## ABSTRACT

COLUCCI, SUSAN JEAN. Host Range, Fungicide Resistance and Management of *Pseudoperonospora cubensis*, causal agent of Cucurbit Downy Mildew. (Under the direction of Gerald J. Holmes).

In 2004, downy mildew (causal agent *Pseudoperonospora cubensis*) re-emerged as an important foliar disease on cucumber after decades of control with resistant, commercially available cultivars. Because the new strain of *P. cubensis* had increased aggressiveness on cucumber, it was hypothesized that it may also have a different host range than the previously described populations in the United States. To determine host range, leaf disks of 12 cucurbit host differentials were inoculated with 32 isolates of *P. cubensis* collected from the eastern United States, including two isolates collected prior to the 2004 epidemic. The result was 32 different host range patterns. *Cucumis* spp., including cucumber, were the most susceptible hosts. The least susceptible differentials were *Citrullus lanatus*, *Lagenaria siceraria*, *Luffa cylindrica*, *Benincasa hispida* and *Cucurbita pepo* var. *pepo*. Natural populations of the pathogen were evaluated in field experiments in Florida, South Carolina, Georgia, North Carolina, Delaware, Maryland, New York, Michigan and Ohio. *Cucumis* and *Cucurbita* were the most susceptible genera, and *Benincasa hispida* and *Luffa cylindrica* were the least susceptible.

Because the commercially available resistant cucumber cultivars are no longer effective in controlling downy mildew, fungicides are necessary to manage the disease. A fungicide efficacy trial in 2004 in Sampson County, North Carolina indicated a reduced efficacy of mefenoxam and the QoI fungicides, azoxystrobin and pyraclostrobin. To confirm resistance, the sensitivity of *P. cubensis* isolates to treatments of mefenoxam (0.01, 0.1, 1.0, 10, 100 µg/ml) and the QoI fungicide, azoxystrobin (0.001, 0.01, 0.1, 1.0, 10, 100 µg/ml)

was determined using a whole cucumber plant assay with inoculum derived from 24 single leaf isolates collected in 2004 through 2007 from the eastern United States. Seven additional isolates and four isolates from the original assay were tested with mefenoxam at 1 and 100 µg/ml and azoxystrobin at 1 and 100 µg/ml, as well as positive (fluopicolide) and non-treated control. Insensitivity (less than 25% disease control) to all mefenoxam and azoxystrobin concentrations was demonstrated in 27 out of 31 (87%) of the total isolates assayed. Practical resistance of *P. cubensis* populations to mefenoxam and pyraclostrobin was evaluated in Florida, South Carolina, North Carolina, Delaware and New York. There was no reduction in disease severity compared to the non-treated control at all locations and marketable yield was significantly reduced in mefenoxam and pyraclostrobin plots in all locations except Florida.

Fungicide efficacy trials in Sampson County, NC in 2005, 2006 and 2007 indicate that fungicides with active ingredients famoxadone, cymoxanil, zoxamide, propamocarb hydrochloride, cyazofamid and fluopicolide are the most efficacious at managing downy mildew on cucumber. Treatments involving these fungicides result in lower disease severity and higher yield than the non-treated control. In addition, a locally systemic + protectant fungicide program was compared to a protectant-only program with respect to delay of fungicide application. Results indicated that the locally systemic + protectant program was more effective than the protectant only program when applied before disease detection. If efficacious fungicides are not applied within 2 to 3 weeks of initial detection, downy mildew control was not possible.

Host Range, Fungicide Resistance and Management of *Pseudoperonospora cubensis*, Causal Agent of Cucurbit Downy Mildew.

by
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## DEDICATION

This work is dedicated to my parents, Frank and Linda Colucci, who always taught me to work hard and let me believe that I could achieve anything. Though I don't think they envisioned me as a plant pathologist, they continue to support me by eating a cucumber everyday.

## BIOGRAPHY

Susan Jean Colucci was born on April 27, 1982 in Kent County, RI. She was raised in Coventry, RI. After graduating from Coventry High School as president of her class, she attended the University of Rhode Island (URI) in Kingston, RI. At URI Susan took an interest in plant pathology and mycology working on projects involving arbuscular mycorrhizae with Dr. Richard Koske and plant pathogens of ornamentals and turf with Dr. Larry Englander and Dr. Nathaniel Mitkowski. In May 2004 Susan graduated from URI with a bachelors degree in Biological Sciences with a minor in Anthropology. In 2005 Susan accepted a graduate student assistantship from the Department of Plant Pathology at North Carolina State University where she pursued a Master's of Science degree in Plant Pathology. She completed her degree in August of 2008 under the direction of Dr. Gerald Holmes.

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Cindy Richardson Decker, the woman who can figure anything out in Microsoft office.

The wise fortune cookie that once said, “It is not in your character to give up.”

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# CHAPTER 1 – REVIEW OF THE LITERATURE

## SECTION 1 – DOWNY MILDEW AND ITS CAUSAL AGENT

**Distribution, importance and host range.** *Pseudoperonospora cubensis* (Berkeley & Curtis) Rostovtsev is the causal agent of downy mildew of plants in the Cucurbitaceae (gourd family), which includes cucumber, watermelon, squash, gourds and melons (Palti, 1975). Berkeley and Curtis first reported the disease in 1868 from Cuba (Berkeley and Curtis, 1868). Downy mildew of cucurbits is found in temperate areas, such as the Americas, Europe, Japan, Australia and South Africa, tropical regions internationally and some semi-arid regions, such as the Middle East. The disease affects cucurbits in the open field as well as those under plastic or in greenhouses and is especially damaging in those areas that have a warm, humid climate in which the pathogen thrives (Cohen, 1981; Holmes et al., 2004; Palti and Cohen, 1980).

*P. cubensis* is an obligate parasite, requiring live cucurbit host tissue to grow and reproduce (Palti, 1975; Waterhouse, 1973). Forty species in 20 genera within Cucurbitaceae are known to be hosts to *P. cubensis* (Palti and Cohen, 1980). Downy mildew on *Cucumis* alone has been accounted for in 70 countries (Cohen, 1981). Palti (1974) presented the distribution of *P. cubensis* on its crop hosts in the genera *Cucumis*, *Cucurbita*, *Citrullus* and *Luffa*. While it has the widest geographic distribution on *Cucumis*, it is common on *Cucurbita* crops in Australia and the Pacific region, is less common in Asia, Africa and the Americas, and is virtually absent from *Cucurbita* in Europe. Downy mildew is widely distributed on *Citrullus* in the Americas, but scattered elsewhere and totally absent in Europe

and the Middle East, where the climate is similar to areas where the disease is a severe problem. The disease is extensive on *Luffa* only in Southeast Asia.

It is interesting to note that two species of *Plasmopara*, *Plasmopara australis* and *P. orientalis* have been recorded on cucurbit hosts *Sicyos angulata* (burr cucumber) and *Cyclanthera* sp., respectively, and more recently *P. australis* was reported from Brazil on *L. cylindrica* (Palti and Kenneth, 1981; Soares et al., 2006).

**Taxonomy, nomenclature and evolution.** Berkeley and Curtis first documented downy mildew of cucurbits in 1868 in Cuba. They named the pathogen *Peronospora cubensis* (Berkeley and Curtis, 1868). In 1902 Berlese subdivided the genus *Plasmopara* Schroeter, placing *Peronospora cubensis* Berk. & Curt. in the subgenus *Peronoplasmopara*. The subgenus was based on the branching of sporangiophores, similar to that of *Peronospora*, and the presence of sporangia that germinate by releasing zoospores, similar to the reproductive unit of *Plasmopara*. In 1903 Rostovtsev conducted a critical study of the downy mildew pathogen of cucumber in Russia. Like Berlese, Rostovtsev noted the similarities between the cucurbit downy mildew pathogen and *Peronospora* and *Plasmopara*. In his report, Rostovtsev proposed the new genus *Pseudoperonospora* and never mentioned Berlese's 1902 report. Because of Rostovsev's complete description, intricate drawings and Article 42 of the International Code of Botanical Nomenclature 1978, the genus *Pseudoperonospora* is the accepted and proper name (Waterhouse and Brothers, 1981).

*Pseudoperonospora, Plasmopara* and *Peronospora* are three genera that are part of the Peronosporaceae (the downy mildews) in the order Peronosporales within the class Oomycetes. Other members of the Peronosporales are *Albuginaceae,* haustoria forming,

obligate parasites of plants with unbranched sporangiophores, and *Pythiaceae,* a family that includes saprobes and non-obligate plant parasites that do not produce haustoria (Waterhouse, 1973).

The sporangiophores of *Pseudoperonospora* branch at acute angles and the tips of the branches are pointed and thus resemble the conidiophores of *Peronospora*. The conidiophore of *Peronospora* branches dichotomously, while those of *Pseudoperonospora* branch irregularly for the first one or two branches with the later branch as being dichotomous. Rarely, branching of some *Peronospora* conidiophores is irregular (Waterhouse, 1973; Waterhouse and Brothers, 1981). Unlike *Pseudoperonospora*, the sporangiophore of *Plasmopara* usually branches at right angles, has truncated (blunt) tips and the branches have cross walls (Waterhouse and Brothers, 1981).

*Pseudoperonospora* has true sporangia with a poroid apex that germinate via zoospores (Waterhouse and Brothers, 1981). By Shaw's definition, true sporangia have a distinct apical modification of the wall, are operculate, and the operculum is lens-shaped. As the sporangium matures the operculum swells and becomes completely detached due to the increasing osmotic pressure inside the sporangium. The enlarging operculum results in the papillate appearance at the apex of the sporangium. This increase in osmotic pressure occurs prior to the initial swarming and fleeing of zoospores. The empty sporangium is broadly poroid after zoospore release. Unlike *Pseudoperonospora* (and *Plasmopara*), *Peronospora* does not produce sporangia. The asexual spore has lost the operculum and therefore lost the ability to produce zoospores. As a result, *Peronospora* produces conidia that germinate via a germ tube. The germ tube is not limited to the apical region and can emerge from any point

on the circumference of the conidium (Shaw, 1981).

Sporangia of *P. cubensis* measure 20-40 x 14-25 μm in diameter (Palti, 1975; Palti and Cohen, 1980). The sporangia are pale grayish- to olivaceous-purple, lemon-shaped, have a papilla at the distal end, a poroid apex and the periplasm is persistent and conspicuous (Palti, 1975; Waterhouse, 1973). Sporangia appear smooth under the compound microscope, but with the scanning electron microscope, the finely decorated surface of the sporangium is evident. Lange et al. (1989a) hypothesized that the ornamentation on the outer thin layer of sporangia may aid in its ability to withstand unfavorable conditions (up to 3-4 months of -18°C) and possibly act as a resting structure.

The sporangia are borne singly on the pointed tips of sporangiophores that branch at acute angles (Palti, 1975; Waterhouse, 1973). The sporangiophore ranges from 180-600 μm in height, 20 μm in diameter and 5-7 μm in width (Lange et al., 1989a, Palti and Cohen, 1980).

The sporangia germinate via zoospores (Palti, 1975; Waterhouse, 1973). Zoospores measure 10-13 μm in diameter and zoospore germ tubes reach lengths of 50-95 μm (Palti, 1975; Palti and Cohen, 1980). The zoospores of *P. cubensis* are ovoid and biflagellate, with one posterior whiplash and one anterior tinsel flagellum (Cohen, 1981; Lange et al., 1989c; Palti, 1980).

The intercellular mycelium is hyaline and coenocytic. The mycelium develops in the mesophyll, but also penetrates the palisade tissue. The diameter of hyphae is 5.4-7.2 μm (Palti, 1975; Palti and Cohen, 1980). The haustoria are varied and appear stunted, inflated or as branched clusters of hyphae (Lange et al., 1989b, Waterhouse, 1973).

The presence of oospores is rare for *P. cubensis*. The production of oospores has been reported in Russia, China, Japan, India and Italy (Lange et al., 1989a, 1989c; Palti and Cohen, 1980; Waterhouse and Brothers, 1981). In 1932, Doran explored the over-wintering of the pathogen in Massachusetts. Though he was able to find some oospores in tissue of infected and buried cucumbers, he was unable to germinate them and unable to positively identify them as *P. cubensis* (Doran, 1932).

**Life cycle and epidemiology.** Infection is initiated by sporangia that are transported from infected plants through the air and travel to local or distant places via wind currents (Palti, 1975; Waterhouse, 1973). Symptomatic plants with yellow lesions have the greatest sporulating capacity. Optimal temperature for sporulation is 15°C and requires as little as 6 hours of moist conditions. The sporulating capacity of purely necrotic lesions is low and that of yellow-necrotic lesions is intermediate, with optimal temperature being 20°C (Cohen and Rotem, 1969).

Sporangia and sporangiophores are greatly affected by changes in temperature and humidity. Warming and drying of the atmosphere, typical of early morning hours, causes twisting of the sporangiophores, which may be of importance for the detachment of sporangia (Lange et al., 1989a). Spore traps in Florida trapped sporangia from infected watermelon fields in abundance from 06.00 to 10.00 h after dew had dried and surface winds increased. Sporangia were not collected after 15.00 to 16.00 h or in the evening hours (Schenck, 1968). In the semi-arid climate of Israel, Cohen and Rotem (1971) found that intensive dispersal started between 06.00 and 07.00 h, reached peak around 08.00 h, and subsided by noon in infected cucumber plantings.

Moisture, as leaf wetness is required for the sporangia to release 2-15 zoospores. It is also important for zoospore movement and penetration of host tissue by germ tubes (Cohen, 1981; Lange et al., 1989c; Palti and Cohen, 1980). However, moisture may reduce the duration of sporangia viability (Palti and Cohen, 1980). Zoospores are released through an exit pore or a rupture of the sporangial wall. The zoospores may adhere for a short time before they become motile (Lange et al., 1989a). Minimum and optimum temperatures for zoospore release is relative and dependent upon the time of incubation, but occur within the range of 5-28°C. If incubation lasts for only 1 h the minimum temperature is 10°C and the optimum is 15°C. For a 2 h incubation the minimum is 5°C and the optimum 15°C (Cohen, 1981).

Zoospores must encyst on a stomatal opening to cause infection (Cohen, 1981; Lange et al., 1989c; Palti, 1980; Palti and Cohen, 1980). High temperatures induce immediate cyst formation. The optimum temperature for cyst formation is 25°C (Cohen, 1981). The cyst will then form a germ tube and enter the host tissue via the stomata opening (Palti, 1975; Waterhouse, 1973). Intercellular hyphae form haustoria in order to receive essential nutrients for survival (Palti, 1975; Waterhouse, 1973).

New sporangiophores, differentiated from the mycelium, then emerge singly or in tufts from the epidermis usually via the stomata opening (Waterhouse, 1973). Emergence of sporangiophores does not occur until the air over the lesion is moisture saturated. Due to the higher frequency of hyphae in the spongy parenchyma most sporangia are produced on the lower leaf surface (Cohen, 1981).

**Dispersal.** Because the members of the Cucurbitaceae are sensitive to frost, the pathogen cannot survive in areas where the temperatures are low enough to destroy cucurbit crops (Holmes et al., 2004; Thomas and Jourdain, 1992). In the U.S., *P. cubensis* can survive in extreme southern areas (below the 30$^{th}$ latitude), such as southern Florida, as active mycelium in cultivated or wild species of cucurbits (Bains and Jhooty, 1976; Doran, 1932; Cohen, 1981; Holmes et al., 1998).

Sporangia of *P. cubensis* are thought to travel from southern areas, where the organism over-winters, to northern areas in the spring and summer with the cultivation of host plants. Doran (1932) discussed the likelihood of the movement of sporangia from Florida to Georgia to the Carolinas and so on northward. In 1943, Nusbaum, with the help of collaborators along the Atlantic Coast, tracked the progression of the disease. Though there was a drought that year, disease spread through Florida in late April and early May moved to South Carolina in early June, North Carolina by late June, Virginia and Delaware in July, Connecticut in early August and Massachusetts in late August (Nusbaum, 1944). Nusbaum published the results of a similar study in 1948. Contrary to 1943, the disease was more widespread to inland areas in Virginia, New York and Pennsylvania, appeared earlier and caused considerable damage. This was possibly due to the presence of more favorable environmental conditions (Nusbaum, 1948).

In 1998, Holmes and Main of North Carolina State University began a forecasting system to track the long-distance movement of the pathogen from known sources of inoculum to potential sites of infection based on large-scale weather systems. This differs from other disease forecasting systems that assume the host and inoculum are present and

efforts are based on the environmental factors that favor growth and development of the pathogen. In addition, like Nusbaum, this system helps track and monitor the location and progression of the disease (Holmes et al., 1998, 2004).

**Plant Parts Affected and Symptomology.** *Pseudoperonospora cubensis* is an obligate parasite and therefore requires live cucurbit host tissue to grow and reproduce (Palti, 1975; Waterhouse, 1973). The pathogen almost exclusively attacks the host leaf. Downy mildew may appear on the cotyledons, but is rare on the very young true-leaves when they are in the process of unfurling (Palti and Cohen, 1980; Van Haltern, 1933). In heavily infected cantaloupe vines, Van Haltern (1933) reported the presence of sporangiophores on the stems, leaf petioles, tendril, and the peduncles of blossoms. When the runners were sectioned, haustoria and mycelium were found in the cortex, but the pathogen was not found in the fruit.

On most cucurbits, symptoms first appear as small, slightly chlorotic to yellow areas on the upper leaf surface. Lesions first appear on the older leaves and appear progressively on the younger, more distal leaves as these leaves expand. As the lesions expand, they become brighter yellow and eventually become necrotic and brown. On cucumber, squash and sometimes melon, lesions appear angular because they are bound by leaf veins. When conditions favor sporulation, such as humid or wet conditions, the production of sporangia occurs usually on the lower leaf surface beneath the lesions. Sporulation ranges from colorless to grey to dark-purple and appears as felt or down. As lesions expand, they often coalesce, resulting in the necrosis of larger leaf areas and the eventual death of the leaf. On watermelon, lesions may be angular but are often irregular in shape. These lesions will

eventually turn brown to black in color and an exaggerated upward leaf curl will occur. Severe infection on all cucurbits can cause death of the foliage. Death of leaves increases the occurrence of sunscald on fruit, which results in reductions in both quality and quantity of marketable yield (Thomas, 1996).

**Host range.** *Pseudoperonospora cubensis* is known to exhibit host specialization within the Cucurbitaceae (Doran, 1932; Ellis, 1951; Hughes and Van Haltern, 1952; Palti, 1974; Palti and Cohen, 1980). In 1932, Doran explored this subject by comparing the susceptibility of different cucurbit hosts in Massachusetts. A variety of cucurbits were planted, including cucumber, melons, gourds, squash and pumpkin, and artificially inoculated with a spore suspension of *P. cubensis* collected from cucumber. Doran found that downy mildew was severe on cucumber, moderate on muskmelon, barely detected on watermelon, and a few of the other gourds, and absent on squash and pumpkin (Doran, 1932).

Hughes and Van Haltern (1952) conducted a host range experiment with cucumber, cantaloupe and watermelon in Georgia and South Carolina. They used two isolates of the pathogen, one collected from cucumber and the other from watermelon. The isolate collected from cucumber caused severe damage on cucumber and melon, though the disease developed slower on cantaloupe, and watermelon exhibited only mild symptoms of downy mildew. Lesions on watermelon were few and atypical and did not sporulate. The isolate from watermelon caused moderately severe disease on cucumber and cantaloupe and considerable damage on watermelon with typical large and irregular lesions (Hughes and Van Haltern, 1952). In addition, the possibility of the presence of races of the pathogen was

supported by the sudden breakdown of the cucumber cultivar Palmetto, which was previously regarded as highly resistant to downy mildew (Barnes and Epps, 1952).

Palti (1974) summarized the cucurbit/*P. cubensis* cross-inoculation studies conducted previously, including Doran (1932) and Hughes and Van Haltern's (1952) studies, research from Japan by Iwata (1941, 1942) and in Israel from Yigel Cohen's Ph.D. thesis (1969). Palti concluded that the divergences could be attributed to physiological races. He concluded that there were at least two races in Japan and two races in the U.S., and that the disease is less common on *Cucurbita* and *Citrullus* in Europe and in the Mediterranean. As a result, Palti concluded that precautions must be taken not to transfer cucurbits infected downy mildew from one region to the next and that varieties that are bred for resistance in one area must be retested in the region where they will be introduced. In addition, Palti (1974) discussed that the diversity in *P. cubensis* must not be attributed solely to diverse races and environmental and biotic conditions must also be considered because of the positive reactions of some crops under optimum conditions in countries where the disease is not found in the open field (Palti, 1974). Thomas et al. (1987) believed that "when host and pathogen are present, meteorological conditions determine if infection can occur, but the virulence of the pathogen population to the specific host encountered determines if it will occur".

From 1972-1974 Bains and Jhooty (1976) surveyed muskmelon-growing regions in India for other cucurbit crops infected with downy mildew. In addition, they inoculated different cucurbit hosts with an isolate of *P. cubensis* collected from muskmelon. They found that this isolate was not pathogenic on *Benincasa hispida* (ash gourd) and *Cucurbita moschata* (a type of squash). These two hosts were only slightly infected in the field.

In order to unify the research on this topic and solidify the theory on host range, Thomas et al. (1987) conducted a host range study in three countries, Japan, Israel and the U.S. Each collaborator challenged 26 cucurbit species with isolates from their respective country. The results of this test showed that the intensity of sporulation on all hosts was not equal, but that all plants within a host genotype reacted uniformly to each isolate. They also found that *Cucumis sativus* (cucumber) 'Sagamihanjiro' and *Cucumis melo* var. *reticulatus* (cantaloupe) 'Ananas Yokneam' were highly compatible with all *P. cubensis* isolates tested. The reaction of the remainder of cucurbit species with the isolates varied (Thomas et al., 1987).

Based on highly compatible reactions at the genus, species, and subspecies level of hosts, pathotype designations were proposed for *P. cubensis*. As a result, five pathotypes of the pathogen were described (Table 1.1). The length and width of sporangia and the length of sporangiophores were not significantly different among isolates (Thomas et al., 1987).

The data by Thomas et al. (1987) validated the existence of distinct physiological forms of *P. cubensis* that can be distinguished based on host genus, species, and subspecies specificities. Like Crute (1981), Thomas believed that referring to these forms as *forma speciales* is a misleading oversimplification. Evidence for this is the loss of virulence of an isolate of *P. cubensis* collected from *Cucurbita pepo*. Lesion development was poor and sporulation sparse when this isolate was cultured on *Cucumis melo* var. *reticulatus* for 61 generations and then re-inoculated on *Cucurbita* spp. Thomas et al. (1987) believed that the pathotype concept for this pathosystem was not presented as the entire, definitive picture of the physiological specialization of *P. cubensis* and that more isolates need to be tested in

more locations on a combined, international collaboration.

Because the studies conducted previously do not include European isolates, Lebeda in the Czech Republic has conducted numerous studies on this topic (Lebeda and Gadasová, 2002; Lebeda and Widerlechner, 2003; Lebeda and Urban, 2004a, 2004b). In 2002, Lebeda proposed a broader set of cucurbit differentials as an appropriate baseline for more research of the *P. cubensis* population structure (Table 1.2). Lebeda (2002) found that the European populations of *P. cubensis* are highly variable and generally do not conform to the model of Thomas et al. (1987), though he felt this was due to the new set of differentials used in his study.

In 2001, the only crop infected with *P. cubensis* in the Czech Republic was *Cucumis sativus* (cucumber). The twelve cucurbit crops were challenged by 42 isolates of *P. cubensis* collected from *C. sativus* in 2001. Lebeda described 34 different pathotypes and attributed the ability of isolates to infect cucurbit hosts that are not commonly cultivated in the Czech Republic (*Citrullus lanatus*, *Benincasa hispida*, *Lagenaria siceraria*) to favorable laboratory conditions (2004b).

In 2003 a new pathotype was described by Cohen et al. (2003) in Israel. Based on Thomas et al. (1987), pathotype 3 (compatible exclusively with *Cucumis sativus* and *Cucumis melo*) was the only known pathotype in Israel. In 2002, an outbreak of downy mildew occurred on *Cucurbita moschata*, and *Cucurbita pepo* var. *pepo*, in open field in Israel. Based on a host range study it was determined that this is a new pathotype that is able to infect *Cucumis sativus*, *C. melo* and *Cucurbita* spp., but not *Citrullus lanatus* (watermelon) (Cohen et al., 2003). Also, Cappelli and Buonaurio (2003) in Italy reported

that, contrary to previous studies, pathotype 5 (able to cause disease on squash) is present.

## SECTION 2 – THE CUCUMBER (*CUCUMIS SATIVUS* L.) AND THE CUCURBITACEAE (GOURD FAMILY)

**Cucurbitaceae: Their importance, origin and geographical distribution.** The Cucurbitaceae is one of the most important families of plants that supply man with edible fruit and valuable fibers. Though they are not nearly as important on a worldwide scale as the cereals and legumes, in the tropics, subtropics and milder portions of the temperate zones, their importance is extraordinary (Whitaker and Davis, 1962).

The Cucurbitaceae is comprised of about ninety genera and 750 species are cultivated(Whitaker and Davis, 1962). Only twelve of the 750 species (Sitterly, 1978). Beside tomato and onion, cucumber and melon may be the most widely cultivated vegetable species in the world (Pitrat et al., 1999).

The members of Cucurbitaceae are frost-sensitive and produce predominantly tendril-bearing vines. Ecologically, the family is dichotomous. Many genera include aggressive climbers that thrive in the humid tropics, while others are native to the arid regions of Africa and North America (Robinson and Decker-Walters, 1997). Only seven genera are common to both hemispheres (Whitaker and Davis, 1962).

***Cucumis sativus* L: origin and growth habits.** *Cucumis sativus* L., the garden cucumber, is of Asiatic origin. *C. sativus* var. *hardwickii,* a wild cucumber, was first found in the foothills of the Himalayas in Nepal (Robinson and Decker-Walters, 1997; Whitaker and Davis, 1962). The remains of cucumber found in Iran have been dated back to the third

millennium BC and cultivation of cucumber goes back at least 3000 years in India and 2000 years in China. Early travelers brought cucumber to Mediterranean countries 3000-4000 years ago. In the fourteenth century, cucumber plants were cultivated in the U.K. Portuguese explorers brought the plants to West Africa and Columbus is credited with bringing the cucumber to the New World, planting it in Haiti in 1494 (Robinson and Decker-Walters, 1997).

Most cucumber plants are indeterminate and produce a small trailing vine (1-3 m long), though cucumbers with compact plant habit have been developed for home growers and once-over machine harvesting. A single tendril develops at each leaf axil and trichomes occur on the stems and leaves. The leaves are triangularly ovate with 3 to 5 lobes (Robinson and Decker-Walters, 1997).

*C. sativus* was originally monoecious (both male and female flowers on same plant), but gynoecious (plants with only female flowers) and andromonoecious (plants with bisexual and male flowers) cultivars have been developed. In monoecious cultivars, female and male flowers are borne on different nodes with the female at the more distant nodes. Usually just a single female flower is found per node. Fruits are round to oblong or cylindrical and green at the edible stage. Apparent on the rind of the fruit are small warts and spines of trichome origin. Fruit flesh is typically white and crisp. Seeds are small, white and flat (Robinson and Decker-Walters, 1997).

Cucumbers are mainly used for culinary purposes, however there are a few non-food uses. Cucumbers may be used for health and beauty reasons in such products as perfumes, lotions, soaps and shampoos. Indigenous practitioners used the roots, leaves, stems and

seeds in mixtures used for medical purposes (Robinson and Decker-Walters, 1997).

Cucumbers are mostly used for food and can be used fresh or pickled. Fresh cucumbers, referred to as slicers, are most often found in salads, whereas picklers are fermented. Generally, picklers have smaller fruits, usually with more warts. After harvest, slicers are graded and waxed before going to market. Picklers are prepared for fresh pack (unpasteurized, refrigerated dills) and for brined and fermented products (Robinson and Decker-Walters, 1997). In 2006, approximately 65% of the cucumbers grown in the U.S. were for processing (Anonymous, 2008).

**Cucumber production in the United States and North Carolina.** According to the USDA QuickStats (Anonymous, 2006) there were 166,800 acres of cucumbers planted in 2006 for a total value of production of nearly 400 million USD. In North Carolina 18,100 acres, 7,000 fresh market and 11,100 processing, were planted for a total value of production just over 23.5 million USD. North Carolina is second to Michigan where 39,600 acres were planted in 2006 (5,600 for fresh market and 34,000 for processing).

**Downy mildew resistance in cucumber.** Breeding efforts in the 1940s led to the release of cultivar Palmetto in 1948 with resistance to downy mildew (Barnes, 1948; Van Vliet and Meysing, 1977). Barnes describes the performance of 'Palmetto' against downy mildew as not being immune, but as highly resistant. This resistance allowed 'Palmetto' to be grown in the fall without the use of fungicides. During the fall crop the disease is present throughout the season (Barnes, 1948). The resistance, referred to as "yellow lesion type", resulted in very few lesions and the yellow lesions were small and produced few sporangia. As a result, secondary infection was slowed and even though there was an early season

presence of the disease, no damage resulted (Epps and Barnes, 1952).

In 1950 and 1951, there was a sudden breakdown of resistance in 'Palmetto' in South Carolina. Plantings of Palmetto were severely diseased with downy mildew. Lesions resembled those of susceptible varieties and sporulation was profuse. Epps and Barnes hypothesized that this breakdown was caused by the presence of a new and more aggressive race of the pathogen (Epps and Barnes, 1952).

Barnes and Epps also discovered a new source of resistance (brown lesion type) in PI 197087 in the early 1950s. This line originally sparked interest due to its apparent immunity to anthracnose (*Colletotrichum lagenarium*). After inoculation with a "heavy spore suspension" of *P. cubensis*, PI 197087 produced irregularly shaped brown lesions that frequently appeared water-soaked then rapidly dried out. Under natural conditions the lesions remained small, circular and brown. These brown lesions dried more rapidly and infected plants were rated as moderately diseased in the early season. As the season progressed, the plants of PI 197087 were rated as highly resistant (Barnes and Epps, 1954). PI 197087 and cultivar Chinese Long are the sources of downy mildew resistance in current commercially available cultivars (Sitterly, 1972).

In 1966, cultivar Poinsett was released. Poinsett proved to be highly resistant to downy mildew. Early research on downy mildew resistance in cucumbers concluded that at least one single recessive gene, *dm*, controlled resistance in Poinsett (Van Vliet and Meysing, 1977). As cucurbit breeding progressed through the twentieth century, it was determined that several genes are actually involved in downy mildew resistance (Pierce and Wehner, 1990). Doruchowski and Lakowska-Ryk (1992) reported that three recessive genes, *dm-1*, *dm-2* and

*dm-3* control resistance in cucumber cultivar Wisconsin-4783. In addition, a single dominant gene, *Dm-3*, and two complementary genes, *Dm-1* and *Dm-2*, control susceptibility of cultivar SMR-18.

