ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF CUCURBIT POWDERY MILDEW IN NORTH CENTRAL FLORIDA

By

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To my husband Alberto Azeredo and my daughter Anna Azeredo
To my mother Arlete Silveira

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ISOLATION, IDENTIFICATION AND CHARACTERIZATION OF CUCURBIT POWDERY MILDEW IN NORTH CENTRAL FLORIDA

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Powdery mildew is a common and economically important foliar disease in vegetable production throughout the world. On cucurbitaceous crops, the disease can reduce yield by decreasing fruit size, number of fruits, and length of time fruits can be harvested. Fruit quality and marketability can also be affected due to premature leaf senescence causing fruits to become exposed and more susceptible to sunburn. Additionally, powdery mildew infection can predispose cucurbit plants to other diseases. Cucurbit powdery mildew is most frequently caused by two obligate fungal pathogens, *Podosphaera xanthii* [(Castagne) U. Braun & Shishkoff 2000] and *Golovinomyces cichoracearum* [(DC.) V.P. Heluta 1988]. The most commonly identified pathogen; particularly in warmer production regions has been *P. xanthii*. Recently, there has been an increase in occurrence and severity of the disease in Florida, resulting in heightened concern with fungicide resistance and potentially a shift or displacement of the pathogen population. In this study, we identified and characterized single colony isolates of cucurbit powdery mildew from multiple sites, dates, and cucurbit hosts. A method for living culture maintenance of cucurbit powdery mildew isolates was determined. Two butternut winter squash (‘Butterbush’) fields at Live Oak and Citra, FL, were sampled

during spring and fall 2009. For comparison, additional cucurbit isolates were collected from south west and north east FL. Microscopic observations of all 297 isolates sampled from butternut winter squash ‘Butterbush’ at Live Oak and Citra revealed hyaline conidia, ellipsoid to ovoid in shape, with conidial dimensions of 31-44 x 15-24 µm (n = 100) and footcells of 45-67 x 10-13 µm (n = 25). Conidial length to width ratios varied from 1.4-2.6. All isolates exhibited fibrosin bodies and conidia edge lines were crenate. Commercial winter squash resistance lines were evaluated for disease response and pathogen characterization. Isolates from butternut winter squash (‘Butterbush’) and additional cucurbit hosts from varied dates and locations around Florida (FL) were subjected to multiplex polymerase chain reactions (PCR) with species-specific primers S1/S2 (for *P. xanthii*) and G1/G2 (for *G. cichoracearum*). With S1/S2, a specific PCR product of 454 bp (base pairs) was amplified from genomic DNA of most isolates. In total, based on morphological and genetic analysis, all cucurbit powdery mildew isolates were identified as *Podosphaera xanthii*.

CHAPTER 1
INTRODUCTION

Historically, cucurbits (Cucurbitaceae) have been important to mankind, especially as a food source. There is great diversity among consumed cucurbitaceous crops and production regions around the world (276). Cucurbit crops continue to be developed and produced for market niches worldwide (290, 291).

Cucurbits include a number of species in three distinct genera in the family Cucurbitaceae. The genus *Cucumis* includes cucumber and many types of melon. Squash and pumpkin are found in a range of cultivated species of *Cucurbita* and watermelon is in the genus *Citrullus*. Worldwide, 7 species are economically important and include: *Citrullus lanatus* (Thunb.) Matsum. & Nakai (watermelon), *Cucumis sativus* L. (cucumber), *Cucumis melo* L. (cantaloupe), *Cucurbita pepo* L. (field pumpkin), *Cucurbita maxima* Duchesne (winter squash), *Cucurbita moschata* Duchesne. (crookneck squash or calabaza) and *Lagenaria vulgaris* (Molina) Standl. (bottle gourd) (263). In the United States, the most commonly cultivated and consumed cucurbits are fresh market and processing cucumber, many types of squash (acorn, butternut, kabocha, yellow squash and zucchini), various types of melons (muskmelon, cantaloupe and honeydew), pumpkins (*Cucurbita pepo* and *Cucurbita maxima*) and watermelon (295).

Cucurbit crops have global economic importance. Over 1 billion tons of vegetables (including cucurbits), were produced worldwide in 2009, with a total harvested area of more than 53 million hectares and a calculated yield of over 190 thousand hectogram/hectare. Among the estimates, cucurbits including watermelon,

cucumber, melons, pumpkin, squash and gourds accounted for over 206 million tons corresponding to roughly 20% of the total vegetables produced worldwide (81).

In 2010, the total U.S. average acreage of the major classes of cucurbits was around 185,000 hectares, yielding 4 million metric tons of produce with a value of US$1.5 million. The major cucurbit producing states in the U.S. are California, Florida, Georgia, Texas, Arizona, Illinois and Michigan. While California and Arizona lead production of honeydews and cantaloupe, Florida and Georgia lead in production of cucumber for fresh market and of watermelon. Michigan was the leader in production of processed cucumbers (for pickles) and of squash. In most years, Illinois leads pumpkin production (327).

According to 2009 data from the Florida Department of Agriculture and Consumer Service (FDACS), Florida's key cucurbit crops contributed a value of nearly US$290 million to the state's economy, which represented 14% of Florida's vegetable industry (233). Cucurbit production in Florida is critical as it supplies the national demand for cucurbits during the winter months. These vine crops are well adapted to production in Florida during the spring, early summer, and fall seasons. During the winter month's production of cucurbits is limited to the warmest southern growing areas of the state (238).

The causal agents of powdery mildew diseases include a diverse range of pathogenic species under the broad order Erysiphales in the phylum Ascomycota (27). Worldwide, approximately 500 powdery mildew species are able to infect over 10,000 distinct plant species (312). However, recent phylogenetic research reported the increase in the number of recognized powdery mildew species to about 820 (26).

Among the economically important plant species susceptible to powdery mildew are cereals (wheat, barley), members of the families Solanaceae (tomato, potato), Cucurbitaceae (melon, squash, cucumber, pumpkin, and watermelon), Rosaceae (apple, strawberry, cherry), Fabaceae (pea, bean), Asteraceae (lettuce, artichoke), Vitaceae (grape), and many ornamental plants from varied plant families.

Cucurbit powdery mildew is particularly prevalent in tropical and some temperate climates where it frequently causes significant reductions in product quality and yield. The disease has been reported to be the most common, widespread and easily recognized disease of both field and greenhouse grown cucurbit crops (214). This foliar fungal disease is a major cause of crop losses in cucurbit production worldwide, and is among the most intensively studied because of its economic impact on these crops (161). Powdery mildew is a common and serious disease of cucurbit crops in Florida, and the disease occurs on cucumber, melon, squash, zucchini, pumpkin, gourd and more recently, and increasingly, on watermelon crops (235).

Like other powdery mildews, signs and symptoms are characterized by a white, talcum-like fungal growth on leaves, petioles, and stems. Fruit are rarely directly infected by powdery mildew. Under favorable environmental conditions, fungal colonies can coalesce and the host tissue becomes chlorotic, senescing prematurely. This disease is frequently more severe at the end of the vegetative cycle, causing additional losses by shortening the length of harvest time and affecting fruit quality as a result of premature defoliation and sunscald. During the growing season, fungal conidia (asexual spores) are readily disseminated and can quickly move within and among fields and inside greenhouses aerially.

The two main causal agents of cucurbit powdery mildew are *Podosphaera xanthii* [(Castag.) U. Braun & N. Shish], formerly known as *Sphaerotheca fuliginea* (Schlecht ex Fr), and *Golovinomyces cichoracearum* [(DC.) V.P. Heluta] previously referred to as *Erysiphe cichoracearum* DC ex Merat. Studies throughout the world have shown that *P. xanthii* is the species most commonly identified across cucurbit hosts (329). *Podosphaera xanthii* is commonly detected in subtropical and tropical regions as well as in greenhouse grown crops, while *G. cichoracearum* occurs more frequently in temperate and cooler areas under field conditions (143). The two species can occur singly or in mixed infections on cucurbit crops (123, 143, 164). Potentially, all cultivated cucurbitaceous crops can be susceptible to powdery mildew (71), however tolerant and resistant cultigens exist. Some cultivated cucurbits, such as cucumber and melons, have greater resistance and are commercially available.

While *P. xanthii* and *G. cichoracearum* have historically been described as pathogenic on cucurbits, recent reports indicate that *G. cichoracearum* has been documented to cause disease on several members of the Asteraceae family such as lettuce (*Lactuca sativa* L.) in the Czech Republic (160), gerbera daisy (*Gerbera jamesonii* Bolus ex Hook. f.) (324), orange coneflower (*Rudbeckia fulgida* Aiton.) (90), Paris daisy (*Argyranthemum frutescens* (L.) Sch. Bip.) (91) and English daisy (*Bellis perennis* L.) (92) in Italy as well as elegant zinnia (*Zinnia elegans Cav.*) (243) and chamomile (*Matricaria chamomilla* L.) in Korea (242) and in the U.S., on crotalaria (*Crotalaria juncea* L.), a member of the Fabaceae family (95).

Prior to the mid-1990s, powdery mildew was reported as an occasional problem on watermelon (66, 120, 277) but the incidence of powdery mildew outbreaks has

increased and the disease has become an important problem in the major U.S. watermelon production areas (65, 128, 275, 305, 318) and in other parts of the world (46, 144, 239, 268). Currently, only *P. xanthii* has been reported on watermelon (66) and to date, two races of *P. xanthii*, race 1 (1W) and race 2 (2W), have been identified on watermelon in the U.S. (46, 65-68, 201, 319). Recent outbreaks of the disease have been confirmed in South Carolina, Georgia, Florida, Oklahoma, Texas, Maryland, New York, Arizona and California (65, 305).

Detection of powdery mildew on watermelon can be difficult because the presence of the pathogen is less apparent than on other cucurbit crops (66). On watermelon plants, powdery mildew is manifested as chlorotic spots with or without white mycelial and/or conidial development on leaves and stems. In addition to these symptoms, water-soaked areas may appear on petioles of highly susceptible cultigens (319). Fruit infections are rare (66); however they have been detected on young watermelon fruit and are characterized by small circular patches of water-soaked tissue covered by white, talcum-like pathogen sporulation. In late 2010, severe powdery mildew outbreaks on seedless and seeded watermelon fruit on some commercial farms in southwestern Florida were confirmed through microscopic and molecular analysis. Powdery mildew symptoms were mainly observed on immature fruit, but not on mature older fruit or leaves. Orange-to-dark brown chasmothecia (sexual-spore bearing fruiting bodies) containing a single ascus was detected on the surface of some fruit samples. The powdery mildew isolate from watermelon fruit was maintained on cotyledons of several cucurbit and bean cultivars and following artificial inoculation tests, results indicated the profile of *P. xanthii* race 1 (139).

The identification of cucurbit powdery mildew fungi has not always been made accurately. The two major species defined in the literature may have been misidentified and at times, were considered as synonymous (195). The causal agents of cucurbit powdery mildew disease produce identical symptoms and can be difficult to differentiate in the absence of the perfect (sexual or teleomorphic) stage which can be distinguished by the number of asci, ascospores and appendages and presence of the chasmothecia (22). However, morphological features of the anamorphic (asexual) stage of *P. xanthii* differ from those of *G. cichoracearum* and include: size and shape of conidia, presence of fibrosin bodies, immature conidia edge and germ tube morphology.

The two cucurbit powdery mildew fungal pathogens also differ in geographical distribution (10, 41, 71, 101, 144, 218, 221, 255), pathogenicity on cucurbit cultivars (35, 48, 161, 163, 192, 224, 254), temperature requirements (13, 144, 164, 294) and sensitivity to some fungicides (128, 166, 201, 204-206, 210, 213, 296). *Podosphaera xanthii* has been reported to be more aggressive than *G. cichoracearum* (200). A lower temperature optimum has been associated with *G. cichoracearum* since this species is found mainly in cooler regions of the world and in the U.S. has been reported during cooler springs and early summer periods while *P. xanthii* appears to progress most rapidly in warmer regions during the warmest months of the year. The optimum temperature ranges for conidial germination are reported to be between 25-30°C for *P. xanthii* and 15-25°C for *G. cichoracearum* (218).

Podosphaera and *Golovinomyces* are described by broad pathogenic variability characterized as pathotypes and races (190, 322). Races 1 and 2 of *P. xanthii* were first defined in the Imperial Valley of California in 1938 when the pathogen overcame

resistance in ‘PMR-45’ melon. More than 30 years later, in 1976, race 3 of *P. xanthii* was detected in Texas. (193). To date, approximately 30 distinct physiological races of *P. xanthii* (39, 190) and 2 races of *G. cichoracearum* (11, 71, 158, 264, 322) have been identified worldwide. Traditionally, cucurbit powdery mildew races have been defined by the disease response of the pathogen isolate on a set of muskmelon differentials (158). The most frequently used set of melon differentials includes 11 genotypes that can differentiate cucurbit powdery mildew races originating from melon and other cucurbits such as cucumber, *Cucurbita* spp. and watermelon (157).

In spite of the advances in genetic, chemical and biological measures of control, the management of powdery mildew of cucurbit crops worldwide is insufficient. Typically, the control of powdery mildew in susceptible cucurbit cultivars is achieved with use of fungicides (223). Fungicides for control of cucurbit powdery mildew include a variety of active ingredients and modes of action with pre-harvest intervals ranging from 3 to 14 days (34).

The repeated use of site-specific fungicides over time has resulted in powdery mildew resistance to some commercial chemical compounds (30, 112, 118, 166, 179, 203, 236, 326, 340, 341, 347). Good fungicide coverage or fungicides with some mobile (systemic or translaminar) activity are needed to obtain adequate protection on the underside of leaves, where conditions favor disease (199). However these fungicides generally have high risk for developing resistance because of their single-site and specific modes of action (201).

Natural selection within asexual populations followed by a fungicide selection process which favors resistant mutants has been widely reported. Cucurbit powdery

mildew species are known to have high evolutionary potential (163, 196) and are naturally more likely to overcome plant genetic resistance and/or develop fungicide resistance (148, 201).

Cucurbit powdery mildew pathogens are highly variable in their pathogenicity and virulence which is evident by the existence of a large number of different pathotypes and races (190). Distinct physiological pathotypes and races of *P. xanthii* have been detected with resistance to as many as eight classes of fungicides (77, 201, 210, 236, 296, 302). Presence of resistant fungal strains has been associated with lack of powdery mildew control and the relative ease with which fungicide resistant strains can develop in a short period of time (161).

Difficulty in achieving adequate fungicide coverage, public concerns for the environment, likelihood of the development of disease resistance to chemical control and shifts in pathogen virulence indicate that the best method for powdery mildew management would be provided through the use of resistant varieties, with occasional use of chemical control when necessary (321). Resistant cucurbit varieties are being developed and are becoming an increasingly important component of powdery mildew management programs in the U.S. (65, 123, 194, 319) and elsewhere (113, 140, 161, 261, 268, 302, 339, 347). Additionally, integrated pest management (IPM) alternatives continue to be evaluated for efficacy and incorporation into cucurbit powdery mildew disease management programs (16, 72, 97, 135, 208, 223, 245, 280, 281, 283, 306)

To further understand the recent increase in incidence, severity and host range of cucurbit powdery mildew in Florida, this thesis study undertook the following research objectives: (i) to speciate and characterize the prevailing causal agent of cucurbit

powdery mildew in north central Florida through morphological features and DNA analysis; (ii) to develop an efficient technique for *in vivo* establishment and maintenance of the fungal isolates; (iii) to assess the presence of physiological races within cultured isolates via bioassays using detached leaves; and (iv) to evaluate the varietal reactions of *Cucurbita* breeding lines (genetic accessions) for susceptibility to powdery mildew under local (FL) field conditions.

CHAPTER 2
LITERATURE REVIEW

The Cucurbitaceae

Uses and Economic Importance

Cucurbitaceous crops have worldwide economic importance. Statistical data indicated that over 1 billion tons of vegetables including cucurbits were produced worldwide, with a total harvested area of more than 53 million hectares and a calculated yield of over 190 thousand hectogram/hectare in 1999. Among these estimates, cucurbits including watermelon, cucumber and gherkins, melons, pumpkin, squash and gourds accounted for over 206 million tons, representing more than 20% of the total worldwide production of vegetables (81) (Table 2-1).

Worldwide, the most cultivated cucurbit was watermelon with a total production of about 99 million tons, followed by cucumbers and gherkins (61 million tons), melons (26 million tons) and *Cucurbita* spp. (22 million tons) (Table 2-2). The largest cucurbit producer, China, led the production of watermelon with 66 % of the world production. Five countries (China, Turkey, Iran, Brazil and USA) produced about 77% (76 million tons) of the world production of watermelon. Cucumber was the second largest cucurbit produced, with five countries (China, Turkey, Iran, Russia and USA) representing 82 % (50 million tons) of the world production. Five countries (China, Turkey, Iran, USA and Spain) produced 68 % (17 million tons) of the world's melons. The first five producers (China, India, Russia, USA and Egypt) of *Cucurbita* spp. represented about 50% (13 million tons) of the world production (81).

In 2010, the total U.S. field production of the major classes of cucurbits was approximately 4 million metric tons on about 185,000 hectares, with a value of US$ 1.5

million (Table 2-3). The leading states in cucurbit production were California, Florida, Georgia, Texas, Arizona, Illinois and Michigan (327). While California and Arizona lead production of honeydew and cantaloupe; Florida and Georgia were leaders of cucumber for fresh market and of watermelon. Furthermore, Michigan was the leader in production of processed cucumbers (for pickles) and of squash. In addition, Illinois led in U.S. pumpkin production (Table 2-4).

In 2010, Florida ranked first in production of fresh market cucumber and watermelon and was the third largest producer of squash, behind Michigan and California (Table 2-4) (327). Combined, these crops contributed a value of nearly US$290 million to the state's economy which represented 14% of Florida's vegetable industry (Table 2-5). The major vegetables produced in the state of Florida for the 2008-2009 season (79) are presented in Table 2-6. The total Florida production of vegetables, including the major classes of cucurbits was approximately 2.3 million tons on 90,700 hectares (224,000 acres) of harvested area with a total value of US$ 1.9 million dollars (Table 2-6).

Cucurbitaceous crops are cultivated around the world under different environmental conditions, both in field and protected structures (i.e. greenhouse and tunnels) and for several uses and purposes. Globally, cucurbits are primarily used as a source of food, consumed fresh or cooked (353). Cucurbits are mainly cultivated as vegetables and different parts of the plants may be used, including seeds, flowers, very young shoots, tendrils and roots (263). Alternatively, cucurbits are also used for fiber, utensils, containers, floats, sponges, filters, sweeteners, musical instruments, for decoration, and also as succulent and ornamental plants (9, 276, 307). Many species

are known to have medicinal value especially in Asian countries (9). Some cucurbitaceous plants, native to Asia and Australia, are known to be toxic and poisonous to humans (94, 353). Additionally, several cucurbit species are considered noxious invasive weeds in the U.S. (133) and in other parts of the world (228, 244, 310).

Cucurbits are trailing or vining, tendril-bearing annuals (335), typically indeterminate in length and can grow up to 15 m long. Cucurbit leaves are borne singly and can be simple, three or five-lobed and leaf sizes vary between cucurbit species. Flowers vary greatly in size, color and shape depending on the species. Cucurbits bear both perfect (hermaphroditic) and imperfect (pistillate or staminate) flowers and require various insects, especially honey bees (*Apis* spp.), to ensure adequate pollination for both fruit and seed production (353).

Cucurbitaceae are most diverse in tropical and subtropical regions with hotspots in Southeast Asia, West Africa, Madagascar, and Mexico. Cucurbits are of Asian origin and most likely originated in the Late Cretaceous, some 60 million years ago (291). It has been reported that long-distance dispersal of seeds, between continents for at least 10,000 years, has played an important role in the biogeographical history of the Cucurbitaceae family (289).

The family Cucurbitaceae forms a diverse group of species which contains several important fruits and vegetables with a variety of sizes, shapes, colors, textures and flavors (307). Cucurbit fruits, specialized berries called pepo, are multi-seeded. Except for a few types of winter squash and netted melons, which are rich in beta-carotene and vitamin A, cucurbit fruits are generally low in nutritional value, though watermelon is an excellent source of lycopene, a red pigment known to have anticancer properties (335).

Cucurbitacins, compounds responsible for the bitter taste of some cucurbit species, can be highly toxic to humans. Cultivated cucurbits are low in cucurbitacins, while some wild species have larger amounts (up to 1%) in roots and fruits. Studies have determined that these compounds are important in protecting cucurbit plants against insects and herbivores. Cucumber beetles (*Acalymma* spp. Barber. and *Diabrotica* spp. Chev. Dejean) are attracted to cucurbits plants by this class of secondary plant compounds. These beetles are induced to feeding behavior and are capable of consuming these bitter compounds (172). Insect baits and repellents have been developed from crosses between cucurbit cultivars and wild cucurbit relatives (353).

Among the diversity in the Cucurbitaceae family, three genera are of the greatest economic significance throughout the world: *Cucumis, Cucurbita and Citrullus*. The major cultivated genera include: cucumber (*Cucumis sativus* L.), several types of melons (*Cucumis* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. Nakai), and squash and pumpkin (*Cucurbita* L.) (238). A few minor types of cultivated specialty cucurbits include chayote (*Sechium edule* (Jacq.) Swartz), long squash or bottle gourd (*Laginaria siceraria* (Mol.) Standl.), bitter melon (*Momordica charantia* L.), luffa sponge gourd (*Luffa aegyptiaca* Miller), Chinese okra or luffa ridge gourd (*Luffa acutangula* (L.) Roxb.), parvar or snake gourd (*Tricosanthes dioica* Roxb.), wax gourd (*Benincasa hispida* Thumb.), cassabanana (*Sicana odorifera* (Vell.) and tinda (*Praecitrullus fistulosus* Stocks) (9, 238, 262, 353).

Cucurbits are among the first domesticated plant species (263) and have varied centers of origin with representatives native to both the Western and Eastern

hemispheres (109, 353). While *Citrullus* (watermelon) and *Cucumis* (cucumber and melon) are thought to have originated in the Eastern hemisphere, *Cucurbita* (pumpkin and squash) originated in the Western hemisphere. Following European contact, centers of diversity developed in Turkey (*Cucurbita pepo*), India and Burma (*C. maxima*), China and Japan (*C. moschata*). Cucumber originated in India; cantaloupes and melons in Africa; summer squash and butternut squash came from Mexico and Central America; winter squash from South America and watermelon from Central Africa (238, 353).

Cucurbits differ in their ability to tolerate cold and heat, yet all cucurbits are sensitive to frost. The vast majority of species are vining herbaceous annuals. A few cultivars produce bush-like plants (353) and a limited number of species are woody vines in the rainforests of Australia (291). A small number of species are thorny shrubs and one cucurbit specie, endemic to Yemen, grows as a tree (*Dendrosicyos socotranus* Balf. f.) (289).

Taxonomic Classification

Cucurbitaceous crops belong to the order Cucurbitales and family Cucurbitaceae (Juss.). All the cultivated species are found in the subfamily Cucurbitoide. This plant family consists of over 100 genera and more than 800 species distributed largely in tropical and subtropical regions of the world with few representatives in temperate to cooler climates (332). Recent phylogenetic research by Schaefer and Renner (291) based on molecular and morphological data, described a new classification of 95 genera and 950 to 980 species comprising the Cucurbitaceae family.

Cucurbit Breeding and Resistance to Powdery Mildew

While not recognized as modern day breeding for resistance, domestication and selection of cucurbits by inhabitants of caves in Mexico, has been dated back 7,000 to 10,000 calendar years before present, and as suggested by archeological records, predates the domestication of corn (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) by more than 4,000 years (21, 308). Until approximately 70 years ago, *Cucurbita* cultivars were characterized by high genetic variability attributed in part by their natural tendency of outcrossing. However, demand for uniformity and selection for horticultural traits such as fruit size, shape and color, as well as quality and earliness resulted in high homozygosity and true breeding cultivars (21). For nearly 50 years, inbred cucurbit lines have been extensively studied and used to develop hybrids which have the advantage of being more uniform and homogeneous then previous open pollinated cultivars (241).

In the U.S., early cucurbit breeding research and work on sources of resistance were summarized by Peterson (257) and in 2002 was updated by Jahn et al. (123).Today, many cucurbit breeding programs worldwide consider incorporating disease resistance into commercial cultivars while enhancing crop yield and quality, horticultural traits (123) as well as adaptability and marketability (238).

Breeding for resistance to diseases has advanced greatly since the beginning of the 20th century when Biffen (1907) discovered that a particular resistance to yellow rust in wheat plants was controlled by a single recessive gene (20). Selecting and breeding cucurbit cultivars for resistance to powdery mildew has proved to be an effective means against this disease and an alternative that can lead to environmental and economic

advantages as well as a reduction in fungicide usage, resulting in a greater benefit to cucurbits growers.

Worldwide, cucurbit disease resistance breeding greatly advanced with improved understanding of plant genetics. Since the 1930s, international efforts in breeding for resistance to powdery mildew in commercial varieties of melon, cucumber, pumpkin and squash has been ongoing and considerable attention has been directed at breeding cucurbit cultivars with resistance to *P. xanthii* (syn. *S. fuliginea*) (123).

The effectiveness of cucurbit breeding programs depends on germplasm resources. All over the world, breeders are increasingly interested in sources of resistance to powdery mildew and have extensively investigated procedures for assessment of powdery mildew resistance (43, 140, 161, 261, 298, 347, 352). Interspecific hybrids of high resistance to cucurbit powdery mildew have been obtained through crosses and pedigree selection between the cultivar *Cucurbita moschata* and wild *Cucurbita* species (37, 45, 87, 140, 141, 155, 168, 240) as well as species of the genus *Cucumis* (49, 149, 153, 156) and *Citrullus* (66, 67)

Particularly in the U.S., recent outbreaks of powdery mildew on watermelon have contributed to further advances in cucurbit disease resistance breeding (66, 68, 146, 319, 321). Powdery mildew had not been considered a problem in watermelon because older cultivars were resistant to previously described races of *P. xanthii* (64). In1975, susceptibility to powdery mildew was demonstrated in the watermelon plant introduction (PI 269677) accession (277) and was found to be controlled by a single recessive gene *pm* (278). Since then, screening of the USDA-ARS watermelon germplasm, made up of over 1500 accessions, recovered 8 accessions with high levels of resistance to powdery

mildew and another 86 accessions demonstrated intermediate resistance to *P. xanthii* race 1W (66, 318). More recently, over 1600 cultigens (plant introductions, cultivars and breeding lines) were screened in greenhouse tests and from those, 8 cultigens had high resistance and 21 had intermediate resistance to *P. xanthii* 2W (319).

Extensive work in genetic linkage mapping and the understanding of fungus-host as well as fungus resistant-gene interactions in cucurbit powdery mildew resistance mechanisms have been recognized as essential tools for genetic research and breeding for disease resistance (5, 89, 177, 178, 251, 302, 316, 346).

Cucurbit Powdery Mildew

Importance

Plant diseases have had profound effects on mankind as evidenced by numerous Biblical references (about 750 B.C.) to blasts, blights and mildews of plants, found in the Old Testament (3, 220) . Powdery mildew is one of the oldest plant diseases on record. The first historical account of the disease was recorded by the Greek writer and gardener, Theophrastus, who in his studies of botany and plant diseases, described powdery mildew on roses in 300 B.C. (3, 132).

Different powdery mildew genera infect different host plants. As an example, *Erysiphe* (and *Golovinomyces*) spp. cause powdery mildew infections on ornamentals (begonia, chrysanthemum, and dahlia), cucurbits, legumes, crucifers, beets and tomato. *Leveillula* spp. G. Arnoud infects tomato and cucurbits. *Podosphaera* spp. infects apples, pears, stone fruits (apricot and plum), some ornamentals and cucurbits. The genus *Sphaerotheca* spp. Lev. is generally pathogenic on berries (strawberry, gooseberry), roses, some vegetable crops (including cucurbits), and also stone fruits. The genus *Uncinula* (Schwein.) Burrill causes powdery mildew of grapes. *Blumeria* spp.

(DC.) Speer is the causal agent of powdery mildew on cereals and grasses. *Microsphaera* spp. affects many shade trees and woody ornamentals (azalea, lilac, and rhododendron) (3). A wide variety of vegetable crops and herbs are affected by powdery mildews, including artichoke, beans, beets, broccoli, carrot, cauliflower, collard, cucumber, eggplant, lettuce, melons, okra, parsley, parsnips, peas, peppers, potato, pumpkins radicchio, radishes, squash, tomatillo, tomatoes, and turnips (69).

While powdery mildew diseases are very common and usually distinctive, these fungi have been reported to cause less significant losses than those caused by many other important groups of plant pathogens, such as viruses, downy mildews, rusts and root-rotting fungi (342). Moreover, powdery mildews are typically present every production year in varying degrees do disease pressure.

It wasn't until the 1840s that one causal agent of powdery mildew, *Uncinula necator* (syn*. Erysiphe necator* Schwein.), identified in vineyards in France, caused noteworthy economic losses and nearly destroyed grape production in that country. Young grape leaves were becoming covered with small white powdery spots and as the leaves grew and expanded, the white spots also expanded and covered most of the leaf surface. The disease spread onto the grape berries and these developed a gray "dirty" appearance, resulting in withered and cracked berries. Grape leaves would eventually turn brown to black and die while the grape berries remained small and discolored, becoming unfit for wine production or fresh market. By 1854, French wine production was reduced by 80% due to this new disease (3). It was reported that the yield of wine decreased from about 45 million hectoliters in 1850, during the early stages of the

powdery mildew epidemic, to about 10 million hectoliters in 1854 when this new disease of grapes was at its highest (342).

In the U.S., powdery mildew on a cucurbitaceous crop was first noted by Jagger and Scott (121) in 1925, as a destructive disease of melons being grown in the Imperial Valley of California. While screening cantaloupe plant material from all over the world, the researchers found powdery mildew resistance in a seed lot (PI 78374) originated from India (41, 186, 189). More recently, losses to cucurbit powdery mildew have ranged from 30-50% in Chinese produced cucumbers (114) and have affected roughly 70% of the squash acreage in Florida (235).

Cucurbit powdery mildew is likely the most common disease and important limiting factor in all cucurbit-producing areas of the world (41, 150, 218).This cucurbit disease has been reported in North America (82, 103, 189), Caribbean (101), South America (137, 184, 270, 271), Africa (74, 102, 221, 239), Asia (7, 111, 113, 130, 225, 301), Middle East (4, 44, 117, 131, 286), Mediterranean (80, 329), Europe (10, 143, 165, 218, 260, 310, 322, 323, 331), Australia (169) and New Zealand (23, 107, 236).

This potentially devastating disease of cucurbits has been recognized as economically important and has been extensively studied all over the world, since the early 1800s under field and greenhouse conditions (353). Powdery mildew has been shown to reduce yield by decreasing the size and number of fruits, and length of time these fruits can be harvested (214). Fruit quality and marketability are reduced due to premature leaf senescence which renders fruits exposed and susceptible to sunscald (353). Additionally, powdery mildew infection results in premature or incomplete fruit ripening causing poor flavor in melons, reduced storability in squash, and brittle and

bleached stems handles in pumpkin (235, 256). Other imperfections on fruit rinds such as, speckling, raised indentations and edema may occur. Moreover, powdery mildew infection can predispose cucurbit plants to other important diseases such as some viruses and gummy stem blight caused by the fungus *Didymella bryoniae* (200).

Causal Organisms

The two powdery mildew fungi most frequently reported to cause disease on field and greenhouse cucurbit crops are *Podosphaera xanthii*] and *Golovinomyces cichoracearum* (10, 71, 157, 214, 255, 331). Both pathogens belong to the family Erysiphaceae consisting of 16 genera and approximately 650 species (27). A third endoparasitic species of cucurbit powdery mildew, *Leveillula taurica*, has been considered of minor economic importance and occurs only in warmer areas such as countries surrounding the Mediterranean Sea (73, 164, 329).

Powdery mildews are obligate biotrophic pathogens and cannot survive in the absence of a living host, unless in the form of chasmothecia which are the (teleomorphic) overwintering stage (200). Due to the obligate biotrophy of the powdery mildews, these fungi cannot be cultured on artificial media (161) and require living host tissue to grow and sporulate.

These fungi are typically ectoparasites which grow on the host surface, obtaining nutrients from the host epidermal cells through specialized structures called haustoria (104). In general, powdery mildew fungi induce identical distinctive signs on cucurbits, however with the use of standard light microscopy; the organisms can be easily distinguished based on morphological characteristics (27).

The identification of cucurbit powdery mildew fungi has not always been made accurately. In early literature, the two major species may have been misidentified and

were considered as synonymous (195). For example, since the first report (in 1925) of cucurbit powdery mildew on melon in the Imperial Valley of California by Paulus and others (247) the pathogen on melon and other cucurbit species (248) in the U.S. was generally regarded as *Erysiphe cichoracearum*. In 1968, *Sphaerotheca fuliginea* was named as the cause of powdery mildew without mention of *E. cichoracearum* in an article on control of powdery mildew on cucumber and squash (246) and two other reports on genetic resistance referred only to *E. cichoracearum* (77). In 2004, McCreight (189) published an extensive and interesting review on the change of the causal species of cucurbit powdery mildew in the U.S., demonstrating how the taxonomy and nomenclature of these pathogens have been unclear, and at times inaccurate.

In recent literature, the nomenclature of the two main causal agents of cucurbit powdery mildew has been controversial and, not yet standardized. The organism currently designated as *P. xanthii* has formerly been reported as *Sphaerotheca fuliginea* (Schleccht. ex Fr.) Poll. Other synonyms have included *Sphaerotheca fusca* (Fr.) Blumer emend. U. Braun (71, 179), *Sphaerotheca cucurbitae* (Jacz.) Z.Y. Zhao (108, 225) and *Podosphaera fusca* (255, 325). The other cucurbit powdery mildew pathogen, *Golovinomyces cichoracearum*, has previously been referred to as *Erysiphe orontii* Cast. Emend. U. Braun (71), *Erysiphe cichoracearum* (DC ex Merat) (142) and *Golovinomyces orontii* (Castagne) V.P. Heluta (235). Based on scanning electron microscopy of the anamorphic stages and extensive molecular and phylogenetic work, the genus *Sphaerotheca* has become synonymous to *Podosphaera; G. cichoracearum* synonymous with *E. cichoracearum* (27, 41, 325).

Morphology

Morphological characteristics of anamorphs and teleomorphs have been widely used among researchers to distinguish between powdery mildew genera. Hammett (107) argued that conidial dimensions within the Erysiphaceae fungi overlap, and have little diagnostic value. However, conidial length/width ratios have been reported to reliably distinguish between *P. xanthii* and *G. cichoracearum* (269). Furthermore, since ascal structures vary with environmental conditions and do not develop on all hosts, conidial observations are more generally useful (322).

Cook et al. (52) demonstrated the identification and classification of powdery mildew anamorphs using light (LM) and scanning electron microscopy (SEM) as well as host range tests. The authors indicated that previously undescribed features on the surfaces of powdery mildew conidia, revealed by SEM, reinforced differences observed by traditional light microscopy. Description of conidia germination patterns were also proposed as reliable key to aid rapid identification of powdery mildew anamorphs (51).

Both *P. xanthii* and *G. cichoracearum* produce hyaline, septate and thin-walled mycelia. The hyphae are relatively straight and flexuous, and the hyphal cells are uninucleate and vacuolated. Conidia are colorless (hyaline), uninucleate, one-celled and are produced in chains (24) on the conidiophores. The conidia are readily separated from conidiophores and become airborne with moving air.

Podosphaera xanthii conidiophores have foot-cells of 26-86 x 10-16 µm, associated with 1-3 shorter cells. Conidia are hyaline and smooth, with crenate edge lines formed by chained immature conidia (304). Conidia are primarily ellipsoid to ovoid with absolute values of length and width ranging between 20-39 x 12-22 µm (36, 304). Tomason and Gibson reported length/width ratios of 1.38-1.66 (322), while Frolov and

others reported slightly larger ranges of 1.32-1.76 and 1.57-1.79 for this same species (88, 152).

Mature conidia of *P. xanthii* contain well-defined fibrosin bodies (22). These distinctive refractive particles (not present in *G. cichoracearum*) appear yellow or blue when observed in polarized light (107). Fibrosin bodies are rod-shaped structures measuring between 2-8 µm in diameter and easily be visualized using standard light microscopy when fresh conidia are mounted in 3% aqueous KOH (potassium hydroxide) solution (24).The true nature of these fibrosin bodies remains unclear, however they have been considered taxonomically relevant since first detected by Zopf in 1887 (27, 77).

The conidiophores of *G. cichoracearum* are composed of chains of conidia with sinuate edges (304). Basal foot-cells are 40-140 µm long and 9-15 µm wide, and are usually followed by 1-3 shorter cells measuring 10-30 µm. Fresh conidia are ellipsoid-ovoid to cylindrical-doliform, with length of 25-45µm and width of 14-22 µm, and do not contain fibrosin bodies (160). The germ tubes generally terminate in a club-shaped aspersorium (51). Unlike, *P. xanthii* germ tubes are usually short and forked (22, 23). The length to width ratio for *G. cichoracearum* has been described as being greater than 2.0 (127, 152). Other authors have described ranges of 2.04-2.42 (322) and 1.91-1.96 (88).

Powdery mildew fungi reproduce largely asexually. The sexual stage is controlled by a bipolar heterothallic mechanism (198) and therefore requires two compatible hyphae of opposite mating types for reproduction to occur and give rise to a fruiting body (chasmothecium) which contains one or more ascus, bearing the ascospores

(sexual spores). Nevertheless the role of the sexual process in the pathogen's survival and epidemiology remains unclear. Still, this information could prove essential for developing more effective control strategies because the increased genetic diversity resulting from sexual reproduction could produce new combinations of virulence genes and fungicide resistance genes, making cucurbit powdery mildew management more challenging. When found, the chasmothecia (formerly known as cleistothecia) of *P. xanthii* and *G. cichoracearum* are similar. These ascomata are useful diagnostic morphological features when present. Visually, the chasmothecia are globose, dark brown to black and appear embedded superficially on the mycelium. The presence of one or several asci inside each chasmothecium as well as the morphology of its appendages are also important references and can be useful for species identification. Depending on the species of powdery mildew, a variety of appendages may occur on the surface of the chasmothecium and these appendages are thought to act like hooks or adhesive fasteners, attaching these fruiting bodies to the host (333). In *G. cichoracearum*, the average diameter of the chasmothecia is 85-160 µm with 2 to 25 asci containing only 2 ascospores per ascus. The ascospores are one-celled, primarily straight and their size range is 18-30 x 11-20 µm (25). In the case of *P. xanthii*, chasmothecia diameter has been described as 56-80 x 56-70 µm, containing a single globular ascus with dimensions of 52-70 x 44-56 µm bearing eight elliptical ascospores of 12-20 x 10-16 µm in size (36).

Taxonomic classification

Cucurbit powdery mildews are in the Kingdom Fungi; Phylum Ascomycota; Subdivision Pezizomycotina; Class Leotiomycetes; Order Erysiphales and Family Erysiphaceae (33). In 1753, in his *"Species Plantarum"*, Linneaus mentioned the first

binomial species referring to a powdery mildew, *Mucor erysiphe* (currently known as *Phyllactinia guttata*) (342). Nearly 150 years later, in 1900, Salmon (288) published the first comprehensive monograph of the powdery mildews ("The Monograph of the Erysiphaceae"). The second comprehensive taxonomic monograph regarding these pathogens appeared more than 80 years later, when Braun (24) published the "Monograph of the Erysiphales". Since then, information regarding the biology, host range, distribution, phylogeny and taxonomy of the anamorphic as well as teleomorphic stages of these pathogens has increased immensely (26) especially with the advent of molecular techniques and tools.

Currently, a new and updated monograph has been underway by Braun U. & Cook R. T. A., (26). In this new book, scheduled to be launched at the CBS (Fungal Biodiversity Center of the Royal Netherlands) Symposium on April 2012, the number of recognized powdery mildew species has increased from 515, since Braun's first monograph in 1987, up to approximately 820 species including several revisions and description of new species (26).

Signs and symptoms

Signs of cucurbit powdery mildew disease are easily recognized as white to gray talcum-like fungal growth primarily composed of conidia, which may appear on leaf surfaces, petioles and stems. Fungal hyphae and conidia are produced in abundance forming a white powdery mycelium that resembles talcum powder or dust on the plant surface. These numerous conidia are easily carried to adjacent leaves and plants within a field, as well as to those at greater distance via any air movement.

Symptoms usually develop first on the underside of older leaves, lower in the canopy, which is protected from direct sunlight. Initially, small yellow spots may form on

the upper leaf surface opposite to the powdery mildew colonies. As the disease progresses, the individual fungal spots enlarge and coalesce becoming reddish-brown and necrotic. Heavily infected plants become chlorotic. Affected plants eventually senesce and drop their leaves prematurely leaving the fruits more susceptible to damages caused by sunburn. Cucurbit plants in the field are often not affected until after fruit set. Leaves are reported to be most susceptible 16-23 days after unfolding (200).

Powdery mildew on watermelon

Although all cucurbits are susceptible to powdery mildew, watermelon had been considered to be the most resistant (321, 334). Except for a few isolated and minor cases of the disease on watermelon fruit (Maia, personal observation) (120, 215, 277) powdery mildew had been an occasional problem for watermelon production. However, since 1996 the incidence of watermelon powdery mildew outbreaks has increased and the disease has become an important problem in the major U.S. production areas (65, 128, 275, 305, 318) and in other parts of the world (46, 239, 268, 334). To date, just *P. xanthii*, race 1 (1W) and race 2 (2W), have been identified on watermelon in the U.S. (46, 65, 66, 68, 319).

Since 1996, outbreaks of watermelon powdery mildew have been confirmed in South Carolina, Georgia, Florida, Oklahoma, Texas, Maryland, New York, Arizona and California (63, 65, 128, 305, 319). Watermelon lines with resistance to powdery mildew have been demonstrated in the U.S. (64, 66) and worldwide research is currently underway to find plant introductions with new sources of resistance (63, 66-68, 140, 171, 268, 318, 319, 321).

Early detection of powdery mildew on watermelon can be difficult because the presence of the pathogen is less apparent than on other cucurbit crops (66). Watermelon leaves often begin declining prior to obvious detection of fungal mycelia or conidia. On some highly susceptible cultigens, the petioles and stems can show water soaked areas (319) in addition to yellow blotching on leaves with little or no sporulation, accompanied by a small amount of mycelia, and conidial development on either leaf surface without the characteristic chlorotic spots (65).

Epidemiology and disease cycle

Cucurbit powdery mildews have similar disease cycles. Typically, the fungus produces hyphae and asexual spores (conidia) on lower and older leaves during the crop growing season. From infected leaves, conidia are readily disseminated to healthy adjacent leaves and plants by any air movement or water splashing. Characteristically, in FL, in the absence of the sexual cycle (i.e. production of chasmothecia) windborne conidia land on susceptible healthy tissue, germinate, produce an abundance of mycelia and conidiophores which give rise to more conidia and the perpetuation of the disease cycle (Figure 2-1).

The initial source of cucurbit powdery mildew infection can be difficult to determine due to the fact that conidia are readily airborne and can travel long distances. The disease spreads almost exclusively asexually (via conidia). Possible sources of infection could include cucurbit crops grown earlier in the season, inoculum from greenhouse-grown cucurbits, ascospores stored in chasmothecia on crop debris (200), alternate hosts (242, 243, 249, 313, 324) and production regions in proximity or “upwind”. Studies in the U.S. have demonstrated that powdery mildew conidia are dispersed by wind from southern states where cucurbit are grown earlier, into northern regions (200, 353).

Upon landing on a susceptible host, powdery mildew conidia produce a short germ tube (Figure 2-1), ending in a primary appressorium, from which primary haustoria are formed inside host epidermal cells. Primary hyphae arise from primary appressoria or from other poles of the conidia, and form secondary appressoria from which secondary haustoria are formed. At this stage, morphologically distinct conidiophores emerge vertically (Figure 2-1) from secondary hyphae on the surface of host tissue. At the tip of each conidiophore, 5 or more conidia are produced in single chains. The abundance of hyphae and conidia forms the white mycelium on the surface of the plant tissue and this becomes the characteristic talcum-like sign of powdery mildew infection (254).

Chasmothecia are thought to be overwintering structures and sources of cucurbit powdery mildew inoculum (124, 169, 198, 218, 340). In a 3-year study of powdery mildew of grapes, chasmothecia were confirmed to serve as the primary source of inoculum (250). However, in the case of cucurbit powdery mildew, these structures have not been observed or are rarely found (353) in several important cucurbit producing areas in the U.S. and around the world (47, 103, 198, 212, 218).

Within the family Erysiphaceae, several species are heterothallic and therefore require two compatible hyphae of opposite mating types for sexual reproduction to occur. Following the encounter of these opposite mating types, a small round and dark fruiting body termed chasmothecium (formerly known as cleistothecium), is formed which contains one or more asci, bearing the ascospores (sexual spores). While chasmothecia can be obtained under laboratory conditions, their production in the field varies greatly depending on the region. For instance, in New York State (U.S.), chasmothecia were observed every year from 1989 to 1994, while in other U.S. states,

the occurrence was sporadic (198). The same observations were reported in Europe (12, 181, 328).

Ecology

Powdery mildew develops rapidly under favorable conditions, taking 3-7 days from infection to the appearance of the first symptoms (334). A large number of conidia can be produced in a very short time and remain viable for 7-8 days (200).

Unlike other fungal pathogens, powdery mildews are able to survive and sporulate on host tissue without presence of free water (344). In contrast to most fungal spores, powdery mildew conidia are fully hydrated and germination does not require uptake of exogenous water (333). The combination of factors such as temperature, humidity, sun light (radiation), wind, and rainfall influence dissemination and germination of conidia, mycelial growth and fungal sporulation (342). Rain and free moisture on the plant surface are unfavorable; however disease development occurs in presence or absence of dew (200).

