Biology and Control of Pepper Anthracnose

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Characterization and Control of Pepper Anthracnose

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Abstract

Anthracnose (caused by *Colletotrichum capsici* or *C. gloeosporioides*) of bell peppers (*Capsicum annum*) has become a serious problem in recent years on the Eastern Shore of Virginia. The purpose of this research was to characterize isolates of the fungus from the Eastern United States, to compare them with the type species from the American Type Culture Collection, and to evaluate fungicides for disease management. Two cultivars of pepper were inoculated with a conidial suspension, and held in a dew chamber. Lesions were counted and measured every 48 hours. The type species was either not pathogenic or only mildly virulent; most of the virulent isolates originated in areas of intensive pepper production. In addition to pathogenicity experiments and traditional morphology, the Biolog® system was used to compare the ability of fungi to utilize different carbohydrate combinations in 96-well plates. Plates were read at 96 and 168 hours. Analysis of data, by Ward's statistical method, could reliably distinguish field isolates if based on 15 or more replications, but species-level identification was inconsistent. Standard fungicides and new compounds were compared in a field test with four replications of treatments in a randomized complete block design. Fruits were harvested three times, weighed for yield, and the number of marketable and diseased fruit recorded. Aggressive isolates from green pepper were controlled by applications of maneb, or alternation of maneb and strobilurin fungicides.

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Introduction

Pepper anthracnose is a reemerging disease problem for growers on the Eastern Shore of Virginia and elsewhere on the East Coast. In 1998, pepper growers approached the plant pathology department of the Eastern Shore Agricultural Research and Extension Center (ESAREC) with complaints about the difficulty of controlling anthracnose using recommended control practices. Losses due to this disease were estimated as high as 70 percent in some fields. Previously, The Northeast Pepper Integrated Pest Management Manual listed it as a minor disease of pepper (Boucher & Ashley, 2001).

Most anthracnose fungi are members of the genus Colletotrichum; however, the species that causes anthracnose disease on peppers is unclear. *Colletotrichum capsici* (Sydow) Butler et Bisby is known for infection on leaves, stems, and fruit, and *C. gloeosporioides* (Penzig) Penzig et Saccardo is only known for fruit lesions. Both have been found on the Eastern Shore. On seedlings, small brown lesions form on the leaves, but as the plant matures, levels of infection and disease spread are minimal. Diseased fruits have orange, tan, brown, or black, sunken lesions on green, immature and red, mature fruit.

Developing an understanding of pepper anthracnose is needed to assist the pepper growers on the eastern shore. Species identification and differences in virulence, are key points in developing effective control measures for this increasingly destructive disease. The objectives of this research included 1) to characterize and compare isolates from the Eastern United States based on pathogenicity and virulence, 2) to compare regional isolates of type species from the American Type Culture Collection (ATCC), and 3) to evaluate fungicides for control of disease caused by isolates on pepper.

Chapter 1: Biology and Control of Pepper Anthracnose

Literature Review

Importance of Peppers

In 2001, there were 405 hectares of bell peppers planted throughout Virginia, and the majority were grown on the Eastern Shore of Virginia. Nationally in 1997, 18,237 hectares of hot peppers and 24940 hectares of sweet peppers were harvested (NASS, 1997). The value of the Virginia pepper crop in 2001 was 30.00 dollars per hundred weight for a statewide total of around one million dollars (Manheimer & Vick, 2002).

Anthracnose caused by *C. capsici* is considered to be a dry fruit rot (Pearson *et al*., 1984). *C. capsici* and *C. gloeosporioides* are the two main casual agents of pepper anthracnose in the hot humid tropics of Asia. *C. capsici* and *C. gloeosporioides* are the most important *Colletotrichum* spp. in reducing marketable fruit yields of pepper (Manandhar *et al*., 1995). Anthracnose has been found not only on mature fruit but also on seedlings, leaves and immature green fruits (Lee & Chung, 1995). Recently, Park and Kim reported that five anthracnose fungi, *C. gloeosporioides*, *C. dematium* (Persoon: Fries) Grove, *C. coccodes* (Wallr.) S. Hughes, *C. acutatum* Simmonds, and *Glomerella cingulata* (Stoneman) Spaulding & v. Schrenk, were pathogenic to different tissues of pepper plants. Of these anthracnose fungi, *C. gloeosporioides* attacks the fruit at all stages of development, but not the leaves and stems of plants. Leaf anthracnose of pepper seedlings caused by *C. coccodes* was first found in pepper-growing fields in Chungnam province of Korea in 1988 (Hong & Hwang, 1998). Although all ages of pepper fruits were susceptible to infection by *C. gloeosporioides*, purple and ripe red fruits developed more anthracnose than the immature stages (Oh *et al*., 1999).

No significant differences in susceptibility to anthracnose were found among pepper cultivars from Korea, the United States, India, and Thailand or accessions tested, irrespective of genetic or country origin (Hong & Hwang, 1998). Isolates of *C. gloeosporioides* from almond, apple, avocado, and mango, as well as *C. acutatum* from anemone, apple, and peach, colonized fruits including apple, avocado, almond, mango, and nectarines (Freeman *et al*., 1998).

Anthracnose and *Colletotrichum* Disease Epidemiology

C. acutatum, *C. coccodes*, *C. dematium*, *C. gloeosporioides*, and *G. cingulata* have been identified as fungal species responsible for anthracnose of pepper in Korea, and *C. capsici*, *C. gloeosporioides*, and *G. cingulata* in Taiwan. Among these species, *C. gloeosporioides* was the predominant species causing anthracnose on pepper fruits (Oh *et al*., 1999).

C. coccodes can infect pepper seeds, seedling leaves and stems, mature leaves, and sometimes green but not red fruits. In general, pepper plants seem to acquire resistance to *C. coccodes* as they mature, since the anthracnose caused by *C. coccodes* does not readily occur in mature plants. Primary inoculum density of *C. coccodes* seems to be important for producing typical anthracnose lesions on pepper plants. In contrast, anthracnose caused by *C. coccodes* does not result in the severe epidemics in mature leaves and fruits of pepper (Hong & Hwang, 1998).

Conidia do not function as survival structures as their viability declines rapidly. Mycelium, however, may remain viable for long periods in/on colonized seeds, plant debris, or as latent infections in plants not showing any disease symptoms. Microsclerotia, formed sparsely by species such as *C. gloeosporioides* and *C. coccodes*, play an important role in survival (Baxter *et al*., 1985). *C. gloeosporioides* was recovered from leaf spots on sicklepod (*Senna obtusifolia*), and *Colletotrichum* spp. has been reported on sicklepod. *Colletotrichum* spp. has been reported to cause anthracnose of pokeweed (*Phytolacca americana* L.) in Oklahoma, but has not been reported in Georgia (Whiting & Roncadori, 1997). In particular, researchers have noticed that some growers leave infected fruit on the plant when harvesting thus providing an inoculum source for further infection (Pearson *et al*., 1984). *Colletotrichum* spp. can be seed borne in crop plants. *C. capsici* and *C. gloeosporioides* occur either externally or internally in pepper seeds. Survival of mycelia and stromata in colonized pepper seeds have been reported (Manandhar *et al*., 1995). It was shown that the pathogen readily colonizes the seed coat and peripheral layers of endosperm even in moderately colonized seeds. Heavily colonized seeds had abundant inter- and intra-cellular mycelium and acervuli in seed coat endosperm and embryo, showing disintegration of parenchymatous layers of the seed coat and depletion of food material in endosperm and embryo (Chitkara *et al*., 1990). A

separate study clarified that seed-borne *C. gloeosporioides* was transmitted from endosperm tissue to hypocotyls and radicals in red pepper (Lee & Chung, 1995).

Conidia germinate on fruit and produce germ tubes with adhesive appressoria (Manandhar *et al*., 1995). Germination and development of appressoria occurred at 95 to 100% RH and at 20 to 30 degrees C; however, abundant surface moisture was only visible on leaf and fruit surfaces at 100% RH (Dodd *et al*., 1991). The conidia of *C. gloeosporioides* germinated on both green and red fruits within 2 hr after inoculation. Infection of green fruits by the fungus may lead to anthracnose development on immature fruit (Oh *et al*., 1997). On green fruits of pepper, only one isolate caused dark, brown to black lesions 6 days after inoculation. Later, these lesions slowly increased in size and became sunken. On red fruit of pepper, all isolates produced more severe symptoms (Yu *et al*., 1987). The colonizing hyphae grow both intracellularly and intercellularly as a lesion develops. It is during the initial phase of colonization that the resistance responses of the plant may be expressed (Jeffries *et al*., 1990).

Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the produce ripens. *Colletotrichum* and *Glomerella* species are by far the most important pathogens that cause this type of infection. Although these genera have been the subject of numerous investigations, there remains many gaps in our knowledge of the disease process and our understanding of the complex relationships between the various fungi involved (Jeffries *et al*., 1990). Appressoria are known to form adhesive disks for adhering to plant surfaces and remain latent until physiological changes occur in fruits. Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits. The symptoms observed on different age fruit may be due to differences in the formation of infection hyphae (penetration pegs) and not to differences in conidial germination and appressoria formation (Manandhar *et al*., 1995).

Conidia often do not germinate *in situ* because of the presence of germination-inhibitors in the spore matrix, but will germinate after being washed or rain-splash disseminated (Manandhar *et al*., 1995). Under normal conditions, conidia dispersed by rainfall may remain on a plant surface and retain potential to cause disease for periods of over 7 days (Estrada *et al*., 1993). During wet periods, appressoria have been reported to

produce secondary conidia, which may be involved in secondary spread to pepper fruits (Manandhar *et al*., 1995).

Variability in *Colletotrichum*

The reproduction mode in many *Colletotrichum* populations is mainly or exclusively vegetative. In the absence of a sexual stage, the only means of exchanging genetic material between two strains would be anastomosis and heterokaryosis. Microscope examination reveals that anastomosis occurs between lateral branches, which grow out of neighboring hyphae and form anastomosis bridges connecting two hyphae. The resultant fused cells are binucleate and appear not to proliferate, but support adjacent uninucleate cells with genes of either nuclei (Katan, 2000). Heterokaryosis and parasexuality are the crucial factors determining the phenotypic heterogeneity within the group and a thorough analysis at the molecular genetic level is needed. Around 98% of conidia from a single agar culture of *Colletotrichum* are uninucleate, but a small number is always multinucleate. Nuclear heterogeneity can be increased under different environmental conditions and growth in liquid culture can increase the proportion of bi-nucleate conidia to 17% and tri-nucleate conidia to 3-5% in some species (Jeffries *et al*., 1990).

Traditional Identification Methods

The taxonomy of *Colletotrichum* species is in a state of change and remains confusing (Freeman *et al*., 1998). *Colletotrichum* species are highly variable, as manifested by colony morphology, conidial shape, presence and shape of setae and appressoria, pigmentation, fungicide sensitivity, pathogenicity, and other traits (Katan, 2000). Traditional differentiation between *Colletotrichum* species, based on host range or origin, may not be reliable criteria for fungi of this genus since taxa, such as *C. gloeosporioides*, infect a broad range of host plants (Freeman *et al*., 1998). However, the isolates of *C. gloeosporioides* that are specifically pathogenic to distantly related hosts may not be genetically isolated, indicating that the population structure and dynamics of *C. gloeosporioides* are very complex (Cisar *et al*., 1994).

Chapter 1. Literature Review

The identification of species of *Colletotrichum* has relied primarily on morphological differences such as colony color, size, and shape of conidia, optimal temperature, growth rate, presence or absence of setae, and existence of the teleomorph, *Glomerella* (Freeman *et al*., 1998). Conidial morphology has been traditionally emphasized over other taxonomic criteria, although conidia of *Colletotrichum* are potentially variable. In this study, shape of the conidia was a reliable character in speciation only if the conidia were produced on a medium such as strawberry leaf agar (SLA), which ensured uniform conidia within a species. This was particularly true of *C. fragariae* and *C. gloeosporioides* (Gunnell & Gubler, 1992). Differences between isolates are also evident with respect to their relative pathogenicity or virulence (Jeffries *et al*, 1990).

The majority of the conidia of *C. gloeosporioides* were oblong with obtuse ends, and were generally shorter and broader than conidia of *C. fragariae* and *C. acutatum* (Gunnell & Gubler, 1992). In general, conidia of *C. acutatum* are elliptic to fusiform in shape; whereas conidia of *C. gloeosporioides* are oblong with obtuse ends (Freeman *et al*, 1998).

Table 1.1 Measurements of conidia reported for species of *Colletotrichum*.

	Sutton, 1982		Gunnell & Gubler, 1992		Baxter *et al*, 1985	
Species	Length (µm)	Width (µm)	Length (µm)	Width (µm)	Length (µm)	Width (µm)
C. acutatum	8.5-10	4.5-6	15	4	12.9	3.2
C. capsici	18-23	3.5-4				
C. coccodes	16-22	3-4			19.2	3.6
C. dematium	19.5-24	2-2.5			16.7	3.1
C. gloeosporioides	9-24	3-4.5	15	4.3	15.9	4.4

Table 1.2 Cultural characteristics of several *Colletotrichum* species according to B.C. Sutton (1982).

Species	Setae	Sclerotia	Conidia Shape	Colony Color	Appressoria Size (µm)	Appressoria Shape
C. acutatum	Present	Absent	Fusiform, medianly constricted	White to pinkish gray	8.5-10 X 4.5-6	Clavate or irregular
C. capsici	Abundant	Absent	Falcate, fusiform apices acute	White to dark gray	9-14 X 6.5-11.5	Clavate to circular
C. coccodes	Present	Abundant	Fusiform, medianly constricted	White mycelia	11-16.5 X 6-9.5	Long clavate, irregular
C. dematium	Abundant	Abundant	Falcate, fusiform apices acute	White to gray or dark brown	8-11.5 X 6.5-8	Clavate to circular
C. gloeosporioides	Varied	Varied	Straight, obtuse at apex	Varied	6-20 X 4-12	Clavate or irregular

Contemporary methods of identification

In particular, the capacity to accurately identify phenotypically similar strains from diverse sources and to separate them from other phenotypes by molecular markers must be demonstrated. *C. gloeosporioides* is a highly variable species, as shown by morphological characters and molecular markers. As mentioned above, *C. gloeosporioides* from avocado is heterogeneous and genetically complex (Freeman *et al*., 1998). Recently, alternative approaches based on RFLP and PCR-RAPD analysis revealed considerable variation within *C. gloeosporioides*, but it was not possible to relate these to the taxonomic status of species (Sherriff *et al*., 1994).

Biolog® uses a 96 – well microtiter tray containing a range of dehydrated carbon sources for assimilation and oxidation tests. The profile of growth responses provides a metabolic fingerprint for each isolate. Biolog® contains fingerprints for *C. acutatum*, *C. dematium*, *C. gloeosporioides*, and *C. coccodes*. Biolog® has overcome several problems of existing diagnostic kits by providing a large number of tests and a large database. Nevertheless, further refinements to the database and tests used may permit the system to

be used with greater confidence (Praphailong *et al*., 1997). Prescreening on the basis of carbon-source utilization by an automated approach such as the Biolog® plate system might be worthwhile in optimization of fungal screening programs. The correlation with fungal species was not as clear using Biolog®, compared to genetic relatedness as measured by RAPDs (Talbot *et al*., 1996).

Disease Control

Several management strategies have been developed to control quiescent infections in tropical fruit but they often involve the extensive use of fungicides, which are both expensive for growers in developing countries and potentially damaging to the environment (Dodd *et al*., 1991). Differentiating between *Colletotrichum* species responsible for disease epidemics is vital for developing and implementing effective control strategies (Freeman *et al*., 1998).

As the single, marketable organ, the fruit of peppers must be protected from pathogens or abiotic stresses. Pathogenesis-related (PR) proteins and several antifungal proteins that are responsible for protection against pathogens during fruit ripening have been identified. Plant responses to fungal morphogenesis (*Colletotrichum* spp.) during fruit ripening may be more important in determining resistance or susceptible interactions. Hypersensitive responses (HR) cause rapid cell death to halt colonization of tissues by the pathogen. Another array of defense strategies include the production of antimicrobial phytoalexins, pathogenesis-related (PR) proteins, and cysteine proteins, such as lipid transfer protein and thionins (Oh *et al*., 1999). The creation of these anti-fungal compounds can be elicited by using plant activators such as harpin proteins (Messenger 3 WDG, Eden Bioscience, Bothell, WA) and acibenzolar-s-methyl (Actigard 50 WG, Syngenta, Greensboro, N.C.).

Harpin is a heat-stable, cell envelope-associated protein with an apparent molecular mass of 44 kd. In fact, $harpin_{Pss}$ appears to be highly hydrophilic and is a soluble cytoplasmic protein when expressed in *Escherichia coli.* It is argued that the *Pseudomonas syringae* HR elicitor acts in a nonhost as a signal that triggers a plant defense response pathway rather than as a toxic agent that directly kills plant cells. The limited data available suggests that sensitivity to harpins varies among plants without any

obvious correlation to host range. It also appears that harpins delivered by living Hrp+ bacteria are more effective at eliciting the HR than are purified harpins (He *et al*., 1993).

$Harpin_{Pss}$ protein elicited the HR in solanaceous (tomato) plants (He *et al*., 1993). It is shown that harpin induced Systemic Acquired Resistance (SAR) in cucumber to diverse pathogens, including the anthracnose fungus (*C. lagenarium*). Biological induction of SAR is usually associated with cell death during the HR or disease necrosis triggered by avirulent or virulent pathogens (Strobel *et al*., 1996).

Actigard has been reported to induce resistance in wheat against fungal pathogens, in bean against bacterial and fungal infections, and in tobacco and *Arabidopsis thaliana* against fungal, bacterial, and viral infections. Actigard complies with the definition of a SAR inducer. It gives protection to the same spectrum of pathogens, causes the expression of the same molecular and biochemical markers (e.g., pathogenesis-related proteins) as biological inducers, and does not have direct antimicrobial activity. Its use in conjunction with or alternated with traditional fungicides and bactericides may lead to a reduction of the number of applications and perhaps rate (Romero *et al*., 2001). Peppers treated with Actigard at 35 g a.i./ha had slightly lower yields per plant, but not significantly lower than the untreated control. This suggests that using lower rates of Actigard may not significantly affect yield. These results suggest that Actigard can activate resistance not only in leaves, but also in pepper fruits (Kousik & Subramanya, 2001).

Induced resistance in bell pepper is expressed as early as 3 days after treatment and continues for at least 2 weeks. In contrast, Actigard induced resistance in monocots may be considerably longer, lasting the entire growing season in wheat. The sum of the number of opened flowers and flower buds at 5 weeks after transplanting was 0.5 to 26% less for Actigard sprayed plants compared to non-sprayed controls. This could mean that some flower buds did not develop or aborted before counting. Plants sprayed with Actigard alone or combined with copper hydroxide, had the greatest yields in either the second or third harvest, again suggesting a delay in fruit maturity or fruit set. Data supports the hypothesis that there could be a cost when induced resistance is expressed constitutively, evidenced as a reduction or delay in fruit set, maturity, or both (Romero *et*

al., 2001). Plants treated with acibenzolar-s-methyl alone appeared stunted compared to plants in other treatments (Miller *et al*., 1998).

It seemed that the continued application of Actigard may have a horticultural cost manifested as a loss in fruit yield in bell pepper; this was most evident when Actigard was applied weekly. So, if defense is expensive to plants in terms of energy or precursors, conversion of resistance that is inducible to constitutive may result in reduced yields (Romero *et al*., 2001).

Strobilurin compounds, azoxystrobin (Quadris 2.08 SC, Syngenta Crop Protection, Greensboro, N.C., kresoxim methyl (Sovran 50 WG, BASF, Mount Olive, NJ), and trifloxystrobin (Flint 50 WP, Bayer, Leverkusen, Germany), inhibit mitochondrial respiration by blocking quinol oxidation in cytochrome bc_1 complex, thus blocking the production of ATP. The strobilurins form a distinct class of fungicidal compounds based on a chemical that can be isolated from several basidiomycete species that inhabits decay of plant material in woodland soils. The fungicide is used on a preventative basis with surface systemic properties (Ypema & Gold, 1999). Azoxystrobin moves through the leaf blade by leaking through the waxy layers and into adjacent cells where the compound moves systemically. Kresoxim methyl and trifloxystrobin move translaminarly but not systemically (Vincelli, 2002). These two compounds have demonstrated movement by vapor redistribution on the plant epidermis which is termed "mesostemic" and "surface systemic". This vapor redistribution occurs by the vaporization of the chemical and precipitation elsewhere on the leaf. This occurs over a period of several weeks. Recently, resistance to this new fungicide has been discovered in several pathogens; therefore, applications are recommended in rotation with board spectrum fungicides for resistance management. In May of 2001, a section 18 was granted for use of azoxystrobin on peppers to control anthracnose, but only in an alternating fungicide regiment. Currently, azoxystrobin has a full label. (EPA Reg. 100-1098)

Copper sulfate (Cuprofix 20 DF, Cerexagri, Philadelphia, PA) acts to denature general proteins and may be effective as an anti-fungal compound; beyond its usual application for control of disease incited by bacteria. Ethlenebisdithiocarbamate (Maneb 75 DF, Cerexagri, Philadelphia, PA) has non-systemic, protective attributes that acts by

being metabolized to the isothiocyanate radical which then inactivates the SH group in amino acids within fungal cells. Maneb has been typically recommended for control of anthracnose on maturing pepper fruit. The standard recommendation is to start weekly applications prior to fruit ripening.

Chapter 2: Evaluation of Chemicals for Management of Anthracnose on Bell Pepper

Introduction

In 1997, the United States produced 18237 hectares of hot peppers and 24940 hectares of sweet peppers (NASS, 1997). Approximately one million dollars worth of peppers (*Capsicum annum*) are grown in Virginia on 405 hectares annually (Manheimer & Vick, 2002). Prior to the late 1990's, pepper anthracnose was easily controlled with applications of Maneb 75 DF (Cerexagri Inc., King of Prussia, PA) and copper hydroxide (Kocide, Griffin Corp., Valdosta, GA) on the Eastern Shore of Virginia. Since then, there has been a steady increase of pepper anthracnose on mature and immature fruit. By the year 2000, losses from this disease had reached as high as 70% in fields located around the Chesapeake Bay.

The literature on pepper anthracnose indicates that this disease usually attacks mature fruit and is considered of minor economic importance (Boucher & Ashley, 2001). There are several species that cause anthracnose on pepper leaves and stems, but there are two main species that are considered to cause anthracnose on pepper fruit, *Colletotrichum capsici* (Sydow) Butler et Bisby and *C. gloeosporioides* (Penzig) Penzig et Saccardo (Manandhar *et al*., 1995; Hong & Hwang, 1998). *C. capsici* forms a sunken lesion which turns black with growth. *C. gloeosporioides* forms sunken lesions that range in color from orange, tan, brown, and black. The two species can be distinguished from each other based on conidia size and shape (Sutton, 1982).

Conidia do not function as survival structures as their viability declines rapidly. Mycelium, however, may remain viable for long periods in infected seeds or plant debris. Microsclerotia, formed sparsely by a few species can play an important role in survival (Baxter 1985). When the relative humidity of the pepper microclimate ranges around 95 to 100 % and temperatures are 20 to 30 °C, conidia of *Colletotrichum* spp. will germinate and form appressoria in a few hours and anthracnose lesions may ensue (Dodd *et al*., 1991; Estrada *et al*., 1993). Species in this genus can cause latent infections in many crops (Jeffries *et al*., 1990). Upon germination, the conidia produce appressoria on the outer cuticle then cease to develop further (Manandhar *et al*., 1995). Continual

application of fungicides is the most used method of combating latent infections and the development of latent infections into disease (Dodd *et al*., 1991).