Wehner and Shetty (1997) released their findings of a late 1980's field screen of 881 cucumber cultigens for resistance to downy mildew. The cultigens were ranked by mean downy mildew rating. The nine most resistant cultigens were of US origin, one of them being Poinsett 76. It is interesting to note that some cultigens that were considered highly resistance in this test in North Carolina were reported as susceptible in other areas such as the Czech Republic. In addition, PI 197087 was only moderately resistant in the test (Wehner and Shetty, 1997).

During the last several decades the presence of downy mildew on cucumber was inconspicuous and did not result in obvious crop losses (Peterson et al., 2002). In 2004, the occurrence of the disease on cucumber changed dramatically. A new strain of the pathogen more aggressive than previous strains emerged and downy mildew-resistant cultivars were not sufficient to prevent severe economic losses. The disease ravaged cucumber-growing areas of North Carolina, Delaware, Maryland and Virginia in 2004 (Colucci et al., 2006; Holmes et al., 2006).

## SECTION 3 – FUNGICIDE RESISTANCE OF *PSEUDOPERONOSPORA CUBENSIS*

**Fungicides for the control of downy mildews.** Cultural practices and breeding for resistance are very important aspects of controlling downy mildew diseases; however, chemical control is the most successful measure in protecting crops (Gisi, 2002). The

systemic downy mildews caused by the Peronosporales, including *Pseudoperonospora cubensis*, were not affected by chemical control until modern, systemic compounds became available. These pathogens cause economically significant losses. In 1991, 21% of expenses for chemical disease control were allocated to downy mildew and late blight, 30% of this was applied to vegetable crops (Schwinn and Staub, 1995).

In 1996 the global fungicide market was estimated at 7.2 billion SFr (approx. 6.25 billion USD), of which 16.7% were chemicals to control downy mildews (Gisi, 2002). The largest percentage of downy mildew control was for grape downy mildew caused by *P. viticola* (54%) followed by the cucurbit downy mildew caused by *P. cubensis* (10%)(Gisi, 2002).

The most important chemical groups used for downy mildew control include phenylamides (mainly metalaxyl), dithiocarbamates (mainly mancozeb), cymoxanil, copper, chlorothalonil, fosetyl-Al, hymexazol, fentins, dimethomorph, propamocarb, fluazinam and phthalimides. Strobulurins are not included in this list as they were not available for commercial use until only a few years prior to this report (1992-1998) (Gisi, 2002). However, in 1999, sales of strobilurin and strobilurin related fungicides totaled approximately 620 million USD, representing over 10% of the global fungicide market. The strobilurin fungicide azoxystrobin was the leading privately developed fungicide worldwide with sales of 415 million USD (Bartlett et al., 2002; Heaney et al., 2000).

**Fungicide Resistance.** Fungicide resistance may be defined as the stable, inheritable adjustment by a fungus to a fungicide, resulting in a less than normal sensitivity to that fungicide. The term is used for strains of a sensitive fungal species, which have become,

usually by mutation, significantly less sensitive to the fungicide (Dekker, 1987).

Fungicide resistance has developed more slowly than resistance to human antibacterial antibiotics and insecticides. Eckert attributes this phenomenon to the fact that non-selective multi-site fungicides were not used until the mid-1960s. With the inundation of highly selective agricultural fungicides, including the phenylamides, organophosphates, dicarboximides and the sterol inhibitors, which target a specific site in a cell, fungicide resistance reports have accelerated (Eckert, 1988).

Plant pathogen resistance to fungicides can be defined as a combination of:

1. The presence of naturally occurring resistant individuals, initially at a very low frequency, originating from recurrent mutations conferring resistance;
2. The increase in frequency of resistant individuals over time (e.g., throughout season, from year to year) resulting in resistant subpopulations caused by the selection process of fungicide applications and through migration of resistant individuals.
3. A reduction of disease control compared to earlier results and to standard treatments with products of a different mode of action (Gisi et al., 2000).

The Fungicide Resistance Action Committee (FRAC) has categorized *Pseudoperonospora cubensis* as one of the pathogens with the highest risk of developing resistance to fungicides. Other pathogens in this list are *Penicillium* spp., *Mycosphaerella fijiensis*, *Botryotinia fuckeliana (Botrytis cinera)*, *Erisiphe (=Blumeria) graminis, Phytophthora infestans, Pyricularia* spp., *Sphaerotheca fuliginea* and related species and *Venturia* spp. (Russell, 2003).

**Mefenoxam and the phenylamide fungicides.** The phenylamide fungicides can be separated into three groups: the acylanines (metalaxyl, furaxyl and benalaxyl), butyrolactones (ofurace and cyprofuram) and oxazolidinones (oxadixyl) (Davidse, 1995). The phenylamide fungicides hinder ribosomal RNA (rRNA) synthesis, specifically the RNA polymerases (Davidse, 1995; Gisi, 2000, 2002; Schwinn and Staub, 1995). These fungicides act as preventative and curative treatments and are effective against hyphal growth, haustorium and spore formation. They also demonstrate eradicative and anti-sporulant activity, but do not seem to be effective on early developmental stages of disease such as zoospore release, germination or penetration. Because of the many ribosomes that are supporting early growth within the spore, phenylamides are most effective when applied after germination when RNA synthesis is in effect (Schwinn and Staub, 1995).

The phenylamides are effective against pathogens in the Peronosporales. Metalaxyl has the widest range of activity and effects on the different genera of organisms within the order. Schwinn summarized the major biological qualities of metalaxyl as high inherent fungitoxicity, protective and curative activity against all Peronosporales, rapid uptake, high acropetal systemicity, leading to protection of new growth, good persistence in plant tissue allowing extended spray intervals, control of systemic seed-and soil-borne diseases, weak on senescent tissue (Schwinn and Staub 1995). Mefenoxam, metalaxyl-M or the (R)-enantiomer of metalaxyl, was introduced to market as a foliar fungicide, soil fungicide and seed treatment in 1996 as ‘Ridomil Gold’ or ‘Apron XL’. This fungicide was declared a “Reduced Risk” product by the EPA meaning that it poses a low risk to harm humans or the environment and low toxicity to non-target organisms. In addition, the new form of

metalaxyl allows for a reduction in rate of fungicide without loss of disease control (Nuninger et al., 1996). Gisi identifies mefenoxam as the most systemic phenylamide and calls it the most active, versatile, and broadly used molecule within phenylamides against a wide range of foliar diseases (2002).

Phenylamides are absorbed by roots and foliage quickly and move acropetally or apoplastically (xylem-mobile by means of transpiration stream) within leaves and from leaf to leaf (Jacob and St. Neumann, 1987). Metalaxyl and mefenoxam can spread within plants and over short distances in the canopy through vapor phase. Because of this systemicity, phenylamides can protect untreated and young plant parts. The best control practice with the phenylamides involves early season application as apoplastic transport is lessened in senescing tissue and as a result translocation is reduced in a maturing crop. These chemicals supply long lasting disease control, however the threat of resistant subpopulations limits application intervals (10-14 days maximum) and number of sprays per season (2 to 4) (Gisi, 2002).

The mode of resistance for the phenylamide fungicides is not completely understood. The responsible resistance gene(s) and loci of mutations in the genome have not been identified. It is believed that resistance is a monogenic trait. This information results from experiments with *Phytophthora infestans*. Known metalaxyl-sensitive and a metalaxyl-resistant parent were crossed and the result was predominately intermediate progeny (F1). Crosses between the F1 resulted in a 1 sensitive: 2 intermediate: 1 resistant ratio. This segregation pattern reflects monogenic resistance. Shattock also suggests that the resistance is due to a single, incompletely dominant gene (Shattock, 1988; Gisi et al., 2000).

It is known that phenylamides reduce the incorporation of uridine into rRNA (20–60% of the untreated control, depending on the fungal species). However, even at concentrations that fully suppress fungal growth, inhibition of uridine incorporation is incomplete, suggesting that only a part of the cellular RNA synthesis is sensitive to phenylamides. When phenylamides are applied to phenylamide-resistant isolates, this process remains completely unaffected (Davidse, 1988; Gisi and Cohen, 1996).

*P. cubensis* was the first pathogen that was identified as having resistance to metalaxyl. Metalaxyl was first reported by Urech et al. (1977) and was introduced into the market as a single product without any anti-resistance measures (Urech, 1988). Resistant strains of *P. cubensis* were first identified in greenhouses in Israel in 1979 (Reuveni et al., 1980). Since this initial report, resistant isolates have been identified in other countries including Greece (Georgopoulos and Grigoriu, 1981), Italy (D'Ercole and Nipoti, 1985), Russia (Grin'Ko, 1992), Australia (O'Brien and Weinart, 1995) and in an abstract in the U.S. (Moss, 1987). Resistance to metalaxyl has also been identified in other oomycete pathogens including *P. infestans* (Deahl et al., 1993; Gisi and Cohen, 1996; Daayf and Platt, 1999; Shattock, 1988), *Pythium* spp. (Sanders, 1984), *Bremia lactucae* (Crute, 1987) and *Phytophthora capsici* (Parra and Ristaino, 2001).

**The strobilurin fungicides**. The strobilurin fungicides were derived from the antibiotics that are produced naturally in the wood-rotting Basidiomycete fungi, *Oudemansiella mucida* (Schrad. ex. Fr.) Hoehn and *Strobilurus tenacellus* (Pers. ex. Fr.) Singer (Anke and Oberwinkler, 1977; Bartlett, 1977). Two antifungal antibiotics were isolated from the mycelium of *Strobilurus tenacellus* strain No. 21602, a small agaric

(mushroom) forming fungus that inhabits decaying cones of *Pinus sylvestris*. These antibiotics, known as strobilurin A and B, are active against yeast and filamentous fungi (Anke and Oberwinkler, 1977). The two strobilurins differ from each other in their substituents at the benzyl ring (Becker et al., 1981).

These fungicides were originally called moa (E-β-methoxyacrylate)-inhibitors (Becker, 1981). Today this group of fungicides is also known as STAR, strobilurin-type action and resistance group fungicides, QoIs, quinone outside respiration inhibitors or the strobilurins. QoIs inhibit electron transport in mitochondrial respiration by binding to the Qo site of the cytochrome bc1 enzyme complex (complex III, ubiquinol-cytochrome *c* oxidoreductase). Cytochrome bc1 complex is located in the inner mitochondrial membrane of fungi and consists of cytochrome b, cytochrome c and the Rieske iron-sulphur protein (ISP). When the inhibitor binds it halts electron transfer between cytochrome b and cytochrome c1 and disrupts the energy cycle as ATP production is stopped, resulting in the inhibition of fungal growth (Bartlett, 2002; Gisi et al., 2000).

The QoIs, azoxystrobin and kresoxim-methyl, were first commercialized in 1996. That year they were introduced to the European market for the control of wheat powdery mildew (*Erysiphe graminis* f. sp. *tritici)*. Field resistance to strobilurin fungicides was first reported in wheat powdery mildew, in northern Germany in May of 1998 (Heaney et al., 2000). In Japan kresoxim-methyl and azoxystrobin were released commercially in 1997 and 1998, respectively. Although most cucumber growers followed manufacturers usage recommendations to control powdery mildew (*Podosphaera* spp. = *Sphaerotheca* spp.), reduced efficacy was frequently reported in southern Japan. In 1999, reduced efficacy of the

strobilurin fungicides for cucumber downy mildew was reported (Heaney et al., 2000; Ishii et al., 2001). In 2000, twenty-eight populations of *P. cubensis* in Mexico were sensitive to QoI-STAR fungicides, however resistant populations were developed at full field rates (Heaney et al., 2000).

Resistance to the QoI fungicides is known to be a qualitative type of resistance. Qualitative resistance is described as resistant reactions that can be placed in distinct categories, usually conferred by one or few genes (D'Arcy et al., 2001). Because of the site-specific nature of the strobilurin fungicides, they generally possess a high risk of resistance development if resistant isolates are not impaired in their ability to survive and multiply in the agricultural environment (Ishii et al., 2001). It is believed that the source of resistance in most pathogens to the QoIs is a single-point mutation leading to an amino acid change (glycine to alanine) (Gisi et al., 2000). In contrast, it is believed that *Venturia inaequalis* probably carries a mutation conferring resistance in another site in the genome due to the absence of the G143A mutation in resistant isolates (Gisi et al., 2000; Olaya et al., 1998).

The rapid evolution of resistance to the strobilurin fungicides was not anticipated based on the initial studies conducted on *Saccharomyces cerevisiae*. The evidence available from *Erysiphe graminis* f. sp. *tritici* and *Sphaerotheca fulginea* suggest that in these cases resistance evolves by a disruptive selection involving an increase in frequency in the population of a resistance allele arising from a mutation (G143A) of the cytochrome b gene. This mutation confers little noticeable fitness penalty and can spread quickly in large population sizes and efficient dispersal mechanisms. However, this may not always be the case. In other pathosystems, resistance seems to be a local phenomenon even when

pathogens that have large population sizes and efficient dispersal mechanisms (*Mycosphaerella graminicola* and *Uncinula necator*). The G143A mutation itself might not always have the same influence on the fitness of the individual carrying it either. In *Plasmopara viticola*, there is evidence that the mutation results in a fitness penalty even under optimum growth conditions (Heaney et al., 2000).

Molecular assays are available for detection of the G143A mutation, however biological assays should also be completed in order to validate the findings (Gisi et al., 2000; Ishii et al., 2001). In 2001, Ishii et al. characterized strobilurin resistance in *P. cubensis* using molecular techniques. A polymerase chain reaction was used to amplify fragments of the cytochrome b gene from resistant and sensitive isolates of the pathogen. The sequences were then analyzed to elucidate the molecular mechanism of resistance. The single point mutation (GGT to GCT), resulting in a substitution of glycine to alanine at position 143 (G143A) was found in the resistant isolate of *P. cubensis* (Ishii et al., 2001).

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**Table 1.1.** Pathotype designations based on *Pseudoperonospora cubensis* and host compatibility (Thomas et al., 1987).

| | Pathotype | | | | |
|---|---|---|---|---|---|
| Host | 1 | 2 | 3 | 4 | 5 |
| *Cucumis sativus* | +[a] | + | + | + | + |
| *C. melo* var. *reticulatus* | + | + | + | + | + |
| *C. melo* var. *conomon* | − | + | + | + | + |
| *C. melo* var. *acidulus* | − | − | + | + | + |
| *Citrullus lanatus* | − | − | − | + | + |
| *Cucurbita* spp. | − | − | − | − | + |

[a]+ Highly compatible host interaction, -- incompatible or very slightly compatible host-pathogen interaction

**Table 1.2.** Cucurbitaceae differential set for the determination of pathogenic variability in *Pseudoperonospora cubensis* (from Lebeda, A. and Gadasová, V., 2002).

| No. | Taxon | Cultivar name | Country of origin |
|---|---|---|---|
| 1 | *Cucumis sativus* | Marketer 430 | USA |
| 2 | *C. melo* subsp. *melo* | Ananas Yokneam | Israel |
| 3 | *C. melo* var. *conomon* | Baj-Gua | Japan |
| 4 | *C. melo* var. *acidulous* | | Myanmar |
| 5 | *Cucurbita pepo* var. *pepo* | Dolmalik | Turkey |
| 6 | *Cucurbita pepo* var. *texana* | NA | USA |
| 7 | *C. pepo* var. *fraterna* | NA | Mexico |
| 8 | *C. maxima* | Goliáš | Czechoslovakia |
| 9 | *Citrullus lanatus* | Malali | Israel |
| 10 | *Benincasa hispida* | NA | USA |
| 11 | *Luffa cylindrica* | NA | ? |
| 12 | *Lagenaria siceraria* | NA | ? |

NA = Not available; ? = Unknown

# CHAPTER 2 – HOST RANGE OF *PSEUDOPERONOSPORA CUBENSIS* IN THE EASTERN UNITED STATES


## ABSTRACT

After four decades of successful control with resistant cultivars, downy mildew (causal agent *Pseudoperonospora cubensis*) re-emerged as an important disease of cucumber (*Cucumis sativus* L.) in the eastern United States (US) in 2004. Because of the increased severity on cucumber, it was hypothesized that of the new *P. cubensis* strain(s) was a race of the pathogen or had a different host range than previously described isolates in the US. Leaf disks of 12 cucurbit taxa from six genera (*Cucumis*, *Cucurbita*, *Citrullus*, *Benincasa*, *Luffa* and *Lagenaria*) were inoculated with 30 *P. cubensis* isolates collected from 2004 through 2007. Two isolates collected prior to the 2004 epidemic also were used to determine their host range and for comparison to isolates collected after 2004. *Cucumis* was the most susceptible genus and the least susceptible differentials were *Citrullus lanatus*, *Lagenaria siceraria*, *Luffa cylindrica*, *Benincasa hispida* and *Cucurbita pepo* var. *pepo*. Natural populations of the pathogen were tested for their ability to cause disease on the host differentials in field experiments in Florida, South Carolina, Georgia, North Carolina, Delaware, Maryland, New York, Michigan and Ohio. *Cucumis* and *Cucurbita* were the most susceptible genera and *Benincasa hispida* and *Luffa cylindrica* were the least susceptible differentials across all locations. A susceptible and a resistant commercially available cucumber cultivar were inoculated with 11 isolates of *P. cubensis*. The isolates collected between 2004 and 2007 caused equally abundant sporulation on both cultivars and the

historic isolates resulted in more sporulation on the susceptible cultivar. Overall, current populations of *P. cubensis* were more diverse than expected and all commercially cultivated cucurbits are at risk for infection.

## INTRODUCTION

Downy mildew of cucurbits, caused by *Pseudoperonospora cubensis* (Berk. & Curt.) Rostov. is a devastating disease worldwide (Palti and Cohen, 1980). The disease can be found in temperate areas, such as the Americas, Europe, Japan, Australia and South Africa, tropical regions internationally and some semi-arid regions, such as the Middle East. Downy mildew affects cucurbits in the field as well as those under plastic or in greenhouses and is especially damaging in those areas that possess the warm, humid climate in which the pathogen thrives (Cohen, 1981; Holmes et al., 2004; Palti and Cohen, 1980).

*P. cubensis* is characterized by variation in pathogenicity, specificity and host-parasite interactions (Lebeda and Widerlechner, 2003). Palti and Cohen identified 40 species in 20 genera within the Cucurbitaceae that are infected by *P. cubensis* (1980). More recently, downy mildew has been identified on 60 species of cucurbits (Lebeda, 1999). *P. cubensis* is known to exhibit host specialization among these hosts (Doran, 1932; Ellis, 1951; Hughes and Van Haltern, 1952; Palti, 1974; Palti and Cohen, 1980, Thomas et al., 1987) and divergences in host range have been reported within and among countries (Thomas and Jourdain, 1992; Shetty et al., 2002). Efforts to identify host range of *P. cubensis* are important to the management of downy mildew, especially for breeding purposes. Host range of *P. cubensis* has been studied in India (Bains and Sharma, 1986), Israel (Cohen et al.,

2003; Thomas et al., 1987), Japan (Thomas et al., 1987), the United States (US) (Doran, 1932; Hughes and Val Haltern, 1952; Thomas et al., 1987) and the Czech Republic (Lebeda, 1991, 1999; Lebeda and Gadasová, 2002; Lebeda and Urban, 2004a, 2004b).

Thomas et al. (1987) studied physiological specialization of *P. cubensis* on a global basis. Isolates of the pathogen were investigated in Israel, Japan and the US for compatibility to 26 cucurbit cultivars representing 13 species and subspecies and seven genera. They condensed their results and those of previous host range experiments to identify five pathotypes in *P. cubensis*. In the US, two isolates were assayed and two pathotypes, 4 and 5, were described. Pathotype 4, which is compatible with *Cucumis sativus*, *C. melo* subsp. *melo* (syn. *C. melo* var. *reticulatus*), *C. melo* var. *conomon*, *C. melo* var. *acidulus* and *Citrullus lanatus,* was based on an isolate collected from *C. melo* subsp. *melo.* Pathotype 5*,* which is compatible with the same cucurbit hosts as Pathotype 4, but also attacks *Cucurbita* spp., was based on an isolate collected from *Cucurbita pepo* (Thomas et al., 1987).

Recently, Lebeda has conducted studies on the host range of *P. cubensis* populations in the Czech Republic (Lebeda and Gadasová, 2002; Lebeda and Urban, 2004a, 2004b; Lebeda and Widerlechner, 2003) and he has proposed a broader set of cucurbit host differentials. Similar to the work of Thomas et al. (1987), these 12 cucurbit host differentials were chosen to unify the global effort of characterizing populations of *P. cubensis* at the level of pathotype (Lebeda and Gadasová 2002; Lebeda and Urban, 2004a, 2004b).

In the eastern US, downy mildew is an annual problem on cucurbits in the late summer and fall. Cultivar resistance in *Cucumis sativus* L. (cucumber) has controlled the

disease without the use of fungicides since the 1960s (Holmes et al., 2006). However, in 2004 the cucumber crop in North Carolina, Virginia, Delaware, Maryland and New Jersey was devastated by downy mildew resulting in a 40% region-wide loss of the crop (Holmes et al., 2006). The sudden increased severity of *P. cubensis* to cucumber suggested the possibility that a new race of the pathogen might have been introduced into the US. The purpose of this study was to investigate the host range of 30 isolates of *P. cubensis* collected from 2004 through 2007 in the eastern US using the new set of cucurbit host differentials proposed by Lebeda et al. (2002 and 2003). The host range pattern of these isolates were compared to two historic isolates that were collected prior to the 2004 epidemic, including the isolate characterized as Pathotype 4 by Thomas et al. (1987). In addition, the 12-cucurbit hosts were planted in eight US states to assess host range patterns in wild populations. Finally, a susceptible and resistant variety of commercial cucumber was challenged by 11 *P. cubensis* isolates to determine if susceptibility of cucumber cultivars to downy mildew has changed in the US.

## MATERIALS AND METHODS

**Plant materials**. Methods used in the host range assay were based on those used by Lebeda and Gadasová (2002). Four *Cucumis* spp., four *Cucurbita* spp., *Citrullus lanatus*, *Benincasa hispida*, *Luffa cylindrica* and *Lagenaria siceraria* (Table 2.1) were grown in growth chambers under controlled conditions. Plants were grown in 152 mm diameter pots in a standard mix of steam-sterilized, #16 construction grade gravel and commercial peat-lite mixture (Redi Earth, W.R. Grace Company, Cambridge, MA), composed of peat moss and

vermiculite. Plants were watered twice daily with de-ionized water and fertilized as needed. Plants were placed at 22°C, night and 26°C, day with a 12 h photoperiod until leaves were harvested for the host range moist box assays.

***Pseudoperonospora cubensis* isolates**. Isolates of *P. cubensis* were collected from commercial production areas in the eastern half of the US from 2004 through 2007. Sixty isolates were tested and 32 isolates were successfully assayed in moist boxes for host range. The remaining isolates did not establish infection following re-inoculation on host plants and therefore could not be assayed. Of the 32 isolates assayed, 19 isolates were collected from cucumber, three from other *Cucumis* spp., six from *Cucurbita* spp. and three from *Citrullus lanatus*. One historic isolate obtained from Charleston, South Carolina in autumn of 1982 that was collected from *Cucumis melo* subsp. *melo* and is the isolate designated 'C' from Thomas et al. (1987) was used. Another isolate was obtained that has unknown history (location, year and host) but is known to have been collected prior to the 2004 epidemic. It is sensitive to fungicides since and is used in standard fungicide screening assays at DuPont (Newark, DE) (R. Geddens, *personal communication*).

To increase inoculum, sporangia were dislodged from a single, infected leaf using a pressurized narrow stream of $dH_2O$ produced by a Preval Complete Spray Unit (Precision Valve Corporation, Yonkers, NY). The suspension was adjusted to $10^4$ sporangia $ml^{-1}$ and misted onto a highly susceptible cucumber, 'Coolgreen'. Two ml of inoculum was applied to adaxial and abaxial sides of the true leaves and the cotyledons. Plants were placed in moist chambers that were constructed using plastic sheeting and humidifiers (ReliOn Ultrasonic humidifier model H-0695-0, Walmart) and placed at 18°C in darkness. After 24 h, plants

were removed from moist chambers and incubated at 18°C, night and 21°C, day with a 12 h photoperiod. Seven days after inoculation, infected cotyledons or whole leaves were collected from the plants and sporulation was induced by placing the leaves in moist boxes placed at 18°C darkness. After 24 h, leaves were removed and inoculum was prepared (method below) for host range assays.

**Long-term isolate storage and revival.** *P. cubensis* isolates were collected from infected plants in the field and placed in plastic bags. Isolates were stored in the refrigerator (2°C) for up to 5 days after collection before re-inoculating on host plants. Sporulation was induced and plants were inoculated on susceptible cucumber 'Coolgreen' as previously described. Seven days after inoculation, leaves with prolific sporulation, induced by 20 h of darkness at 18°C and high humidity (>80%), were removed from infected plants. The leaves were allowed to dry slightly so visible moisture was no longer present, but not long enough for sporangia liberation (approximately 10 min). The leaves were placed in plastic Petri dishes (100 × 15 mm). The dishes were wrapped in Parafilm and placed in a -20°C freezer for 24 h. After 24 h the cultures were placed in -80°C for long-term storage. Stored isolates remain viable in -80°C for at least six months. We have successfully re-inoculated host plants with isolates stored for 12 months at -80°C. For revival of isolates, cultures were removed from -80°C and allowed to thaw on the laboratory bench. Inoculum was prepared as previously described.

**Host range assays.** Filter paper (Fisherbrand 20.5 cm diameter, Fisher Scientific Pittsburgh, PA) was placed on the bottom of plastic boxes and moistened with $dH_2O$. The oldest leaves were harvested from mature host plants that were 6 to 8 weeks old. Leaf disks

were cut using an 18 mm cork borer. Five leaf disks of each of 12 host differentials were placed abaxial side up on the moist filter paper in the plastic boxes.

Inoculum was prepared by dislodging sporangia of *P. cubensis* from a single, infected cucumber leaf using a pressurized narrow stream of $dH_2O$ produced by the Preval Complete Spray Unit. The concentration of the spore solution was adjusted to $10^4$ sporangia $ml^{-1}$. Each of the four of leaf disks for all 12 differentials were inoculated with four- 10µl droplets of the sporangia suspension. The fifth leaf disk served as a non-inoculated control. Post-inoculation, the plastic boxes were covered and placed in an incubator at 18°C darkness for 24 h. To reduce bacterial contamination, 24 h post-inoculation the sporangial suspension droplet was removed with a pipette. The plastic moist boxes were returned to the incubator programmed for 18°C, night and 21°C, day with a 12 h photoperiod. The boxes were removed from the incubator and examined daily for sporulation from day 4 through 14 using a dissecting microscope. Sporulation was rated qualitatively as compatible (able to sporulate) or incompatible (not able to sporulate). For compatible reactions percent leaf area covered with sporulation was recorded.

**Measuring of sporangia and sporangiophores.** Sporangia and sporangiophores of four *P. cubensis* isolates (Erie County, OH cucumber 2007; Duplin County, NC cucumber 2007; Sampson County, NC watermelon 2005 and the isolate of unknown history collected prior to the 2004 epidemic) were measured. For each isolate the length and width of 25 sporangia and length and width of 10 sporangiophores were measured using a light microscope and ocular micrometer. Mounts were made with water or glycerin. Notes on color, branching and unusual characteristics were recorded.

**Host range field assays.** The 12 host differentials (Table 2.1) were grown using commonly utilized cultural practices (i.e. greenhouse trays, potting mix and nutrient regime) in each collaborating location. When the plants had two true leaves, five healthy plants of each cucurbit differential were transplanted into 11 field plots in eight US states, Georgia (GA), South Carolina (SC), Delaware (DE), New York (NY), Ohio (OH) and two plots each in Florida (FL), North Carolina (NC) and Michigan (MI). Transplants were arranged in a three plot by four-plot grid using a 0.6-m within-row spacing. The differentials were randomized within the grid. In NY, plots consisted of three- 3.05-m rows with two plants each. Plants were at 0.6-m spacing within rows and rows were at 1.7-m spacing. Plots were separated by 9.14 m in the row. Plots were inspected at least once weekly for the presence of symptoms and signs. The presence of the pathogen was verified by visualizing sporangiophores and sporangia using a 20× hand lens or microscope. Quinoxyfen (Quintec, Dow Agro Sciences LLC, Indianapolis, IN) was applied if powdery mildew (causal agent *Podosphaera xanthii*) posed a threat to the plantings. This product has no efficacy against downy mildew.

**Cucumber cultivar comparison.** In order to investigate the susceptibility of commercial cucumber, resistant cucumber 'Poinsett' and susceptible cucumber 'Coolgreen' were inoculated with 11 isolates of *P. cubensis*. Leaf disks were produced using the methods previously described. Leaf disks (20 mm) were arranged in a plastic box, two disks of each cultivar were randomized within four repetitions. In addition, two disks of each cultivar served as an un-inoculated control. Inoculum was prepared and the leaf disks were inoculated as previously described. The boxes were removed from the incubator and

examined for sporulation 3 days after inoculation to determine if there was a difference in timing of onset of disease development. A final rating was taken 7 days after inoculation. This final rating included a combination of percent sporulation (0 = no infection detected, 1 = 1-10% of leaf disk area covered with sporulation, 2 = 11-50% of leaf disk area covered with sporulation, 3 = greater than 50% of leaf disk area covered with sporulation) and leaf color (G = green, C = chlorotic, N = necrotic) for all eight disks of each cultivar.

## RESULTS

**Host range assays.** Out of the 32 isolates of *P. cubensis* tested there were 32 different host range patterns based on sporulation intensity (Table 2.2). All 32 isolates were highly compatible with *C. sativus* (cucumber) and *C. melo* subsp. *melo* (cantaloupe). Other broadly susceptible hosts were *C. melo* var. *conomon* (honeydew melon), *C. melo* var. *acidulus* (bitter melon), *C. pepo* var. *texana* (squash), *C. pepo* subsp. *fraterna* (squash) and *C. maxima* (winter squash) (Table 2.2).

Only four *P. cubensis* isolates were highly compatible with *C. lanatus* (watermelon) and five isolates were compatible with *L. cylindrica*. Seven isolates were highly compatible with both *B. hispida* and *L. siceraria.*

The most striking result was the isolate collected from squash (*Cucurbita* spp.) in Johnston County, NC in 2007 that was highly compatible with all 12 cucurbit hosts. Two isolates were highly compatible with only three of the 12-cucurbit hosts. One of these isolates was collected from cucumber in Central Oceana County, MI, 2005 and the historic isolate collected from cantaloupe in Charleston County, SC in 1982 caused highly

compatible reactions with three *Cucumis* spp. An isolate collected from *C. sativus* in Wicomico County, MD 2007, gave a highly compatible reaction to two *Cucumis* spp. and on *C. pepo* var. *texana*.

**Measuring of sporangia and sporangiophores.** The isolate collected from watermelon was had significantly larger sporangia and sporangiophores than the other isolates (data not presented).

**Host range field assays.** Field results were rated qualitatively as yes (exhibiting symptoms and signs) or no (not exhibiting symptoms or signs) (Table 2.3). Cucumber was the first host to become infected at 10 of the 11 sites. All four *Cucumis* spp. became infected at nine of the 12 sites.