Powdery mildew becomes more severe during periods of low rainfall in the winter and spring months in Florida. The fungus is thought to survive between crop seasons, on wild cucurbit and other weeds year round (235). In successive cucurbit field plantings, older plants planted up-wind, have been reported to serve as source of conidia (200). The role of non-cucurbit hosts as source of inoculum has been insufficiently investigated. An example is a common ornamental plant in the family Verbenaceae, verbena (*Verbena* spp.), which has been reported as a source of inoculum specially for cucurbits transplants grown under greenhouse conditions (59, 200).

Greenhouses and other protective, season extension structures can also allow for the persistence of inoculum between field productions. Greenhouses have been considered sources of powdery mildew since these structures often provide ideal conditions for powdery mildew development due to the microclimate which includes moderate temperatures (20-30°C), high relative humidity (>95%), higher plant density, shading and lower light intensity (245) and constant air movement which favor disease development and spread. In some parts of the world such as Canada (76), Japan (113, 224), Spain (71), Israel (272) and the Netherlands (138, 293), where some types of cucurbit are commercially produced in greenhouses, powdery mildew is a serious concern.

Temperature optimum varies for cucurbit powdery mildew pathogens. Work on conidial germination, carried out by Yarwood and Gardner (345) demonstrated that the temperature optimum for *P. xanthii* ranged from 9-34°C, with 22°C promoting the greatest growth. An isolate from cantaloupe in the hot Imperial Valley of California had an optimum of 25-28°C, whereas an isolate of the same powdery mildew species found in squash in the cooler area of Colma (California) had an optimum of 15°C (124). Nagy (230) showed that the conidial germination of *P. xanthii* was observed at temperatures of 20-30°C with optimum of 22°C and the temperature range for *G. cichoracearum* had a larger interval of 15-30°C with optimum temperature at 25°C. Recently, laboratory experiments established that the temperature range of 15-25°C was optimum for conidial germination of *G. cichoracearum* and 25-30°C for *P. xanthii* (164). Daytime temperatures of 38°C or above have been reported to stop fungal development (200).

Dry atmospheric conditions can favor colonization, sporulation and dispersal of both pathogens, however *P. xanthii* tolerates higher moisture content during the infection process with the highest infection potential at 15°C and 65% relative humidity (14). High relative humidity is favorable for infection and conidial survival but infection can occur at levels as low as 50% (200).

In the Czech Republic, *G. cichoracearum* is the predominant cucurbit powdery mildew pathogen, affecting 70% of field grown cucurbits. In this same country *P. xanthii* has predominated on cucurbits in warmer production areas and glasshouses (143). It has been widely shown that both cucurbit pathogens may occur either singly or together. Mixed infections were found in 10 to 40% of the samples collected in the Czech Republic from 1995 to 2003 (143). In a subsequent study, Kristokova et al. (144) found that *G. cichoracearum* was found with *P. xanthii* as mixed infections in 28% of the locations surveyed in different parts of Europe. Infections by *P. xanthii* alone occurred only in up to 5% of the Czech samples during 1995-2003 periods (143). In contrast, 83% of cucurbit powdery mildew infections detected in France were caused by *P. xanthii* (264). Comparatively, in the U.S., *P. xanthii* alone appears to be the most important cause of powdery mildew on commercial cucurbits (189). In a survey carried out from 2004 to 2006, researchers in India reported the occurrence of cucurbit powdery mildew pathogens on 35 wild plant species (249).

Host range

Powdery mildew pathogens have a broad host range comprised strictly of angiosperm species. These plant pathogenic fungi have been shown to infect leaves, stems, flowers and fruits of nearly 10,000 plant species (98). Conflicting views on host

range and on the species concept within the Erysiphaceae have arisen (27) with recent advances in molecular and phylogenetic analyses (26, 98, 312, 314).

Hosts of *P. xanthii* include economically important families such as Asteraceae (Compositeae), Cucurbitaceae, Laminaceae, Fabaceae, Solanaceae and additionally Scrophulariaceae and Verbenaceae (78, 176, 255, 265). The genus *Golovinomyces* has a broad and overlapping host range including the Asteraceae, Beraginaceae, Scrophulariaceae, Cucurbitaceae, Solanaceae and Laminaceae. Field samples of *G. cichoracearum* from varied hosts belonged to six distinct RFLP haplotypes with each haplotype specific to either a single host or to a set of related host species (160). Recent studies of the phylogenetic relationship between *G. cichoracearum* isolates from Australia and isolates from the northern hemisphere resulted in the distinction of 6 lineage groups based on host range, foot cell morphology and chasmothecia (55).

Reports from the U.S. have also identified *G. cichoracearum* as the causal agent of powdery mildew on *Coreopsis* spp. L. (Asteraceae) (99, 299). In 2004, researchers in California described the first occurrence of *G. cichoracearum* on potato (Solanaceae) plants from three fields, based on morphology and polymerase-chain reaction (PCR) (279). Gevens et al. (95) described the first occurrence of *G. cichoracearum* in sunn hemp (*Crotalaria juncea* L., Fabaceae) in Florida based on morphology and amplification via PCR genomic sequence variation.

Adam et al. (1) reported a single isolate of *G. cichoracearum* recovered from *Arabidopsis thaliana* L. (Brassicaceae), to be pathogenic on five cucurbit species including cucumber, watermelon and pumpkin (189). *Golovinomyces cichoracearum* has also been reported on other cucurbit species such as *Momordica balsamina* L.

(balsam apple), *Trichosanthes dioicia* Roxb. (pointed gourd), *Lagenaria vulgaris* (Molina) Standl. (bottle gourd), *Coccinia cordifolia* (L.) Voigt. (scarlet gourd) and *Benincasa hispida* (Thunb.) Cogn. (wax gourd or winter melon) (322). Recently, reports of *G. cichoracearum* on important ornamentals and medicinal herbs such as zinnia (*Zinnia elegans* Jacq.) (243), gerbera (*Gerbera jamesonnii* Bolus ex Hook. f.) (324) and chamomile (*Matricaria chamomilla* L.) (242) have been confirmed.

Population biology and genetic diversity

Both *G. cichoracearum* and *P. xanthii* are characterized by great pathogenic variability represented by existence of different pathotypes and races (123, 146, 147, 162, 190, 260, 328). To date, the identification of races of *P. xanthii* and *G. cichoracearum* has been based upon the differing disease responses on several muskmelon (*Cucumis melo*) cultigens (41, 158, 163, 320). Eleven muskmelon differentials have been widely used as race differentials, including: Iran H, Vedrantais, Top Mark, Ananas, PMR 45, PMR 5, WMR 29, Edisto 47, PI 414723, PI 124112 and MR-1 (190).

Physiological races of *P. xanthii* on muskmelon were first recognized more than 70 years ago, in the Imperial Valley of California, when races 1 and 2 of the pathogen overcame resistance in ‘PMR-45’ melon (121). In 1976, race 3 of *P. xanthii* was first observed in the U.S. in Texas. (193). Since then, new races continue to be described in the U.S.(188, 190, 191, 193) and all around the world (113, 137, 144, 145, 163, 271, 296, 323, 328).

Currently, close to 30 distinct physiological races of *P. xanthii* (39, 190, 193) and two races of *G. cichoracearum* (11, 158, 162, 264, 322) have been described based on the differing responses of various muskmelon (*Cucumis melo*) cultigens to these

pathogens. Worldwide, many other muskmelon cultigens have been considered as race differentials (41, 185, 188, 190, 298, 347). Recently, watermelon cultigens have been investigated as potential differentials (319, 321). It will be imperative that researchers of watermelon and muskmelon powdery mildew resolve disparities in race identification. In a current review, Lebeda et al. (158) propose that the international cucurbit powdery mildew research, breeding, seed and production communities use a unified and uniform system of cucurbit powdery mildew determination and denomination. Additionally, they propose a uniform screening methodology based on leaf-disk protocol under uniform conditions (161). Two sets of differential cucurbit genotypes for the identification of cucurbit powdery mildew pathotypes and races, as well as an objective, and comprehensive coded system for meaningful and concise designation of pathotypes (sextet code) and races (septet code) (158). The proposed set of differentials for pathotypes screening includes six cucurbit genotypes(*C. melo* 'Vedrantais' and PMR 45, *C. sativus* 'Marketer', *C. pepo* 'Diamant' F1, *C. maxima* 'Golias' and *Citrullus lanatus* 'Sugar Baby'), three cucurbit genera (*Cucumis, Cucurbita, Citrullus*), and five species (*C. sativus, C. melo*, *C. pepo, C. maxima, C. lanatus*), and, ultimately, six unique cucurbit genotypes. Each genotype is arbitrarily assigned a permanent differential order (1-6) and value (1, 2, 4, 8, 16, or 32) for a compatible (i.e. susceptible) interaction. In a given assay with a particular *G. cichoracearum* or *P xanthii* strain, the interactions are scored and then summed to yield a unique sextet code for that strain. Due to their greater number, the proposed set of race differentials is arbitrarily divided into three groups. This set comprises 21 genotypes of the single species, *Cucumis melo*. The race differentials are assigned an arbitrary permanent order with a group prefix (1-3) and

value (1, 2, 4, 8, 16, 32 or 64) within each group. The binary results of any assay are then translated into a triple-part, septet code and one part for each group of seven differentials. The three sums are then presented as a unique code in the format: sum of group 1, sum of group 2 and sum of group 3, which serves as a unique identifier for each race (158). Such detailed and standardized assessment of pathogenic variability will greatly improve our knowledge and exchange of cucurbit powdery mildew resistance. This proposed system gives basis for the application of population biology and genetic studies, which are important for both applied and theoretical research in resistance breeding.

In a survey of the occurrence, distribution and pathogenic variability of cucurbit powdery mildew races in 84 locations in the Czech Republic during the year of 2001, Lebeda et al. (162), reported finding 22 races of *G. cichoracearum* and 4 races of *P. xanthii*. Additionally, they identified 9 different pathotypes, 6 of *G. cichoracearum* and 3 of *P. xanthii*. Sedlakova and Lebeda investigated the dynamics of temporal changes in cucurbit powdery mildew populations from 2001 to 2004. Their findings indicated that among the 180 isolates examined, 16 different pathotypes were found (10 of *G. cichoracearum* and 6 *P. xanthii*); 63 races of *G. cichoracearum* and 26 races of *P. xanthii* (296) were identified. Based on their results, the researchers suggested that pathotypes are distinguished by host range on the most important cultivated cucurbit types and that races are distinguished by the level of virulence on a set of muskmelon differentials which have different resistance factors (158).

The exact number of physiological races of *P. xanthii* varies greatly depending on research groups and the focus of the research program. Inconsistencies in the reactions

of muskmelon cultigens to cucurbit powdery mildew pathogens have led to confusion and misinterpretation of resulting responses. Plant resistance responses can differ with plant age at the time of inoculation (49), purity of the pathogen isolates (234), differences in environmental conditions in the greenhouse versus the open field, cropping season (42), level or concentration of inoculum (234), and shading (167). Furthermore, Bardin et al. (10) emphasized that race determination in powdery mildew can be confusing because most of the differential melon lines appear to possess several resistance genes, some of which have not yet been characterized. Additionally, it has been shown that the level of resistance varies when plants are tested under different environmental conditions and/or geographical areas. Such differences may also be attributed to variability in the virulence and aggressiveness of isolates present in the natural pathogen populations (145).

Powdery Mildew Diagnosis and Research

With the advent of molecular and DNA analysis techniques, especially polymerase chain reaction (PCR) and sequencing technology, the identification and characterization of cucurbit powdery mildew, as well as other fungal plant pathogens, has greatly advanced (182). While assessment of the phenotypic (and morphological) characteristics is still necessary for identification, current technologies can be employed to investigate species identity and to discriminate variations within species (54, 55). Furthermore, molecular techniques have been particularly useful in the identification of obligate pathogens such as, powdery mildews, as well as fungi that grow very slowly in the culture media (231). In contrast to conventional methods, including pathogen isolation and assessment of morphological characteristics using microscopy, molecular

techniques provide accurate, consistent and reproducible results more rapidly, which facilitates early disease management (232).

The development of gene-specific primers for PCR amplification has facilitated the detection and identification of fungal plant pathogens (337). Based on sequence variations of the ITS region, species-specific primers S1/S2 and G1/G2, specific for *P. xanthii* and *G. cichoracearum* respectively, were recently designed by Chen et al. (36). These primers are able to amplify a portion of rDNA of *P. xanthii* equivalent to 454 bp and to 391 bp for *G. cichoracearum*. Development of these primers allowed successful identification *of P. xanthii* and *G. cichoracearum* in mixed infections by means of multiplex PCR which enabled simultaneous amplification of the different pathogens, in a single reaction, by using more than one pair of primers simultaneously (36).

Accurate determination of the pathogen species is very important not only for disease management, but also in plant breeding programs since different resistance genes may confer resistance to different pathogen species and pathotypes (77). With recent advances in biotechnology, molecular genetic markers have been used for rapid identification of some cucurbit powdery mildews (315, 316). The use of molecular characters, especially ITS (internal transcribed spacer) sequence data, has given promising results for species determination in some powdery mildews, (28, 54, 312-314) still to date, molecular tools have yet to differentiate species to race.

Disease Management

An effective method for management of powdery mildew should include IPM (Integrated Pest Management) components combining use of powdery mildew resistant or tolerant cultivars (genetic resistance), use of biorational compounds, biological control agents, use of synthetic and preventive fungicides as well as compounds that

stimulate host defense mechanisms (222). Accurate knowledge about the population structure of a pathogen is essential to improve the design of appropriate disease management programs as well as to reinforce resistance breeding strategies.

Host resistance

The use of resistant varieties is the simplest, effective, eco-compatible and economical means of controlling plant diseases (70, 201, 307). Powdery mildew pathogens are already present in cucurbit producing regions worldwide and the use of resistant plant cultivars would be the most suitable control strategy (251). Resistant varieties are continuously being developed and are becoming an increasingly important component of management programs all over the world (161, 261, 302, 322, 323, 339, 347, 352). In the U.S., cucurbit breeding programs have conducted extensive research in an effort to increase availability of powdery mildew resistant lines. Most resistance squash and pumpkin varieties in the U.S. contain one or two copies of the same major resistance gene form wild cucurbit species (200). Resistance already exists in melon (110, 190), gourds (140), cucumber (22) and watermelon (63, 66-68, 110, 319, 321).

Temporal avoidance (avoiding high risk seasons)

Adjusting planting dates to avoid conditions favorable to the pathogen is important when managing cucurbit powdery mildew. In Florida, warmer temperatures (10-32°C) and drier conditions from fall through spring typically favor the occurrence of *P. xanthii* (330). Since cucurbit production occurs year round in the state of Florida, this has enabled powdery mildew to become endemic in the state, occurring every year to some degree (235, 330).

Scouting and early prevention in high risk areas and crops

Programmed scouting for powdery mildew symptoms is critical to determine appearance of the very first signs and symptoms of the disease. Especially the underside of older leaves as well as petioles and stems lower (shaded areas) in the canopy should be monitored early and frequently (330). Weekly inspections are recommended especially after fruit set, when plants become more susceptible to powdery mildew disease. Preventive fungicide applications should start when cucurbit vines begin to “run” or to produce fruit (200). Early control of powdery mildew is the most effective strategy for effective control and maintenance of yield and quality of cucurbits (65, 200).

Application of fungicides

Biological and organic fungicides: Global desire to reduce total pesticide load in the environment combined with the higher cost of fungicide applications and limitations in use of some resistant cultivars have led to extensive research into alternative methods for powdery mildew control. Biorational, biological and other eco-friendly strategies have been examined and developed all over the world (16, 135, 287). Greenhouse crops may offer the best opportunities for implementation of biological and non-fungicide strategies for control of powdery mildew and other disease (245). The ability to control environmental conditions, restrictions for pesticide usage, and the higher value of greenhouse grown crops make the use of biocontrol and cultural methods more adequate for greenhouse and protected structures (217, 303).

Several biopesticides are registered for conventional and organic control powdery mildew disease in the U.S. Perhaps the most widely studied biofungicide of many powdery mildew fungi, including cucurbit powdery mildew, is a naturally occurring fungal

hyperparasite *Ampelomyces quisqualis* (75, 134, 136, 258, 259). A commercial formulation of this hyperparasite, AQ10, must be applied preventatively along with a mineral oil or other surfactants (136, 208, 283, 297) to be most effective. Studies of AQ10 in combination with chemical fungicides have been conducted (97, 306).

Another biological control agent registered for cucurbit powdery mildew control in the U.S. is the antagonistic bacteria *Bacillus subtilis* (strain QST 713). Commercial formulations, Serenade and Rhapsody, of this bacterial strain have demonstrated adequate control against *P. xanthii* under controlled conditions (97). Additionally, bioassay studies indicated that lipopetides extracts obtained from cultures of two antagonic strains of *B. subtilis* (UMAF6614 and UMAF6639) were able to arrest conidial germination of *P. xanthii* (syn. *P. fusca*) *in vitro* (282).

In greenhouse and field trials, the microbial products Actinovate AG, Companion, BU EXP 1216C, and BU EXP 1216 (the first formulation containing *Streptomyces lydicus* and the other three, *B. subtilis*) were assessed in Florida on summer squash and cantaloupe against powdery mildew. Applications of these inoculants alone or alternated with a half-rate of conventional fungicide Procure 480SC (triflumizole) was evaluated. In greenhouse experiments, the product BU EXP 1216S significantly reduced the disease severity by nearly 70% relative to the water control but was not significantly different from that obtained with Procure 480SC, in two of four greenhouse experiments. The untreated water control, BU EXP 1216C and BU EXP 1216S, when applied alternately with Procure 480SC, consistently promoted plant growth. Alternating applications of all four products with Procure 480SC resulted in significantly less powdery mildew disease than in the water control alone (351).

Field trials conducted in Arizona, comparing the efficacy of the biopesticide Actinovate (*Streptomyces lydicus* Waksman & Henrici), Kaligreen (potassium bicarbonate) and Procure (triflumizole), applied alone or within a rotation program with each other, for control of powdery mildew on cantaloupe showed reduction of disease after five applications of Actinovate or Kaligreen (72 and 59%, respectively) alone at weekly intervals (183).

Cucurbit powdery mildew control with oils and mineral salts has been evaluated. In laboratory and greenhouse studies, JMS Stylet-Oil significantly slowed the expansion of powdery mildew colonies from both natural infections and artificial inoculations (209).Cucumber plants treated with 0.5 and 1% olive oil and with potassium silicate respectively, demonstrated significant powdery mildew control (252). Evaluation of black seed oil (*Nigela sativa L*.) against powdery mildew of cucumber revealed that at a concentration of 0.5%, this oil significantly reduced the severity of *P. xanthii* from 52% in the control plants to nearly 8% on leaves sprayed with black seed oil. Additionally, toxicity tests and microscopic examinations demonstrated that hyphal growth and conidial germination were greatly compromised (105). In greenhouse experiments, mineral oil SunSpray Ultra-fine combined with 0.5% bicarbonate salts (sodium and potassium) significantly reduced powdery mildew (6.1% and 5.9%, respectively) with inhibitory affects lasting for 10 days after treatment (354). Studies demonstrated pre-inoculation with salts (K_2HP0_4, KH_2P04 + KOH, KNO and $NaHCO_3$) controlled powdery mildew on leaves of greenhouse-grown cucumbers as effectively as the systemic fungicide pyrifenox (272). Biocompatible products with low toxicity and adjuvants for control of powdery mildew, specifically on cucurbit crops, have been studied. The

paraffinic oil JMS Stylet Oil suppressed powdery mildew in field grown squash. Powdery mildew severity was significantly lower than non-treated plants for cucurbits receiving applications of 0.75% JMS Stylet-Oil initiated 5 days post inoculation in greenhouse studies and with applications initiated after disease detection in the field (208).

The use of plant extracts and plant compounds to control cucurbit powdery mildew has been investigated. Soil applications of bio-fertilizers combined with organic mulch and foliar spray of fermented garlic were reported to reduce the incidence of powdery mildew on organic melon produced under greenhouse conditions (267). Tests with Milsana (leaf extracts from the giant knot weed *Reynoutria sachalinensis* F. Schmidt ex Maxim. applied to powdery mildew susceptible ('Mustang') and tolerant ('Flamingo') cucumber cultivars, increased the accumulation of phenolic compounds in the cucumber plants and resulted in an improved antifungal activity significantly reducing the incidence of *P. xanthii* (56-58).Similar results were obtained by Fofana et al. Plants treated with Milsana were significantly less infected compared to control plants and this protective effect against powdery mildew was maintained over time (86). Rongai et al. studied the effects of a vegetable fungicide on cucumber powdery mildew (*G. cichoracearum*). Formulations consisting of a dispersion of Brassicaceae meal in vegetable or mineral oils on infected muskmelon plants cultivated under plastic tunnels were tested in comparison to each oil separately. Both formulations containing Brassicaceae meals, caused 94% of powdery mildew conidia to be distorted while for the untreated controls only 2% were distorted (284). Numerous other studies have demonstrated the effectiveness of compounds with antifungal activities which promote

host-induced defenses and could potentially be used to control cucurbit powdery mildews (38, 106, 280, 283, 311)

Foliar applications of soluble silicone (Si) for the control of powdery mildew of several crops have been widely reported (227). An assessment of concentration (0, 250, 500, 750 and 1000 mg l^{-1}), frequency of application (1 to 3 times per week) and runoff (covered and uncovered pots) of soluble silicon (K_2SiO_3) on the severity of *P. xanthii* on zucchini was carried out. Results indicated that in combination with the surfactant Break-Thru, treatments with Si reduced powdery mildew severity significantly. While the effect of concentration was not significant, spray frequency had a significant effect on Si efficacy. Using the same concentration of Si, efficacy was increased initially by 30% and almost doubled when the spray frequency was tripled. An overall increase of 17% (Trial 2) and 18% (Trial 1) in disease reduction occurred on plants in uncovered pots, where Si was allowed to reach the rhizosphere, compared to the covered pots (317). The effects of suppression of powdery mildew caused by *P. xanthii* (syn. *S. fuliginea*) on hydroponically-grown cucumber by addition of silicone to nutrient solution was demonstrated to be inhibited at high temperatures (24 to 32°C) in Florida when compared to the same growing conditions in experiments conducted in Canada (294).

Evaluation of cow's milk and whey for the control of powdery mildew in organic crop productions has been widely investigated. Milk-based foliar sprays have been reported effective against powdery mildew (*Uncinula necator*) on grapes (53). Reports have indicated that milk-based foliar sprays can effectively reduce both the signs and symptoms of natural *P. xanthii* infections on pumpkin and muskmelon (85) and on tomato powdery mildew caused by *Leveillula taurica* in the field (309). In greenhouse

experiments, milk whey sprayed twice a week at concentrations >10% reduced severity on cucumber powdery mildew by 71–94% and zucchini powdery mildew by 81–90% in comparison to 40 and 50%, respectively, in control plants of cucumber and zucchini squash at 15 days after first application (19). Efficacy of different types of cow's milk and combinations of milk and *Lactobacillus* on powdery mildew of pumpkins demonstrated better suppression of *P. xanthii* (syn. *S. fuliginea*) with raw (unpasteurized, unhomogenized) cow's milk alone (348). According to Medeiros (216), the use of milk and whey is, in general, less expensive than fungicides and has the advantage of achieving the same level of control. The viability of whey in disease control is dependent on cost and benefits for the grower, which in turn will depend on the availability of the product and transportation costs from the dairy industries to the farm where the product will be applied (19).

Conventional fungicides: Presently, conventional fungicides are the most effective for managing cucurbit powdery mildew. There are numerous currently-registered fungicides available (34, 200). For conditions in Florida, fungicides should be applied immediately if powdery mildew symptoms are present, reapplying on a 7 day interval. Although fungicides for cucurbit powdery mildew management can limit disease severity, the level of protection in some crops and regions has not been sufficient. Worldwide reports of reduced or lack of powdery mildew control have been indicated (60, 83, 96, 108, 118, 166, 179, 201, 202, 296, 326, 340, 341). This is likely due to the appearance of new pathogen strains insensitive to the single-site mode of action of the synthetic fungicides, which consequently lose their efficacy over time (34, 203). To prevent this, defense strategies that minimize the risk of development of resistant

strains must be adopted. Therefore, the recommendation of rotation of fungicide modes of action and limitation on the number of treatments with the same active ingredient is highly advisable (34).

Fungicide resistance

It is widely known that successful management of powdery mildew in cucurbit crops is challenged by the pathogen's potential for fungicide resistance development and the appearance of new pathogenic strains. Proper timing is critical to ensure that fungicide applications are made at appropriate times. It is important to assess efficacy of fungicides and include protectant fungicides, which are not at risk for resistance development, in a fungicide spray program (207). Fungicides with mobile (systemic, translaminar or volatile) activity are ideally recommended for protection and some residual activity against cucurbit powdery mildew on the underside of leaves, where conditions are more favorable for early disease development (199, 203, 321). Tank mixing with contact fungicides has been recommended in some regions to avoid development of fungicide resistance (203).

Strains of *P. xanthii* have been detected with resistance to as many as eight classes of fungicides (77, 161, 201, 210, 236, 296, 302). In a study on dynamics of fungicide resistance, McGrath and Shishkoff (207) concluded that it was not possible to predict the efficacy of fungicides based on the frequency of resistant strains the previous year. After one application of triadimefon and benomyl, the pathogen population rapidly shifted within two weeks, to predominantly resistant strains and consequently, more than one application did not provide additional disease control

Continued field observations are essential when implementing a fungicide program. If and when poor disease control appears following a spray of high risk-for-

resistance materials, growers should consider seeking assistance from extension crop consultants to determine if resistance has developed.

Table 2-1. Major cucurbit producing countries and production estimates for 2009

Cucumber		Watermelon		Squash, pumpkin and gourds		Melons	
Country	Prod. (tons)	Country	Prod. (tons)	Country	Prod. (tons)	Country	Prod. (tons)
China	44,250,182	China	65,002,319	China	6,506,966	China	12,224,801
Turkey	1,735,010	Turkey	3,810,210	India	4,108,510	Turkey	1,679,190
Iran	1,603,740	Iran	3,074,580	Russia	1,123,360	Iran	1,278,540
Russia	1,132,730	Brazil	2,056,310	U.S.A.	749,879	U.S.A.	1,069,980
U.S.A.	888,180	U.S.A.	1,819,890	Egypt	700,000	Spain	1,007,000
Ukraine	883,000	Egypt	1,500,000	Iran	674,545	India	830,244
Spain	700,000	Russia	1,419,030	Mexico	577,067	Egypt	750,000
Japan	620,200	Uzbekistan	1,071,000	Ukraine	559,900	Morocco	730,000
Egypt	600,000	Algeria	1,034,720	Cuba	413,191	Mexico	552,371
Indonesia	575,995	Mexico	1,007,160	Turkey	411,942	Italy	520,800
Poland	480,553	S. Korea	900,000	Guatemala	455,556	Bangladesh	340,249
Netherlands	435,000	Spain	819,100	Brazil	402,959	Argentina	332,663
Mexico	433,644	Syria	749,695	France	301,724	S. Korea	330,000
Iraq	420,945	Greece	656,379	Pakistan	300,000	Italy	315,700
S. Korea	400,000	Morocco	650,000	Saudi Arabia	243,866	Indonesia	313,611
Total (World)	60,555,572		98,047,947		22,141,401		25,504,704

(Source: FAOSTAT)

Table 2-2. United States cucurbit production in 2010

2010 Crop		Production (tons)	Area harvested (ha)	Value (US$1,000)
Watermelons		1,866,669	53,661	492,035
Cantaloupes		854,477	30,242	314,379
Pumpkins		481,897	19,627	203,592
Cucumbers	(fresh)	384,737	17,766	193,643
	(processed)	542,600	39,457	180,845
Squash		296,740	17,604	116,539
Honeydews		145,331	5,949	49,608
Total (USA)		4,572,451	184,306	1,550,641

(Source: NASS/USDA)

Table 2-3. Comparison of production, area and value of the major U.S. states for cucurbit crops in 2010

2010 Crop	State	Prod. (tons)	Rank	Area (ha)	Rank	Value ($1000)	Rank
Watermelon	Florida	340,330	1	9,956	2	112,545	1
	Georgia	304,814	2	9,713	3	75,936	3
	California	284,402	3	4,452	4	83,391	2
	Texas	282,361	4	10,077	1	52,290	4
	Indiana	128,820	5	2,873	6	32,376	6
	S. Carolina	119,748	6	3,238	5	35,640	5
	Total U.S.A.	1,866,669		53,663		492,035	
Cantaloupe	California	495,323	1	15,783	1	134,316	1
	Arizona	221,716	2	8,418	2	97,271	2
	Georgia	68,039	3	2,024	3	51,000	3
	Colorado	18,960	4	890	6	7,984	5
	Indiana	17,735	5	931	5	6,178	6
	Texas	13,472	6	1,093	4	9,266	4
	Total U.S.A.	854,477		30,243		314,379	
Cucumber	Florida	105,233	1	4,695	1	47,792	2
	Georgia	96,388	2	3,440	2	51,000	1
	Michigan	40,959	3	1,740	4	20,498	3
	California	37,739	4	1,295	5	16,224	5
	New Jersey	30,481	5	1,295	6	15,725	6
	N. Carolina	30,436	6	2,469	3	11,743	7
	Total U.S.A.	384,737		17,766		193,643	
Pumpkin	Illinois	193,865	1	6,111	1	15,667	5
	California	84,368	2	2,509	6	18,786	2
	New York	66,315	3	2,752	4	35,088	1
	Ohio	50,077	4	2,792	2	16,670	3
	Pennsylvania	44,089	5	2,711	5	16,524	4
	Michigan	43,182	6	2,752	3	13,804	6
	Total U.S.A.	481,897		19,628		116,539	
Squash	Michigan	59,874	1	2,671	2	12,144	6
	California	54,431	2	2,428	3	34,017	3
	Florida	49,532	3	3,683	1	56,784	1
	New York	40,687	4	1,862	4	36,777	2
	Georgia	21,772	5	1,619	5	15,360	4
	New Jersey	16,874	6	1,255	7	10,304	7
	Total U.S.A.	296,740		17,604		203,592	
Honeydew	California	107,275	1	4,452	1	31,218	1
	Arizona	28,123	2	1,255	2	12,586	2
	Texas	9,934	3	243	3	5,804	3
	Total U.S.A.	145,331		5,949		49,608	

(Source: NASS/USDA)

Table 2-4. Cucurbit production in Florida for 2008-2009 seasons

2008-2009 Crop		Production (tons)	Area harvested (ha)	Value (US$1,000)
Watermelon		370,993	10,441	135,771
Cucumber	(fresh)	120,474	4,573	78,618
	(processing)	49,000	2,833	22,932
Squash		51,891	3,561	51,480
Total (FL)		592,358	21,408	288,801

(Source: FACS/NASS)

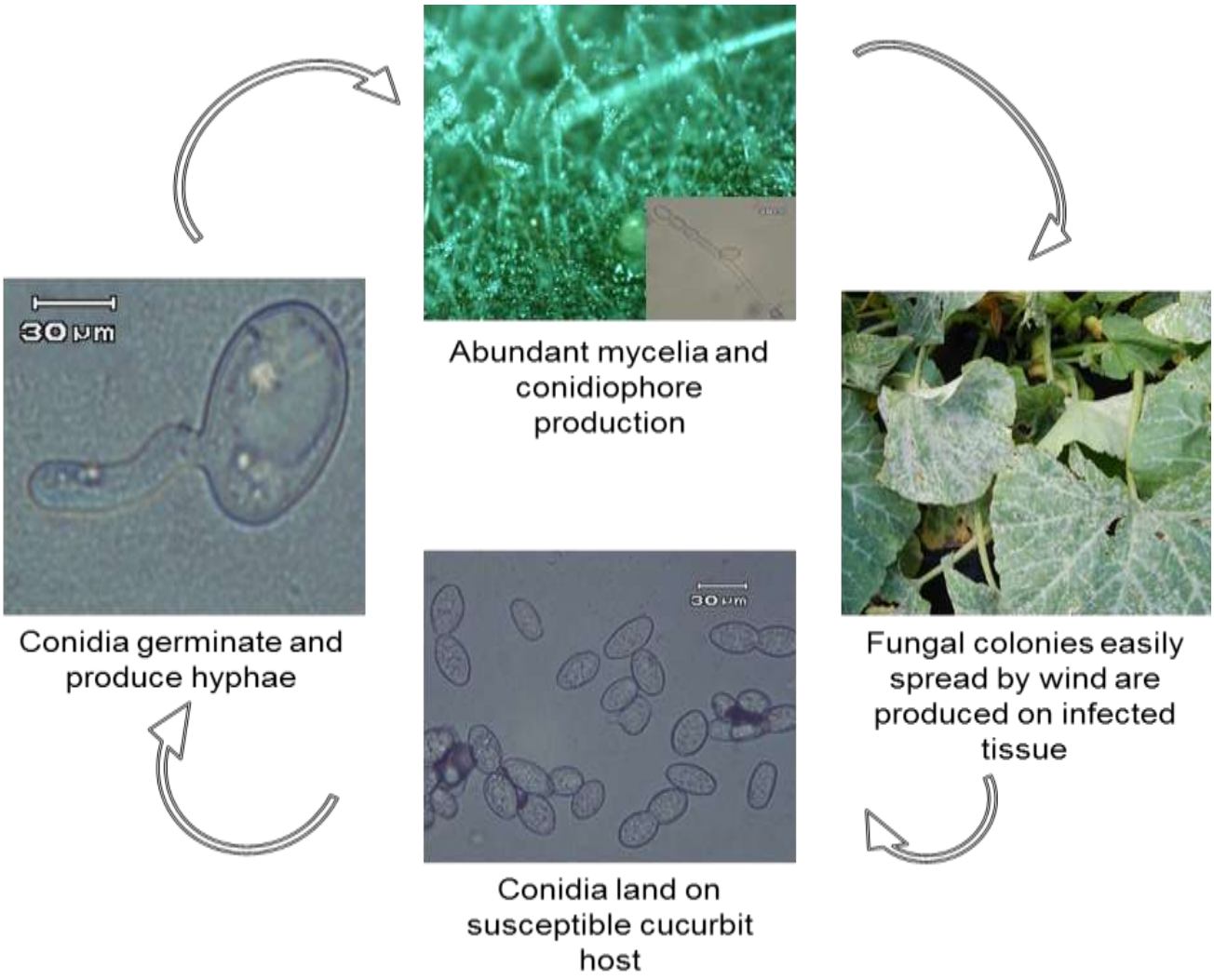

Figure 2-1. Disease cycle of cucurbit powdery mildew, illustrating the asexual stage.

CHAPTER 3
CUCURBIT POWDERY MILDEW ISOLATE COLLECTION, MAINTENANCE AND CHARACTERIZATION

Biotrophic fungi such as rusts, downy mildews and powdery mildews sporulate only on living tissue (285). As an obligate parasite, the cucurbit powdery mildew fungi cannot be cultured *in vitro* on artificial media. These pathogens have been traditionally cultured and maintained by periodical transfers of mycelia, conidia and conidiophores to fresh plant material (253).

The use of leaf disk assays for obligate pathogen maintenance in plant pathology research has been a common practice for many years and in many parts of the world (32, 115, 173, 175, 237, 266, 338). Traditionally, researchers working with cucurbit powdery mildew characterization (80, 174, 229, 230, 305), breeding programs (22, 40, 45, 261) and fungicide resistance (61, 108, 213, 219) have used leaf disks as a fast and convenient bioassay. However in some cases, leaf disks and whole-plant inoculations have yielded inconsistent results (17).

Factors such as host plant age, method of inoculation, and source of inoculum, inoculum density, relative humidity, and light intensity influence colonization and sporulation of powdery mildew fungi. High relative humidity (> 95%), relatively warm temperatures (24-30°C), low light intensity, and vigorous plant growth contribute to pathogen sporulation. In general, though high relative humidity is required, infection has been reported to occur at lower relative humidity and free water on plant surfaces has been considered detrimental (35, 235, 273, 343).

Throughout decades of cucurbit powdery mildew research, some of the most common inoculum application methods have been direct leaf-to-leaf contact (292), use of whole plants as inoculum source (354), and dusting of plants with inoculum (140,

151, 226). Powdery mildew inoculation originating from discrete fungal colonies maintained *in vitro* have been widely demonstrated and used to maintain pathogen cultures which can be considered homogeneous (161).

There are contradictory reports on the harmful effects of suspending powdery mildew conidia in water for use as inoculum (234). Some researchers have had successful inoculation with spore suspensions of *S. fuliginea* (syn. *P. xanthii*) and *E. cichoracearum* (syn. *G. cichoracearum*) (116, 344). Others have found that suspension inoculation methods result in poor uniformity of spore deposition, clumping of spores, and consequently inability to accurately quantify spore deposition.

Limited information is available in the literature on the effect of techniques for *in vitro* production and maintenance of isolates for disease establishment of *P. xanthii* under laboratory conditions. In this research, I developed and optimized protocols for obtaining, producing and maintaining powdery mildew subcultures for assessment of pathogen morphology, genetic variability and race identification collected from cucurbits in northern Florida.

Materials and Methods

Field Site Trials

Field experiments were conducted in north Florida at two University of Florida (UF) research stations. One field site was at the North Florida Research and Education Center – Suwannee Valley (NFREC-SV) in Live Oak in Suwannee County, FL. The second field site was at the Plant Science Research and Education Unit (PSREU) in Citra in Marion County, FL (Figure 3-1).

At both locations, different cucurbit types were planted in adjacent rows on raised beds covered with plastic mulch. At Live Oak, plots consisted of 6 rows spaced 150 cm

apart covered with black plastic mulch. There were 16 plots per row and each plot was 275 cm long. Each plot contained five plants spaced 46 cm apart. Plots were separated by 92 cm between plots in the same row (Figure 3-2). Two susceptible cucurbit hosts, butternut winter squash (*Cucurbita moschata* L. cultivar Butterbush) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. cultivar Mickey Lee) were planted at the ends of each row to serve as inoculum sources. The end plots were considered buffer rows and measured 305 cm long. In addition to the susceptible hosts, 22 advanced *Cucurbita* breeding lines (Rupp Seeds Inc., Wauseon, OH) were evaluated for susceptibility to powdery mildew in FL. All plots were replicated four times in a randomized complete block design.

At Citra, the field trial was comprised of 6 rows, on reflective silver mulch, spaced 200 cm apart and with 3 plots per row (Figure 3-3). Each plot was designed in the same manner as in Live Oak. The powdery mildew susceptible cucurbit hosts, 'Butterbush' and 'Mickey Lee' were planted at Citra in a randomized complete block design with 8 replications.

At both locations, field soil was fumigated prior to planting and row middles were sprayed for weed control, at least one month before planting. During the growing season, insect pests and weeds were managed as needed by mechanical cultivation (no insecticides or fungicides were applied) and hand-weeding of rows was done throughout the experiments. The plots were subsurface drip irrigated and fertilized as needed to maintain plant health.

Plant Material for Field Experiments

Two seeds of each, butternut winter squash ('Butterbush') and watermelon ('Mickey Lee'), were direct-seeded on 31 March 2009 at row ends in Live Oak. Each

alternating row end contained only one species of susceptible cucurbit host. On 16 April 2009, butternut winter squash ('Butterbush') and watermelon ('Mickey Lee') were direct-seeded into all remaining test plots. Due to lack of germination, susceptible cultivars were direct-seeded again on 23 April 2009 at Live Oak.

At Citra, both 'Butterbush' and 'Mickey Lee' were direct seeded into all plots, on 23 April 2009 in the same manner as in Live Oak. Additional seeding was done on 2 June 2009 to compensate for poor germination.

Origin and Collection of Cucurbit Powdery Mildew Samples

Cucurbit powdery mildew isolates used in this study originated from infected leaves of butternut winter squash 'Butterbush', collected at both research stations in north FL (Live Oak and Citra), as well as from additional cucurbit species collected from south west and north east FL (samples sent by Dr. Pamela Roberts and Mr. Gene McAvoy) during 2008 and 2009.

Occurrence of cucurbit powdery mildew was monitored weekly at both research sites in north Florida during spring and fall of 2009. At both field sites, the powdery mildew pathogen population was naturally-occurring.

Over the course of 9 weeks, during the fall of 2009, powdery mildew colonies were collected from the same 2 'Butterbush' plants at each of the 2 research stations in north Florida. From each plant, 3 leaves were excised for the collection of a total of 9 powdery mildew colonies per collection day per site. At the end of a 9 week period, 126 isolates had been sampled from each field site, which represented a total of 252 cucurbit powdery mildew isolates to be maintained *in vitro* at the laboratory, until morphological and genetic analysis were performed.

Weekly sampling was done in the spring season of 2009 during a consecutive 9 week period at Live Oak and 7 week period at Citra. Leaves were removed only after assessment of powdery mildew distribution within each location and disease severity ratings had been recorded for all cucurbit plants. Leaf samples were collected from Live Oak filed site on 19 and 26 May, 2, 16 and 23 June, 1, 10, 15 and 23 July. At Citra, leaves were collected on 23 June, 1, 10, 15, 23 and 28 July and 3 August.

A second set of samples were collected, in the same manner described above, at the Citra field site, during the fall of 2009 for a period of 5 consecutive weeks. Forty-five, powdery mildew isolates were collected during this time period. Fall season samples were collected on 13, 20 and 29 October and 3 and 13 November.

Each field (single colony) isolate was assigned a code detailing location and timing of collection from each field site (LO=Live Oak and Ci=Citra) during each time period (week 1- week 7 and week 8- week 12), according to susceptible host plant (plant 1 or 2), number of leaves sampled per plant (A, B or C) and the number of discrete colonies taken from each leaf (1, 2 or 3) (Table 3-1).

Since different susceptible host plants were grown at each field location, individual leaf samples were placed in separate labeled and sealed plastic bags and analyzed separately in the laboratory. Leaf samples were kept under refrigeration (4°C) until preparation for morphological and genetic analysis of each sample could be completed. For comparison, additional cucurbit samples infected with powdery mildew were collected at other locations in Florida (Table 3-2) and sent or brought to our laboratory. Morphological data were collected and single colony field isolates were frozen (-20°C) for subsequent molecular analysis.

Plant Material for Laboratory Experiments

Since powdery mildew pathogens cannot be cultured on artificial growth media, it was necessary to maintain all fungal isolates on living host tissue. Traditionally, leaf disk bioassays were maintained on amended water agar to prevent early leaf senescence (13, 40). In our work, rather than small leaf disks, we used detached whole leaves (cotyledons and primary leaves) as substrate for powdery mildew maintenance. Within 24 to 48h of field collection, infected leaf tissue ('Butterbush') was inoculated onto detached leaflets and/or cotyledons of various susceptible cucurbit hosts.

Preliminary tests were conducted to determine which commercially available cucurbit cultivars were most susceptible for enhancing powdery mildew sporulation. Depending on seed availability, speed of seed germination and abundance of fungal sporulation produced on host tissue, different cucurbit hosts were grown for periodic isolate transfer, subculture and maintenance. Cucurbitaceous cultivars such as cucumber ('Straight Eight' and 'Poinsett 76'), pumpkin ('Big Max'), watermelon ('Mickey Lee'), muskmelon ('TopMark' and 'Hales Best Jumbo') and *Cucurbita* spp. ('Waltham', 'Table Queen', 'Dark Green Zucchini', 'Butterbush', 'Long Island Cheese' and 'Table Ace') were evaluated.

Cucurbit seedlings for all laboratory experiments, were grown in a 24 ± 3°C growth room in styrofoam growth trays at the University of Florida, Gainesville, FL. Trays were maintained on a bench top under fluorescent lighting with 12 hour photoperiod. Susceptible host seeds were sown every 10 to 15 days to maintain a continuous supply of fresh leaves to be used in laboratory bioassays. Styrofoam tray cells were filled with Fafard Professional 4P Mix potting media (Conrad Fafard Inc. Agawam, MA), and one

seed of each cucurbit type was sown per cell. Throughout the experiments, plants were observed daily and seedlings were watered as needed.

Seedlings were ready for bioassays at cotyledon stage (7 to 10 days old) or at 2 or 3 true-leaf stage (3-4 weeks old), depending on cultivar (Figure 3-6). To further prevent powdery mildew contamination, the air conditioner filter was cleaned regularly and structures resembling cubes made of PVC pipes and covered with organza cloth were built and placed over growing trays. Growth room doors were kept closed at all times while working inside the growth room.

Optimization of Living Culture Technique for Cucurbit Powdery Mildew

Detached intact cucurbit leaflets and cotyledons were used rather than leaf disks to prolong the time in which cultures were viable and available for morphological and molecular characterization. During preliminary studies, we observed that detached leaf tissue could be maintained and rooted on 2% water agar media (Figure 3-4).

In initial tests, aside from maintaining isolates in Petri dishes (10cm in diameter), leaflets were also grown in 1mL test tubes containing water agar. This method as successful as leaflets inserted into test tubes rooted in to the water agar and fungal sporulation was abundant (Figure 3-5). The test tube technique required less media and was space efficient. Additionally, older and larger leaves could be used for inoculations and isolate maintenance (Figure 3-5). Test tubes were kept inside aluminum trays (5 cm deep) lined with paper towels and had moistened cotton balls placed at each corner of the trays to provide humidity (Figure 3-5). Each tray contained a single isolate inoculated onto all leaves. Trays were kept at room temperature on the laboratory bench, were covered with plastic bags and were sealed with twist ties. While effective and space efficient, the use of test tubes was laborious and tubes held only a small

amount of media, which at room temperature tended to dry faster (5-7 days) than Petri Plates resulting in wilting of leaves.

Cucurbit Powdery Mildew Isolation, Multiplication and Maintenance

Monoconidial cultures have been used for *in vitro* multiplication of powdery mildew isolates in past research. Traditionally, a single powdery mildew conidium is taken from an infected sample and is transferred onto healthy susceptible tissue. Artificial inoculation procedures described in the literature have included methods such as dusting of conidia (140), use of sterile brushes (170, 223, 311), rubbing of conidia (298), use of spore suspension (6, 40, 225, 352), use of eyelash or hair method (212) and mass transfer of conidial chains (211).