Plant activators initiate defensive responses in the plant, which may protect against pathogen infection and disease, a process called systemic acquired resistance (SAR). In initiating this process, energy may be directed to increasing the thickness of plant cell walls, increasing phytoalexin concentration, and initiating cell death, which decrease the amount of plant energy put into growth and fruit production (Romero *et al*., 2001). Acibenzolar-S-methyl (Actigard 50 WG, Syngenta, Greensboro, N.C.), a plant activator, has shown a non-significant decrease in yields when used at the rate of 35 g a.i./ha. Flower abortion, fruit drop, chlorosis, stunting, and reduced yield have been reported when acibenzolar-s-methyl is applied on a regular basis, but not at lower rates (Kousik & Subramanya, 2001). The sum of the number of opened flowers and flower buds on pepper plants counted 5 weeks after transplanting was 0.5 to 26% less for acibenzolar-s-methyl sprayed plants than for non-sprayed controls (Romero *et al*., 2001). Several experiments have shown an increase in disease resistance not only on pepper leaves, but in fruit as well (Kousik & Subramanya, 2001).

Strobilurin compounds have shown activity in suppressing many different fungi. These compounds may be useful in reducing anthracnose. Strobilurins inhibit mitochondrial respiration by blocking quinol oxidation in cytochrome bc_1 complex, thus blocking the production of ATP (Ypema & Gold, 1999). This action isn't a lethal effect, but it is inhibitory and may make the fungus more susceptible to parasitism. Azoxystrobin (Quadris 2.08 SC, Syngenta, Greensboro, N.C.) is labeled for control of anthracnose on green pepper, but there is little data showing the efficacy of this product. Kresoxim methyl (Sovran 50 WG, BASF, Mount Olive, NJ) and trifloxystrobin (Flint 50 WP, Bayer, Leverkusen, Germany) have not been labeled for use on peppers for anthracnose, and often are compared to azoxystrobin in field trials.

Maneb has been used for many years to control the development of anthracnose. This product had worked well in reducing economic loss due to anthracnose, but its efficacy has been questionable as a result of problems in disease control. Maneb is usually rotated or combined with copper sulfate or a similar copper compound to control bacterial spot (*Xanthomonas campestris* pv. *vesicatori*) (Miller *et al*., 1998).

Chapter 2. Introduction

To investigate the emerging disease problem with anthracnose, experiments were initiated at the Eastern Shore Agricultural Research and Extension Center in Painter, Virginia. Plant activators, strobilurin compounds, and standard recommended fungicides were applied to fields at various timings, combinations and amounts to determine levels of anthracnose disease suppression. The objective was to develop effective management strategies for this disease.

Materials and Methods

In 2001, 2002, and 2003, four field experiments were conducted in Painter, Virginia. Experiments were conducted as 2001, 2002A, 2002B, and 2003. Seedlings of pepper (*Capsicum annum* 'Paladin') were obtained from a local commercial greenhouse. The plants were transplanted to bare ground that had been prepared with a moldboard plow and fertilized (Appendix 1.1-1.4). The experiment was conducted in a randomized complete block with four replications in a Bojac sandy loam. Plants were spaced 30.48 cm apart within rows spaced 0.914 m apart. The single row plots were 9.12 m long and bordered by guard rows. Ten consecutive plants from the middle of each plot were harvested.

Guard rows were inoculated with a local isolate (VA0110) of *Colletotrichum gloeosporioides* from pepper. The isolate was identified by Dr. Helene R. Dillard and Dr. Peter Oudemans. The fungus was cultured on potato dextrose agar (Difco laboratories, Detroit, MI) in 9-cm-dia. Petri plates for 7 days (Appendix 2.7). Plates were rinsed with distilled water and scraped lightly with a sterile swab to remove conidia. The opaque solution was then filtered through sterile cheesecloth. The number of conidia was estimated using a hemacytometer and adjusted by dilution with distilled water to 7.9×10^6 conidia/ml for 2001, 8.0×10^6 conidia/ml for 2002A and 2002B, 2.2×10^5 conidia/ml for 2003 (Hansen, 2002)(Appendix 2.1). The conidia were applied to a single pinprick on pepper fruits on plants both ends and the middle of each guard row. The lesions that developed acted as a source of inoculum for the plot rows.

Treatments were applied at varying times and at rates listed on the product label. Maneb, azoxystrobin, copper sulfate, and trifloxystrobin were applied every 7 days as soon as fruit began to appear. Combinations, such as azoxystrobin and maneb,

acibenzolar-s-methyl and azoxystrobin, kresoxim-methyl and maneb, and maneb and copper sulfate were alternated every 7 days after fruit development. Acibenzolar-s-methyl and harpin protein (Messenger 3 WDG, Eden Bioscience, Bothell, WA) were applied every 14 days after transplanting. Acibenzolar-s-methyl was applied every 14 days before flowering in combination with azoxystrobin and maneb alternating sprays (Appendix 3). The weather conditions were recorded for every application (Appendix 4).

Treatments were applied with a propane-pressurized backpack sprayer, using a three nozzle boom, 0.914 m wide with outer 22.86 cm drop nozzles. The nozzles contained D4/45 disc, core combination, which delivered 243.1 L/ha at 276 kPa.

The transplants were planted on 21 June 2001, 19 June 2002, and 23 June 2003, respectively. There were three to four harvests of marketable and diseased fruit. Diseased fruit was picked at any stage since it is unmarketable. The number and weight of marketable and diseased fruit were tallied for each harvest. Statistical analyses were performed using Agriculture Research Manager (Gylling Data Management, Inc, Brookings, South Dakota).

Results

Azoxystrobin had the highest total fruit weight per hectare for 2003 but was not significantly different from the control. Throughout the four experiments, maneb as a single treatment had a high number of marketable fruit and marketable fruit weight (Table 2.1). Trifloxystrobin in 2001 and kresoxim-methyl in 2002A had the highest yields, but were not significantly different from the controls. Azoxystrobin and maneb, used as single treatments or in alternations, consistently reduced percentages of diseased fruit with an increase in number of marketable fruit to a significant level (Table 2.2, 2.4, and 2.5). Number of marketable fruit and marketable fruit weight was lower or equal to the control for 2002A, 2002B, and 2003 for acibenzolar-s-methyl at 35 g a.i./ha. In 2003, acibenzolar-s-methyl at lower rates showed a slight increase in yield in kilograms per hectare, percentage of number of fruit diseased, and percentage of weight of fruit diseased compared to acibenzolar-s-methyl at 35 g a.i./ha (Table 2.1, Table 2.4, and Table 2.5). In study 2002A, 2002B, and 2003 acibenzolar-s-methyl yield was lower than the untreated control, while maneb had significantly higher yields than the untreated

control. Harpin protein was among the lowest in number of marketable fruit and marketable fruit weight for all experiments. Copper sulfate showed no activity at all in reducing anthracnose or significantly increasing yields.

Figure 2.1 Symptoms of pepper anthracnose on unmarketable fruit.

Table 2.1 Effect of fungicide treatments on total yield of pepper fruit (kg/ha) in four field trials.

Treatment		**Appl. Timing**	**Rate a.i.**		2001[4]	2002A[5]	2002B[6]	2003[7]
Actigard	50 WG	All season – 14 day	3.5	g/ha	28400 abc[1]			
Actigard	50 WG	All season – 14 day	10.5	g/ha	28766 abc[2]	10782 de		29376 cd
Actigard	50 WG	All season – 14 day	35.0	g/ha		12084 cde	11108 d	26976 d
Actigard BF	50 WG	Pre-bloom– 14 day	10.5	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha	28807 abc	15705 a-d		34137 bcd
Actigard	50 WG	All season – 7 day	10.5	g/ha				
+Quadris	2.08 SC	All season – alt	9.0	ml/ha	25551 c	11392 de		36985 abc
Quadris	2.08 SC	All season – 7 day	9.0	ml/ha	30149 abc	20222 ab	20384 abc	43535 a
Maneb	75 DF	All season – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	All season – alt	9.0	ml/ha	34340 a	13752 a-e	19693 abc	39182 ab
Maneb	75 DF	All season –7 day	1.7	kg/ha	34625 a	19408 abc	23721 a	39914 ab
Maneb	75 DF	All season – 7 day	1.7	kg/ha				
+Cuprofix	20 DF	All season – alt	0.4	kg/ha	29742 abc	10660 de		36374 abc
Cuprofix	20 DF	All season – 14 day	0.4	kg/ha	29417 abc	6266 e		36659 abc
Flint	50 WP	All season – 7 day	140.0	g/ha	34747 a	18309 a-d		
Messenger	3 WDG	All season – 7 day	448.0	g/ha	32794 ab			
Messenger	3 WDG	All season – 14 day	0.7	g/ha	29702 abc	20547 a		
Messenger	3 WDG	All season – 14 day	1.1	g/ha		14322 a-d	20344 abc	
Sovran	50 WG	All season – 14 day	1.8	g/ha	29539 abc[3]	18391 a-d		
Sovran	50 WG	All season – 7 day	448.0	g/ha				
+Maneb	75 DF	All season – alt	1.7	kg/ha		15868 a-d	22297 ab	31980 bcd
Messenger BF	3 WDG	Pre-bloom – 14 day	1.1	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha	32428 abc	12369 b-e		
Control					28237 abc	15787 a-d	19001 bc	35439 a-d
LSD					7039	7925	4517	9041

[1]In 2001, the rate was 7 g a.i./ha for acibenzolar-s-methyl. [2]In 2001, the rate was 21 g a.i./ha for acibenzolar-s-methyl. [3]In 2001, the rate was 25.6 g a.i./ha for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD). [4] In 2001: pre-bloom – 19 Jun, 3, 17 Jul; all season, 14 day – 19 Jun, 2, 17, 28 Jul, 17, 31 Aug; all season, 7-day – 28 Jul, 3, 10, 17, 24, 31 Aug and 8 Sep; all season alt. – 3, 17, and 31 Aug. [5] In 2002A: pre-bloom - 25 Jun, 3, 8, and 15 Jul; all season 14-day – 25 Jun, 3, 8, 15 Jul, 5, 19 Aug, 3, and 17 Sep; all season, 7-day – 29 Jul, 5, 12, 19, 26 Aug, 3, 10, and 17 Sep; all season, alt. – 5, 19 Aug, 3, and 17 Sep. [6] In 2002B, all season 14-day – 26 Jun, 8, 16, 23, Jul, 6, 22 Aug, 6, and 20 Sep; all season, 7-day – 23, 31 Jul, 6, 15, 22, 30 Aug, 6, 12, and 20 Sep; all season, alt. – 23 Jul, 6, 22 Aug, 6, and 20 Sep. [7] In 2003, pre-bloom 26 Jun, 12, 25 Jul; all season 14-day 26 Jun, 12, 25 Jul, 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, 7-day 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, alt. – 6, 20 Aug, 3, and 17 Sep; early fruit 7-day 6, 20 Aug, 3, and 17 Sep; early fruit – alt 13, 20 Aug, 3, and 17 Sep.

Table 2.2 Effect of fungicide treatments on marketable fruit weight (kg/ha).

Treatment			**Rate a.i.**		2001[4]	2002A[5]	2002B[6]	2003[7]
Actigard	50 WG	All season – 14 day	3.5	g/ha	24250 cde[1]			
Actigard	50 WG	All season – 14 day	10.5	g/ha	24941 cde[2]	5330 def		18675 c
Actigard	50 WG	All season – 14 day	35.0	g/ha		5981 c-f	5330 c	20547 c
Actigard BF	50 WG	Pre-bloom– 14 day	10.5	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha	26325 a-e	8422 a-e		24901 abc
Actigard	50 WG	All season – 7 day	10.5	g/ha				
+Quadris	2.08 SC	All season – alt	9.0	ml/ha	24005 cde	6144 c-f		31248 ab
Quadris	2.08 SC	All season – 7 day	9.0	ml/ha	28318 a-d	12939 ab	8951 ab	34381 a
Maneb	75 DF	All season – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	All season – alt	9.0	ml/ha	33079 a	7161 a-f	9033 ab	31980 ab
Maneb	75 DF	All season –7 day	1.7	kg/ha	32794 ab	13020 a	11921 a	27993 abc
Maneb	75 DF	All season – 7 day	1.7	kg/ha				
+Cuprofix	20 DF	All season – alt	0.4	kg/ha	27057 a-e	3703 ef		24778 abc
Cuprofix	20 DF	All season – 14 day	0.4	kg/ha	25918 b-e	1465 f		23761 bc
Flint	50 WP	All season – 7 day	140.0	g/ha	32835 ab	11311 a-d		24087 abc
Messenger	3 WDG	All season – 7 day	448.0	g/ha	25185 cde			
Messenger	3 WDG	All season – 14 day	0.7	g/ha	24697 cde	11230 a-d		
Messenger	3 WDG	All season – 14 day	1.1	g/ha		5656 c-f	7242 bc	
Sovran	50 WG	All season – 14 day	1.8	g/ha	27505 a-e[3]	9073 a-e		
Sovran	50 WG	All season – 7 day	448.0	g/ha				
+Maneb	75 DF	All season – alt	1.7	kg/ha		8911 a-e	10741 a	24005 abc
Messenger BF	3 WDG	Pre-bloom – 14 day	1.1	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha	30678 abc	6591 c-f		
Control					21198 e	7161 a-f	5411 c	23110 bc
LSD					7002	6231	3006	10572.8

[1]In 2001, the rate was 7 g a.i./ha for acibenzolar-s-methyl. [2]In 2001, the rate was 21 g a.i./ha for acibenzolar-s-methyl. [3]In 2001, the rate was 25.6 g a.i./ha for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD). [4] In 2001: pre-bloom – 19 Jun, 3, 17 Jul; all season, 14 day – 19 Jun, 2, 17, 28 Jul, 17, 31 Aug; all season, 7-day – 28 Jul, 3, 10, 17, 24, 31 Aug and 8 Sep; all season alt. – 3, 17, and 31 Aug. [5] In 2002A: pre-bloom - 25 Jun, 3, 8, and 15 Jul; all season 14-day – 25 Jun, 3, 8, 15 Jul, 5, 19 Aug, 3, and 17 Sep; all season, 7-day – 29 Jul, 5, 12, 19, 26 Aug, 3, 10, and 17 Sep; all season, alt. – 5, 19 Aug, 3, and 17 Sep. [6] In 2002B, all season 14-day – 26 Jun, 8, 16, 23, Jul, 6, 22 Aug, 6, and 20 Sep; all season, 7-day – 23, 31 Jul, 6, 15, 22, 30 Aug, 6, 12, and 20 Sep; all season, alt. – 23 Jul, 6, 22 Aug, 6, and 20 Sep. [7] In 2003, pre-bloom 26 Jun, 12, 25 Jul; all season 14-day 26 Jun, 12, 25 Jul, 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, 7-day 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, alt. – 6, 20 Aug, 3, and 17 Sep; early fruit 7-day 6, 20 Aug, 3, and 17 Sep; early fruit – alt 13, 20 Aug, 3, and 17 Sep.

Table 2.3 Effect of fungicide treatments on number of marketable fruit (kg/ha x 1000).

Treatment		**Appl. Timing**	**Rate a.i.**		2001[4]	2002A[5]	2002B[6]	2003[7]
Actigard	50 WG	All season – 14 day	3.5	g/ha	182 d-i[1]			
Actigard	50 WG	All season – 14 day	10.5	g/ha	188 d-i[2]	51 def		142 b
Actigard	50 WG	All season – 14 day	35.0	g/ha		57 c-f	49 c	142 b
Actigard BF	50 WG	Pre-bloom– 14 day	10.5	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha	220 a-f	80 a-e		162 ab
Actigard	50 WG	All season – 7 day	10.5	g/ha				
+Quadris	2.08 SC	All season – alt	9.0	ml/ha	192 c-i	55 c-f		200 ab
Quadris	2.08 SC	All season – 7 day	9.0	ml/ha	216 a-g	132 a	83 ab	212 a
Maneb	75 DF	All season – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	All season – alt	9.0	ml/ha	244 abc	70 b-f	85 ab	216 a
Maneb	75 DF	All season –7 day	1.7	kg/ha	228 a-e	126 ab	111 a	180 ab
Maneb	75 DF	All season – 7 day	1.7	kg/ha				
+Cuprofix	20 DF	All season – alt	0.4	kg/ha	197 b-h	36 ef		177 ab
Cuprofix	20 DF	All season – 14 day	0.4	kg/ha	172 f-i	13 f		160 ab
Flint	50 WP	All season – 7 day	140.0	g/ha	256 a	109 a-d		165 ab
Messenger	3 WDG	All season – 7 day	448.0	g/ha	163 hi			
Messenger	3 WDG	All season – 14 day	0.7	g/ha	160 hi	87 a-e		
Messenger	3 WDG	All season – 14 day	1.1	g/ha		56 c-f	72 bc	
Sovran	50 WG	All season – 14 day	1.8	g/ha	233 a-d[3]	105 a-d		
Sovran	50 WG	All season – 7 day	448.0	g/ha				
+Maneb	75 DF	All season – alt	1.7	kg/ha		91 a-e	100 a	161 ab
Messenger BF	3 WDG	Pre-bloom – 14 day	1.1	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha				
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha				
Control					140 i	78 a-e	48 c	142 b
LSD					40	61	28	59

[1]In 2001, the rate was 7 g a.i./ha for acibenzolar-s-methyl. [2]In 2001, the rate was 21 g a.i./ha for acibenzolar-s-methyl. [3]In 2001, the rate was 25.6 g a.i./ha for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD). [4] In 2001: pre-bloom – 19 Jun, 3, 17 Jul; all season, 14 day – 19 Jun, 2, 17, 28 Jul, 17, 31 Aug; all season, 7-day – 28 Jul, 3, 10, 17, 24, 31 Aug and 8 Sep; all season alt. – 3, 17, and 31 Aug. [5] In 2002A: pre-bloom - 25 Jun, 3, 8, and 15 Jul; all season 14-day – 25 Jun, 3, 8, 15 Jul, 5, 19 Aug, 3, and 17 Sep; all season, 7-day – 29 Jul, 5, 12, 19, 26 Aug, 3, 10, and 17 Sep; all season, alt. – 5, 19 Aug, 3, and 17 Sep. [6] In 2002B, all season 14-day – 26 Jun, 8, 16, 23, Jul, 6, 22 Aug, 6, and 20 Sep; all season, 7-day – 23, 31 Jul, 6, 15, 22, 30 Aug, 6, 12, and 20 Sep; all season, alt. – 23 Jul, 6, 22 Aug, 6, and 20 Sep. [7] In 2003, pre-bloom 26 Jun, 12, 25 Jul; all season 14-day 26 Jun, 12, 25 Jul, 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, 7-day 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, alt. – 6, 20 Aug, 3, and 17 Sep; early fruit 7-day 6, 20 Aug, 3, and 17 Sep; early fruit – alt 13, 20 Aug, 3, and 17 Sep.

Table 2.4 Effect of fungicide treatments on the percentage of diseased fruit.

Treatment		**Appl. Timing**	**Rate a.i.**		2001[4]	2002A[5]	2002B[6]	2003[7]
Actigard	50 WG	All season – 14 day	3.5	g/ha	23.04 de[1]			
Actigard	50 WG	All season – 14 day	10.5	g/ha	21.19 def[2]	15.87 b-e		11.83 ab
Actigard	50 WG	All season – 14 day	35.0	g/ha		17.11 b-e	19.93 bc	8.79 ab
Actigard BF	50 WG	Pre-bloom– 14 day	10.5	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha	13.20 efg	11.52 cde		8.70 ab
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha				
Actigard	50 WG	All season – 7 day	10.5	g/ha	7.61 g	14.58 b-e		8.70 ab
+Quadris	2.08 SC	All season – alt	9.0	ml/ha				
Quadris	2.08 SC	All season – 7 day	9.0	ml/ha	11.83 efg	10.68 de	10.56 d	7.61 ab
Maneb	75 DF	All season – 7 day	1.7	kg/ha	8.45 g	14.98 b-e	6.34 d	4.37 b
+Quadris	2.08 SC	All season – alt	9.0	ml/ha				
Maneb	75 DF	All season –7 day	1.7	kg/ha	11.12 efg	8.78 e	4.72 d	7.79 ab
Maneb	75 DF	All season – 7 day	1.7	kg/ha	16.21 efg	14.52 b-e		14.70 a
+Cuprofix	20 DF	All season – alt	0.4	kg/ha				
Cuprofix	20 DF	All season – 14 day	0.4	kg/ha	28.71 cd	14.45 b-e		15.25 a
Flint	50 WP	All season – 7 day	140.0	g/ha	9.72 fg	11.07 cde		12.96 ab
Messenger	3 WDG	All season – 7 day	448.0	g/ha	44.90 a			
Messenger	3 WDG	All season – 14 day	0.7	g/ha	38.96 abc	23.32 ab		
Messenger	3 WDG	All season – 14 day	1.1	g/ha		29.02 a	27.33 ab	
Sovran	50 WG	All season – 14 day	1.8	g/ha	10.48 fg[3]	19.06 a-e		
Sovran	50 WG	All season – 7 day	448.0	g/ha		13.14 b-e	12.88 cd	9.13 ab
+Maneb	75 DF	All season – alt	1.7	kg/ha				
Messenger BF	3 WDG	Pre-bloom – 14 day	1.1	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha	9.19 fg	14.23 b-e		
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha				
Control					40.27 abc	22.65 abc	32.29 a	13.91 a
LSD					12.19	11.79	9.19	9.38

[1]In 2001, the rate was 7 g a.i./ha for acibenzolar-s-methyl. [2]In 2001, the rate was 21 g a.i./ha for acibenzolar-s-methyl. [3]In 2001, the rate was 25.6 g a.i./ha for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD). [4] In 2001: pre-bloom – 19 Jun, 3, 17 Jul; all season, 14 day – 19 Jun, 2, 17, 28 Jul, 17, 31 Aug; all season, 7-day – 28 Jul, 3, 10, 17, 24, 31 Aug and 8 Sep; all season alt. – 3, 17, and 31 Aug. [5] In 2002A: pre-bloom - 25 Jun, 3, 8, and 15 Jul; all season 14-day – 25 Jun, 3, 8, 15 Jul, 5, 19 Aug, 3, and 17 Sep; all season, 7-day – 29 Jul, 5, 12, 19, 26 Aug, 3, 10, and 17 Sep; all season, alt. – 5, 19 Aug, 3, and 17 Sep. [6] In 2002B, all season 14-day – 26 Jun, 8, 16, 23, Jul, 6, 22 Aug, 6, and 20 Sep; all season, 7-day – 23, 31 Jul, 6, 15, 22, 30 Aug, 6, 12, and 20 Sep; all season, alt. – 23 Jul, 6, 22 Aug, 6, and 20 Sep. [7] In 2003, pre-bloom 26 Jun, 12, 25 Jul; all season 14-day 26 Jun, 12, 25 Jul, 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, 7-day 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, alt. – 6, 20 Aug, 3, and 17 Sep; early fruit 7-day 6, 20 Aug, 3, and 17 Sep; early fruit – alt 13, 20 Aug, 3, and 17 Sep.

Table 2.5 Effect of fungicide treatments on the percentage of diseased fruit by weight.