The variation in this pathosystem was exhibited in the difference of hosts infected among the 11 field plots. For example in Henderson County, NC only two hosts, cucumber and *C. melo* var. *acidulus* became infected. In the Charleston County, SC plot, all 12 hosts eventually became infected. Only the field plots in Wayne County, OH and site two in Collier County, FL showed identical host range patterns.

**Cucumber cultivar comparison.** Most isolates collected from cucumber did not show a discernible difference in percentage of leaf disk covered with sporulation or with density of sporangia (Table 2.4). A difference was seen in chlorosis and necrosis of the leaf disks. 'Coolgreen' became chlorotic and necrotic more rapidly after inoculation than did 'Poinsett' which never became necrotic. All cucumber isolates collected after 2004 had a 3-N (greater than 50% leaf disk area covered with sporulation and tissue necrotic) rating for 'Coolgreen' and 3-C (greater than 50% leaf disk area covered with sporulation and tissue

chlorotic) for ‘Poinsett’. There was no delay in the appearance of symptoms or sporulation seen between the two cultivars.

The isolate collected from squash in 2004 from Johnston County, NC resulted in less sporulation on ‘Poinsett’ than on ‘Coolgreen’ and ‘Poinsett’ maintained its green color. A difference in susceptibility of the two cucumber cultivars to the isolate collected in 1982 was also demonstrated. More leaf disk area was covered with dense sporulation on ‘Coolgreen’ than what was apparent on ‘Poinsett’. In addition, ‘Coolgreen’ became chlorotic more rapidly than ‘Poinsett’. The DuPont isolate responded similarly to the isolates collected from cucumber after 2004.

Un-inoculated ‘Coolgreen’ leaf disks became chlorotic before those of ‘Poinsett’, beginning five days after they were cut. Poinsett leaf disks maintained a healthy, green color for at least seven days after they were cut.

## DISCUSSION

Prior to 2004, cucurbit downy mildew was an annual disease problem for squash and watermelon in the eastern US. In contrast, due to the use of commercially available resistant cultivars, downy mildew was an uncommon presence on cucumber. In 2004, downy mildew re-emerged as one of the most important foliar diseases on cucumber in the eastern US (Holmes et al., 2006). Because of this shift in virulence on previously resistant cultivars of cucumber, it was hypothesized that a new race of *P. cubensis* is present in the US. Upon investigation of 32 isolates of *P. cubensis*, we determined that populations of the pathogen are more diverse than previously described with respect to their host range. Results from

field experiments conducted throughout the eastern US suggest that these diverse populations are present in many areas and can infect a range of cucurbit hosts.

In the US, Doran (1932), Hughes and Van Haltern (1952) and Thomas et al. (1987) have studied host range of *P. cubensis*. In 1932, Doran explored the relative susceptibility of 19 cucurbit hosts in Massachusetts. Field plots of cucumber, melons, squash, pumpkin, watermelon and gourds were artificially inoculated with a sporangial suspension of *P. cubensis* collected from cucumber. Doran found that downy mildew was severe on cucumber, moderate on muskmelon, mild on watermelon and absent on squash and pumpkin (Doran, 1932). In 1952, Hughes and Van Haltern conducted a host range experiment with two isolates of *P. cubensis*, one collected from cucumber and the other from watermelon. The isolate collected from cucumber caused severe damage on cucumber and cantaloupe, though the disease developed slower on cantaloupe, and watermelon exhibited only mild symptoms (e.g., lesions were few and atypical and did not sporulate). The isolate from watermelon caused moderately severe disease on cucumber and cantaloupe and severe damage on watermelon (Hughes and Van Haltern. 1952).

Similarly, a variety of cucurbit hosts were artificially inoculated with isolates of *P. cubensis*. Leaf disks of 12 differentials were inoculated with 30 isolates collected between 2004 and 2007 from various cucurbit crops in downy mildew affected areas from the eastern half of the US, as well as two isolates collected prior to the 2004 epidemic. Similar to the results from the previous studies in the US (Doran, 1932; Hughes and Van Haltern, 1952; Thomas et al., 1987), all of the isolates tested were highly compatible with cucumber and *C. melo* subsp. *melo* (cantaloupe). The remaining *Cucumis* spp. were also highly compatible

with a majority of the isolates tested. Twenty-four out of the 32 isolates assayed (75%) were compatible with *C. melo* var. *conomon* (honeydew melon) and 27 out of 32 (84%) were highly compatible with *C. melo* var. *acidulous* (bitter melon). *Cucumis* has been reported as most highly susceptible genus in the Czech Republic (Lebeda and Gadasová, 2002; Lebeda and Urban, 2004a, 2004b).

Of the *P. cubensis* isolates assayed, 81% and 72% were highly compatible with squash differentials, *Cucurbita pepo* var. *texana* and *C. pepo* subsp. *fraterna*, respectively. Similar to the findings in the Czech Republic (Lebeda, 2002), *C. pepo* var. *pepo* was the most resistant *Cucurbita* genotype, forming highly compatible reactions with only seven of the 32 isolates. Five of the seven isolates were originally collected from *Cucurbita* spp. and the other two isolates were collected from cucumber and melon. We have demonstrated that isolates collected from *Cucumis* spp. are less likely to cause infection on *Cucurbita* spp., but nearly all isolates form highly compatible reactions with cucumber and melon. These results are similar to the results from Thomas et al. (1987) who distinguished the US *P. cubensis* populations based the presence of highly compatible or incompatible reactions to *Cucurbita* spp. (1987). Many of the isolates collected from squash did not establish infection upon re-inoculation in the current study and therefore could not be assayed. To further investigate the differences of host range between squash and cucumber isolates in the US, future studies should include *P. cubensis* isolates collected from a more diverse collection of cucurbit hosts, especially *Cucurbita* spp.

Because watermelon is a typical host for *P. cubensis* in the eastern US, we expected many of the isolates to have a highly compatible reaction to watermelon. Sixteen isolates

showed some level of compatibility to watermelon, but only four were highly compatible. In fact, two isolates collected from watermelon did not even result in highly compatible reactions to the watermelon differential. *P. cubensis* infected watermelon does not produce abundant sporulation compared to cucumber or melon, especially in necrotic lesions (Cohen, 1981; Thomas, 1970). When infection developed on watermelon disks, necrotic lesions were produced and few sporangiophores were typically found. This was the case with the isolate collected from watermelon from Sampson County, NC in 2005. No symptoms or necrotic lesions were found on the watermelon leaf disks inoculated with the isolate collected from watermelon from Charleston County, SC in 2005, however. Therefore, rating *P. cubensis* for compatibility or incompatibility to watermelon using the leaf disk method may need to be adjusted for the presence of lesions in addition to sporulation intensity.

The original isolate classified as Pathotype 4, collected from 1982 was highly compatible with watermelon, cucumber and melon in the original assay (Thomas et al., 1987). In our test we did not see a highly compatible reaction with watermelon. This may be due to the inefficient rating of the leaf disk method or a loss of virulence on that host. Loss of virulence is an occurrence first described by Thomas et al. (1987) who demonstrated that when an isolate collected from *Cucurbita pepo* was maintained on *C. melo* subsp. *melo* for 61 generations that upon re-inoculation on *Cucurbita* spp. poor lesion development and sporulation resulted. The historic isolate has been maintained on its original host *C. melo* subsp. *melo* 'Ananas Yokneam' since the 1980's and, to our knowledge, has not been inoculated on a variety of cucurbit hosts. Loss of virulence highlights the importance of keeping the integrity of each *P. cubensis* isolate in a collection by periodically re-inoculating

plants of the host species that the isolate was collected from and testing isolates within a minimum number of generations after collection.

Previous studies (Bains and Sharma, 1986; Thomas et al., 1987; Cohen et al. 2003) did not find statistically significant differences in dimensions of sporangia and sporangiophores. Though the there were significant differences of the size of sporangia and sporangiophores of the isolate collected from watermelon, our sample size was small. Future studies should look at a larger number of *P. cubensis* isolates to see if there are differences in the dimensions of sporangia and sporangiophores from isolates collected on different hosts.

The field experiments allowed us to examine the dynamics of the local population(s) of *P. cubensis*. Similar to the leaf disk assays, cucumber was infected at every field site and was the first host to exhibit downy mildew symptoms and signs of the pathogen at 10 out of the 11 sites. The remaining *Cucumis* spp. were frequently attacked as well, becoming infected at nine out of the 11 sites. *Cucurbita pepo* var. *texana* was the most frequently affected squash, though the *C. pepo* var. *pepo* and *C. pepo* subsp. *fraterna* did become infected at seven out of the 11 of the sites.

Similar to the leaf disk assay results, watermelon did not become infected as readily as other hosts. Watermelon became infected at only five of the 11 sites. However, these trials were rated qualitatively (yes/no) for the presence of symptoms and signs and the severity of downy mildew was not recorded at every site. For instance, though watermelon in the Johnston County, NC plot was rated as positive to downy mildew infection, only a few lesions were found on one leaf of one watermelon plant. Additionally, watermelon was one of the last hosts to become infected at this location. The lack of infection of watermelon in

the field experiments suggests that the cultivar used may not be representative of watermelon cultivars grown in the US and another genotype may be more appropriate for testing *P. cubensis* host range. Plots should also be rated quantitatively in future studies.

In the leaf disk assays, *C. pepo* var. *pepo* was not as susceptible as it was in the field experiments. *P. cubensis* relies on wind dissemination of sporangia for dispersal (Palti, 1975; Waterhouse. 1973). The susceptibility of *C. pepo* var. *pepo* in the seven out of the 11 field experiments may be due to the mixing of natural populations. As numerous and varied cucurbit crops are cultivated and become infected throughout the growing season, there may be several populations of *P. cubensis* with different host range patterns present in the environment. Therefore, designating pathotypes may be an oversimplification of what occurs in wild populations as host selection pressure changes temporally and geographically. Future studies should track acreage of the different cucurbit crops that the *P. cubensis* populations encounter throughout the season to determine the effects of host selection pressure.

Introduction of a strain of *P. cubensis* from another cucumber producing area that suffers from severe *P. cubensis* infection may be the reason for the downy mildew epidemic of cucumber in 2004. Perhaps, as Thomas et al. (1987) suggested, the new strains may contain a very high and concentrated frequency of virulence factors against cucumber, resulting from selection on that host. To test this we looked at the difference in susceptibility of a resistant cucumber, 'Poinsett', and a highly susceptible cucumber, 'Coolgreen'. The isolates collected from cucumber after 2004 caused an equal amount of sporulation on both of the cultivars, however 'Poinsett' did not go necrotic as rapidly as 'Coolgreen'. This

differs from the isolate collected from cantaloupe in 1982, which was more aggressive on 'Coolgreen' than it was on 'Poinsett', suggesting that there is a genotype of the pathogen currently present in the US that is virulent on the commercially available resistant cultivars of cucumber. It would be informative to compare the US isolates to isolates collected from international areas where downy mildew epidemics occur on cucumber.

There was a difference between the isolate collected from squash in 2004 from Johnston County, NC and the isolates collected from cucumber. The isolate originally collected from squash in 2004 from Johnston County, NC did not cause the same amount of disease on 'Poinsett' as it did on 'Coolgreen'. This isolate caused greater chlorosis/necrosis and sporulation on 'Coolgreen' compared to resistant 'Poinsett'. The other squash isolates caused similar amounts of dense and dark sporulation on both cultivars, however much more rapid necrosis on 'Coolgreen'. Our data suggests that isolates collected from squash are more virulent than those collected from cucumber. For example, the Johnston County, NC isolate collected from squash was compatible with the full complement of cucurbit hosts tested. This is a phenomenon that Lebeda has not recorded with the Czech Republic isolates (Lebeda and Gadasová, 2002; Lebeda and Urban, 2004a, 2004b).

Lebeda found that the European populations of *P. cubensis* are highly variable and generally do not conform to the model of Thomas et al., (1987), though he believes this is due to the new set of differentials (Lebeda and Gadasová, 2002; Lebeda and Urban, 2004b). We were able to compare the new set of differentials to the historic 'C' isolate (collected from Charleston County, SC in 1982) from the original study to provide a direct comparison of the methods. Similar to what Thomas et al. (1987) found, our results indicate that there is

differences within the US populations based on highly compatible reactions to squash (*Cucurbita pepo* var. *pepo*), however our results were not as dramatic as what they reported. We did see a difference in the susceptibility of watermelon from the original assay to the leaf disk method and we have mentioned possible reasons for this above. Overall, the leaf disk method was an appropriate way to rate the susceptibility of cucurbit hosts to a large number of *P. cubensis* isolates based on sporulation and chlorosis.

In this study we looked at 32 *P. cubensis* isolates collected in the US and, similar to what Lebeda found with the Czech Republic isolates (2002, 2004b), there is a wide range of variability in host range among isolates. Therefore, the widely accepted standard of partitioning all *P. cubensis* isolates or populations into five pathotypes based on results from eight *P. cubensis* isolates may be an over- generalization, something Thomas et al. (1987) also considered. Our results illustrate that there is high variability in pathogenicity among the US isolates of *P. cubensis* and defining populations of into specific groups or pathotypes based on host range is a difficult task. It is possible that there could be as many pathotypes as *P. cubensis* isolates or cucurbit hosts challenged. However, similar to what Thomas et al. (1987) found in the US, it seems that there are at least two genotypes that differ in susceptibility to squash. Our results indicate, all commercially grown cucurbit crops are at risk for downy mildew infection in natural populations, though certain genera, such as *Cucumis* spp., are more susceptible than others. Most importantly, our data implicates the need for new and commercially available downy mildew resistant cucumber cultivars in the US.

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**TABLE 2.1.** Differential set of cucurbit taxa for host specialization determination (adapted from Lebeda and Widrlechner, 2003)

| No. | Taxon (common name) | Cultivar name | Country of origin | Donor | EVIGEZ[a] |
|---|---|---|---|---|---|
| 1 | *Cucumis sativus* (cucumber) | Marketer 430 | USA | | H39-0121 |
| 2 | *C. melo* subsp. *melo* (cantaloupe) | Ananas Yokneam | Israel | PI 292008 | H40-1117 |
| 3 | *C. melo* var. *conomon* (honeydew melon) | Baj-Gua | Japan | CUM 238/1974 | H40-0625 |
| 4 | *C. melo* var. *acidulous* (bitter melon) | 95 | Myanmar | PI 200819 | H40-0611 |
| 5 | *Cucurbita pepo* var. *pepo* (squash) | Dolmalik | Turkey | PI 171622 | H42-0117 |
| 6 | *Cucurbita pepo* var. *texana* (squash) | HDW 3173 | USA | PI 614687 | H42-0130 |
| 7 | *C. pepo* subsp. *fraterna*[b] (squash) | 179 | Mexico | PI 532355 | H42-0136 |
| 8 | *C. maxima* (winter squash) | Goliáš | Czechoslovakia | | H42-0137 |
| 9 | *Citrullus lanatus* (watermelon) | Malali | Israel | | H37-0008 |
| 10 | *Benincasa hispida* (wax gourd) | NA | USA | BEN 485 | H15-0001 |
| 11 | *Luffa cylindrica* (luffa) | NA | ? | | H63-0010 |
| 12 | *Lagenaria siceraria* (bottle gourd) | NA | ? | | H63-0009 |

[a]EVIGEZ – Czech Republic gene bank number
[b]Described as *Cucurbita fraterna* by Lebeda and Gadasová, 2002.

**TABLE 2.2.** Reaction of cucurbit host differentials to isolates of *Pseudoperonospora cubensis* collected in the eastern United States as determined by a leaf disk assay.

| Source of isolate | | | Host differentials' reaction to *Pseudoperonospora cubensis*[a] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Host / State** | **County** | **Year** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** |
| *Cucumis sativus* | | | | | | | | | | | | | | |
| North Carolina | Sampson | 2004 | +++ | +++ | +++ | +++ | – | – | – | + | – | + | + | nt[c] |
| | Sampson | 2006 | +++ | +++ | +++ | +++ | – | +++ | +++ | + | – | + | – | – |
| | Hyde | 2006 | +++ | +++ | + | + | – | ± | ± | – | ± | +++ | +++ | – |
| | Johnston | 2007 | +++ | +++ | +++ | – | ± | +++ | +++ | + | – | ± | – | + |
| | Duplin | 2007 | +++ | +++ | +++ | +++ | – | +++ | +++ | +++ | ± | – | + | – |
| | Pender | 2007 | +++ | +++ | +++ | +++ | – | +++ | +++ | +++ | – | + | + | +++ |
| Michigan | Central Oceana | 2005 | +++ | +++ | – | +++ | – | + | + | + | nt | – | – | – |
| | East Oceana | 2005 | +++ | +++ | +++ | +++ | ± | +++ | +++ | +++ | – | +++ | +++ | + |
| | Arenac | 2007 | +++ | +++ | + | +++ | +++ | +++ | +++ | + | – | ± | – | – |
| Virginia | Accomack | 2007 | +++ | +++ | +++ | +++ | – | +++ | +++ | + | + | + | + | – |
| Maryland | Wicomico | 2007 | +++ | +++ | + | + | + | +++ | + | ± | – | – | – | ± |
| Ohio | Erie | 2007 | +++ | +++ | +++ | +++ | ± | +++ | +++ | + | nt | + | – | – |
| | Huron | 2007 | +++ | +++ | – | +++ | – | + | ± | +++ | – | ± | – | – |
| South Carolina | Clarendon | 2007 | +++ | +++ | +++ | +++ | ± | +++ | +++ | + | nt | – | – | +++ |
| | Charleston | 2007 | +++ | +++ | +++ | +++ | + | +++ | +++ | – | +++ | ± | + | – |
| Florida | Collier | 2007 | +++ | +++ | +++ | +++ | ± | +++ | +++ | +++ | ± | + | ± | +++ |
| Kansas | Johnson | 2007 | +++ | +++ | +++ | +++ | + | +++ | +++ | +++ | – | +++ | +++ | +++ |
| New York | Suffolk | 2007 | +++ | +++ | +++ | +++ | ± | +++ | +++ | +++ | +++ | ± | ± | ± |
| Delaware | New Castle | 2007 | +++ | +++ | – | +++ | ± | +++ | + | +++ | – | ± | ± | – |
| *Cucumis melo*[d] | | | | | | | | | | | | | | |
| North Carolina | Sampson | 2007 | +++ | nt | +++ | +++ | ± | +++ | +++ | + | + | – | + | – |
| | Johnston | 2007 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | ± | ± | – | + |
| South Carolina | Charleston | 1982 | +++ | +++ | – | +++ | – | + | + | + | + | – | – | – |
| *Cucurbita* spp.[e] | | | | | | | | | | | | | | |
| North Carolina | Johnston | 2004 | +++ | +++ | +++ | +++ | +++ | +++ | + | + | + | + | – | – |
| | Johnston | 2005 | +++ | +++ | + | +++ | +++ | +++ | +++ | +++ | + | + | – | – |
| | Johnston | 2006 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | + | + | + |
| | Johnston | 2007 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| New York | Suffolk | 2007 | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | + | + | – | +++ |
| New Jersey | Cumberland | 2007 | +++ | +++ | +++ | ± | – | + | + | +++ | – | + | ± | ± |
| *Citrullus lanatus* | | | | | | | | | | | | | | |
| North Carolina | Lenoir | 2007 | +++ | +++ | +++ | +++ | – | +++ | +++ | +++ | +++ | +++ | ± | + |
| | Sampson | 2005 | +++ | +++ | +++ | +++ | ± | +++ | +++ | +++ | + | +++ | +++ | +++ |
| South Carolina | Charleston | 2007 | +++ | +++ | +++ | – | ± | +++ | +++ | +++ | – | + | – | + |
| Unknown[f] | | | | | | | | | | | | | | |
| | Unknown | <2004 | +++ | +++ | +++ | +++ | ± | +++ | +++ | +++ | – | +++ | – | + |

[a] – = No evidence of lesions or sporulation detected, classified as incompatible; ± = sporulation limited to a very few sporangiophores; (lowly compatible); + = sparse sporulation present, less than 20% of leaf disk area (lowly compatible); +++ = abundant sporulation present on more than 50% of leaf disk (highly compatible).
[b] Cucurbit host isolate was originally collected from.
[c] Not tested because of poor seed germination at time of assay.
[d] Isolate collected from *Cucumis melo* subsp. *melo* or *Cucumis melo* var. *acidulus*.
[e] Isolate collected from *Cucurbita pepo* or *Cucurbita maxima*.
[f] Origin of isolate unknown; obtained from DuPont, Wilmington, DE.

**TABLE 2.3.** Reaction of 12 cucurbit host differentials under filed conditions to naturally occurring populations of *Pseudoperonospora cubensis* in eight US states in 2007.

| | Cucurbit downy mildew detection[ab] | | | | | | | | | | | Positive |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Differential Host** | **FL[c]** | **FL[d]** | **GA[e]** | **SC[f]** | **NC[g]** | **NC[h]** | **OH[i]** | **MI[j]** | **MI[k]** | **DE[l]** | **NY[m]** | **Reactions** |
| *Cucumis sativus* | + | +[n] | +[n] | +[n] | +[n] | +[n] | +[n] | +[n] | +[n] | +[n] | +[n] | 11 |
| *C. melo* subsp. *melo* | +[n] | + | + | + | +[n] | – | + | + | +[n] | – | +[n] | 9 |
| *C. melo* var. *conomon* | + | +[n] | + | + | + | – | + | + | +[n] | – | + | 9 |
| *C. melo* var. *acidulus* | +[n] | +[n] | +[n] | + | + | + | + | + | – | – | + | 9 |
| *Cucurbita pepo* var. *pepo* | + | + | +[n] | + | + | – | + | – | – | – | + | 7 |
| *C. pepo* var. *texana* | + | + | +[n] | + | + | – | + | + | – | + | + | 9 |
| *C. pepo* var. *fraterna* | – | +[n] | + | + | + | – | + | + | – | + | – | 7 |
| *Cucurbita maxima* | – | +[n] | +[n] | + | – | – | + | – | – | + | – | 5 |
| *Citrullus lanatus* | + | – | + | + | + | – | – | + | – | – | – | 5 |
| *Benincasa hispida* | – | – | – | + | – | – | – | – | – | + | – | 2 |
| *Luffa cylindrica* | – | + | – | + | – | – | + | – | – | – | – | 3 |
| *Lagenaria siceraria* | +[n] | + | – | + | + | – | + | + | – | – | – | 6 |

[a] Presence of symptoms on day of final inspection.
[b] + = Symptoms and signs present, – = symptoms or signs absent.
[c] Collier County, FL site one, symptoms first detected on 20 April.
[d] Collier County, FL site two, symptoms first detected on 4 April.
[e] Tift County, GA, symptoms first detected on 12 July.
[f] Charleston County, GA, symptoms first detected on 12 July.
[g] Johnston County, NC, symptoms first detected on 23 July.
[h] Henderson County, NC, symptoms first detected on 1 September.
[I] Wayne County, OH, symptoms first detected on 13 August.
[k] Clinton County, MI, symptoms first detected on 24 August.
[j] Ingham County, MI, symptoms first detected on 27 August.
[l] New Castle County, DE, symptoms first detected on 6 August.
[m] Suffolk County, NY, symptoms first detected on 14 August.
[n] First host(s) to become infected by *P. cubensis* at field site.

**Table 2.4**. Reaction of downy mildew susceptible cucumber 'Coolgreen' and downy mildew resistant cucumber 'Poinsett' to 11 isolates of *Pseudoperonospora cubensis*.

| Origin of *P. cubensis* isolate | | | | Sporulation intensity and leaf disk color[a] | |
|---|---|---|---|---|---|
| **County** | **State** | **Host** | **Year** | **Coolgreen** | **Poinsett** |
| Central Oceana | MI | Cucumber | 2005 | 3[b]-N[c] | 3-C |
| Greene | NC | Cucumber | 2006 | 3-N | 3-C |
| Sampson | NC | Cucumber | 2006 | 3-N | 3-C |
| Bay | MI | Cucumber | 2007 | 3-N | 3-C |
| New Castle | DE | Cucumber | 2007 | 3-N | 3-C |
| Sampson | NC | Watermelon | 2005 | 3-N | 3-C |
| Johnston | NC | Squash | 2004 | 1-C | 2-C |
| Johnston | NC | Squash | 2005 | 3-C | 3-G |
| Johnston | NC | Squash | 2006 | 3-C | 3-G |
| Unknown | na | na | na | 3-N | 3-C |
| Charleston | SC | Cantaloupe | 1982 | 3-N | 2-G |

[a] Reaction of cucumber cultivar to isolates of *P. cubensis* based on amount sporulation and color of leaf disk. Rating averaged over 12 leaf disks of each cultivar.
[b]Sporulation rating, 0 = no infection detected, 1 = 1-10% of leaf disk area covered with sporulation, 2 = 11-50% of leaf disk area covered with sporulation, 3 = greater than 50% of leaf disk area covered with sporulation.
[c]Leaf color rating, G = green, C = chlorotic, N = necrotic.

# CHAPTER 3 – MEFENOXAM AND QoI RESISTANCE OF *PSEUDOPERONOSPORA CUBENSIS* IN THE EASTERN UNITED STATES

## ABSTRACT

Downy mildew, caused by *Pseudoperonospora cubensis*, re-emerged as the one of the most important foliar plant pathogens on cucumber in the eastern United States in 2004. A fungicide efficacy trial in 2004 in North Carolina identified a reduced efficacy of mefenoxam and the QoI fungicides, pyraclostrobin and azoxystrobin for disease control. In this study, the sensitivity of *P. cubensis* isolates to mefenoxam (0.01, 0.1, 1.0, 10, 100 µg/ml) and the QoI fungicide azoxystrobin (0.001, 0.01, 0.1, 1.0, 10, 100 µg/ml) was determined using a whole cucumber plant assay with inoculum derived from 24 single leaf isolates of *P. cubensis* collected in 2004 through 2007 from the eastern United States. Seven additional isolates and four isolates from the original assay were also evaluated using mefenoxam at and azoxystrobin at 1 and 100 µg/ml, as well as a positive (fluopicolide) and non-treated control. Insensitivity (less than 25% disease control) to all mefenoxam and azoxystrobin concentrations was observed in 27 out of the 31 (87%) isolates assayed. Practical resistance of *P. cubensis* to mefenoxam and pyraclostrobin was evaluated in Florida, South Carolina, North Carolina, Delaware and New York. There was no reduction in disease severity compared to the non-treated control at all locations and marketable yield was significantly reduced in mefenoxam and pyraclostrobin plots in all locations except Florida. This study suggests that mefenoxam and QoI resistance is prevalent in the eastern United States. Management of cucurbit downy mildew should rely on fungicides other than mefenoxam and

QoIs and anti-resistance strategies should be employed.

## INTRODUCTION

*Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovtsev is the causal agent of cucurbit downy mildew, a disease of international importance (Cohen, 1981; Palti, 1974). *P. cubensis* can be found in temperate areas, such as the Americas, Europe, Japan, Australia and South Africa, tropical regions internationally and some semi-arid regions, such as the Middle East. The disease affects cucurbits in the open field as well as those under plastic or in greenhouses and is especially damaging in those areas that possess the warm, humid climate in which the pathogen thrives, such as the southeastern United States (US) (Cohen, 1981; Holmes et al., 2004; Palti and Cohen, 1980).

When resistant cultivars are not available, fungicides are necessary for the control of downy mildew on cucurbit crops (Sherf and MacNab, 1986; Thomas, 1996). In 1996, the global fungicide market was estimated at approximately 6.25 billion USD, of which 16.7% were fungicides to control members of the Peronosporales (i.e., downy mildews). The largest proportion of the downy mildew market was for grape downy mildew caused by *Plasmopara viticola* (54%) followed by cucurbit downy mildew (10%) (Gisi, 2002).

The phenylamides and QoI fungicides are two of the most important groups of fungicides that are effective against the downy mildews (Gisi, 2002; Heaney, 2000; Sherf and MacNab, 1986). The phenylamides include metalaxyl, described as having the widest range of activity on the different families within the Peronosporales (Schwinn and Staub, 1995), and mefenoxam or metalaxyl-M, the (R)-enantiomer of metalaxyl (Nuninger et al.,

1996). Gisi identified mefenoxam as the most systemic phenylamide and describes it as the most active, versatile, and broadly used phenylamide molecule against a wide range of foliar diseases (2002).

The phenylamides inhibit RNA synthesis by reducing the incorporation of uridine into ribosomal RNA (rRNA). However, even at concentrations that fully suppress fungal growth, inhibition of uridine incorporation is incomplete and messenger RNA and transfer RNA synthesis are unaffected. When phenylamides are applied to phenylamide-resistant isolates, incorporation of uridine into rRNA remains completely unaffected (Davidse, 1983, 1988; Gisi and Cohen, 1996).

Another class of fungicides used for control of the downy mildews, and various other fungal pathogens, is the quinone outside inhibitors (QoIs) or strobilurin-type fungicides. The QoIs represent a highly effective group of broad-spectrum fungicides with a novel mode of action (Ypema and Gold, 1999). The QoIs inhibit mitochondrial respiration by binding to the subunit protein of the cytochrome bc1 enzyme complex (complex III) of the electron transport chain located in the inner-mitochondrial membrane, resulting in a loss of ATP production and inhibiting of fungal growth (Bartlett, 2002; Gisi et al., 2000). The QoIs, azoxystrobin and kresoxim-methyl, were first commercialized in 1996 (Heaney et al., 2000). In 1999, sales of QoI and QoI-related fungicides totaled approximately 620 million USD, representing over 10% of the global fungicide market. Azoxystrobin sales totaled 415 million USD, making it the number one selling fungicide in the world in 1999 (Bartlett et al., 2002; Heaney, 2000).

Historically in the eastern US, downy mildew of cucurbits occurs on squash, pumpkin

and melons and less commonly on watermelon in late summer and fall. In contrast, downy mildew on cucumber (*Cucumis sativus* L.) has been successfully controlled through the use of resistant cultivars since the 1950s (Barnes and Epps, 1954; Colucci et al., 2006b; Holmes et al., 2006; Sitterly, 1972). Although the disease could be found on cucumber in North Carolina during the last several decades, its presence was inconspicuous and did not result in obvious crop losses (Peterson et al., 2002). In 2004, the disease on cucumber changed dramatically when a new race of the pathogen devastated cucumber-growing areas of North Carolina (NC), Delaware (DE), Maryland (MD) and Virginia (VA). Virtually all modern commercially grown cucumber cultivars are downy mildew resistant, but these were not sufficient to prevent severe economic losses estimated at 40% region-wide (Colucci et al., 2006b; Holmes et al., 2006).

Since 2004 downy mildew has remained the most important foliar pathogen on cucumber throughout the eastern United States. Because the disease is no longer controlled by the available resistant commercial cucumber varieties, fungicide applications are necessary. In a fungicide efficacy field experiment conducted in 2004, mefenoxam and the QoI fungicides, azoxystrobin and pyraclostrobin, were not effective at controlling downy mildew on cucumber (Thornton et al., 2005). This finding suggests that there are populations of *P. cubensis* in the eastern US that are resistance to mefenoxam and the QoI fungicides. The purpose of this study was to determine the sensitivity of *P. cubensis* isolates collected from the eastern US in controlled conditions for sensitivity to mefenoxam and azoxystrobin. Fungicide resistance was also evaluated in field trials in NC, DE, Florida (FL), South Carolina (SC) and New York (NY) in 2007.