Preliminary artificial inoculation studies were carried out to evaluate which method would be most efficient for single-spore isolation and conidial transfer for high volume of samples. Initially, an eyelash and hair affixed to a disposable pipette, as described by McGrath et al. (213) was used without consistency. Alternatively, hand-made glass needles produced from a pair of Pasteur pipettes as described by Goh (100) were prepared and experimented, however glass tips were still too thick to transfer a single conidium. Additionally, a small piece of optical fiber was used as a tool to transfer a single conidium; however this material was flexuous and did not provide support for fungal transfer. The optical fiber was kindly provided by Dr. Huikai Xie (Associate Professor, Department of Electrical & Computer Engineering at University of Florida). Mini ophthalmological surgical blades (BD Micro-Sharp blade, 1.5mm depth) were investigated. Under a stereoscope, these mini blades were used to remove a single conidium from the top of a conidiophore, and directly transfer the conidium to healthy tissue. This approach was unsuccessful because blade tips were too large compared to

the size of conidia. Alternatively, these mini blades were used to cut out a small piece of water agar (2%) poured into small Petri plates (6 cm diameter) and had been coated with a suspension of powdery mildew. This suspension was prepared by rinsing, with sterile distilled water, a small amount of conidia from a heavily infected leaf directly onto the surface of the water agar. Most likely, because water agar media and distilled water surrounded the fungal conidia, no conidia germination was observed after 7 days. Powdery mildew suspensions were prepared and applied to 3% water agar in a 10 cm Petri dish. Single conidia along with water agar media were removed with a bacterial loop and transferred to fresh tissue. This approach resulted in low conidial germination on host tissue after 7 days.

After evaluating and adjusting several of the single conidial techniques described in the literature, the decision was made to isolate and maintain colony level isolates rather than single conidial isolates.

Cucurbit powdery mildew isolation from field samples

Cucurbit powdery mildew isolates were obtained from a field-grown susceptible cultivar (Butterbush) and subcultured for identification of phenotypic (morphology) and genotypic (molecular) characteristics. Field isolates were subcultured on detached cucurbit leaflets, in Petri dishes (10 cm in diameter) on 2% water agar as growth medium for detached host tissue.

Within two days after field collection, infected leaf tissue (‘Butterbush’) was used to generate powdery mildew isolates in the laboratory. Three discrete colonies per leaf sample were excised from each leaf with a cork borer (10 mm in diameter). To avoid isolate mixture or contamination, only one bag containing a single field leaf sample was

opened at a time. The cork borer and laminar flow hood were disinfected with 70% ethanol between each isolate transfer.

Freshly cut leaf disks, of the same colony isolate, were placed in covered Petri plates, over moist filter paper, with fungal colony facing upward. Each leaf disk contained only one (discrete) fungal colony (Figure 3-7). Subsequently, infected leaf disks were gently rubbed or pressed onto detached healthy tissue (cotyledons or leaflets) of susceptible cucurbit hosts, until mycelia could be visibly detected on the surface of healthy tissue (Figure 3-8). When field inoculum was abundant, several subcultures were made from the same isolate which would allow multiple Petri plates of the same isolate to be available for further characterization. Fungal isolates were isolated and subcultured, in clean bench (Labconco Corp. Kansas City, MO) under axenic conditions.

Multiplication and maintenance of cucurbit powdery mildew isolates

Powdery mildew subcultures were incubated for 7 to 15 days in the laboratory, in enclosed containers at 22 ± 3°C under ambient lighting, before being transferred to fresh tissue or used in bioassays. The optimum period for incubation was 7days, which was when inoculated cotyledons and primary leaves were mostly covered with sporulating mycelium of the isolate to be tested. When necessary, subcultures were kept for longer than 7 days to allow enough time to prepare subculturing media or when host cotyledons were not yet ready to be used in transfers. Cultures were not kept for longer than 10-15 days before a new transfer was made, to maintain viable plant tissue for culture maintenance. When sporulation was abundant, conidia were readily transferred to multiple cotyledons and leaflets to obtain ample quantity of inoculum for bioassays and to maintain isolates.

Powdery mildew living cultures were incubated at room temperature (26 ± 2°C), under fluorescent lighting for 12 hours photoperiod daily (Figure 3-9).

Cucurbit Powdery Mildew Isolate Characterization

Morphological analysis

Conidia and conidiophores were collected from individual field leaf samples for microscopic morphological characterization. Three randomly selected, discrete fungal colonies were taken per leaf sample. Tape strips were mounted on microscope glass slide and examined in 3% KOH (potassium hydroxide) aqueous solution for profiling of morphological characters. Conidial features such as shape, size, presence or absence of fibrosin bodies, immature conidia edge line and conidiophores (footcell dimensions and number of conidia per chain) were recorded for each colony isolate.

Conidia size was measured for all field samples. The length (L) and width (W) of 100 randomly selected conidia, per fungal colony, were measured by visualization with light microscope (40 x objectives; Olympus BX51, Japan) with an attached ocular reticule. Conidial length was defined as the length of the long axis of the area bounded by the outer conidia edges and width was the length of the short axis between the outer edges. For each fungal isolate, average dimension of conidia length (L), width (W) and length to width ratio (L: W) were subjected to statistical analysis. Footcell dimensions of length and width were also recorded and analyzed (Table 3-3).

Molecular analysis

Conidiophores and conidia from individual field leaf samples were also collected and frozen (-20°C) for later molecular characterization. To obtain fungal material for DNA extraction, mycelia (conidiophores and conidia) was taken from randomly selected discrete colonies, on leaves sampled from each field site. A small piece of clear

adhesive tape was gently pressed over a fungal colony and gently pulled off without removing plant tissue. The piece of adhesive tape with mycelium was put into autoclaved 1 mL snap cap micro test tubes (Eppendorf, Hamburg, Germany) and frozen at -20°C for future DNA extraction. Three discrete colonies were taken from each leaf sample and each colony was put in a separate, labeled micro tube.

Genomic DNA was extracted and amplified from leaf tissue using REDExtract-N-Amp Plant PCR Kit (Sigma-Aldrich, St Louis, MO), according to the instructions supplied by the manufacturer. Fungal DNA was amplified at the Department of Plant Pathology at the University of Wisconsin, Madison.

Micro tubes containing previously frozen fungal samples were briefly thawed and 100 µL of Extraction Solution (REDExtract-N-Amp Plant PCR Kit) was added to each tube and vortexed briefly making sure the piece of tape was covered by the Extraction Solution. Fungal DNA was extracted from each the piece of adhesive tape by incubation in of Extraction Solution in hot water bath at 95°C for 10 minutes. After brief cooling, 100 µL of Dilution Solution (REDExtract-N-Amp Plant PCR Kit) was added to each tube to neutralize inhibitory substances, and the extracted DNA was vortexed again. The diluted DNA sample was ready for PCR (polymerase chain reaction) or was stored at 4°C until next day.

For each fungal isolate, an aliquot (4 µL) of the diluted DNA extract was combined with REDExtract-N-Amp ReadyMix (10 µL), specific primer pairs (2 µL of each) and PCR grade water (4 µL), mixed gently and loaded into thin-walled, 96-well PCR plates with adhesive aluminum foil cover (Bio-Rad Laboratories, Hercules, CA). The provided ReadyMix (REDExtract-N-Amp ReadyMix) contained the necessary buffer solutions,

salts, dNTPs (deoxyribonucleotide triphosphate), Taq polymerase enzyme, JumpStart Taq antibody (used for hot start PCR to enhance specificity) and REDTaq dye to allow direct loading of PCR product onto the agarose gel.

DNA was amplified according to Chen et al. using pairs of primer, S1 (5'-GGATCA TTA CTG AGC GCG AGG CCC CG -3')/S2 (5'- CGC CGC CCT GGC GCG AGA TAC A -3') and G1 (5'- TCC GTA GGT GAA CCT GCG GAA GGA T -3')/G2 (5'-CAA CAC CAA ACC ACA CAC ACG GCG -3'), in a multiplex reaction based on sequence variations of the ITS region and specific to *P. xanthii* and *G. cichoracearum*, respectively (36). DNA amplification was carried out in programmed thermo cycler (Techne TC-512, Keison Products, UK) with heated lid according to the following protocol: initial denaturing cycle at 94 °C for 5 min.; followed by 30 cycles of denaturation at 94 °C for 40 s., annealing at 62 °C for 1 min., extension at 72 °C for 90 s., and a final extension cycle at 72 °C for 5 min. When samples were not amplified on the same day, PCR plates were kept at 4°C until the following day. Amplified PCR products were loaded onto 2% agarose gel and were subjected to electrophoresis in TAE (Tris-acetate-EDTA) buffer. The amplified products were visualized with a trans-illuminator (BIO-RAD Universal Hood II, Bio-Rad Lab., Hercules, CA) after staining with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA). A 1-kb molecular weight marker DNA ladder (Promega, Madison, WI) was used for comparison.

Sterile distilled water and DNA extracted from healthy (powdery mildew free) zucchini squash (*Cucurbita pepo* cv. Dark Green Zucchini) leaves were used as negative controls. Positive controls were generated from plant tissue infected with known species of cucurbit powdery mildew. In the fall season of 2008, during

preliminary studies at the UF/IFAS Cowpen Branch Facility in Hastings in St Johns County, FL, powdery mildew pathogens were isolated from samples of summer squash (*Cucurbita pepo*) and sunn hemp (*Crotalaria juncea* cv. Tropic Sun) respectively. On the basis of morphological characteristics through microscopic examination of the asexual conidia, findings were consistent with published reports of *Podosphaera xanthii* and *Golovinomyces cichoracearum*. The nuclear rDNA internal transcribed spacer regions were amplified by PCR, using universal primers ITS1 and ITS4, and sequenced (GenBank Accession No. FJ479803). ITS sequence data indicated 100% homology with *Golovinomyces. cichoracearum* from *Helianthus annuus* L. (GenBank Accession No. AB077679) and with *Podosphaera xanthii*.

Statistical Analysis

Data were summarized as means for each isolate in each study. Analysis of variance was performed with SAS® (SAS® Institute Inc., Cary, NC). Mean separation was determined by Duncan's multiple range test (P=0.05).

Results and Discussion

Cucurbit Powdery Mildew Isolation, Multiplication and Maintenance

Detached cotyledon and leaflet inoculations of various cucurbit hosts proved to be an efficient *in vitro* assay to reproduce, powdery mildew symptoms observed in the field. Fungal sporulation on some cucurbit host plants ('Dark Green Zucchini' and 'Butterbush') occurred faster and was more abundant. These cucurbit hosts remained alive for longer periods of time after inoculation. Cucumber seeds germinated fastest, resulting in usable seedlings within approximately 7 days, yet cotyledon size was smaller compared to tissue from other hosts. Pumpkin cultivars produced larger cotyledons for fungal sporulation; however seeds took longer to germinate. Squash

cultivars (Butterbush and Dark Green Zucchini) were most adequate for fungal sporulation and required 3-4 weeks for seedlings to become usable (2-3 leaf stage) for inoculation. Melon cultivars did not produce adequate fungal sporulation and were not routinely used. Watermelon cultivars were infrequently used due to leaf shape being irregular (lobed).

Morphological Analysis

To investigate the powdery mildew species causing disease on cucurbit crops in north Florida, a total of 297 single colony isolates were collected from cucurbit fields in Live Oak and Citra, FL. Fungal colonies on infected leaves were slightly raised and were observed on both upper and lower leaf surfaces (Figure 3-8). Signs and symptoms of powdery mildew pathogens on infected plants were characterized by white, powdery-like mycelium on stems, petioles and on leaves (Figure 3-11).

Upon microscopic analyses, the following morphological features were observed: erect conidiophores, conidia produced in chains atop 2-3 mother cells above footcells, immature conidia with crenate edge lines, septate hyphae, ellipsoid to ovoid hyaline conidia, fibrosin bodies and infrequent forked germ tubes (Figure 3-12).

Conidial length (L), width (W) and length to width ratios (L:W) were consistent with previous reports on dimensions of *P. xanthii* (36, 84, 144, 322).

Some colony isolates were lost during the subculturing and maintenance process. Of the 126 samples collected in Live Oak during spring of 2009, 94 samples were analyzed morphologically from Citra, of the total 126 samples collected during spring of 2009, 119 were analyzed. For the isolates collected in Live Oak, the mean values for conidia were 33.6 ± 1.26 µm (length), 19.1 ± 0.79 µm (width) and 1.8 ± 0.11 for the L:W ratio. Footcell measurements were 56.1 ± 3.61 µm (length), 11.5 ± 0.37 µm (width). For

the field site in Citra, the mean values for conidia were 33.5 ± 0.81 µm (length), 18.9 ± 0.62 µm (width) and 1.8 ± 0.05 for the L: W ratio. As for the 45 samples collected at Citra during fall of 2009, the mean values for conidia were 33.9 ± 1.86 µm (length), 19.0 ± 0.41 µm (width) and 1.8 ± 0.09 for the L:W ratio. Mean dimensions of *P. xanthii* conidia on 'Butterbush' at both field locations are summarized in Table 3-4.

Of the 297 colonies isolated from both field site locations during spring and fall seasons of 2009, 258 colonies were morphologically characterized. Conidia had overall mean dimensions of 31-44 x 15-24 µm (L x W). The overall ratio of length to width (L:W) varied from 1.43-2.59 and footcell dimensions varied from 45-67 x 10-13 µm (LxW) (Table 3-4). The range of L:W ratios reported for *P. xanthii* by Frolov (88), Tomason and Gibson (322) and by Kristkova et al. (144) were of 1.0-2.6 respectively and encompassed results observed in our study.

Zeller (349) reported that the size of conidia and the length to width ratio showed a high degree of phenotypic variation within population and, alone should not be considered significant taxonomic features. Studies by Braun (25) reported that powdery mildew conidia which developed on senescent leaf surfaces were smaller than those produced on healthy tissue. In working with powdery mildew of eggplant, Whipps and Helyer (336) indicated that size of conidiophores and conidia were also affected by environmental conditions and by host plant. Tomason and Gibson (322) reported that L:W ratio of conidia, location on conidia where germination occurred (lateral or terminal) and shape of germ tube (clavate or rod) consistently differentiated between *S. fuliginea* (syn. *P. xanthii*) and *E cichoracearum* (*G. cichoracearum*) across locations, years and host species. Nevertheless, all of these results demonstrate significant variation in

conidial morphology between different powdery mildew species. Microscopic analyses of morphological characters indicated that all isolates were *Podosphaera xanthii* (27, 304).

Molecular Analysis

Electrophoretic profiles of PCR products amplified by primer pairs S1/S2 and G1/G2 specific for *P. xanthii* and *G. cichoracearum* respectively were characterized. Standard agarose gel electrophoreses were performed to separate and analyze DNA fragments extracted from cucurbit field samples A 2% agarose gel showed specific PCR detection of *Podosphaera xanthii* from field samples of 'Butterbush' (lanes 6 and 8), watermelon (lanes 2, 3, 4, 5, 9 and 10), muskmelon (lane 11) and 'Table Queen' acorn squash (lane 7) (Figure 3-10). Fragments of expected size for *P. xanthii* (454 bp) were observed in lanes 5, 6, 7, 8, 10, 11 and 12. Lane 14 showed specific detection of two fragments of the expected size (double bands) corresponding to *P. xanthii* and *G. cichoracearum* as a result of multiplex PCR reaction. Lanes 1 and 17 corresponded to 1Kb molecular marker. Lanes 2, 3, 4 and 9 did not detect any PCR products possibly due to poor quality or low quantity of DNA extracted.

PCR products of DNA fragments amplified by multiplex PCR, with DNA extracted from infected 'Butterbush' leaves collected in Citra, were also separated on a 2% agarose gel using the two primer pairs S1/S2 and G1/G2 (Figure 3-11). Bands corresponding to *P. xanthii* were observed in lanes 2 to 56. Isolates Ci-74 and Ci-75 (lanes 57 and 58) resulted in no bands which could possibly indicate that quality of DNA was poor or that quantity of DNA was insufficient. Additionally, the powdery mildew species could have been a species other than *P. xanthii* or *G. cichoracearum* and would not have been detected using the primer pairs S1/S2 and G1/G2. Control lane 59

(double bands) showed fragments of the expected size for *P. xanthii* and *G. cichoracearum* (454 bp and 391 bp respectively) as a result of multiplex reaction. Lanes 1, 20, 21, 40, 41 and 60 corresponded to 1 Kb molecular marker (Figure 3-11). For all samples which yielded DNA fragments as a result of molecular analysis, according to methodology described by Chen et al. (40), isolates were identified as *Podosphaera xanthii*.

Table 3-1. List of powdery mildew isolates from 'Butterbush', from Live Oak and Citra, FL field sites during spring and fall of 2009.

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name
Live Oak	1	5/19	1	1	A	1	1 LO 1(A)1
	2	5/19	1	1	A	2	1 LO 1(A)2
	3	5/19	1	1	A	3	1 LO 1(A)3
	4	5/19	1	1	B	1	1 LO 1(B)1
	5	5/19	1	1	B	2	1 LO 1(B)2
	6	5/19	1	1	B	3	1 LO 1(B)3
	7	5/19	1	1	C	1	1 LO 1(C)1
	8	5/19	1	1	C	2	1 LO 1(C)2
	9	5/19	1	1	C	3	1 LO 1(C)3
	10	5/19	1	2	A	1	1 LO 2(A)1
	11	5/19	1	2	A	2	1 LO 2(A)2
	12	5/19	1	2	A	3	1 LO 2(A)3
	13	5/19	1	2	B	1	1 LO 2(B)1
	14	5/19	1	2	B	2	1 LO 2(B)2
	15	5/19	1	2	B	3	1 LO 2(B)3
	16	5/19	1	2	C	1	1 LO 2(C)1
	17	5/19	1	2	C	2	1 LO 2(C)2
	18	5/19	1	2	C	3	1 LO 2(C)3
	19	6/02	2	1	A	1	2 LO 1(A)1
	20	6/02	2	1	A	2	2 LO 1(A)2
	21	6/02	2	1	A	3	2 LO 1(A)3
	22	6/02	2	1	B	1	2 LO 1(B)1
	23	6/02	2	1	B	2	2 LO 1(B)2
	24	6/02	2	1	B	3	2 LO 1(B)3
	25	6/02	2	1	C	1	2 LO 1(C)1
	26	6/02	2	1	C	2	2 LO 1(C)2
	27	6/02	2	1	C	3	2 LO 1(C)3
	28	6/02	2	2	A	1	2 LO 2(A)1
	29	6/02	2	2	A	2	2 LO 2(A)2
	30	6/02	2	2	A	3	2 LO 2(A)3
	31	6/02	2	2	B	1	2 LO 2(B)1
	32	6/02	2	2	B	2	2 LO 2(B)2
	33	6/02	2	2	B	3	2 LO 2(B)3
	34	6/02	2	2	C	1	2 LO 2(C)1
	35	6/02	2	2	C	2	2 LO 2(C)2
	36	6/02	2	2	C	3	2 LO 2(C)3
	37	6/16	3	1	A	1	3 LO 1(A)1

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Live Oak	38	6/16	3	1	A	2	3 LO 1(A)2
	39	6/16	3	1	A	3	3 LO 1(A)3
	40	6/16	3	1	B	1	3 LO 1(B)1
	41	6/16	3	1	B	2	3 LO 1(B)2
	42	6/16	3	1	B	3	3 LO 1(B)3
	43	6/16	3	1	C	1	3 LO 1(C)1
	44	6/16	3	1	C	2	3 LO 1(C)2
	45	6/16	3	1	C	3	3 LO 1(C)3
	46	6/16	3	2	A	1	3 LO 2(A)1
	47	6/16	3	2	A	2	3 LO 2(A)2
	48	6/16	3	2	A	3	3 LO 2(A)3
	49	6/16	3	2	B	1	3 LO 2(B)1
	50	6/16	3	2	B	2	3 LO 2(B)2
	51	6/16	3	2	B	3	3 LO 2(B)3
	52	6/16	3	2	C	1	3 LO 2(C)1
	53	6/16	3	2	C	2	3 LO 2(C)2
	54	6/16	3	2	C	3	3 LO 2(C)3
	55	7/01	4	1	A	1	4 LO 1(A)1
	56	7/01	4	1	A	2	4 LO 1(A)2
	57	7/01	4	1	A	3	4 LO 1(A)3
	58	7/01	4	1	B	1	4 LO 1(B)1
	59	7/01	4	1	B	2	4 LO 1(B)2
	60	7/01	4	1	B	3	4 LO 1(B)3
	61	7/01	4	1	C	1	4 LO 1(C)1
	62	7/01	4	1	C	2	4 LO 1(C)2
	63	7/01	4	1	C	3	4 LO 1(C)3
	64	7/01	4	2	A	1	4 LO 2(A)1
	65	7/01	4	2	A	2	4 LO 2(A)2
	66	7/01	4	2	A	3	4 LO 2(A)3
	67	7/01	4	2	B	1	4 LO 2(B)1
	68	7/01	4	2	B	2	4 LO 2(B)2
	69	7/01	4	2	B	3	4 LO 2(B)3
	70	7/01	4	2	C	1	4 LO 2(C)1
	71	7/01	4	2	C	2	4 LO 2(C)2
	72	7/01	4	2	C	3	4 LO 2(C)3
	73	7/10	5	1	A	1	5 LO 1(A)1
	74	7/10	5	1	A	2	5 LO 1(A)2
	75	7/10	5	1	A	3	5 LO 1(A)3
	76	7/10	5	1	B	1	5 LO 1(B)1

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Live Oak	77	7/10	5	1	B	2	5 LO 1(B)2
	78	7/10	5	1	B	3	5 LO 1(B)3
	79	7/10	5	1	C	1	5 LO 1(C)1
	80	7/10	5	1	C	2	5 LO 1(C)2
	81	7/10	5	1	C	3	5 LO 1(C)3
	82	7/10	5	2	A	1	5 LO 2(A)1
	83	7/10	5	2	A	2	5 LO 2(A)2
	84	7/10	5	2	A	3	5 LO 2(A)3
	85	7/10	5	2	B	1	5 LO 2(B)1
	86	7/10	5	2	B	2	5 LO 2(B)2
	87	7/10	5	2	B	3	5 LO 2(B)3
	88	7/10	5	2	C	1	5 LO 2(C)1
	89	7/10	5	2	C	2	5 LO 2(C)2
	90	7/10	5	2	C	3	5 LO 2(C)3
	91	7/15	6	1	A	1	6 LO 1(A)1
	92	7/15	6	1	A	2	6 LO 1(A)2
	93	7/15	6	1	A	3	6 LO 1(A)3
	94	7/15	6	1	B	1	6 LO 1(B)1
	95	7/15	6	1	B	2	6 LO 1(B)2
	96	7/15	6	1	B	3	6 LO 1(B)3
	97	7/15	6	1	C	1	6 LO 1(C)1
	98	7/15	6	1	C	2	6 LO 1(C)2
	99	7/15	6	1	C	3	6 LO 1(C)3
	100	7/15	6	2	A	1	6 LO 2(A)1
	101	7/15	6	2	A	2	6 LO 2(A)2
	102	7/15	6	2	A	3	6 LO 2(A)3
	103	7/15	6	2	B	1	6 LO 2(B)1
	104	7/15	6	2	B	2	6 LO 2(B)2
	105	7/15	6	2	B	3	6 LO 2(B)3
	106	7/15	6	2	C	1	6 LO 2(C)1
	107	7/15	6	2	C	2	6 LO 2(C)2
	108	7/15	6	2	C	3	6 LO 2(C)3
	109	7/23	7	1	A	1	7 LO 1(A)1
	110	7/23	7	1	A	2	7 LO 1(A)2
	111	7/23	7	1	A	3	7 LO 1(A)3
	112	7/23	7	1	B	1	7 LO 1(B)1
	113	7/23	7	1	B	2	7 LO 1(B)2
	114	7/23	7	1	B	3	7 LO 1(B)3

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Live Oak	115	7/23	7	1	C	1	7 LO 1(C)1
	116	7/23	7	1	C	2	7 LO 1(C)2
	117	7/23	7	1	C	3	7 LO 1(C)3
	118	7/23	7	2	A	1	7 LO 2(A)1
	119	7/23	7	2	A	2	7 LO 2(A)2
	120	7/23	7	2	A	3	7 LO 2(A)3
	121	7/23	7	2	B	1	7 LO 2(B)1
	122	7/23	7	2	B	2	7 LO 2(B)2
	123	7/23	7	2	B	3	7 LO 2(B)3
	124	7/23	7	2	C	1	7 LO 2(C)1
	125	7/23	7	2	C	2	7 LO 2(C)2
	126	7/23	7	2	C	3	7 LO 2(C)3
Citra	1	6/22	1	1	A	1	1 Ci 1(A)1
	2	6/22	1	1	A	2	1 Ci 1(A)2
	3	6/22	1	1	A	3	1 Ci 1(A)3
	4	6/22	1	1	B	1	1 Ci 1(B)1
	5	6/22	1	1	B	2	1 Ci 1(B)2
	6	6/22	1	1	B	3	1 Ci 1(B)3
	7	6/22	1	1	C	1	1 Ci 1(C)1
	8	6/22	1	1	C	2	1 Ci 1(C)2
	9	6/22	1	1	C	3	1 Ci 1(C)3
	10	6/22	1	2	A	1	1 Ci 2(A)1
	11	6/22	1	2	A	2	1 Ci 2(A)2
	12	6/22	1	2	A	3	1 Ci 2(A)3
	13	6/22	1	2	B	1	1 Ci 2(B)1
	14	6/22	1	2	B	2	1 Ci 2(B)2
	15	6/22	1	2	B	3	1 Ci 2(B)3
	16	6/22	1	2	C	1	1 Ci 2(C)1
	17	6/22	1	2	C	2	1 Ci 2(C)2
	18	6/22	1	2	C	3	1 Ci 2(C)3
	19	7/01	2	1	A	1	2 Ci 1(A)1
	20	7/01	2	1	A	2	2 Ci 1(A)2
	21	7/01	2	1	A	3	2 Ci 1(A)3
	22	7/01	2	1	B	1	2 Ci 1(B)1
	23	7/01	2	1	B	2	2 Ci 1(B)2
	24	7/01	2	1	B	3	2 Ci 1(B)3
	25	7/01	2	1	C	1	2 Ci 1(C)1
	26	7/01	2	1	C	2	2 Ci 1(C)2

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Citra	27	7/01	2	1	C	3	2 Ci 1(C)3
	28	7/01	2	2	A	1	2 Ci 2(A)1
	29	7/01	2	2	A	2	2 Ci 2(A)2
	30	7/01	2	2	A	3	2 Ci 2(A)3
	31	7/01	2	2	B	1	2 Ci 2(B)1
	32	7/01	2	2	B	2	2 Ci 2(B)2
	33	7/01	2	2	B	3	2 Ci 2(B)3
	34	7/01	2	2	C	1	2 Ci 2(C)1
	35	7/01	2	2	C	2	2 Ci 2(C)2
	36	7/01	2	2	C	3	2 Ci 2(C)3
	37	7/10	3	1	A	1	3 Ci 1(A)1
	38	7/10	3	1	A	2	3 Ci 1(A)2
	39	7/10	3	1	A	3	3 Ci 1(A)3
	40	7/10	3	1	B	1	3 Ci 1(B)1
	41	7/10	3	1	B	2	3 Ci 1(B)2
	42	7/10	3	1	B	3	3 Ci 1(B)3
	43	7/10	3	1	C	1	3 Ci 1(C)1
	44	7/10	3	1	C	2	3 Ci 1(C)2
	45	7/10	3	1	C	3	3 Ci 1(C)3
	46	7/10	3	2	A	1	3 Ci 2(A)1
	47	7/10	3	2	A	2	3 Ci 2(A)2
	48	7/10	3	2	A	3	3 Ci 2(A)3
	49	7/10	3	2	B	1	3 Ci 2(B)1
	50	7/10	3	2	B	2	3 Ci 2(B)2
	51	7/10	3	2	B	3	3 Ci 2(B)3
	52	7/10	3	2	C	1	3 Ci 2(C)1
	53	7/10	3	2	C	2	3 Ci 2(C)2
	54	7/10	3	2	C	3	3 Ci 2(C)3
	55	7/15	4	1	A	1	4 Ci 1(A)1
	56	7/15	4	1	A	2	4 Ci 1(A)2
	57	7/15	4	1	A	3	4 Ci 1(A)3
	58	7/15	4	1	B	1	4 Ci 1(B)1
	59	7/15	4	1	B	2	4 Ci 1(B)2
	60	7/15	4	1	B	3	4 Ci 1(B)3
	61	7/15	4	1	C	1	4 Ci 1(C)1
	62	7/15	4	1	C	2	4 Ci 1(C)2
	63	7/15	4	1	C	3	4 Ci 1(C)3
	64	7/15	4	2	A	1	4 Ci 2(A)1
	65	7/15	4	2	A	2	4 Ci 2(A)2

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Citra	66	7/15	4	2	A	3	4 Ci 2(A)3
	67	7/15	4	2	B	1	4 Ci 2(B)1
	68	7/15	4	2	B	2	4 Ci 2(B)2
	69	7/15	4	2	B	3	4 Ci 2(B)3
	70	7/15	4	2	C	1	4 Ci 2(C)1
	71	7/15	4	2	C	2	4 Ci 2(C)2
	72	7/15	4	2	C	3	4 Ci 2(C)3
	73	7/23	5	1	A	1	5 Ci 1(A)1
	74	7/23	5	1	A	2	5 Ci 1(A)2
	75	7/23	5	1	A	3	5 Ci 1(A)3
	76	7/23	5	1	B	1	5 Ci 1(B)1
	77	7/23	5	1	B	2	5 Ci 1(B)2
	78	7/23	5	1	B	3	5 Ci 1(B)3
	79	7/23	5	1	C	1	5 Ci 1(C)1
	80	7/23	5	1	C	2	5 Ci 1(C)2
	81	7/23	5	1	C	3	5 Ci 1(C)3
	82	7/23	5	2	A	1	5 Ci 2(A)1
	83	7/23	5	2	A	2	5 Ci 2(A)2
	84	7/23	5	2	A	3	5 Ci 2(A)3
	85	7/23	5	2	B	1	5 Ci 2(B)1
	86	7/23	5	2	B	2	5 Ci 2(B)2
	87	7/23	5	2	B	3	5 Ci 2(B)3
	88	7/23	5	2	C	1	5 Ci 2(C)1
	89	7/23	5	2	C	2	5 Ci 2(C)2
	90	7/23	5	2	C	3	5 Ci 2(C)3
	91	7/28	6	1	A	1	6 Ci 1(A)1
	92	7/28	6	1	A	2	6 Ci 1(A)2
	93	7/28	6	1	A	3	6 Ci 1(A)3
	94	7/28	6	1	B	1	6 Ci 1(B)1
	95	7/28	6	1	B	2	6 Ci 1(B)2
	96	7/28	6	1	B	3	6 Ci 1(B)3
	97	7/28	6	1	C	1	6 Ci 1(C)1
	98	7/28	6	1	C	2	6 Ci 1(C)2
	99	7/28	6	1	C	3	6 Ci 1(C)3
	100	7/28	6	2	A	1	6 Ci 2(A)1
	101	7/28	6	2	A	2	6 Ci 2(A)2
	102	7/28	6	2	A	3	6 Ci 2(A)3
	103	7/28	6	2	B	1	6 Ci 2(B)1
	104	7/28	6	2	B	2	6 Ci 2(B)2

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Citra	105	7/28	6	2	B	3	6 Ci 2(B)3
	106	7/28	6	2	C	1	6 Ci 2(C)1
	107	7/28	6	2	C	2	6 Ci 2(C)2
	108	7/28	6	2	C	3	6 Ci 2(C)3
	109	8/03	7	1	A	1	7 Ci 1(A)1
	110	8/03	7	1	A	2	7 Ci 1(A)2
	111	8/03	7	1	A	3	7 Ci 1(A)3
	112	8/03	7	1	B	1	7 Ci 1(B)1
	113	8/03	7	1	B	2	7 Ci 1(B)2
	114	8/03	7	1	B	3	7 Ci 1(B)3
	115	8/03	7	1	C	1	7 Ci 1(C)1
	116	8/03	7	1	C	2	7 Ci 1(C)2
	117	8/03	7	1	C	3	7 Ci 1(C)3
	118	8/03	7	2	A	1	7 Ci 2(A)1
	119	8/03	7	2	A	2	7 Ci 2(A)2
	120	8/03	7	2	A	3	7 Ci 2(A)3
	121	8/03	7	2	B	1	7 Ci 2(B)1
	122	8/03	7	2	B	2	7 Ci 2(B)2
	123	8/03	7	2	B	3	7 Ci 2(B)3
	124	8/03	7	2	C	1	7 Ci 2(C)1
	125	8/03	7	2	C	2	7 Ci 2(C)2
	126	8/03	7	2	C	3	7 Ci 2(C)3
	127	10/12	8	1	A	1	8 Ci 1(A)1
	128	10/12	8	1	A	2	8 Ci 1(A)2
	129	10/12	8	1	A	3	8 Ci 1(A)3
	130	10/12	8	1	B	1	8 Ci 1(B)1
	131	10/12	8	1	B	2	8 Ci 1(B)2
	132	10/12	8	1	B	3	8 Ci 1(B)3
	133	10/12	8	1	C	1	8 Ci 1(C)1
	134	10/12	8	1	C	2	8 Ci 1(C)2
	135	10/12	8	1	C	3	8 Ci 1(C)3
	136	10/20	9	1	A	1	9 Ci 1(A)1
	137	10/20	9	1	A	2	9 Ci 1(A)2
	138	10/20	9	1	A	3	9 Ci 1(A)3
	139	10/20	9	1	B	1	9 Ci 1(B)1
	140	10/20	9	1	B	2	9 Ci 1(B)2
	141	10/20	9	1	B	3	9 Ci 1(B)3
	142	10/20	9	1	C	1	9 Ci 1(C)1
	143	10/20	9	1	C	2	9 Ci 1(C)2

Table 3-1. Continued

Field location	Sample No.	Date sampled	Week sampled	Plant	Leaf	Colony	Isolate Name (Code)
Citra	144	10/20	9	1	C	3	9 Ci 1(C)3
	145	10/29	10	1	A	1	10 Ci 1(A)1
	146	10/29	10	1	A	2	10 Ci 1(A)2
	147	10/29	10	1	A	3	10 Ci 1(A)3
	148	10/29	10	1	B	1	10 Ci 1(B)1
	149	10/29	10	1	B	2	10 Ci 1(B)2
	150	10/29	10	1	B	3	10 Ci 1(B)3
	151	10/29	10	1	C	1	10 Ci 1(C)1
	152	10/29	10	1	C	2	10 Ci 1(C)2
	153	10/29	10	1	C	3	10 Ci 1(C)3
	154	11/03	11	1	A	1	11 Ci 1(A)1
	155	11/03	11	1	A	2	11 Ci 1(A)2
	156	11/03	11	1	A	3	11 Ci 1(A)3
	157	11/03	11	1	B	1	11 Ci 1(B)1
	158	11/03	11	1	B	2	11 Ci 1(B)2
	159	11/03	11	1	B	3	11 Ci 1(B)3
	160	11/03	11	1	C	1	11 Ci 1(C)1
	161	11/03	11	1	C	2	11 Ci 1(C)2
	162	11/03	11	1	C	3	11 Ci 1(C)3
	163	11/13	12	1	A	1	12 Ci 1(A)1
	164	11/13	12	1	A	2	12 Ci 1(A)2
	165	11/13	12	1	A	3	12 Ci 1(A)3
	166	11/13	12	1	B	1	12 Ci 1(B)1
	167	11/13	12	1	B	2	12 Ci 1(B)2
	168	11/13	12	1	B	3	12 Ci 1(B)3
	169	11/13	12	1	C	1	12 Ci 1(C)1
	170	11/13	12	1	C	2	12 Ci 1(C)2
	171	11/13	12	1	C	3	12 Ci 1(C)3

Table 3-2. Additional powdery mildew samples, from alternative locations and cucurbit hosts collected for comparison.

Sample No.	Location (County)	Cucurbit Host	Cucurbit species	Cultivar	Date sampled
08-4	Putman	yellow squash	*Cucurbita pepo*	unknown	10/23/08
09-1	Charlotte	watermelon	*Citrullus lanatus*	Crimson	4/100
09-2	Hendry	watermelon	*Citrullus lanatus*	Crimson	4/10
09-3	Collier	yellow squash	*Cucurbita pepo*	unknown	4/13
09-4	Collier	watermelon	*Citrullus lanatus*	Jubilee	4/20
09-5	Collier	watermelon (fruit)	*Citrullus lanatus*	Jubilee	5/06
09-6	Collier	watermelon	*Citrullus lanatus*	Crimson	5/12
09-7	Collier	watermelon	*Citrullus lanatus*	Crimson	5/12
09-9	Marion	acorn squash	*Cucurbita pepo*	Table Queen	5/20
09-20	Suwannee	watermelon	*Citrullus lanatus*	Mickey Lee	6/02
09-21	Suwannee	watermelon	*Citrullus lanatus*	Mickey Lee	6/10
09-22	Putman	muskmelon	*Cucumis melo*	Hales Best Jumbo	6/10
09-23	Marion	acorn squash	*Cucurbita pepo*	Table Queen	6/02
09-25	Suwannee	watermelon	*Citrullus lanatus*	Mickey Lee	7/01
09-26	Suwannee	watermelon	*Citrullus lanatus*	Mickey Lee	7/10
09-27	Marion	watermelon	*Citrullus lanatus*	Mickey Lee	7/10
09-28	Marion	watermelon	*Citrullus lanatus*	Mickey Lee	7/10
09-29	Suwannee	watermelon	*Citrullus lanatus*	Mickey Lee	7/15
09-30	Marion	watermelon	*Citrullus lanatus*	Mickey Lee	7/16
09-33	Suwannee	watermelon	*Citrullus lanatus*	Mickey Lee	7/23
09-34	Marion	watermelon	*Citrullus lanatus*	Mickey Lee	7/23
09-35	Marion	watermelon	*Citrullus lanatus*	Mickey Lee	7/28
09-36	Marion	watermelon	*Citrullus lanatus*	Mickey Lee	7/28
09-38	Marion	squash (27.12.2)	breeding line	Not yet assigned	10/13
09-39	Marion	squash (27.12.A1)	breeding line	Not yet assigned	10/13
09-43	Marion	squash (27.12.2)	breeding line	Not yet assigned	10/20
09-44	Marion	squash (27.12.A1)	breeding line	Not yet assigned	10/20
09-45	Citra	squash (27.16.6)	Rupp breeding line	Not yet assigned	10/20
09-46	Citra	muskmelon	*Cucumis melo*	Hales Best Jumbo	10/29

Table 3-2. Continued

Sample No.	Location	Cucurbit Host	Cucurbit species	Cultivar	Date (sampled)
09-49	Citra	squash (27.12.a1	breeding line	Not yet assigned	11/03
09-50	Citra	squash (27.12.2)	breeding line	Not yet assigned	11/03
09-51	Citra	squash (27.16.6)	breeding line	Not yet assigned	11/03
09-52	Citra	gourd	*C. okeechobeensis*	Not yet assigned	11/03
09-54	Citra	gourd	*C. okeechobeensis*	Not yet assigned	11/13
09-55	Citra	squash (27.12.a1)	breeding line	Not yet assigned	11/13
09-56	Citra	squash (27.16.6)	breeding line	Not yet assigned	11/13
09-57	Citra	squash (27.12.2)	breeding line	Not yet assigned	11/13

Table 3-3. Mean powdery mildew conidia length (µm), width (µm), length to width ratio and conidiophore footcell length(µm) and width(µm) of isolates from 'Butterbush' collected in Live Oak and Citra, FL during spring and fall of 2009.

