temperatures lower than 10°C results in decreased vigor. Coleus can survive in

most sunlight conditions; however, foliage becomes more vibrant when shaded. Coleus prefers moist to very moist soil conditions. To achieve larger, fuller plants, the growing tips are removed after either the fourth or fifth pair of leaves (Moore, 2015). This pinching also delays the production of the undesirable flower spikes. Leaf abscission occurs readily with coleus and the plant is often studied to determine the effect of auxins on plant development, which includes leaf abscission. When the coleus shoot terminates into a flower spike the further development of leaves is limited (Pedley and Pedley, 1974). Coleus can be propagated using seeds or cuttings.

Native to tropical, subtropical, and temperate regions of Africa and Asia, impatiens are found at elevations between 1,000 and 3,000 m (Morgan, 2007). Although considered an herbaceous perennial, they are treated as an annual in regions that encounter frost (Kindersley and Brickell, 2008). Most species are found in shady, damp environments among ferns and mosses, near rivers or streams. The majority of impatiens species do not fare well in temperatures above 25°C, or in constant sunlight (Morgan, 2007). Leaves are elliptic to ovate (5.5-8.8 x 2.5-3 cm) with petioles 0.1-0.2 cm long, with 4-cm wide flowers (Hyland et al., 2010). All commercially available single-flowered cultivars are F1 hybrids and are cultivated from seed while double impatiens can be cultivated from seed or cuttings (Kessler, 2005). Impatiens seeds germinate best with a moist, light medium. For best results, pH of the medium should be kept between 5.5 and 6.0, and temperature between 21°C and 24°C (Kaczperski and Carlson, 1989). The genus name "*Impatiens*" derives from the explosiveness of the plant's seed capsule. Once ripe, the seed is forcefully ejected from the capsule upon touch. The organism's sensitivity to touch has earned it the name "touch-me-not." (Morgan, 2007). Impatiens plugs are transplanted into cell packs or pots which are sold to the public and are generally compact, self-branching and produce flowers continuously during the summer months.

DISEASES OF COLEUS AND IMPATIENS

Before the discovery of downy mildew, viruses were the most common pathogens associated with coleus. In 1945, a report described a disease-causing mosaic symptoms on coleus (Creager, 1945). Symptoms of this disease included oak-leaf patterns and ring spots and was observed on over 40 different cultivars. In 1989, plants showing these identical symptoms were identified as being infected by a strain of cucumber mosaic virus (Holcomb and Valverde, 1991). Although viroids have been detected in both the foliage and seed of coleus, symptoms have not been observed on infected plants. Coleus are not susceptible to many of the fungal pathogens associated with greenhouse bedding plants with Pirone (1978) listing root pathogens *Pythium* spp. and *Rhizoctonia solani* and foliar pathogens *Botrytis cinerea, Alternaria* sp., and *Phyllosticta* sp. Overall, very little research has been conducted concerning fungal pathogens of coleus, possibly due to the low susceptibility of the plant to pathogens often associated with more popular bedding annuals.

Aside from downy mildew, the *Tospovirus* impatiens necrotic spot virus (INSV), which is formally called tomato spotted wilt virus-Impatiens strain (TSWV-1), is the most serious pathogen affecting impatiens throughout the world (Windham et al., 2015). The virus, which was first detected on *Impatiens walleriana* in the United States in 1989, now has host range of several hundred-plant species (Law and Moyer, 1990). Vectored by the western flower thrip (*Frankliniella occidentalis*), once a plant is infected with INSV, no suitable treatment is available and it is recommended that the plant is destroyed. Symptoms of INSV include stunting, ringspots, and flower breaking (Windham et al., 2015). Alternaria leaf spot (*Alternaria* sp.) is a common foliar pathogen of impatiens, however, effective fungicides can be used to manage the pathogen in the greenhouse (Moorman, 2017). *Pseudomonas syringae*, a bacterial

pathogen of *I. wallerana*, can cause water-soaked and necrotic lesions on leaves (Cooksey and Koike, 1990).

DOWNY MILDEW

The downy mildews are obligate pathogens that are economically significant on several important crops grown throughout the world including tobacco, grapes, hops, sugar beets, sunflowers, soybeans, and brassicas (Viennot-Bourgin, 1981). Once grouped with fungi, oomycetes are now classified in the kingdom chromista (Van de Peer, 1997); this classification is a result of oomycetes phylogenetic relation to stamenpillous protists that produce hairy, motile spores (Hardham, 2006). Recent research has expanded this classification to the Chromalveolate "superkingdom" clade which includes algal and protist organisms (Beakes et al., 2012). All downy mildews are classified in the group Peronosporaceae, which include *Bremiella, Plasmopara, Pseudoperonospora* and *Peronospora* spp. While *Bremiella* and *Plasmopara* spp. produce sporangia containing zoospores, *Pseudoperonospora* and *Peronospora* spp. reproduce asexually and produce sporangia which germinate by germ tubes (Shaw, 1981). Sporangiophores, produced during darkness, are projected through the stomatal opening. These sporangiophores bear primary and secondary branches that narrow at the point and bear single terminal sporangia. Sporulation occurs on the adaxial leaf surface; however, under favorable environmental conditions other *Peronospora* spp. can sporulate on the abaxial leaf surface. Pinckard (1942) observed that sporangiophores of several *Peronospora* spp. reacted to dry air by twisting counter-clockwise and the spores were ejected by the spring-like action of the entangled sporangiophores. The downy mildews are obligate pathogens that are economically significant on several important crops grown throughout the world including tobacco, grapes, hops, sugar

beets, sunflowers, soybeans, and brassicas (Viennot-Bourgin, 1981). Multiple races of both *Peronospora* spp. and *Plasmopara* spp. have been isolated from infected spinach and grapevines, however, at this time, no research has been conducted for the species that infect coleus and impatiens (Rivera et al., 2016; Molinero-Ruiz et al., 2002). Downy mildew pathogens usually have restricted host ranges, however, recent discoveries of *Plasmopara halstedii* races that can infect multiple hosts have been observed in several countries (Rivera et al., 2016). Although little is currently understood about the overwintering capability of *Plasmopara obducens*, oospores have been observed on infected stems and leaves. Oospores produced by other *Plasmopara* species, such as *P. viticola,* are considered the primary inoculum for future outbreaks (Carisse, 2016). *Plasmopara obducens* oospores have been observed in impatiens stems and leaf tissue, however, little research has been conducted on the ability of the pathogen to overwinter in colder climates (Palmateer and Lopez, 2013).

DOWNY MILDEW ON COLEUS AND IMPATIENS

Downy mildew on coleus was first reported in the United States by Daughtrey et al. (2006). These first samples were submitted to diagnostic labs by commercial growers after observing stunting, leaf distortion, and leaf abscission. Downy mildew-like growth was observed on infected leaves. Koch's postulates were completed successfully. Since its initial discovery in 2005, coleus downy mildew been reported throughout the United States (Rivera, 2016; Pundt, 2017). In 2006 Thines et al. (2006) classified the downy mildew on coleus as *Peronospora belbahrii*, the same species a affecting basil, however some disagreement remains among the scientific community on this classification. Currently, the *Peronospora* sp. that infects coleus remains unclassified.

Two downy mildews have been observed on *Impatiens walleriana*; *Plasmopara obducens* L (Schröt), the causal agent of the recent outbreaks, and the far less common *Bremiella sphaerosperma* L. (Savulescu) (Constantinescu, 1991). *P. obducens* was initially described on wild and cultivated impatiens in 1974 by Sohi and Tyagi (1974). In 2003, downy mildew was observed on impatiens in the United Kingdom with greater than 80% losses of impatiens plantings reported (Lane, 2004). In 2004, *Impatiens walleriana* 'Sparkler Hot Pink' and 'Fiesta' showing symptoms of downy mildew were observed in a commercial greenhouse in California (Wegulo, 2004). Incidence was nearly 100% with white growths observed on the underside of the leaves, meaning that sporangiophores had emerged from the stomata. Sporangiophore branching was monopodial, and sporangia were ovoid and hyaline. Based on the symptoms and morphology of the observed organisms, researchers concluded the pathogen to be *Plasmopara obducens*. Between 2005 and 2010, there were no published reports of impatiens downy mildew in the United States. In 2011, reports of impatiens defoliating in the landscape from downy mildew infection were reported across multiple states, including California, Illinois, New York, Minnesota, and Wisconsin (Anonymous, 2012). The following year, downy mildew outbreaks continued with notable losses in the landscape observed in Florida and Michigan (Palmateer and Lopez, 2013; Hausbeck, 2013). Reports of the disease continued through 2014 with infected plants observed throughout the United States including the Hawaiian Islands (Crouch et al., 2014). As homeowners and landscapers continued to observe their impatiens plantings defoliated from downy mildew the wholesale value of impatiens dropped from over $133 million in 2009 to $91 million in 2014 (USDA, 2014).

INFLUENCE OF ENVIROMENTAL FACTORS ON DOWNY MILDEW

Disease epidemics are a result of a release of wind-dispersed sporangia (Ronzon-Tran, 1988). The effect of temperature on sporangial dispersion has not been studied on *Plasmopara obducens* or on the *Peronospora* sp. that infects coleus. However, research conducted on other species gives some insight on the relation of temperature, relative humidity, and leaf wetness on infection and sporulation. In work completed by Byrne et al. (2005) with the species *Peronospora antirrhini* on snapdragon, temperature did not have a significant correlation with sporangial release while leaf wetness and rainfall were correlated to the release of sporangia. Decreased sporulation of *Peronospora destructor* was observed with relative humidity below 100% and temperatures above 22°C (Yarwood, 1943; Palti, 1988). Temperature gradients for other *Peronospora* spp. have shown a range of optimum temperatures depending on the species. The minimum temperatures range from 2°C to 7°C while maximum temperatures are between 22°C and 27°C for *Peronospora* (Yarwood, 1943).

The frequency of *Plasmopara* sporulation on grape has been correlated with >3 hours of leaf wetness and relative humidity >80% (Caffi, et al., 2013). Increased temperatures >42.8°C have permanently reduced the pathogen's ability to sporulate from existing lesions, even after a return to weather conducive to sporulation (Kennelly, 2007). In a study conducted by Williams et al. (2007), the influence of temperature was more highly correlated to colonization compared to relative humidity. At temperatures below 10°C infection was greatly reduced, while the most severe infection was observed on inoculated grapes at 20°C. At temperatures of >30°C, no infection from *Plasmopara* was observed.

DOWNY MILDEW MANAGEMENT

All of the currently published research for the control of *Peronospora* sp. on coleus has concentrated on greenhouse production. Greenhouse studies on coleus conducted at Michigan State University have shown that preventive foliar applications of registered fungicides dimethomorph + ametoctradin, dimethomorph, azoxystrobin, fluopicolide, or a single drench application of mefenoxam can be very effective in limiting infection of coleus downy mildew (Harlan and Hausbeck, 2011; Warfield et al., 2007). Research conducted by Ivors et al. (2010) on coleus showed that the non-registered biopesticide, extract of *Reynoutria sachalinensis*, was moderately effective and limited *Peronospora* sporulation by 90% compared to the untreated inoculated control. Very little research has been conducted looking at efficacy of fungicides against *Peronospora* spp. on ornamentals in the landscape. Rose downy mildew, *Peronospora sparsa,* was successfully controlled at a commercial nursery with spray applications of registered fungicides azoxystrobin, dimethomorph, mefenoxam, chlorothalonil, and mancozeb (Gevens et al., 2006). Although no resistance of *Peronospora* sp. on coleus has been reported, in other cropping systems, resistance is not uncommon and the availability of multiple effective products is necessary as frequent rotation between chemical class and mode of action is necessary to prevent resistance over time (Wong and Wilcox, 2000).