## MATERIALS AND METHODS

**Fungicides.** For field experiments, the following formulated products were used: pyraclostrobin (Cabrio 20EG, 0.2 kg a.i./ha, BASF Corp., Research Triangle Park, NC) and mefenoxam (Ridomil Gold 4EC, 0.14 kg a.i./ha, Syngenta Crop Protection, Greensboro, NC). In addition, a positive control fungicide program was used which consisted of the rotation of famoxadone + cymoxanil (Tanos 50DF 2.3 famoxadone kg/ha + 2.3 cymoxanil kg/ha) tank mixed with mancozeb (Manzate Pro-Stick 75DG 2.5 lb a.i./ha, DuPont, Wilmington, DE) alternated weekly with propamocarb hydrochloride (Previcur Flex 6F, 0.9 liter a.i./ha, Bayer CropScience, Research Triangle Park, NC) tank mixed with mancozeb.

Technical grade azoxystrobin and mefenoxam (Syngenta Crop Protection, Greensboro, NC) were used in the fungicide sensitivity growth chamber experiments. To make stock solutions the fungicides were dissolved in acetone. These stock solutions were stored in microcentrifuge tubes at -20°C and diluted in 0.02% Tween 20 (Sigma-Aldrich, St. Louis) in $dH_2O$ upon use. Fluopicolide (38-41% active ingredient, Valent USA Corp., Walnut Creek, CA) as formulated product was used as a positive effective *P. cubensis* control for some assays.

**Collection and maintenance of *P. cubensis* isolates.** Eighty-three whole leaf isolates infected by *P. cubensis* were collected from the 13 eastern United States from 2004 through 2007. Of the 83 isolates collected, 60 were tested and 31 were successfully assayed for sensitivity to mefenoxam and azoxystrobin (Table 3.1). The remaining isolates did not establish disease when re-inoculated on host plants and therefore could not be assayed. The history of fungicide use is not known for all isolates, however most came from areas that

were not treated with fungicides, such as the non-treated areas in fungicide efficacy experiments.

To increase inoculum, sporangia were dislodged from one infected leaf using a Preval Sprayer Complete Unit 267 (Precision Valve Corporation, Yonkers, NY) containing $dH_2O$. The suspension was adjusted to $10^4$ sporangia $ml^{-1}$ and misted onto highly susceptible cucumber, 'Coolgreen'. Two ml of inoculum was applied to axial and abaxial sides of the true leaves and the cotyledons. Plants were placed in moist chambers that were constructed using plastic sheeting and humidifiers (ReliOn Ultrasonic humidifier model H-0695-0, Walmart) and placed at 18°C in darkness. After 24 h, plants were removed from moist chambers and conditions were set to 18°C, night and 21°C, day with a 12 h photoperiod.

Seven days after inoculation, infected cotyledons or whole leaves were collected from the plants and sporulation was induced by placing the leaves in moist chambers placed at 18°C in darkness. After 24 h leaves were clipped from the plants and removed from moist chambers for the excess moisture to evaporate. The leaves were placed in plastic Petri dishes (100 × 15 mm) and sealed with Parafilm. These leaf cultures were stored for 24 h in -20°C then moved to -80°C for long-term storage. Isolates remain viable at -80°C for at least 6 months.

*P. cubensis* isolates assayed for fungicide sensitivity were from freshly infected leaves or whole leaf cultures stored in -80°C. Frozen isolates used for fungicide resistance assays were removed from -80°C, allowed to thaw and inoculum was prepared by dislodging sporangia from leaves using the method mentioned above.

**Field tests.** In 2007, field trials were completed in FL, SC, NC, DE and NY.

Pyraclostrobin, mefenoxam, and the positive control treatment of famoxadone + cymoxanil tank mixed with mancozeb and alternated weekly with propamocarb hydrochloride tank mixed with mancozeb were tested. Cucumber 'Lafayette' was either transplanted or seeded directly to the plots. Plots were 20 ft bare ground single rows with 5 ft between plots and treated rows were bordered by non-treated rows. Plots were arranged in a randomized complete block design with four replicates. In NY, plots were 3 parallel 15-ft rows (45-ft total length of cucumber plants in each plot).

Fungicides were applied using a $CO_2$ backpack sprayer or a tractor-mounted boom sprayer. Treatments were initiated before downy mildew symptoms were detected in the trial and applied on a 5- to 7-day interval beginning at the two-true leaf stage. Disease severity (DS), as percent foliar necrosis was rated weekly. Fruit were hand harvested and harvest data (number and weight of fruit) was collected. Fruit was separated into marketable and unmarketable yield based on shape. Fruit that are straight or have only a slight curvature (<45°) are considered marketable, fruit that are bent or misshaped are considered unmarketable. Yield data from all harvests were combined at each location. Area under the disease progress curve (AUDPC) was calculated and statistical analysis (Proc GLM) was conducted using SAS version 9.1 (SAS Institute, Cary, NC). Analysis of variance and least significant difference (LSD) means separation were calculated ($\alpha = 0.05$) for each location. Data from NY was adjusted to resemble the other locations.

**Fungicide sensitivity assays- experiment 1.** Because *P. cubensis* is a biotroph, it is necessary to perform fungicide sensitivity experiments on live host tissue (O'brien and Weinart, 1995). Highly susceptible cucumber, 'Coolgreen', was grown to the two-true-leaf

stage at 22°C, night and 26°C, day with a 12 h photoperiod.

The true leaves and cotyledons were misted with a total of 2 ml of mefenoxam or azoxystrobin at rates of 0.01, 0.1, 1.0, 10, 100 µg/ml and 0.001, 0.01, 0.1, 1.0, 10, 100 µg/ml, respectively. In addition, a non-treated control consisting of 0.02% Tween 20 in $dH_2O$ was used. Fungicides were applied using the Preval Complete Spray Unit. Three plants were tested for each fungicide concentration. Twenty-four h after plants were treated, each leaf was inoculated with a *P. cubensis* suspension ($5 \times 10^3$ sporangia/ml) to the axial and abaxial sides using the Preval Complete Spray Unit (total 2 ml suspension per plant). Plants were placed in moist chambers at 18°C darkness. After 24 h, plants were removed from moist chambers and growth chamber conditions were set to 18°C, night and 21°C, day with a 12 h photoperiod.

Seven days after inoculation the plants were rated for DS based on percentage of necrotic leaf area using a 0-100% scale. In addition, DS values for each isolate were adjusted to percent control of their respective non-treated control. This value was calculated using the following formula: [(DS non-treated - DS treated)/DS non-treated] × 100. Statistical analysis (PROC GLM) was conducted using SAS version 9.1 (SAS Institute, Cary, NC). Analysis of variance and least significant difference (LSD) means separation were calculated ($\alpha$=0.05).

**Fungicide sensitivity assays- experiment 2.** Because of consistent results among treatments and isolates and to reduce plant material and the space required for screening, a reduced assay was developed using fewer fungicide concentrations. Eleven isolates of *P. cubensis* were tested using six treatments; mefenoxam at 1 and 100 µg/ml, azoxystrobin at 1 and 100 µg/ml, fluopicolide at 0.024 µl a.i./ml (equivalent to 7.41 fl oz a.i./ha) and a non-

treated control. Five of the isolates tested in experiment 1 were repeated and six new isolates were tested. All experiments took place in growth chambers using three replications of each treatment. The method for fungicide and inoculum application, growth chamber conditions, DS ratings and analysis of data were the same as stated previously.

## RESULTS

**Field tests.** Downy mildew was present in all field plots and disease pressure was high in FL, NC, DE and NY with final DS ratings of 80% or greater in the non-treated control plots. In SC, the final DS rating for the non-treated control plots was less than 40% (data not presented). Mefenoxam and pyraclostrobin were ineffective at controlling *P. cubensis* and as a result the AUDPC value for mefenoxam and pyraclostrobin treatments were not significantly different from the non-treated control in SC, NC, DE and NY (Fig. 3.1). In FL, the AUDPC value of the pyraclostrobin treatment was significantly greater than the non-treated control and there was no significant difference between the mefenoxam treatment and non-treated control. The positive control treatment had a significantly lower AUDPC value at all locations.

The effect of downy mildew on cucumber yield is evident from the data. In SC, NC and DE marketable yield was reduced to almost zero in the non-treated control and mefenoxam and pyraclostrobin treated plots. Marketable yield from the mefenoxam and pyraclostrobin treated plots were not significantly different from the non-treated control in all locations except FL (Fig. 3.2). Yield for all treatments were much higher for the FL and NY field trials (Fig. 3.2). Because of a late appearance of downy mildew at the FL experiment,

downy mildew did not have a significant effect on marketable cucumber yield. The positive control treatment had a greater marketable yield than the other treatments at all locations except FL.

**Fungicide sensitivity assays- experiment 1.** Seventeen out of the 24 *P. cubensis* isolates showed no statistically significant differences at any concentration of mefenoxam or azoxystrobin used (Table 3.1). Seven isolates showed significant differences for mefenoxam (isolate 17), azoxystrobin (isolate 31), or both (isolates 1, 2, 7, 8, 23). Of the seven isolates where treatment differences were found, three isolates, 17, 23 and 31, did not provide greater than 16% downy mildew control at any concentration of mefenoxam and azoxystrobin (Table 3.2). Therefore, 20 isolates (83.3%) were insensitive to both mefenoxam and azoxystrobin at all concentrations.

Two isolates collected prior to the 2004 epidemic, isolates 1 and 2, were controlled at azoxystrobin concentrations above 1μg/ml and 0.1 μg/ml, respectively. Downy mildew infection caused by isolate 1 was controlled 72% and 90% by 1μg/ml and 10 μg/ml, respectively. Two isolates collected after the 2004 epidemic, isolates 7 and 8, showed some sensitivity to mefenoxam and azoxystrobin. Isolate 7, collected from cantaloupe in 2007 from Sampson County, NC was 100% controlled by 10 and 100 μg/ml mefenoxam and 56% and 85% by 10 and 100 μg/ml azoxystrobin, respectively. Isolate 8, collected from winter squash in Johnston County, NC in 2004 was controlled 94% by 100 μg/ml mefenoxam and 93% and 89% by 10 and 100 μg/ml azoxystrobin, respectively.

**Fungicide sensitivity assays- experiment 2.** Because of the consistency of the results in experiment 1 for all concentrations of mefenoxam and azoxystrobin, we were able

to reduce the number of treatments to investigate sensitivity to these fungicides. Five isolates from experiment 1 were repeated in experiment 2 and similar results were found. Of the 11 total isolates tested, only three isolates, 1, 2 and 7 demonstrated significant treatment differences. These isolates also showed sensitivity to both mefenoxam and azoxystrobin in this reduced assay. These isolates also demonstrated sensitivity to mefenoxam and azoxystrobin in experiment 1. In addition, the isolates that demonstrated no treatment differences in experiment 1 performed similarly in experiment 2. The additional seven isolates demonstrated insensitivity to both mefenoxam and azoxystrobin.

Results of individual isolate reactions to the 1 and 100 μg/ml levels of azoxystrobin and mefenoxam from experiment 1 and 2 are listed in Table 3.3. The 100 μg/ml mefenoxam treatment did not control isolate 1 as well in experiment 1 as it did in experiment 2. Overall, results from experiment 1 and experiment 2 suggest that 27 out of the 29 (93%) post-2004 *P. cubensis* isolates assayed are not controlled by any concentration of mefenoxam and azoxystrobin tested. Fluopicolide provided 100% disease control against all of the 11 *P. cubensis* isolates tested.

## DISCUSSION

The Fungicide Resistance Action Committee (FRAC) has categorized *Pseudoperonospora cubensis* as a plant pathogen with high risk of development of resistance to fungicides (Russell, 2003). Through field trials in five eastern US states and fungicide sensitivity assays with 31 isolates, the present study demonstrates that resistance to mefenoxam and QoI fungicides is prevalent and widespread among populations of *P.*

*cubensis* in the eastern US. Because fungicides are now required to control downy mildew on cucumber, these results have direct implications for the management of *P. cubensis* populations in the US.

Metalaxyl was first reported by Urech et al. in 1977 and was introduced to the market as a single product without anti-resistance measures (Urech, 1988). Upon release of metalaxyl, treatment of plants with 500 µg formulated product Ridomil/ml provided 100% downy mildew control (Reuveni and Cohen, 1979). Just one year after the release of metalaxyl, *P. cubensis* was the first pathogen to demonstrate metalaxyl resistance in a greenhouse in Israel in 1979. These resistant isolates were not controlled with concentrations of metalaxyl at 500 µg per leaf (Reuveni et al., 1980). Since the initial report, metalaxyl resistant populations of *P. cubensis* have been identified in Greece (Georgopoulos and Grigoriu, 1981), Italy (D'Ercole and Nipoti, 1985), Russia (Grin'Ko, 1992), Australia (O'Brien and Weinart, 1995) and the Czech Republic (Urban and Lebeda, 2006). Resistance to metalaxyl and/or mefenoxam has been identified in other oomycete pathogens including *Phytophthora infestans* (Daayf and Platt, 1999; Deahl et al., 1993; Gisi and Cohen, 1996; Shattock, 1988), *Pythium* sp. (Sanders, 1984), *Bremia lactucae* (Crute, 1987), *Phytophthora capsici* (Parra and Ristaino, 2001) and *Pseudoperonospora humuli* (Klein, 1994).

In the US, reduced efficacy of metalaxyl for the control of *P. cubensis* was detected in research plots in FL on squash and cucumber in the 1983 and 1984 (Morton and Urech, 1988). In 1987 Moss published a similar observation on the reduced efficacy of metalaxyl in an abstract (1987). Laboratory tests were not performed to confirm metalaxyl resistance and, in 1988, resistance was not thought to be widespread in the US (Morton and Urech, 1988).

Similar to metalaxyl and mefenoxam, QoI fungicides generally possess a high risk of resistance development and it is believed that selection for resistant populations can occur quickly (Brent and Holloman, 2007b; Ishii et al., 2001). Rapid selection for QoI resistance occurred in Germany where they were introduced for the control of powdery mildew (*E. graminis* f. sp. *tritici*) on wheat. Two years after introduction, QoI resistant populations of *E. graminis* f. sp. *tritici* were detected (Heaney et al., 2000). Similarly in Japan, soon after the commercial introduction of kresoxim-methyl and azoxystrobin for control of cucurbit powdery mildew (*Podosphaera* spp. = *Sphaerotheca* spp.) and downy mildew in 1997 and 1998, resistant populations were identified. Although most cucumber growers followed manufacturers usage recommendations, control failure of powdery mildew was frequently reported in southern Japan and in 1999, reduced efficacy of the QoI fungicides for *P. cubensis* was reported (Heaney et al., 2000; Ishii et al., 2001). Populations of *P. cubensis* with practical resistance to QoI fungicides have also been identified in Europe (Heaney et al., 2000). The term practical resistance is preferred over field resistance because it indicates consequent, observable loss of disease control by a fungicide. Field resistance indicates merely the presence of resistant variants in field populations (at whatever frequency or severity) (Brent and Hollomon, 2007a).

In the US, reduced efficacy of mefenoxam, and QoI fungicides for the control of cucumber downy mildew was initially observed in a fungicide efficacy field trial in Sampson County, NC in 2004 (Thornton et al., 2005). Since the unprecedented 2004 epidemic of downy mildew on cucumber, yearly fungicide efficacy experiments from 2004 through 2007 indicate mefenoxam and QoI fungicides have reduced efficacy for the control of downy

mildew on cucumber in NC (Colucci et al., 2006a, 2007, 2008a, 2008b). The current study is the first investigation of mefenoxam and QoI resistance of *P. cubensis* on a regional scale and we have demonstrated that resistance to these fungicides is widespread in the eastern US.

Results from the five field experiments in FL, SC, NC, DE and NY demonstrate widespread control failure of mefenoxam and pyraclostrobin for the control of *P. cubensis*. Disease severity ratings from the mefenoxam and pyraclostrobin treated plots were not significantly different or less than the ratings from the non-treated control. These results indicate that fungicides with active ingredients mefenoxam and pyraclostrobin are not effective at controlling downy mildew and resistance problems exist. The QoI fungicides are in the same cross-resistance group and should be managed accordingly (Gisi, 2002; Heaney et al., 2000).

Severe infection by downy mildew results in misshaped fruit and lack of fruit production. The drastic effect that downy mildew has on cucumber yield, especially when it appears early in the season, was demonstrated in the field experiments. Marketable yield was significantly reduced in the non-treated, mefenoxam and pyraclostrobin treated plots at all locations except FL. In fact, marketable yield was reduced to nearly zero for the SC, DE and NC experiments. In NY, only plots treated with the positive control program had fruit at the last harvest. Marketable yield ratings were not different among treatments at the FL experiment due to delayed appearance of downy mildew. These results emphasize the importance of early detection and early fungicide application for the control of downy mildew and improved yields.

The positive control, famoxadone + cymoxanil alternated with propamocarb

hydrochloride, demonstrated effective downy mildew control resulting in lower disease severity at all locations. The success of the positive control program stresses the importance of applying anti-resistance strategies with fungicides of different modes of action. Famoxadone is QiI (quinone inside inhibitor) fungicide and it has been shown that cross-resistance does not exist between the QoI and QiI fungicides (Gisi, 2002).

In our laboratory experiments, 27 out of 29 (93%) isolates of *P. cubensis* collected post-2004 epidemic on cucumber were not controlled at any concentration of mefenoxam or azoxystrobin used. No isolates collected from cucumber showed sensitivity to the fungicides and there was no reduction of disease from the non-treated control. This confirms that in addition to practical resistance, resistance to mefenoxam and the QoI fungicides can be demonstrated under controlled conditions at a range of fungicide concentrations.

Two *P. cubensis* isolates, 1 and 2, were collected prior to the 2004 epidemic. These isolates served as both controls and for comparison, as widespread mefenoxam resistance and QoI resistance was not reported in the US prior to 2004. Isolate 1, collected from Charleston County, South Carolina in 1982 was definitely collected prior to the introduction of the QoI fungicides. Isolate 2 is known to be sensitive to fungicides since it is used in standard fungicide screening assays at DuPont. Both isolates 1 and 2 were sensitive to mefenoxam and azoxystrobin, at 1 μg/ml, though it was interesting that they were not sensitive at lower concentrations of azoxystrobin. Though we do not know why the virulence on *P. cubensis* changed on cucumber in 2004, our data suggests that the new race of the pathogen on cucumber could have been introduced from a production area where downy mildew infected cucumber crops were treated with mefenoxam and the QoI fungicides.

In 1984, Samoucha and Cohen reported that metalaxyl resistant isolates of *P. cubensis* produced disease at concentrations as high as 750 μg/ml and they reported $ED_{50}$ values between 500 and 750 μg/ml. In the Czech Republic, Urban and Lebeda reported that profuse sporulation was present on plants treated with 800 μg/ml (2004, 2006). In Ishii's study only 100 μg/ml azoxystrobin was tested which provided 100% control of the sensitive *P. cubensis* isolate and 0% control of the resistant isolate (2001). Future studies using higher concentrations of mefenoxam and azoxystrobin may be conducted to determine if there is a concentration of these fungicides that reduces disease caused by the US *P. cubensis* isolates. In addition, Samoucha and Cohen reported that after the reintroduction of metalaxyl in Israel, resistant strains of *P. cubensis* were the dominant phenotype after three years, even in crops that were not treated with the fungicide (1985). Gent et al. reported a similar phenomenon in the *Pseudoperonospora humuli*/ hops pathosystem where phenylamide resistance is a stable phenotype (2007). Determining and comparing the $EC_{50}$ values of the contemporary and historic isolates may provide interesting information about the persistence and dynamics of sensitive and resistant *P. cubensis* populations. This information will be helpful in the monitoring of mefenoxam and QoI resistance among *P. cubensis* populations in the US.

Empirical evidence suggests that the source of resistance in most pathogens to the QoIs is one of two single-point mutations. The F129L mutation, results from a shift from phenylalanine to lysine at residue 129 (Bartlett et al., 2002) and the G143A mutation leading to an amino acid change from glycine to alanine at residue 143 of the cytochrome *b* gene (Bartlett et al., 2002; Gisi et al., 2000). The G143A mutation has been identified in fungal pathogens in the US including *Alternaria alternata, A. tenussima, A. arborescens* on

pistachio (MA, 2003, 2004), *A. mali* on apple (Lu, 2003), *A. solania* on solanaceous crops (Rosenzwieg et al., 2008), *Colletotrichum graminicola* on turf (Avila-Adame et al., 2003), *Didymella bryoniae* on cucurbits (Langston 2002), *Erysiphe necator* on grape (Anonymous, 2007), *Pyricularia griseae* on turf (Kim 2003), and *Venturia pirina* on pear (Anonymous, 2007). The G143A mutation is also associated with QoI resistance in *P. cubensis* (Ishii et al., 2001).

Results from our study demonstrate that resistance to QoI fungicides is widespread in isolates from south to north in the eastern US. The molecular detection of the G143A mutation could be used to confirm this resistance, track the QoI-resistance of *P. cubensis* isolates from southern FL to northern areas like Michigan to see how the population changes with selection pressure and to provide information on the future use of the QoI fungicides.

The widespread occurrence of resistance to mefenoxam and the QoI fungicides may be a result of the mechanism of dispersal of *P. cubensis*. Because oospores are extremely rare and their role in reproduction and survival is uncertain (Lange et al., 1989a, 1989b; Palti and Cohen, 1980; Waterhouse and Brothers, 1981), sporangia must travel from locations where a frost sufficient to kill the host plant does not occur. *P. cubensis* can over-winter in the US as active mycelium on wild or cultivated cucurbits in areas that experience mild winters, such as southern Florida (Bains and Jhooty, 1976). Over-wintering areas can act as a harbor for resistant populations at the end of the production season and provide a source of resistant populations the following season resulting in the widespread and yearly occurrence of mefenoxam and QoI resistance. For example, in 2005 downy mildew caused severe crop losses and economic repercussions in Michigan, the number one pickling cucumber

producing state in the US, for the first time in decades. Immediately following the arrival of *P. cubensis* in Michigan, reports of reduced efficacy of mefenoxam and QoIs were reported (Gevens and Hausbeck, 2006). Regardless of the spread and development of QoI and mefenoxam resistance, these fungicides should not be used to control downy mildew on cucumber, however they do play a useful role in the control of other cucurbit plant pathogens, as well as in other pathosystems.

Because fungicides are required to manage downy mildew on cucumber and other cucurbit crops, the results from this study have significant practical implications on the management of *P. cubensis*. Resistance to mefenoxam and QoI fungicides has been identified in field experiments, in planta and by detection of genetic mutation, therefore applications of mefenoxam and QoI fungicides, even in mixtures with fungicides of different modes of action, are not recommended for the control of downy mildew on cucumber. To control downy mildew on cucumber, fungicides with different modes of action should be applied. Finally, because of the rapid development of fungicide resistance of *P. cubensis* and the spread of the pathogen by windborne sporangia, strict anti-resistance strategies should be followed.

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**Table 3.1.** List of *Pseudoperonospora cubensis* isolates tested for fungicide resistance to mefenoxam and azoxystrobin.

| | Isolate Origin | | | | Experiment | |
|---|---|---|---|---|---|---|
| **No.** | **State** | **County** | **Year** | **Host** | **1** | **2** |
| 1 | South Carolina | Charleston | 1982 | Cantaloupe | +[y] | +[y] |
| 2 | NA[z] | NA | NA | NA | +[y] | +[y] |
| 3 | North Carolina | Sampson | 2004 | Cucumber | + | − |
| 4 | North Carolina | Sampson | 2005 | Cucumber | + | − |
| 5 | North Carolina | Sampson | 2006 | Cucumber | + | − |
| 6 | North Carolina | Sampson | 2007 | Cucumber | + | − |
| 7 | North Carolina | Sampson | 2007 | Cantaloupe | +[y] | +[y] |
| 8 | North Carolina | Johnston | 2004 | Winter Squash | +[y] | − |
| 9 | North Carolina | Johnston | 2007 | Cucumber | + | − |
| 10 | North Carolina | Johnston | 2007 | Squash | − | +[y] |
| 11 | North Carolina | Johnston | 2007 | Melon | − | + |
| 12 | North Carolina | Henderson | 2007 | Cucumber | + | − |
| 13 | North Carolina | Duplin | 2007 | Cucumber | + | + |
| 14 | North Carolina | Pender | 2007 | Cucumber | − | + |
| 15 | North Carolina | Lenoir | 2005 | Watermelon | − | + |
| 16 | North Carolina | Hyde | 2006 | Cucumber | − | + |
| 17 | Michigan | Clinton | 2007 | Cucumber | +[y] | − |
| 18 | Michigan | Arenac | 2007 | Cucumber | + | − |
| 19 | Michigan | Oceana (central) | 2005 | Cucumber | + | − |
| 20 | Michigan | Oceana (east) | 2005 | Cucumber | − | + |
| 21 | Florida | Indian River | 2006 | Cucumber | + | − |
| 22 | Florida | Collier | 2007 | Cucumber | − | + |
| 23 | Indiana | Knox | 2005 | Pumpkin | +[y] | − |
| 24 | Indiana | LaPorte | 2007 | Pumpkin | + | − |
| 25 | South Carolina | Clarendon | 2005 | Cucumber | + | − |
| 26 | Ohio | Erie | 2007 | Cucumber | + | − |
| 27 | Maryland | Wicomico | 2007 | Cucumber | + | − |
| 28 | Delaware | New Castle | 2007 | Cucumber | + | − |
| 29 | Kansas | Johnson | 2007 | Cucumber | + | − |
| 30 | New Jersey | Cumberland | 2007 | Pumpkin | + | − |
| 31 | New York | Suffolk | 2007 | Cucumber | +[y] | − |

[y] F-test of fungicide sensitivity assay was significant at $p < 0.05$, resulting in statistically significant treatment differences (PROC GLM, SAS v. 9.1, Cary, NC).
[z] NA= information not available, isolate was obtained from DuPont and was collected prior to 2004 epidemic.

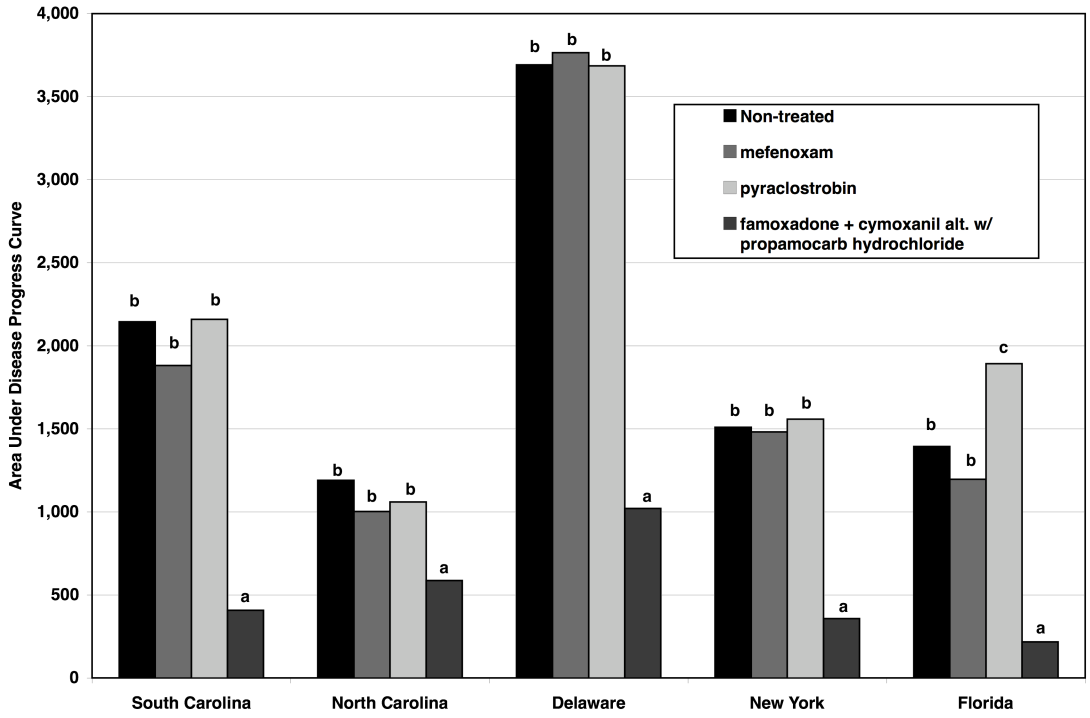


**Fig. 3.1.** Area under the disease progress curve (AUDPC) values for downy mildew severity on cucumber recorded in SC, NC, DE, NY and FL in 2007. Fungicide programs of mefenoxam, pyraclostrobin, famoxadone + cymoxanil alternated with propamocarb hydrochloride and a non-treated control were applied on a 5- to 7-day schedule and disease severity ratings were taken weekly. Bars with the same letter above are not significantly different according to the least significant difference (LSD) means separation test ($\alpha = 0.05$) for each location.

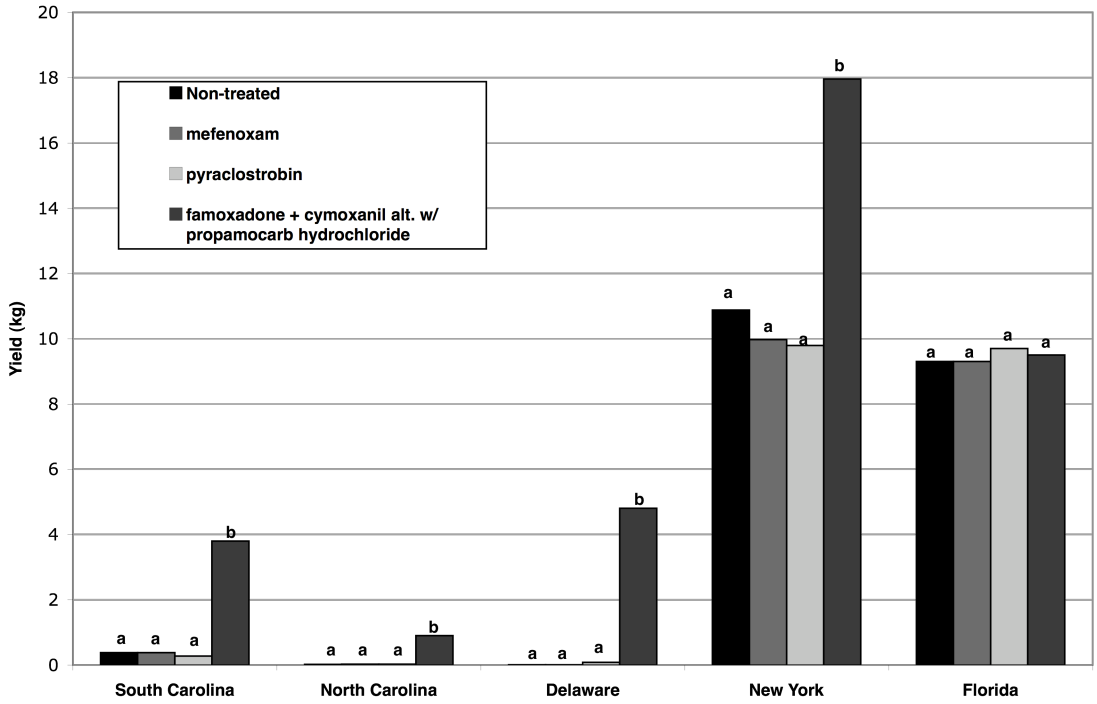


**Fig. 3.2.** Total marketable cucumber yield (kg) from fungicide efficacy field experiments for the control of downy mildew in SC, NC, DE, NY and FL in 2007. Fungicide programs of mefenoxam, pyraclostrobin, famoxadone + cymoxanil alternated with propamocarb hydrochloride and a non-treated control were applied on a 5- to 7-day schedule. Plots were harvested weekly at least twice throughout the season and data represents the combined marketable yield from all harvests. Bars with the same letter above are not significantly different according to the least significant difference (LSD) means separation test ($\alpha = 0.05$) for each location.