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
1 LO 1(A)1	39	15	2.59	59	11
1 LO 1(A)2	38	19	2.05	51	12
1 LO 1(B)2	33	19	1.75	53	12
1 LO 1(C)1	33	19	1.80	45	12
1 LO 1(C)2	31	19	1.64	52	11
1 LO 2(A)1	33	19	1.77	53	11
1 LO 2(B)1	34	19	1.75	60	11
1 LO 2(C)1	32	19	1.66	55	11
1 LO 2(C)2	31	19	1.64	54	12
1 LO 2(C)3	34	19	1.81	56	11
2 LO 1(A)1	34	20	1.72	55	11
2 LO 1(B)1	33	19	1.75	52	11
2 LO 1(C)2	34	20	1.71	60	11
2 LO 2(A)1	33	19	1.74	57	11
2 LO 2(A)2	38	19	1.99	57	11
2 LO 2(B)1	33	19	1.71	53	12
2 LO 2(B)3	33	19	1.73	58	11
2 LO 2(C)1	33	19	1.73	56	11
2 LO 2(C)3	34	19	1.79	54	11
3 LO 1(A)1	33	19	1.78	55	11
3 LO 1(A)2	33	19	1.73	54	12
3 LO 1(A)3	32	19	1.72	54	11
3 LO 1(B)3	36	20	1.76	58	12
3 LO 1(C)3	33	19	1.79	51	12
3 LO 2(A)2	34	19	1.75	56	11
3 LO 2(A)3	33	19	1.74	57	11
3 LO 2(B)1	33	19	1.72	57	12
3 LO 2(B)2	33	19	1.74	58	12
3 LO 2(B)3	33	18	1.91	57	11
3 LO 2(C)3	34	19	1.84	54	11
4 LO 1(A)1	34	20	1.75	58	11
4 LO 1(A)2	34	19	1.75	48	10
4 LO 1(A)3	34	19	1.76	57	11
4 LO 1(B)1	34	19	1.74	56	11
4 LO 1(B)2	34	20	1.74	62	11
4 LO 1(B)3	34	19	1.77	56	11

Table 3-3. Continued

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
4 LO 1(C)1	34	19	1.76	61	12
4 LO 1(C)2	34	19	1.78	58	11
4 LO 1(C)3	34	20	1.72	55	11
4 LO 2(A)1	34	19	1.76	58	11
4 LO 2(A)2	35	20	1.74	57	11
4 LO 2(A)3	34	19	1.78	59	12
4 LO 2(B)1	34	20	1.74	56	12
4 LO 2(B)2	34	19	1.78	60	11
4 LO 2(B)3	34	19	1.77	58	12
4 LO 2(C)1	34	19	1.76	53	11
4 LO 2(C)2	33	19	1.76	53	11
4 LO 2(C)3	33	19	1.77	61	12
5 LO 1(A)1	34	18	1.84	55	11
5 LO 1(A)2	33	19	1.79	56	11
5 LO 1(A)3	33	19	1.78	55	12
5 LO 1(B)1	33	19	1.79	58	12
5 LO 1(B)2	34	19	1.76	55	12
5 LO 1(B)3	33	19	1.72	61	11
5 LO 1(C)1	34	18	1.86	57	12
5 LO 1(C)2	34	19	1.81	49	11
5 LO 1(C)3	34	19	1.76	58	12
5 LO 2(A)1	33	18	1.82	62	11
5 LO 2(A)2	33	19	1.74	64	12
5 LO 2(B)1	34	19	1.81	59	12
5 LO 2(B)2	33	18	1.78	55	11
5 LO 2(B)3	32	18	1.77	53	11
5 LO 2(C)1	33	19	1.75	57	11
5 LO 2(C)2	34	19	1.80	55	12
5 LO 2(C)3	33	19	1.79	59	11
6 LO 1(A)1	33	23	1.46	62	12
6 LO 1(A)2	33	19	1.72	67	12
6 LO 1(B)2	34	18	1.84	55	11
6 LO 1(B)3	33	19	1.74	60	11
6 LO 1(C)1	37	20	1.82	59	12
6 LO 2(A)1	33	19	1.79	59	12
6 LO 2(A)3	34	19	1.74	59	11
6 LO 2(B)2	36	20	1.75	55	11
6 LO 2(C)1	33	19	1.76	58	12

Table 3-3. Continued

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
6 LO 2(C)2	36	21	1.75	56	12
6 LO 2(C)3	34	19	1.80	55	12
7 LO 1(A)1	34	19	1.78	49	11
7 LO 1(A)2	34	19	1.75	52	11
7 LO 1(A)3	33	19	1.79	52	11
7 LO 1(B)1	33	19	1.70	55	12
7 LO 1(B)2	34	19	1.80	58	12
7 LO 1(B)3	34	20	1.72	60	12
7 LO 1(C)1	33	18	1.81	58	12
7 LO 1(C)2	32	20	1.57	52	12
7 LO 1(C)3	33	20	1.68	56	12
7 LO 2(A)1	34	19	1.75	52	12
7 LO 2(A)2	34	21	1.66	55	12
7 LO 2(A)3	33	19	1.75	54	11
7 LO 2(B)1	32	18	1.73	61	12
7 LO 2(B)2	31	19	1.65	55	12
7 LO 2(B)3	33	20	1.67	54	12
7 LO 2(C)1	34	19	1.80	52	11
7 LO 2(C)2	34	19	1.79	49	11
7 LO 2(C)3	33	19	1.77	61	11
1 Ci 1(A)1	33	19	1.75	60	11
1 Ci 1(A)2	34	19	1.77	57	12
1 Ci 1(A)3	33	19	1.75	55	12
1 Ci 1(B)1	33	18	1.77	57	11
1 Ci 1(B)2	34	19	1.78	59	11
1 Ci 1(B)3	33	19	1.75	55	11
1 Ci 1(C)1	33	19	1.73	55	12
1 Ci 1(C)2	33	19	1.72	57	12
1 Ci 1(C)3	34	19	1.78	64	12
1 Ci 2(A)1	33	19	1.75	58	12
1 Ci 2(A)2	34	19	1.78	59	11
1 Ci 2(A)3	33	19	1.75	57	11
1 Ci 2(B)1	34	18	1.84	49	11
1 Ci 2(B)2	33	19	1.74	56	12
1 Ci 2(B)3	33	19	1.75	54	11
1 Ci 2(C)1	34	19	1.80	57	11
1 Ci 2(C)2	33	19	1.76	53	12
1 Ci 2(C)3	34	19	1.81	61	11

Table 3-3. Continued

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
2 Ci 1(A)1	34	20	1.74	59	11
2 Ci 1(A)2	33	20	1.70	62	11
2 Ci 1(A)3	33	19	1.75	58	11
2 Ci 1(B)1	34	19	1.80	57	12
2 Ci 1(B)2	34	19	1.79	54	11
2 Ci 1(B)3	33	19	1.77	59	11
2 Ci 1(C)1	34	19	1.81	55	11
2 Ci 1(C)2	33	19	1.77	56	11
2 Ci 1(C)3	33	19	1.79	56	11
2 Ci 2(A)1	34	19	1.73	56	11
2 Ci 2(A)2	34	19	1.73	61	12
2 Ci 2(A)3	33	19	1.79	57	11
2 Ci 2(B)1	33	19	1.79	57	11
2 Ci 2(B)2	32	18	1.76	63	12
2 Ci 2(B)3	33	18	1.80	56	11
2 Ci 2(C)1	34	19	1.81	63	12
2 Ci 2(C)2	33	19	1.80	63	11
2 Ci 2(C)3	33	18	1.82	62	11
3 Ci 1(A)1	33	19	1.79	62	11
3 Ci 1(A)2	34	19	1.82	60	11
3 Ci 1(A)3	34	19	1.78	59	11
3 Ci 1(B)1	34	19	1.79	56	11
3 Ci 1(B)2	34	19	1.80	57	11
3 Ci 1(B)3	34	18	1.82	63	11
3 Ci 1(C)1	34	19	1.80	58	10
3 Ci 1(C)2	34	19	1.78	58	11
3 Ci 1(C)3	33	18	1.79	58	11
3 Ci 2(A)1	33	19	1.80	58	11
3 Ci 2(A)2	33	19	1.77	57	11
3 Ci 2(A)3	33	19	1.76	52	11
3 Ci 2(B)1	33	18	1.84	57	10
3 Ci 2(B)2	34	19	1.77	56	11
3 Ci 2(B)3	34	19	1.77	57	11
3 Ci 2(C)1	33	19	1.79	58	11
3 Ci 2(C)2	34	19	1.76	58	11
3 Ci 2(C)3	33	19	1.77	57	11
4 Ci 1(A)1	34	18	1.86	55	12
4 Ci 1(A)2	33	19	1.79	59	11

Table 3-3. Continued

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
4 Ci 1(A)3	33	19	1.76	61	12
4 Ci 1(B)1	33	19	1.76	57	12
4 Ci 1(B)2	33	19	1.77	58	11
4 Ci 1(B)3	33	19	1.74	60	11
4 Ci 1(C)1	34	19	1.77	53	11
4 Ci 1(C)2	33	19	1.79	56	11
4 Ci 1(C)3	34	19	1.73	53	12
4 Ci 2(A)1	34	19	1.76	54	11
4 Ci 2(A)2	34	19	1.79	66	11
4 Ci 2(A)3	34	19	1.80	55	12
4 Ci 2(B)1	33	19	1.75	57	11
4 Ci 2(B)2	34	19	1.81	57	11
4 Ci 2(B)3	33	19	1.73	57	12
4 Ci 2(C)1	33	19	1.81	54	11
4 Ci 2(C)2	33	19	1.79	59	11
4 Ci 2(C)3	34	19	1.80	59	11
5 Ci 1(A)1	33	18	1.85	50	12
5 Ci 1(A)2	34	19	1.79	56	12
5 Ci 1(A)3	33	18	1.83	57	11
5 Ci 1(B)1	34	19	1.76	58	12
5 Ci 1(B)2	35	19	1.80	56	12
5 Ci 1(B)3	34	19	1.76	61	12
5 Ci 1(C)1	33	19	1.74	55	11
5 Ci 1(C)2	33	19	1.78	57	12
5 Ci 1(C)3	33	20	1.70	57	12
5 Ci 2(A)1	33	19	1.72	62	12
5 Ci 2(A)2	34	19	1.72	58	11
5 Ci 2(A)3	33	19	1.79	58	12
5 Ci 2(B)1	33	19	1.78	59	12
5 Ci 2(B)2	33	18	1.80	59	11
5 Ci 2(B)3	33	19	1.79	56	10
5 Ci 2(C)1	33	19	1.75	52	12
5 Ci 2(C)2	34	19	1.79	60	12
5 Ci 2(C)3	34	19	1.76	56	13
6 Ci 1(A)1	33	19	1.76	59	12
6 Ci 1(A)2	34	19	1.73	53	12
6 Ci 1(A)3	33	19	1.71	56	12
6 Ci 1(B)1	35	20	1.79	54	11

Table 3-3. Continued

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
6 Ci 1(B)2	33	19	1.71	58	12
6 Ci 1(B)3	34	19	1.75	58	11
6 Ci 1(C)1	33	19	1.73	59	12
6 Ci 1(C)2	33	19	1.76	65	12
6 Ci 1(C)3	33	19	1.73	60	11
6 Ci 2(A)1	33	19	1.72	55	12
6 Ci 2(A)2	32	19	1.75	55	11
6 Ci 2(A)3	34	19	1.76	54	12
6 Ci 2(B)1	34	19	1.79	58	12
6 Ci 2(B)2	33	19	1.73	55	11
6 Ci 2(B)3	33	18	1.82	50	12
6 Ci 2(C)1	33	19	1.73	54	11
6 Ci 2(C)2	33	19	1.77	54	11
6 Ci 2(C)3	33	19	1.78	58	11
7 Ci 1(A)1	33	19	1.75	60	11
7 Ci 1(A)2	33	19	1.76	58	11
7 Ci 1(A)3	36	19	1.92	62	12
7 Ci 1(B)3	35	24	1.43	58	11
7 Ci 1(C)2	39	20	1.90	56	11
7 Ci 2(A)2	36	19	1.89	54	11
7 Ci 2(A)3	33	19	1.75	59	12
7 Ci 2(B)1	33	18	1.78	56	12
7 Ci 2(B)2	33	19	1.78	58	12
7 Ci 2(B)3	33	19	1.75	59	11
7 Ci 2(C)3	37	20	1.84	55	11
8 Ci 1(A)1	35	20	1.79	60	11
8 Ci 1(A)2	34	20	1.73	56	12
8 Ci 1(A)3	34	19	1.75	60	12
8 Ci 1(B)1	33	18	1.81	57	12
8 Ci 1(B)2	34	19	1.75	56	12
8 Ci 1(B)3	35	20	1.76	58	11
8 Ci 1(C)1	34	20	1.71	61	12
8 Ci 1(C)2	44	20	2.26	57	11
8 Ci 1(C)3	34	19	1.75	61	11
9 Ci 1(A)1	35	19	1.81	56	11
9 Ci 1(A)2	33	18	1.78	56	12
9 Ci 1(A)3	33	19	1.76	57	11

Table 3-3. Continued

Isolate Name (Code)	Conidia length (L) (n=100)	Conidia width (W) (n=100)	Conidia L:W (n=100)	Footcell length (n=25)	Footcell width (n=25)
9 Ci 1(B)1	34	20	1.75	58	11
9 Ci 1(B)2	33	19	1.78	59	12
9 Ci 1(B)3	34	19	1.79	57	12
9 Ci 1(C)1	33	19	1.74	52	12
9 Ci 1(C)2	33	19	1.75	55	12
9 Ci 1(C)3	34	19	1.75	59	11
10 Ci 1(A)1	32	19	1.71	55	12
10 Ci 1(A)2	33	19	1.76	60	12
10 Ci 1(A)3	34	19	1.79	56	11
10 Ci 1(B)1	33	19	1.73	55	12
10 Ci 1(B)2	34	19	1.76	54	12
10 Ci 1(B)3	34	19	1.76	57	12
10 Ci 1(C)1	34	19	1.78	56	12
10 Ci 1(C)2	34	19	1.78	57	12
10 Ci 1(C)3	33	19	1.74	52	11
11 Ci 1(A)1	34	19	1.80	54	12
11 Ci 1(A)2	35	19	1.82	55	12
11 Ci 1(A)3	34	19	1.79	56	12
11 Ci 1(B)1	34	19	1.73	55	11
11 Ci 1(B)2	33	19	1.74	55	11
11 Ci 1(B)3	34	19	1.79	56	12
11 Ci 1(C)1	33	19	1.75	59	12
11 Ci 1(C)2	38	19	2.00	58	12
11 Ci 1(C)3	34	19	1.77	55	12
12 Ci 1(A)1	33	18	1.80	55	12
12 Ci 1(A)2	34	19	1.79	54	12
12 Ci 1(A)3	33	19	1.77	57	12
12 Ci 1(B)1	33	19	1.77	55	12
12 Ci 1(B)2	32	19	1.69	56	12
12 Ci 1(B)3	34	19	1.76	57	12
12 Ci 1(C)1	32	18	1.77	50	11
12 Ci 1(C)2	34	19	1.80	53	12
12 Ci 1(C)3	33	18	1.81	52	11

Table 3-4. Dimensions of fresh conidia of *Podosphaera xanthii* from leaf tissue of *Cucurbita moschata* ('Butterbush') collected from Live Oak and Citra, FL field sites during spring and fall of 2009.

Host Species (number of samples)	Size of fresh conidia		
	Length (µm)	Width (µm)	Length: width ratio (µm)
Live Oak, FL (spring) (n=94)			
Mean value	33.6	19.1	1.8
Standard deviation	1.26	0.79	0.11
Minimum	39	23	2.6
Maximum	31	15	1.5
Citra, FL (spring) (n=119)			
Mean value	33.5	18.9	1.8
Standard deviation	0.81	0.62	0.05
Minimum	39	24	1.9
Maximum	32	18	1.4
Citra, FL (fall) (n=45)			
Mean value	33.9	19.0	1.8
Standard deviation	1.86	0.41	0.09
Minimum	44	20	2.3
Maximum	32	18	1.7
Total (both locations)(n=258)			
Mean value	33.6	19.0	1.8
Standard deviation	1.22	0.66	0.08
Minimum	44	24	2.6
Maximum	31	15	1.4

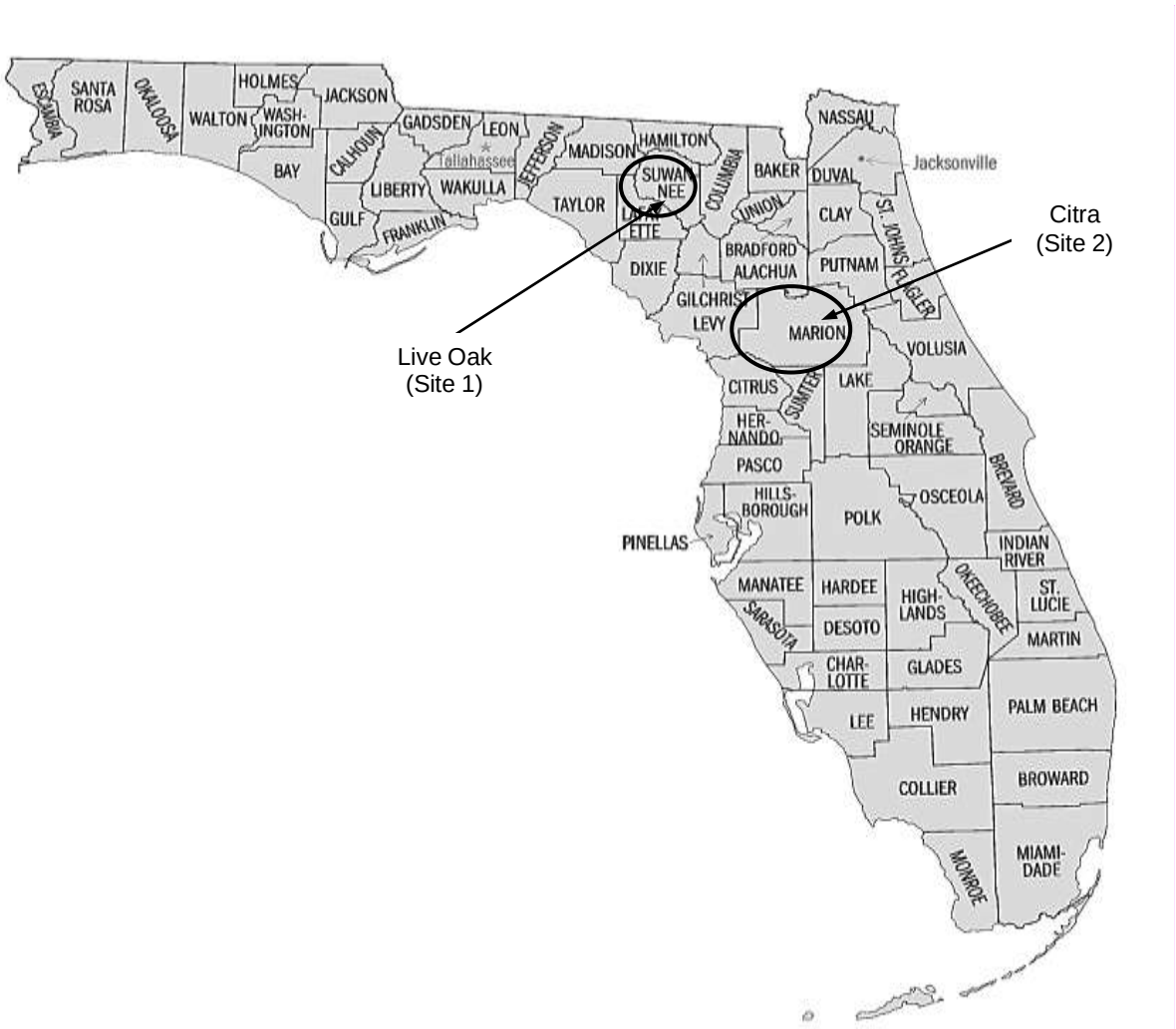

Figure 3-1. Florida sites of cucurbit powdery mildew collection from spring to fall of 2009.

Figure 3-2. (A) Field plot at Live Oak, FL (13 May 2009) Plots consisted of 6 rows covered with black plastic mulch. There were 16 plots per row and each plot contained five plants/ plot. Plots were replicated 4 times in a complete randomized plot design. (B) Field plot at Citra, FL (16 June 2009). Plots consisted of 6 rows covered with reflective silver plastic mulch. There were 3 plots per row and each plot contained five plants/ plot. Plots were replicated 8 times in a complete randomized plot design.

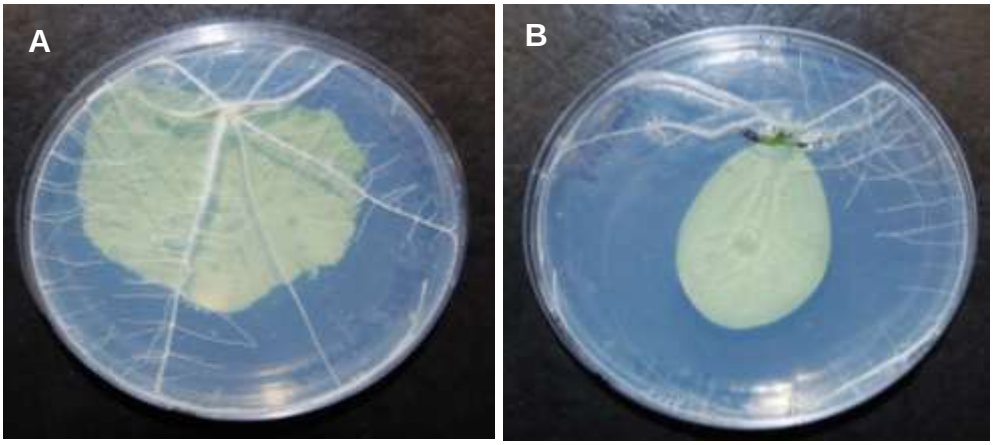

Figure 3-3. Primary leaf (A) and cotyledon (B) of butternut winter squash ('Butterbush') rooting in 2% water agar media. Living host tissue was maintained as substrate for powdery mildew single colony isolates.

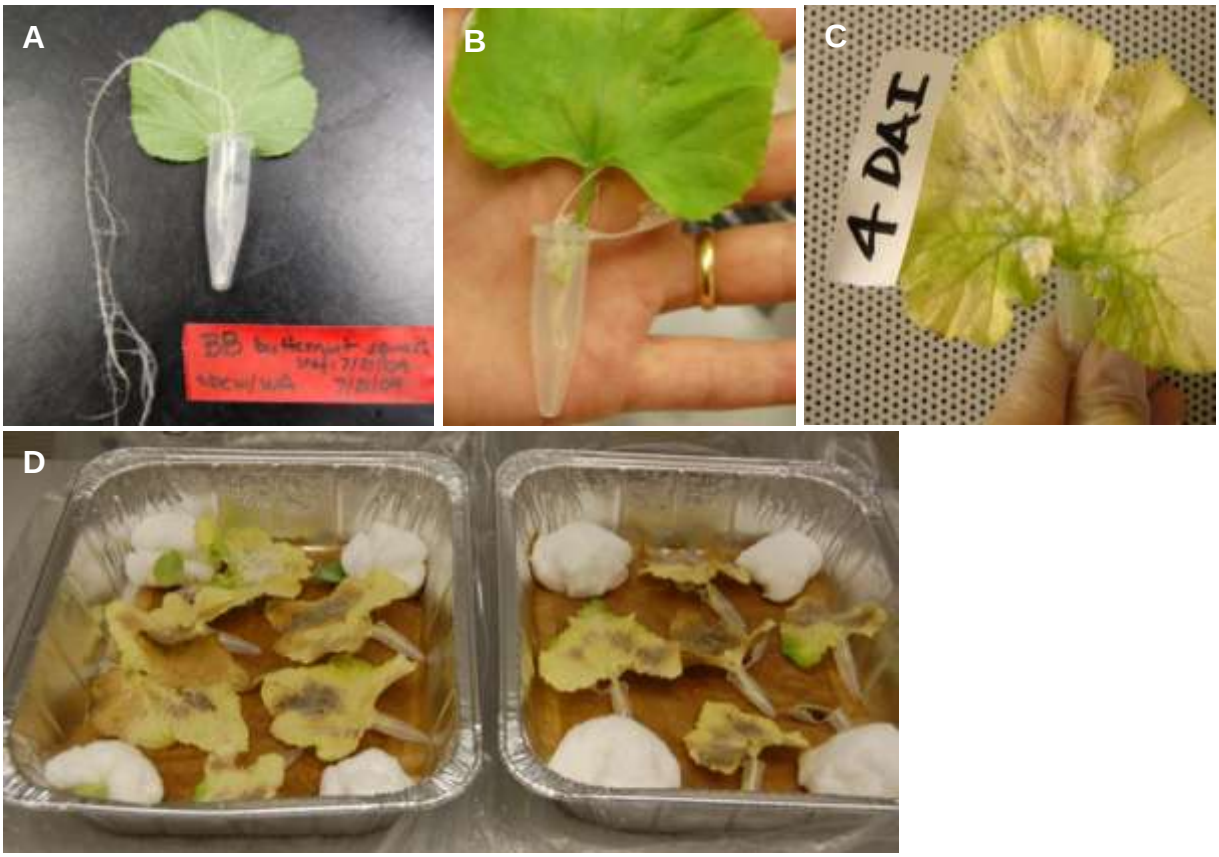

Figure 3-4. (A) Butternut winter squash (’Butterbush’) primary leaf (3-4 weeks old) rooting in 1mL test tube containing 2% water agar. (B) Cultivar ‘Butterbush’ leaflet 18 days in tube containing 2% water agar. (C) Zucchini (’Dark Green’) leaf maintained in 1mL test tubes, 4 days post inoculation with powdery mildew. (D) Set up of aluminum trays used to keep a single powdery mildew isolate growing on ‘Dark Green’ in 1mL test tubes, 7 days post inoculation.

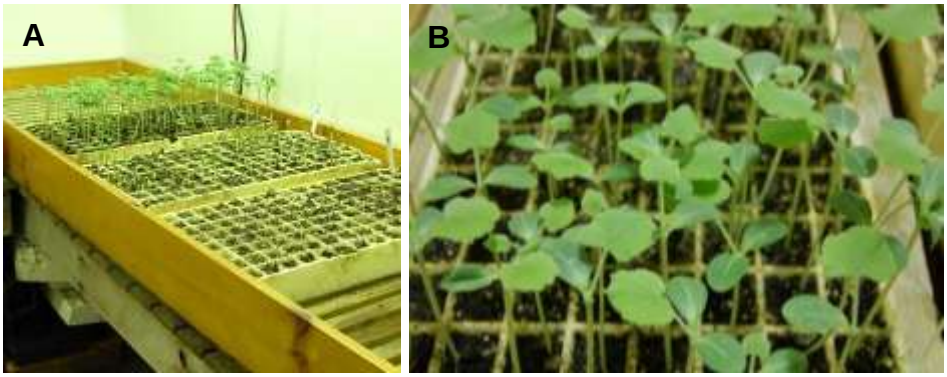

Figure 3-5. (A) Cucurbit seedlings cultivated in growth room. (B) Fresh host tissue (‘Butterbush’) at 1-2 true leaf stage used in periodic fungal isolate maintenance and detached leaf bioassays.

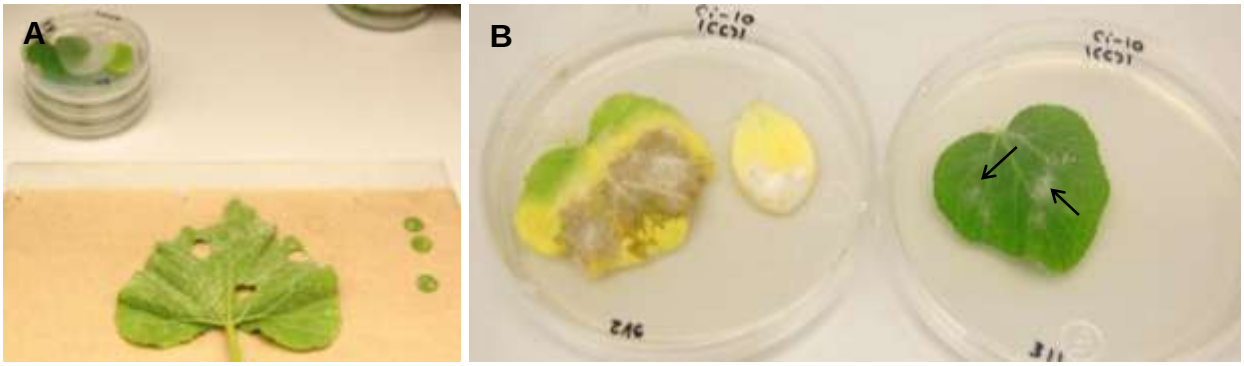

Figure 3-6. (A) Leaf disks containing discrete powdery mildew colonies cut from infected ('Butterbush') leaf sample. (B) Powdery mildew isolate (7-10 days old) transferred to fresh healthy tissue. Signs (arrows) of white mycelia/conidia on fresh green tissue ('Butterbush') after transfer by gently touching infected leaflets (left) onto a healthy leaflet (right).

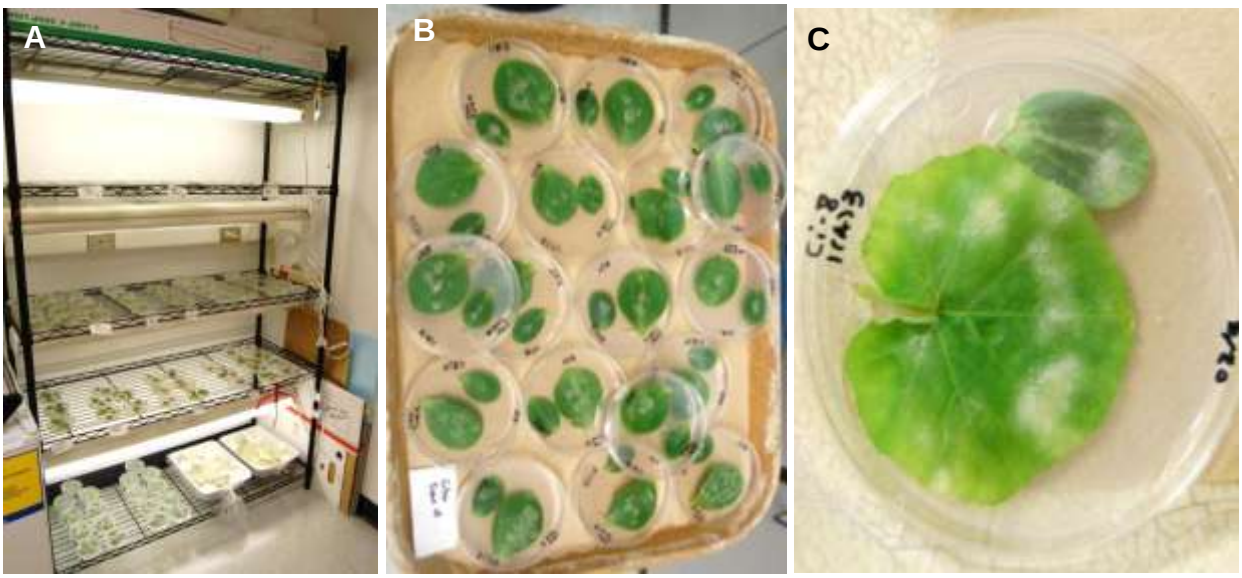

Figure 3-7. Cucurbit powdery mildew isolates growing on different cucurbit hosts after periodic isolate transfer. (A) Overview of isolate maintenance under fluorescent lighting. (B) Cotyledons of 'Mickey Lee' and 'Butterbush' inoculated with powdery mildew isolates, growing on 2% water agar media. (C) Close up of 'Big Max 'leaflet and 'Butterbush' cotyledon 4 days post inoculation with isolate Ci-8 from Citra, FL.

Figure 3-8. Characteristic powdery mildew signs and symptoms on susceptible cucurbits cultivars. (A) Discrete fungal colonies (arrows) on watermelon fruit. (B) Lower leaf surfaces of ‘Mickey Lee’ watermelon infected. (C) Powdery mildew on petioles of acorn squash ‘Table Queen’. (D) Fungal colonies on stems of butternut winter squash ‘Butterbush’. (E) Discrete fungal colonies on leaf undersurface of ‘Butterbush’. (F) Heavy powdery mildew infection on lower canopy and upper leaf surface of ‘Big Max’ pumpkin. At Citra field site, premature yellowing and senescence of ‘Butterbush’ exposing fruit to sunburn as a result of severe powdery mildew infection (G) and severe powdery mildew infection and defoliation (H).

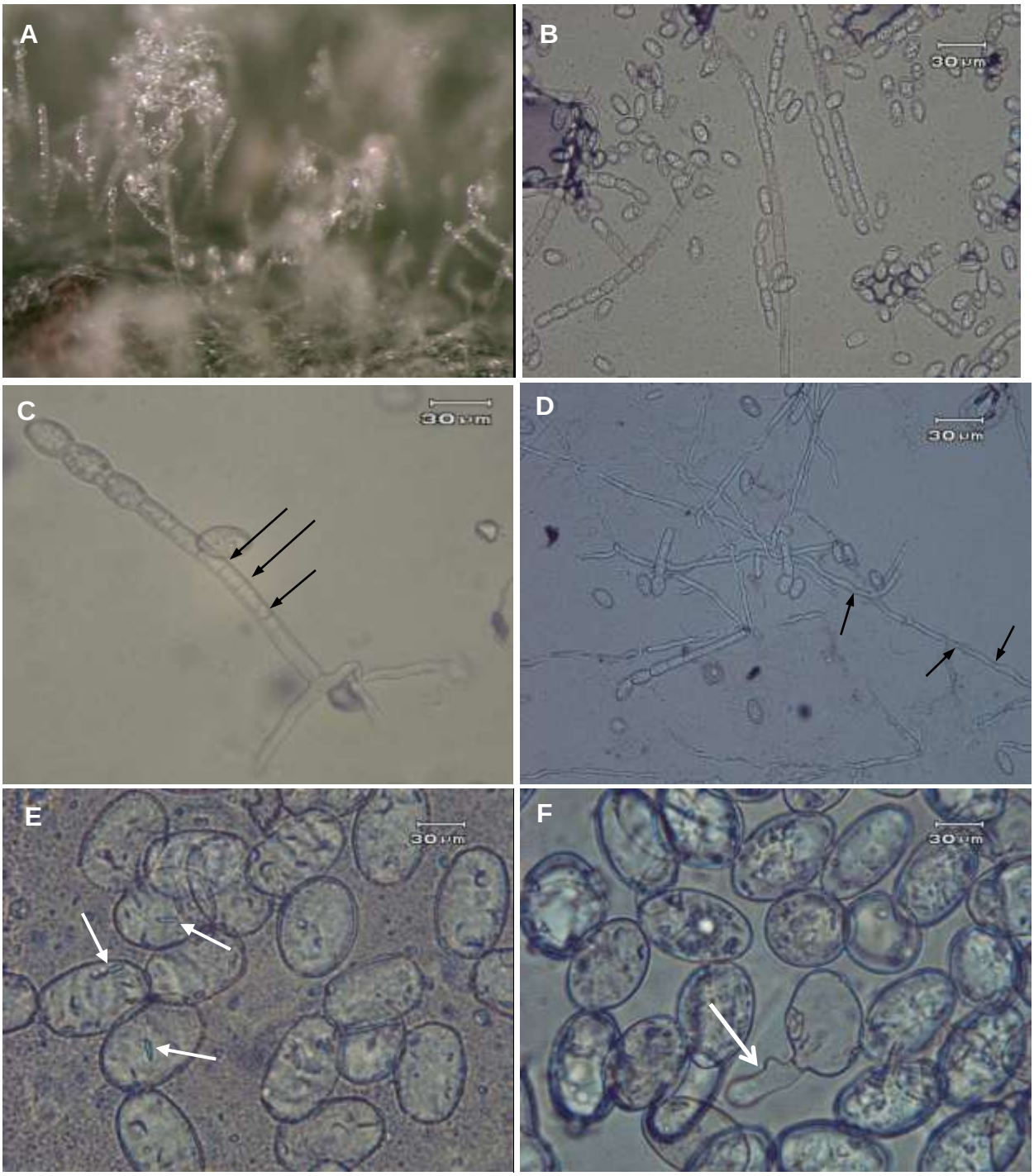

Figure 3-9. Characteristic *Podosphaera xanthii* morphological features. (A) Erect conidiophores on leave surface of butternut winter squash 'Butterbush' seen under stereoscope. (B) Conidia produced in chains following 2-3 mother cells above the foot-cells. (C) Immature conidia with crenate (arrows) edges. (D) Septate hyaline hyphae (arrows) and conidiophores. (E) Hyaline conidia; ellipsoid to ovoid in shape and with presence of reflective fibrosin bodies (arrows). (F) Forked germ tubes (arrow), were infrequently observed.

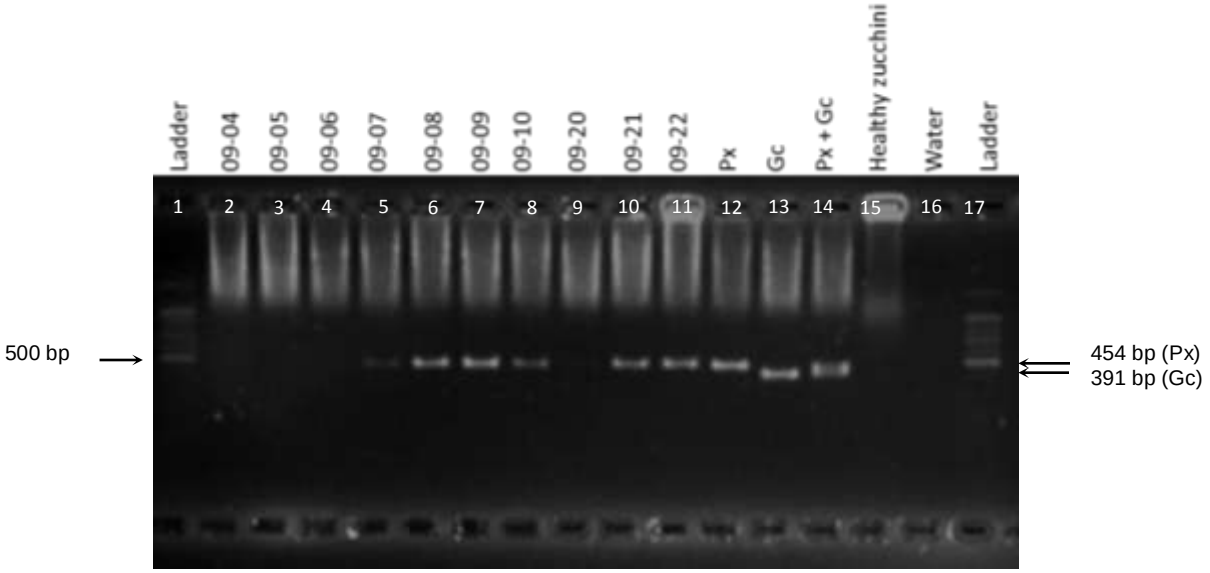

Figure 3-10. Electrophoretic profile of PCR products amplified by primers S1/S2 and G1/G2. Specific PCR detection of *Podosphaera xanthii* (Px) from field samples of various cucurbit hosts. Fragments of the expected sizes (454 bp and 391bp) are observed in lanes 12 (*P. xanthii*) and 13 (*Golovinomyces cichoracearum*) respectively. Lane 14 shows specific PCR detection of a fragment of the expected size (double bands) corresponding to multiplex PCR product. Lanes 1 and 17 correspond to 1Kb molecular marker. Cucurbit cultivars included: butternut winter squash 'Butterbush' (lanes 6 and 8), watermelon (lanes 2, 3, 4, 5, 9 and 10), muskmelon (lane 11) and 'Table Queen' acorn squash (lane 7). Px= *P. xanthii*; Gx= *G. cichoracearum* and Px+Gx= multiplex reaction

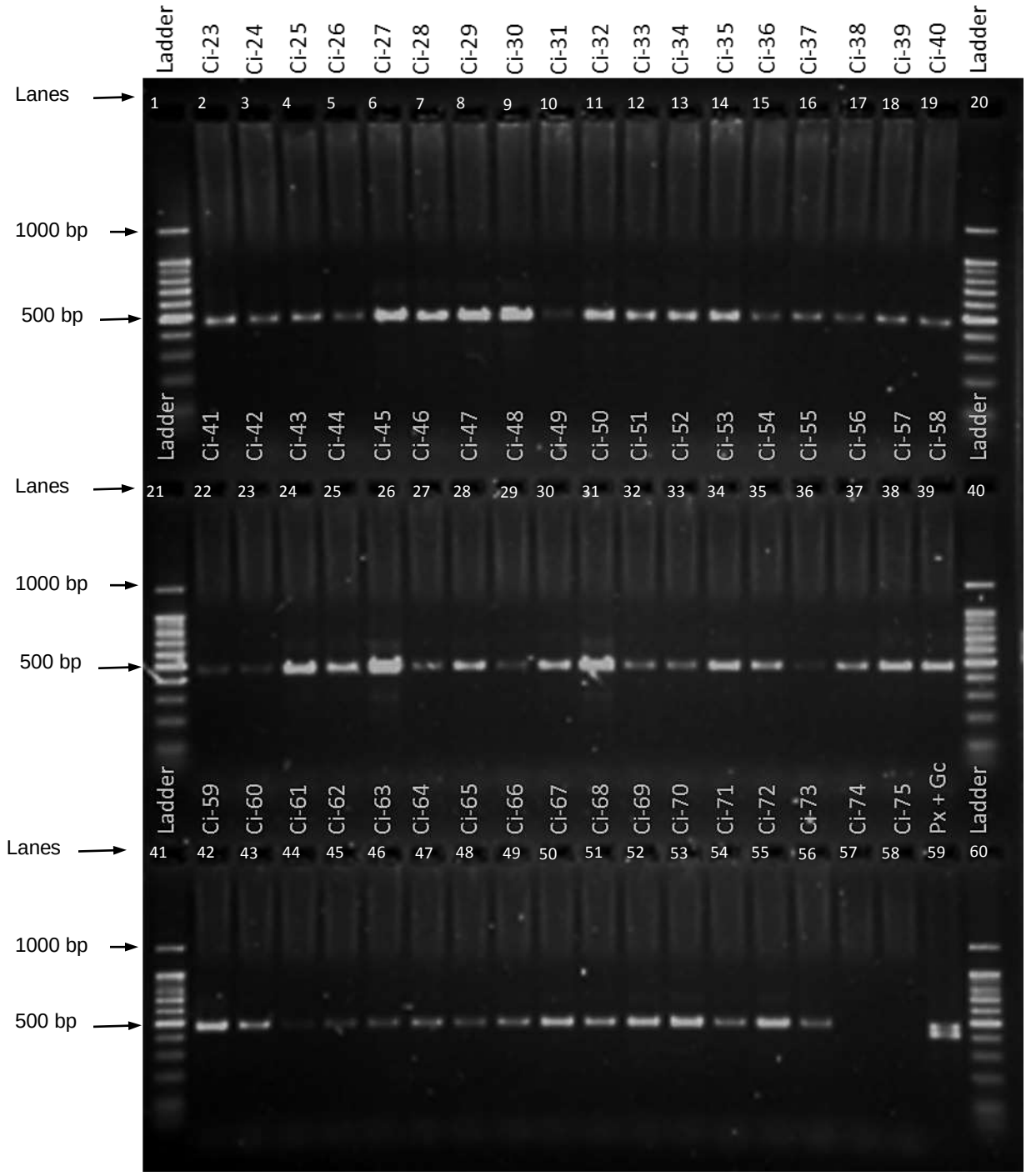

Figure 3-11. Analysis of DNA fragments amplified by a multiplex PCR with DNA extracted from powdery mildew colonies from infected 'Butterbush' leaves.. Two primer pairs (S1/S2 and G1/G2) for *Podosphaera xanthii* and *Golovinomyces cichoracearum*, respectively, were used. Lanes 2 – 56 correspond to P. xanthii (454 bp). Lane 59 shows two fragments of the expected size for *P. xanthii* (454 bp) and *G. cichoracearum* (391 bp) respectively. Lanes 1, 20, 21, 40, 41 and 60 correspond to 1Kb molecular marker.

CHAPTER 4
DETERMINATION OF POWDERY MILDEW PHYSIOLOGICAL RACES

Powdery mildew is an important disease of cucurbit crops worldwide. It is primarily caused by one of two fungi, *Podosphaera xanthii* or *Golovinomyces cichoracearum*. The most commonly found pathogen, *P. xanthii*, is known to occur in several physiological races in the United States and worldwide (163, 190). These physiological races are most frequently distinguished using muskmelon differential genotypes (188). And, to a lesser extent, other cucurbit species such as cucumber, watermelon and *Cucurbita* spp. have been used to differentiate cucurbit powdery mildew physiological races (161, 180).

When breeding crops for resistance to disease, it is fundamental to know the species and races of the causal agent present, since resistance genes are often specific to the prevailing pathogenic race (158, 195). Search for resistance genes to be used in developing resistant cucurbit breeding lines is the focus of many research programs worldwide (43, 123, 157, 339). International effort has been dedicated to breeding for resistance to cucurbit powdery mildew, especially *P. xanthii*. In melon cultivar PMR 45, resistance has been attributed to a single dominant gene. However, a more complex scenario has been reported for other cucurbit cultivars, with control being governed by various combinations of several dominant, recessive and modifier genes (129).

Knowledge of the prevalence and distribution of cucurbit powdery mildew races are essential for the deployment of suitable resistant commercial lines, as well as for choice of appropriate disease management strategies, implementation of molecular techniques and tools, and continued breeding resistance efforts. Screening protocols require efficient methods which can distinguish pathogenic variations among cucurbit powdery mildew pathogens at the level of pathotypes as well as races (10, 12). Lebeda

et al. (161) emphasized that pathotypes express variation at the host range level, while races represent the level of virulence on a set of selected genotypes of one host species, traditionally, muskmelon cultigens.

Because pathogenic variants can impact host range and disease management, an improved understanding of powdery mildew pathogenic strains in FL was necessary. For this reason I undertook the following research objectives: (1) identify and subculture the prevailing causal agent of cucurbit powdery mildew in north central Florida; (2) characterize fungal isolates through morphological features and DNA analysis; (3) assess the presence of physiological races within cultured isolates via bioassays using detached leaves; and (4) evaluate the varietal reactions of *Cucurbita* breeding lines for susceptibility to powdery mildew under local (FL) field conditions.

Materials and Methods

Plant Material and Growth Conditions

A differential set of twelve muskmelon (*Cucumis melo*) genotypes with varying levels of resistance to cucurbit powdery mildew were tested and used for race determination based on modifications of protocols described by McCreight J. D. (188) and Lebeda et al. (161). In our study, the following muskmelon lines were inoculated a sub-set of powdery mildew isolates: Edisto 47, Iran H, MR-1, PI 124111, PI 124112, PI 313970, PI 414723, PMR-45, PMR-5, TopMark, Vedrantais, WMR-29. Seeds were kindly provided by Dr. James D. McCreight (United States Department of Agriculture) and by Dr. Eileen Kabelka (formerly of University of Florida, Department of Horticulture Science, currently with Harris Moran Seed Co., CA).

Muskmelon plants were grown in 3.8 liter plastic pots (Figure 4-1) containing potting medium (Fafard Professional 4P Mix), in a powdery mildew-free greenhouse.

During development in the greenhouse, plants did not receive any fungicide treatments since this application could influence susceptibility to powdery mildew. Throughout cultivation in the greenhouse, plants were continuously monitored for unwanted powdery mildew infection. Day length ranged from 10 to 12 hours and temperature was maintained at 23 ± 5°C. Temperatures inside the greenhouse were recorded continuously (data not shown) over the length of the experiment using a HOBO Microstation Data Logger (Onset Computer Corp., Bourne, MA). Temperature was adjusted as needed to maintain ideal growing conditions. Plants were watered and fertilized with water soluble fertilizer Peters Professional 20-10-20 Peat-lite (Scotts International B.V., Netherlands) and Osmocote Plus controlled release fertilizer (Scott-Sierra Horticultural Products Company, Marysville, OH) as needed.

Muskmelon differentials were maintained in the greenhouse for 6 to 8 weeks (until 3 to 6 true-leaf stage), after which, older plants were discarded. Rather than using plants at the seedling stage, older cucurbit plants are recommended for resistance screening against powdery mildew because expression of resistance typically appears at more mature developmental stages (153). Seeds of each muskmelon type were planted, every 10 to 15 days, to provide a fresh supply of host tissue for detached leaf bioassays. During laboratory experiments, healthy whole leaves from each muskmelon type were removed, using sterile scalpel blades, and taken to the laboratory in individual labeled and sealed plastic bags. While being harvested in the greenhouse, leaves were kept inside a cooler to avoid premature wilting of tissue.

Sample Collection and Pathogen Identification

To determine powdery mildew pathogen races, a sub-set of powdery mildew isolates was screened using detached leaf assays. Isolates tested were selected to

represent specific locations, hosts and times of year. Fully expanded muskmelon leaves (3-6 true-leaf stage from 6-8 weeks old plants) were excised from the middle portion of at least two plants per muskmelon genotype.

Five powdery mildew isolates (Table 4-1) used in this experiment were collected from naturally infected field or greenhouse samples as described in Chapter 3. The following isolates were tested: LO-6 1(A) 1 collected from 'Butterbush' at Live Oak, FL on 20 June 2009; isolate Ci 6 1(A) 1 from 'Butterbush' at Citra FL, on 23 June 2009; isolate 10-02 from muskmelon in greenhouse at UF in Gainesville, FL on 16 April 2010; isolate 10-08 from squash at Immokalee FL; on 10 May, 2010, and isolate10-09 from squash at Dover, FL on 6 June 2010. Prior to race typing, the identification of the powdery mildew species was confirmed by microscopic examination of the morphological characters of fresh conidia in 3% aqueous KOH as previously defined in Chapter 3.

Cotyledons and first true leaves of susceptible cucurbit cultivars (Butterbush, Dark Green Zucchini, Mickey Lee, Straight Eight and Waltham) were used for powdery mildew inoculum maintenance and propagation, incubated for 7-10 days as previously described in Chapter 3. Preliminary tests indicated that certain muskmelon genotypes ('Vedrantais', 'Iran H' and 'TopMark') were highly susceptible to all powdery mildew isolates. As such, healthy muskmelon leaves (3-8 weeks old) were also used to enhance inoculum production

Host Reaction

Pathogenic variation of a select sub-set of fungal isolates was determined by analyzing the reactions of 12 muskmelon differential lines to each powdery mildew isolate, as described by several authors (18, 161, 190).

Detached leaf assays were based on modifications and adaptations of the methods described by McCreight et al. (190), Kristkova et al. (145), Vakalounakis and Klimonomou (328), Cohen et al. (40), and Lebeda et al. (158). According to several reports the detachment of leaves rather than whole plant inoculation, is a common practice and does not seem to affect race determination. In all race determination experiments, each muskmelon genotype was represented by 2 replicates (leaf A and B). Leaves were excised from each muskmelon type and inoculated with a predetermined powdery mildew isolate. Muskmelon differential leaves were not surface sterilized prior to inoculation. Each powdery mildew isolate was inoculated onto a total of 24 detached muskmelon leaves (two leaves per isolate). Each muskmelon differential leaf was placed, adaxial leaf surface face-up, in a petri dish (10 or 15 cm in diameter Petri plates depending on leaf size.) which contained a layer of 2% water agar to provide support and moisture (Figure 4-2).

Each muskmelon leaf was inoculated by gently pressing infected tissue onto a healthy leaf. During inoculation, muskmelon genotypes were intercalated so that all genotypes of the first repetition were inoculated, followed by all genotypes of the second repetition to avoid dilution of the inoculum for the subsequent different genotypes. Inoculated leaves as well as controls (mock-inoculated leaves) were incubated under fluorescent lighting (12 hours per day) at room temperature (22 to 25°C) on a laboratory bench, for 10-15 days. The first powdery mildew symptoms appeared at 3-5 dpi (days post inoculation). Detached muskmelon leaves were observed under 60x magnification and photographed at 3, 7 and 10 days post inoculation. QImaging Digital camera and

software Q-Capture Pro (Surrey, BC, Canada) were used for observation and analysis of infected leaf tissue.