Due to the severity of the symptoms observed by homeowners and landscapers the management of impatiens downy mildew focuses on residual control (Suarez et al., 2015). The use of systemic fungicides, such as mefenoxam, fluopicolide, and oxathiapiprolin are considered highly effective against downy mildews on important crops worldwide (Kennelly et al., 2007; Cohen, 2015). A 2012 study from the University of Florida revealed that impatiens incorporated with granular mefenoxam remained healthy for sixty days. Later experiments identified

fluopicolide, various phosphonates, and oxathiapiprolin as promising for long residual control on impatiens (>30 days) (Suarez et al., 2015). A study conducted by Harlan and Hausbeck (2014) noted that greenhouse drench applications of systemic fungicides, such as mefenoxam and fluopicolide, greatly reduced *P. obducens* incidence and severity on impatiens in the landscape for six weeks post application and transplanting. Phosphonate products, which are considered reduced-risk by the U.S. Environmental Protection Agency, have been noted to be effective at eradicating downy mildew symptoms after infection (Suarez, et al. 2015). Oxathiapiprolin, a newly registered fungicide that was shown to be highly efficacious in other cropping systems, was tested against *P. obducens* by Saurez et al. (2016) and provided excellent control when used alone and in weekly rotations. The mechanism of *P. obducens* resistance to the phenylamides, the class of fungicides that includes mefenoxam, has not been studied. Resistance of downy mildew to the phenylamides can occur quickly, with published reports of *Pseudoperonospora cubensis* resistance to mefenoxam just two years after its registration on cucumber (Reuveni et al. 1980). Due to reports of *P. obducens* resistance to mefenoxam in Europe, Warfield (2012) tested multiple isolates from the Netherlands and from various states with all of the isolates still being completely sensitive to the fungicide. However, two years later in 2014, Warfield (2014) repeated these studies with two newly collected isolates and observed complete resistance of both isolates to fluopicolide and moderate resistance of both isolates to mefenoxam. The testing of newly registered and experimental fungicides will be an important aspect of impatiens continuing to be a popular plant among homeowners.

CHAPTER 1. EPIDEMIOLOGY AND MANAGEMENT OF DOWNY MILDEW, A NEW PATHOGEN OF COLEUS IN THE UNITED STATES

ABSTRACT

Coleus (*Solenostemon scutellarioides*), a herbaceous bedding plant, has been prized by gardeners for its bright colorful foliage since Victorian times, and in recent years has seen a resurgence in popularity in the United States. Coleus plants exhibiting disease symptoms were observed in New York and Louisiana in 2005 and throughout much of the United States by 2006. This pathogen was determined to be *Peronospora* sp. Epidemiological studies were conducted at Michigan State University to determine the optimal environmental conditions for sporangial release in the greenhouse. Additionally, host resistance and reduced-risk fungicides were investigated as management tools. Concentrations of airborne sporangia were monitored by placing a 7-day volumetric spore sampler in a greenhouse with coleus infected with *Peronospora* sp. Coincident hourly leaf wetness, temperature, and relative humidity data were collected. Two years of spore trapping in a greenhouse with downy mildew-infected coleus showed high relative humidity (>95%) followed by lower relative humidity prompted the release of especially high numbers of sporangia into the atmosphere. Two experiments screening coleus for susceptibility to downy mildew included 28 cultivars which were rated for percentage of leaves sporulating and density of pathogen sporulation. 'Volcano' and 'Black Dragon' consistently showed susceptibility to downy mildew. None of the cultivars included in the study showed disease resistance. 'Freckles' had limited downy mildew symptoms and pathogen sporulation. Reduced-risk fungicides that controlled downy mildew on inoculated plants when compared with untreated inoculated plants included fenamidone, mandipropamid, and azoxystrobin. Selected, new and experimental fungicides that are not yet labeled were also effective. The results of an experimental approach that defines the environmental parameters that favor downy mildew sporulation,

identifies coleus cultivars that offer some disease resistance, and tests reduced-risk fungicides will offer growers an effective management program.

INTRODUCTION

Coleus (*Solenostemon scutellarioides* L. (Codd)) is a popular foliage plant grown in gardens, containers, and interiorscapes throughout the United States and Europe. Downy mildew on coleus was first reported in the United States in 2005 (Daughtrey, 2006) and the pathogen was initially identified as a *Peronospora* sp. based on morphological characteristics. Plant stunting, leaf distortion, and leaf abscission are commonly-occurring symptoms (Fig. 1). Downy mildew-like growth may be observed on the undersides of infected leaves.

Figure 1. *Peronospora* sp. infection on 'Color Pride' coleus; symptoms include leaf abscission and necrotic lesions.

Historically, coleus has not been considered to be susceptible to the pathogens commonly associated with bedding plants and foliar fungicide applications have not been necessary (Pirone,

1978). When downy mildew has occurred on greenhouse ornamentals or vegetable crops in the field, growers in the United States have relied on older fungicides such as mancozeb to protect against downy mildew. However, mancozeb is classified as a B2 carcinogen by the United States Environmental Protection Agency and additional fungicides/management strategies that are active against oomycetes are needed. Downy mildew of rose, incited by *Peronospora sparsa*, may be managed with fungicides applied as foliar sprays or soil drenches (Gevens, 2006). In recent years, fungicides classified as reduced-risk by the EPA have been introduced and may be active against downy mildew. An alternative to fungicide applications is identification of coleus germplasm resistant to downy mildew. Although there are hundreds of coleus varieties, certain types are especially popular within the United States. A comparative downy mildew assessment of widely-grown varieties, such as 'Black Dragon' and 'Wizard Rose,' to other varieties could help growers reduce the amount of fungicides needed to produce a healthy, salable plant. Information regarding the epidemiology of this coleus pathogen is lacking. Growers could benefit from knowing the environmental conditions that influence atmospheric concentrations of sporangia and drive disease development. Research conducted on *Peronospora antirrhini,* the pathogen that incites downy mildew on snapdragon, indicated that temperature was not significantly correlated with sporangial release (Byrne, 2005). However, leaf wetness and rainfall were correlated to increased concentrations of sporangia in the atmosphere. In other *Peronospora* spp. studied, relative humidity was considered to be a critical factor. On onion, decreased sporulation of *Peronospora destructor* was observed when relative humidity was less than 100% (Yarwood, 1943).

The goal of our research is to develop an integrated strategy for the management of downy mildew on coleus through the following objectives: i.) identify effective reduced-risk fungicides,

ii.) identify tolerant or resistant coleus germplasm, and iii.) identify the environmental conditions associated with an increased concentration of airborne sporangia.

MATERIALS AND METHODS

Concentrations of airborne sporangia were monitored in a greenhouse using a 7-day volumetric spore sampler (Burkard Mfg. Co. Ltd., Rickmansworth, Herfordshire, England) placed between two greenhouse benches in 2006 and 2007. The spore sampler was positioned so that the orifice was at the height of the crop canopy (20 mm from bench top). Infected coleus plants were purchased from local commercial greenhouses and transplanted into 10.2-cm plastic pots containing Baccto Professional Potting Mix (Michigan Peat Company, Houston, TX, USA) and placed onto benches located on two sides of the spore sampler. The spore sampler was operated at a flow rate of 10 liters/min and the orifice was positioned against the prevailing air movement in the greenhouse as determined by the exhaust fans. Sporangia 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 (28 mg of aniline blue, 20 ml of distilled water, 0.01 ml glycerol, and 10 ml of 85% lactic acid, diluted with five drops to 25 ml of distilled water), and mounted on glass slides beneath 22 x 50-mm coverslips. Under a compound microscope (x100), sporangia of a *Peronospora* sp. were observed. The numbers of sporangia sampled during each 1-hour period were recorded. Counts were converted to numbers of sporangia per cubic meter of air sampled per hour. PROC ARIMA (SAS Institute Inc., Cary, NC, USA) was used to determine any positive or negative correlations among atmospheric sporangial concentration, temperature and humidity for each growing season.

A 288-cell flat of coleus 'Volcano' seedlings was purchased from a commercial greenhouse and transplanted into 10.2-cm plastic pots containing Baccto Professional Potting Mix. Plants were fertilized weekly with 200 ppm of Peter's water-soluble fertilizer (The Scotts Company, Marysville, OH, USA). Several fungicides classified as 'reduced-risk' by the United States EPA were included in this experiment. Fungicides were applied either as a drench or as a spray using a hand-pumped compressed air sprayer. Six single-plant replicates per variety were arranged in a completely randomized design on a greenhouse bench under 73% woven shade fabric. Greenhouse temperatures ranged from a low of 19°C at night to a high of 26°C during the day. A sporangial suspension was prepared by placing leaf tissue with the sporulating pathogen into distilled water and agitating to release spores. This sporangial suspension was diluted to a concentration of 4.4×10^{6} sporangia/ml. The sporangial suspension was sprayed onto the plants using a janitorial spray bottle, after which the plants were immediately enclosed in translucent plastic bags (20 x 10 x 46 cm) for 7 days. The plants were removed from the bags for 7 days and then placed into baskets covered in translucent plastic for increased humidity. After 7 days in the covered baskets, the number of leaves on each plant and the number of leaves with visible pathogen sporulation were counted, and the percentage of the infected leaf tissue with the sporulating pathogen (per plant) was estimated.

Coleus varieties were screened for resistance to the downy mildew pathogen. In Trial I, heirloom cultivars 'Beauty,' 'Beckwith's Gem,' 'Cristata,' 'Duke Yellow,' 'Etna,' 'Freckles,' 'Glory of Luxemborg,' 'Harlequin,' 'Pegasus,' 'Pineapple Beauty,' 'Russet,' 'Tapestry,' 'White Gem,' and 'W-1977' were received as rooted cuttings from the USDA North Central Regional Plant Introduction Station. The standard variety used to determine relative susceptibility was 'Black Dragon.' All plants used in the screening were observed to be free of downy mildew signs

prior to inoculation and were transplanted into 10.2-cm pots. 'Black Dragon' seedlings sown into a 288-cell flat were transplanted into 10.2-cm pots.

In Trial II, seeded flats of commercially popular coleus varieties (Table 2) in 288-cell flats were transplanted into 10.2-cm pots. For both experiments, plants were fertilized weekly with 200 ppm of water soluble fertilizer. Six single-plant replicates per variety were arranged in a completely randomized design on a greenhouse bench under 73% woven shade fabric. Greenhouse temperatures ranged from a low of 19°C at night to a high of 26°C during the day. Plants were inoculated and pathogen sporulation induced as described for the fungicide efficacy experiment. The total number of leaves (healthy + diseased) and the number of leaves with the sporulating pathogen were counted on each plant and percentage of leaves with the sporulating pathogen (per plant) was determined. Data for fungicide efficacy and germplasm trials were analyzed using SAS PROC GLM and statistical differences were compared using the Fisher's Protected Least Significant Differences test (P=0.05).

RESULTS AND DISCUSSION

High concentrations of sporangia were detected in the atmosphere of a greenhouse containing coleus infected with the downy mildew pathogen. Like other *Peronospora* spp., sporangial release events followed a daily periodicity (Pinkard, 1942). Sporangial counts were highest between 1000 and 1500 hr. High relative humidity (>95%) levels followed by a decrease preceded a sporangial release event (Fig. 2). The largest sporangial concentrations occurred following periods of extended high relative humidity conditions. The highest sporangial concentrations occurred in May and June with concentrations decreasing by August. Limiting the relative humidity in the greenhouse could limit downy mildew development. We hope to expand

our epidemiology studies so that the environmental conditions can be used to forecast downy mildew development. A forecasting tool could be used to alert growers if and when a fungicide treatment is needed. In the absence of a disease forecasting system and/or resistant cultivars, it may be prudent to apply a fungicide in a preventive manner.