**Table 3.2.** Percent downy mildew control of isolates of *Pseudoperonospora cubensis* to concentrations of mefenoxam and azoxystrobin.

| **Fungicide** | **Percent control of *P. cubensis* isolate** | | | | | | |
|---|---|---|---|---|---|---|---|
| **(µg/ml)** | **1**[y] | **2**[y] | **7**[y] | **8** | **17** | **23** | **31** |
| 0 | 0 d[z] | 0 cde | 0 cde | 0 e | 0 b | 0 c | 0 b |
| Mefenoxam | | | | | | | |
| 0.01 | -10.13 d | 11.7 cd | -13.7 f | 84 a | 1.8 b | 0 c | 1.8 b |
| 0.1 | 3.8 d | 65.4 ab | 65.4 ab | 14.2 de | 0 b | 0 c | 1.8 b |
| 1.0 | 72 a | 48.9 abc | 27.5 def | 14.5 de | -0.1 b | 0 c | 0 b |
| 10 | 90.6 a | 94.12 a | 100 a | 9 e | -1.9 b | 0 c | 0 b |
| 100 | 53.7 abc | 71.9 ab | 100 a | 94.5 a | 11 a | 10.5 a | 0 b |
| Azoxystrobin | | | | | | | |
| 0.001 | 19.8 bcd | -4.3 de | -3.6 ef | 21.5 de | -3.7 b | 0 c | 0 b |
| 0.01 | 19.8 bcd | -38.9 e | 43.1 bcd | 32.4 cd | -1.9 b | 0 c | 0 b |
| 0.1 | 12.6 cd | 69.7 ab | 73 abc | 46.3 bc | -3.7 b | 0 c | 15.8 a |
| 1 | 53.7 abc | 21.5 bcd | 38.2 cde | 92.6 a | -1.9 b | 0 c | 0 b |
| 10 | 67.9 ab | 72.8 a | 56.2 bcd | 56.2 b | -3.7 b | 3.5 b | 0 b |
| 100 | 92.1 a | 65.6 ab | 85.2 ab | 89 a | -3.7 b | 0 c | 0 b |
| LSD | 49.6 | 50.6 | 42.5 | 22.6 | 9.1 | 3.0 | 4.9 |
| P-value | 0.0009 | 0.0002 | <0.0001 | <0.0001 | 0.026 | <0.0001 | <0.0001 |

[y] Isolates tested in two experiments.

[z] Values are means of three replicates or observations. Percent of disease control calculated as [(DS non-treated - DS treated)/DS non-treated] X 100. Means within columns followed by the same letter are not significantly different according to least significant difference (LSD) means separation test, $\alpha = 0.05$.

**Table 3.3**. Percent reduction of foliar necrosis from two concentrations of mefenoxam and azoxystrobin to 31 isolates of *Pseudoperonospora cubensis*.

| | Control (%) of *P. cubensis*[x] | | | | |
|---|---|---|---|---|---|
| | Mefenoxam | | Azoxystrobin | | Fluopicolide |
| Isolate Number | 1 µg/ml | 100 µg/ml | 1 µg/ml | 100 µg/ml | 0.024 µl/ml |
| 1[y] | 87.6 a[z] | 77.2 b | 77.1 b | 93 a | 100 |
| 2[y] | 65.8 b | 85.2 ab | 85.2 ab | 80 a | 100 |
| 7 | 36 c | 100 a | 100 a | 87.5 a | 100 |
| 8 | 14.4 d | 94.5 a | 94.5 a | 89.1 a | … |
| 26 | 13.6 de | 15 cd | 15 cd | -3.6 c | … |
| 4 | 7.2 def | 1.5 de | 1.5 de | -5.8 c | … |
| 29 | 7.0 def | 1.8 de | 1.8 de | 7 bc | … |
| 3 | 6.5 def | -4.9 e | -4.9 e | 18.7 b | … |
| 28 | 5.3 def | 0 de | 0 de | 1.8 bc | … |
| 21 | 3.5 def | 3.5 de | 3.5 de | 1.8 bc | … |
| 6 | 3.5 def | 7 cde | 7 cde | 1.8 bc | … |
| 11 | 1.8 def | -0.1 de | -0.1 de | -1.9 c | 100 |
| 9 | 0 def | 0 de | 0 de | 0 c | … |
| 14 | 0 def | 0 de | 0 de | 0 c | 100 |
| 31 | 0 def | 0 de | 0 de | 0 c | … |
| 24 | 0 def | 0 de | 0 de | 0 c | … |
| 13 | 0 def | 3.7 de | 3.7 de | 5.3 bc | 100 |
| 18 | 0 def | 0 de | 0 de | 0 c | … |
| 23 | 0 def | 22.8 c | 22.8 c | 0 c | … |
| 20 | 0 def | 0 de | 0 de | 0 c | 100 |
| 22 | 0 def | 0 de | 0 de | 0 c | 100 |
| 12 | 0 def | 5.3 de | 5.3 de | 0 c | … |
| 10 | 0 def | 1.9 de | 1.9 de | 5.6 bc | 100 |
| 30 | 0 def | 1.8 de | 1.8 de | 1.8 bc | … |
| 17 | -0.1 def | 11 cde | 11 cde | -3.7 c | … |
| 25 | -0.1 def | 3.6 de | 3.6 de | 3.5 bc | … |
| 27 | -1.9 ef | -1.9 e | -1.9 e | -1.9 c | … |
| 5 | -2.0 ef | 1.3 de | 1.3 de | -3.9 c | … |
| 16 | -3.7 f | -3.7 e | -3.7 e | -0.1 c | 100 |
| 19 | -6.1 f | -4.1 e | -4.9 e | 3.5 bc | … |
| 15 | -24.3 g | -27.7 f | -27.7 f | -32.7 d | 100 |
| **LSD** | 15.8 | 16.1 | 21.9 | 17.2 | |

[x] Percent of disease control calculated as [(DS non-treated - DS treated)/DS non-treated] X 100.

[y] Historic isolates collected prior to 2004 epidemic.

[z] Values are means of three replicates or observations. Means within columns followed by the same letter are not significantly different according to least significant difference (LSD) means separation test, $\alpha = 0.05$.

# CHAPTER 4 – FUNGICIDE EFFICACY AND APPLICATION TIMING FOR THE CONTROL OF DOWNY MILDEW ON CUCUMBER IN NORTH CAROLINA, 2005–2007.


## ABSTRACT

In 2004, downy mildew re-emerged as an important foliar disease on cucumber in North Carolina. In order to provide cucumber producers with effective fungicide programs, field experiments were conducted in Sampson County, North Carolina. Eight field experiments were conducted from 2005 through 2007. Four experiments investigated the efficacy of commercial fungicides for the control of downy mildew. Twenty-six commercial products were tested and five fungicides, famoxadone + cymoxanil, cymoxanil, cyazofamid, zoxamide, fluopicolide and propamocarb hydrochloride, reduced area under the disease progress curve (AUDPC) and resulted in significantly greater weight of marketable yield than the non-treated control. Reduced efficacy of mefenoxam, pyraclostrobin and azoyxstrobin was demonstrated, suggesting that practical resistance may be a problem in North Carolina. Delaying fungicide applications was also evaluated. Delay in fungicide application until disease was widespread resulted in greater AUDPC and reduced yields. A locally systemic + protectant program was compared to a protectant-only program and the locally systemic + protectant program provided superior disease control when applied within 2 to 3 weeks of *P. cubensis* detection. Finally, when disease severity (area under the disease progress curve) and weight of marketable yield where correlated using PROC CORR, very strong inverse associations (greater than -0.66, $P < 0.0001$) were demonstrated.

## INTRODUCTION

Cucurbit downy mildew caused by *Pseudoperonospora cubensis* is a destructive foliar disease of cucurbit crops worldwide (Cohen, 1981; Palti, 1974; Palti and Cohen, 1980). In the United States, downy mildew on cucumber (*Cucumis sativus* L.) has been successfully controlled through the use of resistant cultivars since the 1950s (Barnes and Epps, 1954; Colucci et al., 2006b; Holmes et al., 2006; Sitterly, 1972). Although the disease could be found on cucumber during the last several decades, its presence was inconspicuous and did not result in obvious crop losses (Peterson et al., 2002). In 2004, the disease on cucumber changed dramatically when a new race of the pathogen devastated cucumber-growing areas of North Carolina (NC), Delaware, Maryland and Virginia. Virtually all-modern commercially grown cucumber cultivars are downy mildew resistant, but these were not sufficient to prevent severe economic losses estimated at 40% region-wide (Colucci et al., 2006b; Holmes et al., 2006).

Because downy mildew is no longer controlled by the available commercial cucumber cultivars, fungicide applications are necessary (Sherf and MacNab, 1986; Thomas, 1996). To provide cucumber producers with the most appropriate and effective fungicide program for the management of downy mildew, a total of 26 commercial fungicides representing 22 different active ingredients were evaluated in 2005, 2006 and in two field experiments in 2007. In addition, annual field experiments were designed to evaluate the effect of delaying fungicide application for the management of downy mildew and to compare a locally systemic + protectant (LS + P) program to a protectant-only (P) program. In this chapter the data from all eight-field experiments is presented and the results are

summarized in an effort to provide recommendations to cucumber producers in NC and throughout the eastern United States for the management of downy mildew.

## MATERIALS AND METHODS

**Experimental design.** Fungicide efficacy and delay of application for the control of downy mildew on cucumber were evaluated in field experiments in 2005, 2006 and in two locations in 2007. Experiments were conducted in commercial cucumber production fields in Sampson County, North Carolina with the exception of one of the 2007 experiments, which took place at the North Carolina State Horticultural Crops Research Station in Clinton, NC, also located in Sampson County, NC. Efficacy and delay-of-application experiments took place side-by-side in the same fields. All experiments used the same experimental design with treatments established in four randomized complete blocks. Plots were bare-ground single rows and rows were 20 ft long with 5 ft borders on each end. Treated rows were alternated with non-treated border rows. Fungicide treatments were applied using a $CO_2$-pressurized backpack sprayer and handheld boom equipped with hollow cone nozzles (TXVS-26) calibrated to deliver 373.8 liters/ha at 45 psi. When plant vines began to run, applications were made using a 2-nozzle (0.48 m spacing) handheld boom with one pass.

Disease severity (percent of necrotic leaf area) ratings were taken weekly and area under the disease progress curve (AUDPC) was calculated. Fruit were harvested and yield was recorded. Fruit were separated as marketable and unmarketable (culls) based on shape. Misshaped fruit and fruit with >45° curvature are considered unmarketable.

**Fungicide efficacy experiments.** Overall, 26 commercial fungicides (Table 4.1)

were evaluated. Fungicides were applied as single commercial products, tank-mixed or alternated with other fungicides according to manufacturers' recommendations. Rates for all treatments are located in each results table (Tables 4.2 through 4.9).

**Delay of fungicide application.** In 2004, the LS + P program of famoxadone + cymoxanil (Tanos 50DF, 5.6 kg/ha) + mancozeb (Manzate 75DG, 2.24 kg/ha) alternated with propamocarb hydrochloride (Previcur Flex 6F, 1.4 liters/ha) + chlorothalonil (Bravo Weather Stik 6SC, 2.3 liters/ha) was found to be an effective fungicide program for the control of downy mildew on cucumber (Thornton et al., 2005). The delay of application studies were designed to compare the efficacy of the LS + P to the P (weekly applications of mancozeb applied as Manzate 75DG, 2.24 kg/ha) program in relation to application timing. Treatments were designed so that every week an additional application of each fungicide program was initiated. For example, in the first week of fungicide applications two treatments (one LS + P and one P) were applied, four treatments were applied in week two, six treatments were applied in week three, eight in the fourth week.

**2005 experiments.** The experiments were conducted in a commercial cucumber field near Spivey's Corner, NC (coordinates N35°11.603', W078°27.500'). Seed of cucumber cultivar Feisty (Harris Moran) was planted on 27 Aug. No irrigation was used. Plots were bare-ground single rows on approximately 1.1-m centers. Treatments were initiated at vine tip-over stage of plant growth. The first application was made with one pass with the single-nozzle boom, and the remaining applications were made using the 2-nozzle boom. Treatments were applied on approximately 7-day intervals with applications made on 20, 26 Sep, 4, and 11 Oct. Disease severity was rated on 28 Sep, 5, 12, 18 Oct. Fruit were

harvested on 11 and 18 Oct.

**2006 experiment.** The experiments were conducted in a commercial cucumber field near Spivey's Corner, NC (N35°11.367' W078°29.274'). Seed of cucumber cultivar Feisty was planted on 14 Aug. No irrigation was used. Plots were bare-ground single rows on approximately 0.8-m centers. For the fungicide efficacy trial, the first two applications were made with one pass with the single-nozzle boom, and the remaining applications were made using the 2-nozzle boom. Treatments were initiated when the majority of plants had three true-leaves. Treatments were applied on approximately 7-day intervals with application made on 29 Aug, 5, 11, 18, and 25 Sep. Disease severity was rated on 22, 25 and 29 Sep. Fruit were harvested on 2 Oct. For the delay of application experiment, the first three applications were made with one pass using the single-nozzle boom while the remaining applications were made using the 2-nozzle boom. Treatments were initiated one week after planting when plants had one true-leaf. Treatments were applied on approximately 7-day intervals with applications made on 21, 28 Aug, 5, 11, 18 and 25 Sep. Disease severity was rated on 22, 25 and 29 Sep. Fruit were harvested on 2 Oct.

**2007 experiment – Newton Grove, NC.** The experiment was conducted on a commercial cucumber field near Newton Grove, NC (N35°09.936', W78°23.645'). Seed of cucumber cultivar Sassy (Harris Moran) was planted on 8 Aug. Plots were irrigated once on 8 Sep. Plots were bare-ground single rows on approximately 0.6-m centers. The first three applications were made with the single-nozzle boom, and the remaining applications were made using the 2-nozzle boom. Treatments were initiated when the majority of plants had two true-leaves. Treatments were applied on approximately 7-day intervals with applications

made on 29 Aug; 5, 12, 19, 26 Sep; 3, 10 and 17 Oct. Fruit from the main harvest (1 Oct) were inadvertently harvested by the grower. Fruit from the fungicide efficacy trial were harvested on 24 Sep and 9 Oct. Fruit for the delay of application experiment were harvested on 21 Sep and 9 Oct.

**2007 experiments – Clinton, NC.** The experiment was conducted at the Horticultural Crops Research Station in Clinton, NC (N35°01.35', W78°17.11'). Seed of cucumber cultivar Lafayette (Nunhems) was planted on 29 Aug. Irrigation by linear sprinkler system was used as needed. Plots were bare-ground single rows on approximately 0.9-m centers. The first three applications were made with the single-nozzle boom, and the remaining applications were made using the 2-nozzle boom. Treatments were initiated when the majority of plants had two true-leaves. Treatments were applied on approximately 7-day intervals with applications on 12, 19, 26 Sep, 3, 10, and 17 Oct. Disease severity was rated on 26 Sep; 10, 17, and 24 Oct. Fruit were harvested on 15 and 29 Oct.

**Statistical analysis.** Statistics were calculated using ARM software (version 7.4.2, Gylling Data Management, Brookings, SD) and means separation was conducted using Student Newman Keuls (2005 and 2006) or Waller-Duncan k-ratio t-test (k=100) (2007).

SAS (version 9.1.3, Cary, NC) (PROC GLM) was used to determine the effects of fungicide program, application timing and the fungicide by timing interaction in the delay-of-fungicide application timing experiments. Box and whisker plots were created for the results of the delay of fungicide application experiments with PROC BOXPLOT. Correlations between disease severity (AUDPC) and marketable yield for both the fungicide efficacy and delay of application experiments were determined using PROC CORR.

## RESULTS

**Fungicide efficacy.** Downy mildew was detected on the day of the first fungicide application in 2005, on the second fungicide application in 2006 and on the week of the third fungicide application in the 2007 experiments. Signs and symptoms were initially detected at low levels (less than 1% incidence) in each experiment and disease progressed quickly, especially when environmental conditions were favorable (i.e. cool nights and/or abundant moisture due to rain, dew or humidity) for the development of disease. Plants never reached optimum health in the 2006 experiment because of heavy rains and problems with plant stand.

In all four of the field experiments, treatments that resulted in the lowest AUDPC values or percent disease included propamocarb hydrochloride, famoxadone + cymoxanil, cymoxanil, cyazofamid and zoxamide (Table 4.2, 4.3, 4.4 and 4.5). In the 2007 experiments, the new fungicide fluopicolide was identified as one of the best treatments. When propamocarb hydrochloride was applied alone it did not perform as well as it has as part of combination programs (Table 4.4 and 4.5).

Downy mildew had a dramatic impact on cucumber yield and the proportion of marketable fruit (by weight) generally decreased as the level of disease increased. Treatments that resulted in the lowest percentage of disease also resulted in the greatest weight of marketable yield, including fluopicolide, and rotations involving propamocarb hydrochloride, famoxadone + cymoxanil, cymoxanil, cyazofamid and zoxamide (Tables 4.2, 4.3, 4.4 and 4.5). In 2005, the best yielding treatment, involving cymoxanil and propamocarb hydrochloride, produced 67.4 times greater marketable fruit weight than the non-treated control. This treatment also resulted in a decrease in disease of 71.6% compared to the non-treated control.

In 2006, 20.2% of the yield from the program involving propamocarb hydrochloride alternated with cyazofamid was unmarketable fruit compared to 100% in the non-treated control. Treatments including mefenoxam, pyraclostrobin, azoxystrobin, fluazinam and mandipropamid, did not yield statistically different from the non-treated control, which didn't produce any marketable fruit (Table 4.3). In the 2007 experiment in Clinton, NC, yield from the non-treated control was 3.4% marketable fruit compared to 45.7% marketable fruit from the fluopicolide treatment.

Correlations between disease severity (AUDPC) and weight of marketable yield were -0.80, -0.66 and -0.79 ($P < 0.0001$) for 2005, 2006 and 2007-Clinton, NC experiments, respectively (Figs. 4.1, 4.2 and 4.3). This data demonstrates a very strong inverse association of AUDPC and marketable yield. In the 2007 Newton Grove experiment, no difference in yield among treatments was detected (data not presented) because of the inadvertent harvest.

**Delay of fungicide application.** Fungicide program had a significant effect on AUDPC in 2006 and both 2007 experiments (*F* test significant at $P <0.0001$). Fungicide program also had a significant effect on marketable yield in 2006 and the 2007 Clinton, NC experiment ($P <0.0001$) and the 2005 experiment ($P = 0.053$). In 2005, disease severity and marketable yield of the LS + P program was superior to the P program, especially when fungicides were applied earlier in the season (Table 4.6). In 2006, the LS + P program was superior to the P program when fungicides were applied within 2 to 3 weeks of disease detection (Table 4.7). In the 2007 Newton Grove experiment, the LS + P program was superior to the P program when the treatment was initiated within one week of downy mildew detection or earlier (Table 4.8).

In 2005 downy mildew was detected on the day of the first fungicide application and the earlier treatments were initiated, the greater the disease control, regardless of materials used. If plants were not treated within 2 to 3 weeks of disease detection, treatments had little to no effect on disease control. A 6-day delay in initiating the LS + P program resulted in a 54% reduction in yield of marketable fruit (Figs. 4.4–4.7).

In contrast, in 2006 even though the first application of the LS + P was made early, the LS + P treatment that was initiated on the second application date yielded significantly greater and had less disease present on the last rating (Table 4.7, Fig. 4.3). Disregarding the first application of the combination treatment, if plants were not treated within 2 to 3 weeks of disease detection, treatments had little or no effect on disease control compared to the non-treated control.

In the 2007 Newton Grove, NC experiment disease progressed rapidly after the third application, when environmental conditions were favorable. The third fungicide initiation date of the LS + P program had less disease present at the final disease rating and the lowest AUDPC value (Table 4.8, Fig. 4.10). If plants were not treated within 2 to 3 weeks of disease detection, treatments had little or no effect on disease control. Because data from the main harvest was lost, differences in yield among treatments were not as dramatic as in previous tests (Table 4.8, Fig. 4.6).

In the 2007 Clinton, NC experiment, downy mildew was widespread throughout the plots by the fourth application. By waiting one week after detection, total yield was reduced by 47.1%. The LS + P program outperformed the P program when applications started on the fourth application date or earlier (Table 4.9). The LS + P program yielded 41% greater

than the P program when both received six weekly applications starting on 12 Sep, this trend can be seen in Fig. 4.7.

Application timing had a significant effect on AUDPC in all four experiments (F test significant at $P < 0.0001$) and on marketable yield in all experiments ($P < 0.0001$) except the 2007 Newton Grove, NC trial. These trends can be seen in the box and whisker plots (Figs. 4.4–4.11).

Correlation between disease severity (AUDPC) and marketable was $r = -0.84$, $-0.86$, and $-0.83$ ($p < 0.0001$) for 2005 (Fig. 4.12), 2006 (Fig. 4.13) and 2007 (Fig. 4.14), respectively, demonstrating a very strong inverse association of AUDPC and marketable yield.

## DISCUSSION

In order to provide growers in NC with effective fungicide programs, we tested the efficacy of commercially available fungicides marketed for the control of downy mildew on cucumber in three consecutive seasons in Sampson County, NC. Most fungicides were applied in combination or in rotation with fungicides of different modes of action according to manufacturers' recommendations. The effect of delaying fungicide applications was also evaluated in order to determine how early growers should begin spraying to manage downy mildew in their cucumber fields.

Because downy mildew disease pressure is great during the second, or fall, cucumber crop in NC (planted in late July through September), all field experiments were conducted during the fall cropping cycle. All cucumber cultivars used were provided by and planted by

the grower whose field was utilized for the experiments, with the exception of the Clinton, NC experiment. Cultivars Feisty (Harris Moran), Sassy (Harris Moran) and Lafayette (Nunhems), have a typical level of resistance to downy mildew available in modern cultivars, but this resistance was inadequate to control the disease.

With no fungicide applications, *P. cubensis* caused severe foliar disease (greater than 83% necrosis in all experiments) and reduced the weight of marketable yields. In several experiments not one marketable fruit was harvested in the non-treated plots. Of the 26 fungicides tested, six stood out as the most efficacious, resulting in the lowest amount of downy mildew disease severity and highest marketable yields. Fungicide programs involving famoxadone + cymoxanil (Tanos), cymoxanil (Curzate), propamocarb hydrochloride (Previcur Flex), cyazofamid (Ranman), zoxamide (Gavel) and fluopicolide (Presidio), resulted in greatly reduced downy mildew severity and increased yields compared to the non-treated control. These fungicides resulted in significantly less downy mildew severity and increased yields compared to fungicides with other active ingredients.

Products that were expected to provide control of downy mildew, including mefenoxam (Ridomil Gold) and the QoI fungicides, azoxystrobin (Quadris), pyraclostrobin (Cabrio), did not provide an effective level of downy mildew control in all four-field experiments, even in rotation with other classes of fungicides. In 2005, the best treatment, involving rotations with propamocarb hydrochloride and cymoxanil, provided 71.6% control compared to the non-treated control while pyraclostrobin provided 24.3% downy mildew control and mefenoxam + chlorothalonil provided 39.1% control. In 2007, mefenoxam and pyraclostrobin did not provide significantly better downy mildew management than the non-

treated control. Results indicate that practical resistance to these fungicides is widespread in the eastern United States (Chapter 3 of this thesis) and these products should not be used to control downy mildew on cucumber. The term practical resistance indicates consequent, observable loss of disease control by a fungicide and is preferred to field resistance which indicates merely the presence of resistant variants in field populations (at whatever frequency or severity) (Brent and Hollomon, 2007).

The delay of application experiments emphasized the importance of early detection, early application of fungicides, and the proper fungicide program for successful management of downy mildew. Results from 2005 demonstrated that by delaying application of the LS + P program one week from symptom detection, marketable yield was reduced 53.5%. By delaying two weeks, marketable yield was reduced by 93.6%. Three years of data also indicate that a LS + P program is superior to the P program. In each experiment marketable yield was greater and disease severity was reduced in the LS + P program when compared to the P program. In 2006, the best performing treatment was the LS + P treatment that was initially applied on 28 Aug (Table 4.7 and Figs. 4.5 and 4.9). In this treatment famoxadone + cymoxanil tank-mixed with mancozeb was present when the pathogen is believed to have arrived (incubation period 4-12 days). This treatment performed better than the LS + P treatment that was initially applied a week earlier and had propamocarb hydrochloride tank-mixed with chlorothalonil present when the pathogen is first thought to have appeared. This indicates that certain fungicides have better preventative control, than others. Finally, if applications are not applied prior within two to three weeks after downy mildew detection, no fungicide program will effectively manage the disease.

When epidemics appear early in the reproductive phase of cucurbit plant growth, yield losses can be great (Keinath, 2001). Results from the fungicide efficacy and delay of fungicide application experiments show that disease severity and marketable yield are strongly inversely correlated. This suggests that in order to receive the best yields possible, fungicide applications must be made prior to widespread downy mildew development. Future studies should aim to define and quantify the ability of cucumber to tolerate a certain amount of foliage loss due to downy mildew without reducing yields.

Since 2004, downy mildew of cucumber continues to be one of the most important foliar disease problem on cucumber in the eastern US. Because commercially available cucumber cultivars will not manage downy mildew alone, fungicides are necessary for management. Fungicides with the active ingredients famoxadone, cymoxanil, propamocarb hydrochloride, cyazofamid, zoxamide and fluopicolide have been shown to be effective at managing downy mildew on cucumber. Manufacturers' recommendations and strict anti-resistance measures should be adhered to in order to make sure that resistance problems don't occur in the future. Our results also indicate that fungicides should be applied in a timely manner, before the disease becomes widespread to effectively manage the disease and obtain maximum yields. Because of the imminent threat of downy mildew in the southeast US, especially crops of cucumber grown in the mid- to late summer and fall, it is important to scout fields for downy mildew symptoms and signs of the pathogen, communicate with cucumber-producing neighbors and to follow the cucurbit downy mildew forecasts generated by the North American Plant Disease Forecast Center (http://www.ces.ncsu.edu/depts/pp/cucurbit/).

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**Table 4.1.** Fungicides tested in North Carolina for efficacy to downy mildew, 2005 through 2007.

| | | | Year(s) Tested | | |
|---|---|---|---|---|---|
| **Manufacturer** | **Active ingredient(s)** | **Trade name** | **2005** | **2006** | **2007** |
| Syngenta Crop Protection | Mefenoxam + chlorothalonil | Ridomil Gold Bravo | + | + | + |
| | Mefenoxam | Ridomil Gold | − | − | + |
| | Chlorothalonil | Bravo Weather Stick | + | + | + |
| | Mandipropamid | Revus | + | + | + |
| | Azoxystrobin + chlorothalonil | Quadris Opti | − | + | − |
| | Azoxystrobin | Quadris | − | + | + |
| BASF Ag Products | Dimethomorph | Forum | + | − | − |
| | Pyraclostrobin | Cabrio | + | + | + |
| | Pyraclostrobin + boscalid | Pristine | − | − | + |
| | Boscalid | Endura | − | − | + |
| DuPont Crop Protection | Cymoxanil + famoxadone | Tanos | + | + | + |
| | Mancozeb | Manzate Pro-Stick | + | + | + |
| | Cymoxanil | Curzate | + | + | + |
| | Copper hydroxide | Kocide 3000 | − | − | + |
| Bayer CropScience | Propamocarb hydrochloride | Previcur Flex | + | + | + |
| | Fenamidone | Reason | + | − | − |
| Dow AgroSciences | Zoxamide | Gavel | + | + | + |
| | Mancozeb | Dithane | + | − | − |
| AgraQuest, Inc. | *Bacillus pumulis* QST 2808 | Sonata | + | − | − |
| | Alkylbenzene sulfonate | Biotune | + | − | − |
| FMC Corp. | Cyazofamid | Ranman | + | + | + |
| Helena Chemical Company | Potassium phosphite | Helena ProPhyt | + | − | − |
| ORO Agri, Inc. | Sodium tetraborohydrate decahydrate | Prev-Am | + | − | − |
| Biagro Western Sales, Inc. | Nitrogen, phosphoric acid, soluble potash | Nutri-Phite Magnum | + | − | − |
| Valent | Fluopicolide | Presidio | − | − | + |

**Table 4.2.** Results of 2005 fungicide efficacy experiment for the control of downy mildew on cucumber, Sampson County, NC. (Modified from Colucci et al., 2006b).

| | Disease severity | | Total yield | |
|---|---|---|---|---|
| **Treatment, rate of product per acre** | **18 Oct [z]** | **AUDPC [x]** | **Marketable(kg/plot)** | **% Cull** |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Curzate 60DF, 3.2 oz + Manzate Pro-Stick 75DG, 2lb | 26.3 h | 445.2 j | 7.28 ab | 28.78 fg |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Gavel 75 DF, 2 lb | 32.5 gh | 526.7 ij | 7.04 ab | 31.62 fg |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt | 31.3 gh | 550.2 ij | 8.13 a | 25.28 g |
| Ranman 400SC, 2.75 fl oz + Silwet L-77, 2 fl oz alt. w/ Tanos 50DF, 8 oz + Bravo Weather Stik 6SC, 2 pt | 45.0 e-h | 663.2 hij | 6.08 abc | 32.69 fg |
| Curzate 60DF, 3.2 pt + Manzate Pro-Stick 75DG, 2lb | 38.8 fgh | 707.4 hij | 5.55 a-d | 34.33 fg |
| Tanos 50DF, 8 oz + Gavel 75DF, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Curzate 60DF, 3.2 oz | 37.5 fgh | 715.5 hij | 5.04 b-e | 42.25 efg |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt w/ Manzate Pro Stick 75DG 2 lb + Curzate 60DF, 3.2 oz | 41.3 fgh | 767.0 g-j | 4.64 b-f | 44.34 efg |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Gavel 75DF, 2 lb | 46.3 d-g | 795.1 f-j | 4.24 b-g | 42.14 efg |
| ProPhyt 4L, 4 pt + Bravo Weather Stik 6SC, 2 pt. | 48.8 d-g | 876.0 e-i | 3.72 c-h | 35.00 fg |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Ridomil Gold Bravo 76.5WP, 2 lb. | 56.3 c-f | 1013.0 d-h | 3.22 c-i | 49.62 d-g |
| Forum 550SC, 6.14 fl oz + Dithane 75DF Rainshield, 2 lb alt. w/ Bravo Weather Stik 6SC, 2 pt | 63.8 cd | 1088.0 c-g | 2.15 e-i | 57.26 b-f |
| Reason 500SC, 5.5 fl oz + Bond 480SL, 0.1% v/v alt. w/ Previcur Flex 6F, 1.2 pt. | 61.3 cde | 1122.4 c-f | 2.51 d-i | 54.10 c-g |
| Manzate Pro-Stick 75DG, 2 lb | 70.0 bc | 1201.2 b-e | 2.35 d-i | 52.34 d-g |
| Prev-Am, 2.5 pt/100gal + Nutri-Phite Magnum, 5 pt/100 gal (5-7 d) | 72.5 bc | 1233.9 bcd | 0.97 ghi | 72.56 a-d |
| Bravo Weather Stik 6SC, 2 pt. | 68.8 bc | 1300.7 bcd | 1.70 e-i | 65.96 b-e |
| BAS 536 00F, 26 oz alt. w/ Bravo Weather Stik 6SC, 2 pt. | 82.5 ab | 1340.4 bcd | 1.33 f-i | 68.36 a-e |
| Cabrio 20EG, 8 oz + Bravo Weather Stik 6SC, 2 pt alt. w/ Forum 550SC, 6.14 fl oz + Bravo Weather Stik 6SC, 2 pt | 70.0 bc | 1389.1 bc | 0.97 ghi | 74.37 a-d |
| Sonata, 3 qt + Biotune, 0.2% v/v | 92.5 a | 1528.0 ab | 0.52 hi | 83.06 ab |
| NOA 446510, 8.2 fl oz | 83.8 ab | 1535.0 ab | 0.50 hi | 80.18 abc |
| Non-treated | 92.5 a[y] | 1789.8 a | 0.11 i | 93.65 a |
| LSD (*P*=0.05) | 12.4 | 233.7 | 2.08 | 17.58 |

[z] Disease rating scale based on percentage of necrotic foliage (0-100).