Treatment		**Appl. Timing**	**Rate a.i.**		2001[4]	2002A[5]	2002B[6]	2003[7]
Actigard	50 WG	All season – 14 day	3.5	g/ha	15.10 cd[1]			
Actigard	50 WG	All season – 14 day	10.5	g/ha	13.14 c-f[2]	13.17 b-e		12.46 abc
Actigard	50 WG	All season – 14 day	35.0	g/ha		13.38 b-e	15.33 bc	7.03 bcd
Actigard BF	50 WG	Pre-bloom– 14 day	10.5	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha	9.08 d-h	9.13 de		7.43 a-d
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha				
Actigard	50 WG	All season – 7 day	10.5	g/ha	6.35 fgh	9.30 de		4.96 cd
+Quadris	2.08 SC	All season – alt	9.0	ml/ha				
Quadris	2.08 SC	All season – 7 day	9.0	ml/ha	6.22 gh	6.93 e	9.52 cd	4.44 d
Maneb	75 DF	All season – 7 day	1.7	kg/ha	3.77 h	7.99 e	5.17 d	3.83 d
+Quadris	2.08 SC	All season – alt	9.0	ml/ha				
Maneb	75 DF	All season –7 day	1.7	kg/ha	5.78 gh	6.94 e	4.98 d	6.76 bcd
Maneb	75 DF	All season – 7 day	1.7	kg/ha	9.48 d-h	10.35 cde		9.42 a-d
+Cuprofix	20 DF	All season – alt	0.4	kg/ha				
Cuprofix	20 DF	All season – 14 day	0.4	kg/ha	12.29 c-g	8.82 e		15.27 a
Flint	50 WP	All season – 7 day	140.0	g/ha	5.58 gh	7.05 e		8.62 a-d
Messenger	3 WDG	All season – 7 day	448.0	g/ha	24.00 ab			
Messenger	3 WDG	All season – 14 day	0.7	g/ha	17.51 bc	25.61 a		
Messenger	3 WDG	All season – 14 day	1.1	g/ha		20.42 abc	20.47 ab	
Sovran	50 WG	All season – 14 day	1.8	g/ha	6.61 fgh[3]	14.64 a-e		
Sovran	50 WG	All season – 7 day	448.0	g/ha		10.29 cde	13.13 c	6.02 bcd
+Maneb	75 DF	All season – alt	1.7	kg/ha				
Messenger BF	3 WDG	Pre-bloom – 14 day	1.1	g/ha				
Maneb	75 DF	Early fruit – 7 day	1.7	kg/ha	5.48 gh	8.91 de		
+Quadris	2.08 SC	Early fruit – alt	9.0	ml/ha				
Control					26.25 a	22.08 ab	27.13 a	13.73 ab
LSD					6.91	11.06	7.11	7.99

[1]In 2001, the rate was 7 g a.i./ha for acibenzolar-s-methyl. [2]In 2001, the rate was 21 g a.i./ha for acibenzolar-s-methyl. [3]In 2001, the rate was 25.6 g a.i./ha for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD). [4] In 2001: pre-bloom – 19 Jun, 3, 17 Jul; all season, 14 day – 19 Jun, 2, 17, 28 Jul, 17, 31 Aug; all season, 7-day – 28 Jul, 3, 10, 17, 24, 31 Aug and 8 Sep; all season alt. – 3, 17, and 31 Aug. [5] In 2002A: pre-bloom - 25 Jun, 3, 8, and 15 Jul; all season 14-day – 25 Jun, 3, 8, 15 Jul, 5, 19 Aug, 3, and 17 Sep; all season, 7-day – 29 Jul, 5, 12, 19, 26 Aug, 3, 10, and 17 Sep; all season, alt. – 5, 19 Aug, 3, and 17 Sep. [6] In 2002B, all season 14-day – 26 Jun, 8, 16, 23, Jul, 6, 22 Aug, 6, and 20 Sep; all season, 7-day – 23, 31 Jul, 6, 15, 22, 30 Aug, 6, 12, and 20 Sep; all season, alt. – 23 Jul, 6, 22 Aug, 6, and 20 Sep. [7] In 2003, pre-bloom 26 Jun, 12, 25 Jul; all season 14-day 26 Jun, 12, 25 Jul, 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, 7-day 6, 13, 20, 27 Aug, 3, 11, 17 and 24 Sep; all season, alt. – 6, 20 Aug, 3, and 17 Sep; early fruit 7-day 6, 20 Aug, 3, and 17 Sep; early fruit – alt 13, 20 Aug, 3, and 17 Sep.

Table 2.6 Product cost (dollars/ha) throughout season and yield of marketable fruit (kg/ha).

				2001		2002A		2002B		2003	
Treatment		**Rate a.i.**		Cost	Yield	Cost	Yield	Cost	Yield	Cost	Yield
Actigard[1]	50 WG	3.5	g/ha	30.60	24250						
Actigard[2]	50 WG	10.5	g/ha	91.80	24941	91.80	5330			107.10	18675
Actigard	50 WG	35.0	g/ha			306.00	5981	408.00	5330	357.00	20547
Actigard BF	50 WG	10.5	g/ha								
Maneb	75 DF	1.7	kg/ha								
+Quadris	2.08 SC	9.0	ml/ha	106.89	26325	142.52	8422			127.22	24901
Actigard	50 WG	10.5	g/ha								
+Quadris	2.08 SC	9.0	ml/ha	87.39	24005	116.52	6144			162.42	31248
Quadris	2.08 SC	9.0	ml/ha	110.64	28318	110.64	12939	124.47	8951	110.64	34381
Maneb	75 DF	1.7	kg/ha								
+Quadris	2.08 SC	9.0	ml/ha	60.99	33079	81.32	7161	95.15	9033	81.32	31980
Maneb	75 DF	1.7	kg/ha	52.00	32794	52.00	13020	58.50	11921	52.00	27993
Maneb	75 DF	1.7	kg/ha								
+Cuprofix	20 DF	0.4	kg/ha	32.00	27057	34.00	3703			34.00	24778
Cuprofix	20 DF	0.4	kg/ha	14.00	25918	16.00	1465			16.00	23761
Flint	50 WP	140.0	g/ha	409.60	32835	409.60	11311			409.60	24087
Messenger	3 WDG	448.0	g/ha	33.00	25185						
Messenger	3 WDG	0.7	g/ha	33.00	24697	33.00	5656				
Messenger	3 WDG	1.1	g/ha			33.00	9073				
Sovran[3]	50 WG	1.8	g/ha	313.60	27505	313.60	11230	44.00	7242		
Sovran	50 WG	448.0	g/ha								
+Maneb	75 DF	1.7	kg/ha			182.80	8911	189.30	10741	182.80	24005
Messenger BF	3 WDG	1.1	g/ha								
Maneb	75 DF	1.7	kg/ha								
+Quadris	2.08 SC	9.0	ml/ha	63.99	30678	103.32	6591				

[1]In 2001, the rate was 7 g a.i./ha for acibenzolar-s-methyl. [2]In 2001, the rate was 21 g a.i./ha for acibenzolar-s-methyl. [3]In 2001, the rate was 25.6 g a.i./ha for kresoxim-methyl. Prices were obtained from dealers on the Eastern Shore of Virginia in November of 2003. Prices reflect the cost for the year for a single hectare. The yield is marketable fruit weight as kg/ha.

Discussion

In 2001, the weather conditions were very favorable for disease development and spread (Appendix 5.1). There were several short periods of rainfall in July which helped in the dissemination of inoculum, and the mean temperature in August was beneficial for infection at 25.2 °C. Weather conditions in 2002 were not as conducive for anthracnose development (Appendix 5.2). Although temperatures were around 26.6 °C during the growing season, there was little rain to transfer the inoculum and initiate the infection process. In 2003, weather conditions were ideal for disease development because of the high amounts of rainfall late in the season and warm temperatures (Appendix 5.3). During the end of the 2003 growing season, the temperature increased to 25.6 °C, which enhanced pepper fruit growth and disease incidence to greatly increase over a short period. The data from this experiment showed little differences in yield for all treatments. This can be attributed to the quick manner that anthracnose spread throughout the field late in the season.

When azoxystrobin was applied every 7 days, disease incidence was suppressed significantly. Because of concerns about fungicide resistance developing to azoxystrobin, the chemical must be alternated with other fungicides and used no more than a maximum number of three applications. Data from this study suggests this system of applications was effective in reducing anthracnose. Other strobilurins such as, kresoxim-methyl and trifloxystrobin, were also effective in preventing anthracnose development when applied on a 7-day schedule; however, the best performing products ranged each year between the strobilurin compounds applied every 7 days and the azoxystrobin and maneb alternated treatment applied every 7 days. The strobilurin compounds, kresoxim-methyl, and trifloxystrobin, were quite costly when compared to azoxystrobin as a replacement. The azoxystrobin was about 110.64 per hectare while the trifloxystrobin was 409.60 dollars per hectare and kresoxim-methyl was 313.60 dollars per hectare. Maneb as a weekly application was very effective in reducing disease development at a cost of about 52.00 dollars per hectare. With maneb applied every 7 days was effective, it appears to be unnecessary to alternate with azoxystrobin for anthracnose control unless anthracnose begins to increase late in the season and a swift reduction of inoculum is needed.

Chapter 2. Discussion

Acibenzolar-s-methyl did not protect pepper fruit from anthracnose in all experiments. This treatment reduced yields, number and weight of marketable fruit for 2002A, 2002B, and 2003. The higher rates slightly increased yields when compared to applications of lower rates. Because of phytotoxic effects, flower abortions and chlorosis, treatments were applied before flowering to avoid negative effects on fruiting. The data showed no beneficial effect for any of the acibenzolar-s-methyl treatments before flowering. The other plant activator, Messenger 3 WDG, proved to be ineffective in protecting the fruit from anthracnose. Also, yields as number and weight of marketable fruit were not increased significantly at $P \leq 0.05$ when compared to the control.

Throughout the experiments, there were slight variations in timings of applications and rates. In 2001, the rates of acibenzolar-s-methyl 3.5 g a.i./ha, acibenzolar-s-methyl 10.5 g a.i./ha and kresoxim-methyl at 488 g a.i./ha were doubled. No effect was observed when rates were increased. Acibenzolar-s-methyl was applied every 7 days before flowering instead of the 14-day schedule used in other years. Again, no effect was observed.

During all seasons maneb worked effectively, and addition of azoxystrobin supplied better disease control and increased yield. Both plant activators were ineffective in controlling pepper anthracnose, and acibenzolar-s-methyl had low levels of marketable fruit weights. Maneb alternated with azoxystrobin provided the best management strategy for pepper anthracnose. The most effective strategy was to use maneb until the disease occurred, and then start alternating with azoxystrobin.

Chapter 3: Characterization of Pepper Anthracnose Isolates from the Eastern United States

Introduction

The history of pepper anthracnose in the United States reports that this disease is known for colonizing ripe fruit and playing a minor role in yield loss (Boucher & Ashley, 2001). On the Eastern Shore of Virginia, pepper anthracnose has steadily increased in intensity and incidence. In 1999, anthracnose appeared on immature fruit even when the recommended fungicide program of maneb and a copper-based fungicide was used. There are two species of *Colletotrichum* that are reported to cause anthracnose on pepper fruit, *C. capsici* (Sydow) Butler et Bisby and *C. gloeosporioides* (Penzig) Penzig et Saccardo (Manandhar et al, 1995). *C. capsici* forms sunken black lesions with large amounts of setae and colonizes ripe or weakened/damaged fruit. The conidia are falcate and range from 18 to 23 µm in length and 3.5 to 4 µm in width (Sutton, 1982). *C. gloeosporioides* forms sunken lesions ranging in colors from orange, tan, brown, and black. In Taiwan and Korea, *C. gloeosporioides* colonizes immature and/or mature fruit (Hong & Hwang, 1998) (Oh *et al*., 1999). The conidia are straight with obtuse ends constricting in the center and ranges from 9 to 24 µm and 3 to 4.5 µm in width (Sutton, 1982).

Although these two species can be easily distinguished based on the morphology of conidia and colonies in culture, the taxonomy of *C. gloeosporioides* is in a state of flux and is not easily distinguishable from other species based on morphology (Freeman et al, 1998). This confusion makes it difficult to accurately diagnose what is occurring on the Eastern Shore of Virginia and describe the epidemiology of disease. Experiments were performed to characterize isolates across the eastern half of the United States for comparison of differences in morphology, pathogenicity, and utilization of carbon sources.

Materials and Methods

Isolates were collected from Florida, Ohio, New Jersey, North Carolina, Georgia, and portions of Virginia where peppers are grown. Isolates from the American Type Culture Collection (ATCC) as type species included: *C. acutatum* Simmonds (#26255),

C. capsici (Sydow) Butler et Bisby (#48574), *C. dematium* (Persoon: Fries) Grove (#58111), and two isolates of *C. gloeosporioides* (Penzig) Penzig et Saccardo (#58692 & #58693). Each isolate originated from a single spore on 0.15% Streptomycin water agar which was incubated at 26 °C +/- 2 °C for 6-8 hours. The germinated single spores were transferred to potato dextrose agar (PDA, Difco laboratories, Detroit, MI), incubated at 26 °C +/- 2 °C for 7 days, and then lyophilized and stored at -80 °C in 10% glycerol as PDA plugs covered with the fungus (Appendix 2.5, 2.3, 2.7). The isolates were characterized based on traditional morphological characteristics, pathogenicity and virulence on detached pepper fruit. Carbohydrate utilization was determined using Biolog® (Hayward, California). Biolog® is a 96-well, titer plate containing a different carbon substrate within each well. As the fungus grows, it will either metabolize the carbon source producing a color and turbidity change or no growth producing no color change. The differences in carbon utilization will produce a "fingerprint" among the 96 wells and possibly show differences between species or isolates.

Culture characteristics. The plugs were removed from the -80 °C freezer and grown on PDA in Petri plates. The fungal isolates were transferred by lightly dragging a sterile dissecting needle over the fungal growth then punching the center of a new PDA plate. The PDA plates were incubated in plastic boxes at 26 °C +/- 2 °C for 7 days then measured. Two, 20-watt fluorescent full spectrum bulbs provided light to the cultures for 12 hr intervals. A section of the fungal growth, was placed on a wet mount and the structures observed (Appendix 2.2). Thirty conidia were measured and photographed at 400X magnification with a BX41 Olympus microscope. The microscope eyepiece was calibrated using a micrometer (Graticules LTD, London, England). Colonies were measured in two directions (length and width) then averaged. One PDA Petri plate comprised a replication, and five replications of each isolate were measured. Each culture, both top and bottom, were scanned with an HP750 flat bed scanner to record images of the cultures (Hewlett Packard, Palo Alto, CA).

Pathogen diagnostics. Green peppers (*Capsicum annum* 'Enterprise') were obtained from research plots by Jim Gilreath in Manatee County, Florida. The peppers were treated with a standard fungicide regimen of maneb and Kocide (Appendix 8.1, 8.2). They were stored at 4 °C for 4 days for the first experiment and 20 days for the

second repetition of the experiment. The peppers were removed from storage, washed in a 10% sodium hypochlorite solution, and allowed to dry and warm on a greenhouse bench. A 16-gauge needle was used to puncture the epidermal layers on both sides of each pepper fruit ranging from 6-7 cm apart depending on the size of the pepper. A 25 µl droplet of inoculum was placed once on each pin prick puncture. Inoculum was prepared by placing 1 ml of sterile water on a 7 to 10-day-old fungal culture grown on PDA and lightly scraping the fungal growth with a pipette tip. The inoculum was estimated by use of a Bright-line hemacytometer (Hausser Scientific, Horsham, PA) and adjusted to 3.6-8.2 $x10^4$ +/- 1.3 and 4.2-9.0 x 10^4 +/- 1.8 for the 'Enterprise' experiments and 1.6 x 10^4 – 1.0 x 10^5 +/- 1.6 and 1.3-9.8 x 10^4 +/- 1.3 for the 'Paladin' experiments (Appendix 2.1, 8.3). The control was inoculated with distilled water. Three inoculated peppers were placed in a tray representing one replication with four replications for each isolate. The peppers were kept in a Percival Dew Chamber Model I-60DL (Percival Corp., Ames, IA) at 28 °C and 70 - 95% relative humidity. Twelve fluorescent 60-watt bulbs provided light on 12 hr cycles. Lesion length and width were measured at 48 hr intervals starting 4 days after inoculation. Lesions that coalesced or lacked a definable edge were not measured. Lesion data were summarized as a mean of the height and width of the lesion. That mean value was then averaged with the mean value of the opposing lesion on the same pepper. From the possible six lesions per tray, the lesion sizes were integrated to achieve an average per tray then a mean value per repetition. The data was analyzed by the ANOVA method using JMP statistical software (SAS Institute Inc., Cary, NC).

Biolog. Isolates were transferred to 2% malt extract agar (Oxoid Inc., Hampshire, England) and placed in an incubator for 7 days. Replications consisted of a succession of five plates transferred and measured every week. The conidia were extracted by submerging the mycelium covered malt agar plates with sterile Biolog® filamentous fungi inoculating fluid, then rolling a sterile cotton swab across the surface of the colony. The filamentous fungi inoculating fluid consisted of 0.25% Phytagel and 0.03% Tween 40. The spore-coated swab was placed into a test tube containing 5 ml of inoculating fluid for measurement by a spectrophotometer. The number of conidia in the inoculation process was estimated by comparing the turbidity with a known standard at 77 to 81 percent transmittance. Aliquots of 100 µl were placed in each well of the Biolog® plate. Plates for

the trial in Blacksburg were incubated at 26 °C +/- 2 °C under ambient light conditions provided on a 12 hr light/dark cycle by two daylight spectrum fluorescent bulbs, but the Plates for the trial in Painter trial were incubated in the dark.

All plates were read by a Biolog® Microstation plate reader at 24, 48, 96, 168, and 240 hours after inoculation. The optical density values were transformed into a positive (1), negative (0), or intermediate (0.5) value for color and turbidity based on the optical density of the control well (water). Data were presented as the most probable species to the unknown. Species level identification occurred when the Similarity Index Value (SIM) was close to 1.0 and above 0.5. The SIM states the similarity of each well, on the plate read, to the carbohydrate utilization profile from species in a database. The Biolog® experiments were performed in Painter, Virginia with an overlapping and smaller set in Blacksburg, Virginia. The Painter series were performed for three single spore isolates of each isolate and merged for statistical analysis. The Blacksburg series consisted of one single spore isolate from each isolate collected. The carbohydrate utilization was able to provide distinguishable color growth patterns which were placed in dendrograms by using the Ward statistical method. The color data, not the turbidity data, was analyzed. Isolates sorted according to the time of each test and location of experiments.

Results

Measurements of conidia showed several differences among isolates and type species. The two North Carolina isolates and the New Jersey isolate were similar in length of conidia, and one North Carolina isolate had wider conidia (Table 3.1). Conidia of all isolates had measurements comparable to *C. acutatum* (*C. act*) and *C. gloeosporioides* (*C.g.* 693), but distinctly different from *C. capsici* (*C. cap*) and *C. dematum* (*C. dem*) in both size and shape (Fig. 3.1). Colony sizes differed also with a Virginia isolate growing the least and the Georgia isolate growing the most (Table 3.1) (Fig. 3.2 & 3.3). All isolates were similar in virulence at 10 days after inoculation on 'Paladin' and 'Enterprise' (Fig. 3.4 & 3.6). Two trends were apparent in the number of lesions on 'Enterprise'. The group causing high number of lesions on 'Enterprise' included: FL0101, NC0106, NC0206, OH0209, VA0110, and VA0908. (Fig. 3.5 & 3.7). The group causing the lowest number of lesions on 'Enterprise' included: *C.g.* 693, *C.*

cap, Control, GA0107, and NJ0102. This grouping on isolates was different on 'Paladin'. Ga0107 produced a high number of lesions on 'Paladin', whereas VA0110 caused a low number of lesions. The Georgia isolate produced the largest lesions on 'Paladin', but lesions were small on 'Enterprise'. The New Jersey isolate was least virulent in both number of lesions and size. *C. act*, *C. dem*, and *C.g.* 692 were avirulent throughout all experiments and therefore not shown. *C.g.* 693 was avirulent on 'Paladin', but mildly virulent on 'Enterprise'.

The 168-hr Painter dendrograms separated all the ATCC cultures at a distinct point early in the dendrogram . This did not occur with the 96-hr Painter dendrogram nor the 168-hr Blacksburg dendrogram (Fig. 3.8). The North Carolina isolates were closely paired with the Eastern Shore isolate in both the Painter dendrograms and the 96-hr Blacksburg dendrogram. Florida and North Carolina isolates were paired within the 96-hr Blacksburg dendrogram and closely for the 168-hr Blacksburg dendrogram (Fig. 3.9). Ohio isolates were closely paired within the 168-hr Painter dendrogram.

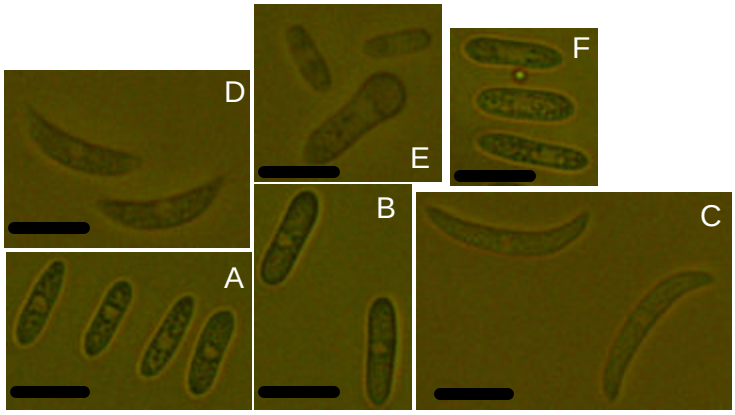

Figure 3.1 Conidia produce in 7-day-old cultures on potato dextrose agar. A. OH02-09. B. VA01-10. C. *C. cap*. D. *C. dem.* E. *C.g.* 693. F. *C. act.* The black line at the lower left signifies 2.4 µm.

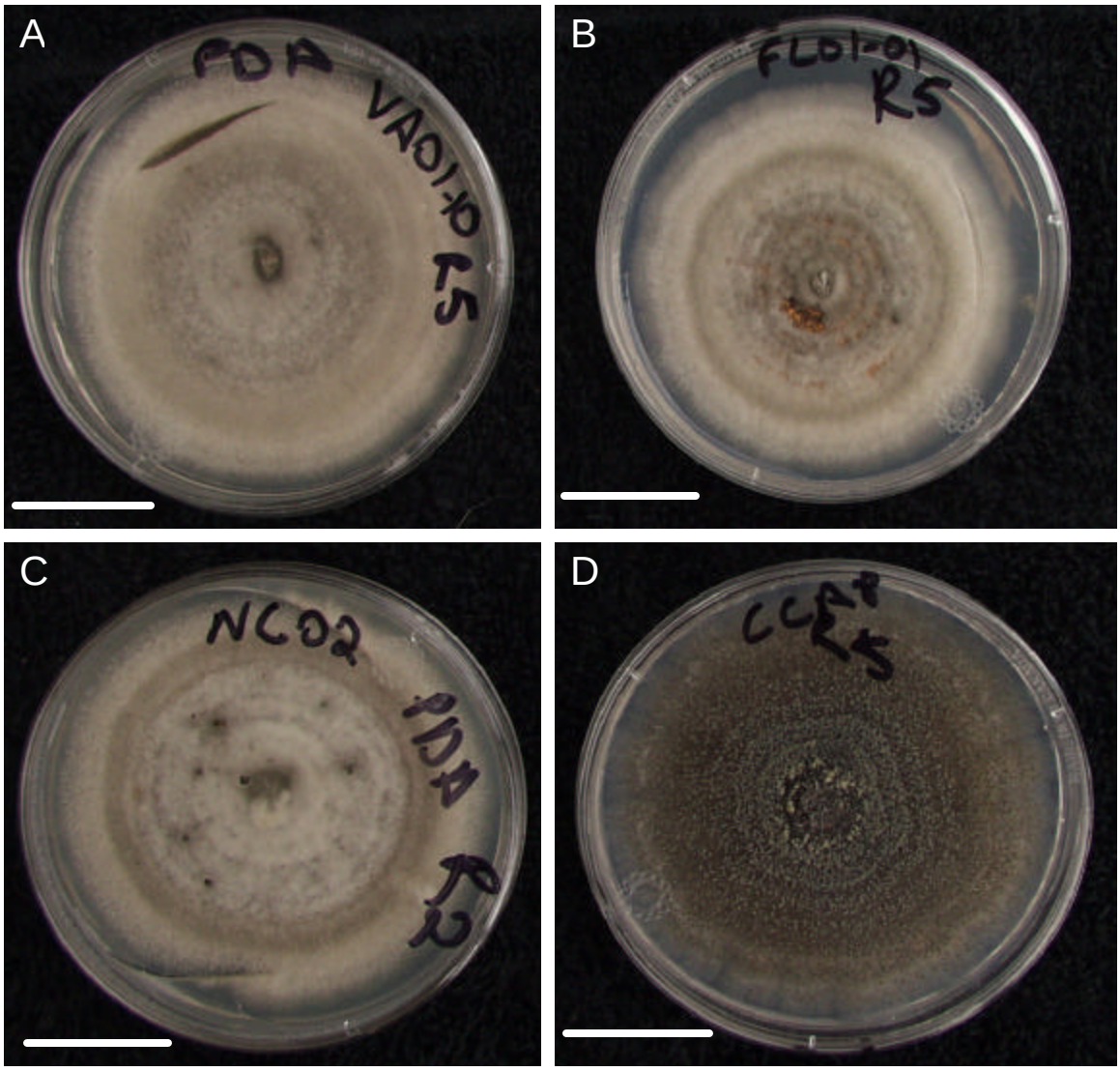

Figure 3.2 Colony growth after 7 days on potato dextrose agar. A. VA0110. B. FL0101. C. NC0206. D. *C. cap*.