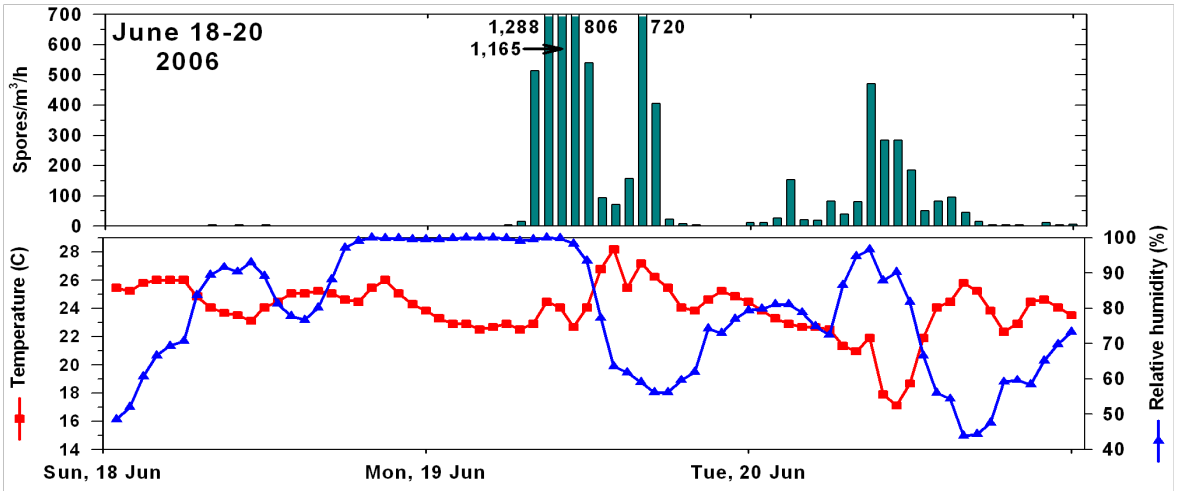

Figure 2. Spore trapping results in a greenhouse with downy mildew infected coleus.

Disease pressure was significant in the fungicide trial and the inoculated control plants averaged 10.3 symptomatic leaves per plant (Fig. 3). Although all treatments limited disease symptoms compared to the control, differences were observed among the products tested. Dimethomorph (alone or in combination with ametoctradin), fenamidone, and mefenoxam were the only treatments that completely prevented infection. Reduced-risk fungicides fenamidone, mandipropamid, and azoxystrobin were very effective and limited infection significantly compared to the untreated control. Although the biological fungicide extract of *Reynoutria sachalinensis* (both rates) limited infection compared to the untreated control, the number of diseased leaves would be unacceptable to growers. Phytotoxicity was not observed on any of the treated plants. Many of the new products developed for oomycetes in recent years are targeted for

root rot pathogens, *Phytophthora* and *Pythium*, and their ability to control downy mildew, a foliar blight, has not been widely studied. Extract of *Reynoutria sachalinensis*, the only biopesticide included in the trial, did have some efficacy against downy mildew, and a rate response trend was observed between the 189 ml and 378 ml/37.8 L rate. A future study to determine consistency of product performance would be helpful in providing robust recommendations to growers. Since all treatments were applied prior to inoculation, necrotic lesions and pathogen sporulation were prevented. Plants displaying downy mildew disease symptoms prior to fungicide treatment will likely remain unmarketable.

Prior to the occurrence of this new downy mildew pathogen, coleus varieties were selected based on consumer preference, and ease of propagation. Today, the difference in susceptibility to downy mildew disease among coleus varieties may play a role as to which ones can be offered commercially. If a grower chooses a variety that is downy mildew susceptible, additional inputs including disease scouting, environmental monitoring, and fungicide applications may be necessary. In Cultivar Trial I, although no variety was completely resistant to the downy mildew pathogen, significant differences were observed in the percentage of leaves with the sporulating pathogen between the cultivars tested (Table 1). The cultivars received from the North Central Regional Plant Introduction Station showed a higher level of resistance compared to the commercial standard included in the experiment. 'Freckles' was the least susceptible variety with just two of the six plants in the trial each having one leaf with the sporulating pathogen. Cultivar Trial II included many of the more commonly grown commercial varieties (Table 2). 'Black Dragon,' 'Wizard Color Sunrise,' and 'Color Pride' (Fig. 1) were most susceptible. 'Wizard Pineapple' had the fewest leaves sporulating at 2.9%. Results from variety trials provide insight into the wide range of susceptibility that propagators, growers, and breeders may encounter. The

heirloom varieties obtained from the USDA were less susceptible to the downy mildew pathogen compared to the commercial standard variety included in the trial. This information may assist breeders in the development of more tolerant germplasm. A shift to the more resistant cultivars on a commercial level could reduce the threat from this pathogen.

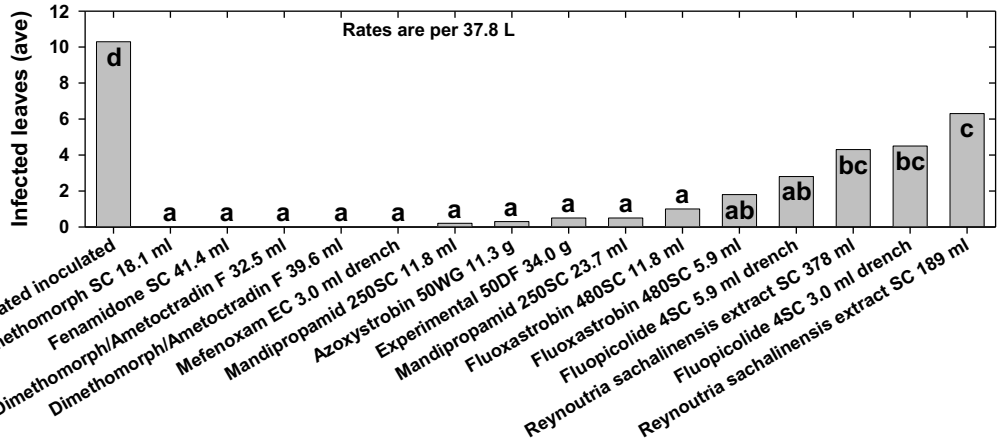

Figure 3. Greenhouse experiment testing fungicides for the control of downy mildew on coleus 'Volcano.'

Table 1. Susceptibility to downy mildew of coleus cultivars received from the USDA North Central Regional Plant Introduction Station.

Cultivar	Leaves with sporulating *Peronospora* (%)	Leaf area with *Peronospora* sporulation (%)[z]
Freckles	0.6 a[y]	33.3 ab
Beauty	1.3 a	20.0 a
W-1977	1.4 a	45.0 ab
Russet	2.0 a	33.3 ab
Harlequin	2.3 a	18.5 a
Pegasus	4.4 a	55.0 ab
Tapestry	5.6 a	58.3 ab
Etna	5.9 a	40.0 ab
Pineapple Beauty	6.1 a	40.0 ab
Glory of Luxemborg	7.0 a	46.7 ab
Beckwith's Gem	9.6 a	61.7 ab
Duke Yellow	29.8 b	79.2 ab
White Gem	41.2 c	75.0 ab
Cristata	41.8 c	80.0 ab
Black Dragon	70.3 d	91.7 b

[z]Based on a visual estimation of percentage of diseased leaves with sporulation.
[y]Column means with a letter in common are not significantly different (Student-Newman-Keuls; P=0.05).

Table 2. Susceptibility of commercially available coleus cultivars to downy mildew.

Cultivar	Leaves with sporulating *Peronospora* (%)		Leaf area with *Peronospora* sporulation (%)[z]	
Wizard Pineapple	2.9	a	9.2	a
Wizard Scarlet	5.5	ab	31.7	abc
Festive Dance	6.6	ab	35.0	abc
Fairway Yellow	8.4	ab	39.2	abc
Fairway Orange	8.8	ab	34.2	abc
Wizard Sunset	12.2	abc	28.3	ab
Wizard Pastel	13.2	abc	44.2	abcd
Wizard Pink	28.1	bc	60.8	bcde
Volcano	29.6	bc	66.7	cde
Wizard Jade	33.3	c	56.7	bcde
Black Dragon	59.2	d	75.0	de
Color Pride	64.2	de	80.8	e
Wizard Color Sunrise	79.1	e	85.0	e

[z]Based on a visual estimation of percentage of diseased leaves with sporulation.

[y]Column means with a letter in common are not significantly different (Student-Newman-Keuls; P=0.05).

CHAPTER 2: EPIDEMIOLOGY AND MANAGEMENT OF IMPATIENS DOWNY MILDEW IN THE UNITED STATES

ABSTRACT

Impatiens (*Impatiens walleriana*), a flowering annual bedding plant, is a highly popular shade plant that is revered for its season-long flowering and bright colors. Downy mildew, caused by the oomycete *Plasmopara obducens*, can rapidly defoliate and kill impatiens. Epidemics have been observed throughout the United States (U.S.) in greenhouses and landscapes and control strategies are needed. The influence of temperature and leaf wetness duration on germination of sporangia and infection of impatiens by *P. obducens* was examined in controlled environmental studies. Impatiens plants were inoculated with *P. obducens* sporangia and immediately placed into a growth chamber set at 15°C, 20°C, 25°C, or 30°C. A greater proportion of leaves hosted sporangia following incubation at 20°C than the other temperatures studied. Inoculated plants incubated at 15°C and 25°C yielded a similar proportion of infected leaves. No pathogen sporulation was observed on plants incubated at 30°C. A leaf wetness period ≥6 hours yielded a greater proportion of leaves hosting sporangia than a leaf wetness period of 3 hours. Conventional and reduced-risk fungicides were evaluated for efficacy. Reduced-risk products cyazofamid, mandipropamid and mefenoxam significantly reduced infection compared to the untreated inoculated plants. An experimental fungicide, oxathiapiprolin, was especially efficacious and provided protection from *P. obducens* in the landscape for >12 weeks after the final greenhouse application. Environmental manipulation coupled with effective reduced-risk fungicides can aid growers and ensure the continued planting of impatiens in U.S. landscapes.

INTRODUCTION

Impatiens bedding plants (*Impatiens walleriana*) produce flowers in a wide array of colors, profusely bloom over a long period of time, and are valued for providing long-lasting color in

shady areas of the garden. Downy mildew, an emerging and widespread disease caused by the water-mold pathogen *Plasmopara obducens,* was observed on impatiens in California, USA in 2004 (Wegulo et al., 2004). This pathogen rapidly deflowers, defoliates and kills impatiens. Downy mildew is a challenge for greenhouse producers of impatiens, landscapers, and

Figure 4. (A) Underside of an impatiens leaf with sporulating *Plasmopara obducens*. (B) A commercial planting of impatiens defoliated by downy mildew.

homeowners (Daughtrey and Palmer, 2014). Symptoms of infection by *P. obducens* include chlorotic mottling of the leaves, downward curling of the foliage and plant stunting. The causal pathogen reproduces rapidly, producing a vast quantity of sporangia on diseased tissue (Fig. 4A). Infected plants eventually become defoliated and die (Fig 4B). This pathogen is thought to cause quiescent infections in plant tissue that remain undetected until favorable environmental conditions occur (Hausbeck, personal observation). As a result, healthy-appearing impatiens can be sold to garden centers, big box stores, retailers, landscapers, and homeowners, only for disease symptoms/pathogen signs to develop if the weather becomes humid and wet. Impatiens production in the United States has been steadily declining as a result of downy mildew; the industry was

valued at $62.9 million in 2014, approximately half of the 2010 industry ($112 million) (USDA, 2014).