[y] Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Student-Newman-Keuls).

[x] AUDPC = Area under disease progress curve.

**Table 4.3.** Results of 2006 fungicide efficacy experiment for the control of downy mildew on cucumber, Sampson County, NC. (Modified from Colucci et al., 2007a).

| | Disease severity[z] | | Total yield | |
|---|---|---|---|---|
| **Treatment, rate of product per acre** | **29 Sep** | **22 Sep** | **Marketable (kg/plot)** | **% Cull** |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/Ranman 400SC, 2.75 fl oz + Silwet L-77, 2 fl oz | 41.3 g[y] | 40.0 d | 2.02 ab | 20.2 e |
| Tanos 50DF, 8 oz + Bravo Weather Stik 6SC, 2 pt alt. w/ Ranman 400SC, 2.75 fl oz + Silwet L-77, 2 fl oz | 47.5 fg | 42.5 cd | 1.84 ab | 33.1 e |
| Curzate 60DF, 3.2 oz + Bravo Weather stik 6SC, 2 pt alt. w/ Ranman 400SC, 2.75 fl oz + Silwet L-77, 2 fl oz | 47.5 fg | 42.5 cd | 2.48 a | 30.0 e |
| Previcur Flex 6F, 1.2pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Gavel 75 DF, 2 lb | 50.0 efg | 45.0 cd | 1.67 ab | 50.6 cde |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Quadris Opti. | 50.0 efg | 43.8 cd | 1.44 abc | 40.3 de |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Curzate 60DF, 3.2 oz + Manzate Pro-Stick 75DG, 2lb | 51.3 efg | 48.8 bcd | 1.13 bc | 45.9 cde |
| Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb | 56.3 def | 46.3 cd | 0.86 bc | 54.4 b-e |
| Curzate 60DF, 3.2 pt + Manzate Pro-Stick 75DG, 2lb | 57.5 c-f | 51.3 bcd | 0.77 bc | 71.7 a-d |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Cabrio 20 EG, 8 oz + Bravo Weather Stik 6SC, 2 pt | 61.3 b-e | 51.3 bcd | 1.04 bc | 53.0 b-e |
| Ridomil Gold Bravo 76.5WP, 2 lb alt. w/ Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb. | 63.8 bcd | 50.0 bcd | 0.41 c | 76.4 a-d |
| Cabrio 20 EG, 8 oz alt. w/ Bravo Weather Stik 6SC, 2 pt. | 67.5 bcd | 62.5 abc | 0.05 c | 92.0 ab |
| Quadris 2.08SC, 11 fl oz alt. w/ Bravo Weather Stik 6SC, 2 pt. | 68.8 bcd | 58.8 a-d | 0.09 c | 93.8 ab |
| Bravo Weather Stik 6SC, 2 pt. | 68.8 bcd | 57.5 a-d | 0.13 c | 86.5 abc |
| Omega 500F, 5.5 fl oz | 68.8 bcd | 68.8 ab | 0.13 c | 81.0 abc |
| Revus 250SC, 8.2 fl oz + Activator 90L, 0.125% v/v | 70.0 bcd | 58.8 a-d | 0.13 c | 93.8 ab |
| Ridomil Gold Bravo alt. w/ Bravo Weather Stik 6SC, 2 pt. | 71.3 bc | 57.5 a-d | 0.22 c | 86.0 abc |
| Manzate Pro-Stick 75DG, 2 lb | 73.8 b | 67.5 ab | 0.09 c | 81.8 abc |
| Non-treated | 83.8 a | 75.0 a | 0.0 c | 100.0 a |
| LSD (*P*=0.05) | 8.58 | 12.03 | 0.81 | 24.98 |

[z] Disease rating scale based on percentage of necrotic foliage (0-100).

[y] Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Student-Newman-Keuls).

**Table 4.4.** Results of 2007 fungicide efficacy experiment for the control of downy mildew on cucumber, Newton Grove, Sampson County, NC. (Modified from Colucci et al., 2008a).

| | Disease severity[z] | | | |
|---|---|---|---|---|
| **Treatment, rate of product per acre** | **26 Sep** | **3 Oct** | **9 Oct** | **AUDPC** |
| Ranman 400SC, 2.75 fl oz + Silwet L-77, 2 fl oz (1,3,5,7)[y] alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 5.0 f | 35.0 k | 53.8 i | 458.75 h[x] |
| Curzate 60DF, 3.2 oz + Manzate Pro-Stick 75DG, 3lb (1,3,5,7) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 5.0 f | 36.3 jk | 58.8 ghi | 481.9 gh |
| Manzate Pro-Stick 75DG, 3 lb (1,2,3); Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 3 lb (4,6) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (5,7) | 2.5 f | 45.0 hij | 66.3 fgh | 526.2 fgh |
| Presidio 4SC, 3 fl oz (1-7). | 7.5 ef | 38.8 ijk | 57.5 hi | 529.4 fgh |
| Gavel 75DF, 2 lb (1,3,5,7) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 5.0 f | 43.8 h-k | 67.5 fgh | 556.9 fgh |
| Kocide 3000 46DF, 1 lb + Bravo Weather Stik 6SC, 2 pt | 5.0 f | 43.8 h-k | 67.5 fgh | 556.9 fgh |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 3 lb (1,3,5,7) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 6.3 f | 45.0 hij | 68.8 efg | 586.2 fgh |
| Manzate Pro-Stik 75DG, 3 lb (1-7) | 5.0 f | 51.3 fgh | 70.0 def | 613.1 f |
| Bravo Weather Stik 6SC, 2 pt (1-7) | 8.8 def | 46.3 ghi | 68.8 efg | 629.4 f |
| Ridomil Gold Bravo 76.5WP, 2lb (1,3,5,7) alt. w/ Bravo Weather Stik, 6SC, 2 pt (2,4,6) | 8.8 def | 46.3 ghi | 70.0 def | 633.1 f |
| Cabrio 20EG, 8 oz (1,3,5,7) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6) | 8.8 def | 47.5 ghi | 70.0 def | 641.25 ef |
| Kocide 46DF 3000, 1 lb (1-7). | 8.8 def | 63.8 cde | 81.3 bc | 780.63 de |
| Endura 70WG, 2 pt (1,3,5,7) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6) | 13.8 cde | 55.0 efg | 82.5 abc | 797.5 d |
| Quadris 2.08SC, 11 fl oz (1,3,5,7) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6). | 15.0 cd | 55.0 efg | 78.8 cde | 803.7 d |
| Pristine 6SC, 2 pt (1,3,5,7) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6) | 13.8 cde | 57.5 ef | 80.0 bcd | 806.2 d |
| Previcur Flex 6F, 1.2 pt (1-7) | 8.8 def | 67.5 bcd | 90.0 ab | 831.2 d |
| Revus 250SC, 8 oz + Activator 90, 0.125% v/v (1-7).. | 18.8 bc | 60.0 def | 85.0 abc | 907.5 cd |
| Ridomil Gold 4EC, 0.25 pt (1-7) | 17.5 c | 73.8 ab | 92.5 a | 1001.9 bc |
| Cabrio 20EG, 14 oz (1-7) | 25.0 ab | 70.0 abc | 85.0 abc | 1060.0 ab |
| Non-treated | 30.0 a | 77.5 a | 88.8 abc | 1190.0 a |
| LSD (*P*=0.05) | 6.9 | 10.5 | 11.7 | 157.2 |

[z] Disease rating scale based on percentage of necrotic foliage (0-100). [y]Numbers represent weeks that fungicides were applied.

[x] Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Waller-Duncan k=100).

**Table 4.5.** Results of 2007 fungicide efficacy experiment for the control of downy mildew on cucumber, Clinton, Sampson County, NC. (Modified from Colucci et al., 2008b).

| Treatment, rate of product per acre | AUDPC | Disease severity[z] 24 Oct | Total yield | |
|---|---|---|---|---|
| | | | Total (kg/plot) | % cull |
| Presidio 4SC, 3 fl oz (1-6)[y] | 708.7 h[x] | 56.3 j | 3.6 a | 54.3 k |
| Curzate 60DF, 3.2 oz + Manzate Pro-Stick 75DG, 3lb (1,3,5) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 883.7 g | 65.0 i | 3.1 ab | 57.3 ijk |
| Ranman 400SC, 2.75 fl oz + Silwet L-77, 2 fl oz (1,3,5) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 927.5 fg | 66.3 hi | 2.9 bc | 62.8 h-k |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 3 lb (1,3,5) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 988.7 fg | 70.0 ghi | 2.7 bc | 68.3 g-j |
| Gavel 75DF, 2 lb (1,3,5) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (2,4,6) | 1006.2 fg | 71.3 fgh | 2.5 c | 62.2 h-k |
| Manzate Pro-Stick 75DG, 3 lb (1,2); Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 3 lb (3,5) alt. w/ Previcur Flex 6F, 1.2 pt + Manzate Pro-Stick 75DG, 3 lb (4,6) | 1071.9 ef | 71.3 fgh | 2.7 bc | 56.3 jk |
| Bravo Weather Stik 6SC, 2 pt (1-6) | 1168.1 de | 76.3 ef | 1.5 de | 75.8 efg |
| Manzate Pro-Stik 75DG, 3 lb (1-6). | 1181.2 cde | 77.5 de | 1.7 de | 71.6 fgh |
| Cabrio 20EG, 8 oz (1,3,5) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6). | 1181.2 cde | 78.8 cde | 1.3 ef | 97.4 a |
| Previcur Flex 6F, 1.2 pt (1-6). | 1190.1 cde | 75.0 efg | 1.5 de | 81.2 c-f |
| Endura 70W, 2 pt (1,3,5) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6) | 1198.7 cde | 80.0 b-e | 1.2 ef | 83.9 cde |
| Pristine 38WG 2 pt (1,3,5) alt w/ Bravo Weather Stik 6SC, 2 pt (2,4,6) | 1225.0 cde | 77.5 de | 1.4 de | 81.8 c-f |
| Ridomil Gold Bravo 78WP, 2lb (1,3,5) alt. w/ Bravo Weather Stik, 6SC, 2 pt (2,4,6) | 1242.5 cd | 78.8 cde | 1.9 d | 68.8 ghi |
| Kocide 3000 46DF, 1lb + Bravo Weather Stik 6SC, 2 pt (1-6) | 1268.7 cd | 77.5 de | 1.4 de | 78.6 d-g |
| Quadris 2.08SC, 11 fl oz (1,3,5) alt. w/ Bravo Weather Stik 6SC, 2 pt (2,4,6). | 1308.1 bcd | 82.5 a-d | 1.3 def | 89.8 a-d |
| Kocide 3000 46DF, 1lb (1-6) | 1330.0 bc | 80.0 b-e | 1.2 ef | 84.5 b-e |
| Revus 250SC, 8 oz + Activator 90, .125% v/v (1-6). | 1426.2 ab | 82.5 a-d | 0.8 fg | 92.9 abc |
| Cabrio 20EG, 14 oz (1.6) | 1531.25 a | 83.8 abc | 0.7 fg | 97.4 a |
| Ridomil Gold 4EC, .25 pt (1-6). | 1548.7 a | 83.8 abc | 0.54 g | 96.2 ab |
| Non-treated | 1548.7 a | 86.3 a | 0.6 g | 96.6 a |
| LSD (*P*=0.05) | 169.13 | 6.0 | 0.6 | 12.78 |

[z] Disease rating scale based on percentage of necrotic foliage (0-100).
[y] Numbers in parentheses represent the weeks that fungicides were applied.
[x] Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Waller-Duncan k=100).

**Table 4.6.** Results of 2005 delay of fungicide application experiment, Sampson County, NC. (Modified from Colucci et al., 2006c).

| | Disease severity | | Total yield | |
|---|---|---|---|---|
| **Treatment, rate of product per acre** | **18 Oct[z]** | **AUDPC[w]** | **Marketable (kg/plot)** | **% Cull** |
| Non-treated | 85.0 a[y] | 1563.4 a | 0.30 c | 87.9 a |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (1,2,3,4)[x] | 28.8 c | 603.7 e | 6.90 a | 27.3 c |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (2,3,4) | 37.5 bc | 872.0 de | 3.21 b | 42.1 bc |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (3,4) | 53.8 b | 1216.0 bc | 0.44 c | 87.5 a |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (4) | 83.8 a | 1543.2 a | 0.38 c | 86.4 a |
| Manzate Pro-Stick 75DG, 2lb (1,2,3,4) | 50.0 b | 779.6 e | 3.77 b | 40.6 bc |
| Manzate Pro-Stick 75DG, 2lb (2,3,4) | 56.3 b | 1076.6 cd | 2.50 bc | 54.7 b |
| Manzate Pro-Stick 75DG, 2lb (3,4) | 72.5 a | 1329.7 abc | 0.51 c | 79.9 a |
| Manzate Pro-Stick 75DG, 2lb (4) | 82.5 a | 1441.1 ab | 0.69 c | 82.2 a |
| LSD (*P*=0.05) | 14.2 | 223.3 | 1.70 | 17.6 |

[z]Disease rating scale based on percentage of necrotic foliage (0-100).
[y]Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Student-Newman-Keuls).
[x]Numbers in parentheses represent the weeks that fungicides were applied.
[w]AUDPC = Area under disease progress curve.

**Table 4.7.** Results of 2006 delay of application experiment, Sampson County, NC. (Modified from Colucci et al., 2007b).

| | Disease severity[z] | | Total yield | |
|---|---|---|---|---|
| **Treatment, rate of product per acre** | **29 Sep** | **22 Sep** | **Marketable (kg/plot)** | **% Cull** |
| Non-treated | 83.8 a[y] | 76.3 a | 0.04 b | 94.2 a |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (1,2,3,4,5,6)[x] | 58.8 bc | 58.8 ab | 0.77 b | 47.9 cd |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (2,3,4,5,6) | 42.5 d | 38.8 c | 1.94 a | 28.0 d |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (3,4,5,6) | 52.5 cd | 53.8 b | 1.49 a | 34.5 d |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (4,5,6) | 51.3 cd | 61.3 ab | 0.63 b | 59.9 bc |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (5,6) | 67.5 b | 67.5 ab | 0.04 b | 86.8 ab |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (6) | 70.0 b | 72.5 ab | 0.01 b | 96.9 a |
| Manzate Pro Stick 75DG, 2lb (1,2,3,4,5,6) | 67.6b | 68.8 ab | 0.18 b | 86.7 ab |
| Manzate Pro Stick 75DG, 2lb (2,3,4,5,6) | 65.0 b | 61.3 ab | 0.41 b | 69.0 abc |
| Manzate Pro Stick 75DG, 2lb (3,4,5,6) | 63.8 b | 63.8 ab | 0.23 b | 83.7 ab |
| Manzate Pro Stick 75DG, 2lb (4,5,6) | 71.3 b | 67.5 ab | 0.05 b | 94.5 a |
| Manzate Pro Stick 75DG, 2lb (5,6) | 67.5 b | 67.5 ab | 0.09 b | 90.0 ab |
| Manzate Pro Stick 75DG, 2lb (6 ) | 72.5 b | 67.5 ab | 0.0 b | 100.0 a |
| LSD (*P*=0.05) | 8.7 | 11.78 | 0.46 | 0.206 |

[z]Disease rating scale based on percentage of necrotic foliage (0-100).

[y]Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Student-Newman-Keuls).

[x]Numbers in parentheses represent the weeks that fungicides were applied.

**Table 4.8.** Results of 2007 delay of fungicide application experiment, Newton Grove, Sampson County, NC. (Modified from Colucci et al., 2008b).

| | Disease severity[z] | | Yield, 9 Oct harvest (kg/plot) | |
|---|---|---|---|---|
| **Treatment, rate of product per acre (week applied)** | **9 Oct** | **AUDPC** | **Marketable** | **Cull** |
| Non-treated | 91.3 a | 1042.5 a[y] | 0.04 d | 1.23 a |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (1,2,3,4,5,6,7)[x] | 71.3 e | 462.5 efg | 0.83 a | 1.73 a |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb (2,3,4,5,6,7) | 68.8 e | 445.6 fg | 0.56 a | 1.36 a |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt+ Bravo Weather Stik 6SC, 2 pt (3,4,5,6,7) | 60.0 f | 386.9 g | 0.64 a | 1.44 a |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb (4,5,6,7) | 70.0 e | 527.5 def | 0.59 ab | 1.50 a |
| Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (5,6,7) | 78.8 d | 875.0 b | 0.18 cd | 1.16 a |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro Stick 75DG, 2 lb (6,7) | 92.5 a | 1028.74 a | 0.16 cd | 1.33 a |
| Manzate Pro-Stick 75DG, 2lb (1,2,3,4,5,6,7) | 78.8 d | 550.0 de | 0.14 cd | 1.16 a |
| Manzate Pro-Stick 75DG, 2lb (2,3,4,5,6,7) | 82.5 cd | 601.9 d | 0.28 cd | 1.38 a |
| Manzate Pro-Stick 75DG, 2lb (3,4,5,6,7) | 85.0 bcd | 620.0 d | 0.32 bc | 1.30 a |
| Manzate Pro-Stick 75DG, 2lb (4,5,6,7) | 86.3 abc | 766.9 c | 0.13 cd | 1.63 a |
| Manzate Pro-Stick 75DG, 2lb (5,6,7) | 86.3 abc | 910.0 b | 0.12 cd | 1.40 a |
| Manzate Pro-Stick 75DG, 2lb (6,7) | 87.5 abc | 950.0 ab | 0.15 cd | 1.37 a |
| LSD (*P*=0.05) | 7.46 | 112.4 | 0.28 | 0.78 |

[z]Disease rating scale based on percentage of necrotic foliage (0-100).

[y]Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Waller-Duncan k=100).

[x]Numbers in parentheses represent the weeks that fungicides were applied.

**Table 4.9.** Results of 2007 delay of fungicide application experiment, Clinton, Sampson County, NC. (Modified from Colucci et al., 2008d).

| | Disease severity[z] | | Total yield | |
|---|---|---|---|---|
| **Treatment, rate of product per acre** | **AUDPC** | **24 Oct** | **Total (kg/plot)** | **% cull** |
| Non-treated | 1540.0 ab[y] | 85.0 ab | 0.6 g | 96.4 abc |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (1,2,3,4,5,6)[x] | 896.9 f | 61.3 f | 2.2 a | 63.3 f |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb (2,3,4,5,6) | 984.4 ef | 62.5 f | 2.3 a | 63.0 f |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (3,4,5,6) | 1071.9 e | 58.8 f | 1.9 b | 70.5 ef |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb (4,5,6) | 1242.5 d | 71.3 e | 1.2 cd | 87.3 cd |
| Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb alt. w/ Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt (5,6) | 1522.5 ab | 81.3 a-d | 0.7 fg | 99.4 ab |
| Previcur Flex 6F, 1.2 pt + Bravo Weather Stik 6SC, 2 pt alt. w/ Tanos 50DF, 8 oz + Manzate Pro-Stick 75DG, 2 lb (6) | 1645.0 a | 86.3 a | 0.6 g | 98.1 ab |
| Manzate Pro-Stick 75DG, 2lb (1,2,3,4,5,6) | 1229.4 d | 71.3 e | 1.3 c | 84.2 d |
| Manzate Pro-Stick 75DG, 2lb (2,3,4,5,6) | 1325.6 cd | 77.5 d | 1.3 c | 79.0 de |
| Manzate Pro-Stick 75DG, 2lb (3,4,5,6) | 1312.5 d | 80.0 bcd | 1.1 cde | 73.0 ef |
| Manzate Pro-Stick 75DG, 2lb (4,5,6) | 1448.1 bc | 78.8 cd | 0.9 def | 89.2 bcd |
| Manzate Pro-Stick 75DG, 2lb (5,6) | 1566.2 ab | 80.0 bcd | 0.8 fg | 99.3 ab |
| Manzate Pro-Stick 75DG, 2lb (6) | 1566.2 ab | 83.8 abc | 0.8 efg | 100.0 a |
| LSD (*P*=0.05) | 142.3 | 6.6 | 0.33 | 11.0 |

[z]Disease rating scale based on percentage of necrotic foliage (0-100).

[y]Treatments followed by the same letter(s) within a column are not significantly different (*P*=0.05, Waller-Duncan k=100).

[x]Numbers in parentheses represent the weeks that fungicides were applied.

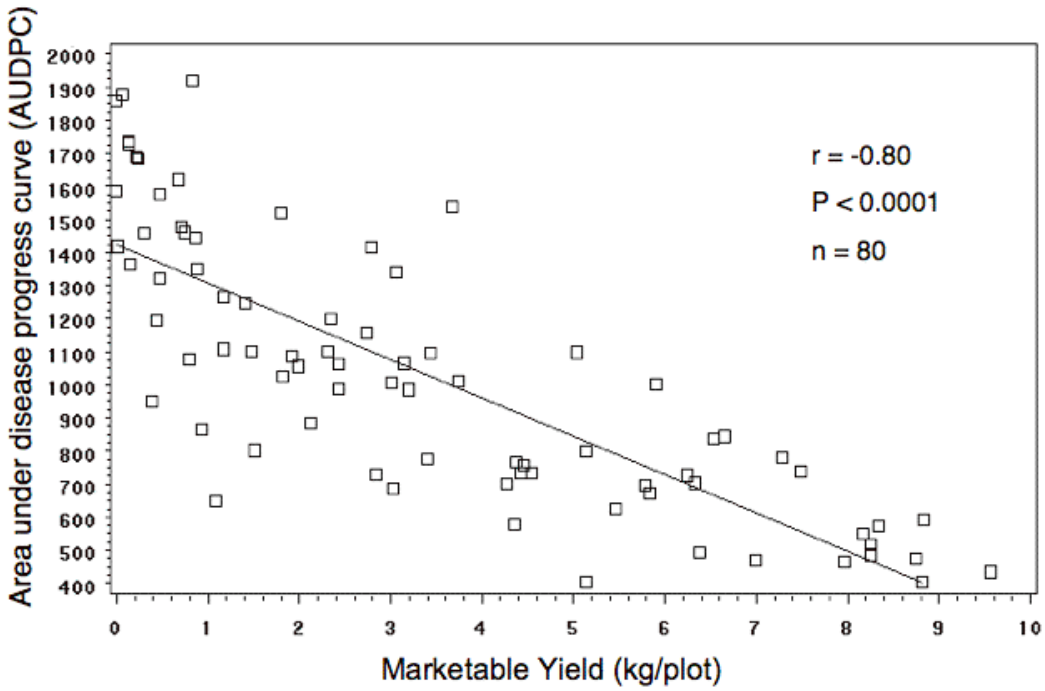


**Fig. 4.1.** Correlation data between disease severity (AUDPC) and weight of marketable yield from the fungicide efficacy experiment in 2005 in Sampson County, NC.

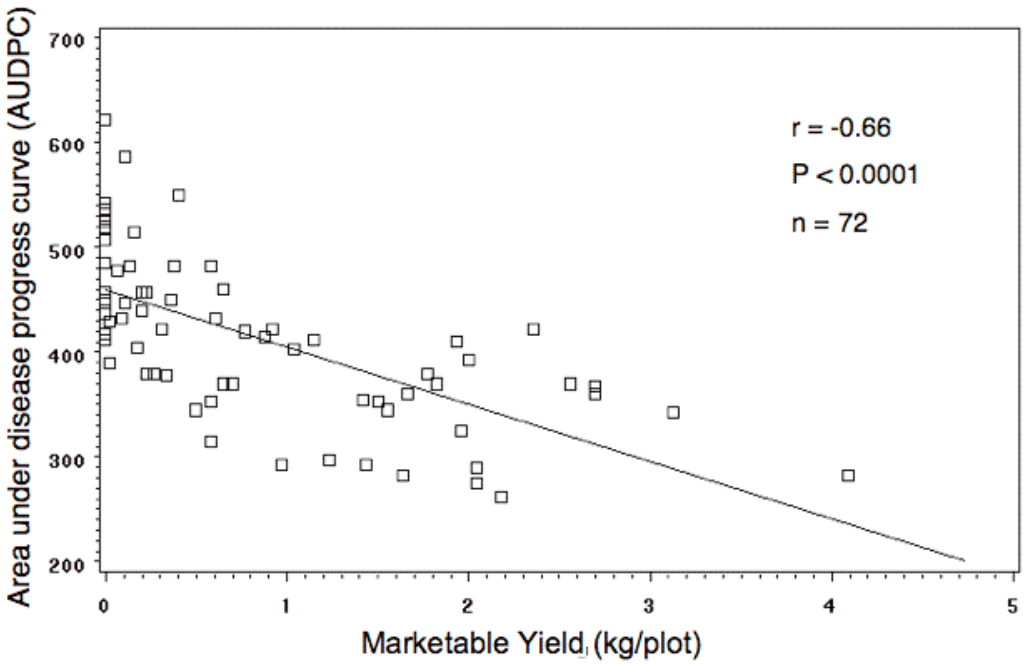


**Fig. 4.2.** Correlation data between disease severity (AUDPC) and weight marketable yield from the fungicide efficacy trial in 2006 in Sampson County, NC.

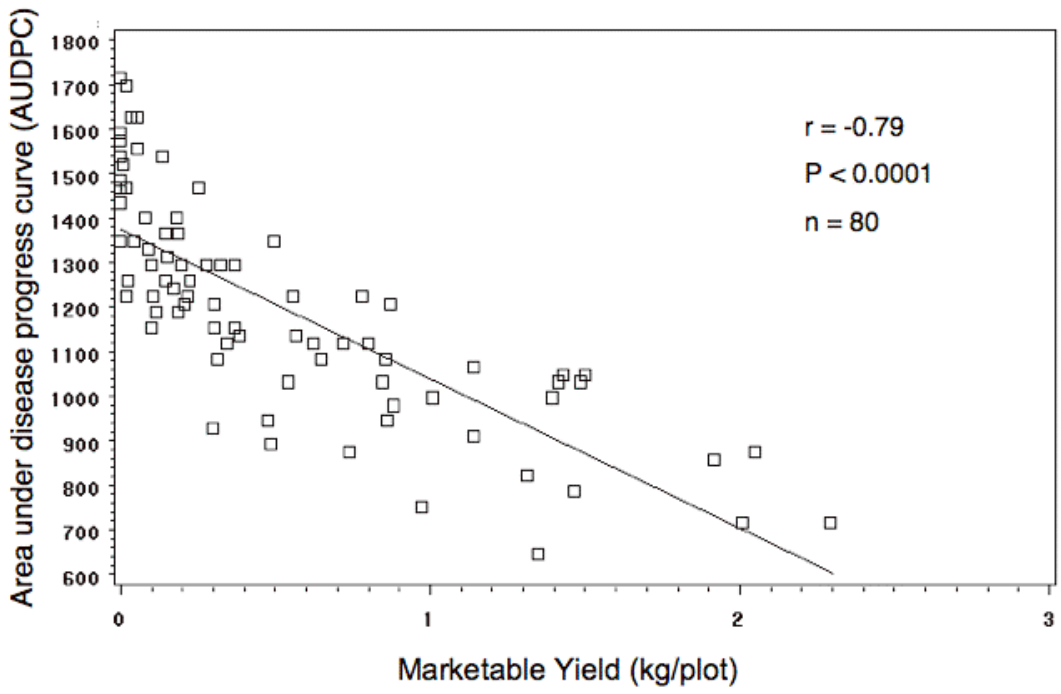


**Fig. 4.3.** Correlation data between disease severity (AUDPC) and weight of marketable yield from the fungicide efficacy trial in 2007 in Clinton, Sampson County, NC.

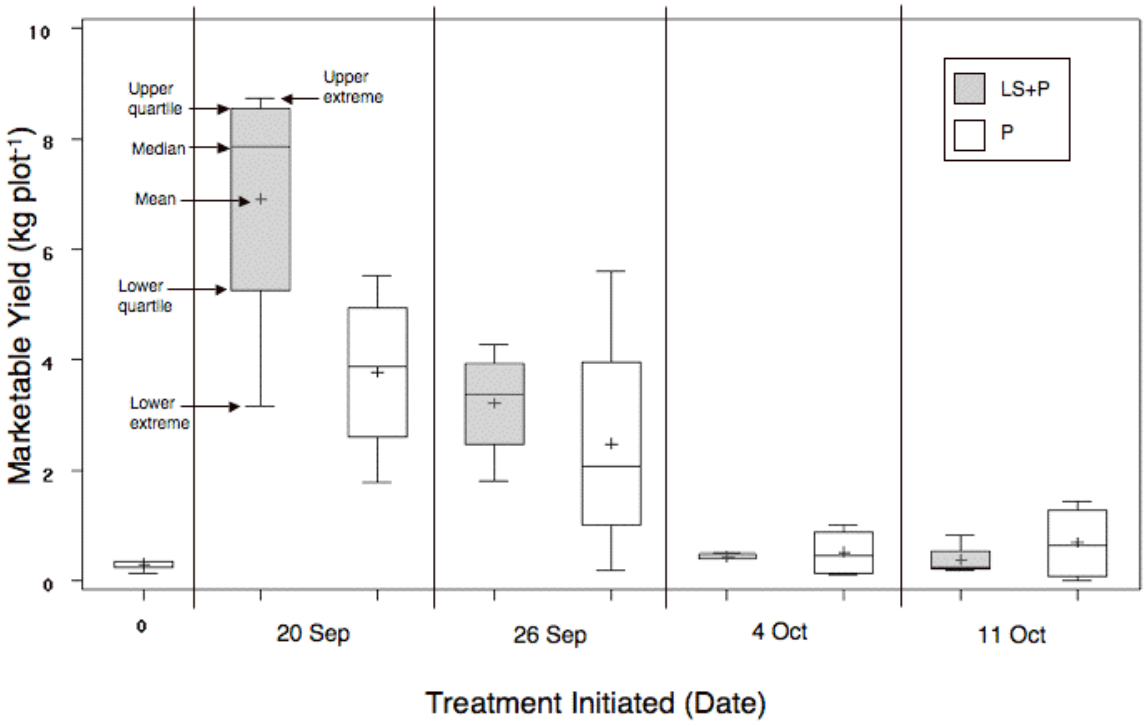


**Fig. 4.4.** Box and whisker plots of marketable yield and treatment initiation date from the 2005 delay of application experiment in Sampson County, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 20 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb.