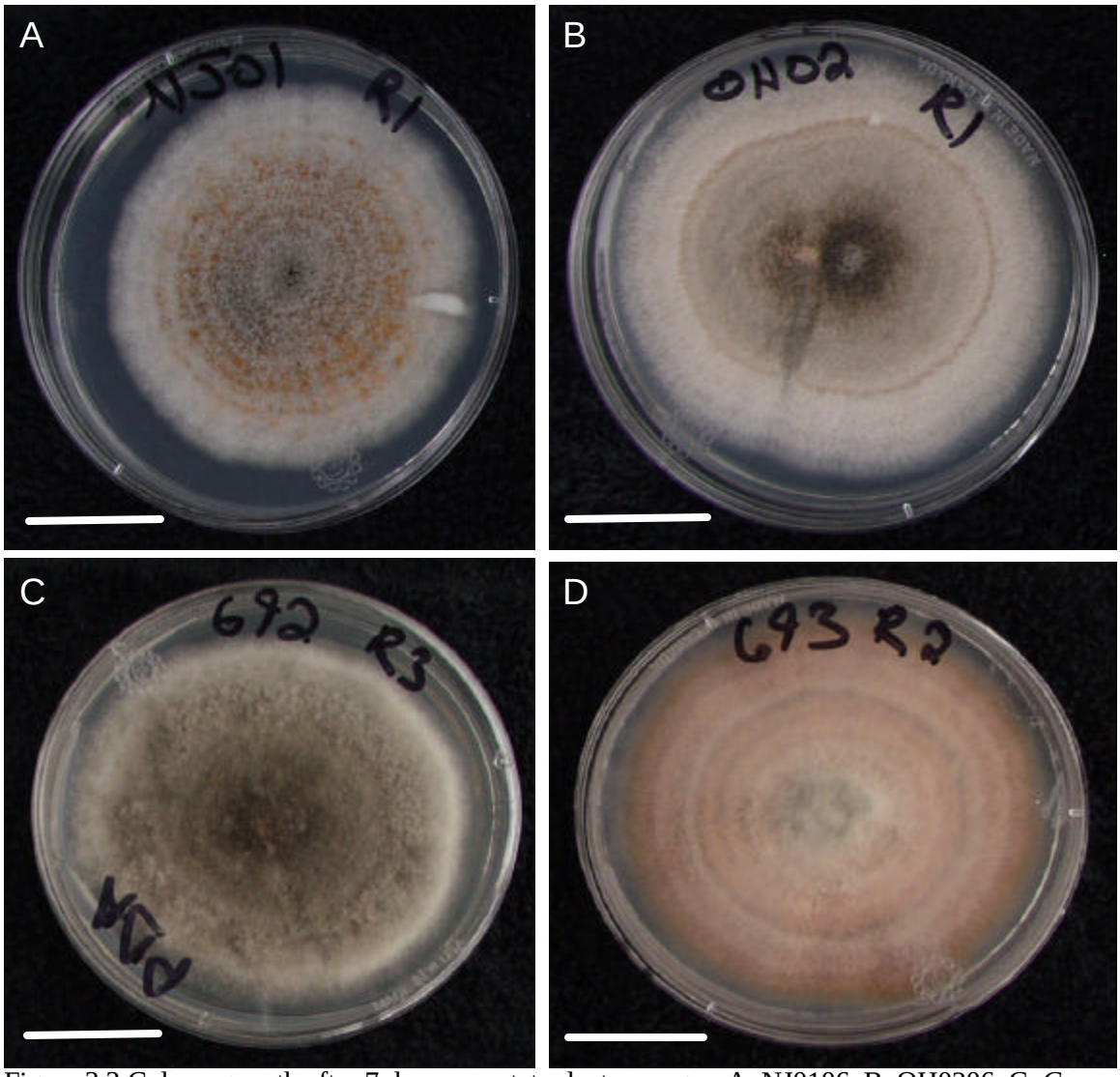

Figure 3.3 Colony growth after 7 days on potato dextrose agar. A. NJ0106. B. OH0206. C. *C.g.* 692. D. *C.g*. 693.

Table 3.1 Characteristics of isolates after 7 days on potato dextrose agar.

Type Species[1]	Conidia Length (µm) [2]	Conidia Width (µm) [3]	Colony size (cm) [4]	Sclerotia present	Colony color
C. act	13.94 +/- 0.20	4.22 +/- 0.10	4.72 +/- 0.03	None	Pink
C. cap	24.02 +/- 0.37	4.26 +/- 0.38	6.26 +/- 0.32	None	Gray, Dark Spots
C. dem	20.67 +/- 0.24	4.49 +/- 0.02	7.39 +/- 0.44	None	Black
*C.g.*692	9.98 +/- 0.46	4.95 +/- 0.05	4.99 +/- 0.10	Sparse	White, Pink
*C.g.*693	11.06 +/- 2.18	4.25 +/- 0.64	5.39 +/- 0.41	None	White, Pink
Isolate[1]					
FL0101	12.69 +/- 0.32	4.43 +/- 0.09	4.74 +/- 0.43	Present	Gray, White
FL0106	12.42 +/- 0.41	4.26 +/- 0.01	4.43 +/- 0.32	Present	Gray, White
GA0107	12.79 +/-0.18	4.47 +/- 0.08	5.39 +/- 0.32	Present	Gray, White
NC0106	12.18 +/- 0.19	4.58 +/- 0.11	4.94 +/- 0.25	Present	Gray, White
NC0206	11.96 +/- 0.19	4.29 +/- 0.11	4.94 +/- 0.33	Present	Gray, White
NJ0102	12.07 +/- 0.39	4.22 +/- 0.02	4.71 +/- 0.20	Present	Gray, White
OH0209	13.50 +/- 0.40	4.23 +/- 0.06	5.28 +/- 0.25	Present	Orange, White
VA0110	13.17 +/- 0.32	4.76 +/- 0.03	4.88 +/- 0.37	Present	Gray, White
VA0908	12.13 +/- 0.53	4.38 +/- 0.07	4.19 +/- 0.60	Present	Gray, White

[1]Standard Deviation of means for each isolate and type species shown as +/- value. [2] The LSD for the conidia length of only the isolates is equal to 1.04. [3] The LSD for the conidia width of only the isolates is equal to 0.28. [4] The LSD for the conidia size of only the isolates is equal to 0.47.

Table 3.2 The number of replications for Biolog® trials and isolate origin.

Isolate	Painter Reps	Blacksburg Reps	Original Host	Collection Location
C. act	5	5	Tomato	#26255
C. cap	6	5	Hot Pepper	#48574
C. dem	4	5	Tomato	#58111
*C.g.*692	4	5	Tomato	#58692
*C.g.*693	4	5	Tomato	#58693
FL01	16	5/5[1]	Pepper	DelRay Beach, FL
GA01	17	5	Pepper	Grady, Co., GA
NC01	14	4	Pepper	Selma, NC
NC02	12	6	Pepper	Union Co., NC
NJ01	17	5	Pepper	Southern New Jersey
OH01	17	5	Pepper	Western Ohio
OH02	14	5	Pepper	Western Ohio
VA01	11	5	Pepper	Painter, VA
VA09	15	5	Pepper	Montgomery Co., VA

[1]Five replicates were completed for FL0101 and five for FL0106.

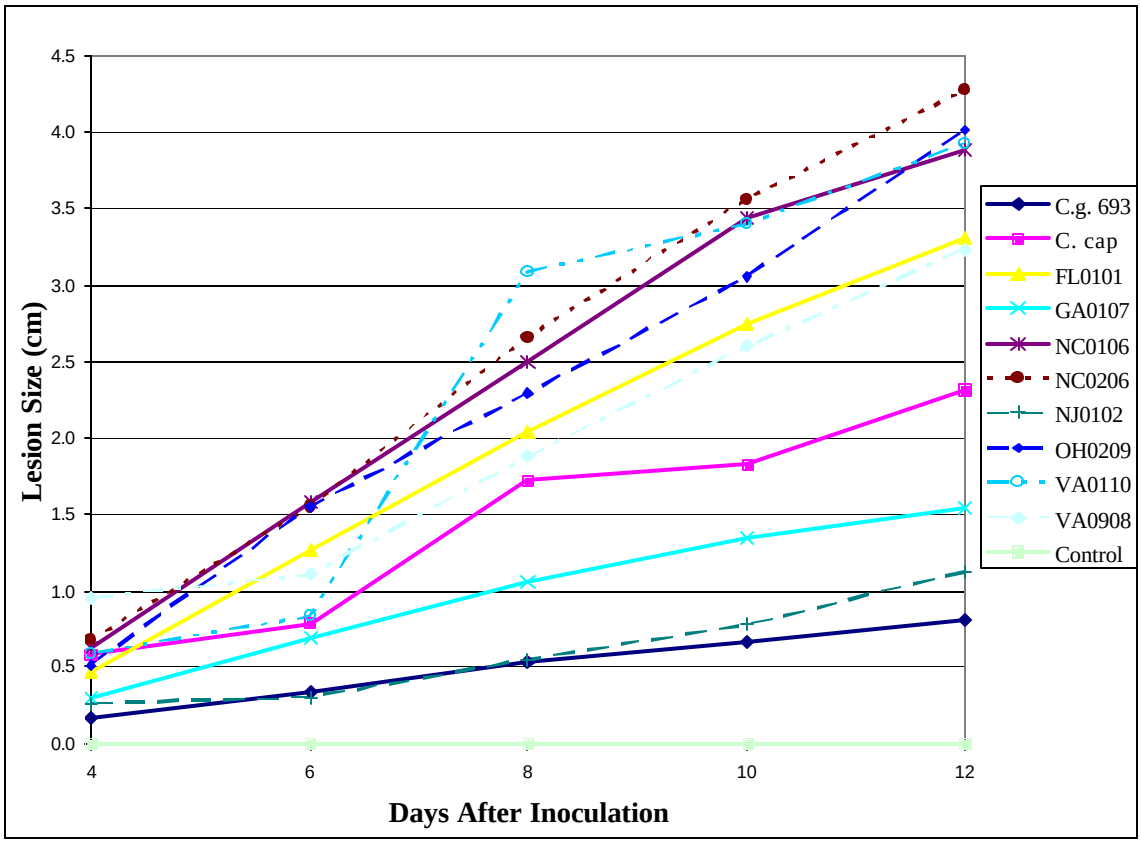

Figure 3.4 Size of lesions on pepper fruit of 'Enterprise' after inoculation.

C.g. 692 and *C. dem* were not included due to low virulence. *C.g.* 692 came from the ATCC (#58692). *C.g.* 693 came from the ATCC (#58693). *C. cap* came from the ATCC (#48574). *C. dem* came from the ATCC (#58111). FL01 came from Delray Beach, FL, GA01 came from Grady County, GA. NC01 came from Selma, N.C., NC02 came from Union County, N.C. NJ01 came from southern New Jersey. OH02 came from western Ohio. VA0110 came from Painter, VA. VA0908 came from Montgomery County, VA.

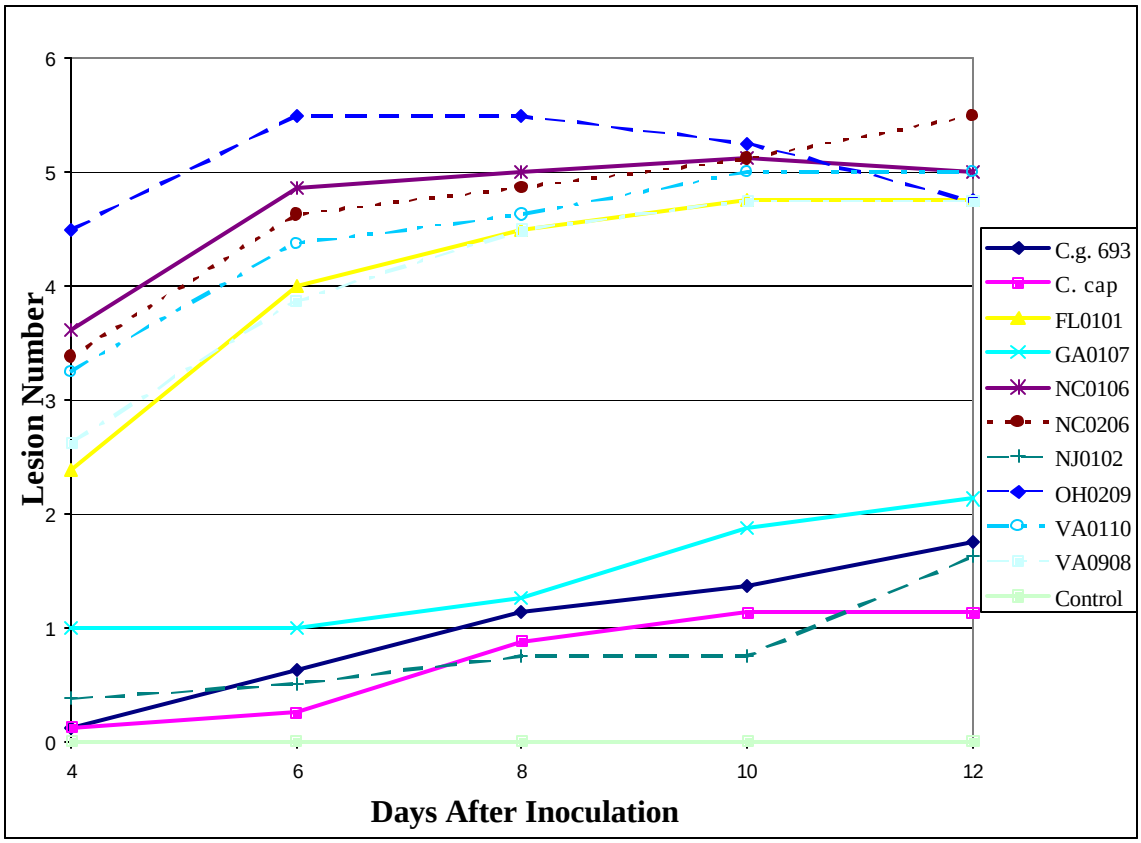

Figure 3.5 Number of lesions on pepper fruit of 'Enterprise' after inoculation.

C.g. 692 and *C. dem* were not included due to low virulence. *C.g.* 692 came from the ATCC (#58692). *C.g.* 693 came from the ATCC (#58693). *C. cap* came from the ATCC (#48574). *C. dem* came from the ATCC (#58111). FL01 came from Delray Beach, FL, GA01 came from Grady County, GA. NC01 came from Selma, N.C., NC02 came from Union County, N.C. NJ01 came from southern New Jersey. OH02 came from western Ohio. VA0110 came from Painter, VA. VA0908 came from Montgomery County, VA.

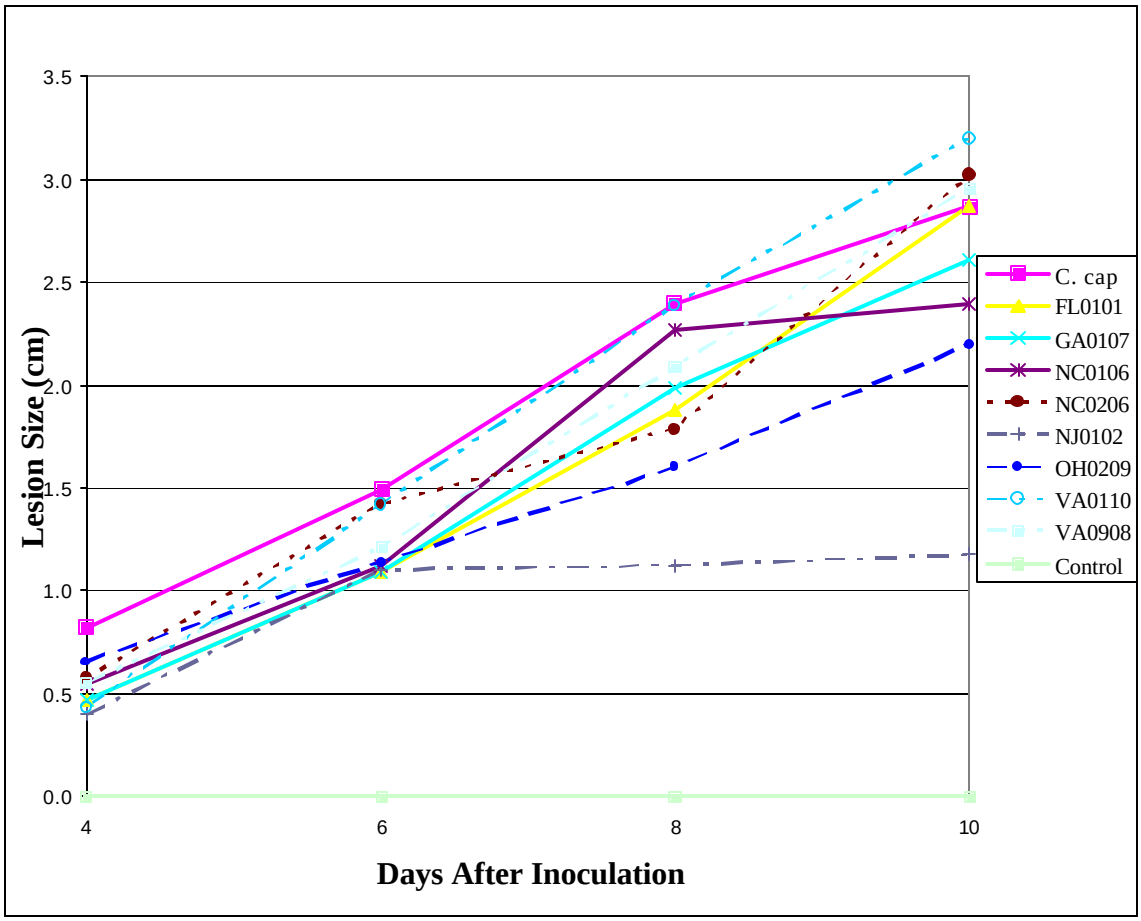

Figure 3.6 Size of lesions on pepper fruit of 'Paladin' after inoculation.

C. act and *C. dem* were not included due to low virulence. *C. act* came from the ATCC (#26255). *C. cap* came from the ATCC (#48574). *C. dem* came from the ATCC (#58111). FL01 came from Delray Beach, FL, GA01 came from Grady County, GA. NC01 came from Selma, N.C., NC02 came from Union County, N.C. NJ01 came from southern New Jersey. OH02 came from western Ohio. VA0110 came from Painter, VA. VA0908 came from Montgomery County, VA.

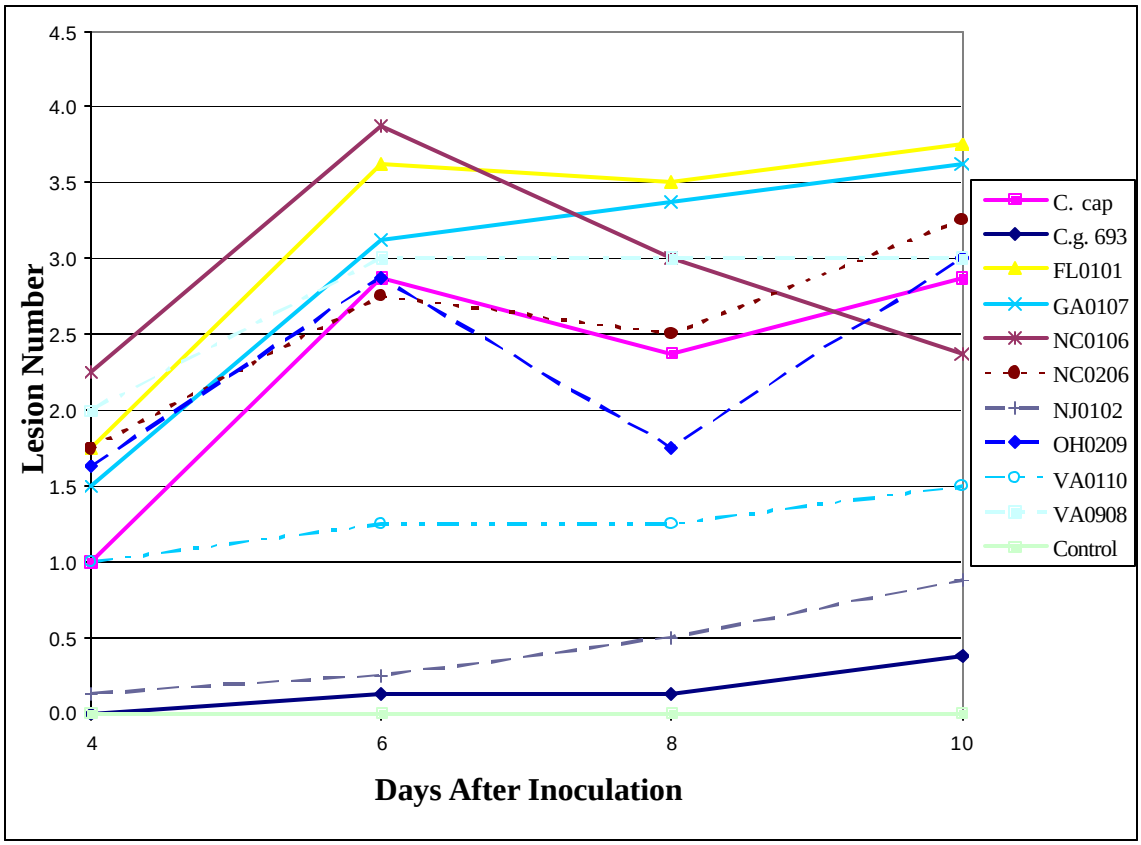

Figure 3.7 Number of lesions on pepper fruit of 'Paladin' after inoculation.

C. act and *C. dem* were not included due to low virulence. *C. act* came from the ATCC (#26255). *C. cap* came from the ATCC (#48574). *C. dem* came from the ATCC (#58111). FL01 came from Delray Beach, FL, GA01 came from Grady County, GA. NC01 came from Selma, N.C., NC02 came from Union County, N.C. NJ01 came from southern New Jersey. OH02 came from western Ohio. VA0110 came from Painter, VA. VA0908 came from Montgomery County, VA.