Disease Evaluation

The development of powdery mildew hyphae and conidia on detached muskmelon leaves was evaluated at 3, 7 and 10 days post inoculation. Inoculated detached leaves were maintained *in vitro* for up to 15 days, the time at which infected leaf tissue became too decomposed for further analysis. Disease evaluations were recorded as soon a first powdery mildew signs were observed with unaided eyes. Assessment of powdery mildew for race typing was evaluated by visually estimating the percent leaf area covered by the pathogen using a standard stereoscope. A pre-determined circumference (10 mm diameter disk) was cut off a piece of plastic film and was placed over an infected leaf area and used as a standard template for disease severity and pathogen development assessment (Figure 4-3).

Assessment of powdery mildew disease on detached muskmelon leaves was evaluated by the quantification of disease severity and the quality of pathogen development (pathogen status). Both measures were used to separate the response of the host (resistant or susceptible) from the reaction of the pathogen (virulent or avirulent) and eventually evaluate if both ratings correlated.

Pathogen status

Powdery mildew pathogen development (sporulation) on each muskmelon detached leaf was rated using a 0-4 scale as described by McGrath et al. (213) as follows: (0) =no fungal growth (noted presence of inoculum); (1) = very little mycelial growth with no conidiophores; (2) = little mycelial growth with few conidiophores; (3) = fairly good mycelial growth with scattered conidiophores and (4) = heavy sporulation. A

genotype was considered be susceptible when the isolate tested produced conidiophores (rating of 2, 3 or 4) on both inoculated leaves of the same genotype (Figure 4-4).

Disease severity

Disease severity (percentage of 10-mm diameter circular leaf area covered by mycelia/conidia) was classified into 5 categories using a modification of the scale described by Kristkova et al.(142), where: 0 = no symptoms of infection on circular area, 1 = less than 25% of circular area covered with mycelia, 2 = 25-50% of circular area covered with mycelia, 3 = 50-75% of circular area covered with mycelia and 4 = over 75% of circular area covered with mycelia. On each differential muskmelon genotype, isolates with an average severity (2 inoculated leaves) of mycelial growth of 0 or 1 were classified as avirulent and those with ratings of 2, 3 or 4 were considered virulent (Figure 4-4).

Results and Discussion

Powdery Mildew Identification

On the basis of morphological and molecular analysis, the isolates tested in this study were determined to be *Podosphaera xanthii*. Characteristics features such as conidia shape and dimensions (length and width), presence of fibrosin bodies and crenate immature conidia edge line were consistent with published reports for *P. xanthii*. Molecular analysis through PCR confirmed presence of *P. xanthii* as previously described in Chapter 3.

Effect of Cucurbit Powdery Mildew on Detached Muskmelon Leaves

Detached leaf inoculation proved to be an efficient *in vitro* assay to test the pathogenic variability (races) of powdery mildew isolates collected from greenhouse and

field samples in north Florida. Mock inoculated muskmelon leaves (negative controls) remained green and healthy for up to 20 days (Figure 4-5). Some detached muskmelon inoculated leaves developed comparable and reproducible disease symptoms on the upper leaf surface. When a muskmelon genotype was susceptible, percentages of leaf area covered by powdery mildew typically showed extensive interwoven mycelium and abundant conidiophore production (Figure 4-6).

Previous studies regarding cucurbit powdery mildew race determination traditionally use leaf disks (161, 190, 192, 218), or entire plants (66, 319). Leaf disks being smaller than whole leaves, allow for greater number of replications and could yield better comparison between isolates and possibly a more accurate representation of pathogen variability. In our studies, two replicates (2 leaves per muskmelon type) may have been insufficient for a conclusive *P. xanthii* race determination but were adequate for a preliminary assessment. In addition, during this study, inoculum production decreased over time. A possible explanation might be that isolates had been cultured *in vitro* for too long and possibly decreased virulence. Furthermore, toward the end of race typing studies, it had become more difficult to increase inoculum proliferation and produce enough inoculum to infect more than 2 leaflets with each isolate.

Disease Evaluation

A disk outline (10-mm diameter circle) was used as a standard template for disease severity and pathogen development assessment (Figure 4-7). This method was chosen so that, for all muskmelon leaves, inoculated with each isolate, the same leaf area was assessed for comparison.

Reactions of 12 muskmelon genotypes to 5 powdery mildew isolates rated 10 days post inoculation (dpi) indicated variability in isolate virulence (Figures 4-8 to 4-12). Images show detail of powdery mildew infection within pre-determined circumference and the corresponding detached muskmelon leaf. Disease severity ratings, corresponding pathogen development (status) ratings, and the type of reaction observed are summarized in Table 4-2.

For each differential muskmelon genotype, isolates with severity rating of 0 or 1 were classified as avirulent and those with ratings greater than 2 were considered virulent (Figure 4-4). A genotype was considered be susceptible to the pathogen when the isolate being tested produced conidiophores (rating ≥ 2) on both inoculated leaves (Table 4-2). The production of conidiophores and consequently fungal sporulation was an indication that infection had progressed (Figure 4-8 to Figure 4-12).

There was good linear correlation (R^2=0.80) between disease severity and pathogen status ratings indicating that as disease severity increased, pathogen development also increased. Isolate 10-02 presented the highest correlation coefficient (R^2=0.93) followed by isolate LO 6 1(A) 1 (R^2=0.89), isolate 10-08 (R^2=0.84) and isolate 10-09 (R^2=0.84). However isolate Ci 6 1(A) 1 presented the lowest correlation (R^2=0.62).

Muskmelon genotypes ‘Edisto 47’, PI 124111 and PMR-5 were resistant to all 5 powdery mildew isolates tested. In contrast, genotypes ‘Iran H’, PMR-45, ‘TopMark’ and ‘Vedrantais’ were universally susceptible to all fungal isolates tested. Genotypes MR-1 and PI 313970 revealed resistance to 2 out of 5 isolates; while PI 124112 and WMR-29 presented resistance to 4 out of 5 isolates and genotype PI 414723 demonstrated

resistance to 3 out of 5 isolates. In this evaluation, ‘Edisto 47’, PI 12411 and PMR-5 plants appeared to be among the most resistant of the 12 muskmelon genotypes tested, never showing more than 25% of leaf area covered with powdery mildew disease symptoms. Susceptible genotypes ‘Iran H’, ‘TopMark’ and ‘Vedrantais’ had ratings as high as 75% coverage and consistently had disease symptoms over 50% of the leaf area assessed. (Table 4-2 and Figure 4-8 to Figure 4-12)

A list of currently known races of *P. xanthii* is presented in Figure 4-13 (personal communication and graciously provided by Dr. James D. McCreight of USDA-ARS, Salinas, CA). In comparison to this list, our race typing assays indicated that we may have found race 2F as its profile is consistent with the isolate from Citra (Figure 4-13). The isolate from Live Oak may be consistent with the profiles described for race 2Z. The other 3 isolates tested (UF, IM and DO) were undefined to date and were not consistent with any of the previously described races of *P. xanthii* (Figure 4-13). In addition, some of the previously defined cucurbit powdery mildew races were incompletely tested (represented by gray color) and therefore cannot be disregarded as possible matches to the results obtained during our studies (Figure 4-13).

It has been widely reported that biotic and abiotic factors may alter host response and subsequently affect race profiling. Factors such as environmental conditions (light intensity, temperature and humidity) as well as age of plant at time of inoculation and purity of inoculum may affect disease severity and adversely influence race identification by masking host resistance or pathogen virulence factors (41, 190).

In our studies, it is possible that we had isolated mixed colonies, indicating a mixture of races. An alternative explanation would be that during sampling, we may

have isolated a novel *P. xanthii* pathogenic race (or races) present in north Florida. Furthermore, differences in environmental conditions specific to Florida (field sites and greenhouse) as well as conditions in the laboratory could have altered host response and/or pathogen virulence during our studies. Additionally, due to our low number of leaf replicates, our results may be inconclusive. Further testing, with larger number of replicates, is necessary.

Table 4-1. Sub-set of six Florida powdery mildew isolates, used for race typing bioassays.

Isolate No.	Cucurbit host	Collection time	Inoculation Date	Origen	Fungal species*
Ci 6 1(A)1	Butterbush	Fall 2009	5/04/10	Citra	*P. xanthii*
10-08	Summer squash	Spring 2010	5/21/10	Immokalee	*P. xanthii*
LO 6 1(A)1	Butterbush	Fall 2009	5/27/10	Live Oak	*P. xanthii*
10-02	TopMark	Spring 2010	6/11/10	Gainesville	*P. xanthii*
10-09	Summer squash	Spring 2010	6/18/10	Dover	*P. xanthii*

**P. xanthii* isolates were confirmed by microscopic analysis of morphological features and molecular analyses.

Table 4-2. Reaction (10 days post inoculation) of some muskmelon (*Cucumis melo*) genotypes to sub-set of powdery mildew isolates.

Muskmelon genotype	Powdery mildew isolate	No. inoc. leaves	Leaves with sympt.	Inoc. presence	affected leaf area (%)	DS rating	PS rating	Type of reaction
Edisto 47	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	1-25	1	1	R
	10-08	2	2	+	1-25	1	1	R
	10-09	2	2	+	1-25	1	1	R
PI 124111	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	1-25	1	1	R
	10-08	2	2	+	1-25	1	1	R
	10-09	2	2	+	1-25	1	1	R
PMR-5	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	1-25	1	1	R
	10-08	2	2	+	1-25	1	1	R
	10-09	2	2	+	1-25	1	1	R

Table 4-2. Continued

Muskmelon genotype	Powdery mildew isolate	No. inoc. leaves	Leaves with sympt.	Inoc. presence	affected leaf area (%)	DS rating	PS rating	Type of reaction
Iran H	LO-6	2	2	+	51-75	3	4	S
	Ci-6	2	2	+	51-75	3	3	S
	10-02	2	2	+	51-75	3	4	S
	10-08	2	2	+	>75	4	4	S
	10-09	2	2	+	51-75	3	3	S
PMR-45	LO-6	2	2	+	1-25	1	2	S
	Ci-6	2	2	+	1-25	1	2	S
	10-02	2	2	+	51-75	3	4	S
	10-08	2	2	+	51-75	3	2	S
	10-09	2	2	+	1-25	1	2	S
TopMark	LO-6	2	2	+	>75	4	4	S
	Ci-6	2	2	+	26-50	2	4	S
	10-02	2	2	+	51-75	3	4	S
	10-08	2	2	+	51-75	3	4	S
	10-09	2	2	+	>75	4	4	S
Vedrantais	LO-6	2	2	+	51-75	4	4	S
	Ci-6	2	2	+	51-75	3	3	S
	10-02	2	2	+	26-50	3	4	S
	10-08	2	2	+	>75	4	4	S
	10-09	2	2	+	51-75	3	3	S
MR-1	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	26-50	2	3	S
	10-08	2	2	+	26-50	2	2	S
	10-09	2	2	+	1-25	1	2	S

Table 4-2. Continued

Muskmelon Genotype	Isolate	No. Inoc. leaves	Leaves with sympt.	Inoc. present	affected leaf area (%)	DS rating	PS rating	Type of reaction
PI 313970	LO-6	2	2	+	26-50	2	3	S
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	1-25	1	1	R
	10-08	2	2	+	1-25	1	2	S
	10-09	2	2	+	26-50	2	3	S
PI 124112	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	1-25	1	2	S
	10-08	2	2	+	1-25	1	1	R
	10-09	2	2	+	1-25	1	1	R
WMR-29	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	2	S
	10-02	2	2	+	1-25	1	1	R
	10-08	2	2	+	1-25	1	1	R
	10-09	2	2	+	1-25	1	1	R
PI 414723	LO-6	2	2	+	1-25	1	1	R
	Ci-6	2	2	+	1-25	1	1	R
	10-02	2	2	+	1-25	1	2	S
	10-08	2	2	+	1-25	1	1	R
	10-09	2	2	+	1-25	1	2	S

Number of inoculated leaves= 2
+= Presence of inoculum noted on leaf surface at day of disease assessment;
DS= Disease severity rating
PS=Pathogen status rating
Type of reaction: R= resistant; S=susceptible

Figure 4-1. Set of healthy muskmelon genotypes growing in powdery mildew-free greenhouse.

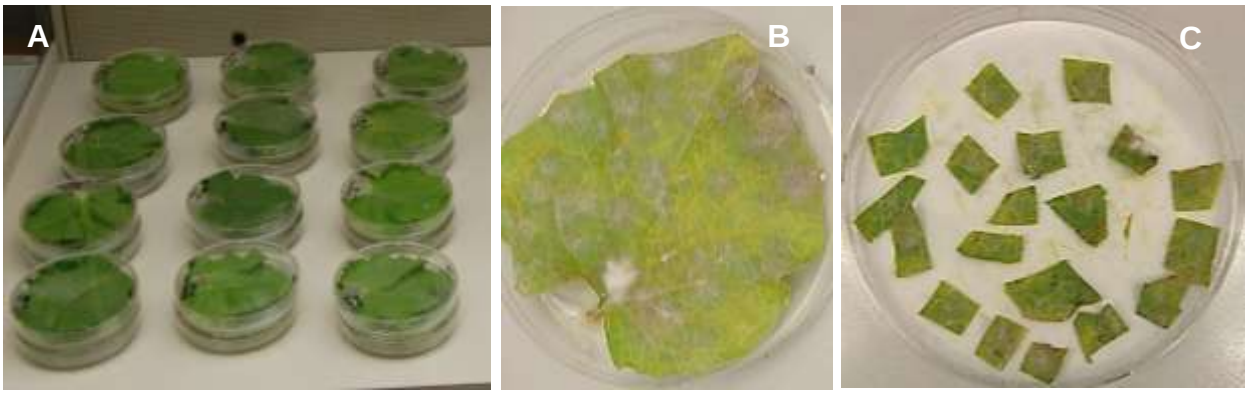

Figure 4-2. Demonstration of muskmelon differential inoculation process. (A) Healthy leaves from a set of twelve muskmelon genotypes awaiting inoculation in detached leaf assay. (B) Detail of infected susceptible muskmelon leaf ('Vedrantais') used to propagate a powdery mildew isolate (C) Leaf pieces originated from whole leaf infected with powdery mildew inoculum used to inoculate healthy muskmelon detached leaves.

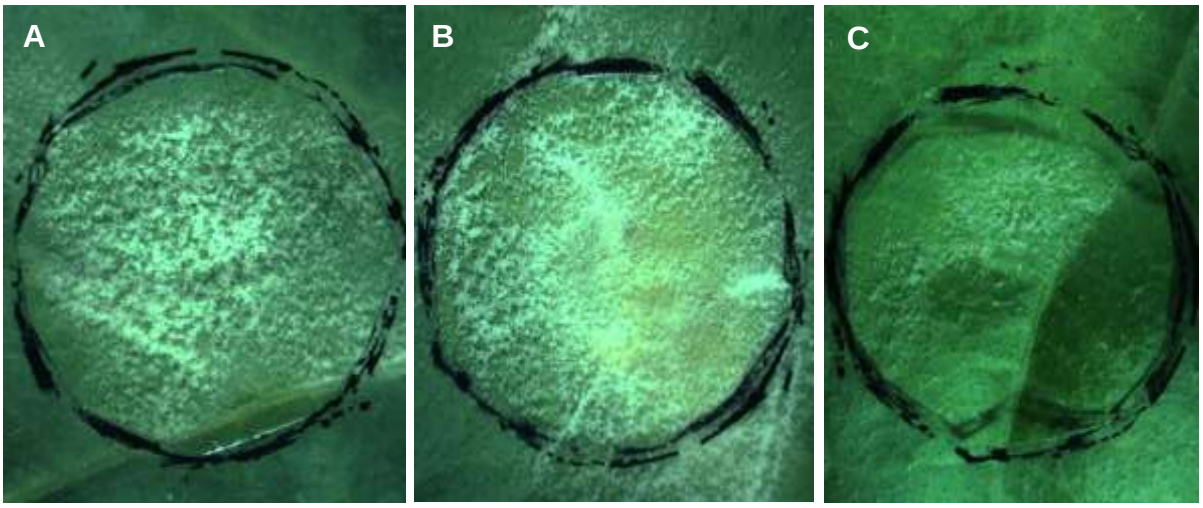

Figure 4-3. A 10-mm diameter circumference was visually assessed for powdery mildew. Powdery mildew isolate, [Ci-6 1(A)1] sporulating on different muskmelon genotypes (A) TopMark (B) Iran H (C) Vedrantais. (Magnification 200x)

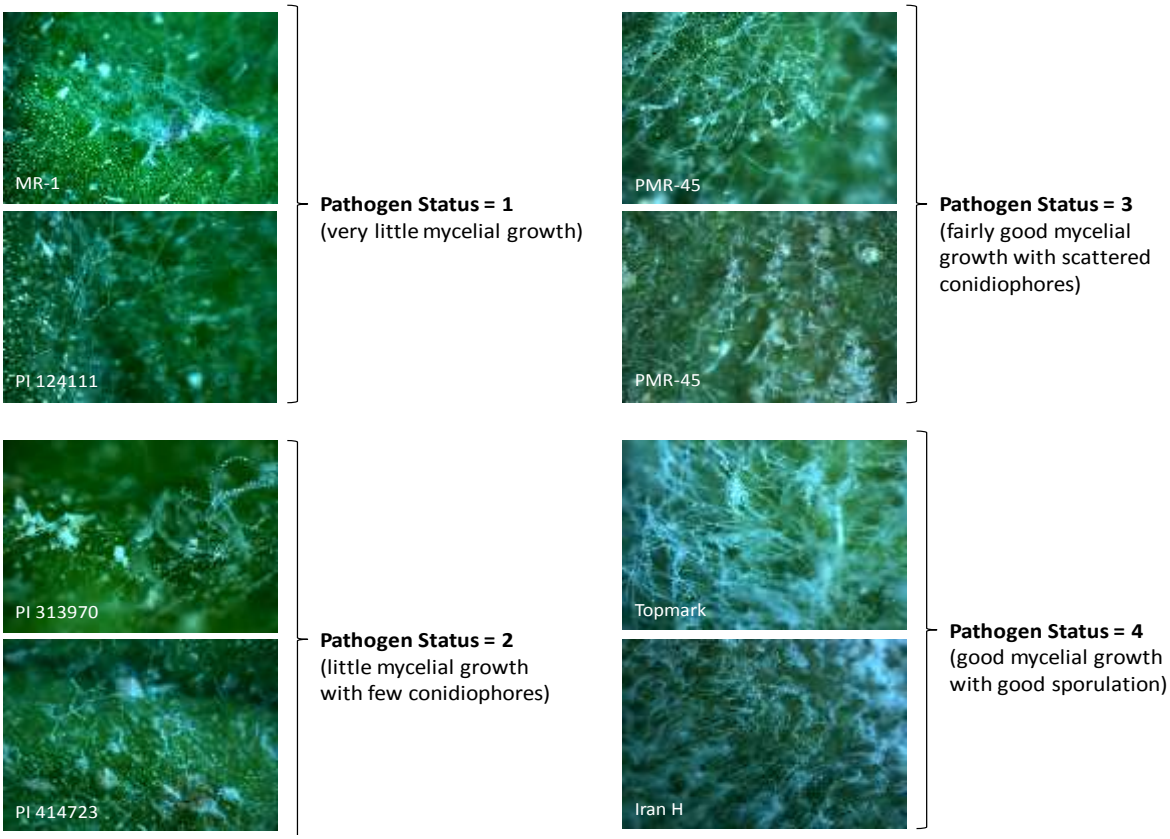

Figure 4-4. Scale used for pathogen status classification. Categories ranged from 1 to 4 according to fungal sporulation and conidiophore production on leaf surface of different muskmelon genotypes. Isolates were considered virulent with ratings of 0 (no fungal growth) and 1and were avirulent with ratings of 2-4.

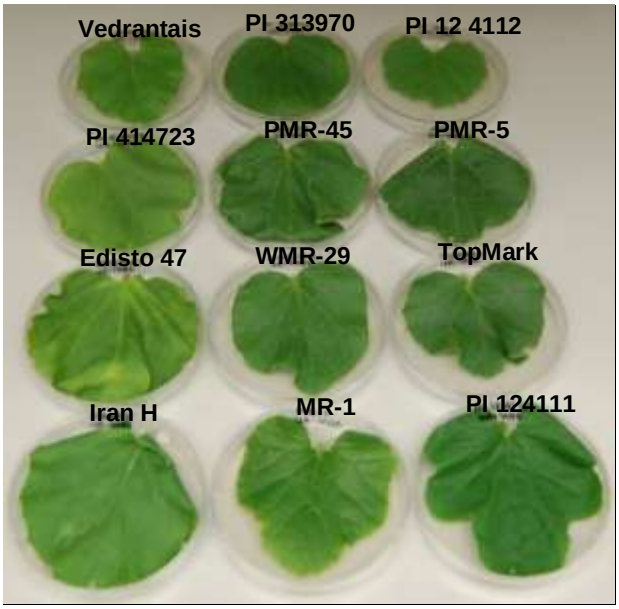

Figure 4-5. Healthy leaves (mock inoculated) of 12 muskmelon genotypes, 17 days post mock inoculation with un-infected ‘Butterbush’ cotyledons.

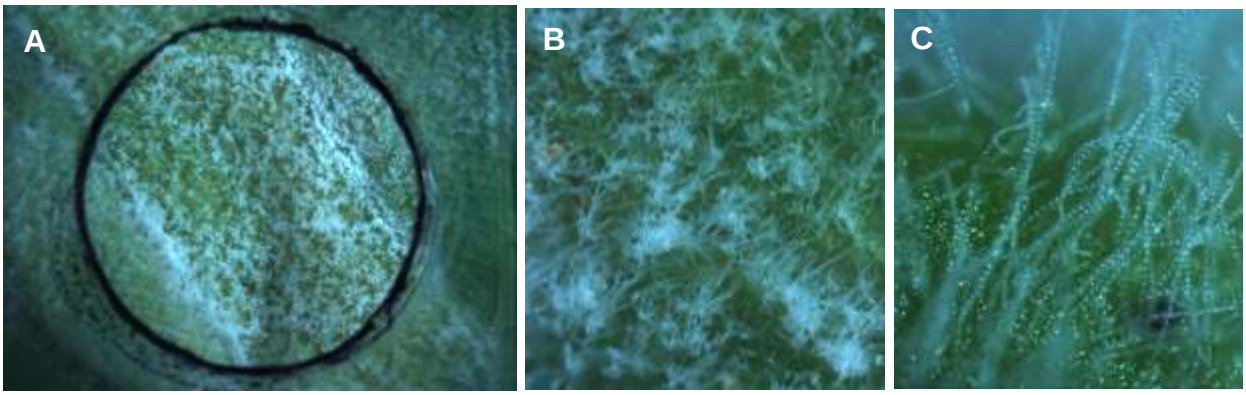

Figure 4-6. Detached muskmelon (‘Iran H’) leaf area covered by powdery mildew, 7 days after pathogen inoculation, showing extensively interwoven mycelium and abundant conidiophore production. (A) Magnification of 200x. (B) Magnification of 450x. (C) Magnification of 630x.

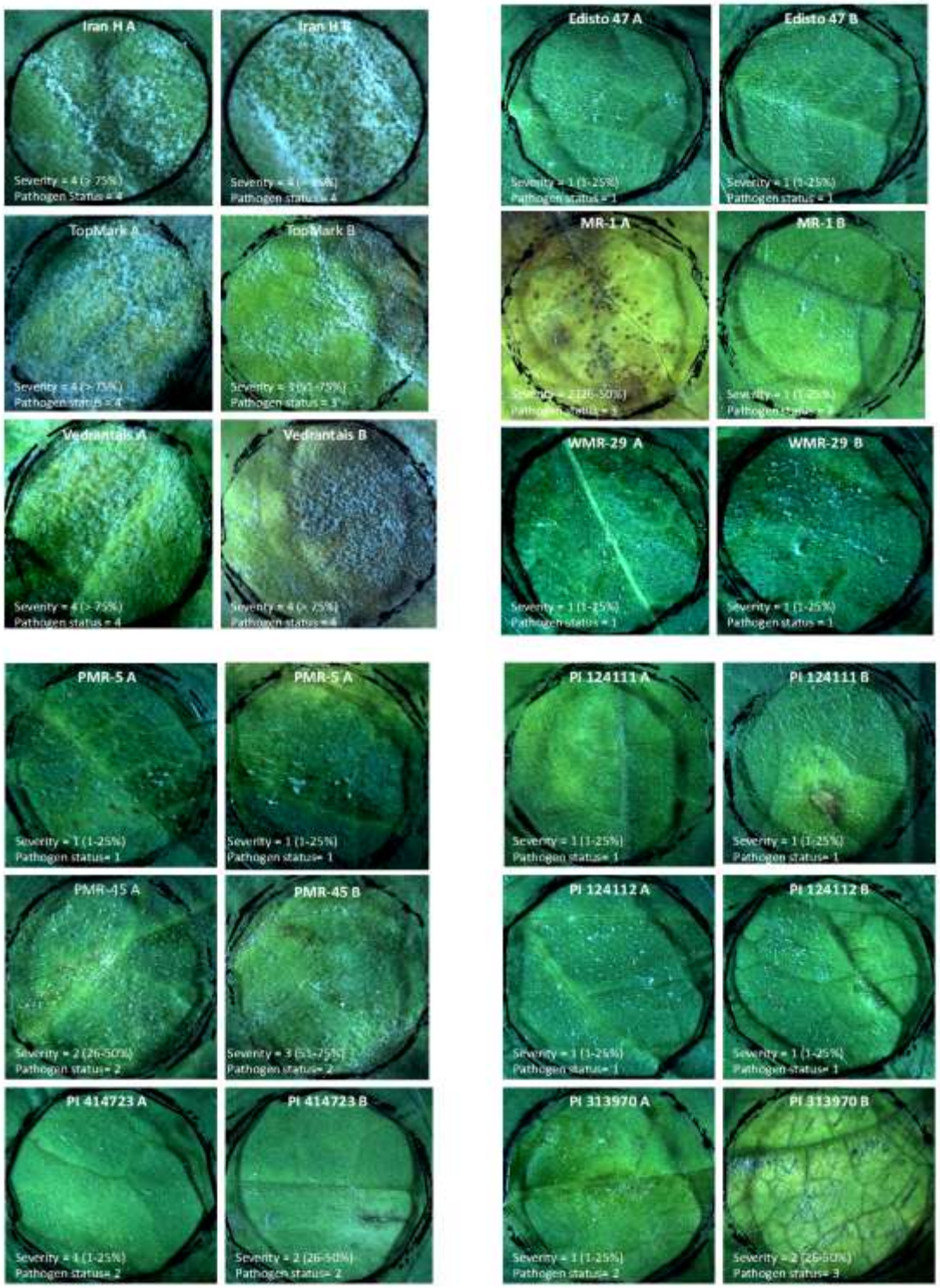

Figure 4-7. Reaction of a set of 12 muskmelon genotypes to powdery mildew isolate from summer squash. Percentage ratings of disease severity (0 to 100%) and pathogen development status (scale 0-4) were recorded 10 days post inoculation. (Magnification 200x)

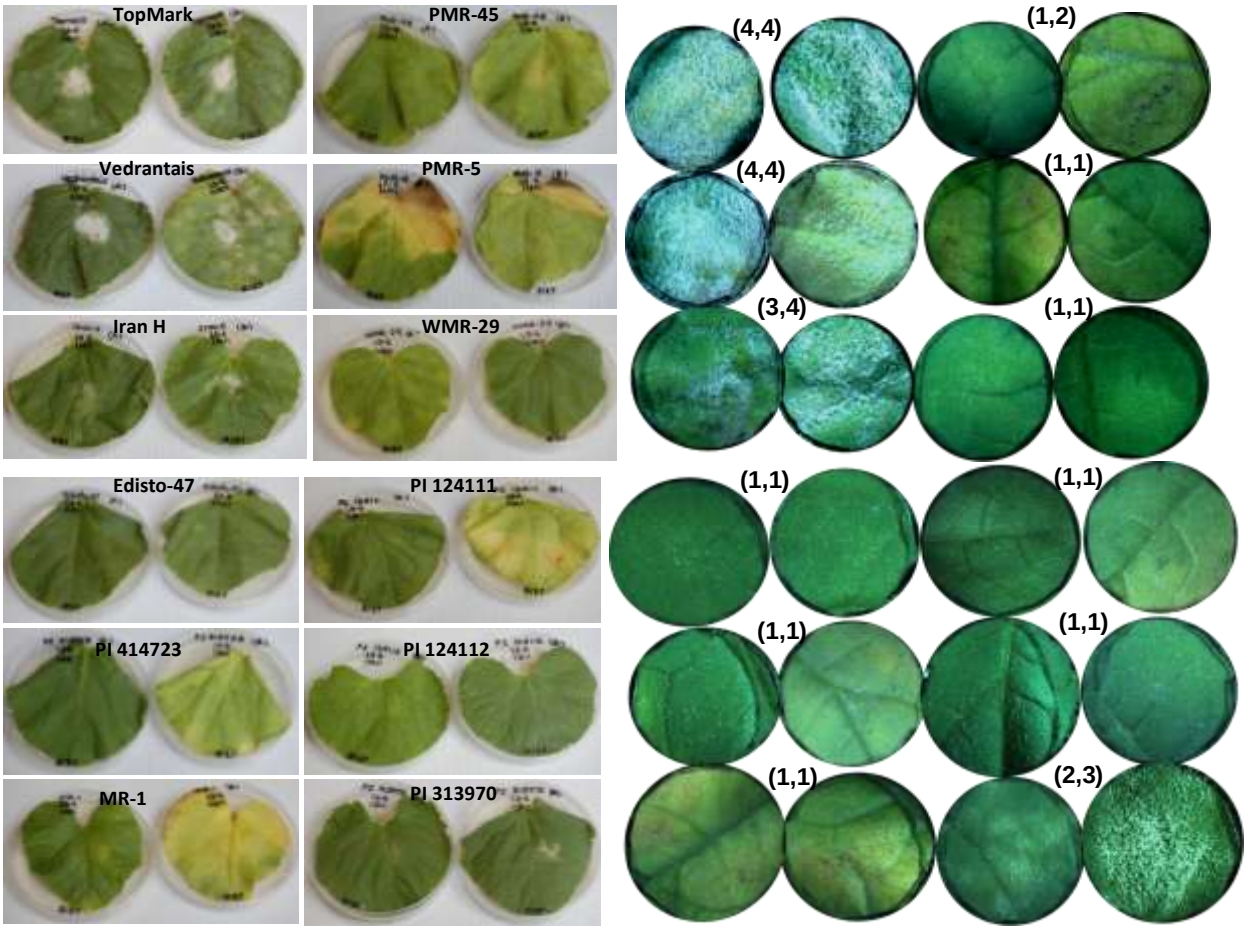

Figure 4-8. Reactions of 12 muskmelon genotypes to *P. xanthii* isolate LO-6 1(A)1 from 'Butterbush' collected at Live Oak, FL (10 dpi). Images on the right show close-up of percentage of powdery mildew infection on corresponding detached leaf (left). Fungal sporulation was recorded and photographed. Average disease severity and pathogen status ratings are expressed between parenthesis (right).Disease severity was categorized by: (0) = circular leaf area without symptoms of infection; (1) = less than 25% of circular area covered with mycelia; (2) = 26-50% of circular area covered with mycelia; (3) = 51-75% of circular area covered with mycelia and (4) = over 75% of circular area covered with mycelia. Pathogen status was rated as: (0) =no fungal growth (noted presence of inoculum); (1) = very little mycelial growth with no conidiophores; (2) = little mycelial growth with few conidiophores; (3) = fairly good mycelial growth with scattered conidiophores and (4) = heavy sporulation.

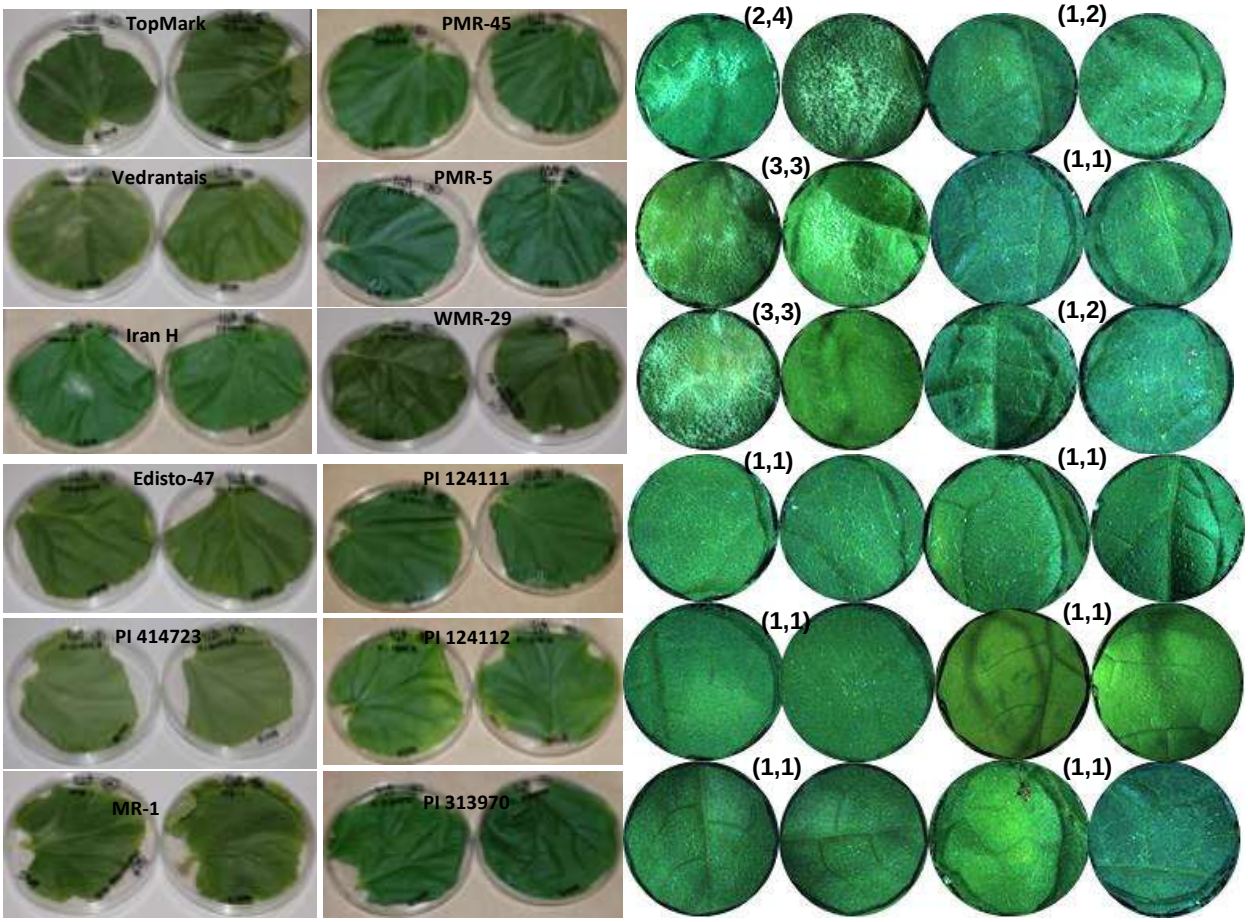

Figure 4-9. Reactions of 12 muskmelon genotypes to *P. xanthii* isolate Ci-6 1(A)1 from 'Butterbush' collected at Citra, FL (10 dpi). Images on the right show close-up of percentage of powdery mildew infection on corresponding detached leaf (left). Fungal sporulation was recorded and photographed. Disease severity and pathogen status ratings are expressed between the parenthesis (right).Disease severity was categorized by: (0) = circular leaf area without symptoms of infection; (1) = less than 25% of circular area covered with mycelia; (2) = 26-50% of circular area covered with mycelia; (3) = 51-75% of circular area covered with mycelia and (4) = over 75% of circular area covered with mycelia. Pathogen status was rated as: (0) =no fungal growth (noted presence of inoculum); (1) = very little mycelial growth with no conidiophores; (2) = little mycelial growth with few conidiophores; (3) = fairly good mycelial growth with scattered conidiophores and (4) = heavy sporulation.

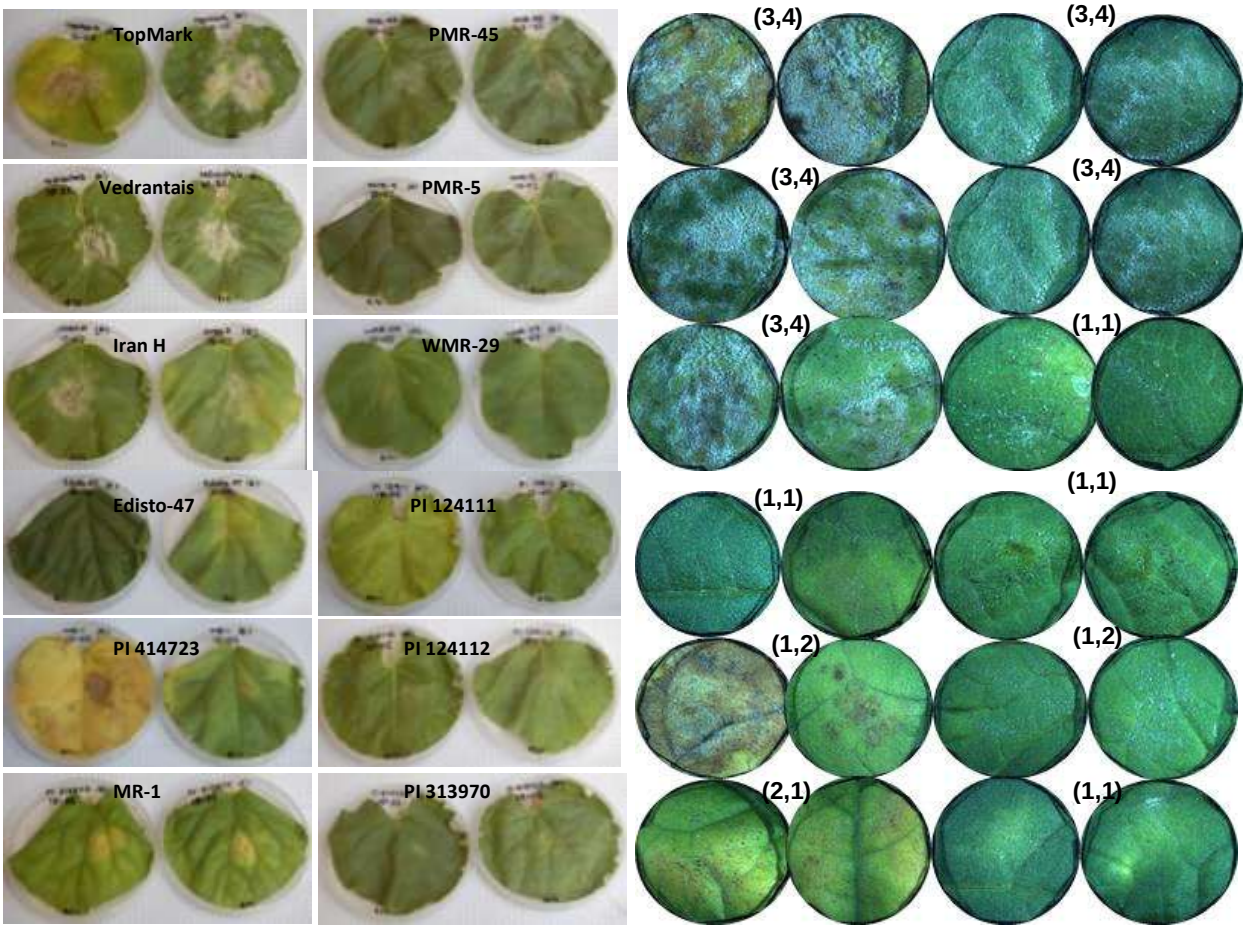

Figure 4-10. Reactions of 12 muskmelon genotypes to *P. xanthii* isolate 10-02 from muskmelon with natural powdery mildew infection in greenhouse at UF, Gainesville, FL (10 dpi). Images on the right show close-up of percentage of powdery mildew infection on corresponding detached leaf (left). Fungal sporulation was recorded and photographed. Disease severity and pathogen status ratings are expressed between the parenthesis (right).Disease severity was categorized by: (0) = circular leaf area without symptoms of infection; (1) less than 25% of circular area covered with mycelia; (2) = 26-50% of circular area covered with mycelia; (3) = 51-75% of circular area covered with mycelia and (4) = over 75% of circular area covered with mycelia. Pathogen status was rated as: (0) =no fungal growth (noted presence of inoculum); (1) = very little mycelial growth with no conidiophores; (2) = little mycelial growth with few conidiophores; (3) = fairly good mycelial growth with scattered conidiophores and (4) = heavy sporulation.

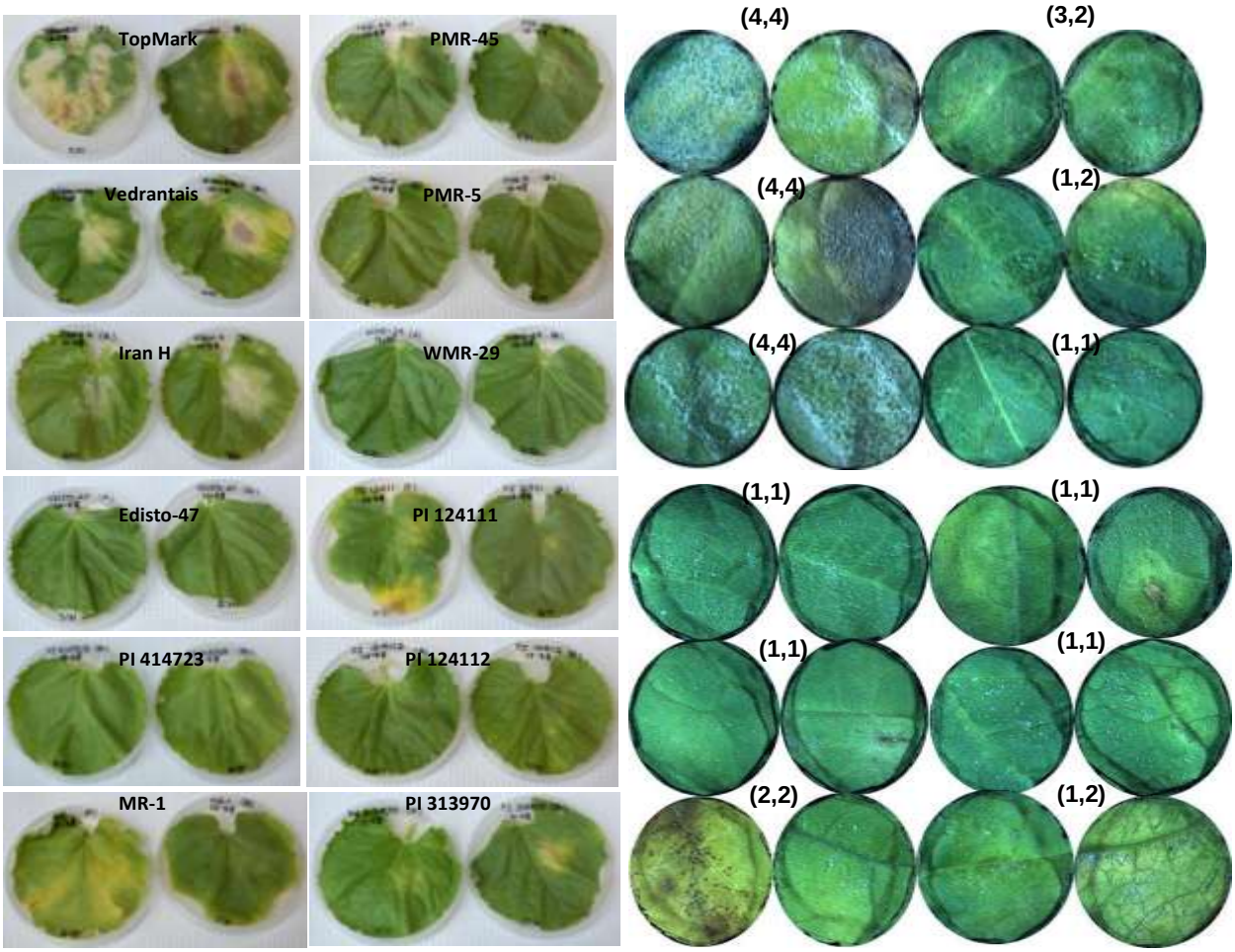

Figure 4-11. Reactions of 12 muskmelon genotypes to *P. xanthii* isolate 10-08 from squash collected in Immokalee, FL (10dpi). Images on the right show close-up of percentage of powdery mildew infection on corresponding detached leaf (left). Fungal sporulation was recorded and photographed. Disease severity and pathogen status ratings are expressed between the parenthesis (right).Disease severity was categorized by: (0) = circular leaf area without symptoms of infection; (1) = less than 25% of circular area covered with mycelia; (2) = 26-50% of circular area covered with mycelia; (3) = 51-75% of circular area covered with mycelia and (4) = over 75% of circular area covered with mycelia. Pathogen status was rated as: (0) =no fungal growth (noted presence of inoculum); (1) = very little mycelial growth with no conidiophores; (2) = little mycelial growth with few conidiophores; (3) = fairly good mycelial growth with scattered conidiophores and (4) = heavy sporulation.