Although impatiens producers were initially reluctant to apply fungicides due to cost and labor, it has become necessary due to the pandemic nature of the pathogen in the United States. Growers had relied on the broad-spectrum fungicides mancozeb and chlorothalonil to manage the disease; both products are classified as B2 carcinogens by the United States Environmental Protection Agency. Reduced-risk fungicides have shown the ability to control downy mildew in other floriculture crops (Harlan and Hausbeck, 2012). There is a critical need to offer growers a reduced-risk approach to controlling *P. obducens* as consumers prefer agricultural products that have been grown using environmentally friendly practices. Additionally, downy mildew pathogens are very adept at developing resistance to commonly used fungicides and it is recommended to use multiple modes of action within a comprehensive disease management program to delay the development of resistance (Gisi and Sierotzki, 2008). In 2014, five isolates obtained from impatiens growing in Michigan, U.S. were tested for mefenoxam resistance; one isolate exhibited resistance to this fungicide which is the most efficacious and commonly used product (Hausbeck, unpublished data). Due to the cost of fungicides and the labor required to apply them, few impatiens are treated once they are placed in the landscape. Therefore, long-lasting protection against downy mildew must be in place as the impatiens leave the greenhouse as homeowners are unlikely to purchase impatiens if they do not remain disease free during the growing season.

Limiting downy mildew in the greenhouse could include cultural strategies such as manipulating greenhouse environmental conditions to reduce the relative humidity and duration of leaf wetness. However, since P. obducens is a relatively new pathogen, little research has

focused on *P. obducens* biology. Research conducted on *Peronospora antirrhini,* the pathogen that incites downy mildew on snapdragon, concluded that leaf wetness was correlated to increased concentrations of sporangia in the atmosphere (Byrne et al., 2005). Understanding the epidemiology of *P. obducens* may offer strategies to reduce downy mildew epidemics and limit the fungicide applications needed to produce a healthy crop.

The goal of our research is to develop a multifaceted approach to controlling downy mildew including defining the environmental conditions required for disease development and identifying fungicides that are efficacious over an extended period. The ability of growers to provide the public with healthy plants that will remain disease free in the landscape could allow impatiens to remain a foundation of shade gardens in the future.

MATERIALS AND METHODS

Epidemiological studies: 'Accent Premium White' impatiens were grown from seed in 288 cell flats containing Suremix Perlite (Michigan Grower Products, Inc., Galesburg, MI) and grown in a glasshouse located on the campus of Michigan State University until transplanting. Plants were transplanted to 10-cm square plastic pots containing Suremix Perlite and inoculated four weeks following transplanting. Plants were watered as needed. Prior to inoculation, each pot was placed in an open plastic bag and watered heavily. Treatments were laid out in a split-plot design with temperature as the whole plot treatment. Growth chambers were set at 15, 20, 25, or 30°C with a 16/8 h (light/dark) photoperiod. Within each growth chamber, plants were arranged in a randomized complete block design with five blocks (rows within the growth chamber). Hence, five replicate plants were used for each temperature-leaf wetness duration combination for each experiment. Plants were preconditioned at the desired temperature overnight prior to inoculation.

Leaves with visible *P. obducens* sporangia were removed from stock plants and placed into sterile distilled water in a beaker and gently agitated to release sporangia from the leaves. The leaves were removed from the suspension, and the concentration was quantified using a hemacytometer. Suspensions were adjusted to 6×10^4 sporangia ml^{-1}. The sporangial suspension was applied to both surfaces of the tagged leaves using a hand-held plastic janitorial spray bottle until leaves were saturated. Immediately after inoculation, each plant was enclosed in a plastic bag that was immediately sealed and was returned to the appropriate growth chamber. Control plants were misted with the same quantity of sterile distilled water using a hand-held plastic spray bottle, enclosed in plastic bags that were sealed using rubber bands, and returned to the appropriate growth chamber for 24 h before being air dried and treated as outlined below.

After the designated leaf wetness period (3, 6, 12, or 24 h) was reached within each growth chamber (temperature), the bags were opened and rolled below the top of the pot; the growth chamber fan dried the leaves. Leaves appeared to be completely dried within 20 min of opening the bags. Six days following inoculation, the bags were again closed and sealed. Nine days following inoculation the number of leaves with *P. obducens* sporulation and the total number of leaves of each plant were counted. The temperature and relative humidity in each incubation chamber was monitored for the duration of each experiment using WatchDog model 450 data loggers (Spectrum Technologies, Plainfield, IL, USA). Data loggers were set to record temperature (°C) and relative humidity (%) at 10 min intervals.

Statistical analyses of the epidemiological studies were performed using the SAS statistical package version 9.3 (SAS Institute, Inc., Cary, NC). A split plot design was used with temperature as the main plot effect and leaf wetness period as the subplot effect. The Proc Glimmix procedure was used to determine differences in the proportion of leaves with *P. obducens* sporulation with a

binomial error distribution and a logit link function ($P<0.05$). Because infection was not observed at 30°C, these data were removed from the dataset prior to analysis.

Fungicide Studies: Trials were conducted to determine whether fungicides applied to impatiens while growing in the greenhouse could provide extended protection from downy mildew when the treated plants are transplanted into the landscape. Impatiens 'Accent Premium White' and 'Accent Premium Red' were seeded into 288-cell flats and placed in a glass greenhouse on the campus of Michigan State University (East Lansing, MI, USA). After six weeks the impatiens seedlings were transplanted into 10-cm pots containing Suremix Perlite (Michigan Grower Products, Inc., Galesburg, MI, USA). Plants were fertilized weekly with 200 ppm of Peters water soluble fertilizer (The Scotts Company, Marysville, OH, USA). Greenhouse temperatures ranged from a low of 20°C at night to a high of 28°C during the day. Six single-plant replicates per variety were arranged in a completely randomized design on a greenhouse bench under 73% woven shade fabric. The fungicides mefenoxam (reduced-risk status and applied at 28-day intervals), fluopicolide (applied at 14-day intervals), oxathiapiprolin (an experimental fungicides applied at 14-day intervals), and ethaboxam (an experimental fungicide applied at 14-day intervals) were all tested. Three control programs were developed and included the previously mentioned products along with the biopesticide phosphorus acid and reduced-risk fungicides azoxystrobin and cyazofamid in combination and/or alternation. The alternating program treatments were applied at 7-day intervals over a 5-week period (Figure 5). Fungicides were applied as a drench (150 ml per pot), with the exception of cyazofamid, that was applied as a foliar spray using a hand-pumped compressed air sprayer. Applications were initiated on May 9th and reapplied at 7- or 14-day intervals with the final application on June 2nd. Landscape sites were located in Ottawa County, MI, U.S. (Site A) and Kent County, MI, U.S. (Site B) and were selected based on prior confirmation

of downy mildew observed during the previous year. Impatiens plants were transplanted June 4, 2014 at Site A and June 9, 2014 at Site B. The impatiens at each site were observed weekly for signs of downy mildew infection beginning one week after transplanting. The percentage of leaves hosting pathogen sporulation was estimated for each plant. Data for the landscape trials were analyzed using SAS PROC GLM and statistical differences were compared using the Fisher's Protected Least Significant Differences test (P=0.05).

RESULTS AND DISCUSSION

The effects of both temperature (P<0.0001) and leaf wetness (P=0.0209) were significant for the mean proportion of leaves with *P. obducens* sporulation, but the interaction between temperature and leaf wetness was not significant (P=0.8757). Pathogen sporulation was not observed on any inoculated plants incubated at 30°C (Table 3). Sporulation was observed on inoculated plants at all other temperatures tested. Following the initial rating, plants that had been incubated at 30°C were moved to the 20°C incubator to determine if plants had a latent infection, but the pathogen did not sporulate indicating that infection had not occurred at the higher temperature. A greater proportion of leaves hosted pathogen sporulation following incubation at 20°C than at all of the other temperatures tested. Plants incubated at 15°C or 25°C yielded a similar proportion of infected leaves. Growing impatiens at temperatures ≤15°C and ≥20°C can reduce *P. obducens* sporulation. A leaf wetness period of ≥6 hours yielded a greater proportion of leaves supporting sporangial production than a leaf wetness period of 3 hours (Table 3). A leaf wetness period of 12 hours resulted in the highest proportion of leaves with pathogen sporulation. These data confirm studies conducted with other downy mildew pathogens in relation to leaf wetness and temperature effects on germination and infection (Neufeld and Ojiambo, 2012).

Defining environmental conditions associated with optimal disease development can alert growers to scout during these periods. Growers can limit the duration of leaf wetness by timing irrigation at a time of day when the leaves can dry rapidly. Venting the greenhouse during periods of high relative humidity may limit pathogen infection. Manipulating environmental conditions may not completely prevent infection and sporulation of *P. obducens* but could reduce the number of fungicide applications needed.

Table 3. Mean proportion of leaves with *P. obducens* sporulation (infected) for impatiens incubated under varying temperatures and leaf wetness duration following inoculation. The interaction between temperature and leaf wetness was not significant.

Temperature (°C)	Proportion infected*	Leaf Wetness (h)	Proportion infected*
15	0.02 b	Control	0.04 c
20	0.59 a	3	0.21 b
25	0.05 b	6	0.25 ab
30	0.00	12	0.30 a
		24	0.28 ab

*Means with the same letter are not statistically different ($P<0.05$). Incubation at 30°C did not result in pathogen sporulation; these data were excluded from statistical analysis.

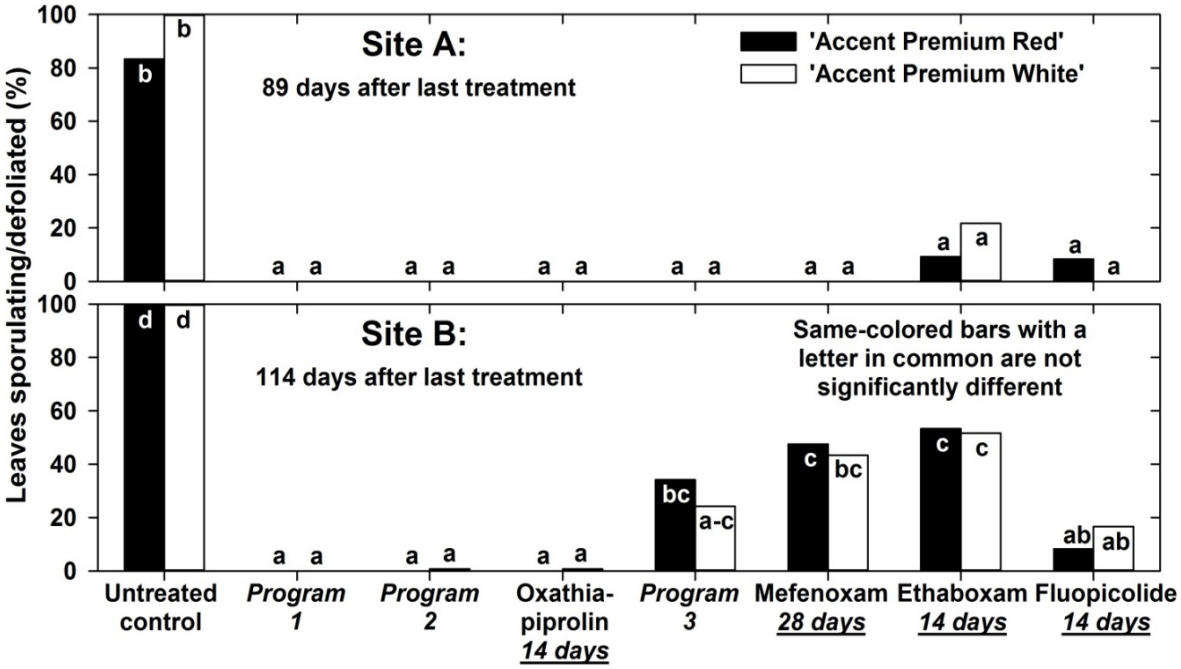

Figure 5. Efficacy of fungicides 89 or 114 days after the last application to two impatiens cultivars while in the greenhouse prior to planting in the landscape Sites A and B. oxa=oxathiapiprilon, phos=phosphorous acid, fluo=fluopicolide, azo=azoxystrobin, cya=cyazofamid, mef=mefenoxam, eth=ethaboxam. *Program 1*: (oxa + phos)/*week 1*, (fluo + azo)/*week 2*, (cya)/*week 3*, (phos)/*week 4*, (oxa + fluo)/*week 5*. *Program 2*: (mef + phos)/*week 1*, (fluo + azo)/*week 2*, (oxa)/*week 3*, (phos + eth)/*week 4*, (mef + fluo)/*week 5*. *Program 3*: (mef + phos)/*week 1*, (fluo + azo)/*week 2*, (cya)/*week 3*, (phos acid)/*week 4*, (mef + fluo)/*week 5*. All fungicides were drenched except cyazofamid, which was applied as a foliar spray. Fungicide rates per L were: azoxystrobin 0.07 g, cyazofamid 0.27 ml, ethaboxam 0.31 ml, fluopicolide 0.08 ml, mefenoxam 0.08 ml, oxathiapiprolin 0.75 ml, and phosphorous acid 1.0 ml.