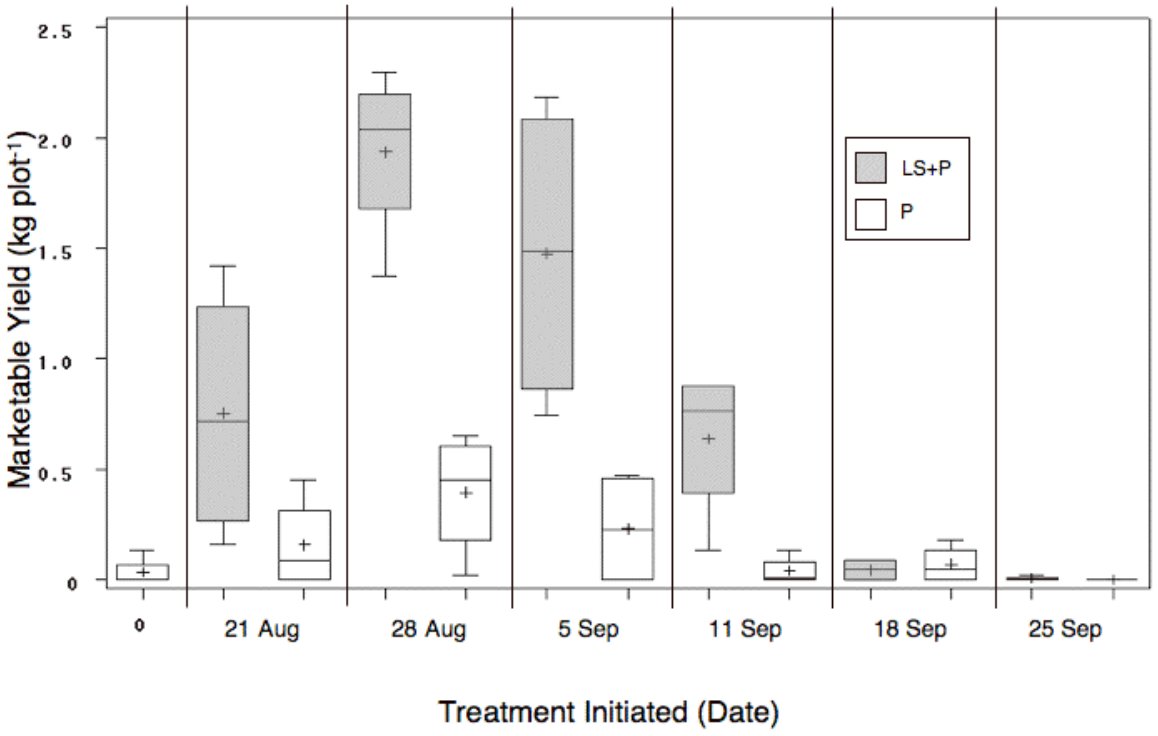


**Fig. 4.5.** Box and whisker plots of marketable yield and treatment initiation date from the 2006 delay of application experiment in Sampson County, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 5 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Explanation of box and whisker plots can be found in Fig. 4.4.

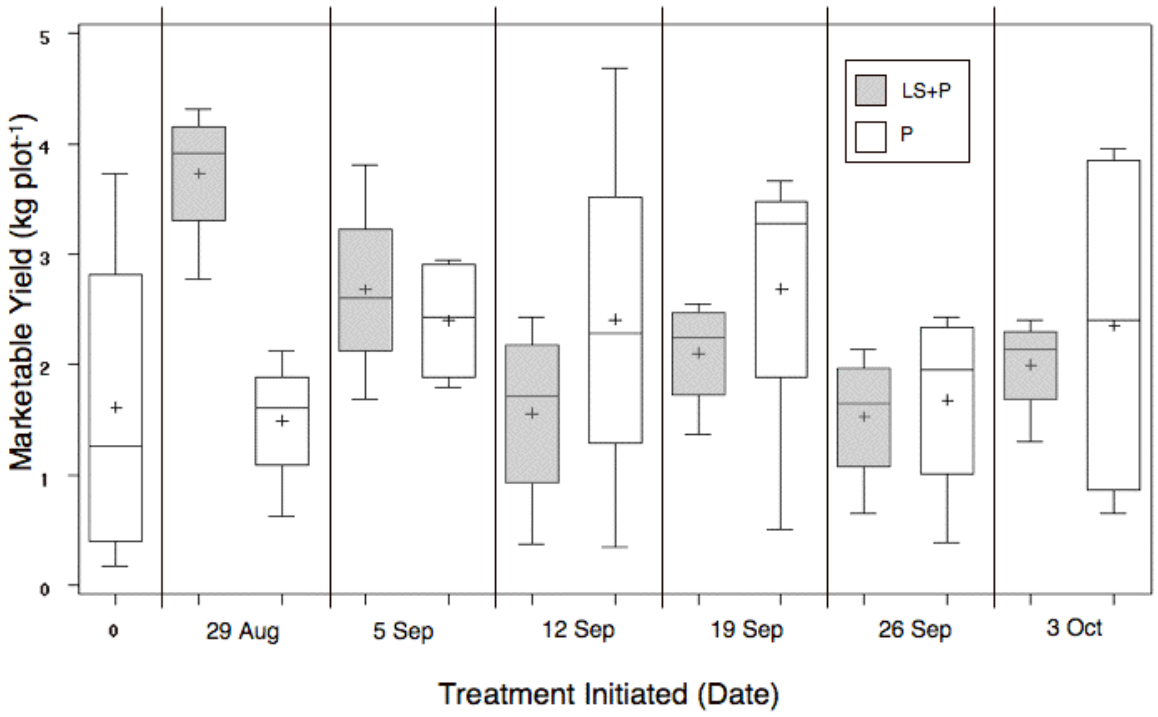


**Fig. 4.6.** Box and whisker plots of marketable yield and treatment initiation date from the 2007 delay of application experiment in Newton Grove, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 12 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Because the main harvest (1 Oct) was inadvertently harvested by the grower, there were no significant differences in marketable yield. Explanation of box and whisker plots can be found in Fig. 4.4.

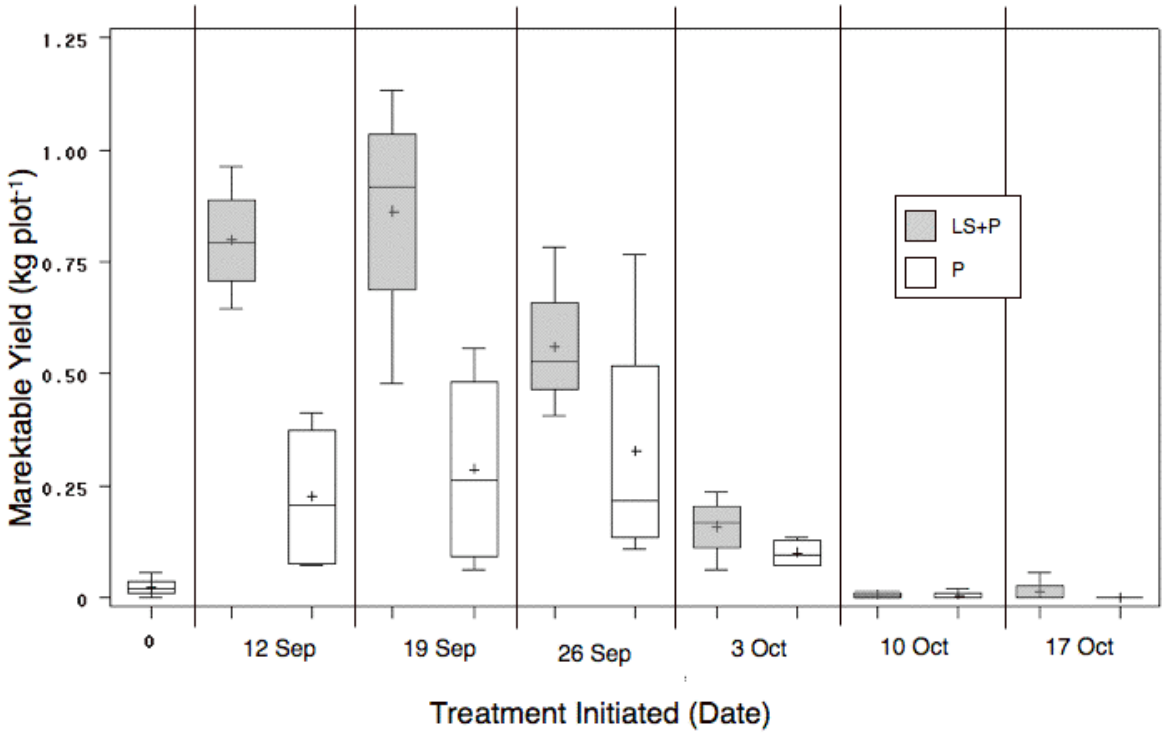


**Fig. 4.7.** Box and whisker plots of marketable yield and treatment initiation date from the 2007 delay of application experiment in Clinton, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 26 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Explanation of box and whisker plots can be found in Fig. 4.4.

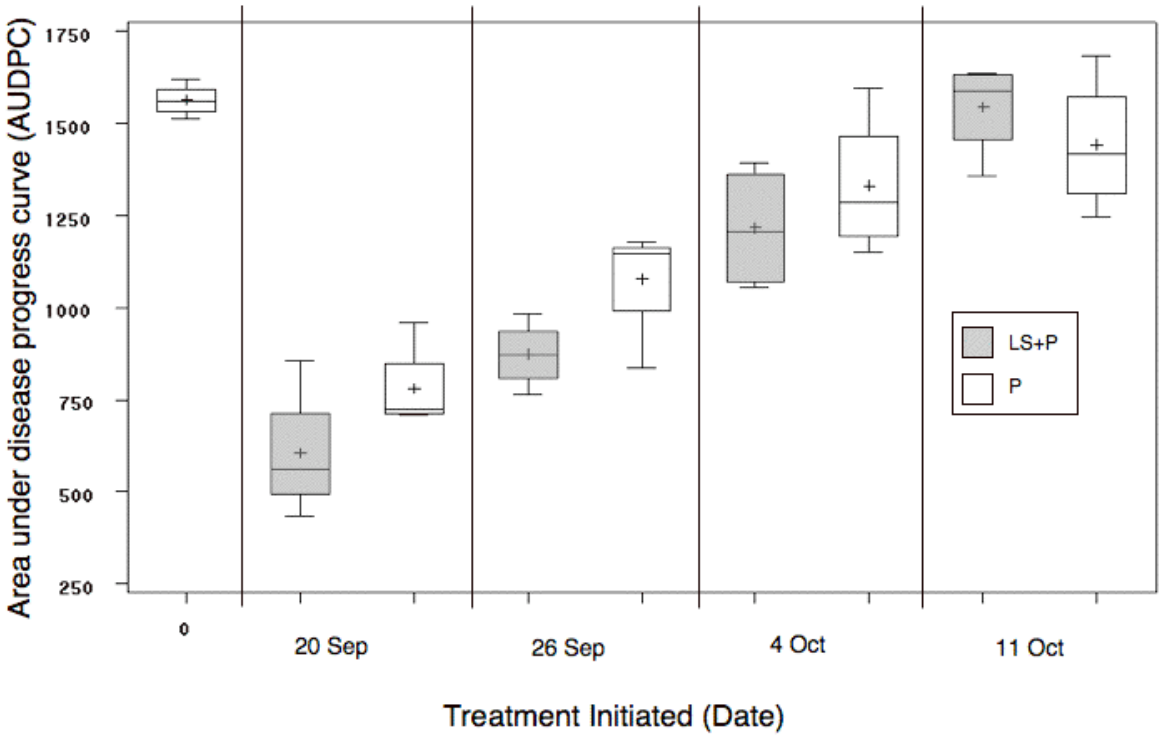


**Fig. 4.8.** Box and whisker plots of area under the disease progress curve (AUDPC) from the 2005 delay of fungicide application experiment in Sampson County, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 20 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Explanation of box and whisker plots can be found in Fig. 4.4.

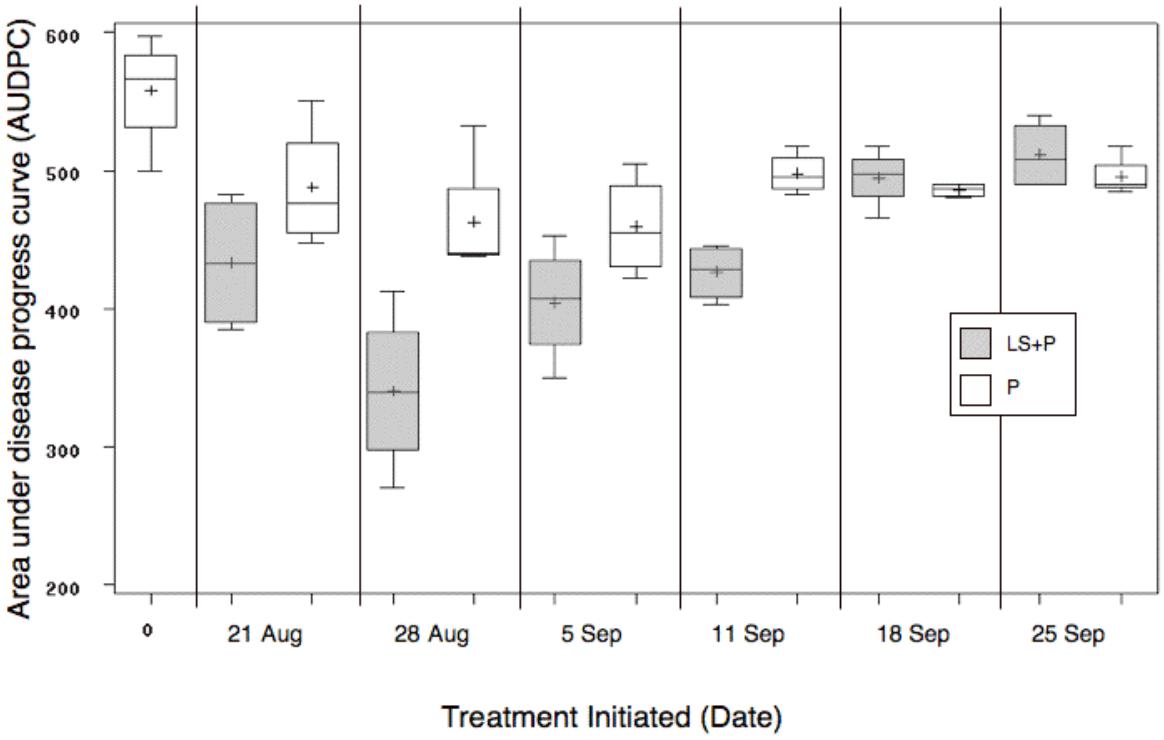


**Fig. 4.9.** Box and whisker plots of area under the disease progress curve (AUDPC) from the 2006 delay of fungicide timing experiment in Sampson County, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 5 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Explanation of box and whisker plots can be found in Fig. 4.4.

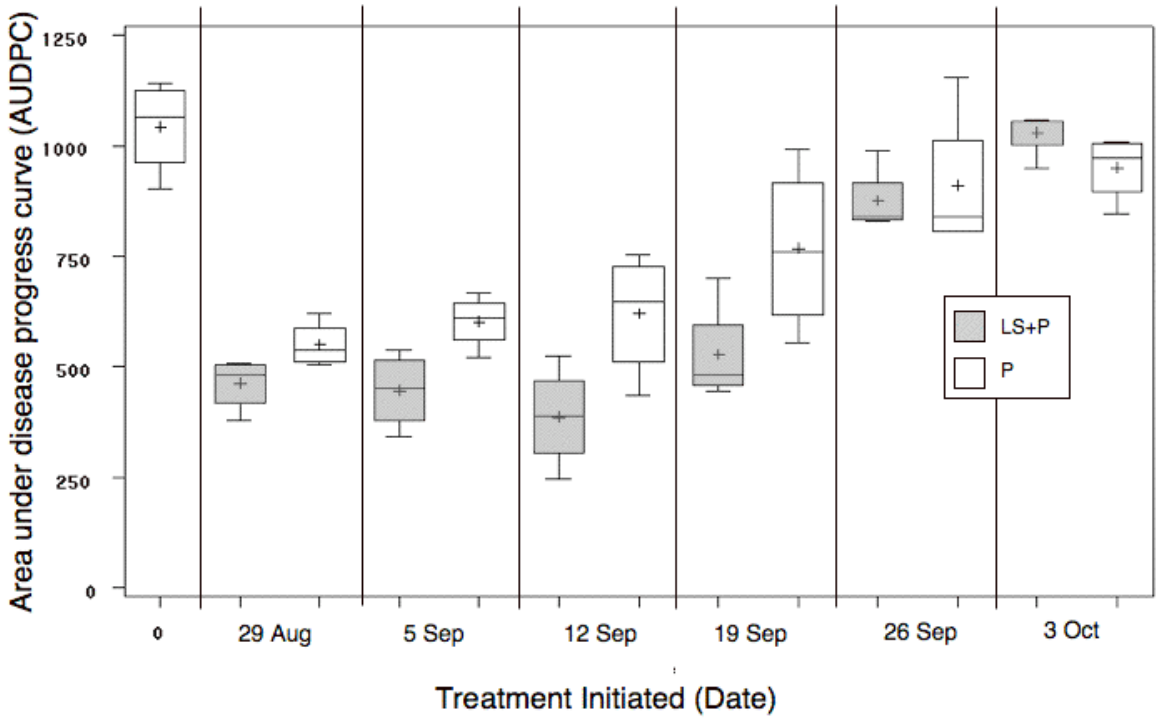


**Fig. 4.10.** Box and whisker plots of area under the disease progress curve (AUDPC) from the 2007 delay of fungicide timing experiment in Newton Grove, Sampson County, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 12 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Explanation of box and whisker plots can be found in Fig. 4.4.

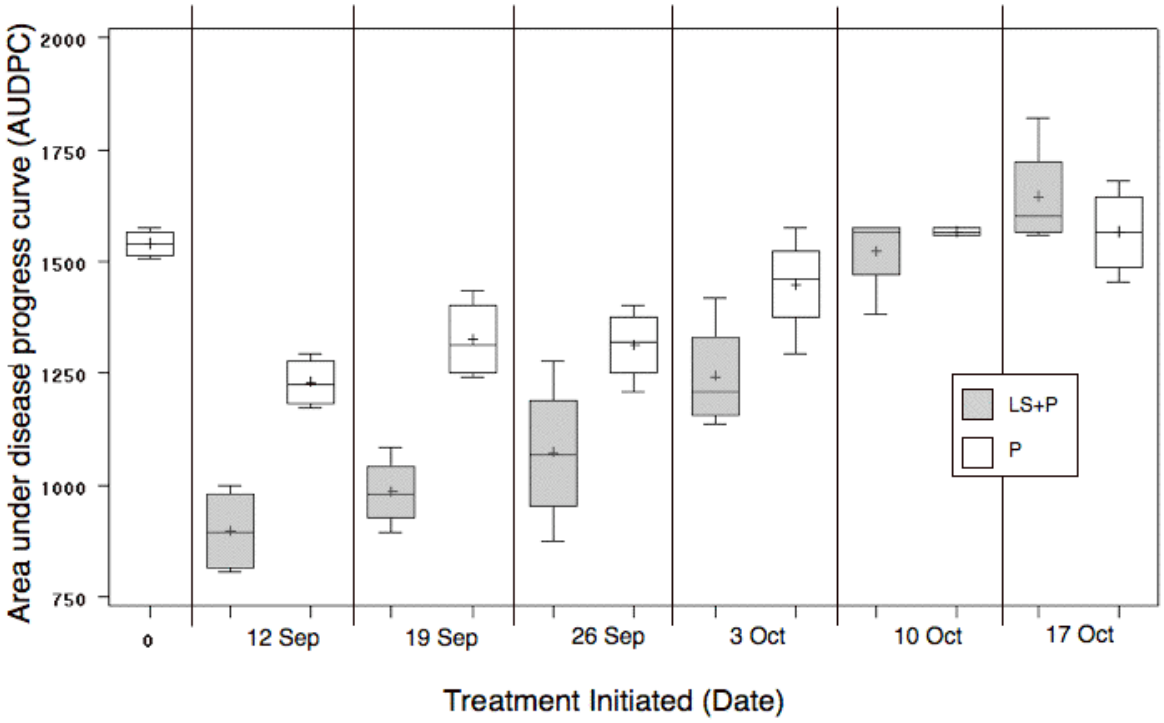


**Fig. 4.11.** Box and whisker plots of area under the disease progress curve (AUDPC) from the 2007 delay of fungicide timing experiment in Clinton, Sampson County, NC. Treatments were applied on an approximately weekly schedule beginning on the treatment initiation date. Downy mildew was detected in the plots on 26 Sep. LS+P is the locally systemic + protectant program of famoxadone + cymoxanil tank-mixed with mancozeb alternated weekly with propamocarb hydrochloride tank-mixed with mancozeb. P is the protectant-only program of weekly application of mancozeb. Explanation of box and whisker plots can be found in Fig. 4.4.

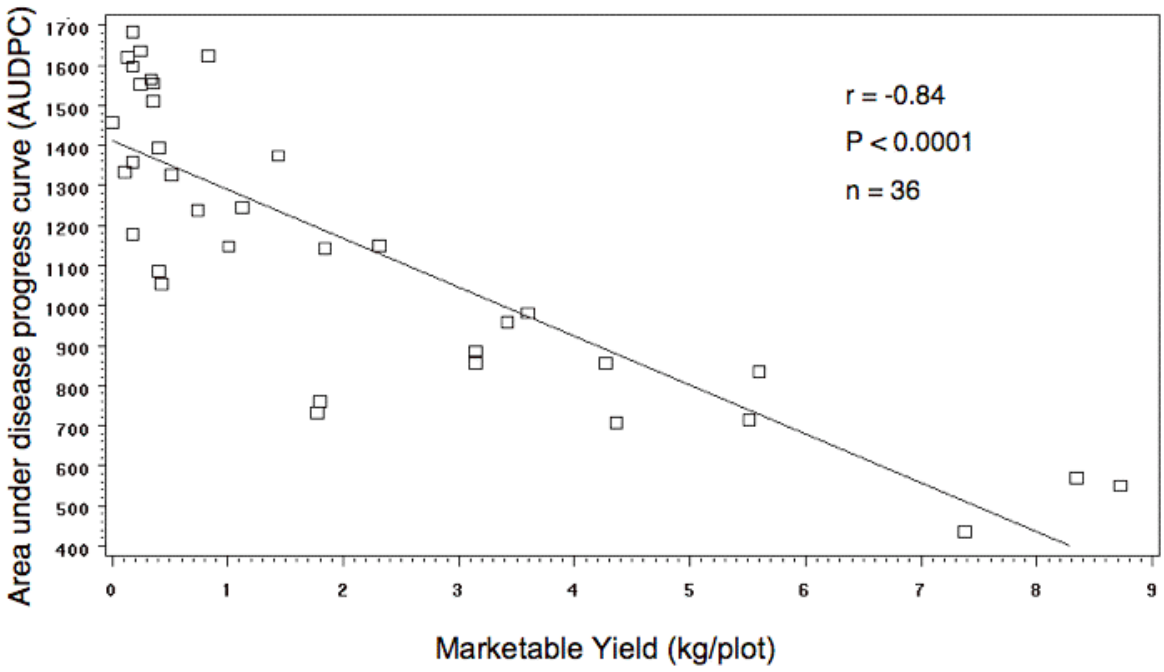


**Fig. 4.12.** Correlation data between disease severity (AUDPC) and weight of marketable yield from the delay of fungicide application experiment in 2005 in Sampson County, NC.

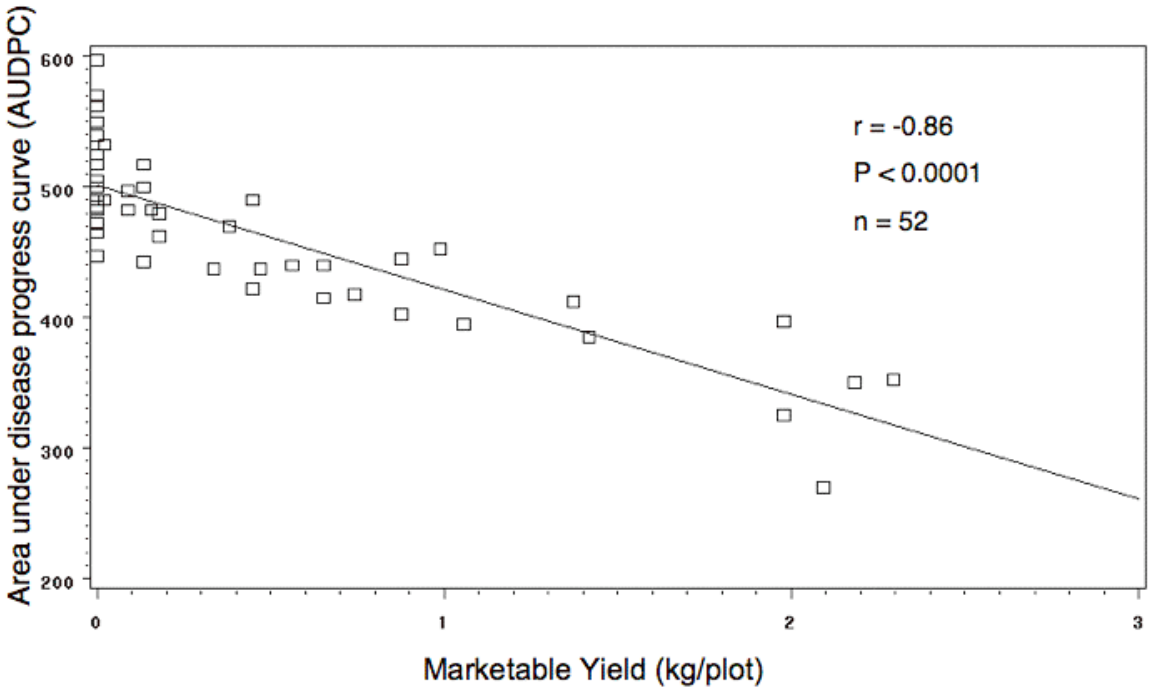


**Fig. 4.13.** Correlation data between disease severity (AUDPC) and weight of marketable yield from the delay of fungicide application experiment in 2006 in Sampson County, NC.

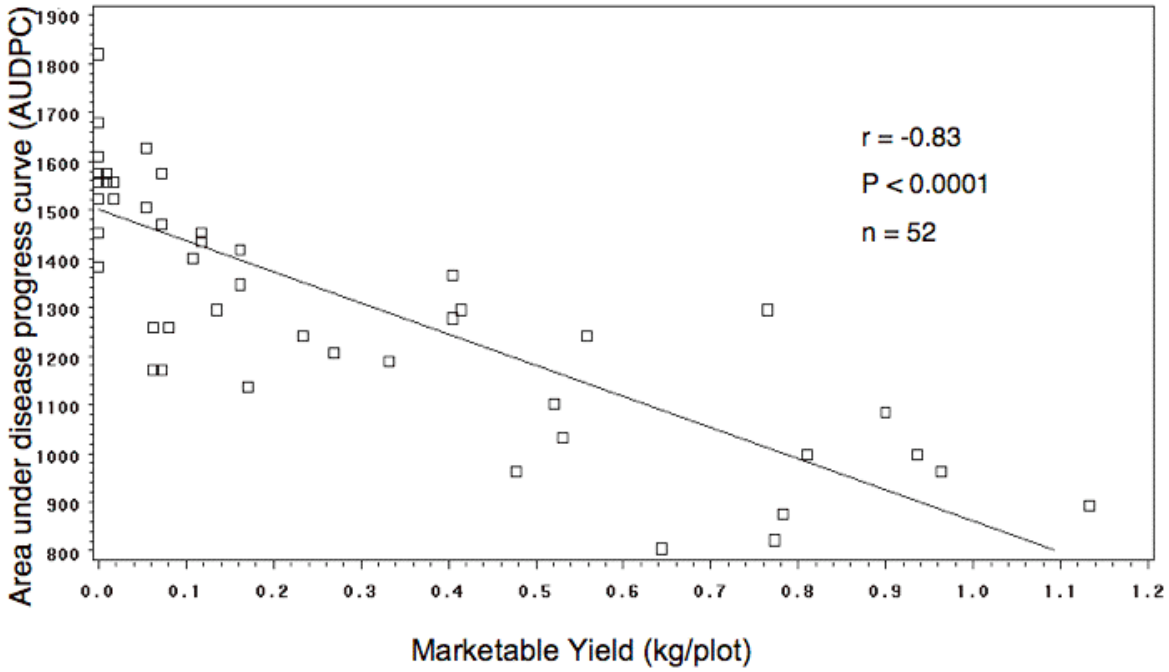


**Fig. 4.14.** Correlation data between disease severity (AUDPC) and weight marketable yield from the delay of fungicide application experiment in 2007 in Clinton, Sampson County, NC.

# CHAPTER 5 – SCANNING ELECTRON MICROSCOPY OF *PSEUDOPERONOSPORA CUBENSIS*

## ABSTRACT

Traditional light microscopy is a powerful tool for the visualization of minute objects or organisms. However, traditional light microscopy is limited in that in only allows for objects to be viewed in one dimension. Scanning electron microscopy is a technique that allows for detailed, three-dimensional images of microscopic objects or organisms to be achieved. This technique was used to provide high quality and detailed images of the oomycete plant pathogen, *Pseudoperonospora cubensis*. Because of the fragile nature of *P. cubensis*, its sporangia often separate from sporanigophores in traditional microscope mounts. The method of fixation of samples in scanning electron microscopy allows for such structures to remain in tact. Eight unique images are presented in this chapter that illustrate the characteristics of *P. cubensis* structures including the papillate nature of the sporangia, the finely pitted sporangia wall, dichotomously branched sporangiophores and twisting of sporangiophores due to humidity changes around the leaf surface.