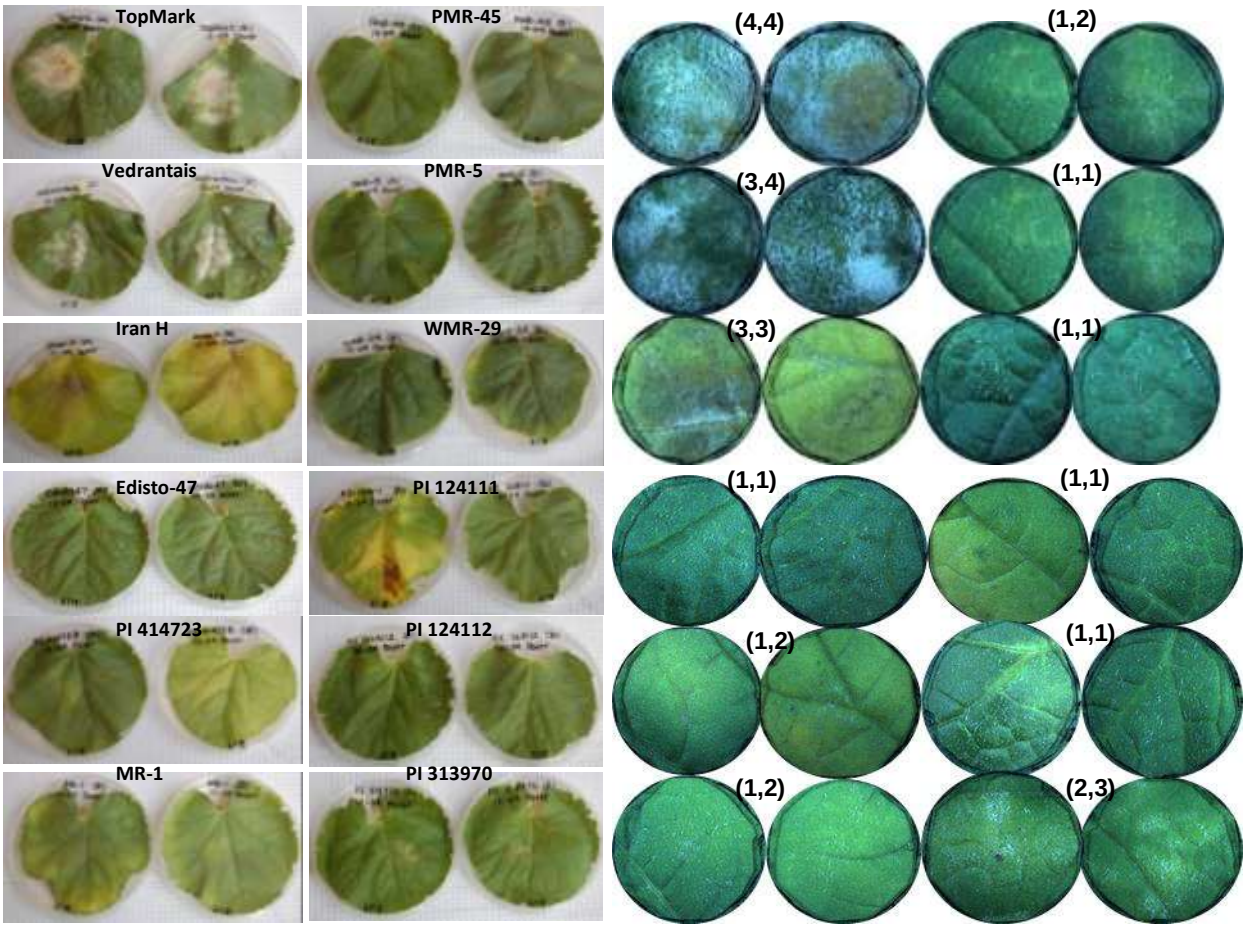

Figure 4-12. Reactions of 12 muskmelon genotypes to *P. xanthii* isolate 10-09 from summer squash collected in Dover, FL (10 dpi). Images on the right show close-up of percentage of powdery mildew infection on corresponding detached leaf (left). Fungal sporulation was recorded and photographed. Disease severity and pathogen status ratings are expressed between the parenthesis (right).Disease severity was categorized by: (0) = circular leaf area without symptoms of infection; (1) = less than 25% of circular area covered with mycelia; (2) = 26-50% of circular area covered with mycelia; (3) = 51-75% of circular area covered with mycelia and (4) = over 75% of circular area covered with mycelia. Pathogen status was rated as: (0) =no fungal growth (noted presence of inoculum); (1) = very little mycelial growth with no conidiophores; (2) = little mycelial growth with few conidiophores; (3) = fairly good mycelial growth with scattered conidiophores and (4) = heavy sporulation

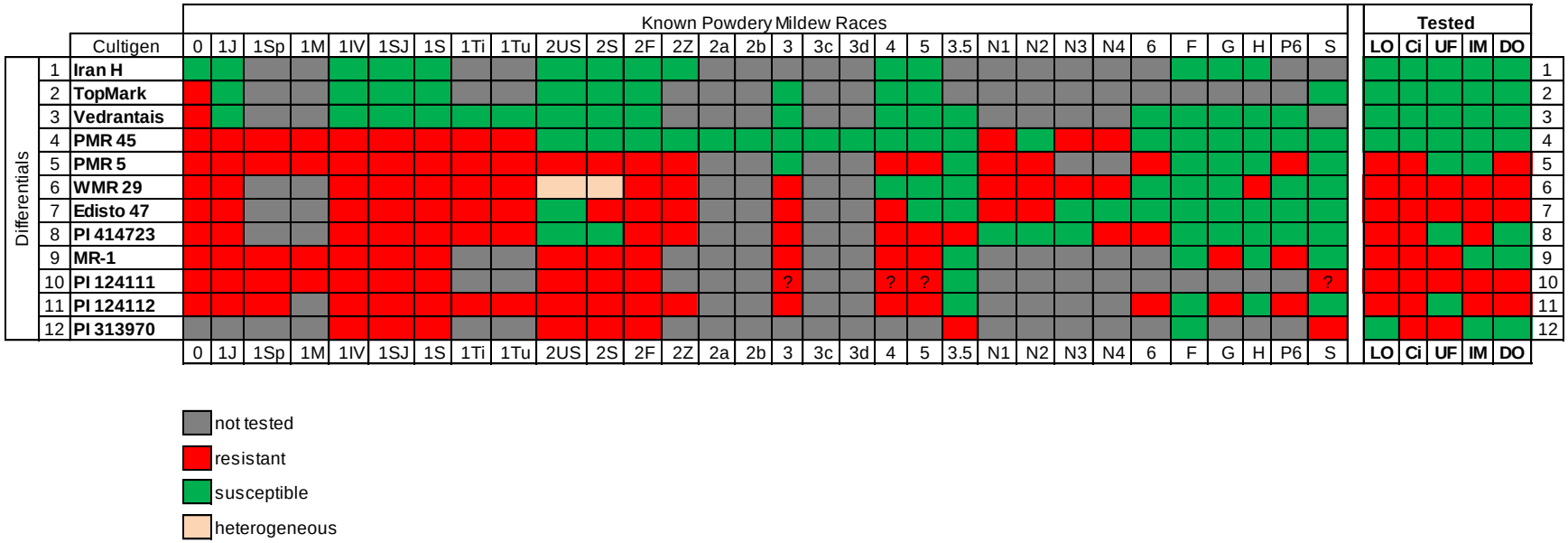

Figure 4-13. Expected reaction of 12 muskmelon genotypes to 31 known races of powdery mildew (*P. xanthii*) found in the U.S. and around the world. Different colors (red, grey, green and salmon) represent reaction of host plant to pathogen inoculation. (Table prepared by Dr. James D. McCreight, USDA, personal communication on 11 August, 2009). Table includes 5 powdery mildew races (far right) tested in this study. Isolates tested were: LO= LO-6 1(A) 1 collected from 'Butterbush' at Live Oak, FL; isolate Ci= Ci 6 1(A) 1 collected from 'Butterbush' at Citra FL; isolate UF= 10-02 collected from muskmelon in greenhouse at the University of Florida in Gainesville, FL; isolate IM= 10-08 collected from squash at Immokalee FL and isolate DO= 10-09 collected from squash at Dover, FL.

CHAPTER 5
RUPP'S BREEDING LINE DISEASE REPORT

Cucurbit powdery mildew pathogens, *Podosphaera xanthii* and *Golovinomyces cichoracearum*, have been considered "high risk" pathogens since these fungi have high evolutionary potential according to McDonald and Linde (196) and are more likely to overcome plant genetic resistance and/or develop fungicide resistance (29, 197). Factors such as mixed reproduction cycles (asexual and sexual), asexual spores (conidia) that are easily disseminated over long distances, the possibility of gene-for-gene interactions with their host (30) and resistance to some current fungicide chemistries have enabled cucurbit powdery mildews to become highly variable in their pathogenicity and virulence represented by the existence of several pathotypes and races (31, 159, 190).

In cucurbits, powdery mildew disease results in moderate to severe damage to the foliage, as well as a considerable reduction in yield and fruit quality (214). The disease is adequately controlled with fungicides, nonetheless, an increase in the number of reports of resistance to some recommended fungicides, and the difficulty of fungicide application on the underside of leaves where conditions are more favorable for disease development, have required more resourceful methods of disease management (128, 159, 201). As part of a successful integrated disease management program, breeding cucurbit crops for resistance to powdery mildew offers a more economical and safer method to considerably reduce disease pressure however, incorporating resistance into all horticultural cucurbit types would be a nearly impossible task, demanding great time and effort. Thus the use of plant disease resistance is not a viable option for all cucurbit

growers. In addition, resistant cultivars don't often provide complete disease control when used as the sole management practice.

Breeding of cucurbit crops for powdery mildew resistance has been effective especially in resources of race-specific resistance in muskmelon (8, 77, 110, 137, 149, 190, 305, 347). Sources of resistance have also been described in pumpkin, gourds and squash (15, 119, 123, 140, 154, 155, 352) and in cucumber (5, 22, 153, 156, 177, 178). Additionally, sources of resistance to powdery mildew in watermelon have been identified (63, 66-68, 171, 318, 319, 321, 350).

Genes for resistance to powdery mildew in melon have been widely studied. PMR 45 has a single dominant gene for powdery mildew resistance (77, 122). Moreover, most reported genotypes of melon resistant to powdery mildews include several genes (187, 264) and the exact number of genes involved differs according to the study (77, 129) and to the strain (race) of cucurbit powdery mildew tested (195). In most cases, monogenic or digenic dominant control has been reported (251). In watermelon, findings suggest that resistance is controlled by multiple genes which are expressed as degrees of tolerance (62, 64).

The development of cucurbit varieties displaying resistance to powdery mildew (mainly *P. xanthii*) has been one of the major aims of cucurbit breeding programs worldwide. In this study we evaluated the powdery mildew resistance response of 22 elite breeding lines developed by Rupp Seeds which contained two sources (*Cucurbita lundelliana* Bailey *and C. okeechobeensis* (Small) Bailey) of resistance to powdery mildew. Resistance to powdery mildew in the wild species, *C. lundelliana*, is conferred by a single dominant gene (274) and can be transferred to *C. moschata*. Resistance in

the other wild species *C. okeechobeensis* was reported to be conferred by a single dominant or incomplete dominant gene which is subject to the influence of modifier genes affecting the level of resistance (50). Resistance in the cultivated species *C. moschata* is conferred by either of two genes, designated *pm-1*^{L} and *pm-2*^{S} (2).

The pedigree of the Rupp breeding lines presented in Figure 5-6 provides additional information about the parentage of each line. *Cucurbita lundelliana* and *C. okeechobeensis* are wild *Cucurbita* species, known to have natural resistance to powdery mildew disease. Each Rupp breeding line with both sources of resistance has 12.5% of *C. lundelliana* genome and 12.5% of *C. okeechobeensis* genome. The rest of the genome of each of the breeding lines is 75% *Cucurbita moschata* (winter butternut squash type). Each line contained different proportions of each of the two wild species.

The purpose of this study was to assess the resistance of Rupp cucurbit breeding material to powdery mildew in comparison to two cucurbit cultivars (Butterbush and Mickey Lee) known to be highly susceptible to powdery mildew in Florida. Weekly evaluations, on leaf and whole plants, were recorded based on disease severity (percentage of infection) after the initial disease find in field plots.

Materials and methods

Plant Material

Field experiments were conducted at the North Florida Research and Education Center – Suwannee Valley (NFREC-SV) in Live Oak, FL. During spring of 2009, seedlings of 22 *Cucurbita* Rupp elite breeding lines were planted in adjacent rows on raised beds covered with black plastic mulch, as descried in Chapter 3. Additionally, two susceptible cultivars (Butterbush and Mickey Lee), were planted at Live Oak to promote

disease and serve as inoculum sources. The same two susceptible cultivars were also planted at Citra for disease assessment and comparison.

Seeds of each breeding line were kindly supplied by Mr. Duane Bell, cucurbit breeder with Rupp Seeds Inc. A list of the breeding lines is presented in Table 5. Seeds were direct-seeded into styrofoam growing trays on 25 March 2009. One seed of each breeding line was planted into each tray cell using potting media Fafard Professional 4P Mix (Fafard Company, Agawam, MA). A total of 25 seeds of each breeding line were sown. Seedlings were grown in a greenhouse at UF, for 30-45 days (1 to 2 true-leaf stages). Subsequently, seedlings were acclimatized for one week in a shaded and naturally ventilated greenhouse at Live Oak field station, before transplant into field plots. During acclimatization, seedlings were watered as needed. Five seedlings of each breeding line were transplanted into field plots on 23 April 2009. Some seedlings were replanted on 29 April 2009 due to lack of plant growth. All plots were replicated four times in a randomized complete block design as previously described in Chapter 3.

Powdery Mildew Disease Assessment in Field Trial

Occurrence of cucurbit powdery mildew was assessed on ‘Butterbush’ and ‘Mickey Lee’ in field plots at Live Oak. Watermelon plants (‘Mickey Lee’) were not assessed at Citra due to lack of seed germination when this cultivar was direct seeded in the field. Consequently, disease occurrence was monitored only on Butterbush plants (at each location) based on weekly evaluations of leaves, until disease on-set. Powdery mildew disease severity of the 22 elite breeding lines was assessed for a period of 9 consecutive weeks at Live Oak. Disease evaluations were based on average percentage of leaf area infected on both adaxial (upper) and abaxial (lower) leaf surfaces. Three leaves per plant per plot were assessed each week. The average

disease severity for whole plants, for each breeding line, was recorded based on the individual leaf assessments (3 leaves per plant) and on total appearance of plant health (green, senescing or necrotic). Disease severity ratings of all breeding lines were compared to both susceptible cultivars Butterbush and Mickey Lee at Live Oak. Disease severity evaluations for both susceptible cultivars were recorded from two plants per plot, at each location, on two randomly selected lower leaves per plant. Disease severity ratings at Live Oak, were recorded on 13, 19 and 26 May, 2, 10, 16 and 23 June, 1, 10, 15 and 23 July 2009. Ratings at Citra were noted on 26 May, 2, 10, 16 and 22 June, 1, 10, 15, 23 and 28 July and 3 August 2009.

The time from transplanting in the field to first disease symptoms was approximately 30 days. Powdery mildew disease symptoms appeared first at the Live Oak site, on 13 May 2009 and at Citra symptoms were first noted on 16 June 2009. Powdery mildew severity evaluations were conducted, starting from the time of appearance of the first signs of disease and upon confirmation of powdery mildew presence via microscopic examination of leaf samples as described in Chapter 3. First disease symptoms were defined as having at least one visible (unaided eye) fungal colony on any leaf of any susceptible plant, indicating naturally-occurring presence of the pathogen in each field site. Each week, infected ‘Butterbush’ and ‘Mickey Lee’ leaf samples were randomly collected from each location, on the same day as disease severity ratings were recorded. Individual leaves were placed in separate labeled and sealed plastic bags, kept in coolers and taken to the laboratory for morphological analysis and powdery mildew species identification within 24 to 48 hours, as described in Chapter 3.

Weekly assessment of powdery mildew disease severity was evaluated by visual observations based on disease grading scale with 11 categories ranging from 0-100% total leaf area infected (covered by mycelia), as follows: (0) = 0% leaf area infected; (1) =1 to 9%; (2) =10 to 19%; (3) =20 to 29%; (4) =30 to 39%; (5) =40 to 49%; (6) =50 to 59%; (7) =60 to 69%; (8) =70 to 79%; (9) =80 to 89%; (10) =90 to 99% and (11) =100% of leaf area infected. Breeding lines with severity ratings ≥4 were considered susceptible to powdery mildew. Lines with ratings of ≤3 were considered resistant to powdery mildew.

Statistical Data Analysis

Data were summarized as means (of 5 plants per plot) for each Rupp breeding line and for two susceptible standards (Table 5-2). Analysis of variance (ANOVA) was performed with SAS® (SAS® Institute, Cary, NC). Numerical data were compared using the Duncan's test (α=0.05). In the breeding line screening experiment, disease severity was scored each week, up to 9 consecutive weeks, starting from first appearance of disease symptoms.

The AUDPC (area under the disease progress curve) is an integrated measure which can be calculated directly from observed data or from the logistic function of estimated parameters. It is an approach for assessing plant disease epidemic as well as a useful tool for evaluating treatment effectiveness and for the development of disease control strategies (126). Additionally, AUDPC has served for the assessment of quantitative resistance in breeding programs (125) and has been reported as a reliable parameter to estimate and rank the performance of host genotypes according to their ability to retard the rate of disease development (93). In this study, AUDPC was calculated directly from observed field data (disease severity) and was chosen as a way

to summarize disease progress over time. The total plant disease severity (% leaf area affected assessed on 2 leaves per plant and 5 plants per plot) was calculated as the average of weekly ratings starting from time of appearance of first disease symptoms at each location. AUDPC values were calculated according to Equation 5-1.

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i) \quad (5\text{-}1)$$

Where *t* is time (in days) at the i^{th} observation (rating), *y* is the percentage of affected foliage (powdery mildew severity) at the i^{th} observation and *n* is the total number of observations (300).

Results and Discussion

Powdery Mildew Pathogen Characterization

Upon morphological and molecular analysis of powdery mildew samples collected randomly, each week, from susceptible types (‘Butterbush’ and ‘Mickey Lee’) and from some of the Rupp breeding lines, isolates tested in this study were determined to be *Podosphaera xanthii*. Characteristic morphological features such as erect conidiophores, immature conidia with crenate edge lines, ellipsoid to ovoid hyaline conidia, presence of fibrosin bodies and conidia dimensions (length and width) were consistent with published reports for *P. xanthii* and comparable to observations made previously in Chapter 3.

Disease Severity Evaluation

Powdery mildew infection at both field sites in north Florida (Live Oak and Citra) was characterized by a wide range of reactions. Symptoms ranged from little fungal sporulation, to 100% coverage of mycelia on susceptible plants; moderate yellowing to senescing leaves, and premature plant senescence and defoliation which caused fruit to

be exposed and suffer sun damage. Powdery mildew disease symptoms progressed over a period of 9 weeks at Live Oak and of 6 weeks at Citra. Highly susceptible cultivars (Butterbush and Mickey Lee) became infected first at Live Oak on 13 May 2009 (Figure 5-2) and four weeks later (16 June 2009), disease symptoms were observed at Citra (Figure 5-3). Initial disease severity ratings, for both field locations, were visually assessed and recorded at the appearance of first disease symptoms observed.

There were no significant differences ($P>0.05$) in disease severity (% leaf area infected) on 'Butterbush' in Live Oak and Citra (Figure 4-5). Overall, ratings taken on a weekly basis were continually increasing to harvest. At both locations, disease increased from 0 to 76% leaf area infected, with variability in rate of disease progress.

In contrast, cumulative AUDPC values were significantly different ($P<0.05$) between locations (Table 5-3 and Figure 5-5 and). This may have been explained by the fact that AUDPC values represent a buildup of disease, at each location, over time (9 or 6 weeks for Live Oak and Citra respectively) and is not based on single ratings. Dissimilarities in plant maturity when powdery mildew infection initially occurred and the presence of downy mildew (*Pseudoperonospora cubensis* (Berk. & M.A. Curtis) Rostovzev) and aphids (*Myzus* spp. Sulzer and *Aphis* spp.) at Citra may have led to premature plant decline and affected disease severity assessment.

Before powdery mildew disease peaked at Live Oak, 'Butterbush' plants thrived and were not severely impacted by downy mildew and aphids in the same way as the plants at the other location. Butterbush' plots were planted later and plants were younger when first disease symptoms were observed at Citra. Susceptible plants were

healthier and survived longer at Live Oak which could explain why there was gradual disease pressure build up at Live Oak compared to Citra (Figure 5-5).

Of the 22 Rupp breeding lines evaluated, a total of 19 lines were monitored. Some Rupp plots could not be assessed during the 9 week period due to severe downy mildew infection, confirmed via microscopic examination of leaf samples, which killed plants prematurely. No data were collected from three Rupp lines (lines 13, 14 and 15) which were omitted due to poor seed germination and/or viability. Upon analysis of disease severity for each Rupp line, 10 breeding lines had disease severity ≥ 6, represented by ratings of at least 50% of mycelia coverage on plants and indicating susceptibility to powdery mildew. Nine breeding lines had disease severity ratings of 4 or 5 indicated by 30 to 49% leaf area covered by mycelia. Two breeding lines were statistically different from the rest ($P<0.05$) and had disease severity rating ≤ 3, which represented some resistance to the pathogen (Table 5-2). According to disease severity ratings, susceptible cultivars (Butterbush and Mickey Lee) were not statistically different ($P>0.05$) from some Rupp breeding lines (Table 5-2).

According to our calculated AUDPC values, most breeding lines were not significantly different ($P>0.05$) in disease from susceptible controls ‘Butterbush’ and ‘Mickey Lee’ (Figure 5-5). Breeding lines 12 (08-CvU3124) and 5(08-CqU2659-6) were statistically different ($P<0.05$) from the rest and displayed the highest level of resistance to powdery mildew. The identification of powdery mildew resistance in Rupp lines 5 and 12 indicates that deployment of these lines under local (north Florida) conditions could result in a healthier crop with less reliance on fungicide applications. A few cultigens (lines 3, 7, 1 and 9) demonstrated some level of resistance that was better than the

susceptible controls but not statistically significant (*P*>0.05). No significant difference (*P>0.05*) was detected in disease severity between ‘Butterbush’ and ‘Mickey Lee’, suggesting that these plants were good control checks in this study. However, lines 21(SB368-3) and 2 (06-CvU2029-29-7) showed statistically higher (*P<0.05*) susceptibility to powdery mildew compared to all other cultigens tested (Figure 5-5).

If a particular line was considered susceptible to powdery mildew (in Live Oak, FL) it may have been that it did not carry the correct wild genome segment (the 12.5% from either wild parent). Additionally, the susceptibility of a particular line may also have been explained by the fact that the two wild species’ resistance was not to a specific powdery mildew race present in Florida at the time of disease assessments. The powdery mildew pathogen characterized in this study may have differed in pathogenicity and virulence to the breeding lines evaluated at Live Oak. We expected to find one or more of the breeding lines to be resistant or tolerant to the pathogen population present in the field during the spring of 2009 however more studies are needed to definitely confirm which race or races of cucurbit powdery mildew are present in Florida and to establish if and which specific plant resistance genes were effective against single or select genotypes of powdery mildew pathogens.

Table 5-1. List of 22 elite breeding lines supplied by Rupp Seeds. Cultigens were evaluated for resistance to cucurbit powdery mildew present in north Florida

No.	Breeding Lines	Cucurbit Specie
1	01-CsC460-1-5-4-4-3-9	*Cucurbita* sp.
2	06-CvU2029-29-7	*Cucurbita* sp.
3	08-CqU2659-3	*Cucurbita* sp.
4	08-CqU2659-5	*Cucurbita* sp.
5	08-CqU2659-6	*Cucurbita* sp.
6	08-CqU2659-7	*Cucurbita* sp.
7	08-CqU2659-8	*Cucurbita* sp.
8	08-CqU2659-9	*Cucurbita* sp.
9	08-CvU3090	*Cucurbita* sp.
10	08-CvU3091	*Cucurbita* sp.
11	08-CvU3123	*Cucurbita* sp.
12	08-CvU3124	*Cucurbita* sp.
13	08-CvU3125	*Cucurbita* sp.
14	08-CvU3139	*Cucurbita* sp.
15	08-CvU3163	*Cucurbita* sp.
16	08-CvU3164	*Cucurbita* sp.
17	08-CvU3253	*Cucurbita* sp.
18	08-CzU3089	*Cucurbita* sp.
19	08-CzU3130	*Cucurbita* sp.
20	SB359-7	*Cucurbita* sp.
21	SB368-3	*Cucurbita* sp.
22	SD358	*Cucurbita* sp.
23	SQ4-10 (‘Butterbush’)	*Cucurbita moschata*
24	WM23-20 (‘Mickey Lee’)	*Citrullus lanatus*

Commercially available cultivars Butterbush (23) and Mickey Lee (24) are known to be highly susceptible to powdery mildew in Florida.

Table 5-2. Evaluation of Rupp elite breeding lines represented by percent leaf area affected and disease severity rating caused by powdery mildew at Live Oak, FL during spring 2009.

Breeding Line Cultigens	% leaf area affected *	Average disease severity rating
21	72 ± 24.82 A	8
2	69 ± 23.73 AB	7
20	58 ± 22.92 ABC	6
8	56 ± 14.08 BCD	6
22	54 ± 20.28 BCDE	6
18	54 ± 20.61 BCDE	6
16	54 ± 19.96 BCDE	6
23 (‘Butterbush’)	54 ± 17.7 BCDE	6
11	52 ± 23.06 CDEF	6
17	52 ± 22.6 CDEF	6
19	49 ± 19.24 CDEF	5
6	48 ± 17.68 CDEF	5
10	48 ± 14.36 CDEFG	5
24 (‘Mickey Lee’)	43 ± 17.96 CDEFG	5
4	43 ± 14.59 CDEFG	5
3	41 ± 18.45 CDEFG	5
7	40 ± 15.86 DEFG	5
1	37 ± 14.42 EFG	4
9	36 ± 12.53 FG	4
5	30 ± 12.82 GH	3
12	18 ± 10.85 H	2

Data represents the mean of n=32. Values followed by the same letters are not statistically significant (Duncan, α=0.05). Disease severity ratings are visual estimates taken based on grading scale with 11 categories where: (0)= 0% leaf area infected; (1)=1 to 9%; (2)=10 to 19%; (3)=20 to 29%; (4)=30 to 39%; (5)=40 to 49%; (6)=50 to 59%; (7)=60 to 69%; (8)=70 to 79%; (9)=80 to 89%; (10)=90 to 99%; (11)=100% of leaf area infected. Breeding lines with severity ratings ≥6 were considered susceptible to powdery mildew. Lines with ratings of 4 or 5 were considered moderately resistant and lines with ratings of ≤3 were resistant to powdery mildew. Description of each cultigen is presented in Table 5-1.

* Values represent % leaf area affected followed by the standard deviation.

Table 5-3. Evaluation of powdery mildew susceptible cultivar Butterbush represented by AUDPC (area under disease progress curve) and average disease severity (% leaf area affected) at Live Oak and Citra, FL during spring 2009.

Cultivar Butterbush (location)	AUDPC *	Average disease severity (% leaf area affected) *
23 (Live Oak)	2735 ± 478.7 A	53.6 ± 17.7 A
23 (Citra)	1951 ± 206.4 AB	47.0 ± 32.6 A

Data represents the mean of n=32 for Live Oak and n=56 for Citra. Values followed by the same letters are not statistically significant (Duncan, α=0.05). Average disease severity based grading scale with 11 categories ranging from 0 to 100%. AUDPC was calculated directly from observed field data (disease severity) and was chosen as a way to summarize disease progress over time. The total plant disease severity (% leaf area affected assessed on 2 leaves per plant and 5 plants per plot) was calculated as the average of weekly ratings starting from time of appearance of first disease symptoms at each location.

* Values represent AUDPC and average disease severity followed by the standard deviation.

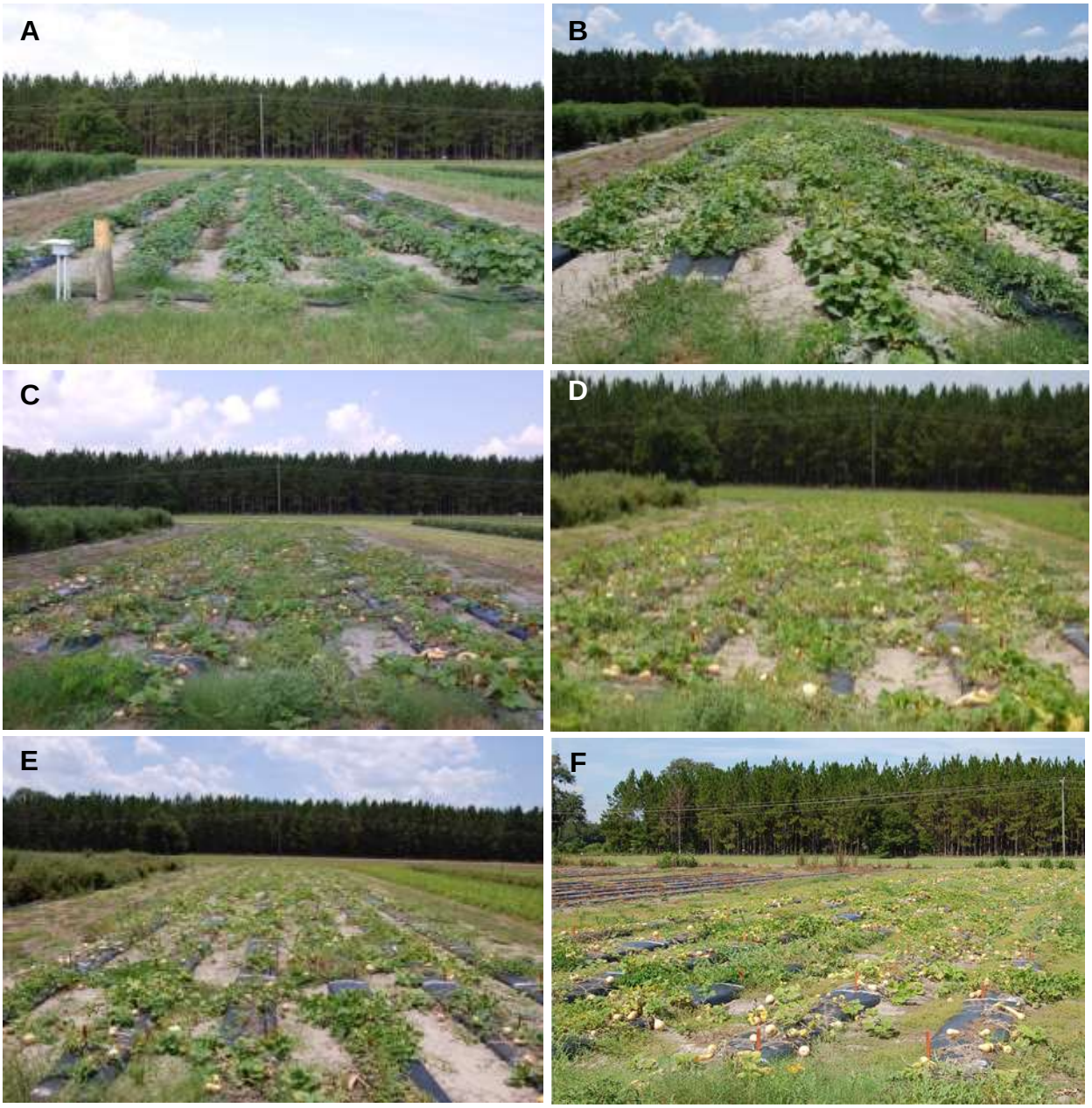

Figure 5-1. Powdery mildew disease on 22 Rupp breeding lines (*Cucurbita* spp.) and on powdery mildew susceptible cultivars Butterbush and Mickey Lee, at Live Oak, FL field site during spring 2009. Increase powdery mildew disease resulted in premature plant senescence, defoliation and exposure of fruit to sunscald. Disease severity assessed on (A) 26 May (B) 10 June (C) 16 June (D) 23 June (E) 01 July (F) 23 July.

Figure 5-2. Detail of powdery mildew disease on the same plant of susceptible cultivar Butterbush at Live Oak, FL field site during spring 2009. Increase powdery mildew disease resulted in premature plant senescence, defoliation and exposure of fruit to sunscald. Disease severity assessed on (A) 13 May (B) 26 May (C) 10 June (D) 23 June (E) 10 July (F) 23 July 2009

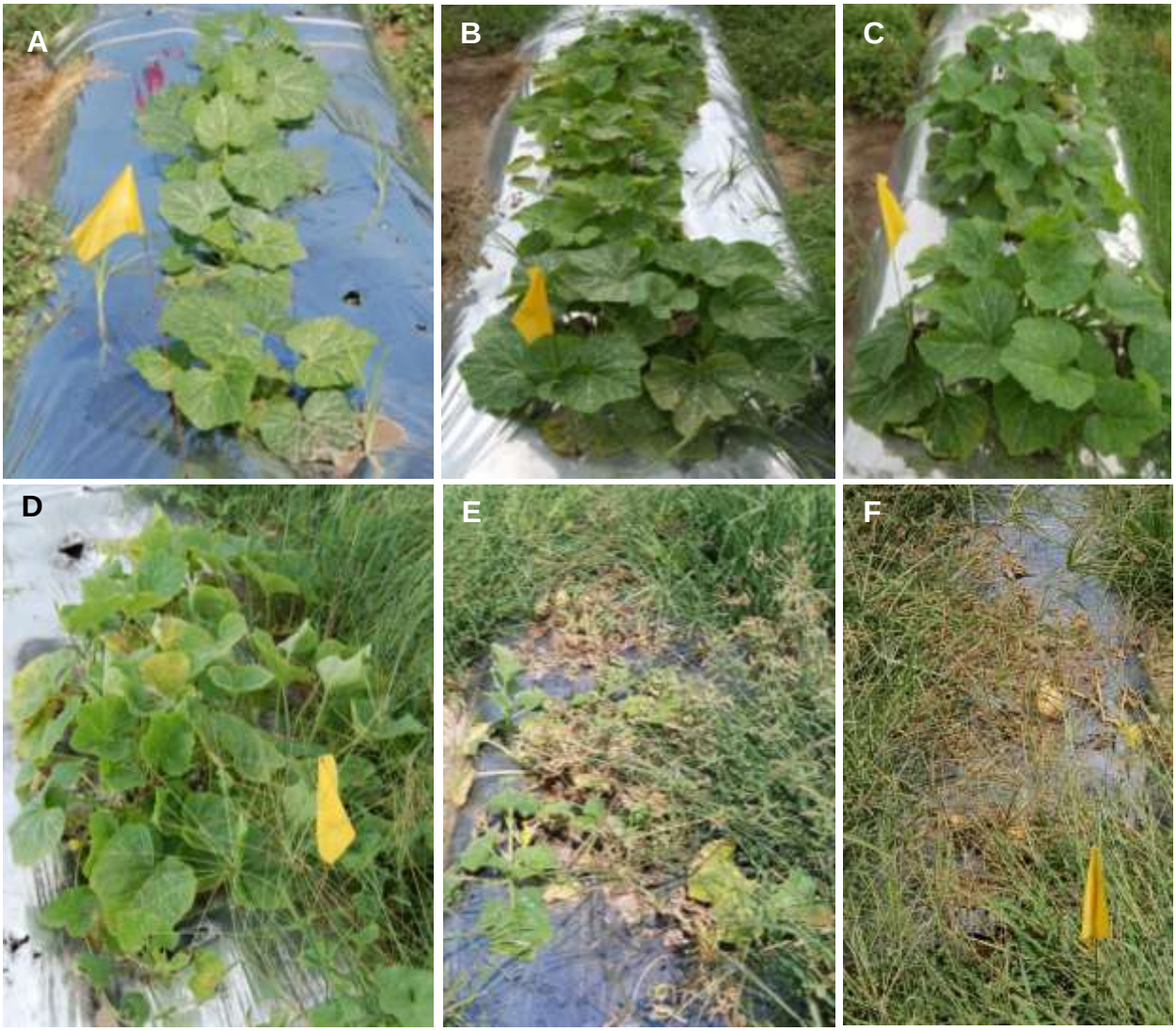

Figure 5-3. Detail of powdery mildew disease on the same plant of susceptible cultivar Butterbush at Citra, FL, field site during spring 2009. Increase powdery mildew disease resulted in premature plant senescence, defoliation and death. In addition to powdery mildew, plants at Citra were heavily affected by downy mildew (*Pseudoperonospora cubensis*) and by aphid pests (*Myzus* spp. and *Aphis* spp.) before fruit set. Disease severity assessed on (A) 16 June (B) 23 June (C) 01 July (D) 10 July (E) 23 July (F) 28 July 2009.

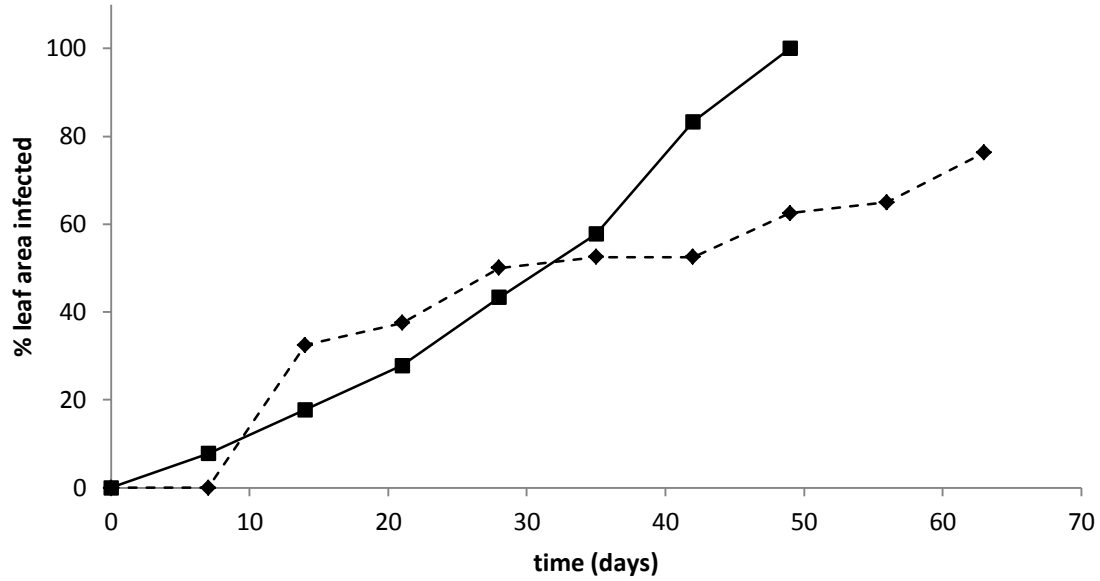

Figure 5-4. Disease severity (percent leaf area infected) of powdery mildew on susceptible 'Butterbush' assessed over 9 weeks (Live Oak) and 6 weeks (Citra). At Live Oak, day 0 corresponded to 13 May 2009 and at Citra day 0 was 16 June 2009. Live Oak values are represented by dotted line and Citra by solid line.

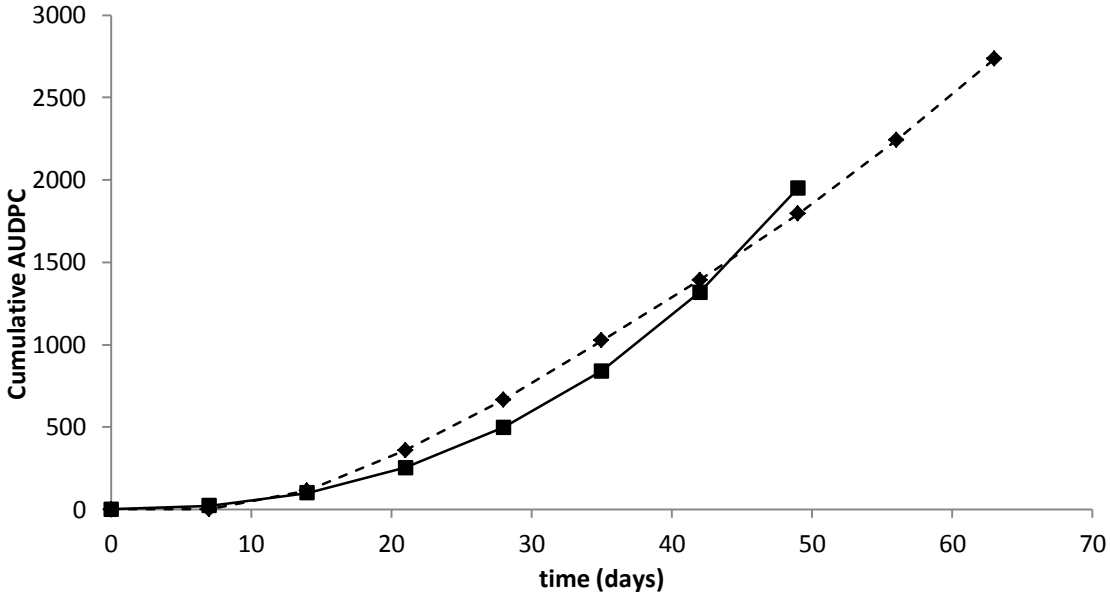

Figure 5-5. The cumulative AUDPC (area under disease progress curve) of weekly powdery mildew assessment on susceptible 'Butterbush' infected at Live Oak (dotted line) and at Citra (solid line). AUDPC values were calculated from disease severity data presented in Figure 5-4.Time 0 (zero) represents the week prior (no symptoms) to powdery mildew appearance at each field site.

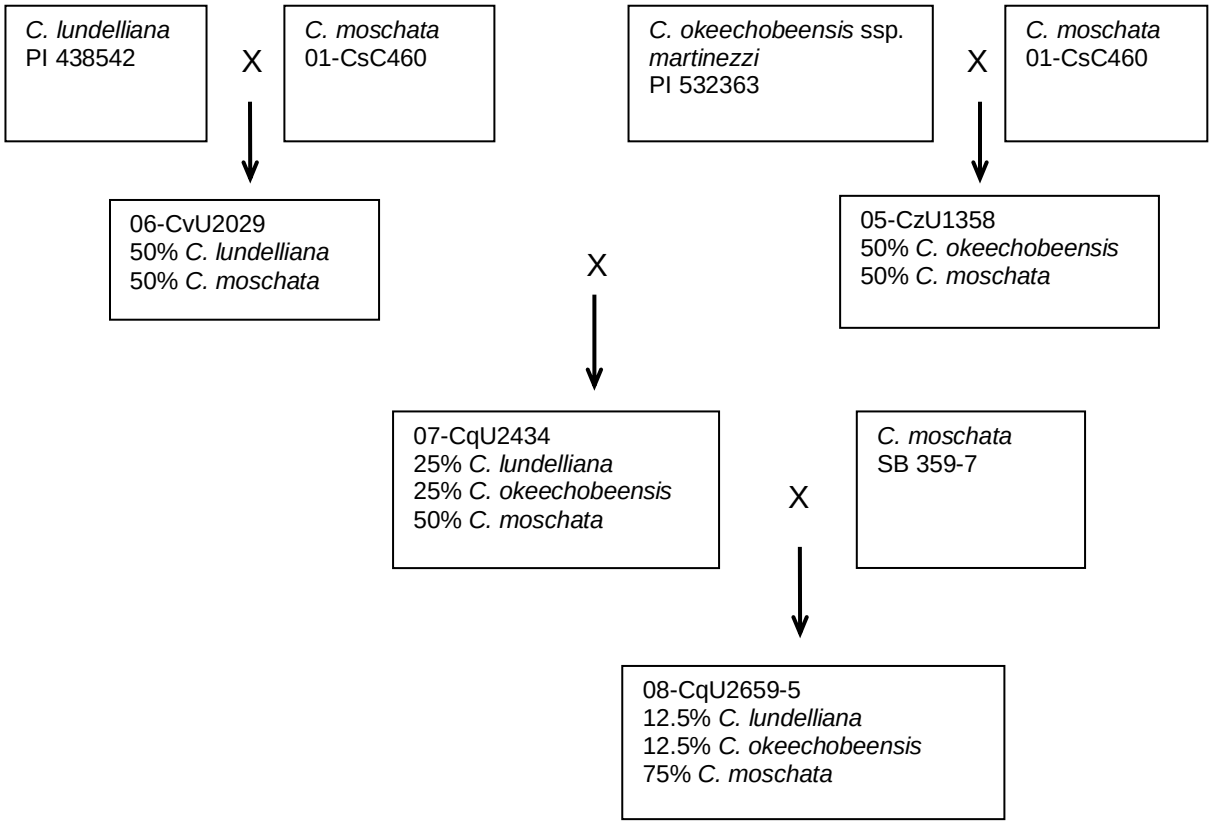

Figure 5-6. Pedigree of Rupp powdery mildew breeding lines evaluated at Live Oak, FL during field trial in spring of 2009 (Pedigree prepared by Dr. Eileen Kabelka, formerly of UF Department of Horticulture Science, currently with Harris Moran Seed Co., CA)

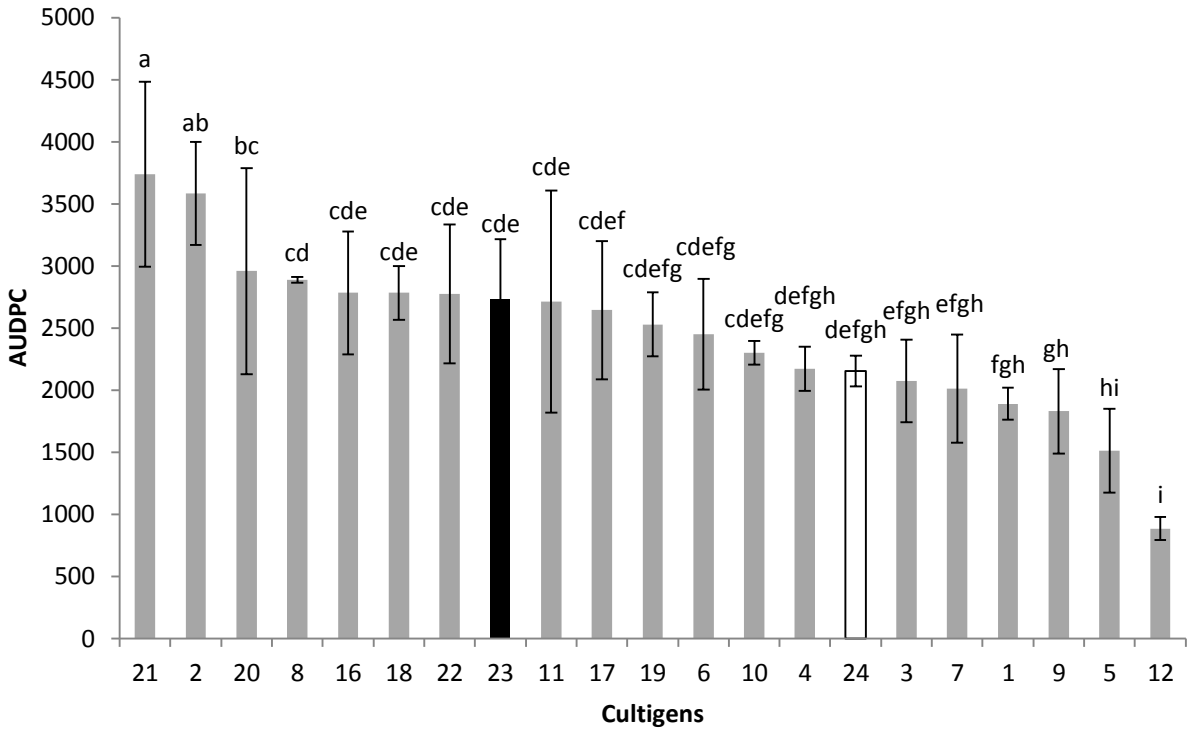

Figure 5-7. The cumulative area under the disease progress curve of powdery mildew on 22 elite breeding lines (Rupp Seeds Inc.) inoculated by natural infection at Live Oak, FL during spring of 2009. Data represents the mean of n=4. Values with similar letters are not significantly different (Duncan,α= 0.05). The black bar (cultigen 23) represents susceptible winter butternut squash 'Butterbush' and white bar (cultigen 24) represents susceptible watermelon 'Mickey Lee'. Area under the disease progress curve was calculated using the formula $AUDPC = \sum_{i=1}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$, where *t* is time in days between each evaluation, *y* is the percentage of affected foliage at each rating time and *n* is the number of ratings. Description of each cultigen is presented in Table 5-1.