Disease pressure was severe at both landscape sites. While the industry standard fluopicolide provided excellent protection from downy mildew at both sites, mefenoxam-treated plants at Site B had >40% leaves with sporulation at the end of the study (Figure 5). The isolate from Site B was tested for mefenoxam sensitivity and results showed that this particular isolate was resistant to the fungicide. The resistance of *P. obducens* to mefenoxam, which is considered the most effective registered fungicide, confirms the need for additional downy mildew fungicides. The experimental fungicide ethaboxam was moderately effective and could be used in a disease control program. Oxathiapiprolin, an experimental fungicide that has shown excellent efficacy

against other oomycete pathogens was very effective when applied as a drench with just one leaf on one plant supporting pathogen sporulation by the end of the study. With the discovery of a mefenoxam resistant *P. obducens* isolate in Michigan, a priority was placed on testing effective experimental products in control programs. When oxathiapiprolin was used in place of mefenoxam in a control program, a drop in efficacy was not observed at either site. Overall, the 5-week control program applied while impatiens were growing in the greenhouse offered excellent protection against downy mildew in the landscape with the vast majority of plants being symptom free >89 days post treatment. The ability to offer consumer's impatiens that will remain healthy in their landscape is essential for the future of this well regarded annual shade plant.

FUTURE WORK

Unfortunately, compared to more economically important crops, such as those in the cucurbit or vitis family, which product food and libation for the world's population, very little research is being published on the downy mildew pathogens of ornamentals. This lack of research has resulted in a knowledge deficiency on the basic biology of the pathogens. I believe the future work for each pathogen, the *Peronospora* sp. that infects coleus and *Plasmopara obducens*, overlaps and can be segmented into; i.) biology, ii.) genetic diversity, and iii.) host resistance.

i.) Biology – To this point, no research has been published looking at the biology of the *Peronospora* sp. that infects coleus. Currently, we do not have any knowledge in regards to oospores production by this pathogen and if they are could be a vessel for epidemics in landscape beds. We also do not understand the role of zoospores in this host pathogen complex. Lastly, heterothallism or homothallism and if mating types persist in the population needs to be determined.

ii.) Genetic Diversity – Some preliminary work has been conducted at the USDA-ARS Laboratory in Beltsville, MD to determine the genetic diversity of *Plasmopara obducens* in the United States. The presentation I observed showed a high level of diversity from year to year and region to region. I believe this work should be expanded to isolates collected from across the world with a hope of determining if different races are present and if these races are a determining factor in virulence.

iii.) Host Resistance – New coleus varieties are developed and released annually. I would like to see resistance screening continue as a high variability in susceptibility was observed in previous experiments.

APPENDIX

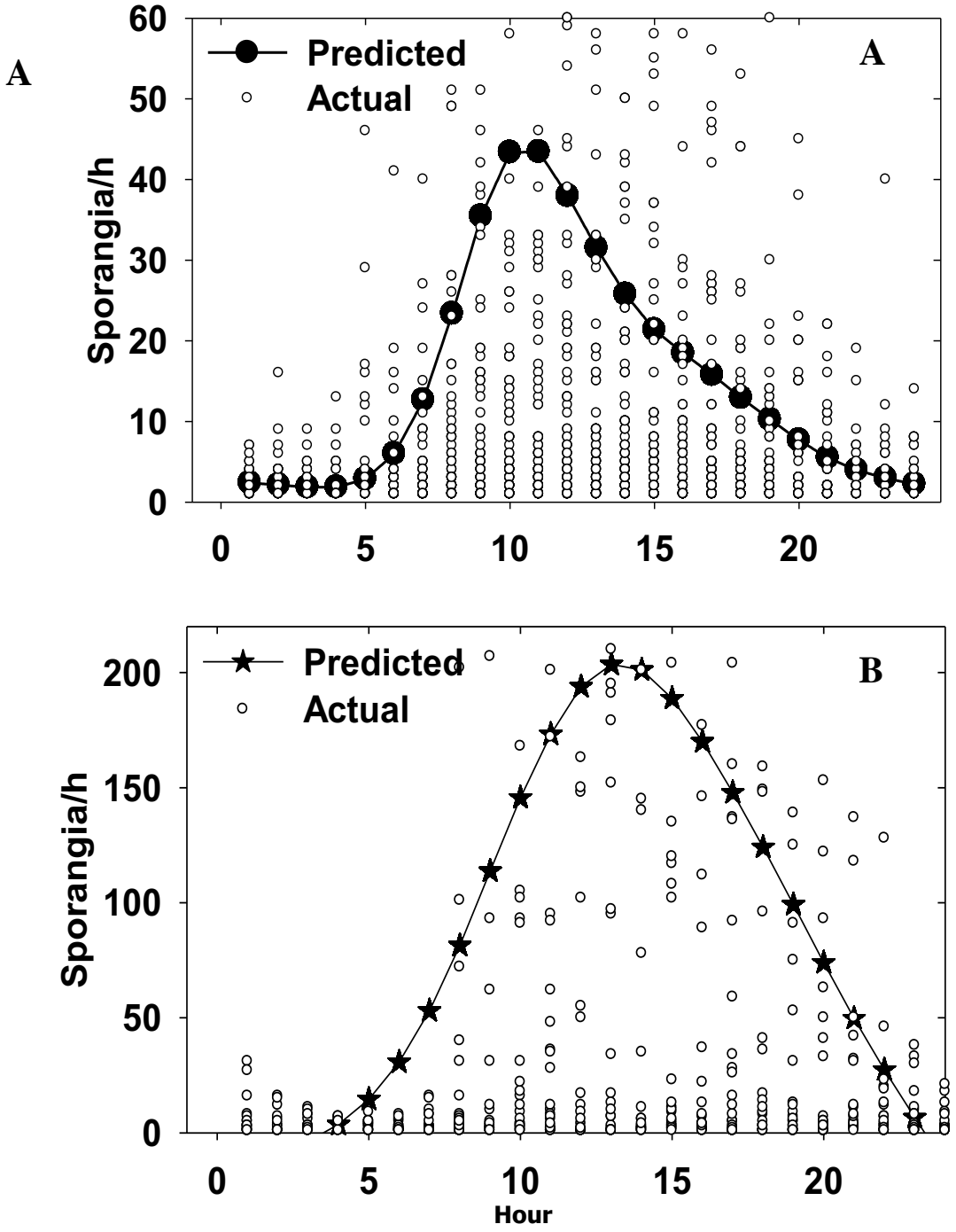

Figure 6. Hourly average predicted vs. actual concentrations of airborne conidia of *Peronospora* sp. from coleus in the greenhouse in 2006 (A) and 2007 (B). PROC REG procedure, SAS statistical package version 9.3.

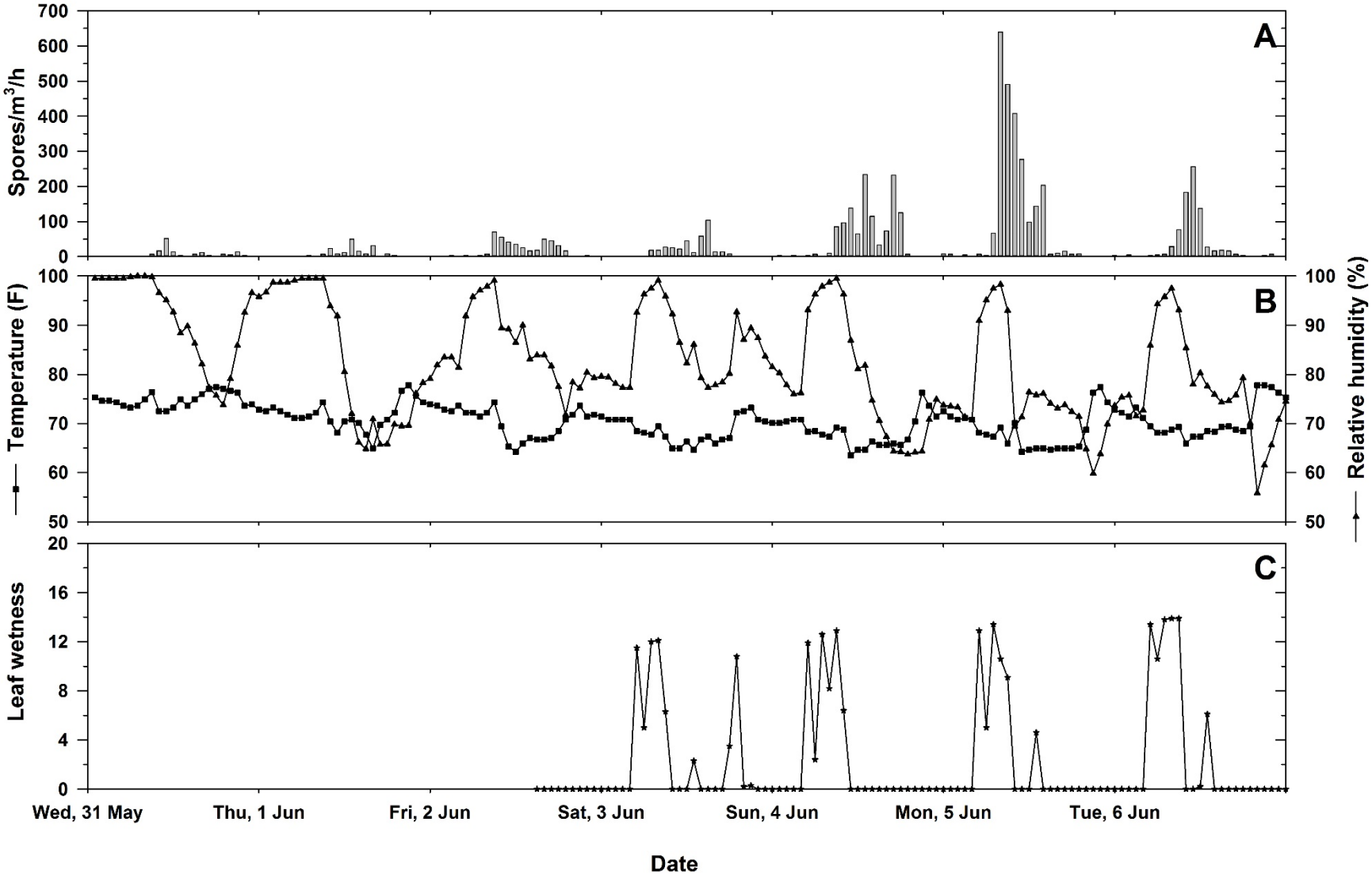

Figure 7. (A) Concentration (spores/m³/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 31 May to 6 June 2006.