## INTRODUCTION

*Pseudoperonospora cubensis,* causal agent of cucurbit downy mildew, is an obligate parasite, requiring live cucurbit host tissue to grow and proliferate (Palti, 1974; Waterhouse, 1973). Downy mildew is a yearly problem on cucurbits, including squash, cucumber and watermelon, in North Carolina. The disease is typically more severe in the fall crop of

cucumber and squash. Symptoms and signs of downy mildew infection are apparent on the leaf. Downy mildew may appear on the cotyledons, but is rare on the very young true-leaves when they are in the process of unfurling (Palti and Cohen, 1980, Van Haltern, 1933).

Disease symptoms first appear as small, slightly chlorotic to bright yellow areas on the upper leaf surface. Lesions first appear on the older leaves and appear progressively on the younger, more distal leaves as these leaves expand. As the lesions expand, they may remain chlorotic or become necrotic (Palti and Cohen, 1980; Van Haltern, 1933). When conditions favor sporulation, the production of sporangia and sporangiophores occurs usually on the lower leaf surface. This sporulation ranges from colorless to grey to dark-purple and appears as a felt or downy growth (Thomas, 1996).

Because of the fragile nature and the biotrophic lifestyle of the pathogen, high quality images of *P. cubensis* are limited. In traditional compound light microscopy, the structures of *P. cubensis* can only be viewed in one dimension limiting the detail that can be obtained. In addition, when preparing a traditional mount using a slide and slide cover, the fragile nature of the pathogen results in the sporangia being dislodged from the sporangiophores. Scanning electron microscopy (SEM) allows for the detailed visualization of a subject in three dimensions. SEM was used to take an intricate look at *P. cubensis* structures and the relationship between the pathogen and its host. SEM allowed for the sporangia and sporangiophores to remain intact during preparation, resulting in images of the entire reproductive structure.

## MATERIALS AND METHODS

***Pseudoperonospora cubensis* isolate and maintenance**. An isolate of *P. cubensis* collected from a commercial cucumber field in Sampson County, North Carolina in 2004 was used for microscopy and photography. The isolate was maintained on *Cucumis sativus* (cucumber) 'Coolgreen' grown 22°C, night and 26°C, day with a 12 h photoperiod to the two-true-leaf stage in growth chambers in the North Carolina State University Phytotron. 'Coolgreen' was inoculated with a $5x10^3$ sporangia/ml suspension of *P. cubensis* applied using a Preval Complete Spray Unit 267 (Precision Valve Corporation, Yonkers, NY). After inoculation, plants were placed in growth chambers at 18°C darkness and >90% relative humidity. After 24 h, plants were removed from moist chambers and growth chamber conditions were set to 18°C, night and 21°C, day with a 12 h photoperiod. After 7 days leaves were harvested and placed in moist chambers for 24 h at 18°C darkness to promote sporulation.

**Material preparation for visualization using scanning electron microscopy**. Selected areas of infected 'Coolgreen' cucumber leaf with abundant sporulation were excised and placed in a glass Petri dish containing a moistened piece of filter paper and a few drops of 4% aqueous osmium tetroxide in a small glass vial. Samples were fixed with osmium vapor for 24 h at 4°C in darkness then rinsed briefly in two changes of cold distilled water, dehydrated with a graded ethanol series to 100% ethanol, critical point dried in a Samdri-795 (Tousimis Corp., Rockville MD) with liquid $CO_2$, mounted on stubs with double-stick tape and silver paint and coated with approximately 50 Angstroms of gold/palladium using a Hummer 6.2 coating system (Anatech USA, Hayward CA). Samples were viewed in a JEOL

JSM-5900LV Scanning electron microscope at 10kV (JEOL USA, Peabody, MA).

## RESULTS

Twenty images were produced using SEM, eight of these images are presented. The papillate nature of sporangia and the dichotomous branching of sporangiophores was evident (Figs. 5.1, 5.2, 5.5). In Fig. 5.2, sporangia, which appear smooth under the compound microscope, have a finely decorated or pitted (rugose) wall. Prolific sporangiophore production was evident at the junctions of leaf veins (Fig. 5.3). The size of sporangiophores and sporangia in relation to trichomes is also seen in Fig. 5.3. More than one sporangiophore is capable of exiting a single stomate (Fig. 5.4). A film appears to be peeling from the sporangial surface in Figs. 5.1 and 5.5. Because the method of fixation used is not known to cause peeling it may be part of the pathogen structure. Twisting and contorting of sporangiophores can be seen in Figs. 5.6 and 5.7. In Fig. 5.8 direct germination of the sporangiophore is evident over a stomate.

## DISCUSSION

Twenty high-magnification images were generated using SEM. These images provided great detail and a greater depth of field than with compound light microscopes. Using this technology we were able to generate detailed images of the structures of biotrophic plant pathogen, *P. cubensis* and its host, *Cucumis sativus* L. (cucumber). In addition, these pictures provide information into the relationship between the host and the pathogen.

Through the images created with SEM, ornamentation on the sporangia was evident. Lange et al. (1989a) hypothesized that the ornamentation on the outer thin layer of the sporangia may aid in its ability to withstand unfavorable conditions (up to 3-4 months of -18°C) and possibly act as a resting structure.

Our images revealed twisted and contorted sporangiophores. Sporangia and sporangiophores are greatly affected by changes in temperature and humidity. Warming and drying of the atmosphere, typical of early morning hours, causes twisting of the sporangiophores, which may be of importance for the detachment of sporangia (Lange et al., 1989a). In order to limit this twisting from occurring and to obtain images with reduced contortion of the sporangiophore and intact sporangia, fastidious tissue preparation is necessary. Samples were exposed to a change in humidity as they were removed from moist chambers for preparation. More intact structures would have been obtained if the humidity remained at a more constant and high relative humidity.

Not only are the structures of *P. cubensis* of great interest, but also the topography of the cucumber leaf surface. It is known that the zoospores must encyst on a stomatal opening to cause infection (Cohen, 1981; Lange et al., 1989b; Palti and Cohen, 1980). Plant surface topography and its function in directing germ tube growth and the differentiation of infection structures have been well documented (Heath, 2000). For example, Hoch et al. (1987) found that a simple ridge on the plant surface with a height of 0.5 μm acts as a growth orientation and infection structure formation signal for the rust fungus *Uromyces appendiculatus* and concluded that the fungus is able to distinguish small differences in leaf surface topography in order to infect the host plant. Such detailed studies have not been conducted with *P.*

*cubensis*, but it would be interesting to determine the requirements for zoospore cyst formation.

Taking high quality and high-magnification images is an excellent method to study the biology, taxonomy and nomenclature, as well as the relationships between plant pathogens and their hosts. These images provide details that cannot be visualized otherwise that may help to answer important questions and pose new questions and theories. The scanning electron microscope images of *P. cubensis* provided detailed images of the morphology of the organism, as well as the plant host surface. These images also raise questions about topography requirements for zoospore recognition, direct germination by sporangiophores and morphology associated with sporangia survival and liberation.

**ACKNOWLEDGEMENTS**

The author would like to acknowledge Valerie Knowlton of the Department of Microbiology at North Carolina State University for her work in fixing, preparing and microscopy of the samples that were used in this chapter.

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**Fig. 5.1.** Scanning electron micrograph of the papillate sporangia of *P. cubensis* that are borne on the tips of dichotomously branched sporangiophores. The sporangium wall appears rugose or finely pitted.

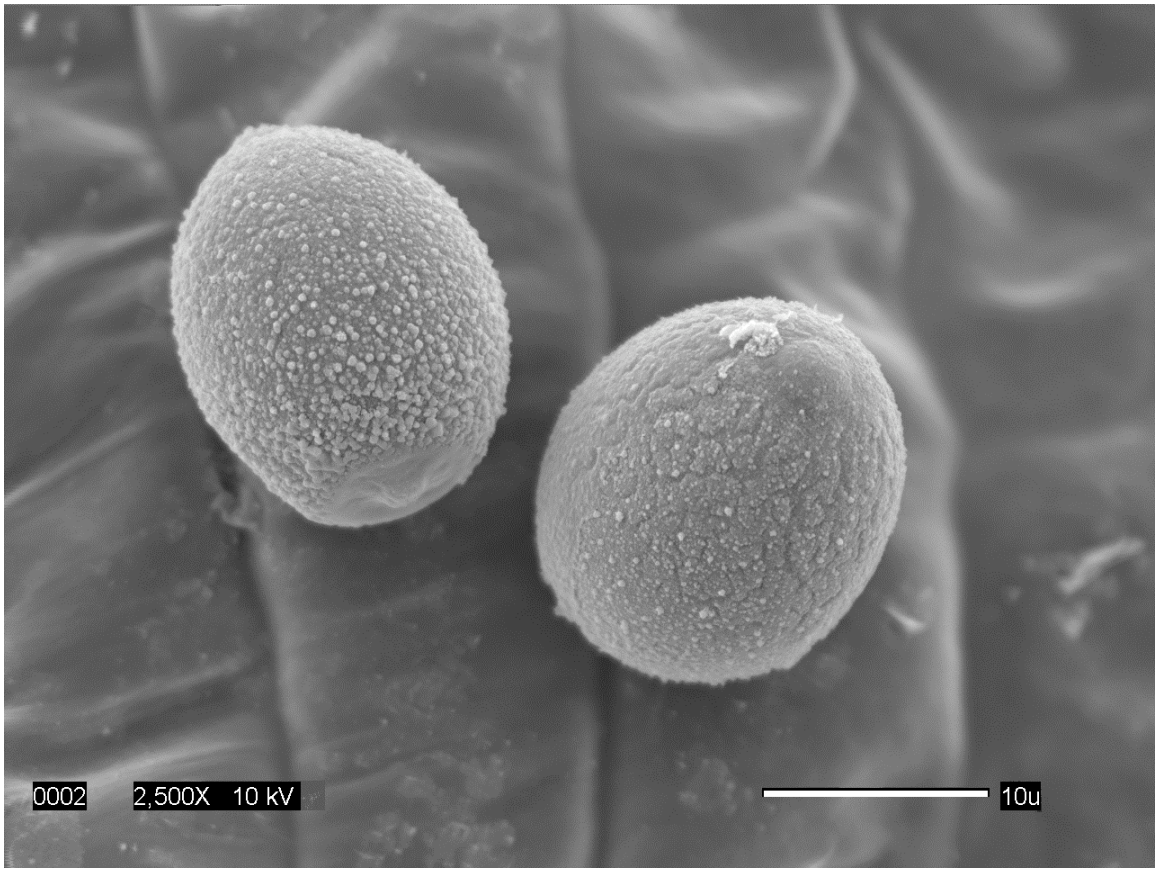


**Fig. 5.2.** Scanning electron micrograph of *P. cubensis* sporangia. The sporangia are papillate and the sporangium wall has a rugose or finely pitted appearance.

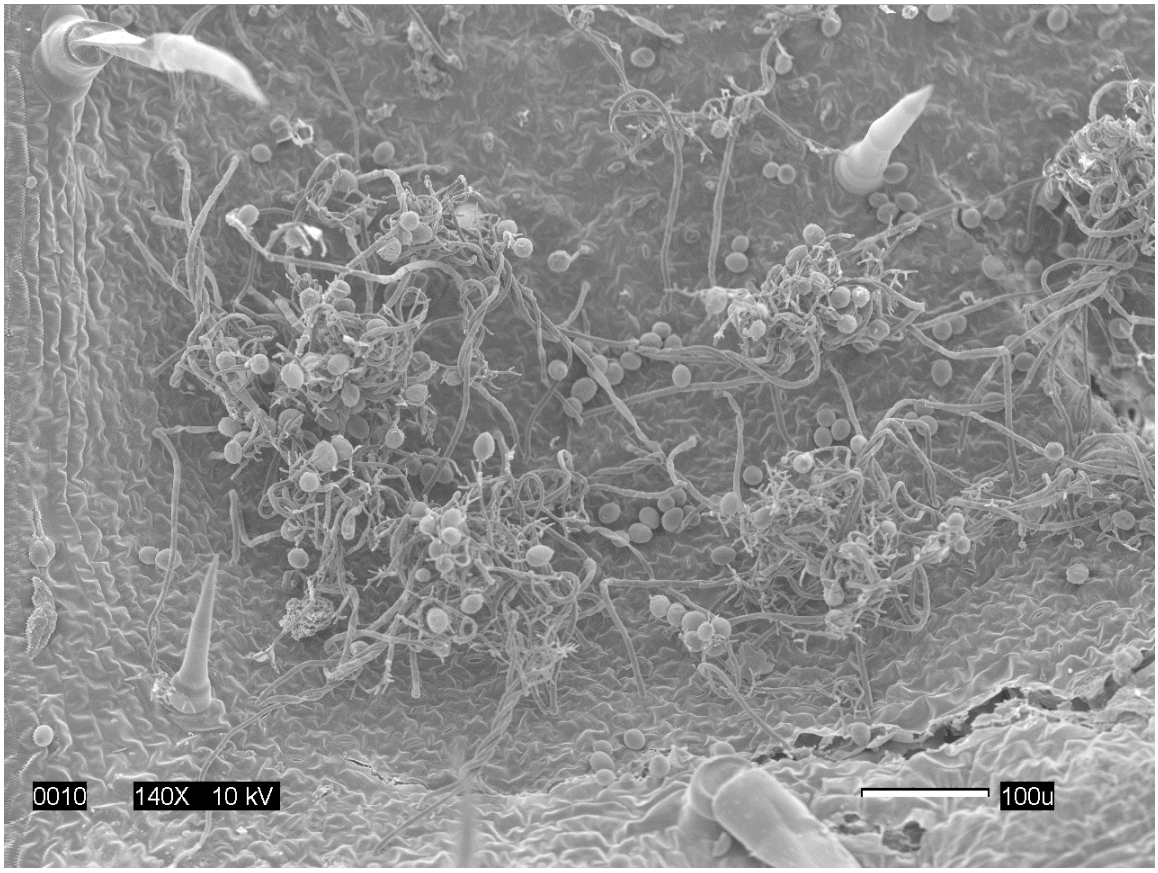


**Fig. 5.3.** Scanning electron micrograph of the prolific sporangiophore and sporangia production of *P. cubensis* found at the junction of two leaf veins.

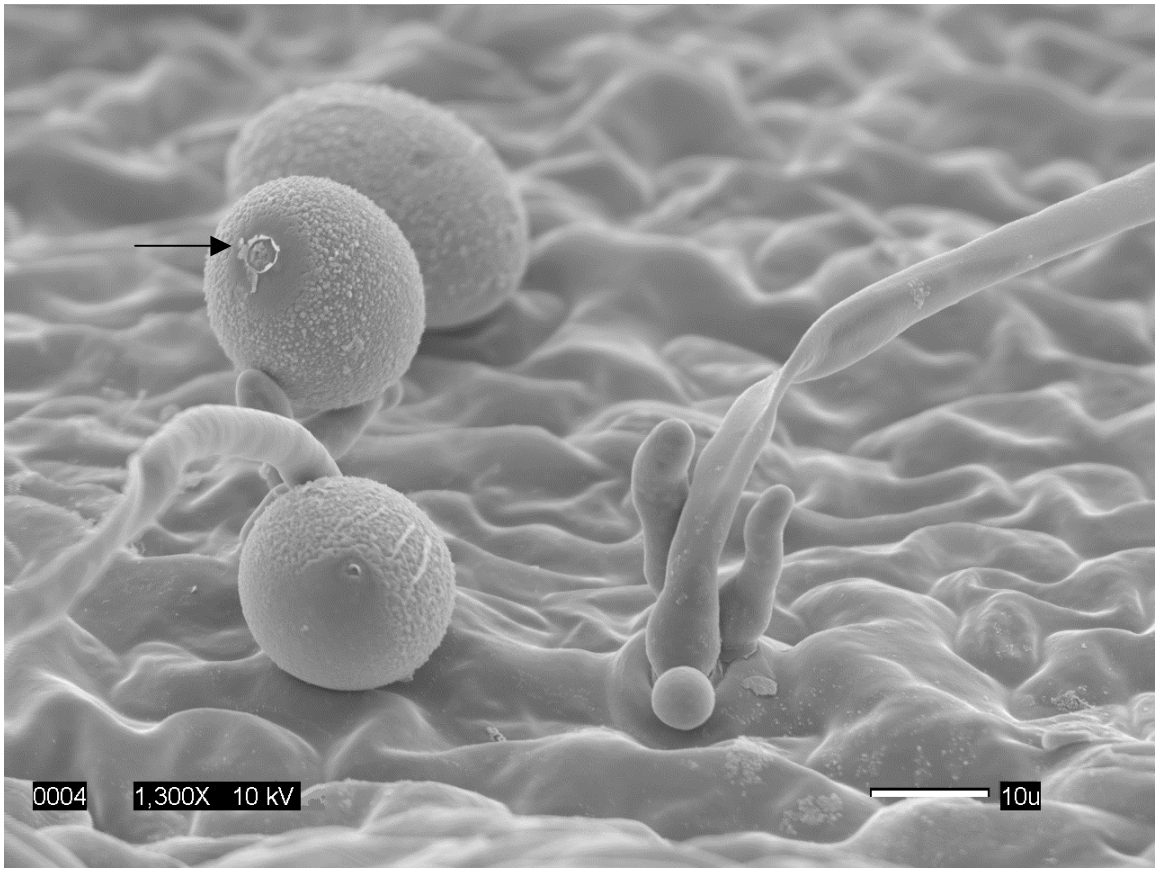


**Fig. 5.4.** Scanning electron micrograph of multiple *P. cubensis* sporangiophores exiting one stomate. Also apparent is the point on the sporangia at which it breaks off the sporangiophore (arrow).

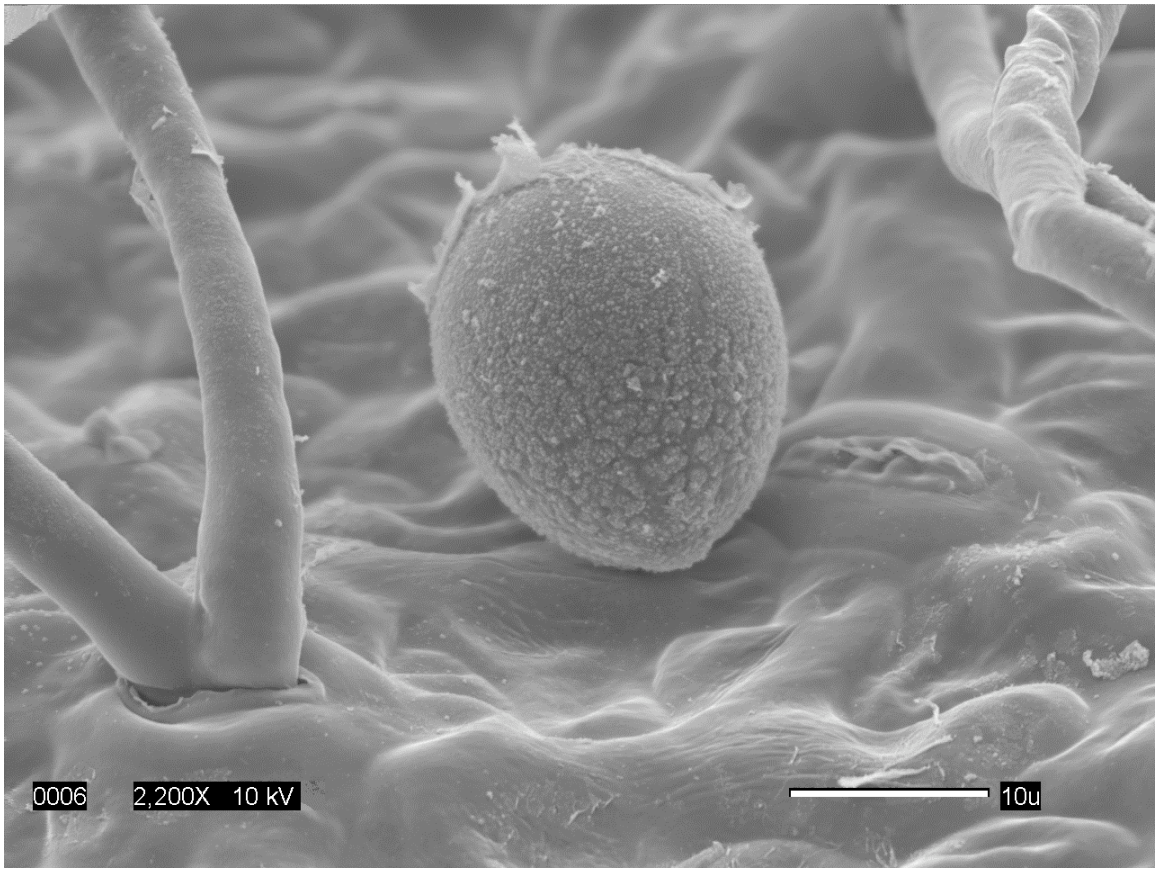


**Fig. 5.5.** Scanning electron micrograph of two *P. cubensis* sporangiophores exiting a single stomata. The papillate, finely pitted or rugose sporangium is evident.

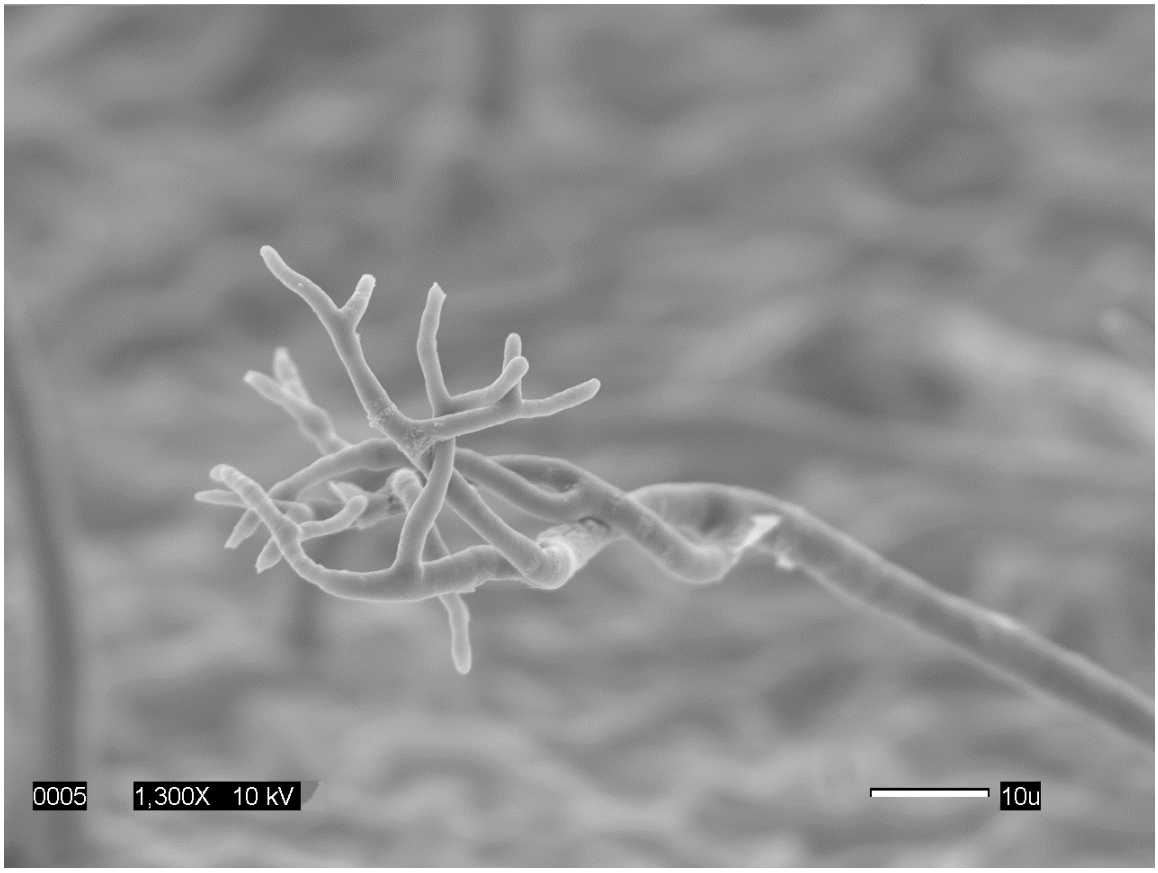


**Fig. 5.6**. Scanning electron micrograph of the twisting and contorting of dichotomously branched *P. cubensis* sporangiophores.

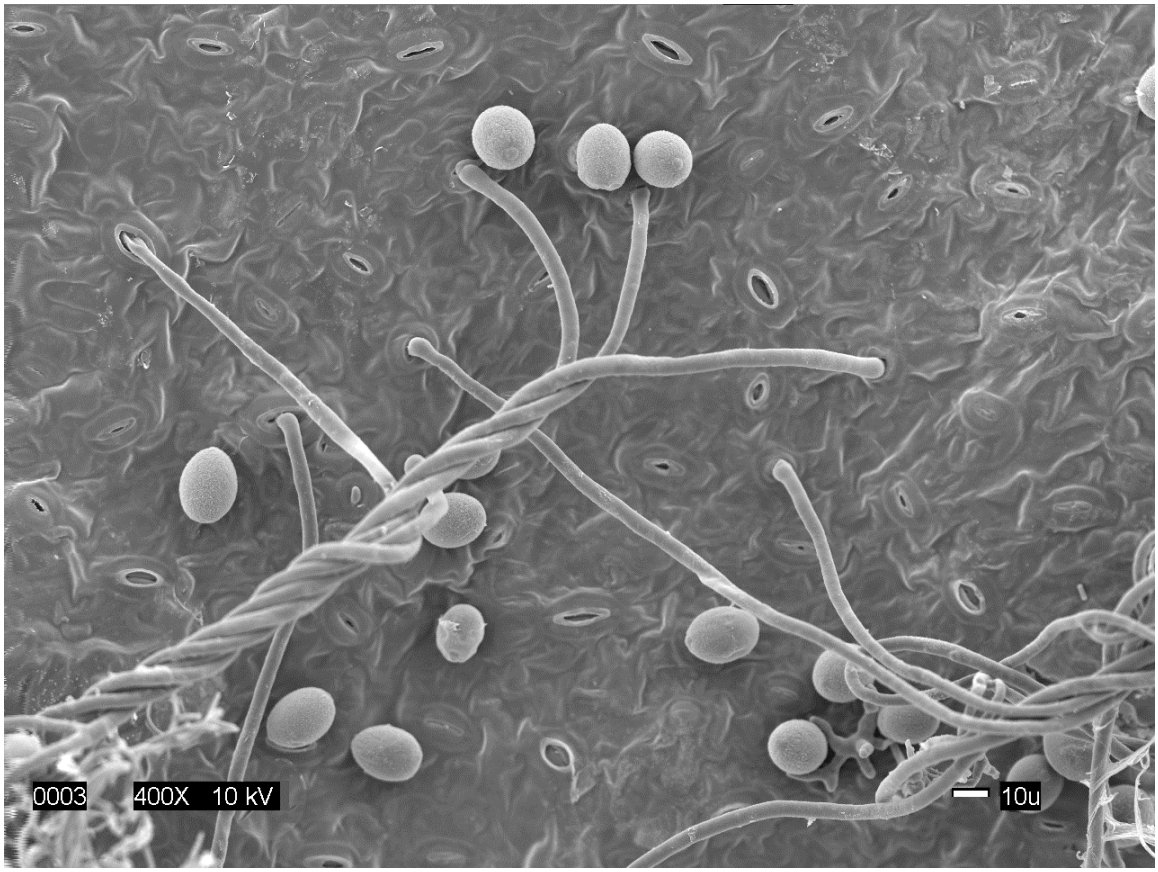


**Fig. 5.7.** Scanning electron micrograph of twisting and entangled *P.cubensis* sporangiophores emerging singly from multiple stomata.

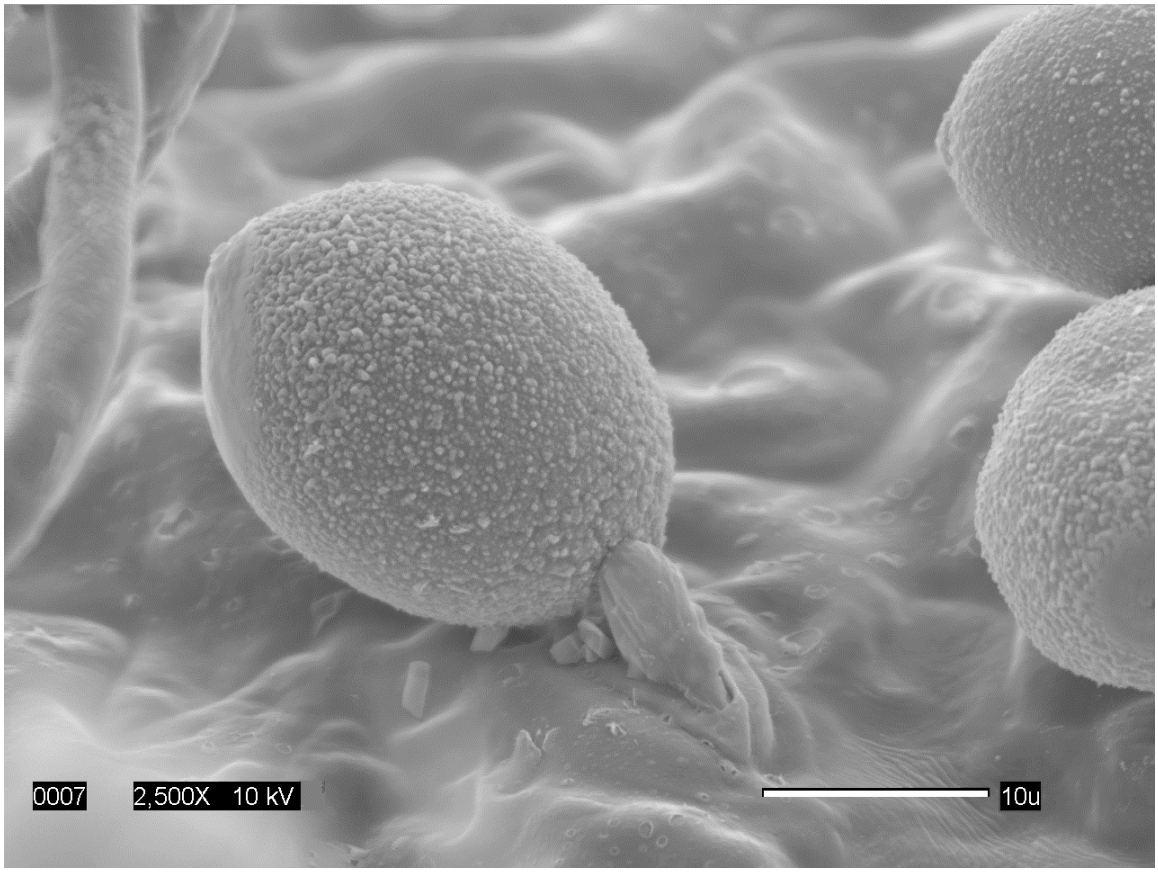


**Fig. 5.8.** Scanning electron micrograph of either direct germination of sporangia over a stomate.