CHAPTER 6
SUMMARY AND CONCLUSIONS

Cucurbit powdery mildew caused by two obligate fungal pathogens, *Podosphaera xanthii* and *Golovinomyces cichoracearum*, has been reported to cause crop losses in cucurbit production worldwide (218). The disease can significantly reduce yield by decreasing fruit size, number of fruits, and length of time fruits can be harvested (353). Fruit quality and marketability can also be affected due to premature leaf senescence causing fruits to become exposed to sunscald (214). Recently, there has been an increase in occurrence and severity of powdery mildew in Florida, resulting in heightened concern with fungicide resistance and potentially a change in the race composition. Cucurbit powdery mildew occurs on cucumber, melon, squash, zucchini, pumpkin, gourd and more recently, on watermelon where the incidence of outbreaks has increased and the disease has become an important problem in the major U.S. production areas. This study focused on investigating the recent increase in incidence, severity and host range of cucurbit powdery mildew in Florida. An efficient technique for *in vivo* establishment and maintenance of cucurbit powdery mildew isolates collected throughout Florida was developed. The first objective was to identify and characterize the prevailing causal agent of cucurbit powdery mildew in north central Florida through morphological features and DNA analysis. The second objective was to assess the presence of physiological races within cultured isolates using detached muskmelon leaf bioassays. The third objective was to evaluate the varietal reactions of 22 *Cucurbita* elite breeding lines (Rupp Seed Inc.) for susceptibility to powdery mildew under local (Florida) field conditions.

Cucurbit Powdery Mildew Isolate Collection, Maintenance and Characterization

The causal agents of cucurbit powdery mildew disease produce identical symptoms and can be difficult to differentiate in the absence of the teleomorphic stage. However morphological features of *P. xanthii* differ from those of *G. cichoracearum* at the anamorphic (asexual) stage and include: size and shape of conidia, presence of fibrosin bodies, immature conidia edge morphology and germ tube morphology (22, 24, 36, 304).

In this study, we identified and characterized single colony isolates of cucurbit powdery mildew from multiple field sites, dates, and cucurbit hosts. Two butternut winter squash cultivar Butterbush fields in north central Florida (Live Oak and Citra) were sampled and additional cucurbit isolates were collected from southwest and northeast FL. Microscopic observations of isolates sampled from 'Butterbush' revealed hyaline conidia, ellipsoid to ovoid in shape, with conidial dimensions of 31-44 x 15-24 µm (n = 100) and footcells of 45-67 x 10-13 µm (n = 25). All isolates exhibited fibrosin bodies and conidia edge lines were crenate. Isolates were subjected to multiplex polymerase chain reactions (PCR) with species-specific primers S1/S2 (for *P. xanthii*) and G1/G2 (for *G. cichoracearum*) and a specific PCR product of 454 bp was amplified from genomic DNA of most isolates. Based on morphological and molecular analyses, all cucurbit powdery mildew isolates were identified as *P. xanthii*. Additionally, in this study, a method for culture maintenance of cucurbit powdery mildew isolates on cotyledons and first leaflets of some cucurbit types was developed. Previous studies had demonstrated that isolates can be maintained using leaf disks (161, 192, 218) or whole plants (66, 319) and we showed that detached leaflets can be an efficient substrate for powdery mildew isolate maintenance and for *in vivo* bioassays.

Determination of Powdery Mildew Physiological Races

To date, approximately 30 distinct physiological races of *P. xanthii* (39, 190) and 2 races of *G. cichoracearum* (71, 158, 322) have been identified worldwide. Traditionally, cucurbit powdery mildew races have been defined by the disease response of the pathogen isolate on a set of muskmelon differentials which can differentiate cucurbit powdery mildew races originating from melon, cucumber, *Cucurbita* spp. and watermelon (157).

In this study we evaluated the reactions of 12 muskmelon genotypes to 5 powdery mildew isolates (10 days post inoculation). Our observations indicated variability in isolate virulence. When a muskmelon genotype was susceptible to a certain isolate, percentages of leaf area covered by powdery mildew typically showed extensive interwoven mycelium and abundant conidiophore production. Three muskmelon genotypes were resistant to all 5 powdery mildew isolates tested, exhibiting less than 25% of leaf area infected. In contrast, three other genotypes were susceptible to all fungal isolates and had ratings as high as 75% leaf area covered with mycelia. Based on currently recognized races of *P. xanthii* using a differential profile on muskmelon (Dr. James D. McCreight, personal communication) our isolates did not belong to any of the currently described races of *P. xanthii*. Our results indicated that we may have found race 2F or race 2Z or race 3. Three isolates tested (UF, IM and DO) were undefined to date and not consistent with any of the previously described race profiles of *P. xanthii*. A different *P. xanthii* pathogenic race may be present in the field sites sampled throughout Florida or a mixed race colonies were isolated during our sampling process. Further replication and careful monitoring or environmental conditions during inoculation experiments are suggested approaches for future work. Further characterization studies

of the powdery mildew pathogen population in Florida should be performed to determine if other pathogenic strains could be present at different times of the year and in different regions. By identifying these pathogen populations researchers will be able to distinguish and recommend appropriate control strategies for powdery mildew disease in Florida and better understand and match host resistance to local pathogen populations. This information is important for cucurbit breeders, plant pathologists and extensionists, as well as cucurbit growers.

Rupp's Breeding Line Disease Report

Typically, the control of powdery mildew in susceptible cucurbit cultivars is achieved with the use of fungicides. However, the repeated use of site-specific products over time has resulted in powdery mildew resistance to some commercial chemical compounds. Distinct physiological pathotypes and races of *P. xanthii* have been detected with resistance to as many as eight classes of fungicides (302). Presence of resistant fungal strains has been associated with lack of powdery mildew control (161). Resistant cucurbit varieties are being developed and are becoming an increasingly important component of powdery mildew management programs in the U.S. (123) and elsewhere (347).

In this study, we evaluated the powdery mildew resistance response of 22 elite breeding lines developed by Rupp Seed Inc. (Wauseon, OH) which contained parentage from two wild cucurbit species (*Cucurbita lundelliana and C. okeechobeensis*) with known resistance to powdery mildew. Disease response was measured by pathogen development, disease severity and AUDPC (area under the disease progress curve). Powdery mildew infection at two field sites in north central Florida (Live Oak and Citra) was characterized by little fungal sporulation to 100%

coverage of mycelia on susceptible plants; moderate yellowing to senescing leaves, and premature plant senescence and defoliation which caused fruit to be exposed to sunscald. Analysis of disease severity indicated that 10 breeding lines had disease severity greater than 50% indicating susceptibility to powdery mildew and two breeding lines were statistically different from the rest and demonstrated some level of resistance to powdery mildew. Susceptible cultivars (Butterbush and Mickey Lee) known to be highly susceptible to powdery mildew in Florida, were not statistically different from some of the susceptible breeding lines. If a particular breeding line was considered susceptible to powdery mildew it may have been that it did not carry the correct wild genome segment (from either wild parent) or it may have been because the source of resistance was not to a specific powdery mildew race present in Florida at the time of disease assessments. AUDPC values for most breeding lines were not significantly different from the susceptible cultivars. Two breeding lines presented lower calculated AUDPC and were statistically different from the rest. Two other lines showed statistically higher AUDPC compared to all other cultigens tested. A possible explanation would be that AUDPC values represent a buildup of disease over time (9 or 6 weeks for Live Oak and Citra respectively) and is not based on a single rating. Dissimilarities in plant maturity and the presence of downy mildew and aphids at Citra may have led to premature plant decline and adversely affected powdery mildew disease assessments. Before powdery mildew disease peaked at Live Oak, ‘Butterbush’ plants thrived and were not severely impacted by other disease or pests in the same way as plants at the other location. Butterbush plots were planted later and plants were younger when first disease symptoms were observed at Citra. Susceptible plants were healthier and

survived longer at Live Oak which could explain why there was gradual disease pressure build up compared to Citra. Additional studies with less variation which caused plants to senesce prematurely, before complete powdery mildew assessment would be recommended for a more comparable evaluation between locations. Ideally plants evaluated at each location should have been planted at the same time so that possible effects such as plant maturity and interference of other diseases would be reduced. Further studies are needed to confirm which race or races of cucurbit powdery mildew are present in Florida and to establish which specific plant resistance genes would be effective against single or select genotypes of powdery mildew pathogens.

Discovery, deployment, and adoption of host resistance to powdery mildew will potentially advance the profitability and reduce reliance upon fungicides in Florida cucurbit crops.

APPENDIX
LIST OF POWDERY MILDEW SUSCEPTIBLE HOST

Table A-1. List of powdery mildew-susceptible cucurbit hosts used throughout this research

	PI (Plant Introduction)	Cultivar	Cucurbit specie	Cucurbit type
SQ4-10		Butterbush	*Cucurbita moschata*	butternut squash
WM23-20		Mickey Lee	*Citrullus lanatus*	watermelon
		All Sweet	*Citrullus lanatus*	watermelon
PMR 45	Ames 26811		*Cucumis melo*	muskmelon
PMR 5	Ames 26809		*Cucumis melo*	muskmelon
MR-1	Ames 8578		*Cucumis melo*	muskmelon
	PI 414723		*Cucumis melo*	muskmelon
Edisto 47	NSL 34600		*Cucumis melo*	muskmelon
Topmark	NSL 30032		*Cucumis melo*	muskmelon
		Athena Hybrid	*Cucumis melo*	cantaloupe
		Best Jumbo	Cucumis melo	cantaloupe
	SQ72-20	Table Ace	*Cucurbita pepo*	acorn squash
	PM2-10	Big max	*Cucurbita pepo*	pumpkin
		Long Island Cheese	*Cucurbita moschata*	pumpkin
	CU57-20	Poinsett 76	*Cucumis sativus*	cucumber
	CU34-20	Straight 8	*Cucumis sativus*	cucumber
	SMR-58	Wisconsin	*Cucumis sativus*	cucumber
		Waltham	*Cucurbita moschata*	butternut squash

LIST OF REFERENCES

1. Adam, L., Ellwood, S., Wilson, I., Saenz, G., Xiao, S., Oliver, R. P., Turner, J. G., and Somerville, S. 1999. Comparison of *Erysiphe cichoracearum* and *E. cruciferarum* and a survey of 360 *Arabidopsis thaliana* accessions for resistance to these two powdery mildew pathogens. Mol. Plant-Microbe Interact. 12:1031-1043.

2. Adeniji, A. A., and Coyne, D. P. 1983. Genetics and mature resistance to powdery mildew in crosses of butternut with calabaza squash and 'Seminole pumpkin' Journal of the American Society for Horticultural Science 108:360-368.

3. Agrios, G. N. 1997. Plant Pathology. 4th ed. Academic Press, San Diego, CA.

4. Al-Kherb, S. M. 1994. Prevalent races of cucurbit powdery mildews in Riyadh region of Saudi Arabia. Assiut Journal of Agricultural Sciences 25:249-252.

5. Alfandi, M., Ji, Y., Shen, L., Qi, X., Xu, Q., and Chen, X. 2010. Construction of genetic linkage map and localization of QTLs for powdery mildew resistance in cucumber (*Cucumis sativus* L.). Acta Horticulturae 871:33-42.

6. Alvarez, J. M., Gomez-Guillamon, M. L., Tores, N. A., Canovas, I., and Floris, E. 2000. Virulence differences between two Spanish isolates of *Sphaerotheca fuliginea* race 2 on melon. 67-69 In: Proceedings of the 7th EUCARPIA Meeting on Cucurbit Breeding and Genetics, Cucurbitaceae 2000 Acta Horticulturae, Ma'ale Ha Hamisha, Israel.

7. Amano, K. 1986. Host Range and Geographical Distribution of the Powdery Mildew Fungi. Japan Scientific Societies Press, Tokyo, Japan.

8. Anagnostou, K., Jahn, M., and Perl-Treves, R. 2000. Inheritance and linkage analysis of resistance to *zucchini yellow mosaic virus*, *watermelon mosaic virus*, *papaya ringspot virus* and powdery mildew in melon. Euphytica 116:265-270.

9. Andres, T. C., and Decker-Walters, D., Cucurbitaceae. Cucurbit Network. Retrieved 6 July 2011, from http://www.cucurbit.org/family.html

10. Bardin, M., Carlier, J., and Nicot, P. C. 1999. Genetic differentiation in the French population of *Erysiphe cichoracearum*, a causal agent of powdery mildew of cucurbits. Plant Pathology 48:531-540.

11. Bardin, M., Dogimont, C., Nicot, P., and Pitrat, M. 1997. Genetic analysis of resistance of melon line PI 124112 to *Sphaerotheca fuliginea* and *Erysiphe cichoracearum* studied in recombinant inbred lines. 163-168 In: Proceedings of the First International Symposium on Cucurbits International Society of Horticulture Science, Adana, Turkey.

12. Bardin, M., Nicot, P. C., Normand, P., and Lemaire, J. M. 1997. Virulence variation and DNA polymorphism in *Sphaerotheca fuliginea*, causal agent of powdery mildew of cucurbits. Eur J of Plant Pathol 103:545-554.

13. Bardin, M., Suliman, M. E., Sage-Palloix, A. M., Mohamed, Y. F., and Nicot, P. C. 2007. Inoculum production and long-term conservation methods for cucurbits and tomato powdery mildews. Mycol. Res. 111:740-747.

14. Bashi, E., and Aust, H. J. 1980. Quality of spores produced in cucumber powdery mildew compensated for their quantity Journal of Plant Diseases and Protection 87:594-599.

15. Behera, T. K., Behera, S., Bharathi, L. K., John, K. J., Simon, P. W., and Staub, J. E. 2010. Bitter Gourd: Botany, Horticulture, Breeding. Pages 101-141 in: Horticultural Reviews, vol. 37. J. Janick, ed. Wiley-Blackwell

16. Belanger, R. R., and Labbe, C. 2002. Control of Powdery Mildews Without Chemicals: Prophylactic and Biological Alternatives for Horticultural Crops. Pages 256-267 in: The Powdery Mildews: A Comprehensive Treatise, vol. 1. R. R. Belanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

17. Bertrand, F., and Pitrat, A. 1989. Screening of a muskmelon germplasm for susceptibility to 5 pathotypes of powdery mildew. 140-142 In: Proceedings of Cucurbitaceae: Evaluation and Enhancement of Cucurbit Germplasm ARS/USDA, Charleston, SC.

18. Bertrand, F., Pitrat, M., Glandard, A., and Lemaire, J. M. 1992. Diversity and variability of fungi responsible for powdery mildew on Cucurbitaceae. Phytoma 438:46-49.

19. Bettiol, W., Silva, H. S. A., and Reis, R. C. 2008. Effectiveness of whey against zucchini squash and cucumber powdery mildew. Scientia Horticulturae 117:82-84.

20. Biffen, R. H. 1907. Studies in the inheritance of disease-resistance. J. Agric. Sci. 2:109-128.

21. Bisognin, D. A. 2002. Origin and evolution of cultivated cucurbits. Ciencia Rural 32:715-723.

22. Block, C. C., and Reitsma, K. R. 2005. Powdery mildew resistance in the US National Plant Germplasm System cucumber collection. Hortscience 40:416-420.

23. Boesewinkel, H. J. 1980. The morphology of the imperfect states of powdery mildew (Erysiphaceae) Bot. Rev. 46:167-224.

24. Braun, U. 1987. A Monograph of the Erysiphales (Powdery Mildews). J. Cramer, Berlin, Germany.

25. Braun, U. 1995. The Powdery Mildews (Erysiphales) of Europe. VEB Gustav Fischer Verlag, Jena, Germany.

26. Braun, U. 2011. The current systematics and taxonomy of the powdery mildews (Erysiphales): an overview. Mycoscience 52:210-212.

27. Braun, U., Cook, R. T. A., Inman, A. J., and Shin, H. D. 2002. The Taxonomy of the Powdery Mildew Fungi. Pages 13-55 in: The Powdery mildews: A Comprehensive Treatise, vol. 1. R. R. Belanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

28. Braun, U., and Takamatsu, S. 2000. Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysipheae) and *Cystotheca, Podosphaera, Sphaerotheca* (Cystotheceae) inferred from rDNA ITS sequences: some taxonomic consequences. Schlechtendalia:1-33.

29. Brent, K. J., and Hollomon, D. W. 2007. Fungicide Resistance: The Assessment of Risk. 2nd edition. Fungicide Resistance Action Committee, Bristol, UK.

30. Brown, J. K. M. 2002. Comparative Genetics of Avirulence and Fungicide Resistance in the Powdery Mildew Fungi. Pages 56-65 in: The Powdery mildews: A Comprehensive Treatise, vol. 1. R. R. Belanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

31. Bryson, R. J. 2006. Fungicide Resistance: Are We Winning the Battle But Losing the War? Association of Applied Biologists, Wellesbourne, UK.

32. Bussey, M. J., and Stevenson, W. R. 1991. A leaf disk assay for detecting resistance to early blight caused by *Alternaria solani* in juveline potato plants. Plant Dis. 75:385-390.

33. CABI, IndexFungorum. CAB International. Retrieved 12 December 2009, from http://www.indexfungorum.org/names/names.asp

34. Camele, I., Campanelli, G., Ferrari, V., Viggiani, G., and Candido, V. 2009. Powdery mildew control and yield response of inodorus melon. Italian Journal of Agronomy 4:19-26.

35. Cheah, L. H., Page, B. B. C., and Cox, J. K. 1996. Epidemiology of powdery mildew (*Sphaerotheca fuliginea)* of squash. 147-151 In: Proceedings of the Forty Ninth New Zealand Plant Protection Conference New Zealand Plant Protection Society, Nelson, New Zealand.

36. Chen, R. S., Chu, C., Cheng, C. W., Chen, W. Y., and Tsay, J. G. 2008. Differentiation of two powdery mildews of sunflower (*Helianthus annuus*) by a PCR-mediated method based on ITS sequences. Eur. J. Plant Pathol. 121:1-8.

37. Cho, M., Om, Y., Huh, Y., Mok, I., and Park, H. 2003. Two oriental squash varieties resistant to powdery mildew bred through interspecific crosses. Cucurbit Genetics Cooperative Report 26:40-41.

38. Clément-Mathieu, G., Chain, F., Marchand, G., and Bélanger, R. R. 2008. Leaf and powdery mildew colonization by glycolipid-producing *Pseudozyma* species. Fungal Ecology 1:69-77.

39. Coffey, M. D., McCreight, J. D., and Miller, T. 2006. New races of the cucurbit powdery mildew *Podosphaera xanthii* present in California. Phytopathology 96:S25-S25.

40. Cohen, R. 1993. A leaf disk assay for detection of resistance of melons to *Sphaerotheca fuliginea* race 1. Plant Dis. 77:513-517.

41. Cohen, R., Burger, Y., and Katzir, N. 2004. Monitoring physiological races of *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*), the causal agent of powdery mildew in cucurbits: factors affecting race identification and the importance for research and commerce. Phytoparasitica 32:174-183.

42. Cohen, R., Burger, Y., Shraiber, S., Elkind, Y., and Levin, E. 1996. Influence of the genetic bakcground and environmental conditions on powdery mildew of melons. 162 In: The 17th Congress of the Israel Phytopathological Society The Israeli Phytopathological Soceity, Bet Dagan, Israel.

43. Cohen, R., Hanan, A., and Paris, H. S. 2003. Single-gene resistance to powdery mildew in zucchini squash (*Cucurbita pepo*). Euphytica 130:433-441.

44. Cohen, R., Katzir, N., Schreiber, S., Greenberg, R., and Yarden, O. 1996. Occurrence of *Sphaerotheca fuliginea* race 3 on cucurbits in Israel. Plant Dis. 80:344.

45. Cohen, R., Leibovich, G., Shtienberg, D., and Paris, H. S. 1993. Variability in the reaction of squash (*Cucurbita pepo*) to inoculation with *Sphaerotheca fuliginea* and methodology of breeding for resistance. Plant Pathology 42:510-516.

46. Cohen, Y., Baider, A., Petrov, L., Sheck, L., and Voloisky, V. 2000. Cross-infectivity of *Sphaerotheca fuliginea* to watermelon, melon, and cucumber. 85-88 In: Proceedings of the 7th EUCARPIA Meeting on Cucurbit Breeding and Genetics, Cucurbitaceae 2000 Acta Horticulturae, Ma'ale Ha Hamisha, Israel.

47. Cohen, Y., and Eyal, H. 1986. *Sphaerotheca fuliginea* sexual reproduction on cucurbits in Israel. Phytopathology 76:1082-1082.

48. Cohen, Y., and Eyal, H. 1988. Pathogenicity of *Erysiphe cichoracearum* to cucurbits. Pages 87-90. North Carolina State University, Raleigh, NC.

49. Cohen, Y., and Eyal, H. 1995. Differential expression of resistance to powdery mildew incited by race 1 or race 2 of *Sphaerotheca fuliginea* in *Cucumis melo* genotypes at various stages of plant development. Phytoparasitica 23:223-230.

50. Contin, M., and Munger, H. M. 1977. Inheritance of powdery mildew resistance in interspecific crosses with *Cucurbita martinezii*. Hortscience 12:397-397.

51. Cook, R. T. A., and Braun, U. 2009. Conidial germination patterns in powdery mildews. Mycol. Res. 113:616-636.

52. Cook, R. T. A., Inman, A. J., and Billings, C. 1997. Identification and classification of powdery mildew anamorphs using light and scanning electron microscopy and host range data. Mycol. Res. 101:975-1002.

53. Crisp, P., Wicks, T. J., Troup, G., and Scott, E. S. 2006. Mode of action of milk and whey in the control of grapevine powdery mildew. Australasian Plant Pathology 35:487-493.

54. Cunnington, J., Lawrie, A., and Pascoe, I. 2004. Molecular determination of anamorphic powdery mildew fungi on the Fabaceae in Australia. Australasian Plant Pathology 33:281-284.

55. Cunnington, J. H., Lawrie, A. C., and Pascoe, I. G. 2010. Genetic characterization of the *Golovinomyces cichoracearum* complex in Australia. Plant Pathology 59:158-164.

56. Daayf, F., Ongena, M., Boulanger, R., El Hadrami, I., and Belanger, R. R. 2000. Induction of phenolic compounds in two cultivars of cucumber by treatment of healthy and powdery mildew-infected plants with extracts of *Reynoutria sachalinensis*. J. Chem. Ecol. 26:1579-1593.

57. Daayf, F., Schmitt, A., and Belanger, R. R. 1995. The effects of plant-extracts of *Reynoutria sachalinensis* on powdery mildew development and leaf physiology of long english cucumber. Plant Dis. 79:577-580.

58. Daayf, F., Schmitt, A., and Belanger, R. R. 1997. Evidence of phytoalexins in cucumber leaves infected with powdery mildew-following treatment with leaf extracts of *Reynoutria sachalinensis*. Plant Physiology 113:719-727.

59. Daughtrey, M. L., and Hausbeck, M. K. Verbenas and Powdery Mildew. Retrieved 17 May 2010 from http://www.gpnmag.com/Pests-Diseases-Verbenas-Powdery-Mildew-article7004

60. Davey, J., and McGrath, M. T. 2006. Baseline sensitivity of cucurbit powdery mildew (*Podosphaera xanthii*) to the fungicide quinoxyfen in New York State. Resistant Pest Management Newsletter 15:47-49.

61. Davey, J. F., and McGrath, M. T. 2006. Sensitivity to the fungicide quinoxyfen of powdery mildew isolates collected from pumpkin in New York in 2004. Phytopathology 96:177.

62. Davis, A., Bruton, B. D., and Pair, S. D. 2001. Potencial for selecting powdery mildew resistance germplasm in watermelon. 97-100 In: 20th Horticulture Industries Conference.

63. Davis, A., Wehner, T., Amnon, L., and Stephen, K. 2005. Update on powdery mildew resistance screening in watermelon. 871 In: Proceedings of Southern Region 65th Anuual Meeting American Society of Horticulture Science, Little Rock, AK.

64. Davis, A. R., Bruton, B. D., and Pair, S. D. 2002. Powdery mildew resistance in watermelon. 178-181 In: Proceedings of 21st Annual Horticulture Industry Conference USDA-ARS, Lane, OK.

65. Davis, A. R., Bruton, B. D., Pair, S. D., and Thomas, C. E. 2001. Powdery mildew: an emerging disease of watermelon in the United States. Cucurbit Genetics Cooperative Report 24:42-48.

66. Davis, A. R., Levi, A., Tetteh, A., Wehner, T., Russo, V., and Pitrat, M. 2007. Evaluation of watermelon and related species for resistance to race 1W powdery mildew. Journal of the American Society for Horticultural Science 132:790-795.

67. Davis, A. R., Levi, A., Wehner, T., and Pitrat, M. 2006. PI525088-PMR, a melon race 1 powdery mildew-resistant watermelon line. Hortscience 41:1527-1528.

68. Davis, A. R., Tetteh, A., Wehner, T. C., Levi, A., and Pitrat, A. 2006. Watermelon resistance to powdery mildew race 1 and race 2. 412-420 In: Cucurbitaceae 2006 Universal Press, Ashville, NC.

69. Davis, R. M., and Gubler, W. D. 2008. Powdery mildew on vegetables. University of California Statewide IPM Program. Online publication. P 7406.

70. De Giovanni, C., Dell' Orco, P., Bruno, A., Ciccarese, F., Lotti, C., and Ricciardi, L. 2004. Identification of PCR-based markers (RAPD, AFLP) linked to a novel powdery mildew resistance gene (ol-2) in tomato. Plant Sci. 166:41-48.

71. del Pino, D., Olalla, L., Perez-Garcia, A., Rivera, M. E., Garcia, S., Moreno, R., de Vicente, A., and Tores, J. A. 2002. Occurrence of races and pathotypes of cucurbit powdery mildew in southeastern Spain. Phytoparasitica 30:459-466.

72. Dik, A. J., Verhaar, M. A., and Belanger, R. R. 1998. Comparison of three biological control agents against cucumber powdery mildew (*Sphaeroteca fuliginea*) in semi-commercial-scale glasshouse trials. Eur. J. Plant Pathol. 104:413-423.

73. El-Ammari, S. S., and Khan, M. W. 1983. *Leveillua taurica* powdery mildew on greenhouse cucumbers in Lybia. Plant Dis. 67:553-556.

74. El-Ammari, S. S., and Khan, M. W. 1987. Observations on the survival of *Sphaerotheca fuliginea* and recurrence of powdery mildew on cucurbits in Libya. Arab Journal of Plant Protection 5:88-86.

75. Elad, Y., Kirshner, B., Yehuda, N., and Sztejnberg, A. 1998. Management of powdery mildew and gray mold of cucumber by *Trichoderma harzianum* T39 and *Ampelomyces quisqualis* AQ10. BioControl 43:241-251.

76. Elmhirst, J. 2006. Crop profile for greenhouse cucumber in Canada. Online publication. 01B68-3-0042.

77. Epinat, C., Pitrat, M., and Bertrand, F. 1993. Genetic analysis of resistance of five melon lines to powdery mildews. Euphytica 65:135-144.

78. Erysiphales, Erysiphales Database. Washington State University. Retrieved October 12 2011, from http://www.erysiphales.wsu.edu/displayE.aspx?eID=69

79. FACS. 2010. Vegetables, Melons and Berries. Florida Agriculture Statistical Directory, Florida Department of Agriculture and Consumer Services. Online publication. DACS-P-01304.

80. Fanourakis, N., Tsekoura, Z., and Nanou, E. 2000. Morphological characteristics and powdery mildew resistance of *Cucumis melo* land races in Greece. 241-245 In: Proceedings of the 7th EUCARPIA meeting on cucurbit breeding and genetics, Cucurbitaceae 2000 Acta Horticulturae, Ma'ale Ha Hamisha, Israel.

81. FAOSTAT, Crops. Food and Agriculture Organization of the United Nations. Retrieved 8 July 2011, from http://faostat.fao.org/site/567/default.aspx#ancor

82. Felix-Gastelum, R., Apodaca-Sanchez, M. A., Martinez-Valenzuela, M. d. C., and Espinosa-Matias, S. 2005. *Podosphaera* (sect. *Spaerotheca*) *xanthii* (Castagne) U. Brawn and N. Shishkoff on cucurbits in northern Sinaloa, Mexico. Revista Mexicana de Fitopatologia 23:162-168.

83. Fernandez-Ortuno, D., Perez-Garcia, A., Lopez-Ruiz, F., Romero, D., Vicente, A. d., and Tores, J. A. 2006. Occurrence and distribution of resistance to QoI fungicides in populations of *Podosphaera fusca* in south central Spain. Eur. J. Plant Pathol. 115:215-222.

84. Fernandez-Ortuno, D., Tores, J. A., de Vicente, A., and Perez-Garcia, A. 2008. First report of powdery mildew elicited by *Podosphaera fusca* (synonym *Podosphaera xanthii*) on *Euryops pectinatus* in Spain. Plant Dis. 92:835-835.

85. Ferrandino, F. J., Smith, V. L., and Cecarelli, R. 2004. Evaluation of milk as a foliar spray for control of powdery mildew on pumpkin. Phytopathology 94:S164-S164.

86. Fofana, B., McNally, D. J., Labbé, C., Boulanger, R., Benhamou, N., Séguin, A., and Bélanger, R. R. 2002. *Milsana*-induced resistance in powdery mildew-infected cucumber plants correlates with the induction of chalcone synthase and chalcone isomerase. Physiol. Mol. Plant Pathol. 61:121-132.

87. Formisano, G., Paris, H. S., Frusciante, L., and Ercolano, M. R. 2010. Commercial *Cucurbita pepo* squash hybrids carrying disease resistance introgressed from *Cucurbita moschata* have high genetic similarity. Plant Genetic Resources 8:198-203.

88. Frolov, V. V. 1996. Species structure of powdery mildew fungi in melon in Kherson. 114-117 In: International Conference "Selection" and technology: cucurbits production Ukraine Science Research Institute of Southern Vegetables and Melon Crops, Kherson, Russia.

89. Fukino, N., Ohara, T., Monforte, A., Sugiyama, M., Sakata, Y., Kunihisa, M., and Matsumoto, S. 2008. Identification of QTLs for resistance to powdery mildew and SSR markers diagnostic for powdery mildew resistance genes in melon (*Cucumis melo* L.). Theor. Appl. Genet. 118:165-175.

90. Garibaldi, A., Bertetti, D., Frati, S., and Gullino, M. L. 2008. First report of powdery mildew caused by *Golovinomyces cichoracearum* on orange coneflower (*Rudbeckia fulgida*) in Italy. Plant Dis. 92:975-975.

91. Garibaldi, A., Bertetti, D., Frati, S., Minuto, A., and Gullino, M. L. 2008. Powdery mildew caused by *Golovinomyces cichoracearum* on Paris daisy (*Argyranthemum frutescens*) in Italy. Plant Dis. 92:1135-1135.

92. Garibaldi, A., Minuto, A., and Gullino, M. L. 2008. First report of powdery mildew caused by *Golovinomyces cichoracearum* on English daisy (*Bellis perennis*) in Italy. Plant Dis. 92:484-484.

93. Gawande, V. L., and Patil, J. V. 2003. Genetics of powdery mildew (*Erysiphe polygoni* DC) resistance in Mungbean (*Vigna radiata* (L.) Wilczek). Crop Protection 22:567-571.

94. Gemmill, C. L. 1972. Robley Dunglisons Dictionary of Medical Science. 5th ed. Charles Bowen, Charlottesville, VA.

95. Gevens, A. J., Maia, G., and Jordan, S. A. 2009. First report of powdery mildew caused by *Golovinomyces cichoracearum* on *Crotalaria juncea* ('Tropic Sun' Sunn hemp). Plant Dis. 93:427-427.

96. Gevens, A. J., and Maia, G. S. 2009. Characterization of cucurbit powdery mildew in north Florida. Phytopathology 99:198.

97. Gilardi, G., Manker, D. C., Garibaldi, A., and Gullino, M. L. 2008. Efficacy of the biocontrol agents *Bacillus subtilis* and *Ampelomyces quisqualis* applied in combination with fungicides against powdery mildew of zucchini. Journal of Plant Diseases and Protection 115:208-213.

98. Glawe, D. A. 2008. The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. Annu. Rev. Phytopathol. 46:27-51.

99. Glawe, D. A., Grove, G. G., and Nelson, J. 2006. First report of powdery mildew of *Coreopsis* spcecies caused by *Golovinomyces cichoracearum* in the Pacific Northwest. Plant Management Network. Online publication. doi:/10.1094/PHP-2006-0405-01-BR.

100. Goh, T.-K. 1999. Single-spore isolation using a hand-made glass needle. Fungal Divers 2:47-63.

101. González Morejón, N. 2010. Mildiu polvoriento en las cucurbitáceas. Revista de Protección Vegetal 25:44-50.

102. Gorter, G. J. M. 1966. Powdery mildew fungus on cucurbits in Transvaal Province of South Africa. Nature 209:938.

103. Grand, L. F. 1987. Teleomorph of *Sphaerotheca fuliginea* on cucurbits in North Carolina. Mycologia 79:484-486.

104. Green, J. R., Carver, T. L. W., and Gurr, S. J. 2002. The Formation and Function of Infection Feeding Structures. Pages 66-68 in: The Powdery Mildew: A Comprehensive Treatise, vol. 1. R. R. Belenger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

105. Hafez, Y. M. 2008. Effectiveness of the antifungal black seed oil against powdery mildews of cucumber (*Podosphaera xanthii*) and barley (*Blumeria graminis* f.sp. *hordei*). Acta Biologica Szegediensis 52:17-25.

106. Hammami, W., Castro, C. Q., Remus-Borel, W., Labbe, C., and Belanger, R. R. 2011. Ecological basis of the interaction between *Pseudozyma flocculosa* and powdery mildew fungi. Appl. Environ. Microbiol. 77:926-933.

107. Hammett, K. R. W. 1977. Taxonomy of Erysiphaceae in New Zealand. N. Z. J. Bot. 15:687-711.

108. Haramoto, M., Hamamura, H., Sano, S., Felsenstein, F. G., and Otani, H. 2006. Sensitivity monitoring of powdery mildew pathogens to cyflufenamid and the evaluation of resistance risk. J. Pestic. Sci. 31:397-404.

109. Hargraves, O. 2004. Cucurbits. Cambrigde University Press. Online publication. doi:/10.1017/S0266078404004092.

110. Henning, M. J., Munger, H. M., and Jahn, M. M. 2005. 'Hannah's Choice F-1': a new muskmelon hybrid with resistance to powdery mildew, *Fusarium* race 2, and *Potyviruses*. Hortscience 40:492-493.

111. Hirata, K. 1968. Notes on host range and geographic distribution of the powdery mildew fungi. Transactions of the Mycological Society of Japan 9:73-88.

112. Hollomon, D. W., and Wheeler, I. E. 2002. Controlling Powdery Mildews with Chemistry. Pages 249-255 in: The Oowdery Mildews: A Comprehensive Treatise, vol. 1. R. R. Belanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

113. Hosoya, K., Narisawa, K., Pitrat, M., and Ezura, H. 1999. Race identification in powdery mildew (*Sphaerotheca fuliginea*) on melon (*Cucumis melo*) in Japan. Plant Breed. 118:259-262.

114. Hou, F. 1999. Powdery mildew of Cucumber. Pages 756-778 in: Cucumber (*Cucumis sativus* L.), vol. 1. D. Yin, ed. Tian Jing Technology Publising Company, Tian Jing, China.

115. Hsieh, T., and Huang, J. 2001. Leaf-disk method for assessment of disease severity of lily leaf blight caused by *Botrytis elliptica*. Plant Pathol. Bull. 10:37-44.

116. Huggenberger, F., Collins, M. A., and Skylakakis, G. 1984. Decreased sensitivity of *Sphaerotheca fuliginea* to fenarimol and other ergosterol biosynthesis inhibitors. Crop Protection 3:137-149.

117. Ibrahim, I. F., Kadhim, L. J., and Mahmood, A. H. 1985. A study of some biological characters for *Sphaerotheca fuliginia* as a new powdery mildew causative agent on cucumber in Iraq. Journal of Biological Sciences Research 16:1-12.

118. Ishii, H. 2009. QoI Fungicide Resistance: Current Status and the Problems Associated with DNA-Based Monitoring. Pages 37-45 in: Recent Developments in Management of Plant Diseases, vol. 1. U. Gisi, I. Chet and M. L. Gullino, eds. Springer, Netherlands.

119. Ishii, H., Yano, K., Date, H., Furuta, A., Sagehashi, Y., Yamaguchi, T., Sugiyama, T., Nishimura, K., and Hasama, W. 2007. Molecular characterization and diagnosis of QoI resistance in cucumber and eggplant fungal pathogens. Phytopathology 97:1458-1466.

120. Ivanoff, S. S. 1957. Powdery mildew intumescences on watermelon fruits. Phytopathology 47:525-525.

121. Jagger, I. C. 1926. Powdery mildew of muskmelons in the Imperial Valley of California in 1925. Phytopathology 16:1009-1010.

122. Jagger, I. C., and Scott, G. W. 1937. Development of powdery mildew resistant cantaloup No. 45. U.S. Dep. Agric. Circul. 441:5.

123. Jahn, M., Munger, H. M., and McCreight, J. D. 2002. Breeding Cucurbit Crops for Powdery Mildew Resistance. Pages 239-248 in: The Powdery Mildews: A Comprehensive Treatise, vol. 1. R. R. Belenger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

124. Jarvis, W., G., G. W., and G., G. G. 2002. Epidemiology of Powdery Mildew in Agricultural Ecosystems. Pages 169-199 in: The Powdery Mildew: A Comprehensice Treatise, vol. 1. R. R. Belenger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. The Americal Phytopathological Society Press, Saint Paul, MN.

125. Jeger, M. J. 2004. Analysis of disease progress as a basis for evaluating disease management practices. Annu. Rev. Phytopathol. 42:61-82.

126. Jeger, M. J., and Viljanen-Rollinson, S. L. H. 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theor. Appl. Genet. 102:32-40.

127. Kaur, P., Li, C. X., Barbetti, M. J., You, M. P., Li, H., and Sivasithamparam, K. 2008. First report of powdery mildew caused by *Erysiphe cruciferarum* on *Brassica juncea* in Australia. Plant Dis. 92:650-651.

128. Keinath, A. P., and DuBose, V. B. 2004. Evaluation of fungicides for prevention and management of powdery mildew on watermelon. Crop Protection 23:35-42.

129. Kenigsbuch, D., and Cohen, Y. 1992. Inheritance and allelism of genes for resistance to races 1 and 2 of *Sphaerotheca fuliginea* in muskmelon. Plant Dis. 76:626-629.

130. Khan, M. W., and Salam, M. A. 1990. Occurance and identity of powdery mildew of cucurbits in Andaman Islands India. International Journal of Tropical Plant Diseases 8:157-160.

131. Khodaparast, S. A., and Abbasi, M. 2009. Species, host range, and geographical distribution of powdery mildew fungi in Iran. Mycotaxon 108:213-216.

132. Kiani, M., Zamani, Z., Nikkhah, M. J., and Fatahi, R. 2008. Screening of damask rose genotypes for powdery mildew resistance. 171-174 In: V International Symposium on Rose Research and Cultivation International Society for Horticultural Science (ISHS), Gufi, Japan.

133. Kirkbride, J. H., Gunn, C. R., and Dallwitz, M. J., Cucurbitaceae. Systematic Mycology and Microbiology Laboratory, ARS, USDA. Retrieved 3 July 2011, from http://nt.arsgrin.gov/sbmlweb/OnlineResources/SeedsFruits/rptSeedsFruitsFamData.cfm?thisFamily=Cucurbitaceae

134. Kiss, L. 1998. Natural occurrence of *Ampelomyces* intracellular mycoparasites in mycelia of powdery mildew fungi. New Phytol. 140:709-714.

135. Kiss, L. 2003. A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Management Science 59:475-483.

136. Kiss, L., Russell, J. C., Szentivanyi, O., Xu, X., and Jeffries, P. 2004. Biology and biocontrol potential of *Ampelomyces* mycoparasites, natural antagonists of powdery mildew fungi. Biocontrol Sci. Technol. 14:635-651.

137. Kobori, R. F., Suzuki, O., Wierzbicki, R., Della Vecchia, P. T., and Camargo, L. E. 2004. Occurrence of *Podosphaera xanthii* race 2 on *Cucumis melo* in Brazil. Plant Dis. 88:1161-1161.

138. Kooistra, E. 1968. Powdery mildew resistance in cucumber. Euphytica 17:236-&.

139. Kousik, C. S., Donahoo, R. S., Webster, C. G., Turechek, W. W., Adkins, S. T., and Roberts, P. D. 2011. Outbreak of cucurbit powdery mildew on watermelon fruit caused by *Podosphaera xanthii* in southwest Florida. Plant Dis. 95:1586-1586.

140. Kousik, C. S., Levi, A., Ling, K. S., and Wechter, W. P. 2008. Potential sources of resistance to cucurbit powdery mildew in U.S. plant introductions of bottle gourd. Hortscience 43:1359-1364.

141. Kristkova, E., and Lebeda, A. 1999. Disease resistance of *Cucurbita pepo* and *C. maxima* genetic resources. Cucurbit Genetics Cooperative 22:53-54.

142. Kristkova, E., and Lebeda, A. 2000. Powdery Mildew Field Infection on Leaves and Stems of *Cucurbita pepo* Accessions. 61-66 In: Proceedings of the 7th EUCARPIA Meeting on Cucurbit Genetics and Breeding Acta Horticulturae, Ma'ale HaHamisha, Israel.

143. Kristkova, E., Lebeda, A., and Sedlakova, B. 2007. Temporal and spatial dynamics of powdery mildew species on cucurbits in the Czech Republic. 337-343 In: Proceedings of the Third International Symposium on Cucurbits, Townsville, Australia, 11-17 September 2005. International Society for Horticultural Science (ISHS).

144. Kristkova, E., Lebeda, A., and Sedlakova, B. 2009. Species spectra, distribution and host range of cucurbit powdery mildews in the Czech Republic, and in some other European and Middle Eastern countries. Phytoparasitica 37:337-350.

145. Krístková, E., Lebeda, A., and Sedláková, B. 2004. Virulence of Czech cucurbit powdery mildew isolates on *Cucumis melo* genotypes MR-1 and PI 124112. Scientia Horticulturae 99:257-265.

146. Kristkova, E., Sedlakova, B., Vinter, V., and Lebeda, A. 2003. Aggressiveness of *Erysiphe cichoracearum* isolates pathotype AB1B2CCm on cucumber and watermelon. Czech Mycology 55:127.

147. Kristkova, E., and Vinter, V. 2002. Aggressiveness Vvriation in Czech isolates of *Erysiphe cichoracearum* pathotype AB1B2CCm. Cucurbit Genetics Cooperative Report 25:58-62.

148. Kuck, K. H., and Russell, P. E. 2006. FRAC: combined resistance risk assessment. Aspects of Applied Biology 78:3-10.

149. Kuzuya, M., Hosoya, K., Yashiro, K., Tomita, K., and Ezura, H. 2003. Powdery mildew (*Sphaerotheca fuliginea*) resistance in melon is selectable at the haploid level. Journal of Experimental Botany 54:1069-1074.

150. Kuzuya, M., Yashiro, K., Tomita, K., and Ezura, H. 2006. Powdery mildew (*Podosphaera xanthii*) resistance in melon is categorized into two types based on inhibition of the infection processes. Journal of Experimental Botany 57:2093-2100.

151. Laws, F. A., Walmsleywoodward, D. J., and Whittington, W. J. 1982. Germination and growth of single-pustule isolates of *Erysiphe gramis* tolerant and sensitive to tridemorph and ethrimol. Annals of Applied Biology 100:253-262.

152. Lebeda, A. 1983. The genera and species spectrum of cucumber powdery mildew in Czechosovakia. Phytopathologische Zeitschrift (Journal of Phytopatology) 108:71-79.

153. Lebeda, A. 1984. Screening of wild *Cucumis* species for resistance to cucumber powdery mildew (*Erysiphe cichoracearum* and *Sphaerotheca fuliginea*). Scientia Horticulturae 24:241-249.