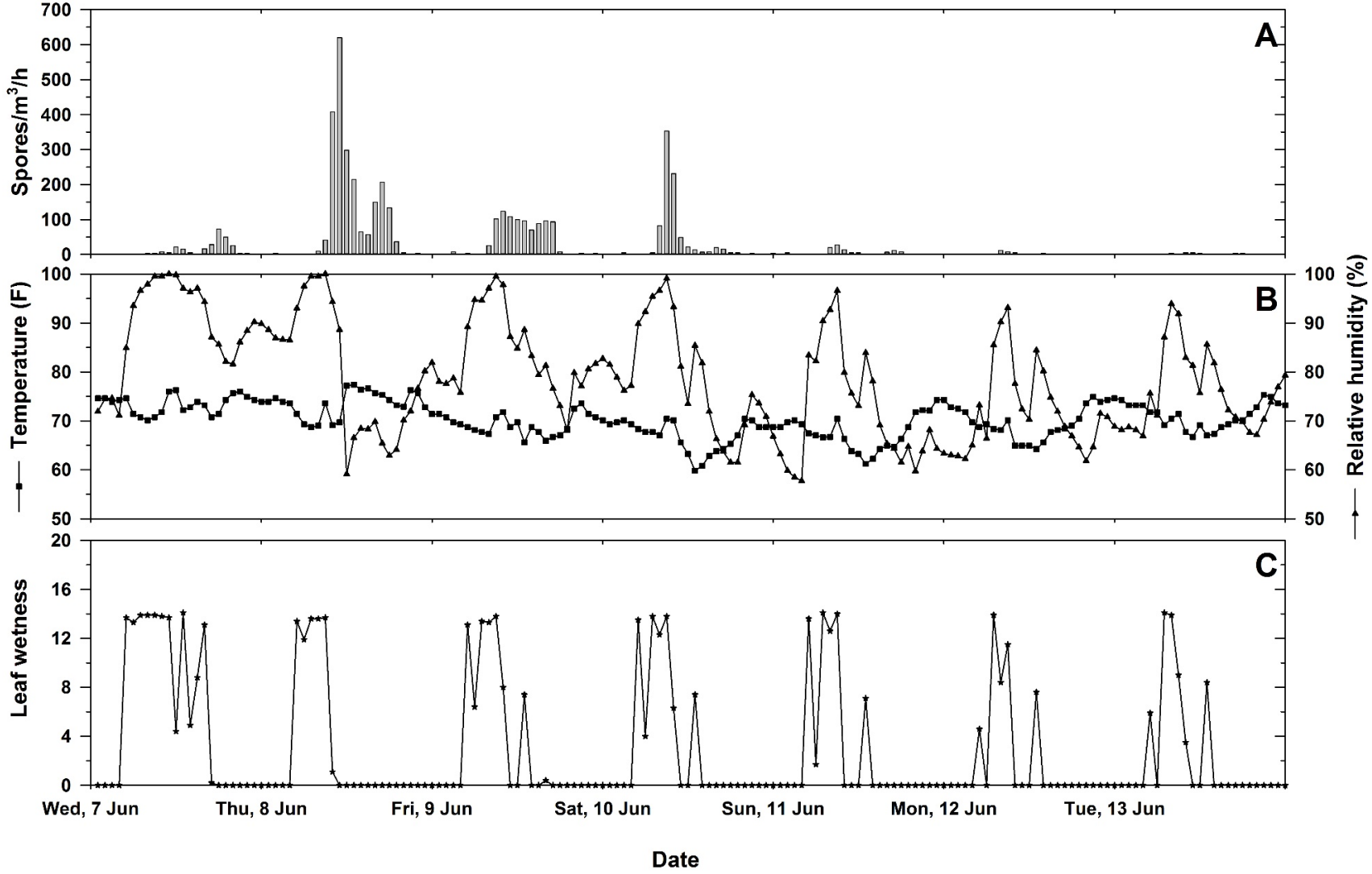

Figure 8. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 7 to 13 June 2006.

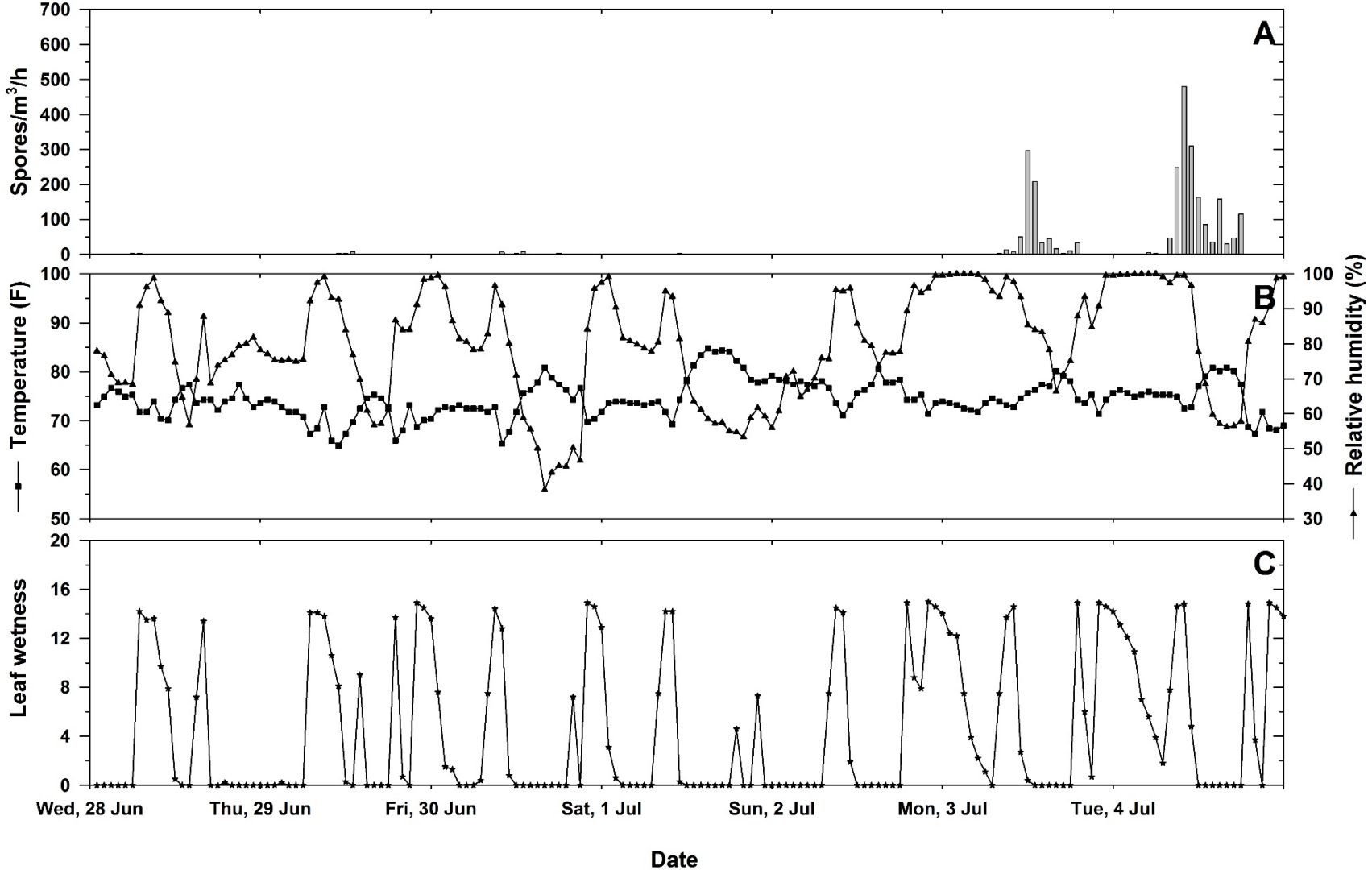

Figure 9. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 28 June to 4 July 2006.

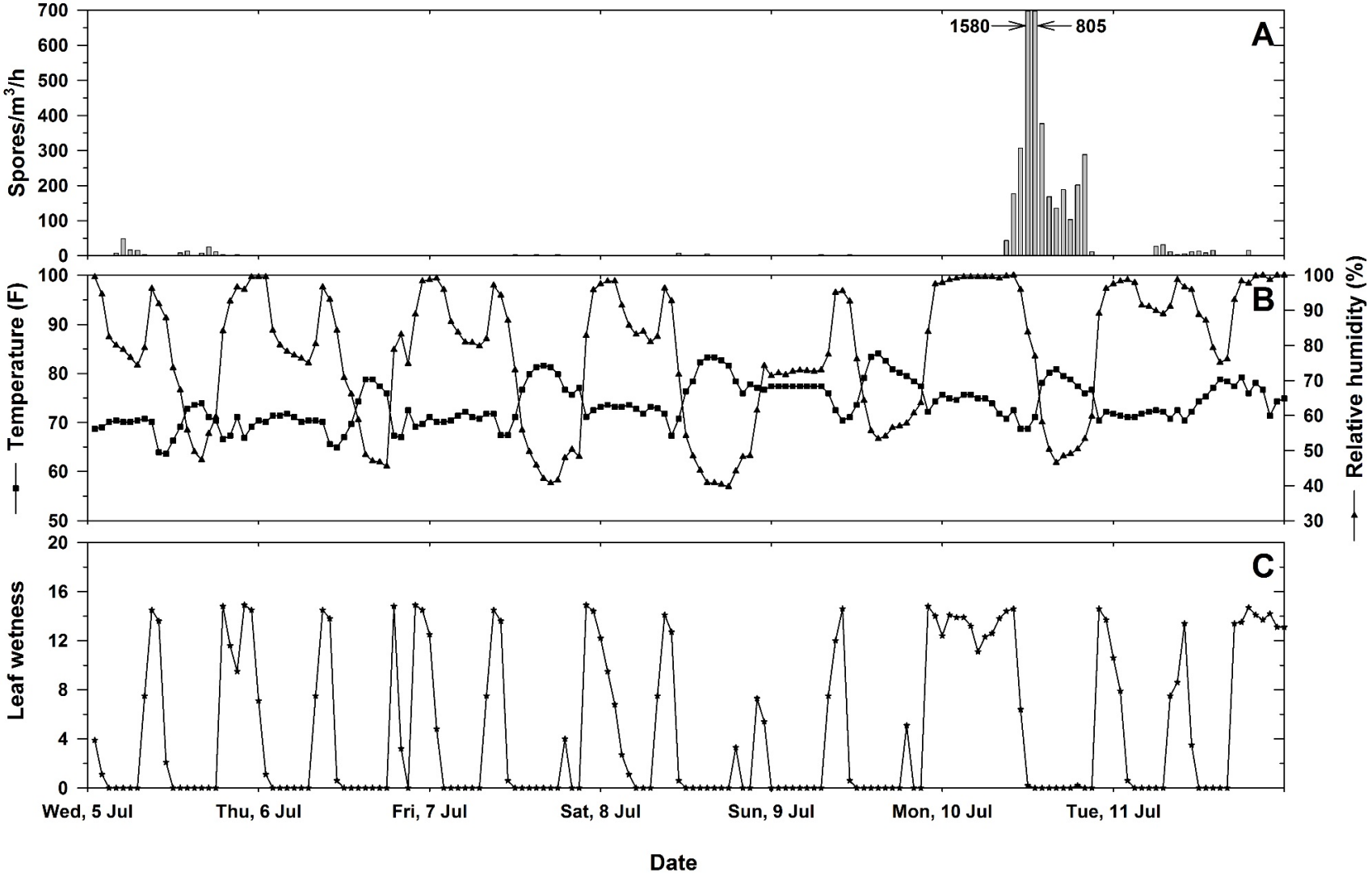

Figure 10. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 5 to 11 July 2006.