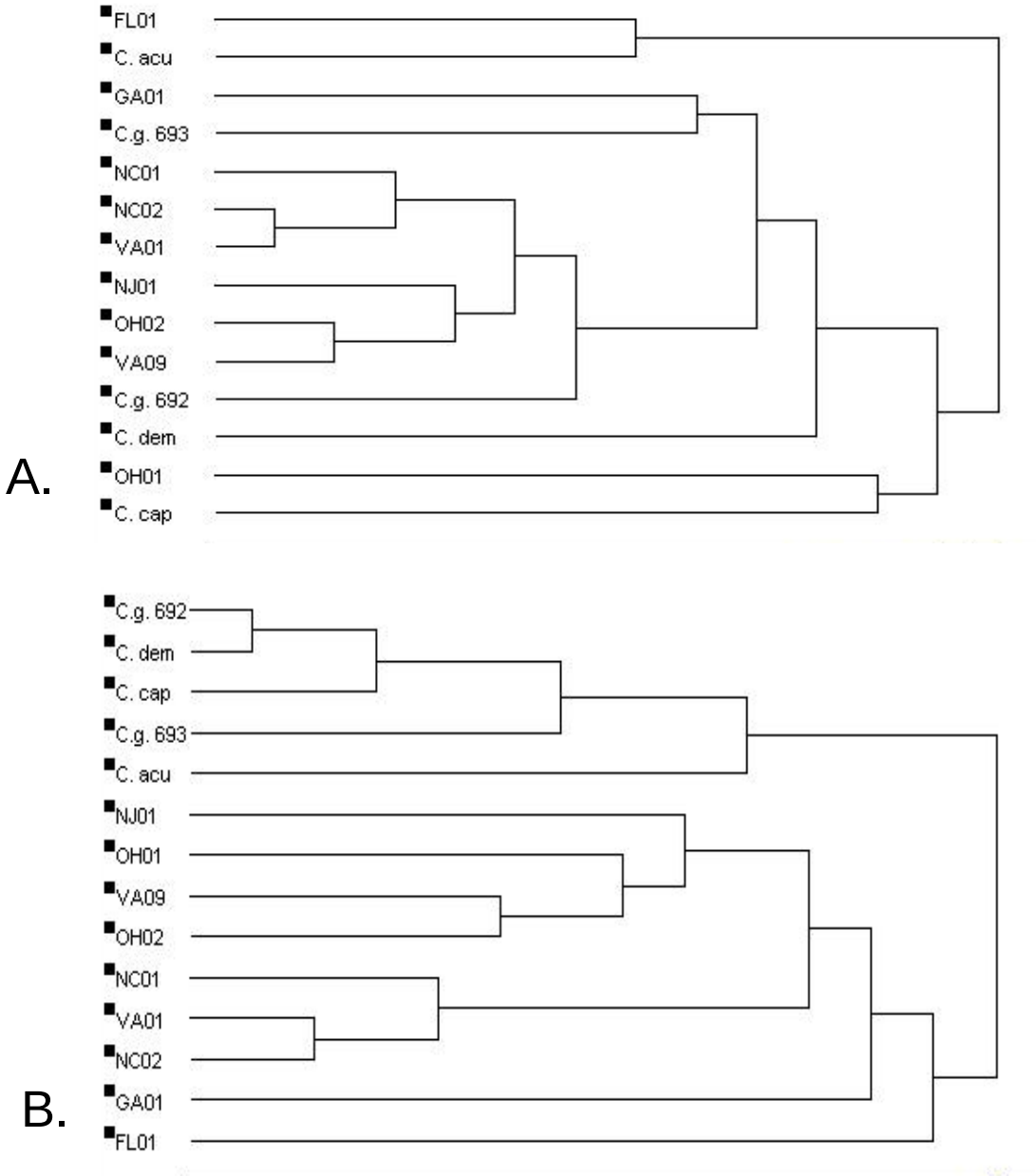

Figure 3.8

A. Dendrogram from the percentage of positives in all replications read at 96 hr after inoculation at Painter, VA.

B. Dendrogram from the percentage of positives in all replications read at 168 hr after inoculation at Painter, VA.

C. act came from the ATCC (#26255). *C.g.* 692 came from the ATCC (#58692). *C.g.* 693 came from the ATCC (#58693). *C. cap* came from the ATCC (#48574). *C. dem* came from the ATCC (#58111). FL01 came from Delray Beach, FL, GA01 came from Grady County, GA. NC01 came from Selma, N.C., NC02 came from Union County, N.C. NJ01 came from southern New Jersey. OH01 and OH02 came from western Ohio. VA0110 came from Painter, VA. VA0908 came from Montgomery County, VA.

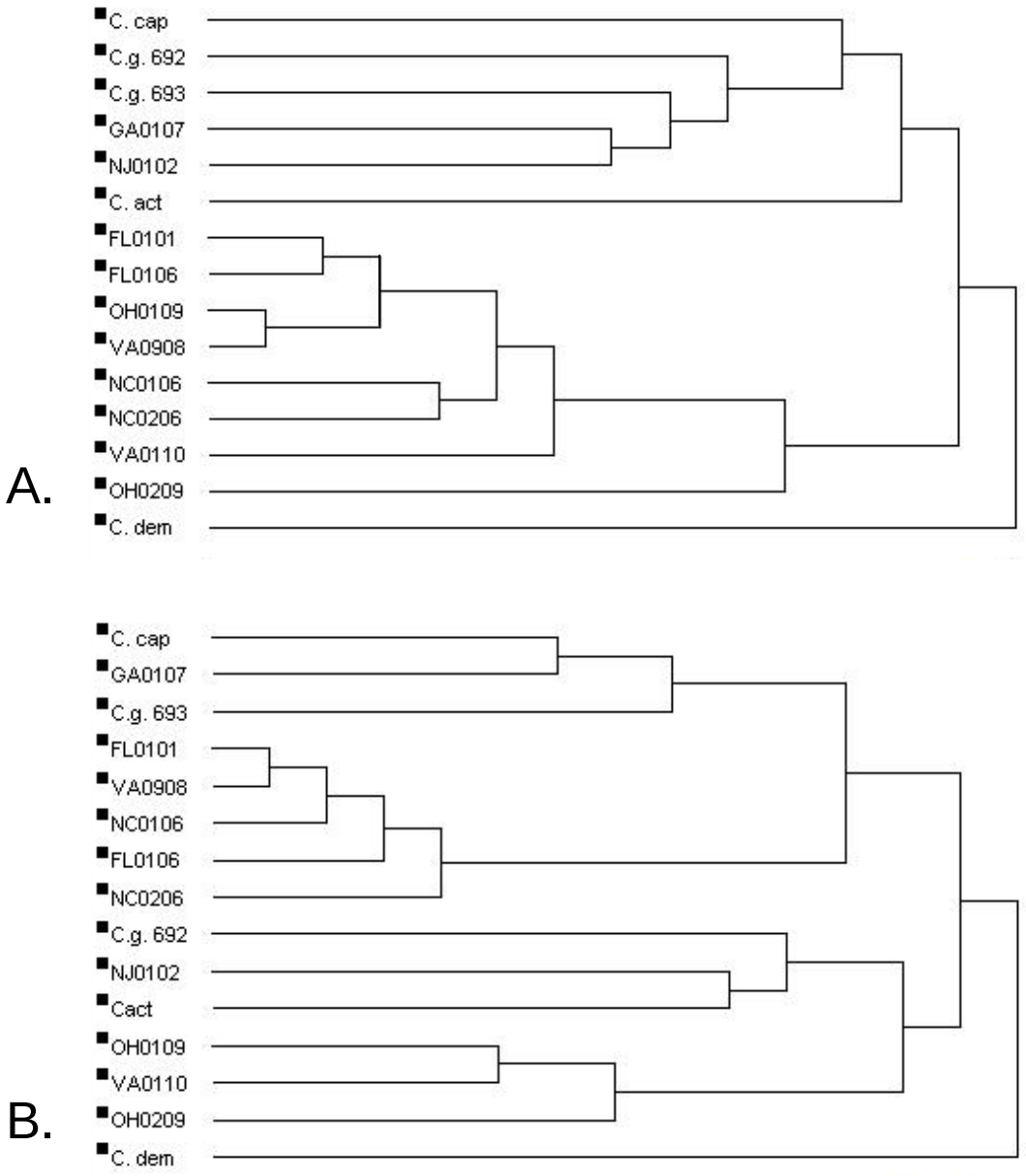

Figure 3.9

A. Dendrogram from the percentage of positives in all replications read at 96 hr after inoculation at Painter, VA.

B. Dendrogram from the percentage of positives in all replications read at 168 hr after inoculation at Blacksburg, VA.

C. act came from the ATCC (#26255). *C.g.* 692 came from the ATCC (#58692). *C.g.* 693 came from the ATCC (#58693). *C. cap* came from the ATCC (#48574). *C. dem* came from the ATCC (#58111). FL01 came from Delray Beach, FL, GA01 came from Grady County, GA. NC01 came from Selma, N.C., NC02 came from Union County, N.C. NJ01 came from southern New Jersey. OH01 and OH02 came from western Ohio. VA0110 came from Painter, VA. VA0908 came from Montgomery County, VA.

Discussion

The dimensions of conidia were variable, and did not show any relationship tp their location of origin. Similarities of the type species, such as *C. act*, *C.g.* 692, and *C.g.* 693, to the isolates are apparent. The *C. cap* and *C. dem* showed strong differences from each other and the collected isolates. The Georgia isolate from pepper (GA0107) was one of the fastest growing isolates, whereas the isolate from the Eastern Shore of Virginia (VA0110) was the slowest. Both of these isolates exhibited moderate to high in virulence on pepper fruit.

The difference in disease severity among cultivars by isolate is slight and could not be used to designate a cultivar effect. The Virginia isolate (VA0110) from the Eastern Shore had the largest lesion size on 'Paladin' of all the isolates tested and among the largest lesion sizes on 'Enterprise'. The other Virginia isolate (VA0908) was typically less virulent on 'Paladin', but was moderately virulent on 'Enterprise'. Isolates from Ohio, the Eastern Shore of Virginia, North Carolina, and Florida were highly virulent. The longer storage time in the 'Enterprise' experiments may have caused more ripeness and damage to the fruit, and thus directly increased the virulence of isolates that were capable of pathogenic and saprophytic growth.

The Painter series of experiments had more replications because of the merged profiles of three single spore cultures of the same isolate. This may have increased the accuracy of carbohydrate patterns when compared to the Blacksburg series. The Blacksburg series of Biolog® experiments were of one single spore culture from one isolate. Low replication or single readings were unreliable and usually inconsistent in identification. The 96 hr and 168 hr readings were focused on for this study. The 96 hr reading gave a relatively high level of Species level identification when compared with the other timed readings. The 168 hr reading is recommended as the end point or identification point in the Biolog® protocol. The ATCC cultures were separated out in the 168 hr Painter dendrogram which shows the specific isolate fingerprints were in the Biolog® database. This may be remedied with the selective rating for wells since each isolate contained examples of extreme differences from 0% growth and 100% growth throughout all replications. The Ward method is affected less by outliers and is more representative when the sample sizes are the same. The differences in each branch were

high for each dendrogram starting from high percentages and being quickly reduced after the first two to three separations.

Variability in the carbohydrate utilization over time may have caused some inconsistency in Biolog® test results. Additional variability could be attributed to the multi-nucleate state that is known to occur in this genus. Small percentages of conidia may contain up to three nuclei. The conidia could select for genes, hidden in its genome, for nuclei capable of utilizing a nutrient source. The 168 hr dendrogram from the Painter test delineated between the ATTC type species cultures and the pepper isolates. Biolog® may not be able to consistently distinguish species within the *Colletotrichum* genus which was evident when no identification occurred upon comparing a specific reading to a database made from that isolate. These differences of carbohydrate utilization and pathogenicity experiments between the type species and the isolates suggest different species, but this genus relies on morphology and taxonomy for speciation. If these variations can not be distinguished based on the morphology and taxonomy or this species, then the methods used to distinguished species may not be valid. DNA homology may have to be determined in order to recognize species limits. The conidial morphological characteristics observed show no distinct separation from both *C. gloeosporioides* type species and the isolates that were collected, but variable taxonomic characteristics, sclerotia presence and colony color, were different. The pathogenicity/virulence experiment displayed the greatest difference between the closest taxonomic type specie and the isolates. *C. gloeosporioides* proved to be weakly virulent or non-pathogenic, but a majority of the pepper isolates were highly virulent. B.C. Sutton (1982) states that the species *C. gloeosporioides* is a collection of multiple species, and this data supports that differences exist within the species. A molecular study, using DNA- DNA homology, may determine if these highly virulent isolates are a separate species.

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Appendix 1: Field preparation for all experiments

1.1 Preparation for field experiment 2001.
The field trial had been planted with sweet potatoes in the summer of the previous year. The field consisted of a Bojac sandy loam soil type which was amended with 1,000 lb/A of 10-10-10 broadcast incorporated fertilizer on 6/1/01. The field was prepared to suppress weeds by applying Treflan HFP 1 pt/A on 6/4/01 and Sencor DF 0.33 lb/A on 6/22/01. Pepper plants were planted on 6/21/01. Insect populations were suppressed by using Orthene 97S 1.0 lb/A on 8/31/01. Irrigation was required on 7/13/01 for 0.5 in. of water and 8/9/01 for 0.25 in. of water. The guard rows were inoculated on 8/14/01 with a conidial spore suspension of 7.9×10^6 spores/ml. The fruit was harvested on 8/20/01, 9/10/01, and 9/28/01.

1.2 Preparation for field experiment 2002A.
The field trial had been planted with bell peppers in the summer of the previous year. The field consisted of a Bojac sandy loam soil type which was amended with 1,000 lb/A of 10-10-10 broadcast incorporated fertilizer on 6/17/02. The field was prepared to suppress weeds by applying Treflan HFP 1 pt/A with Command 4EC 12 oz/A pre-plant incorporated on 6/17/02. Pepper plants were planted on 6/19/02. Insect populations were suppressed by using Spintor 2SC 6 oz/A on 8/7/02, 8/26/02 and 9/9/02 and Orthene 97S 0.75 lb/A on 8/16/02 and 8/23/02. Irrigation was required on 6/24/02, 6/28/02, 7/17/02, 8/13/02, and 8/20/02 for 1.0 in. of water and 9/13/02 for 0.75 in. of water. The guard rows were inoculated on 8/01/02 with a conidial spore suspension of 8.0×10^6 spores/ml. The The fruit was harvested on 8/15/02, 9/5/02, and 9/24/02.

1.3 Preparation for field experiment 2002B.
The field trial had been planted with bell peppers in the summer of the previous year. The field consisted of a Bojac sandy loam soil type which was amended with 1,000 lb/A of 10-10-10 broadcast incorporated fertilizer on 6/17/02. The field was prepared to suppress weeds by applying Treflan HFP 1 pt/A with Command 4EC 12 oz/A pre-plant incorporated on 6/17/02. Pepper plants were planted on 6/19/02. Insect populations were suppressed by using Spintor 2SC 6 oz/A on 8/7/02, 8/26/02 and 9/9/02 and Orthene 97S 0.75 lb/A on 8/16/02 and 8/23/02. Irrigation was required on 8/13/02 for 0.5 in. of water. The guard rows were inoculated on 8/01/02 with a conidial spore suspension of 8.0×10^6 spores/ml. The fruit was harvested on 8/16/02, 9/3/02, and 9/23/02.

1.4 Preparation for field experiment 2003.
The field trial had been planted with bell peppers in the summer of the previous year. The field consisted of a Bojac sandy loam soil type which was amended with 1,000 lb/A of 10-10-10 broadcast incorporated fertilizer on 6/16/03. The field was prepared to suppress weeds by applying Treflan HFP 1 pt/A with Command 4EC 12 oz/A pre-plant incorporated on 6/17/03. Pepper plants were planted on 6/23/03. Insect populations were suppressed by using Orthene 97S 0.75 lb/A every seven days from 08/06/03 to 9/24/03. Irrigation was required on 6/27/03 and 6/28/03 for 0.5 in. of water and 7/9/03 for 1 in. of water. The guard rows were inoculated on 8/19/03 with a conidial spore suspension of 9.5

x 10^4 spores/ml and 8/28/03 with a conidial spore suspension of 2.2 x 10^5 spores/ml. The fruit was harvested on 8/21/03 and 9/5/03, 9/11/03.

Appendix 2: Laboratory techniques

2.1 Use of the Hemacytometer
Place 50 µl of spore suspension on one side of a Brightline hemacytometer. Count the conidia in the four corners of the grid. Repeat the procedure on the second grid. Multiply the number counted by 2,000 according to Hansen (2002).

2.2 Making a Wet Mount
Place one to two drops of distilled water on a glass slide. Place specimen on the water droplets. Drop a cover slip over the specimen with one side touching first.

2.3 Making 10% gycerol
Add 10ml gycerol (Sigma inc., St. Louis, MO) to 90ml of dH_2O. Stir vigorously. Place 2ml aliquots into 3ml cryogenic tubes. Place cap on loosely. Autoclave at 15 lbs pressure and 121 °C for 20 minutes.

2.4 Lyophilization Toothpicks
Add 24 g potato dextrose broth (Difco laboratories, Detroit, MI) to 1 liter of dH_2O. Add 5 boxes of toothpicks (Forster, Wilton, Maine) to solution. Autoclave at 15 lbs pressure and 121 °C for 20 minutes and cool to 50°C. Place toothpicks in Petri plate.

2.5 Antibiotic Water Agar
Add 30 g agar to 1 liter dH_2O. Autoclave at 15 lbs pressure and 121 °C for 20 minutes and cool to 50°C. Add 0.015g of Streptomycin sulfate while stirring. Pour into Petri plates.

2.6 2% Malt Extract Agar
Add 20 g Oxoid Malt extract and 18 g of Bacterical Grade Agar to 1 Liter of dH_2O. Autoclave 15 lbs pressure and 121 °C for 20 minutes and cool to 50 °C. Gently stir then pour into Petri plates.

2.7 Acidified Potato Dextrose Agar
Add 39 g potato dextrose agar (Difco laboratories, Detroit, MI) to 1 liter dH_2O. Autoclave at 15 lbs pressure and 121 °C for 20 minutes and cool to 50 °C. Add 18 ml tartaric acid while stirring.

2.8 Biolog® Inoculating Fluid
Add 2.5 g Gellan Gum and 0.3 g Tween 40 to boiling dH_2O. Cool and stir liquid before dispensing 20 ml into 20 x 150 ml tubes. Autoclave tubes at 15 lbs pressure and 121 °C for 30 minutes.

Appendix 3: Application dates for all experiments
3.1 Application dates for field experiment 2001

Actigard	6/19	7/2	7/17	7/28			8/17		8/31		
Actigard	6/19	7/2	7/17	7/28			8/17		8/31		
Actigard	6/19	7/3	7/17								
+ Maneb				7/28		8/10		8/24			
+ Quadris					8/3		8/17		8/31		
Actigard				7/28		8/10		8/24			
Quadris					8/3		8/17		8/31		
Quadris				7/28	8/3	8/10	8/17	8/24	8/31	9/3	9/8
Maneb					8/3		8/17		8/31		
+ Quadris				7/28		8/10		8/24			9/8
Maneb				7/28	8/3	8/10	8/17	8/24	8/31	9/3	9/8
Maneb				7/28		8/10		8/24			9/8
+ Cuprofix					8/3		8/17		8/31		
Cuprofix	6/19	7/2	7/17	7/28		8/10		8/24	8/31		9/8
Flint				7/28	8/3	8/10	8/17	8/24	8/31	9/3	9/8
Sovran				7/28	8/3	8/10	8/17	8/24	8/31	9/3	9/8
Messenger	6/19	7/2	7/17	7/28			8/17		8/31		
Messenger	6/19	7/2	7/17	7/28			8/17		8/31		
Actigard	6/19	7/3	7/17								
+ Maneb				7/28		8/10		8/24			9/8
+ Sovran					8/3		8/17		8/31		
Messenger	6/19	7/3	7/17								
+ Maneb				7/28		8/10		8/24			9/8
+ Quadris					8/3		8/17		8/31		
Control											

3.2 Application dates for field experiment 2002A

Actigard	6/25		7/8		7/29		8/12		8/26		9/10	
Actigard	6/25		7/8		7/29		8/12		8/26		9/10	
Actigard BF	6/25	7/3	7/8	7/17								
+ Maneb					7/29		8/12		8/26		9/10	
+ Quadris						8/5		8/19		9/3		9/17
Actigard					7/29		8/12		8/26		9/10	
+ Quadris						8/5		8/19		9/3		9/17
Quadris					7/29	8/5	8/12	8/19	8/26	9/3	9/10	9/17
Maneb						8/5		8/19		9/3		9/17
+ Quadris					7/29		8/12		8/26		9/10	
Maneb						8/5		8/19		9/3		9/17
+ Sovran					7/29		8/12		8/26		9/10	
Maneb					7/29	8/5	8/12	8/19	8/26	9/3	9/10	9/17
Maneb					7/29		8/12		8/26		9/10	
+ Cuprofix						8/5		8/19		9/3		9/17
Cuprofix					7/29	8/5	8/12	8/19	8/26	9/3	9/10	9/17
Flint					7/29	8/5	8/12	8/19	8/26	9/3	9/10	9/17
Sovran					7/29	8/5	8/12	8/19	8/26	9/3	9/10	9/17
Messenger	6/25		7/8		7/29		8/12		8/26		9/10	
Messenger	6/25		7/8		7/29		8/12		8/26		9/10	
Actigard BF	6/25	7/3	7/8	7/17								
+ Maneb					7/29		8/12		8/26		9/10	
+ Sovran						8/5		8/19		9/3		9/17
Messenger BF	6/25	7/3	7/8	7/17								
+ Maneb					7/29		8/12		8/26		9/10	
+ Quadris						8/5		8/19		9/3		9/17
Control												

3.3 Application dates for field experiment 2002B

Actigard	6/28	7/8	7/16	7/23		8/6		8/22		9/6		9/20
Messenger	6/28	7/8	7/16	7/23		8/6		8/22		9/6		9/20
Quadris				7/23	7/31	8/6	8/15	8/22	8/30	9/6	9/12	9/20
Maneb				7/23	7/31	8/6	8/15	8/22	8/30	9/6	9/12	9/20
Maneb					7/31		8/15		8/30		9/12	
+ Quadris				7/23		8/6		8/22		9/6		9/20
Maneb					7/31		8/15		8/30		9/12	
+ Sovran				7/23		8/6		8/22		9/6		9/20
Control												

3.4 Application dates for field experiment 2003

Actigard	6/26	7/12	7/25	8/6	8/13	8/20	8/27	9/3	9/11	9/17	9/24
Actigard	6/26	7/12	7/25	8/6	8/13	8/20	8/27	9/3	9/11	9/17	9/24
Actigard	6/26	7/12	7/25								
+ Maneb				8/6		8/20		9/3		9/17	
+ Quadris					8/13		8/27		9/11		9/24
Actigard	6/26	7/12	7/25		8/13		8/27		9/11		9/24
Quadris				8/6		8/20		9/3		9/17	
Quadris				8/6	8/13	8/20	8/27	9/3	9/11	9/17	9/24
Maneb					8/13		8/27		9/11		9/24
+ Quadris				8/6		8/20		9/3		9/17	
Maneb				8/6	8/13	8/20	8/27	9/3	9/11	9/17	9/24
Maneb				8/6		8/20		9/3		9/17	
+ Cuprofix					8/13		8/27		9/11		9/24
Cuprofix				8/6	8/13	8/20	8/27	9/3	9/11	9/17	9/24
Flint				8/6	8/13	8/20	8/27	9/3	9/11	9/17	9/24
Sovran				8/6		8/20		9/3		9/17	
+ Maneb					8/13		8/27		9/11		9/24
Control											

Appendix 4: Weather conditions for applications of each experiment

4.1 Application conditions for 2001

Date	Temp.°F	%Relative Humidity	Wind Speed	%Cloud Cover	Growth Stage; Anthracnose Infection level
6/19	85	49	4-6	0	Transplants; no infection
7/3	72	56	5-8	75	Pre-bloom; no infection
7/17	83	60	3-5	40	Plants 12” height, bloom; no infection
7/28	85	70	3-5	60	Early fruit; no infection
8/3	80	64	4-8	0	Fruit-set no infection
8/10	87	73	3-5	0	Mature fruit; infection mostly in guard rows, increasing in plot rows
8/17	83	67	8-12	0	Mature green fruit; anthracnose increasing throughout
8/24	84	63	8-10	0	Mature green fruit; anthracnose moderate throughout
8/31	81	77	3-5	80	Mature green fruit; anthracnose severe throughout
9/7	78	57	0-2	0	Mature green fruit; anthracnose severe throughout