154. Lebeda, A., and Kristkova, E. 1994. Field resistance of *Cucurbita* species to powdery mildew (*Erysiphe cichoracearum*) Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz (Journal of Plant Diseases and Protection) 101:598-603.

155. Lebeda, A., and Kristkova, E. 1996. Genotypic variation in field resistance of *Cucurbita pepo* cultivars to powdery mildew (*Erysiphe cichoracearum*). Genetic Resources and Crop Evolution 43:79-84.

156. Lebeda, A., and Kristkova, E. 1997. Evaluation of *Cucumis sativus* L. germplasm for field resistance to the powdery mildew. Acta Phytopathol. Entomol. Hung. 32:299-305.

157. Lebeda, A., Kristkova, E., Sedlakova, B., Coffey, M. D., and McCreight, J. D. 2011. Gaps and perspectives of pathotype and race determination in *Golovinomyces cichoracearum* and *Podosphaera xanthii*. Mycoscience 52:159-164.

158. Lebeda, A., Kristkova, E., Sedlakova, B., McCreight, J. D., and Coffey, M. D. 2008. New concept for determination and denomination of pathotypes and races of cucurbit powdery mildew. 125-134 In: Cucurbitaceae 2008. Proceedings of the IXth EUCARPIA meeting on genetics and breeding of cucurbitaceae, Avignon, France, 21-24 May 2008. Institut National de la Recherche Agronomique (INRA).

159. Lebeda, A., McGrath, M. T., and Sedlakova, B. 2009. Fungicide Resistance in Cucurbit Powdery Mildew. Pages 221-246 in: Fungicides, vol. 1. O. Carisse, ed. InTech, Rijeka, Croatia.

160. Lebeda, A., and Mieslerová, B. 2011. Taxonomy, distribution and biology of lettuce powdery mildew (*Golovinomyces cichoracearum sensu stricto*). Plant Pathology 60:400-415.

161. Lebeda, A., and Sedlakova, B. 2010. Screening for Resistance to Cucurbit Powdery Mildew (*Golovinomyces cichoracearum*, *Podosphaera xanthii*). Pages 295-307 in: Mass Screening Techniques for Selection Crops Resistant to Disease, vol. 1. M. Miranda and M. M. Spencer, eds. International Atomic Energy Agency (FAO), Vienna.

162. Lebeda, A., Sedlakova, B., and Kristkova, E. 2004. Distribution, harmfulness and pathogenic variability of cucurbit powdery mildew in the Czech Republic. Acta fytotechnica et zootechnica 7:174-176.

163. Lebeda, A., Sedlakova, B., and Kristkova, E. 2007. Temporal changes in pathogenicity structure of cucurbit powdery mildew populations. 381-388 In: Proceedings of the Third International Symposium on Cucurbits, Townsville, Australia, 11-17 September 2005. International Society for Horticultural Science (ISHS).

164. Lebeda, A., Sedlakova, B., Kristkova, E., and Vysoudil, M. 2009. Long-lasting changes in the species spectrum of cucurbit powdery mildew in the Czech Republic: influence of air temperature changes or random effect? Plant Protection Science 45:S41-S47.

165. Lebeda, A., Sedlakova, B., and Kristova, E. 2004. Distribution, harmfulness and pathogenic variability of cucurbit powdery mildew in the Czech Republic. 174-176 In: International XVI Slovak and Czech Plant Protection Conference Acta fytotechnica et zootechniva, Nitra, Slovakia.

166. Lebeda, A., Sedlakova, B., Pejchar, M., and Jerabkova, H. 2010. Variation for fungicide resistance among cucurbit powdery mildew populations in the Czech Republic. Acta Horticulturae:465-476.

167. Leibovich, G., Cohen, R., Paris, H. S., and Dinoor, A. 1993. Effect of environmental conditions on the development of powdery mildew in summer squash. Phytoparasitica 21:136.

168. Leibovich, G., Elkind, Y., Dinoor, A., and Cohen, R. 1995. Quantitative genetic analysis of *Sphaerotheca fuliginea* sporulation in *Cucurbita pepo*. Plant Breed. 114:460-462.

169. Letham, D. B., and Priest, M. J. 1989. Occurrence of cleistothecia of *Sphaerotheca fuliginea* on cucurbits in South Australia and New South Wales. Australasian Plant Pathology 18:35-37.

170. Leus, L., Van Huylenbroeck, J., Van Bockstaele, E., and Hofte, M. 2002. Powdery Mildew on Roses: Pathotype Screening. 91-95 In: Proceedings of the Twentieth International Eucarpia Symposium - Section Ornamentals Leuven, Melle. Belgium.

171. Levi, A., Thomas, C. E., Keinath, A. P., and Wehner, T. C. 2001. Genetic diversity among watermelon (*Citrullus lanatus* and *Citrullus colocynthis*) accessions. Genetic Resources and Crop Evolution 48:559-566.

172. Lewis, P. A., Lampman, R. L., and Metcalf, R. L. 1990. Kairomonal attractants for Acalymma vittatum (Coleoptera, Chrysomelidae) Environ. Entomol. 19:8-14.

173. Li, Y., Windham, M., Trigiano, R., Reed, S., Rinehart, T., and Spiers, J. 2009. Assessment of resistance components of bigleaf hydrangeas (*Hydrangea macrophylla*) to *Erysiphe polygoni* in vitro. Revue Canadienne De Phytopathologie (Canadian Journal of Plant Pathology) 31:348-355.

174. Li, Y. H., Windham, M. T., Trigiano, R. N., Fare, D. C., Spiers, J. M., and Copes, W. E. 2005. Spore germination, infection structure formation, and colony development of *Erysiphe pulchra* on dogwood leaves and glass slides. Plant Dis. 89:1301-1304.

175. Li, Y. H., Windham, M. T., Trigiano, R. N., Fare, D. C., Spiers, J. M., and Copes, W. E. 2007. Evaluation for resistance to powdery mildew in *Cornus* species and hybrids using a leaf disk assay. Journal of Environmental Horticulture 25:131-133.

176. Liberato, J. R., Shivas, R. G., and Cunnington, J. H. 2006. *Podosphaera xanthii* on *Euryops chrysanthemoides* in Australia. Australasian Plant Pathology 35:739-741.

177. Liu, L. Z., Cai, R., Yuan, X. J., He, H. L., and Pan, J. S. 2008. QTL molecular marker location of powdery mildew resistance in cucumber (*Cucumis sativus* L.). Science in China Series 51:1003-1008.

178. Liu, L. Z., Yuan, X. J., Cai, R., Pan, J. S., He, H. L., Yuan, L. H., Guan, Y., and Zhu, L. H. 2008. Quantitative trait loci for resistance to powdery mildew in cucumber under seedling spray inoculation and leaf disk infection. Journal of Phytopathology 156:691-697.

179. López-Ruiz, F. J., Pérez-García, A., Fernández-Ortuño, D., Romero, D., García, E., de Vicente, A., Brown, J. K., and Torés, J. A. 2010. Sensitivities to DMI fungicides in populations of *Podosphaera fusca* in southcentral Spain. Pest Management Science 66:801-808.

180. Ma, Q., Zhao, X. M., Sun, H., and Shang, H. S. 2011. Ultrastructural study on induced resistance of cucumber plants against *Sphaerotheca fuliginea* by oligochitosan. Plant Pathology J 27:8-13.

181. Marrras, F., and Corda, P. 1976. About powdery mildew of cucurbits in Sardinia (Italy). 421-428 In: Intern. Symp. Vegetable Crops under glass protection Acta Horticulturae (ISHS), Bucharest.

182. Martínez-culebras, P. V., Abad-campos, P., and García-jiménez, J. 2004. Molecular characterization and PCR detection of the melon pathogen *Acremonium cucurbitacearum*. Eur. J. Plant Pathol. 110:801-809.

183. Matheron, M. E., and Porchas, M. 2008. Effectiveness of the biopesticides Actinovate and Kaligreen within a management program for powdery mildew on cantaloupe. Phytopathology 98:99-100.

184. Mazzanti de Castanon, M. A., Alvarez, R. E., and Cabrera de Alvarez, M. G. 1987. Contribution to the knowledge of the aetiology of powdery mildew of Cucurbitaceae grown in northeast Argentina. Fitopatologia 22:21-29.

185. McCreight, J. D. 2001. Resistance to powdery mildew in melon PI 313970. Hortscience 36:455.

186. McCreight, J. D. 2002. Powdery mildew race 1 in Imperial Valley of California. Cucurbit Genetics Cooperative Report 25:20-21.

187. McCreight, J. D. 2003. Genes for resistance to powdery mildew races 1 and 2US in melon PI 313970. Hortscience 38:591-594.

188. McCreight, J. D. 2003. Reactions of 20 melon cultigens to powdery mildew race 1 in field and growth chamber tests. Hortscience 38:735.

189. McCreight, J. D. 2004. Notes on the change of the causal species of cucurbit powdery mildew in the U.S. Cucurbit Genetics Cooperative Report 27:8-23.

190. McCreight, J. D. 2006. Melon-powdery mildew interactions reveal variation in melon cultigens and *Podosphaera xanthii* races 1 and 2. Journal of the American Society for Horticultural Science 131:59-65.

191. McCreight, J. D., and Coffey, M. D. 2007. Resistance to a new race of the cucurbit powdery mildew present in Arizona and California. Hortscience 42:1013.

192. McCreight, J. D., and Coffey, M. D. 2011. Inheritance of resistance in melon PI 313970 to cucurbit powdery mildew incited by *Podosphaera xanthii* race S. Hortscience 46:838-840.

193. McCreight, J. D., Coffey, M. D., Turini, T. A., and Matheron, M. E. 2005. Field evidence for a new race of powdery mildew on melon. Hortscience 40:888.

194. McCreight, J. D., and Pitrat, M. 1993. Club mildew: working group on resistance of melon to powdery mildew. Cucurbit Genetics Cooperative Report 16:39-40.

195. McCreight, J. D., Pitrat, M., Thomas, C. E., Kishaba, A. N., and Bohn, G. W. 1987. Powdery mildew resistance genes in muskmelon. Journal of the American Society for Horticultural Science 112:156-160.

196. McDonald, B. A., and Linde, C. 2002. Pathogen population genetics, evolutionary potential, and durable resistance. Annu. Rev. Phytopathol. 40:349-379.

197. McDonald, B. A., and Linde, C. 2002. The population genetics of plant pathogens and breeding strategies for durable resistance. Euphytica 124:163-180.

198. McGrath, M. T. 1994. Heterothalism in *Sphaerotheca fuliginea*. Mycologia 86:517-523.

199. McGrath, M. T. 1996. Successful management of powdery mildew in pumpkin with disease threshold-based fungicide programs. Plant Dis. 80:910-916.

200. McGrath, M. T. 1997. Powdery mildew of cucurbits. Cooperative Extension, Cornell University. Online publication. PP 732.30.

201. McGrath, M. T. 2001. Fungicide resistance in cucurbit powdery mildew: experiences and challenges. Plant Dis. 85:236-245.

202. McGrath, M. T. 2006. Occurrence of fungicide resistance in *Podosphaera xanthii* on Long Island, NY, in 2004 and impact on cucurbit powdery mildew control. Phytopathology 96:180-181.

203. McGrath, M. T. 2007. Managing cucurbit powdery mildew and fungicide resistance. 211-216 In: Proceedings of the Third International Symposium on Cucurbits, Townsville, Australia, 11-17 September 2005. International Society for Horticultural Science (ISHS).

204. McGrath, M. T., Egel, D. S., Jasinski, J., Miller, S. A., Rhodes, L. H., and Precheur, R. 2009. Fungicide sensitivity and resistance of the cucurbit powdery mildew pathogen in New York, Pennsylvania, Ohio, and Indiana in 2008. Phytopathology 99:82.

205. McGrath, M. T., and Hausbeck, M. K. 1993. Insensitivity of *Sphaerotheca fuliginea* to triadimefon in a commercial pumpkin field in Michigan. Plant Dis. 77:319.

206. McGrath, M. T., and Miazzi, M. M. 2008. Sensitivity of *Podosphaera xanthii* to registered fungicides at-risk for resistance related to their efficacy for powdery mildew in pumpkin. Phytopathology 98:S102-S102.

207. McGrath, M. T., and Shishkoff, N. 1997. Dynamics of fungicide resistance and impact on control of cucurbit powdery mildew. Resistant Pest Management 9:16-17.

208. McGrath, M. T., and Shishkoff, N. 1999. Evaluation of biocompatible products for managing cucurbit powdery mildew. Crop Protection 18:471-478.

209. McGrath, M. T., and Shishkoff, N. 2000. Control of cucurbit powdery mildew with JMS Stylet-Oil. Plant Dis. 84:989-993.

210. McGrath, M. T., and Shishkoff, N. 2003. First report of the cucurbit powdery mildew fungus (*Podosphaera xanthii*) resistant to strobilurin fungicides in the United States. Plant Dis. 87:1007-1007.

211. McGrath, M. T., and Staniszewska, H. 1996. Management of powdery mildew in summer squash with host resistance, disease threshold-based fungicide programs, or an integrated program. Plant Dis. 80:1044-1052.

212. McGrath, M. T., Staniszewska, H., Shishkoff, N., and Casella, G. 1996. Distribution of mating types of *Sphaerotheca fuliginea* in the United States. Plant Dis. 80:1098-1102.

213. McGrath, M. T., Staniszewska, H., Shishkoff, N., and Casella, G. 1996. Fungicide sensitivity of *Sphaerotheca fuliginea* populations in the United States. Plant Dis. 80:697-703.

214. McGrath, M. T., and Thomas, C. E. 1996. Powdery Mildew. Pages 28-30 in: Compendium of Cucurbit Diseases, vol. 1. T. A. Zitter, D. L. Hopkins and C. E. Thomas, eds. The American Phytopathological Society Press, Saint Paul, MN.

215. McLean, D. M. 1970. Powdery mildew on watermelon fruits. Plant Dis. Reptr. 54:372-373.

216. Medeiros, F. H. V. 2005. Mecanismo de acao e atividade de fracao do leite no controle biologico do oidio da abobrinha. Maters Thesis. Universidade Federal de Lavras, Lavras, Brazil.

217. Menzies, J. G., and Belanger, R. R. 1996. Recent advances in cultural management of diseases of greenhouse crops. Revue Canadienne De Phytopathologie (Canadian Journal of Plant Pathology). 18:186-193.

218. Miazzi, M., Laguardia, C., and Faretra, F. 2011. Variation in *Podosphaera xanthii* on cucurbits in southern Italy. Journal of Phytopathology 17:1-8.

219. Miyamoto, T., Ishii, H., and Tomita, Y. 2010. Occurrence of boscalid resistance in cucumber powdery mildew in Japan and molecular characterization of the iron-sulfur protein of succinate dehydrogenase of the causal fungus. J. Gen. Plant Pathol. 76:261-267.

220. Moham, S. K. 2005. Plant Disease Diagnosis and Managment. Pages 159-170 in: Idaho Master Gardner Program Handbook, vol. 2. R. R. Tripepi, R. C. Wilson, S. M. Bell, J. Robbins and W. B. Jones, eds. Educational Communications, University of Idaho, Moscow, ID.

221. Mohamed, Y. F., Bardin, M., Nicot, P. C., and Pitrat, M. 1995. Causal agents of powdery mildew of cucurbits in Sudan. Plant Dis. 79:634-636.

222. Momol, T., Marois, J., Pernezny, K., and Olson, S. M. 2007. Integrated disease management for vegetable crops in Florida. Pages 89-93 in: Vegetable Production Handbook, vol. Plant Pathology Deptarment, UF/IFAS, FL Coop Ext. Serv, Gainesville, FL.

223. Moret, A., Munoz, Z., and Garces, S. 2009. Control of powdery mildew on cucumber cotyledons by chitosan. J. Plant Pathol. 91:375-380.

224. Morishita, M., Sugiyama, K., Saito, T., and Sakata, Y. 2002. An improved evaluation method for screening and selecting powdery mildew resistant cultivars and lines of cucumber (*Cucumis sativus* L.). J Jpn Soc Hortic Sci 71:94-100.

225. Morishita, M., Sugiyama, K., Saito, T., and Sakata, Y. 2003. Powdery mildew resistance in cucumber. Jarq - Jpn. Agric. Res. Q. 37:7-14.

226. Moseman, J. G., Macer, R. C. F., and Greeley, L. W. 1965. Genetic studies with cultures of *Erysiphe graminis* f. sp. *hordei* virulent on *Hordeum spontaneum*. Trans Brit Mycol Soc 48:479-489.

227. Moyer, C., Peres, N. A., Datnoff, L. E., Simonne, E. H., and Deng, Z. 2008. Evaluation of silicon for managing powdery mildew on gerbera daisy (*Gerbera jamesonii*). J. Plant Nutr. 31:2131-2144.

228. Muniappan, R., Reddy, G. V. P., and Raman, A. 2009. *Coccinia grandis* (L.) Voigt (Cucurbitaceae). Pages 175-182 in: Biological control of tropical weeds using arthropods, vol. 1. R. Muniappan, G. V. P. Reddy and A. Raman, eds. Cambridge University Press, Cambridge, UK.

229. Nagy, G. S. 1972. Studies on powdery mildews of cucurbits: host range and maintenance of *Sphaerotheca fuliginea* and *Erysiphe* sp. under laboratory and glass-house conditions. Acta Phytopathologica Academiae Scientiarum Hungaricae 7:415-420.

230. Nagy, G. S. 1976. Studies on powdery mildews of cucurbits: life cycle and epidemiology of *Erysiphe cichoracearum* and *Sphaerotheca fuliginea*. Acta Phytopathologica Academiae Scientiarum Hungaricae 11:205-210.

231. Narayanasamy, P. 2008. Molecular Techniques for Detection of Microbial Pathogens. Pages 7-158 in: Molecular Biology in Plant Pathogenesis and Disease Management, vol. 1. Springer, Netherlands.

232. Narayanasamy, P. 2011. Detection of Fungal Pathogens in Plants. Pages 5-199 in: Microbial Plant Pathogens-Detection and Disease Diagnosis:, vol. 1. Springer, Netherlands.

233. NASS, Florida Agriculture and Statistical Directory. National Agricultural Statistics Service, USDA. Retrieved 5 June 2011, from http://www.Florida-Agriculture.com

234. Nicot, P. C., Bardin, M., and Dik, A. J. 2002. Basic methods for epidemiological studies of powdery mildews: culture and preservation of isolates, production and delivery of inoculum and disease assessment. Pages 83-99 in: The Powdery Mildews, A Comprehensive Treatise, vol. 1. R. R. Belenger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathological Society Press, Saint Paul, MN.

235. Nunez-Palenius, H. G., Hopkins, D., and Cantliffe, D. J. 2006. Powdery mildew of cucurbits in Florida. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. Online publication. HS-1067.

236. O'Brien, R. G. 1994. Fungicide resistance in populations of cucurbit powdery mildew (*Sphaerotheca fuliginea*). New Zealand Journal of Crop and Horticultural Science 22:145-149.

237. Olmstead, J. W., Lang, G. A., and Grove, G. G. 2000. A leaf disk assay for screening sweet cherry genotypes for susceptibility to powdery mildew. Hortscience 35:274-277.

238. Olson, S. M., Simonne, E. H., Stall, W. M., Roberts, P. D., Webb, S. E., and Smith, S. A. 2010. Cucurbit Production in Florida. in: Vegetable Production Handbook, vol. S. M. a. S. Olson, E. H. , ed. Horticultural Sciences Departement, UF/IFAS, FL Coop Ext. Serv, Gainesville, FL.

239. Omara, S. K., and Taha, M. 1995. Powdery mildew attacks commercial watermelon cultivars in Sudan. Cucurbit Genetics Cooperative Report 18:55.

240. Paris, H., and Cohen, R. 2002. Powdery mildew-resistant summer squash hybrids having higher yields than their susceptible, commercial counterparts. Euphytica 124:121-128.

241. Paris, H. S. 1989. Historical records, origins, and development of the edible cultivar groups of *Cucurbita pepo* (Cucurbitaceae). Econ. Bot. 43:423-443.

242. Park, M. J., Choi, Y. J., Han, J. G., and Shin, H. D. 2010. First report in Korea of powdery mildew of *Matricaria chamomilla* caused by *Golovinomyces cichoracearum*. Plant Pathology 59:801-801.

243. Park, M. J., Park, J. H., Kim, H. G., Lee, S. G., Koh, Y. J., Kim, B. S., Cha, B., Lee, H. B., and Shin, H. D. 2011. Outbreak of powdery mildew on *Zinnia elegans* by *Golovinomyces cichoracearum* in Korea. Plant Pathology J 27:85-88.

244. Parsons, W. T., and Cuthbertson, E. G. 2001. Noxious Weeds of Australia. CSIRO Publishing, Collingwood, Australia.

245. Paulitz, T. C., and Belanger, R. R. 2001. Biological control in greenhouse systems. Annu. Rev. Phytopathol. 39:103-133.

246. Paulus, A. O., Shibuya, F., Osgood, J., Bohn, G. W., Hall, B. J., and Whitaker, T. W. 1969. Control of powdery mildew of cucurbits with systemic and non-systemic fungicides. Plant Dis. Reptr. 53:813-816.

247. Paulus, A. O., Shibuya, F., Whitaker, T. W., Hall, B. J., Bohn, G. W., and Little, T. M. 1967. Control of powdery mildew on cantaloupe. California Agriculture 21:12-15.

248. Paulus, A. O., Shibuya, F., Whitaker, T. W., Hall, B. J., Bohn, G. W., and Little, T. M. 1968. Control of powdery mildew in cucumber and squash. California Agriculture 22:10-11.

249. Pawar, V. P., and Patil, V. A. 2011. Occurrence of powdery mildew on some wild plants from Khandesh region of Maharashtra state. Recent Research in Science and Technology 3:94-95.

250. Pearson, R. C., and Gadoury, D. M. 1987. Cleistothecia, the source of primary inoculum for grape powdery mildew in New York. Phytopathology 77:1509-1514.

251. Perchepied, L., Bardin, M., Dogimont, C., and Pitrat, A. 2005. Relationship between loci conferring downy mildew and powdery mildew resistance in melon assessed by quantitative trait loci mapping. Phytopathology 95:556-565.

252. Perez-Angel, R., Garcia-Estrada, R. S., Carrillo-Fasio, J. A., Angulo-Escalante, M. A., Valdez-Torres, J. B., Muy-Rangel, M. D., Garcia-Lopez, A. M., and Villarreal-Romero, M. 2010. Powdery mildew (*Sphaerotheca fuliginea* Schlechtend.: Fr, Pollaci) control with vegetable oils and mineral salts on cucumber growing in greenhouse in Sinaloa, Mexico. Revista Mexicana de Fitopatologia 28:17-24.

253. Perez-Garcia, A., Mingorance, E., Rivera, M. E., Del Pino, D., Romero, D., Tores, J. A., and De Vicente, A. 2006. Long-term preservation of *Podosphaera fusca* using silica gel. Journal of Phytopathology 154:190-192.

254. Pérez-Garcia, A., Olalla, L., Rivera, E., Del Pino, D., Cánovas, I., De Vicente, A., and Torés, J. A. 2001. Development of *Sphaerotheca fusca* on susceptible, resistant, and temperature-sensitive resistant melon cultivars. Mycol. Res. 105:1216-1222.

255. Perez-Garcia, A., Romero, D., Fernandez-Ortuno, D., Lopez-Ruiz, F., De Vicente, A., and Tores, J. A. 2009. The powdery mildew fungus *Podosphaera fusca* (synonym *Podosphaera xanthii*), a constant threat to cucurbits. Mol. Plant Pathol. 10:153-160.

256. Pernezny, K., Maynard, D., and Stall, W. M. 2008. Powdery mildew of vegetables. Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciecne, University of Florida. Online publication. PP-14.

257. Peterson, C. E. 1975. Plant introductions in the improvement of vegetable cultivars Hortscience 10:575-579.

258. Philipp, W. D., Beuther, E., Hermann, D., Klinkert, F., Oberwalder, C., Schmidtke, M., and Straub, B. 1990. Formulation of the powdery mildew hyperparasite *Ampelomyces quisqualis* CES. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz (Journal of Plant Diseases and Protection) 97:120-132.

259. Philipp, W. D., and Hellstern, A. 1986. Biological control of powdery mildew by Ampelomyces quisqualis under reduced air humidity conditions. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz (Journal of Plant Diseases and Protection) 93:384-391.

260. Pino, D. d., Olalla, L., Perez-Garcia, A., Rivera, M. E., Garcia, S., Moreno, R., Vicente, A. d., and Tores, J. A. 2002. Occurrence of races and pathotypes of cucurbit powdery mildew in southeastern Spain. Phytoparasitica 30:459-466.

261. Pitrat, M., and Besombes, D. 2008. Inheritance of Podosphaera xanthii resistance in melon line '90625'. 135-142 In: Proceedings of the 9th EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae, Cucurbitaceae 2008 INRA Centre de Recherche d'Avignon, Unite Genetique et Amelioration des Fruits et Legumes, Avignon, France.

262. Pitrat, M., Chauvet, M., and Foury, C. 1997. Diversity, history and production of cultivated cucurbits. 21-28 In: Proceedings of the First International Symposium on Cucurbits. Acta Horticulture, Adana, Turkey.

263. Pitrat, M., Chauvet, M., and Foury, C. 1999. Diversity, history, and production of cultivated cucurbits. Acta Hort 492:21 - 28.

264. Pitrat, M., Dogimont, C., and Bardin, M. 1998. Resistance to fungal diseases of foliage in melon. 167-173 In: Cucurbitaceae 98 Evaluation and Enhancement of Cucurbits Germplasm Cucurbit Genetic Cooperative, Pacific Grove, CA.

265. Plantwise. Powdery mildew (*Golovinomyces cichoracearum*). CAB International. Retrieved 17 October 2011 from http://www.plantwise.org/?dsid=22065&loadmodule=plantwisedatasheet&page=4270&site=234.

266. Pratt, R. G., and Rowe, D. E. 2002. Enhanced resistance to *Sclerotium rolfsii* in populations of alfalfa selected for quantitative resistance to *Sclerotinia trifoliorum*. Phytopathology 92:204-209.

267. Qin, F. F., Xu, H. L., and Xu, Q. C. 2009. Control of powdery mildew in organic greenhouse melon cultivation. 1223-1230 In: International Symposium on High Technology for Greenhouse Systems: GreenSys2009 International Society for Horticultural Science, Quebec, Canada.

268. Queiroz, M. A. d., Dias, R. d. C. S., Souza, F. d. F., Ferreira, M. A. J. F., and Borges, R. M. E. 2000. Watermelon breeding in Brazil. 105-112 In: Proceedings of the 7th EUCARPIA meeting on cucurbit breeding and genetics, Cucurbitaceae 2000 Acta Horticulturae, Ma'ale Ha Hamisha, Israel.

269. Rankovic, B. 2003. Powdery mildew fungi (order Erysiphales) on plants in Montenegro (Chernogoria). Mikologiya i Fitopatologiya (Russian Acad. of Sci., Leninngrad Sci. Publ) 37:42-52.

270. Reifschneider, F. J. B., Boiteux, L. S., and Occhiena, E. M. 1985. Powdery mildew of melon (*Cucumis melo*) caused by *Sphaerotheca fuliginea* in Brazil Plant Dis. 69:1069-1070.

271. Reis, A., and Buso, J. A. 2004. Preliminary survey of *Sphaerotheca fuliginea* races occurring in cucurbits in Brazil. Horticultura Brasileira 22:628-631.

272. Reuveni, M., Agapov, V., and Reuveni, R. 1996. Controlling powdery mildew caused by *Sphaerotheca fuliginea* in cucumber by foliar sprays of phosphate and potassium salts. Crop Protection 15:49-53.

273. Reuveni, R., and Rotem, J. 1974. Effect of humidity on epidemiological patterns of the powdery mildew *Sphaerotheca fuliginea* on squash. Phytoparasitica 2:25-33.

274. Rhodes, A. M. 1964. Inheritance of powdery mildew resistance in the genus Cucurbita. Plant Dis Reptr 48:54-55.

275. Roberts, P. D., and Kucharek, T. 2009. Florida Plant Disease Management Guide: Watermelon. Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciecne, University of Florida. Online publication. PDMG-V3-55.

276. Robinson, R. W., and Decker-Walters, D. S. 1997. Cucurbits. CAB International, New York, NY.

277. Robinson, R. W., and Provvidenti, R. 1975. Susceptibility to powdery mildew in *Citrullus lanatus* (Thunb.) Matsum. & Nakai. Journal of the American Society for Horticultural Science 100:328-330.

278. Robinson, R. W., Provvidenti, R., and Shail, J. W. 1975. Inheritance of susceptibility to powdery mildew in watermelon. J. Hered. 66:310-311.

279. Romberg, M. K., Nunez, J. J., and Farrar, J. J. 2004. First report of powdery mildew on potato caused by *Golovinomyces cichoracearum* in California. Plant Dis. 88:309-309.

280. Romero, D., Perez-Garcia, A., Rivera, M. E., Cazorla, F. M., and de Vicente, A. 2004. Isolation and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca*. Appl. Microbiol. Biotechnol. 64:263-269.

281. Romero, D., Rivera, M. E., Cazorla, F. M., De Vicente, A., and Pérez-garcía, A. 2003. Effect of mycoparasitic fungi on the development of *Sphaerotheca fusca* in melon leaves. Mycol. Res. 107:64-71.

282. Romero, D., Vicente, A. d., Olmos, J. L., Davila, J. C., and Perez-Garcia, A. 2007. Effect of lipopeptides of antagonistic strains of *Bacillus subtilis* on the morphology and ultrastructure of the cucurbit fungal pathogen *Podosphaera fusca*. Journal of Applied Microbiology 103:969-976.

283. Romero, D., Vicente, A. d., Zeriouh, H., Cazorla, F. M., Fernandez-Ortuno, D., Tores, J. A., and Perez-Garcia, A. 2007. Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. Plant Pathology 56:976-986.

284. Rongai, D., Cerato, C., and Lazzeri, L. 2009. A natural fungicide for the control of *Erysiphe betae* and *Erysiphe cichoracearum*. Eur. J. Plant Pathol. 124:613-619.

285. Rotem, J., Cohen, Y., and Bashi, E. 1978. Host and environmental influences on sporulation *in vivo*. Ann. Rev. of Phytopathol. 16:83-101.

286. Rudich, J., Karchi, Z., and Eshed, N. 1969. Evidence for 2 races of pathogen causing powdery mildew of muskmelon in Israel. Israel Journal of Agricultural Research 19:41-&.

287. Salmeron, J., Vernooij, B., Lawton, K., Kramer, C., Frye, C., Oostendorp, M., Knauf-Beiter, G., and Staub, T. 2002. Powdery Mildew Control Through Transgenic Expression of Antifungal Proteins, Resistance Genes, and Systemic Acquired Resistance. Pages 268-287 in: The Powdery Mildews: A Comprehensive Treatise, vol. 1. R. R. Belanger, W. R. Bushnell, A. J. Dik and T. L. W. Carver, eds. American Phytopathogical Society Press, Saint Paul, MN.

288. Salmon, E. S. 1900. A Monograph of the Erysiphaceae. Bulletin of the Torrey Botanical Club, New York, NY.

289. Schaefer, H., Heibl, C., and Renner, S. S. 2009. Gourds afloat: a dated phylogeny reveals an Asian origin of the gourd family (Cucurbitaceae) and numerous oversea dispersal events. Royal Society Publishing. Online publication. doi:/10.1098/rspb.2008.1447.

290. Schaefer, H., Kocyan, A., and Renner, S. S. 2008. Linnaeosicyos (Cucurbitaceae): a new genus for *Trichosanthes amara*, the Caribbean sister species of all Sicyeae. Syst. Bot. 33:349-355.

291. Schaefer, H., and Renner, S. S. 2011. Phylogenetic relationships in the order Cucurbitales and a new classification of the gourd family (Cucurbitaceae). Taxon 60:122-138.

292. Schepers, H. 1984. Persistance of resistance to fungicides in *Sphaerotheca fuliginea*. Netherlands Journal of Plant Pathology 90:165-171.

293. Schepers, H. T. A. M. 1984. A pattern in the appearance of cucumber powdery mildew in Dutch glasshouses. Eur. J. Plant Pathol. 90:247-256.

294. Schuerger, A. C., and Hammer, W. 2003. Suppression of powdery mildew on greenhouse-grown cucumber by addition of silicon to hydroponic nutrient solution is inhibited at high temperature. Plant Dis. 87:177-185.

295. Schultheis, J. R. 2006. Cucurbits and Their Importance in North Carolina. 265-271 In: Cucurbitaceae 2006 NC State University, College of Agriculture and Life Sciences, Asheville, NC.

296. Sedlakova, B., and Lebeda, A. 2008. Fungicide resistance in Czech populations of cucurbit powdery mildews. Phytoparasitica 36:272-289.

297. Sedlakova, B., and Lebeda, A. 2010. Occurrence of *Ampelomyces quisqualis* on the cucurbit powdery mildew species in the Czech Republic. Acta Horticulturae:477-482.

298. Sedlarova, M., Lebeda, A., Miksikova, P., Duchoslav, M., Sedlakova, B., and McCreight, J. D. 2009. Histological aspects of *Cucumis melo* PI 313970 resistance to *Podosphaera xanthii* and *Golovinomyces cichoracearum*. Journal of Plant Diseases and Protection 116:169-176.

299. Seijo, T. E., Czarnecki, D., Zhanao, D., and Peres, N. A. 2006. First report of powdery mildew caused by *Golovinomyces cichoracearum* on *Coreopsis leavenworthii*. Plant Health Progress:1-2.

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

301. Sharma, G. K., and Khan, M. W. 1991. Observations on occurrence and identity of powdery mildew of cucurbits in Tamil Nadu. Indian Phytopathology 44:45-51.

302. Shashikumar, K. T., Pitchaimuthu, M., Kumar, D. P., and Rawal, R. D. 2011. Heterosis and combining ability for resistance to powdery mildew in adult melon plants. Plant Breed. 130:383-387.

303. Shaw, N. L., and Cantliffe, D. J. 2003. Hydroponically produced mini-cucumber with improved powdery mildew resistance. 58-62 In: Proceedings of the Annual Meeting of the Florida State Horticulture Society Florida State Horticulture Science, Orlando, FL.

304. Shin, H. D., and La, Y. J. 1993. Morphology of edge lines of chained immature conida on condiophores in powdery mildew fungi and their taxonomic significance. Mycotaxon 46:445-451.

305. Shishkoff, N., and McGrath, M. T. 2001. Distribution of cucurbit powdery mildew races 1 and 2 on watermelon and muskmelon. Phytopathology 91:S197.

306. Shishkoff, N., and McGrath, M. T. 2002. AQ10 biofungicide combined with chemical fungicides or AddQ spray adjuvant for control of cucurbit powdery mildew in detached leaf culture. Plant Dis. 86:915-918.

307. Sitterly, W. R. 1972. Breeding for disease resistance in cucurbits. Annu. Rev. Phytopathol. 10:471-&.

308. Smith, B. D. 1997. The initial domestication of *Cucurbita pepo* in the Americas 10,000 years ago. Science 276:932-934.

309. Smith, V. L., Ferrandino, F. J., and Cecarelli, R. 2002. Evaluation of milk as a foliar spray for control of powdery mildew of tomato. The American Phytopathological Society. Online publication. doi:/10.1094/BC16.

310. Stone, O. M. 1962. Alternate hosts of cucumber powdery mildew. Annals of Applied Biology 50:203-210 pp.

311. Suzuki, S., Fuji, S., Furuya, H., and Naito, H. 2006. Cytokinin-mediated suppression of cucumber powdery mildew disease: 6-benzyladenine suppresses the growth of cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. Plant Pathology Journal (Faisalabad) 5:299-306.

312. Takamatsu, S. 2004. Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequences. Mycoscience 45:147-157.

313. Takamatsu, S., Heluta, V., Havrylenko, M., and Divarangkoon, R. 2009. Four powdery mildew species with catenate conidia infect Galium: molecular and morphological evidence. Mycol. Res. 113:117-129.

314. Takamatsu, S., Matsuda, S., Niinomi, S., and Havrylenko, M. 2006. Molecular phylogeny supports a northern hemisphere origin of *Golovinomyces* (Ascomycota : Erysiphales). Mycol. Res. 110:1093-1101.

315. Teixeira, A. M., Barreto, F. A., and Camargo, L. E. 2006. An AFLP marker linked to the Pm-1 gene that confers resistance to *Podosphaera xanthii* in melon. Phytopathology 96:S113-S114.

316. Teixeira, A. P. M., Barreto, F., and Camargo, L. E. A. 2008. An AFLP marker linked to the Pm-1 gene that confers resistance to *Podosphaera xanthii* race 1 in *Cucumis melo*. Genetics and Molecular Biology 31:547-550.

317. Tesfagiorgis, H. B., and Laing, M. D. 2011. Effects of concentration, frequency of application and runoff of foliar-applied solube silicon on powdery mildew of zucchini. African Journal of Agricultural Research 6:2243-2248.

318. Tetteh, A., Wehner, T., and Davis, A. 2007. Screening the watermelon germplasm collection for resistance to powdery mildew race 2. Hortscience 42:896-896.

319. Tetteh, A. Y., Wehner, T. C., and Davis, A. R. 2010. Identifying resistance to powdery mildew race 2W in the USDA-ARS watermelon germplasm collection. Crop Science 50:933-939.

320. Thomas, C. E., Kishaba, A. N., McCreight, J. D., and Nugent, P. E. 1984. The importance of monitoring races of powdery mildew on muskmelon. Cucurbit Genetics Cooperative Report 7:58-59.

321. Thomas, C. E., Levi, A., and Caniglia, E. 2005. Evaluation of US plant introductions of watermelon for resistance to powdery mildew. Hortscience 40:154-156.

322. Tomason, Y., and Gibson, P. T. 2006. Fungal characteristics and varietal reactions of powdery mildew species on cucurbits in the steppes of Ukraine. Agronomy Research 4:549-562.

323. Tores, J. A., Sanchez-Pulido, J. M., Lopez-Ruiz, F., Vicente, A. d., and Perez-Garcia, A. 2009. First occurrence of cucurbit powdery mildew caused by race 3-5 of *Podosphaera fusca* in Spain. Plant Dis. 93:1073.

324. Troisi, M., Bertetti, D., Garibaldi, A., and Gullino, M. L. 2010. First report of powdery mildew caused by *Golovinomyces cichoracearum* on gerbera (*Gerbera jamesonii*) in Italy. Plant Dis. 94:130-130.

325. Uchida, K., Takamatsu, S., Matsuda, S., So, K., and Sato, Y. 2009. Morphological and molecular characterization of *Oidium* subgenus *Reticuloidium* (powdery mildew) newly occurred on cucumber in Japan. J. Gen. Plant Pathol. 75:92-100.

326. Urban, J., and Lebeda, A. 2006. Fungicide resistance in cucurbit downy mildew: methodological, biological and population aspects. Annals of Applied Biology 149:63-75.

327. USDA-NASS, Vegetables. National Agricultural Statistic Service, USDA Retrieved 3 June 2011, from http://usda.mannlib.cornell.edu/usda/nass/VegeSumm//2010s/2011/VegeSumm-01-27-2011_new_format.pdf

328. Vakalounakis, D. J., and Klironomou, E. 1995. Race and mating type identification of powdery mildew on cucurbits in Greece. Plant Pathology 44:1033-1038.

329. Vakalounakis, D. J., Klironomou, E., and Papadakis, A. 1994. Species spectrum, host range and distribution of powdery mildews on Cucurbitaceae in Crete. Plant Pathology 43:813-818.

330. Vallad, G. E. 2010. Powdery mildew of cucurbits. Plant Pathology Department, Florida Cooperative Extension Service, Golf Coast REC, Institute of Food and Agricultural Sciecne, University of Florida. Online publication. GV10242010.

331. Velkov, N., and Masheva, S. 2002. Species and races composition of powdery mildew on cucurbits in Bulgaria. Cucurbit Genetics Cooperative Report 25:7-10.

332. Wang, Y.-H., Joobeur, T., Dean, R. A., and Staub, J. E. 2007. Cucurbits. Pages 315-329 in: Genome Mapping and Molecular Breeding in Plants, vol. 5. C. Kole, ed. Springer, Berling, Germany.

333. Webster, J., and Weber, R. 2007. Introduction to Fungi. 3rd ed. Cambridge University Press, Cambridge, UK.

334. Wehner, T. C. 2008. Watermelon. Pages 381-418 in: Vegetables I, vol. 1. J. Prohens and F. Nuez, eds. Springer, New York, NY.

335. Wehner, T. C., and Maynard, D. N. 2003. Cucurbitaceae (Vine Crops). in: The Encyclopedia of Life, vol. 1. L. John Wiley & Sons, ed. Nature Publishing, London, UK.

336. Whipps, J. M., and Helyer, N. L. 1994. Occurance of powdery mildew on auburgine in west Sussex. Plant Pathology 43:230-233.

337. White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications vol. 1. M. A. Innis, ed. Academic Press, San Diego, CA.

338. Wong, F. P., and Wilcox, W. F. 2002. Sensitivity to azoxystrobin among isolates of *Uncinula necator*: baseline distribution and relationship to myclobutanil sensitivity. Plant Dis. 86:394-404.

339. Wu, M. Z., Yi, H. P., Zhai, W. Q., and Feng, J. X. 2004. Breeding disease resistant Hami-melon. 69-74 In: Proceedings of the 26th International Horticulture Congress: Advances in Vegetable Breeding International Society of Horticultural Science, Toronto, Canada.

340. Wyenandt, C. A., Maxwell, N., and Ward, D. L. 2008. Fungicide programs affect 'practical' resistance development in cucurbit powdery mildew of pumpkin. HortScience 43:1838-1845.

341. Wyenandt, C. A., McGrath, M. T., Rideout, S. L., Gugino, B. K., Everts, K. L., and Mulrooney, R. P. 2009. Fungicide resistance management guidelines for cucurbit downy and powdery mildew control in the mid-Atlantic and northeast regions of the U.S. Phytopathology 99:144.

342. Yarwood, C. 1957. Powdery mildews. The Botanical Review 23:235-301.

343. Yarwood, C. E. 1939. Control of powdery mildews with a water spray. Phytopathology 29:288-290.

344. Yarwood, C. E. 1978. Water stimulates *Sphaerotheca fuliginea*. Mycologia 70:1035-1039.

345. Yarwood, C. E., and Gardner, M. W. 1964. Unreported powdery mildews. Plant Dis. Reptr. 48:310.

346. Yuste-Lisbona, F. J., Capel, C., Sarria, E., Gomez-Guillamon, M. L., Capel, J., Lozano, R., and Lopez-Sese, A. I. 2010. Construction of a genetic linkage map and identification of a major QTL conferring resistance to powdery mildew in the melon genotype TGR-1551. Acta Horticulturae 871:179-186.

347. Yuste-Lisbona, F. J., Lopez-Sese, A. I., and Gomez-Guillamon, M. L. 2010. Inheritance of resistance to races 1, 2 and 5 of powdery mildew in the melon TGR-1551. Plant Breed. 129:72-75.

348. Zatarim, M., Cardoso, A. I. I., and Furtado, E. L. 2005. Effect of types of cow milk on the powdery mildew control of pumpkin under field conditions. Horticultura Brasileira 23:198-201.

349. Zeller, K. A. 1995. Phylogenetic relatedness within the genus *Erysiphe* estimated with morphological characteristics. Mycologia 87:525-531.

350. Zhang, H. Y., Guo, S. G., Gong, G. Y., Ren, Y., Davis, A. R., and Xu, Y. 2011. Sources of resistance to race 2WF powdery mildew in U.S. watermelon plant introductions. HortScience 46:1349-1352.

351. Zhang, S. A., Vallad, G. E., White, T. L., and Huang, C. H. 2011. Evaluation of microbial products for management of powdery mildew on summer squash and cantaloupe in Florida. Plant Dis. 95:461-468.

352. Zhou, J., Hu, H., Li, X., Zhou, R., and Zhang, H. 2010. Identification of a resource of powdery mildew resistance in *Cucurbita moschata*. Acta Horticulturae 871:141-146.

353. Zitter, T. A., Hopkins, D. L., and Thomas, C. E. 1996. Compendium of Cucurbit Diseases. American Phytopatholocical Society Press, Saint Paul, MN.

354. Ziv, O., and Zitter, T. A. 1992. Effects of biocarbonates and film-forming polymers on cucurbit diseases. Plant Dis. 76:513-517.

BIOGRAPHICAL SKETCH

Gabriella Silveira Maia, was born in Vicosa, MG-Brazil, in 1977 to Jaime Maia dos Santos and Arlete Silveira. Gabriella has a younger sister, Fabiola Silveira Maia. Gabriella graduated from UNESP (Universidade Estadual Paulista in Jaboticabal, Sao Paulo, Brazil) in 2003, with a bachelor degree in Agriculture Engineering.

On August 1999, she was awarded a Fulbright Scholarship and spent one year at Texas A&M University as an undergraduate student in Agronomy. On August 2002, Gabriella came to the University of Florida (UF) in Gainesville for a 3 month internship in the Plant Pathology Department. She worked in the laboratory of Dr. Raghavan Charudattan and assisted in research trials concerning biological control of weeds. On July 2003, Gabriella went to Delemont, Switzerland, where she worked with biological management of invasive weeds and insect pests, in the laboratory of Dr. Andre Gassman, at CABI –Europe, until November 2003.

On June 2004 in Vicosa ,Minas Gerais, Brazil, Gabriella married Alberto Azeredo, a Food Engineer. They moved to Brasilandia de Minas and worked at Fuchs Gewürze until February 2005. During this time, Gabriella managed an insect breeding laboratory which focused on biological control of insect pests.

Gabriella returned to Gainesville, in 2005 to work as a research assistant at the Plant Pathology Department at UF. She again, worked in the laboratory of Dr. Charudattan until she became a graduate student in 2008. In August 2010, Gabriella and Alberto had their first daughter, Anna Azeredo. Gabriella finished her MS degree in Plant Pathology in May 2012. She has a strong interest in plant pathology extension and in research, and intends to continue her studies and pursue a Ph.D. degree.