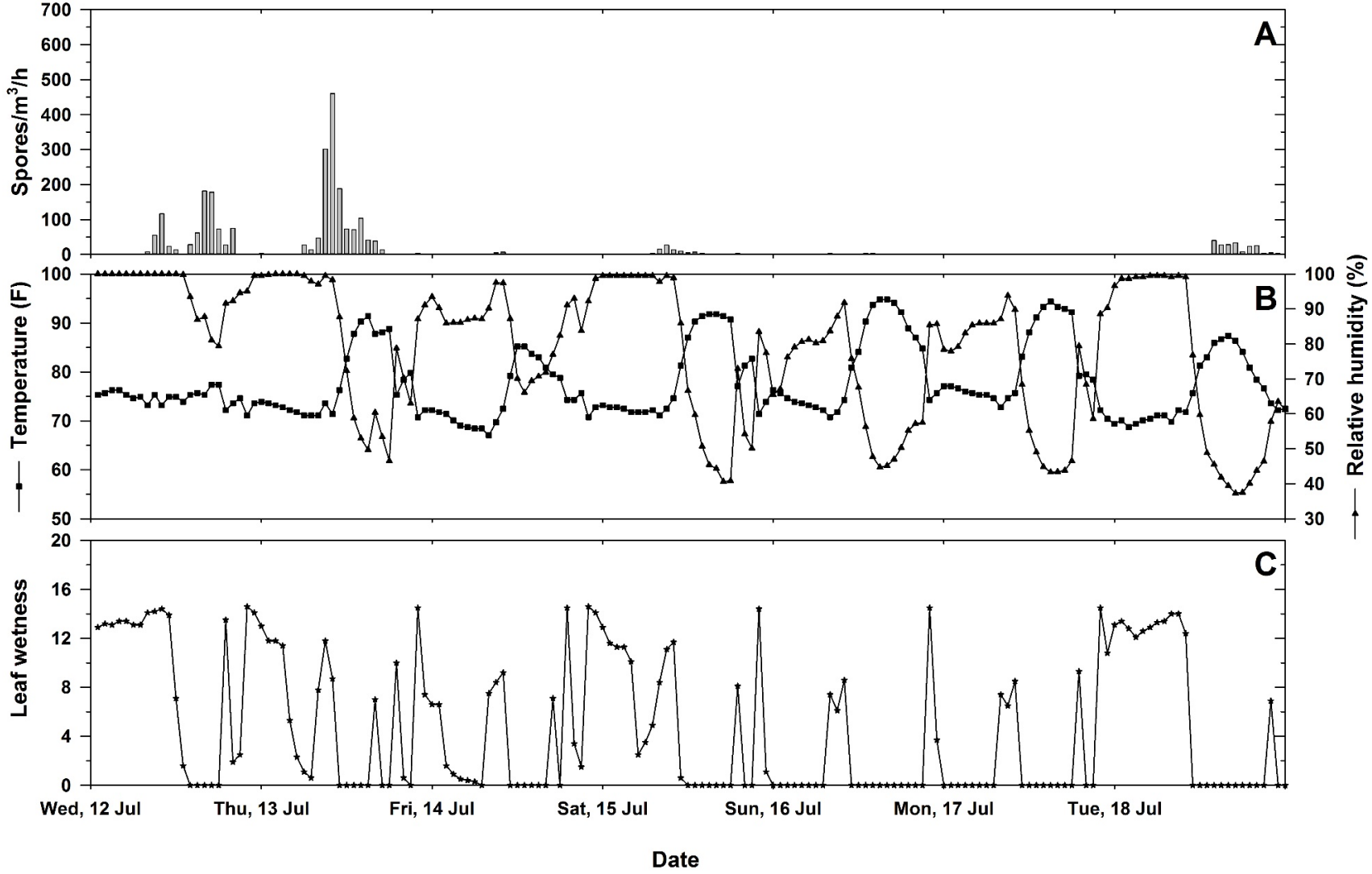

Figure 11. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 12 to 18 July 2006.

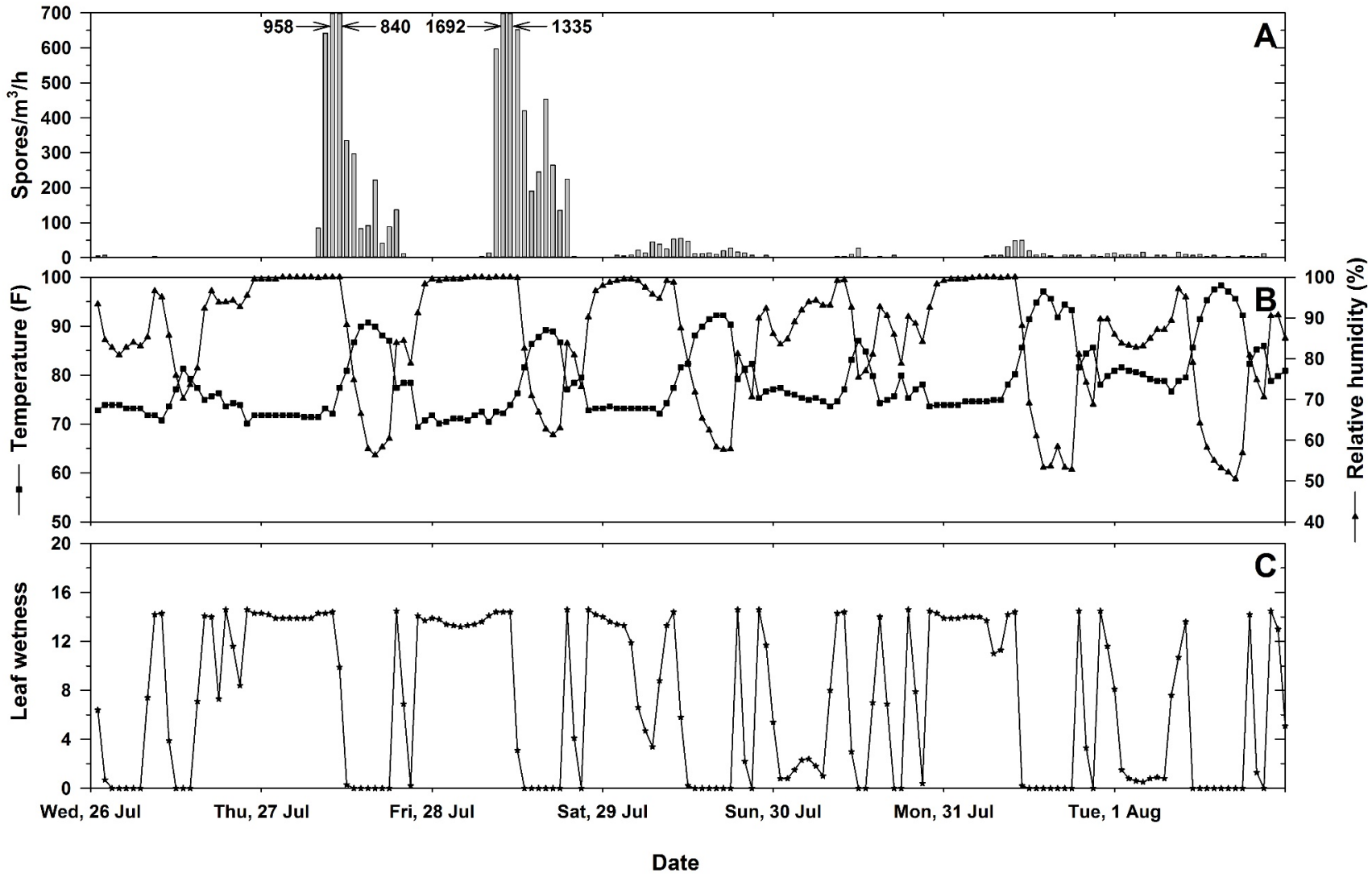

Figure 12. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 26 July to 1 August 2006.

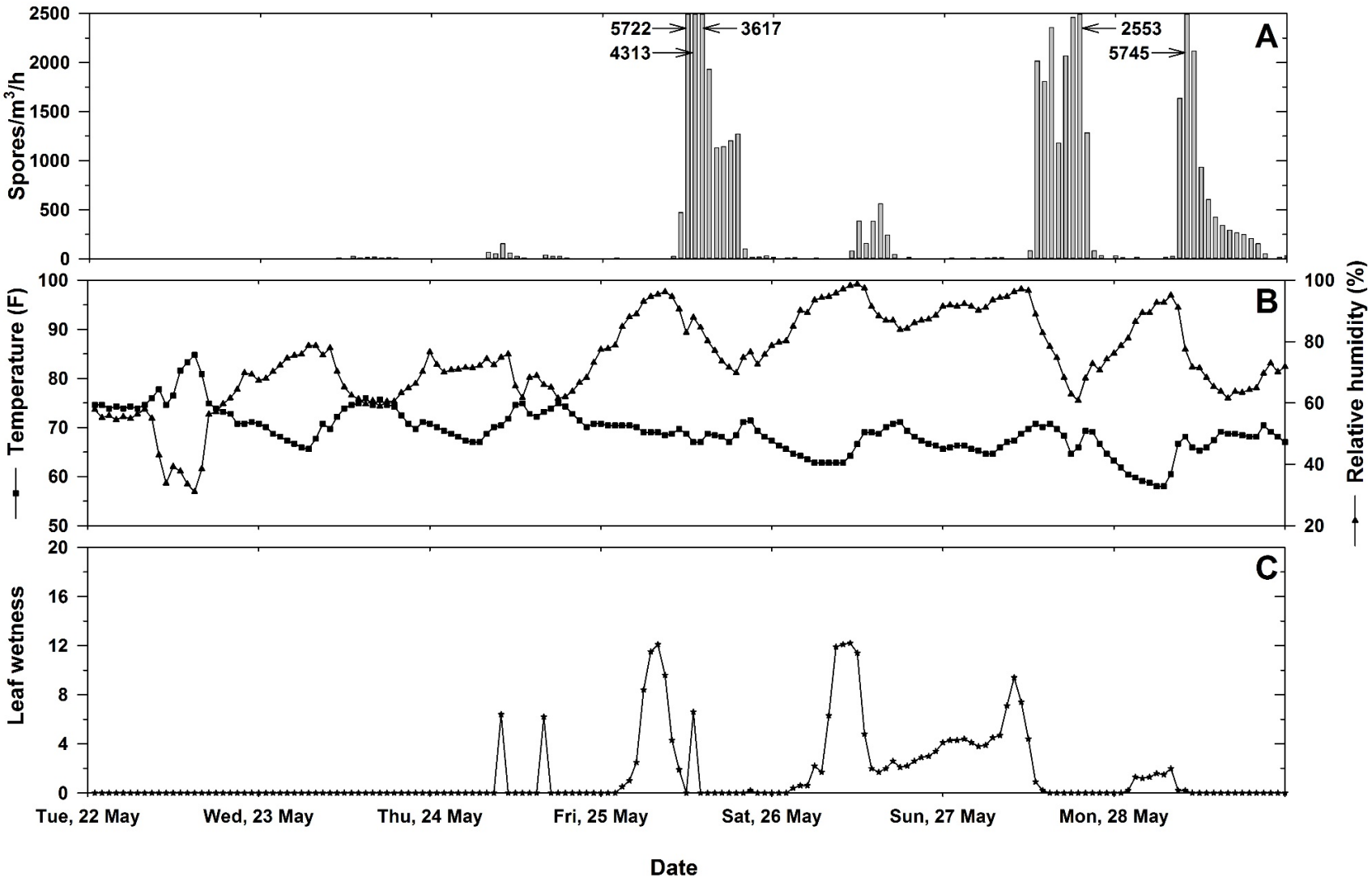

Figure 13. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 22 to 28 May 2007.