4.2 Application conditions for 2002A

Date	Temp.°F	%Relative Humidity	Wind Speed	%Cloud Cover	Growth Stage; Anthracnose Infection level
6/25	89	57	3-6	0	3-4 leaves; no infection
7/3	88	58	0	0	Pre-bloom; no infection
7/8	82	55	8-10	40	Pre-bloom; no infection
7/17	89	49	3-5	0	Pre-bloom; no infection
7/29	91	61	4-6	0	Fruit-set no infection
8/5	85	66	4-6	10	Early fruit; anthracnose starting in guard rows
8/12	83	61	2-4	0	Early fruit; anthracnose 5%-10% in plot rows
8/19	88	68	2-4	0	Mature green fruit; anthracnose 10%-15% in plot rows
8/26	78	75	2-4	100	Mature green fruit; anthracnose 15-20% in plot rows
9/3	76	76	2-4	40	Mature red and green fruit; anthracnose 20-25% throughout
9/10	80	82	5-7	100	Mature red and green fruit; anthracnose 30-35% throughout
9/17	75	70	5-7	0	Mature red and green fruit; anthracnose 35-40% throughout

4.3 Application conditions for 2002B

Date	Temp.°F	%Relative Humidity	Wind Speed	%Cloud Cover	Growth Stage; Anthracnose Infection level
6/28	78	74	3-6	80	6-8 leaves; no infection
7/8	82	55	8-10	0	Pre-bloom; no infection
7/16	85	57	8-10	0	Pre-bloom; no infection
7/23	84	67	8-10	30	Bloom; no infection
7/31	86	52	3-5	0	Early fruit; no infection
8/6	70	59	5-7	0	Early fruit; anthracnose starting in guard rows
8/15	81	75	5-7	20	Mature green fruit; anthracnose 1% in plot rows
8/22	79	60	2-4	100	Mature green fruit; anthracnose 5% in plot rows
8/30	70	78	5-7	100	Mature red and green fruit; anthracnose 10% in plot rows
9/6	78	56	3-5	20	Mature red and green fruit; anthracnose 15-20% throughout
9/12	76	34	6-8	0	Mature red and green fruit; anthracnose 25-30% throughout
9/20	80	62	0-2	20	Mature red and green fruit; anthracnose 30-35% throughout

4.4 Application conditions for 2003

Date	Temp.°F	%Relative Humidity	Wind Speed	%Cloud Cover	Growth Stage; Anthracnose Infection level
6/26	89	55	5-7	0	6-8 leaves; no infection
7/12	76	89	2-4	0	Pre-bloom; no infection
7/21	77	90	1-3	5	Pre-bloom; no infection
8/6	80	76	3-5	80	Early fruit; no infection
8/13	85	72	0-3	85	Early fruit; no infection
8/20	83	66	1-4	10	Mature green fruit; no infection
8/27	86	70	4-6	5	Mature green fruit; anthracnose 5% in plot rows
9/3	82	76	0-3	100	Mature green fruit; anthracnose 10% in plot rows
9/11	69	74	3-5	0	Mature red and green fruit; anthracnose 15-20% throughout
9/17	72	67	4-6	10	Mature red and green fruit; anthracnose 25-30% throughout
9/24	75	54	2-4	5	Mature red and green fruit; anthracnose 35-40% throughout

Appendix 5: Mean weather conditions throughout all seasons

5.1 Mean of the weather conditions for 2001

	Rainfall (in.)	**Mean temperature (F)**
June	5.28	74.7
July	9.29	74.8
August	2.04	77.4
September	2.30	68.8

5.2 Mean of the weather conditions for 2002

	Rainfall (in.)	**Mean temperature (F)**
June	2.15	75.5
July	5.72	80
August	2.73	78.2
September	2.10	72.4

5.3 Mean of the weather conditions for 2003

	Rainfall (in.)	**Mean temperature (F)**
June	2.94	73.4
July	5.34	78.9
August	6.81	78.2
September	5.12	72

Appendix 6: Seasonal weather data summaries.
6.1 2001

June					July				August				September			
Day	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain
1	72	51	61.5		91	79	85.0		78	58	68.0		84	71	77.5	0.13
2	80	66	73.0	0.46	88	64	76.0	0.53	81	59	70.0		79	62	70.5	
3	80	70	75.0		76	54	65.0		82	62	72.0		79	61	70.0	
4	78	56	67.0		87	71	79.0		81	70	75.5		82	67	74.5	0.02
5	74	61	67.5	1.01	87	73	80.0		88	70	79.0		81	70	75.5	
6	83	65	74.0	0.02	86	66	76.0	0.62	91	73	82.0		82	57	69.5	
7	84	67	75.5	0.19	79	56	67.5		91	75	83.0		80	55	67.5	
8	79	59	69.0		78	68	73.0	0.03	95	75	85.0		82	60	71.0	
9	83	59	71.0		86	69	77.5		95	76	85.5		83	64	73.5	
10	83	62	72.5		90	66	78.0		92	79	85.5		85	70	77.5	
11	83	60	71.5		91	71	81.0	0.16	90	73	81.5		84	69	76.5	0.28
12	89	71	80.0		85	62	73.5		87	74	80.5	0.37	80	58	69.0	
13	88	70	79.0		85	60	72.5		87	72	79.5	0.46	81	55	68.0	
14	85	72	78.5		85	58	71.5		82	73	77.5	0.33	78	57	67.5	
15	81	74	77.5		83	60	71.5		83	69	76.0		71	53	62.0	

6.1 2001 cont.

Day	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain
16	80	73	76.5	0.17	88	63	75.5		84	61	72.5		73	52	62.5	
17	85	71	78.0	2.53	90	69	79.5		87	70	78.5		77	49	63.0	
18	85	66	75.5		90	74	82.0	0.13	87	72	79.5	0.07	78	51	64.5	
19	83	59	71.0		78	71	74.5	0.02	81	72	76.5	0.17	76	54	65.0	
20	85	68	76.5		77	63	70.0		85	76	80.5		75	64	69.5	0.45
21	85	68	76.5		77	59	68.0		84	69	76.5		78	66	72.0	0.49
22	82	70	76.0		82	58	70.0		86	64	75.0		82	69	75.5	
23	80	70	75.0	0.70	85	68	76.5		86	64	75.0		83	68	75.5	
24	78	68	73.0	0.20	89	73	81.0		83	72	77.5	0.11	81	67	74.0	
25	81	61	71.0		88	76	82.0	0.36	80	65	72.5		76	65	70.5	0.92
26	86	62	74.0		89	77	83.0	0.01	82	61	71.5		71	52	61.5	
27	89	63	76.0		83	66	74.5	3.67	87	63	75.0		73	49	61.0	
28	92	71	81.5		75	57	66.0		85	69	77.0	0.44	73	53	63.0	
29	93	72	82.5		75	61	68.0	2.84	85	69	77.0		67	47	57.0	
30	93	76	84.5		78	67	72.5	0.92	84	69	76.5		66	53	59.5	0.01
31			0.0		78	61	69.5		84	73	78.5	0.09				

6.2 2002

June					July				August				September			
Day	Max.	Min	Mean	Rain	Max.	Min	Mean	Rain	Max.	Min	Mean	Rain	Max.	Min	Mean	Rain
1	90	74	82.0		88	61	74.5		93	72	82.5	0.39	76	69	72.5	0.59
2	90	69	79.5	0.28	90	69	79.5		89	73	81.0	0.05	75	66	70.5	0.01
3	87	64	75.5		97	72	84.5		89	70	79.5		82	61	71.5	
4	77	60	68.5		99	74	86.5		88	70	79.0		90	71	80.5	
5	87	71	79.0		98	76	87.0		91	73	82.0		86	68	77.0	
6	92	74	83.0		93	66	79.5		90	76	83.0	0.02	81	62	71.5	
7	86	63	74.5	0.92	89	59	74.0		82	60	71.0		80	54	67.0	
8	68	53	60.5		90	65	77.5		82	56	69.0		80	62	71.0	
9	75	47	61.0		94	74	84.0		84	57	70.5		82	64	73.0	
10	86	56	71.0		94	75	84.5		84	57	70.5		82	73	77.5	
11	87	65	76.0		87	66	76.5	0.09	87	61	74.0		86	73	79.5	
12	91	73	82.0		80	52	66.0		90	66	78.0		82	56	69.0	
13	91	69	80.0	0.07	82	55	68.5		92	72	82.0		79	51	65.0	
14	85	66	75.5	0.35	79	73	76.0	0.03	89	74	81.5		80	64	72.0	
15	79	64	71.5	0.33	87	68	77.5	0.03	87	73	80.0		84	72	78.0	0.06

6.2 2002 cont.

Day	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain
16	83	57	70.0		94	74	84.0		87	76	81.5	0.01	82	73	77.5	0.37
17	84	65	74.5	0.02	95	64	79.5		94	76	85.0	0.02	81	66	73.5	0.01
18	79	59	69.0		96	75	85.5		95	76	85.5		83	61	72.0	
19	80	62	71.0		95	78	86.5	TR	96	74	85.0		78	57	67.5	
20	81	62	71.5		93	72	82.5	2.34	96	78	87.0		81	60	70.5	
21	81	63	72.0		87	71	79.0		90	72	81.0	0.04	83	63	73.0	
22	81	60	70.5		87	67	77.0		86	63	74.5		82	61	71.5	
23	86	61	73.5		88	77	82.5	0.01	93	76	84.5		82	69	75.5	
24	95	74	84.5		87	73	80.0	1.37	93	76	84.5	0.12	78	61	69.5	
25	96	77	86.5		81	71	76.0	1.42	86	72	79.0	0.25	77	58	67.5	
26	93	77	85.0		76	66	71.0	0.42	84	68	76.0		73	65	69.0	0.61
27	88	76	82.0		84	71	77.5	0.01	81	68	74.5		83	71	77.0	0.45
28	88	73	80.5	0.18	94	73	83.5		76	67	71.5	0.59	83	70	76.5	
29	89	69	79.0		96	81	88.5		75	70	72.5	1.06	77	59	68.0	
30	89	62	75.5		96	80	88.0		74	64	69.0	0.04	76	58	67.0	
31					91	75	83.0		74	63	68.5	0.14				

6.3 2003

June					July				August				September			
Day	Max.	Min	Mean	Rain	Max.	Min	Mean	Rain	Max.	Min	Mean	Rain	Max.	Min	Mean	Rain
1	73	61	67.0	0.60	90	72	81.0		84	73	78.5	0.65	86	69	77.5	0.02
2	69	56	62.5		80	67	73.5	0.23	86	74	80.0	0.22	89	76	82.5	
3	69	51	60.0		82	68	75.0	0.4	84	72	78.0	0.24	88	71	79.5	
4	76	63	69.5	0.08	89	71	80.0	0.02	83	73	78.0		86	72	79.0	0.56
5	76	62	69.0		92	76	84.0		82	72	77.0	1.19	80	69	74.5	0.81
6	78	57	67.5		93	78	85.5		82	67	74.5	0.17	78	60	69.0	
7	78	63	70.5	0.58	91	77	84.0		79	70	74.5	0.6	78	56	67.0	
8	73	61	67.0	0.71	89	75	82.0		82	70	76.0	0.06	78	58	68.0	
9	78	58	68.0	0.01	92	73	82.5		76	72	74.0	Tr	75	68	71.5	0.08
10	79	61	70.0		92	73	82.5	0.07	83	71	77.0	0.1	73	62	67.5	
11	87	66	76.5	0.06	89	77	83.0		79	70	74.5	0.59	75	55	65.0	
12	89	72	80.5		88	72	80.0	0.42	84	71	77.5		73	65	69.0	1
13	88	75	81.5	0.01	88	71	79.5		85	71	78.0		78	71	74.5	0.42
14	88	75	81.5		80	68	74.0	0.64	90	71	80.5		82	64	73.0	
15	86	71	78.5		82	63	72.5	Tr	92	73	82.5		83	71	77.0	

6.3 2003 cont.

Day	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain	Max	Min.	Mean	Rain
16	77	66	71.5	0.02	89	71	80.0		92	75	83.5		80	68	74.0	
17	71	62	66.5	0.04	89	73	81.0	0.56	86	69	77.5	1.47	78	57	67.5	
18	84	62	73.0	0.03	87	64	75.5		86	69	77.5		75	67	71.0	1.31
19	84	70	77.0	0.76	86	68	77.0	0.33	81	67	74.0		85	70	77.5	Tr
20	75	66	70.5		83	66	74.5		83	63	73.0		86	64	75.0	
21	73	60	66.5		86	73	79.5		87	68	77.5		86	63	74.5	
22	73	62	67.5		90	77	83.5		90	74	82.0		80	67	73.5	
23	86	67	76.5		80	71	75.5	1.06	90	72	81.0	0.08	78	65	71.5	0.35
24	88	68	78.0		84	70	77.0	0.14	83	62	72.5		77	58	67.5	
25	91	65	78.0		85	68	76.5		85	58	71.5		81	57	69.0	
26	92	70	81.0		86	63	74.5		89	72	80.5		81	62	71.5	
27	93	76	84.5		89	72	80.5		94	72	83.0		82	64	73.0	
28	93	68	80.5		91	78	84.5		93	71	82.0	0.29	80	68	74.0	0.57
29	88	68	78.0	0.04	90	70	80.0	0.32	91	74	82.5	0.06	74	59	66.5	
30	91	73	82.0		73	68	70.5	0.85	91	78	84.5		68	48	58.0	
31			0.0		83	68	75.5	0.3	91	69	80.0	1.09				

Appendix 7: Field data in English units.

7.1 Total number of fruit per acre (x 1000).

Treatment	**Rate**	**Units**	2001	2002A	2002B	2003
Actigard[1]	0.1	oz/A	95.47 bcd[1]			
Actigard[2]	0.3	oz/A	96.56 a-d[2]	69.33 cd		115.07 bc
Actigard	1.0	oz/A		68.97 cd	68.60 c	102.00 c
Actigard Maneb Quadris	0.6 2.0 6.2	oz/A lb/A fl. oz./A	101.64 a-d	101.28 abc		123.78 abc
Actigard Quadris	0.6 6.2	oz./A fl. oz/A	84.22 d	68.97 cd		117.25 bc
Quadris	6.2	fl.oz/A	98.74 a-d	117.61 a	120.15 ab	134.31 ab
Maneb Quadris	2.0 6.2	lb/A fl. oz/A	107.81 abc	91.11 a-d	123.05 ab	126.69 abc
Maneb	2.0	lb/A	103.09 a-d	104.18 abc	128.05 ab	138.30 ab
Maneb Cuprofix	2.0 1.6	lb/A lb/A	95.47 bcd	80.22 a-d		148.83 a
Cuprofix	1.6	lb/A	100.55 a-d	56.99 d		120.88 abc
Messenger	9.0	oz/100gal	117.61 ab			
Messenger	15.0	oz/100gal	110.71 abc	99.46 abc		
Messenger	24.0	oz/100gal		108.90 ab	129.95 ab	
Messenger Maneb Quadris	9.0 2.0 6.2	oz/100gal lb/A fl. oz/A	111.44 abc	79.14 bcd		
Flint	4.0	oz/A	115.07 abc	100.55 abc		133.58 ab
Sovran[3]	6.4	oz/A	106.00 a-d[3]	112.53 ab		
Sovran Maneb	6.4 4.0	oz/A lb/A		90.75 ab	123.78 ab	122.69 abc
Control			94.02 cd	97.29 abc	135.03 a	133.95 ab
LSD			23.38	37.40	28.70	133.95

[1]In 2001, the rate was 0.2 oz/A for acibenzolar-s-methyl. [2]In 2001, the rate was 0.6 oz/A for acibenzolar-s-methyl. [3]In 2001, the rate was 12.8 oz/A for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD).

7.2 Yield of pepper fruit as bushels per acre[4].

Treatment	**Rate**	**Units**	2001	2002A	2002B	2003
Actigard[1]	0.1	oz/A	844.58 abc[1]			
Actigard[2]	0.3	oz/A	855.47 abc[2]	320.65 de		873.62 cd
Actigard	1.0	oz/A		359.37 cde	330.33 d	802.23 d
Actigard Maneb Quadris	0.6 2.0 6.2	oz/A lb/A fl. oz/A	856.68 abc	467.06 a-d		1015.19 bcd
Actigard Quadris	0.6 6.2	oz/A fl. oz/A	759.88 c	338.80 de		1099.89 abc
Quadris	6.2	fl. oz/A	896.61 abc	601.37 ab	606.21 abc	1294.70 a
Maneb Quadris	2.0 6.2	lb/A fl. oz/A	1021.24 a	408.98 a-e	585.64 abc	1165.23 ab
Maneb	2.0	lb/A	1029.71 a	577.17 abc	705.43 a	1187.01 ab
Maneb Cuprofix	2.0 1.6	lb/A lb/A	884.51 abc	317.02 de		1081.74 abc
Cuprofix	1.6	lb/A	874.83 abc	186.34 e		1090.21 abc
Messenger	9.0	oz/100gal	975.26 ab			
Messenger	15.0	oz/100gal	883.30 abc	425.92 a-d		
Messenger	24.0	oz/100gal		546.92 a-d	605.00 abc	
Messenger Maneb Quadris	9.0 2.0 6.2	oz/100gal lb/A fl. oz/A	964.37 abc	367.84 b-e		
Flint	4.0	oz/A	1033.34 a	544.50 a-d		1056.33 a-d
Sovran[3]	6.4	oz/A	878.46 abc[3]	611.05 a		
Sovran Maneb	6.4 4.0	oz/A lb/A		471.90 a-d	663.08 ab	951.06 bcd
Control			839.74 abc	469.48 a-d	565.07 bc	1053.91 a-d
LSD			209.35	235.70	134.32	268.89

[1]In 2001, the rate was 0.2 oz/A for acibenzolar-s-methyl. [2]In 2001, the rate was 0.6 oz/A for acibenzolar-s-methyl. [3]In 2001, the rate was 12.8 oz/A for kresoxim-methyl. [4]Based on a 30 lb bushel. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD).

7.3 The percentage of diseased pepper fruit

Treatment	**Rate**	**Units**	2001	2002A	2002B	2003
Actigard[1]	0.1	oz/A	23.04 de[1]			
Actigard[2]	0.3	oz/A	21.19 def[2]	15.87 b-e		11.83 ab
Actigard	1.0	oz/A		17.11 b-e	19.93 bc	8.79 ab
Actigard Maneb Quadris	0.6 2.0 6.2	oz/A lb/A fl. oz/A	13.20 efg	11.52 cde		8.70 ab
Actigard Quadris	0.6 6.2	oz/A fl. oz/A	7.61 g	14.58 b-e		8.70 ab
Quadris	6.2	fl. oz/A	11.83 efg	10.68 de	10.56 d	7.61 ab
Maneb Quadris	2.0 6.2	lb/A fl. oz/A	8.45 g	14.98 b-e	6.34 d	4.37 b
Maneb	2.0	lb/A	11.12 efg	8.78 e	4.72 d	7.79 ab
Maneb Cuprofix	2.0 1.6	lb/A lb/A	16.21 efg	14.52 b-e		14.70 a
Cuprofix	1.6	lb/A	28.71 cd	14.45 b-e		15.25 a
Messenger	9.0	oz/100gal	44.90 a			
Messenger	15.0	oz/100gal	38.96 abc	29.02 a		
Messenger	24.0	oz/100gal		23.32 ab	27.33 ab	
Messenger Maneb Quadris	9.0 2.0 6.2	oz/100gal lb/A fl. oz/A	9.19 fg	14.23 b-e		
Flint	4.0	oz/A	9.72 fg	11.07 cde		11.76 ab
Sovran[3]	6.4	oz/A	10.48 fg[3]	19.06 a-e		
Sovran Maneb	6.4 4.0	oz/A lb/A		13.14 b-e	1.88 cd	8.40 ab
Control			40.27 abc	22.65 abc	32.29 a	13.91 a
LSD			12.19	11.79	9.19	9.38

[1]In 2001, the rate was 0.2 oz/A for acibenzolar-s-methyl. [2]In 2001, the rate was 0.6 oz/A for acibenzolar-s-methyl. [3]In 2001, the rate was 12.8 oz/A for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD).

7.4 The percentage of diseased fruit by weight.

Treatment	**Rate**	**Units**	2001	2002A	2002B	2003
Actigard[1]	0.1	oz/A	15.10 cd[1]			
Actigard[2]	0.3	oz/A	13.14 c-f[2]	13.17 b-e		12.46 abc
Actigard	1.0	oz/A		13.38 b-e	15.33 bc	7.03 bcd
Actigard Maneb Quadris	0.6 2.0 6.2	oz/A lb/A fl. oz/A	9.08 d-h	9.13 de		7.43 a-d
Actigard Quadris	0.6 6.2	oz/A fl. oz/A	6.35 fgh	9.30 de		4.96 cd
Quadris	6.2	fl. oz/A	6.22 gh	6.93 e	9.52 cd	4.44 d
Maneb Quadris	2.0 6.2	lb/A fl. oz/A	3.77 h	7.99 e	5.17 d	3.83 d
Maneb	2.0	lb/A	5.78 gh	6.94 e	4.98 d	6.76 bcd
Maneb Cuprofix	2.0 1.6	lb/A lb/A	9.48 d-h	10.35 cde		9.42 a-d
Cuprofix	1.6	lb/A	12.29 c-g	8.82 e		15.27 a
Messenger	9.0	oz/100gal	24.00 ab			
Messenger	15.0	oz/100gal	17.51 bc	25.61 a		
Messenger	24.0	oz/100gal		20.42 abc	20.47 ab	
Messenger Maneb Quadris	9.0 2.0 6.2	oz/100gal lb/A fl. oz/A	5.48 gh	8.91 de		
Flint	4.0	oz/A	5.58 gh	7.05 e		8.62 a-d
Sovran[3]	6.4	oz/A	6.61 fgh[3]	14.64 a-e		
Sovran Maneb	6.4 4.0	oz/A lb/A		10.29 cde	13.13 c	6.02 bcd
Control			26.25 a	22.08 ab	27.13 a	13.73 ab
LSD			6.91	11.06	7.11	7.99

[1]In 2001, the rate was 0.2 oz/A for acibenzolar-s-methyl. [2]In 2001, the rate was 0.6 oz/A for acibenzolar-s-methyl. [3]In 2001, the rate was 12.8 oz/A for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD).

7.5 The weight (lbs.) of marketable fruit.

Treatment	**Rate**	**Units**	2001	2002A	2002B	2003
Actigard[1]	0.1	oz/A	14.90 cde[1]			
Actigard[2]	0.3	oz/A	15.32 cde[2]	3.28 def		12.63 c
Actigard	1.0	oz/A		3.68 c-f	3.27 c	11.48 c
Actigard Maneb Quadris	0.6 2.0 6.2	oz/A lb/A fl. oz/A	16.17 a-e	5.18 a-e		15.30 abc
Actigard Quadris	0.6 6.2	oz/A fl. oz/A	14.75 cde	3.78 c-f		19.20 ab
Quadris	6.2	fl. oz/A	17.40 a-d	7.95 ab	5.50 ab	21.13 a
Maneb Quadris	2.0 6.2	lb/A fl. oz/A	20.33 a	4.40 a-f	5.55 ab	19.65 ab
Maneb	2.0	lb/A	20.15 ab	8.00 a	4.05 a	17.20 abc
Maneb Cuprofix	2.0 1.6	lb/A lb/A	16.63 a-e	2.28 ef		15.23 abc
Cuprofix	1.6	lb/A	15.93 b-e	0.90 f		14.60 bc
Messenger	9.0	oz/100gal	15.48 cde			
Messenger	15.0	oz/100gal	15.17 cde	3.47 c-f		
Messenger	24.0	oz/100gal		5.57 a-e	4.45 bc	
Messenger Maneb Quadris	9.0 2.0 6.2	oz/100gal lb/A fl. oz/A	18.85 abc	4.05 c-f		
Flint	4.0	oz/A	20.17 ab	6.95 a-d		14.80 abc
Sovran[3]	6.4	oz/A	16.90 a-e[3]	6.90 a-d		
Sovran Maneb	6.4 4.0	oz/A lb/A		5.47 a-e	6.60 a	14.75 abc
Control			13.02 e	4.40 a-f	3.32 c	14.20 bc
LSD			4.30	3.83	1.85	6.50

[1]In 2001, the rate was 0.2 oz/A for acibenzolar-s-methyl. [2]In 2001, the rate was 0.6 oz/A for acibenzolar-s-methyl. [3]In 2001, the rate was 12.8 oz/A for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD).

7.6 The number of marketable fruit.

Treatment	**Rate**	**Units**	2001	2002A	2002B	2003
Actigard[1]	0.1	oz/A	50.75 d-i[1]			
Actigard[2]	0.3	oz/A	52.50 d-i[2]	14.25 def		39.50 b
Actigard	1.0	oz/A		16.00 c-f	13.75 c	39.50 b
Actigard Maneb Quadris	0.6 2.0 6.2	oz/A lb/A fl. oz/A	61.25 a-f	22.25 a-e		45.25 ab
Actigard Quadris	0.6 6.2	oz/A fl. oz/A	53.50 c-i	15.25 c-f		55.75 ab
Quadris	6.2	fl. oz/A	60.25 a-g	36.75 a	23.25 ab	59.25 a
Maneb Quadris	2.0 6.2	lb/A fl. oz/A	68.00 abc	19.50 b-f	23.75 ab	60.25 a
Maneb	2.0	lb/A	63.50 a-e	35.25 ab	31.00 a	50.25 ab
Maneb Cuprofix	2.0 1.6	lb/A lb/A	55.00 b-h	10.00 ef		49.25 ab
Cuprofix	1.6	lb/A	48.00 f-i	3.75 f		44.50 ab
Messenger	9.0	oz/100gal	45.50 hi			
Messenger	15.0	oz/100gal	44.75 hi	15.50 c-f		
Messenger	24.0	oz/100gal		24.25 a-e	20.00 bc	
Messenger Maneb Quadris	9.0 2.0 6.2	oz/100gal lb/A fl. oz/A	70.25 a	18.50 b-f		
Flint	4.0	oz/A	71.50 a	30.50 a-d		46.00 ab
Sovran[3]	6.4	oz/A	65.00 a-d[3]	29.25 a-d		
Sovran Maneb	6.4 4.0	oz/A lb/A		25.50 a-e	28.00 a	45.00 ab
Control			39.00 i	21.75 a-e	13.50 c	39.50 b
LSD			11.14	16.90	7.94	16.57

[1]In 2001, the rate was 0.2 oz/A for acibenzolar-s-methyl. [2]In 2001, the rate was 0.6 oz/A for acibenzolar-s-methyl. [3]In 2001, the rate was 12.8 oz/A for kresoxim-methyl. Means within each column followed by the same letter are not significantly different (P = 0.05, LSD).

Appendix 8: Pathogenicity experiment

8.1 Information on pepper 'Enterprise'
Fungicide: Maneb at 3 lb/A on last spray which was 16 May 2003. Kocide also included at 2 lb/A. Volume of 88 gal/A. They were sprayed with maneb only most of the season. The pepper fruit came from research plots, not commercial fields, so likely not sprayed intensively.

8.2 Information on pepper 'Paladin'
Fungicide: The fruit was obtained from the guard rows between the treatments of the 2003 experiment. The pepper fruit were not sprayed with fungicides, but it is possible that drift may have contacted pepper fruit.

8.3 Inoculum used for pathogenicity experiments

	Experiment 1	Experiment 2	Experiment 3	Experiment 4
C. act				9.8×10^4
C. cap	3.6×10^4	7.2×10^4	6.3×10^4	6.5×10^4
C. dem	4.0×10^4	4.8×10^4	5.8×10^4	
C.*g.* 692	4.2×10^4			
C.g. 693	5.2×10^4	8.2×10^4	6.9×10^4	9.3×10^4
FL0101	6.0×10^4	4.2×10^4	7.6×10^4	7.3×10^4
GA0107	5.8×10^4	4.2×10^4	5.5×10^4	6.0×10^4
NC0106	8.2×10^4	7.8×10^4	5.3×10^4	5.8×10^4
NC0206	4.8×10^4	8.2×10^4	9.0×10^4	
NJ0102	4.4×10^4	5.6×10^4	5.5×10^4	8.8×10^4
OH0209		4.5×10^4	5.4×10^4	7.0×10^4
VA0110	6.0×10^4	8.6×10^4	1.0×10^5	8.0×10^4
VA0908	6.4×10^4	4.6×10^4	5.9×10^4	
STDev	1.3×10^4	1.8×10^4	1.6×10^4	1.3×10^4

Appendix 9: Biolog® Information

9.1 Dates Biolog® plates were created in Painter.

	Rep1	Rep2	Rep3	Rep4	Rep5	Rep6
C. act	9/2/02	9/9/02	9/16/02	9/23/02	9/30/02	10/7/02
C. cap	8/12/02	8/19/02	8/26/02	9/2/02	9/16/02	9/23/02
C. dem	10/30/02	11/4/02	11/11/02	11/18/02	11/25/02	
C.g. 692	10/30/02	11/4/02	11/11/02	11/18/02	11/25/02	
C.g. 693	10/30/02	11/4/02	11/11/02	11/18/02	11/25/02	
FL0101	8/12/02	8/19/02	8/26/02	9/2/02	9/9/02	9/16/02
FL0103	8/12/02	8/19/02	8/26/02	9/2/02	9/9/02	
FL0106	8/12/02	8/19/02	8/26/02	9/2/02	9/9/02	
NC0101	5/8/02	5/13/02	5/28/02	6/3/02	6/12/02	
NC0102	5/7/02	5/13/02	5/28/02	6/3/02	6/10/02	
NC0106	5/7/02	5/13/02	5/28/02	6/3/02	6/17/02	
NC0203	5/13/02	5/20/02	5/28/02	6/3/02	6/12/02	
NC0206	5/13/02	5/20/02	5/28/02	6/3/02	6/12/02	
NC0209	5/13/02	5/20/02	5/28/02	6/3/02	6/12/02	
NJ0102	6/17/02	6/24/02	7/15/02	7/22/02	8/12/02	
NJ0106	6/17/02	6/24/02	7/1/02	7/8/02	8/12/02	
NJ0108	6/17/02	8/12/02	8/19/02	8/26/02	9/2/02	
OH0108	6/17/02	6/24/02	7/1/02	7/8/02	7/15/02	7/22/02
OH0109	6/17/02	6/24/02	7/1/02	7/8/02	7/22/02	
OH0110	6/17/02	6/24/02	7/1/02	7/8/02	8/12/02	
OH0202	6/17/02	6/24/02	7/1/02	7/22/02	8/12/02	
OH0204	6/17/02	6/24/02	7/1/02	7/8/02	7/15/02	7/22/02
OH0209	7/8/02	7/15/02	8/12/02	8/19/02		
VA0101	5/7/02	5/13/02	5/28/02	6/3/02	6/12/02	
VA0105	5/7/02	5/13/02	5/28/02	6/3/02	6/12/02	
VA0110	5/7/02	5/13/02	5/28/02	6/3/02	6/12/02	
VA0902	7/8/02	7/15/02	7/22/02	8/12/02	8/19/02	
VA0908	7/8/02	7/15/02	7/22/02	8/12/02	8/19/02	
VA0909	7/8/02	7/15/02	7/22/02	8/12/02	8/19/02	
GA0104	7/8/02					
GA0107	5/6/03	5/13/03	5/20/03	5/27/03	6/3/03	6/30/03
GA0109	5/6/03	5/12/03	5/20/03	5/27/03	6/3/03	6/30/03
GA0110	5/6/03	5/12/03	5/20/03	5/27/03	6/3/03	6/30/03

9.2 Dates Biolog® plates were created in Blacksburg.

	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
C. act	9/22/03	9/30/03	10/20/03	10/27/03	
C. cap	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
C. dem	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
C.g. 692	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
C.g. 693	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
FL0101	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
FL0106	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
GA0107	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
NC0106		6/23/03	6/30/03	7/7/03	7/14/03
NC0206	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
NJ0102	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
OH0109	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
OH0209	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
VA0110	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03
VA0908	6/18/03	6/23/03	6/30/03	7/7/03	7/14/03

9.3 The identification from Biolog® of the Blacksburg experiment at 96 hours.

Blacksburg	Species ID Biolog® database	Species ID: *C. gloeosporioides*	Species ID: *C. acutatum*	Species ID: *coffeanum*	Genus ID Biolog® database	No ID Biolog® database	Species ID User DB	Species ID User DB w/in state	Species ID User DB out state	No ID User database	Species ID ATCC database	Species ID User DB: *C.g.* 692	Species ID User DB: *C.g.* 693	Species ID User DB: *C. cap*	Species ID User DB: *C. act*	Species ID User DB: *C. dem*	Genus ID ATCC database	No ID ATCC database	Total Reps
C. act															3			2	
C. cap						5								4				1	
C. dem		3				2										5			
C.g. 692			1			4						5							
C.g. 693			3			2							4					1	
FL0101		3			1	1				5									
FL0106		3			1	1		1		4									
GA0107			4			1				5									
NC0106		3				1				4									
NC0206		2				2		1		3									
NJ0102		1				4		5											
OH0109		3			1	1				5									
OH0209		3				2		3		2									
VA0110		2				3		2		3									
VA0908		4			1					5									

9.4 The identification from Biolog® of the Blacksburg experiment at 168 hours.

Blacksburg	Species ID Biolog® database	Species ID: *C. gloeosporioides*	Species ID: *C. acutatum*	Species ID: *coffeanum*	Genus ID Biolog® database	No ID Biolog® database	Species ID User DB	Species ID User DB w/in state	Species ID User DB out state	No ID User database	Species ID ATCC database	Species ID User DB: *C. act*	Species ID User DB: *C.g.* 692	Species ID User DB: *C.g.* 693	Species ID User DB: *C. cap*	Species ID User DB: *C. dem*	No ID ATCC database	Total Reps
C. act												3					1	
C. cap						5									6		2	
C. dem						5										6		
C.g. 692			1			4							6					
C.g. 693			3			2								5				
FL0101			2		1	2		1		4								
FL0106			1		2	2		3		2								
GA0107			3		1	1		5										
NC0106		1		1		2		2		2								
NC0206					3	2				5								
NJ0102			4			1		4		1								
OH0109					3	2		1		4								
OH0209			3			2		3		2								
VA0110		1	1		2	1				5								
VA0908					1	4				5								

9.5 The identification from Biolog® of the Painter experiment at 96 hours.

Painter	Species ID Biolog® database	Species ID: *C. gloeosporioides*	Species ID: *C. acutatum*	Species ID: *C. coffeanum*	Species ID: *C. truncatum*	Genus ID Biolog® database	No ID Biolog® database	Species ID User DB	Species ID User DB w/in state	Species ID User DB out state	No ID User database	Species ID ATCC database	Species ID ATTC DB: *C. act*	Species ID ATTC DB: *C.g.* 692	Species ID ATTC DB: *C.g.* 693	Species ID ATTC DB: *C. cap*	Species ID ATTC DB: *C. dem*	No ID ATCC database	Total reps
C. act		1					4						4					1	5
C. cap					1		5									3		3	6
C. dem		2					2										3	1	4
C.g. 692		2				1	1							4					4
C.g. 693		1				3								3				1	4
FL0101		2					4		3	1	2			2				4	6
FL0103			1				4		4	1					1			4	5
FL0106							5		3		2							5	5
GA0107			2			1	3		5		1				4			2	6
GA0109			2				4		5		1				1	2		3	6
GA0110			4				2		4		2				4			2	6
NC0101		1				1	3		3		2			3		1		1	5
NC0102		1				1	3		4		1			2		2		1	5
NC0106			1				4		2		3					1		4	5
NC0203			1				4				5				1			4	5
NC0206		1				2	2		1		4			1		1		3	5
NC0209					1		4		3		2					1		4	5
NJ0102		3					3		4		2			1				4	5
NJ0106		3	1	1		1			4		2					2		4	6
NJ0108		3				2			4		1			2	1	1		1	5
OH0108							5		4	1				1				4	5
OH0109			1			1	1				3					1		2	3
OH0110			1			1	3				5					2		3	5
OH0202		1					3				4			1				3	4
OH0204		1				1	4				6			1		1		4	6
OH0209		2				3			3		2			2		1		2	5
VA0101						2	3			1	4					1		4	5
VA0105		1				1	3		1	1	3							5	5
VA0110						2	3			1	4			1		1		3	5
VA0902		3				1	1			1	4			2				3	5
VA0908		1		1			3		1		4			1		2		2	5
VA0909		2		2			1		2		3			1		1		3	5

9.6 The identification from Biolog® of the Painter experiment at 168 hours.

Painter	Species ID Biolog® database	Species ID: *C. gloeosporioides*	Species ID: *C. acutatum*	Species ID: *C. coffeanum*	Species ID: *C. truncatum*	Genus ID Biolog® database	No ID Biolog® database	Species ID User DB	Species ID User DB w/in state	Species ID User DB out state	No ID User database	Species ID ATCC database	Species ID ATTC DB: *C. act*	Species ID ATTC DB: *C.g.* 692	Species ID ATTC DB: *C.g.* 693	Species ID ATTC DB: *C. cap*	Species ID ATTC DB: *C. dem*	No ID ATCC database	Total reps
C. act			1			1	4						4			1		1	6
C. cap							6									2		4	6
C. dem							4										4		4
C.g. 692						2	2							4					4
C.g. 693			2				2								4				4
FL0101		1		1			4		3	2	1		1					5	6
FL0103		3					2		1	2	2		3		1	1			5
FL0106							5		1	1	3		3					2	5
GA0107		1		2		2	1		4		2		3					3	6
GA0109			1	2		1	2		5		1		2		1	1		2	6
GA0110		1	1	1		2	1		3		3					1		5	6
NC0101		1		1		1	1		2		2		1		2			1	4
NC0102				1		2	2		3	1	1			2		2		1	5
NC0106				1		2	2		4		1		1	2		1		1	5
NC0203				2			2		2		2					2		2	4
NC0206		1		2		1			3		1		1	1				2	4
NC0209				1		1	3		3		2		1	1		2		1	5
NJ0102			4			1				1	4				1	3		1	5
NJ0106						2	4				6					5		1	6
NJ0108						2	3		2	1	2					3		2	5
OH0108		2				1	3		1	1	4			2				4	6
OH0109						3			1		2			1		1		1	3
OH0110				2		2	1		1	1	3		1	2				2	5
OH0202			1			2	1		1		3		1	2				1	4
OH0204		1	2	1		1	1		2		4			3		2		1	6
OH0209		1	1	1			2		1		4		1			2		2	5
VA0101		1		1		1	1			1	3		1	2		1			4
VA0105				1		1	2			2	2		1	2		1			4
VA0110				2		2	1			4	1		1	1		2		1	5
VA0902						5			2		3		1	3		1			5
VA0908						2	3		2		3			1		3		1	5
VA0909			2			2	1		2		3		1	1		1		2	5

9.7 Percentages of nutrient utilization for each well at 168 hours for Painter, VA

	FL01	GA01	NC01	NC02	NJ01	OH01	OH02	VA01	VA09	*C.g*.692	*C.g*.693	*C. dem*	*C. act*	*C. cap*
Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tween 80	63	92	83	71	100	100	100	67	93	87.5	100	100	100	60
N-Acetyl-D-Galactosamine	0	0	0	0	0	0	0	0	0	0	0	0	8	0
N-Acetyl-D-Glucosamine	100	100	100	100	100	100	100	100	100	100	100	100	100	80
N-Acetyl-D-Mannosamine	9	3	0	0	0	0	0	0	0	0	62.5	0	0	30
Adonitol	16	10	3	0	35	0	0	0	17	0	75	12.5	50	30
Amygdalin	89	100	100	100	100	100	100	100	100	100	100	100	75	100
D-Arabinose	55	26	93	100	64	58	83	100	80	50	25	25	67	70
L-Arabinose	28	100	97	100	90	93	92	100	80	100	62.5	100	42	90
D-Arabitol	69	100	100	100	94	77	92	100	83	87.5	75	12.5	42	70
Arbutin	88	82	90	92	100	100	100	86	93	75	75	62.5	50	100
D-Cellobiose	85	100	100	100	97	93	100	100	97	75	50	100	83	90
a-Cyclodextrin	0	7	0	0	0	0	0	0	0	0	0	0	17	0
ß-Cyclodextrin	3	0	0	0	0	0	0	0	0	0	0	0	17	0
Dextrin	97	100	97	100	93	97	100	100	93	100	100	100	92	60
i-Erythritol	100	100	97	100	91	94	100	100	100	100	87.5	87.5	75	60
D-Fructose	16	97	78	63	66	93	79	50	60	87.5	25	75	25	80
L-Fructose	3	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Galactose	79	100	97	100	100	93	96	100	90	100	87.5	0	50	60
D-Galacturonic Acid	100	88	100	100	100	100	100	100	100	100	75	100	100	80
Gentiobiose	86	100	100	100	100	93	96	100	100	100	100	100	83	90
D-Gluconic Acid	100	100	100	100	100	94	100	100	100	75	87.5	25	100	60
D-Glucosamine	61	100	89	92	67	94	93	100	97	100	37.5	87.5	75	100
a-D-Glucose	28	97	43	54	73	82	68	46	63	100	25	100	25	90
Glucose-1-Phosphate	72	100	28	58	50	8	26	28	23	0	100	12.5	75	40
Glucuronamide	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucuronic Acid	100	100	100	100	100	81	100	100	100	100	100	87.5	100	100
Glycerol	100	100	100	100	100	100	100	100	100	100	100	100	100	60
Glycogen	97	100	100	100	100	100	100	100	100	100	100	100	100	80
m-Inositol	88	100	100	100	100	100	100	100	100	100	75	100	92	70
2-Keto-D-Gluconic Acid	100	100	100	100	100	100	100	100	100	100	100	25	92	90
a-D-Lactose	79	100	90	75	97	88	68	78	70	100	100	100	75	80
Lactulose	91	100	97	92	27	80	97	96	100	75	100	12.5	83	90

Maltitol	97	100	97	100	97	97	100	100	100	100	100	0	83	50
Maltose	69	100	93	96	84	100	90	100	83	100	50	100	75	70
Maltotriose	57	100	93	96	82	93	88	90	77	100	50	100	58	70
D-Mannitol	82	100	100	100	100	69	100	100	93	100	75	25	75	90
D-Mannose	48	97	97	100	86	93	100	100	83	100	75	100	50	100
D-Melezitose	18	97	64	63	67	93	67	60	63	100	25	75	58	80
D-Melibiose	85	100	97	100	100	97	100	100	90	100	75	100	50	90
a-Methyl-D-Galactoside	79	86	93	100	73	94	100	96	100	62.5	62.5	0	50	80
ß-Methyl-D-Galactoside	84	100	100	100	100	100	100	100	97	100	100	50	75	80
a-Methyl-D-Glucoside	58	65	86	79	97	89	100	90	90	100	75	0	33	40
ß-Methyl-D-Glucoside	34	100	86	100	90	79	88	88	70	100	62.5	100	58	90
Palatinose	87	100	89	96	94	93	100	96	90	100	75	87.5	67	90
D-Psicose	53	39	86	58	97	78	75	46	87	50	87.5	0	33	90
D-Raffinose	88	100	100	100	100	100	100	100	93	100	100	100	58	100
L-Rhamnose	89	100	93	96	100	93	100	100	93	100	100	100	83	80
D-Ribose	94	93	100	100	100	100	100	100	97	100	100	100	83	100
Salicin	97	100	100	100	100	94	100	100	100	100	100	100	92	90
Sedoheptulosan	44	18	3	4	0	0	0	4	13	0	0	0	25	20
D-Sorbitol	100	94	93	100	100	61	96	100	97	87.5	100	25	100	100
L-Sorbose	28	100	89	100	100	97	92	86	80	100	100	0	17	60
Stachyose	85	94	100	100	100	100	100	100	100	100	100	87.5	83	80
Sucrose	25	100	90	75	97	93	92	58	60	100	87.5	100	58	90
D-Tagatose	76	56	86	54	41	74	72	67	90	25	37.5	0	42	70
D-Trehalose	97	100	100	100	100	97	100	100	100	100	100	100	100	100
Turanose	97	100	100	100	100	100	100	100	100	100	100	100	75	90
Xylitol	94	100	100	100	97	97	100	100	100	100	100	100	75	80
D-Xylose	66	100	100	96	100	93	100	100	83	100	100	100	75	100
?-Amino-butyric Acid	97	97	100	100	91	89	100	100	100	100	100	100	100	60
Bromosuccinic Acid	100	100	100	100	100	78	100	100	100	100	100	100	100	100
Fumaric Acid	100	100	100	100	100	89	100	100	100	100	100	100	100	100
ß-Hydroxy-butyric Acid	28	25	7	4	0	0	0	8	10	0	12.5	0	42	60
?-Hydroxy-butyric Acid	75	94	35	33	56	49	54	28	50	37.5	100	12.5	67	70
p-Hydroxyphenylacetic Acid	100	100	100	100	100	92	100	100	100	100	100	100	100	100
a-Keto-glutaric Acid	100	100	100	100	97	97	100	100	100	100	100	87.5	100	90
D-Lactic Acid Methyl Ester	72	39	54	13	0	26	33	18	30	12.5	25	75	67	60

L-Lactic Acid	69	71	20	4	0	3	10	14	20	12.5	75	37.5	75	40
D-Malic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	80
L-Malic Acid	100	100	100	100	100	94	100	100	100	100	100	100	100	70
Quinic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	80
D-Saccharic Acid	100	100	100	100	100	89	100	100	100	100	100	100	100	80
Sebacic Acid	0	48	10	8	63	35	56	6	53	25	12.5	0	25	40
Succinamic Acid	100	100	100	100	89	81	100	100	100	100	100	100	100	70
Succinic Acid	100	100	100	100	100	83	100	100	100	100	100	100	100	80
Succinic Acid Mono-Methyl Ester	53	100	100	100	52	54	68	100	37	100	100	50	33	30
N-Acetly-L-glutamic Acid	78	62	100	100	23	18	28	100	37	12.5	0	50	67	40
Alaninamide	69	79	38	54	68	19	42	44	37	0	75	25	67	100
L-Alanine	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Alanyl-Glycine	100	100	100	100	100	86	100	100	100	100	100	100	100	100
L-Asparagine	100	100	100	100	100	94	100	100	100	100	100	75	100	70
L-Aspartic Acid	100	100	100	100	100	72	100	100	100	100	100	100	100	100
L-Glutamic Acid	100	100	100	100	100	81	100	100	100	100	100	100	100	80
Glycyl-L-Glutamic Acid	100	100	93	100	100	69	100	100	93	0	87.5	12.5	100	90
L-Ornithine	87	100	53	75	83	40	81	61	90	50	100	100	100	80
L-Phenylalanine	100	100	100	100	100	78	100	100	100	62.5	100	50	100	90
L-Proline	100	100	100	100	100	86	100	100	100	100	100	100	100	100
L-Pyroglutamic Acid	100	100	100	100	100	67	100	100	100	100	100	100	92	80
L-Serine	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Threonine	100	100	100	100	86	81	100	100	100	100	100	100	100	100
2-Aminoethanol	94	100	83	71	94	49	78	63	97	87.5	100	100	92	100
Putrescine	78	97	97	92	44	75	88	86	83	75	100	37.5	100	50
Adenosine	69	57	45	67	8	21	35	50	20	12.5	37.5	12.5	67	50
Uridine	25	12	7	0	0	0	0	0	13	0	0	0	17	40
Adenosine-5'-Monophosphate	62	63	28	33	0	0	14	32	13	0	62.5	12.5	50	60