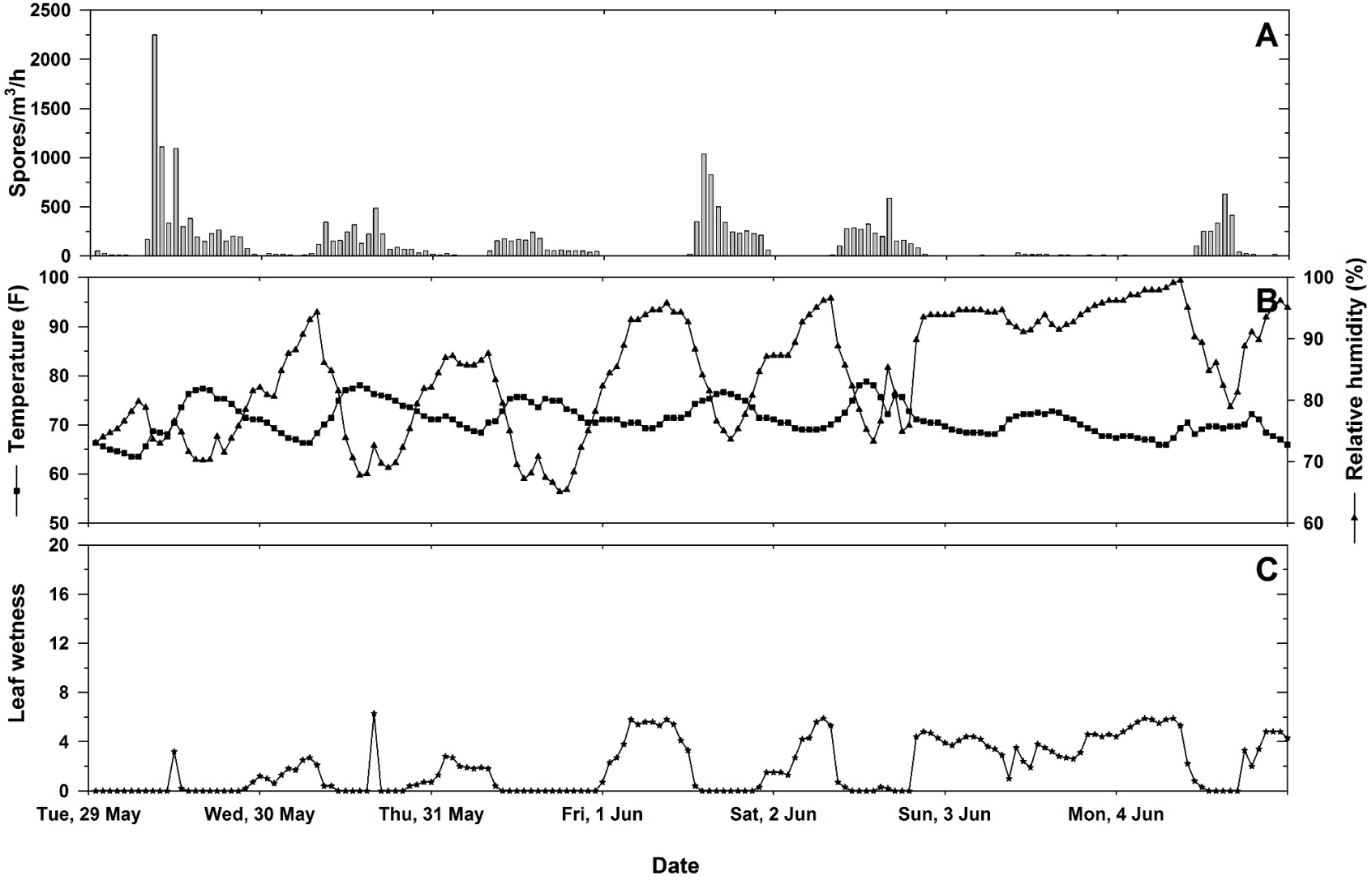

Figure 14. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 29 May to 4 June 2007.

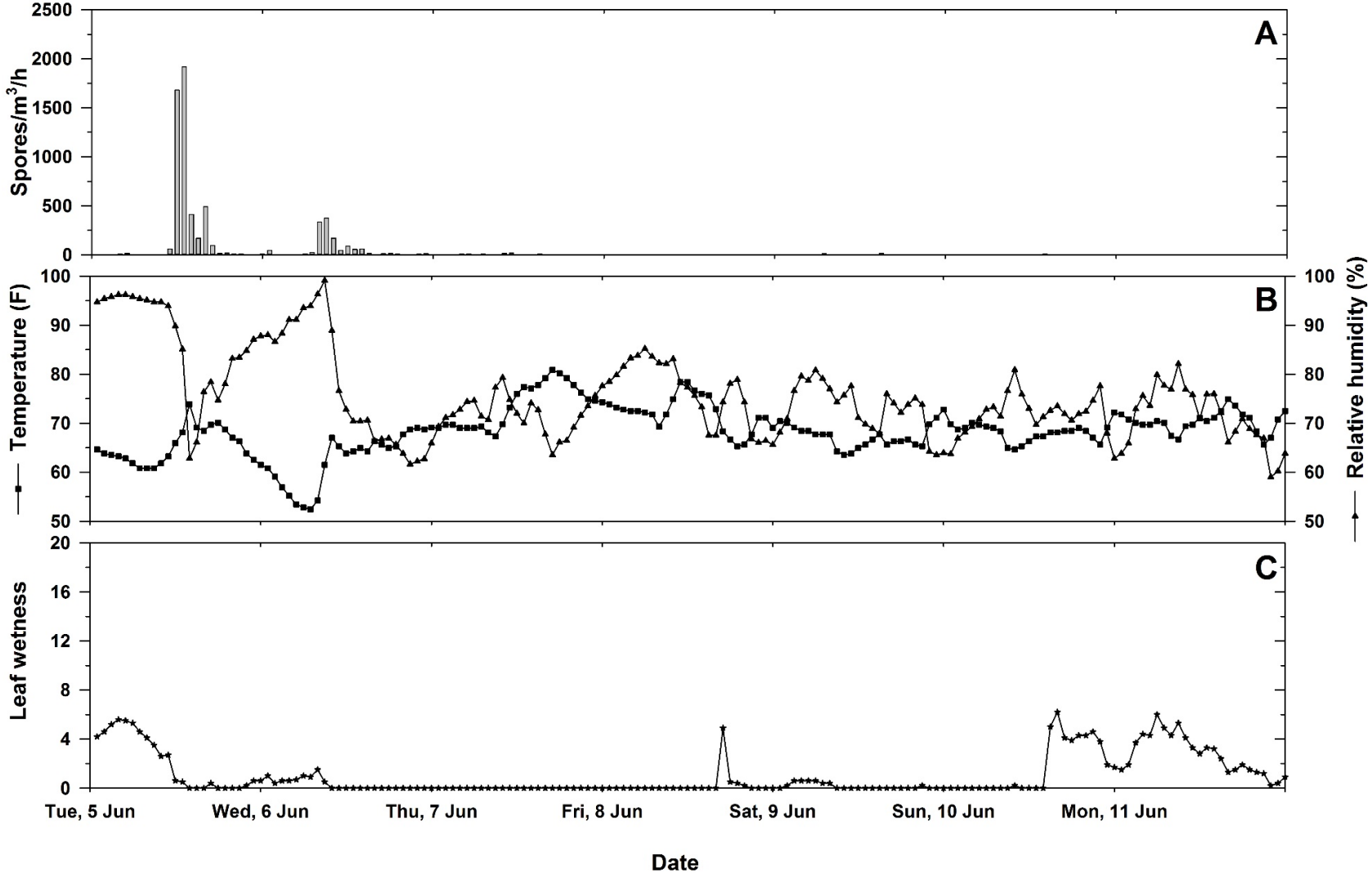

Figure 15. (A) Concentration (spores/m^3/h) of atmospheric sporangia of *Peronospora* sp., (B) temperature (°F) and relative humidity (%), and (C) leaf wetness in greenhouse 2G with coleus infected with downy mildew during 5 to 11 June 2007.

Table 4. Pearson correlation of greenhouse atmospheric *Peronospora* sp. conidia and temperature, relative humidity, and the change in relative humidity (*P*=0.0125). Increased numbers of *Peronospora* sp. conidia were correlated with higher relative humidity in 2006 and 2008, a drop in relative humidity in 2006 and 2007, and higher temperatures in 2008.

	Relative Humidity		Relative Humidity (change)		Temperature	
Year	R	*P*	R	*P*	R	*P*
2006	0.117	<0.001	-0.125	<0.0001	0.042	0.05
2007	0.019	0.6220	-0.16	<0.0001	0.014	0.71
2008	0.116	0.0021	-0.08	0.0043	0.180	<0.0001

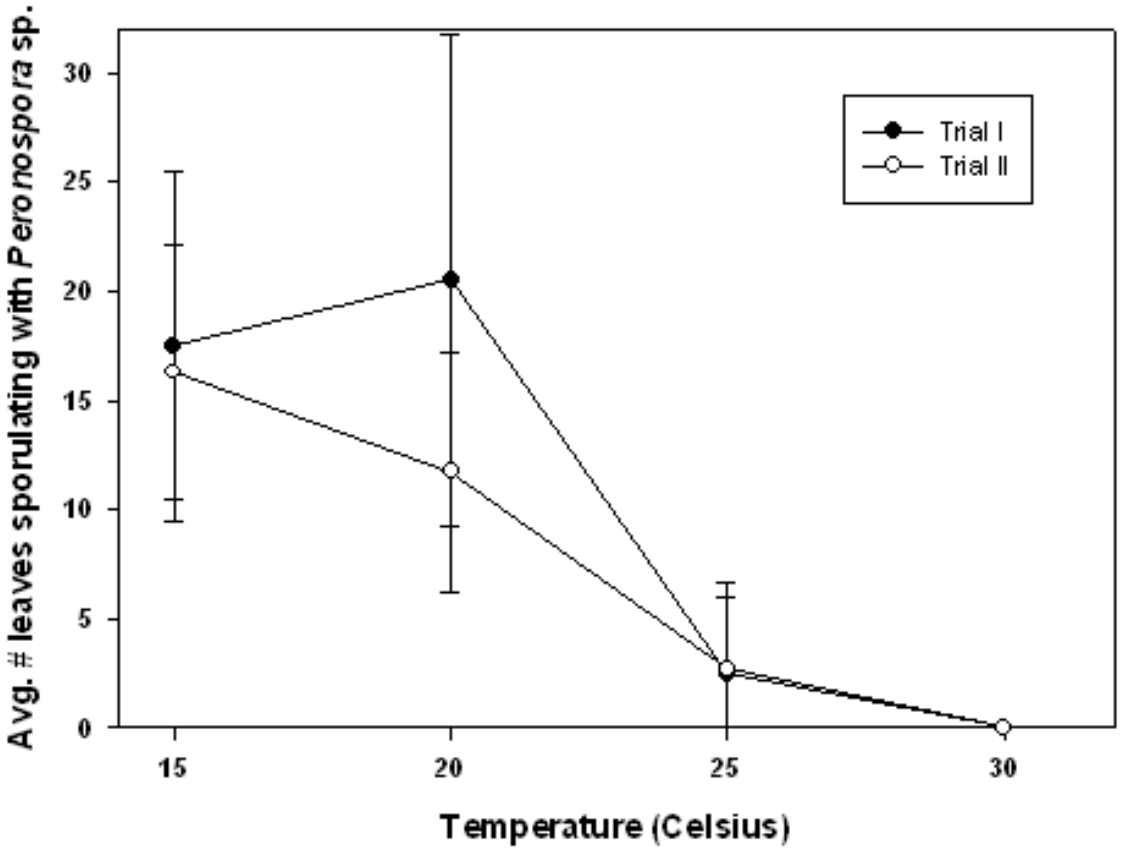

Figure 16. Evaluation of the effect of temperature on *Peronospora* sp. infection of coleus 'Volcano'. Experiment was conducted in growth chambers on the campus of Michigan State University. Plants were inoculated and immediately placed in chambers set at either 15, 20, 25 or 30°C. After one week in growth chambers, the plants were moved to the greenhouse and sporulation was induced. The number of leaves with sporulating downy mildew were counted.

Figure 17. Pictures of the plants from experiment described in Figure 16. Plants incubated at 15°C and 20°C had statistically more leaves with sporulation compared to plants incubated at 25°C and 30°C. No sporulation was observed at 30°C.

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