9.8 Percentages of nutrient utilization for each well at 96 hours for Painter, VA

	FL01	GA01	NC01	NC02	NJ01	OH01	OH02	VA01	VA09	*C.g*.692	*C.g*.693	*C. dem*	*C. act*	*C. cap*
Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tween 80	69	69	73	77	97	100	100	73	93	88	100	100	100	60
N-Acetyl-D-Galactosamine	3	0	0	0	3	0	0	0	3	0	0	0	8	0
N-Acetyl-D-Glucosamine	100	94	100	100	100	80	100	100	97	100	100	100	100	80
N-Acetyl-D-Mannosamine	9	3	0	0	0	0	3	0	0	0	63	0	0	30
Adonitol	7	3	0	0	6	0	0	0	7	0	75	13	50	30
Amygdalin	60	94	97	100	97	93	100	93	97	100	100	100	75	100
D-Arabinose	20	8	80	67	24	37	41	67	30	50	25	25	67	70
L-Arabinose	32	92	100	100	93	93	97	100	73	100	63	100	42	90
D-Arabitol	45	75	57	90	67	40	74	77	53	88	75	13	42	70
Arbutin	78	72	80	77	97	97	97	63	90	75	75	63	50	100
D-Cellobiose	69	94	100	100	97	93	100	100	93	75	50	100	83	90
a-Cyclodextrin	0	6	0	0	0	0	0	0	0	0	0	0	17	0
ß-Cyclodextrin	6	0	0	0	0	0	0	0	0	0	0	0	17	0
Dextrin	84	94	97	100	97	97	100	100	97	100	100	100	92	60
i-Erythritol	84	94	87	100	86	90	97	100	93	100	88	88	75	60
D-Fructose	18	92	77	67	63	93	82	67	57	88	25	75	25	80
L-Fructose	3	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Galactose	61	94	90	100	78	93	97	93	80	100	88	0	50	60
D-Galacturonic Acid	100	72	100	100	100	100	100	100	100	100	75	100	100	80
Gentiobiose	70	94	100	100	100	93	97	100	97	100	100	100	83	90
D-Gluconic Acid	97	94	100	100	91	73	97	100	100	75	88	25	100	60
D-Glucosamine	25	75	57	63	6	90	73	83	53	100	38	88	75	100
a-D-Glucose	31	92	53	73	76	93	80	63	60	100	25	100	25	90
Glucose-1-Phosphate	69	94	27	40	49	0	23	20	20	0	100	13	75	40
Glucuronamide	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucuronic Acid	47	92	87	100	100	67	96	100	87	100	100	88	100	100
Glycerol	100	94	100	100	100	100	100	100	100	100	100	100	100	60
Glycogen	97	86	100	100	100	100	100	100	100	100	100	100	100	80
m-Inositol	72	94	100	100	100	73	94	100	97	100	75	100	92	70
2-Keto-D-Gluconic Acid	100	94	100	100	100	100	100	100	100	100	100	25	92	90
a-D-Lactose	49	64	70	37	52	37	39	47	57	100	100	100	75	80
Lactulose	34	92	17	7	3	3	3	7	23	75	100	13	83	90

Maltitol	88	94	90	100	90	97	100	100	93	100	100	0	83	50
Maltose	66	94	80	100	87	100	91	100	80	100	50	100	75	70
Maltotriose	57	94	83	93	87	93	93	93	77	100	50	100	58	70
D-Mannitol	73	94	67	100	77	50	100	93	77	100	75	25	75	90
D-Mannose	54	86	100	100	92	97	100	100	83	100	75	100	50	100
D-Melezitose	18	92	57	67	70	93	78	60	60	100	25	75	58	80
D-Melibiose	63	94	97	100	94	93	100	100	93	100	75	100	50	90
a-Methyl-D-Galactoside	59	64	47	67	39	87	82	63	87	63	63	0	50	80
ß-Methyl-D-Galactoside	78	94	93	100	94	93	97	100	93	100	100	50	75	80
a-Methyl-D-Glucoside	46	14	87	87	54	93	69	93	57	100	75	0	33	40
ß-Methyl-D-Glucoside	34	94	0	17	44	13	34	7	27	100	63	100	58	90
Palatinose	59	94	73	93	88	93	97	97	83	100	75	88	67	90
D-Psicose	28	6	20	27	43	60	50	20	50	50	88	0	33	90
D-Raffinose	54	94	87	100	97	93	100	100	83	100	100	100	58	100
L-Rhamnose	83	94	93	100	100	93	100	100	90	100	100	100	83	80
D-Ribose	84	89	100	100	86	100	96	100	87	100	100	100	83	100
Salicin	94	92	87	100	97	87	100	100	100	100	100	100	92	90
Sedoheptulosan	41	19	10	7	0	0	4	3	13	0	0	0	25	20
D-Sorbitol	97	94	70	90	94	50	92	93	90	88	100	25	100	100
L-Sorbose	12	75	60	67	36	97	83	43	63	100	100	0	17	60
Stachyose	62	89	97	100	78	97	97	100	87	100	100	88	83	80
Sucrose	22	92	87	70	91	93	89	67	57	100	88	100	58	90
D-Tagatose	63	36	57	60	14	47	53	60	67	25	38	0	42	70
D-Trehalose	91	94	100	100	100	97	100	100	100	100	100	100	100	100
Turanose	88	94	93	100	100	100	100	100	97	100	100	100	75	90
Xylitol	79	94	100	100	97	77	97	100	100	100	100	100	75	80
D-Xylose	59	92	100	97	100	93	100	100	87	100	100	100	75	100
?-Amino-butyric Acid	100	94	100	100	82	73	100	100	100	100	100	100	100	60
Bromosuccinic Acid	100	94	100	100	100	67	100	100	100	100	100	100	100	100
Fumaric Acid	100	94	100	100	100	87	100	100	100	100	100	100	100	100
ß-Hydroxy-butyric Acid	9	17	10	13	0	0	0	7	3	0	13	0	42	60
?-Hydroxy-butyric Acid	34	86	30	33	29	23	37	27	30	38	100	13	67	70
p-Hydroxyphenylacetic Acid	100	94	100	100	100	70	94	100	100	100	100	100	100	100
a-Keto-glutaric Acid	100	94	100	100	97	73	100	100	97	100	100	88	100	90
D-Lactic Acid Methyl Ester	69	39	57	27	3	13	27	17	23	13	25	75	67	60

L-Lactic Acid	69	67	30	13	3	10	16	10	17	13	75	38	75	40
D-Malic Acid	100	94	100	100	100	93	100	100	100	100	100	100	100	80
L-Malic Acid	100	94	100	100	100	77	100	100	100	100	100	100	100	70
Quinic Acid	100	94	100	100	100	100	100	100	100	100	100	100	100	80
D-Saccharic Acid	100	94	100	100	100	87	100	100	100	100	100	100	100	80
Sebacic Acid	0	11	0	0	6	3	17	3	7	25	13	0	25	40
Succinamic Acid	100	94	100	100	92	67	100	100	100	100	100	100	100	70
Succinic Acid	100	94	100	100	100	70	100	100	100	100	100	100	100	80
Succinic Acid Mono-Methyl Ester	3	89	100	100	18	30	22	100	0	100	100	50	33	30
N-Acetly-L-glutamic Acid	84	64	100	100	21	23	40	100	43	13	0	50	67	40
Alaninamide	69	67	37	40	57	7	41	27	23	0	75	25	67	100
L-Alanine	100	94	100	100	100	100	100	100	100	100	100	100	100	100
L-Alanyl-Glycine	100	94	100	100	100	70	100	100	100	100	100	100	100	100
L-Asparagine	100	94	100	100	100	77	100	100	100	100	100	75	100	70
L-Aspartic Acid	100	94	100	100	100	60	100	100	100	100	100	100	100	100
L-Glutamic Acid	100	94	100	100	100	73	100	100	100	100	100	100	100	80
Glycyl-L-Glutamic Acid	100	94	77	97	88	53	93	97	90	0	88	13	100	90
L-Ornithine	72	94	27	40	57	13	35	27	40	50	100	100	100	80
L-Phenylalanine	97	92	80	100	94	53	85	97	93	63	100	50	100	90
L-Proline	100	94	100	100	100	73	100	100	100	100	100	100	100	100
L-Pyroglutamic Acid	100	94	100	100	100	63	100	100	100	100	100	100	92	80
L-Serine	100	94	100	100	100	100	100	100	100	100	100	100	100	100
L-Threonine	100	94	93	100	38	70	100	100	100	100	100	100	100	100
2-Aminoethanol	84	94	57	40	74	23	48	37	63	88	100	100	92	100
Putrescine	68	94	87	70	43	57	74	73	63	100	100	25	100	60
Adenosine	66	53	63	60	28	33	49	43	37	13	38	13	67	50
Uridine	28	11	7	7	0	0	3	0	13	0	0	0	17	40
Adenosine-5'-Monophosphate	62	58	27	33	8	3	14	23	17	0	63	13	50	60

9.9 Percentages of nutrient utilization for each well at 168 hours for Blacksburg, VA

Data	FL0101	FL0106	GA0107	NC0106	NC0206	NJ0102	OH0109	OH0209	VA0110	VA0908	*C.g.*692	*C.g.*693	*C. act*	*C. cap*	*C. dem*
Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tween 80	90	100	100	100	100	70	80	70	80	100	100	100	38	100	80
N-Acetyl-D-Galactosamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Acetyl-D-Glucosamine	100	100	100	100	100	100	100	100	100	100	90	100	100	100	100
N-Acetyl-D-Mannosamine	0	0	0	0	0	0	0	0	0	0	0	40	0	0	0
Adonitol	0	0	0	0	0	10	0	0	0	0	80	20	25	17	10
Amygdalin	100	100	100	100	100	100	100	100	100	100	100	100	75	100	100
D-Arabinose	50	50	0	50	60	0	30	60	60	60	0	0	13	0	20
L-Arabinose	100	100	100	100	100	100	80	80	80	100	90	70	75	100	100
D-Arabitol	100	100	100	100	100	80	80	80	80	100	90	100	75	100	0
Arbutin	100	100	100	100	100	100	100	90	100	100	100	100	100	100	100
D-Cellobiose	100	100	100	100	100	90	80	80	100	100	90	100	100	100	100
a-Cyclodextrin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ß-Cyclodextrin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dextrin	100	100	100	100	100	90	80	80	80	100	100	100	88	100	100
i-Erythritol	100	100	100	100	100	100	100	100	100	100	100	100	100	100	80
D-Fructose	100	100	100	100	100	80	80	80	90	100	90	90	100	100	100
L-Fructose	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
D-Galactose	100	100	100	100	100	90	90	80	90	100	100	80	88	50	0
D-Galacturonic Acid	100	100	100	100	100	90	80	70	80	100	50	20	88	100	80
Gentiobiose	100	100	100	100	100	90	100	100	100	100	100	100	100	100	100
D-Gluconic Acid	100	100	100	100	100	90	90	90	90	100	100	80	100	100	50
D-Glucosamine	100	100	90	88	100	0	90	70	90	100	40	80	38	100	50
a-D-Glucose	100	100	100	100	100	80	80	80	90	100	70	100	100	100	100
Glucose-1-Phosphate	40	40	100	25	40	100	10	10	20	30	100	20	100	0	60
Glucuronamide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucuronic Acid	100	100	100	100	100	100	90	90	90	100	80	100	100	100	90
Glycerol	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Glycogen	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
m-Inositol	100	100	100	100	100	100	100	100	90	100	100	80	75	100	100
2-Keto-D-Gluconic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	20
a-D-Lactose	40	30	100	25	50	60	30	70	40	50	100	80	100	100	100
Lactulose	100	100	100	88	100	40	80	80	80	100	100	90	75	100	40

Maltitol	100	100	100	100	100	100	90	80	100	100	100	100	100	0	50
Maltose	100	100	100	100	100	90	80	80	80	100	100	100	100	100	100
Maltotriose	100	100	100	100	100	90	80	80	80	100	90	90	100	100	100
D-Mannitol	100	100	100	75	90	100	80	90	80	100	100	100	75	33	40
D-Mannose	100	100	100	100	100	90	100	90	90	100	90	100	100	100	100
D-Melezitose	100	100	100	100	100	80	80	80	80	100	90	90	100	100	100
D-Melibiose	100	100	100	100	100	90	100	100	100	100	100	100	88	100	80
a-Methyl-D-Galactoside	100	100	90	100	100	90	90	90	90	100	90	70	75	100	0
ß-Methyl-D-Galactoside	100	100	100	100	100	90	80	90	90	100	100	100	75	100	60
a-Methyl-D-Glucoside	50	40	80	75	50	70	40	0	40	50	60	40	13	17	0
ß-Methyl-D-Glucoside	100	100	100	100	100	90	100	100	100	100	100	100	75	100	100
Palatinose	100	100	100	100	100	90	90	100	80	100	100	80	100	100	100
D-Psicose	90	90	80	88	90	10	80	80	80	90	50	70	25	100	0
D-Raffinose	100	100	100	100	100	90	80	80	80	100	100	90	88	100	100
L-Rhamnose	100	100	100	100	100	100	90	90	80	100	100	100	88	100	100
D-Ribose	100	100	100	100	100	90	90	80	100	100	100	90	100	100	90
Salicin	100	100	100	100	100	100	90	100	100	100	100	100	100	100	100
Sedoheptulosan	0	0	0	0	0	20	0	0	0	0	40	10	25	0	10
D-Sorbitol	100	100	100	100	100	100	90	100	90	100	100	100	100	100	50
L-Sorbose	100	100	100	100	100	90	90	100	90	100	100	90	100	100	0
Stachyose	100	100	100	100	100	90	100	100	100	100	100	80	100	100	90
Sucrose	100	100	100	100	100	90	90	80	90	100	100	100	100	100	100
D-Tagatose	60	40	80	50	40	0	40	80	50	50	40	80	25	100	10
D-Trehalose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Turanose	100	100	100	100	100	100	90	80	100	100	100	100	100	100	100
Xylitol	100	100	100	100	100	100	100	100	100	100	80	100	88	100	100
D-Xylose	100	100	100	100	100	100	90	100	90	100	100	100	100	100	100
?-Amino-butyric Acid	100	100	80	100	100	100	100	100	100	100	100	100	100	100	100
Bromosuccinic Acid	100	100	100	100	100	100	90	90	90	100	100	100	75	100	60
Fumaric Acid	100	100	100	100	100	100	100	100	90	100	100	100	100	100	100
ß-Hydroxy-butyric Acid	0	0	20	25	0	20	20	10	0	0	30	60	50	17	0
?-Hydroxy-butyric Acid	50	50	100	25	30	80	30	80	30	30	100	100	88	100	50
p-Hydroxyphenylacetic Acid	100	100	100	100	100	100	90	100	80	100	100	100	100	100	70
a-Keto-glutaric Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Lactic Acid Methyl Ester	0	0	0	0	0	40	0	0	0	0	80	30	75	50	50

L-Lactic Acid	0	0	20	0	0	20	20	20	20	0	100	100	63	100	60
D-Malic Acid	100	100	100	100	100	100	100	100	90	100	100	100	100	100	100
L-Malic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Quinic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Saccharic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Sebacic Acid	100	70	80	75	50	100	60	90	50	80	40	100	88	100	0
Succinamic Acid	100	100	100	100	80	100	100	100	100	100	100	100	100	100	100
Succinic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Succinic Acid Mono-Methyl Ester	100	100	100	100	100	100	100	100	100	90	80	100	100	100	40
N-Acetly-L-glutamic Acid	10	0	30	25	0	20	30	0	0	10	100	70	75	100	20
Alaninamide	30	60	100	38	40	100	40	30	50	40	100	80	88	83	50
L-Alanine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Alanyl-Glycine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Asparagine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Aspartic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Glutamic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Glycyl-L-Glutamic Acid	100	80	100	100	100	100	100	100	100	100	80	100	100	100	80
L-Ornithine	40	60	100	25	30	100	40	90	30	20	100	100	100	100	100
L-Phenylalanine	100	90	100	100	100	90	100	100	90	100	60	100	100	100	30
L-Proline	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Pyroglutamic Acid	100	100	100	100	100	100	100	100	100	100	100	100	63	100	100
L-Serine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	80
L-Threonine	100	100	100	100	100	80	100	100	100	100	100	100	100	100	100
2-Aminoethanol	30	50	100	13	20	100	0	20	10	30	100	100	88	0	100
Putrescine	100	90	100	88	90	40	80	80	80	100	100	100	75	0	20
Adenosine	50	50	100	25	30	100	30	90	30	20	100	100	75	100	40
Uridine	0	0	0	0	0	20	0	0	0	0	80	20	25	0	50
Adenosine-5'-Monophosphate	20	50	80	13	30	80	20	70	40	30	80	100	75	100	40

9.10 Percentages of nutrient utilization for each well at 96 hours for Blacksburg, VA

	FL0101	FL0106	GA0107	NC0106	NC0206	NJ0102	OH0109	OH0209	VA0110	VA0908	*C.g.*692	*C.g.*693	*C. act*	*C. cap*	*C. dem*
Water	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tween 80	100	100	100	100	100	90	100	80	100	90	100	100	100	100	100
N-Acetyl-D-Galactosamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N-Acetyl-D-Glucosamine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
N-Acetyl-D-Mannosamine	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adonitol	10	0	0	0	0	0	0	0	0	0	40	0	0	33	0
Amygdalin	100	90	90	100	100	100	90	70	90	90	90	100	75	67	100
D-Arabinose	40	30	0	13	50	0	30	30	30	30	0	0	0	0	0
L-Arabinose	100	100	100	100	100	100	100	100	100	100	100	80	100	67	100
D-Arabitol	50	60	80	63	63	50	50	40	50	50	60	80	0	50	88
Arbutin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Cellobiose	100	100	100	100	100	100	100	90	100	100	100	100	100	100	100
a-Cyclodextrin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ß-Cyclodextrin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dextrin	100	100	100	100	100	100	100	100	100	100	100	100	100	83	100
i-Erythritol	100	100	100	100	100	100	100	90	100	100	100	100	25	100	100
D-Fructose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Fructose	0	0	0	0	0	0	0	0	0	0	20	0	0	0	0
D-Galactose	80	80	80	88	100	80	80	50	70	80	90	80	0	67	63
D-Galacturonic Acid	70	80	80	88	100	80	80	60	70	90	40	40	50	100	100
Gentiobiose	100	100	100	100	100	100	100	100	100	100	100	100	100	83	100
D-Gluconic Acid	100	90	90	100	100	100	100	70	90	90	100	80	25	100	100
D-Glucosamine	40	60	0	50	63	0	50	10	50	50	20	60	0	17	100
a-D-Glucose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Glucose-1-Phosphate	40	30	100	25	25	100	30	30	30	40	100	20	50	100	0
Glucuronamide	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D-Glucuronic Acid	90	90	80	100	100	100	80	90	90	80	80	80	50	100	100
Glycerol	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Glycogen	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
m-Inositol	90	90	100	100	100	100	90	70	90	90	100	100	50	83	100
2-Keto-D-Gluconic Acid	100	100	100	100	100	100	100	100	100	100	100	100	0	100	100
a-D-Lactose	10	10	20	13	25	40	0	10	10	10	100	60	75	33	100
Lactulose	20	20	70	13	25	0	20	30	10	10	80	30	0	0	63

Maltitol	100	100	100	100	100	100	90	60	90	90	90	80	13	67	0
Maltose	100	100	100	100	100	100	100	80	100	100	100	100	75	83	100
Maltotriose	100	100	100	100	100	100	100	80	100	100	90	100	88	83	100
D-Mannitol	70	70	100	75	63	100	60	70	70	60	100	100	13	100	38
D-Mannose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Melezitose	100	100	100	100	100	100	100	100	100	100	100	100	50	100	100
D-Melibiose	100	100	100	100	100	100	100	100	100	100	100	100	50	100	100
a-Methyl-D-Galactoside	80	70	80	88	100	70	80	80	80	90	60	50	25	67	100
ß-Methyl-D-Galactoside	90	90	90	100	100	100	80	80	90	90	100	90	25	100	100
a-Methyl-D-Glucoside	0	0	0	0	0	0	0	0	0	0	0	0	0	17	13
ß-Methyl-D-Glucoside	100	100	100	100	100	100	100	100	100	100	100	100	100	83	100
Palatinose	100	100	100	100	100	100	100	100	100	100	100	100	50	100	100
D-Psicose	40	50	10	38	63	0	40	10	40	50	20	20	0	0	100
D-Raffinose	100	100	100	100	100	100	100	80	100	100	100	100	63	100	100
L-Rhamnose	100	100	100	100	100	100	100	90	80	100	100	100	100	100	100
D-Ribose	100	90	90	100	100	90	100	90	100	100	60	70	75	83	100
Salicin	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Sedoheptulosan	0	0	10	0	0	40	0	0	0	0	60	0	0	17	0
D-Sorbitol	70	70	90	75	88	100	70	70	80	70	100	100	13	100	100
L-Sorbose	80	90	80	75	100	40	80	40	80	70	80	50	0	83	100
Stachyose	100	100	100	100	100	100	100	90	100	100	100	80	75	100	100
Sucrose	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Tagatose	30	40	10	50	38	0	40	40	40	40	10	10	0	50	100
D-Trehalose	100	100	100	100	100	100	100	100	100	100	100	100	88	100	100
Turanose	100	100	100	100	100	100	100	100	100	100	100	100	63	100	100
Xylitol	90	90	100	88	100	100	80	80	70	90	100	90	88	83	100
D-Xylose	100	100	100	100	100	100	100	100	100	100	100	100	100	83	100
?-Amino-butyric Acid	100	100	100	100	100	100	100	100	100	100	100	100	88	100	100
Bromosuccinic Acid	100	100	100	100	100	100	100	100	100	100	100	90	50	100	100
Fumaric Acid	100	100	100	100	100	100	100	100	100	100	100	100	75	100	100
ß-Hydroxy-butyric Acid	0	0	0	0	0	0	0	0	0	0	0	0	0	33	0
?-Hydroxy-butyric Acid	40	40	100	13	25	80	30	40	10	20	100	100	25	100	100
p-Hydroxyphenylacetic Acid	100	100	100	100	100	100	100	80	100	100	100	100	50	100	100
a-Keto-glutaric Acid	100	100	100	100	100	100	100	100	90	100	100	100	75	100	100
D-Lactic Acid Methyl Ester	0	0	30	0	0	70	0	20	10	0	90	0	38	50	38

L-Lactic Acid	0	0	30	0	0	20	10	20	0	10	100	100	25	67	100
D-Malic Acid	100	100	100	100	100	100	100	100	100	100	100	100	50	100	100
L-Malic Acid	100	100	100	100	100	100	100	100	100	100	100	100	63	100	100
Quinic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
D-Saccharic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Sebacic Acid	10	10	0	13	13	60	10	20	10	20	20	60	0	33	100
Succinamic Acid	100	100	100	100	100	100	100	100	100	100	100	100	63	100	100
Succinic Acid	100	100	100	100	100	100	100	100	100	100	100	100	88	100	100
Succinic Acid Mono-Methyl Ester	20	20	80	38	25	30	20	20	20	20	80	80	0	33	100
N-Acetly-L-glutamic Acid	40	10	50	13	38	20	20	20	10	30	100	40	13	83	50
Alaninamide	50	60	100	38	25	100	50	40	50	50	100	60	50	100	38
L-Alanine	100	100	100	100	100	100	100	100	100	100	100	100	88	100	100
L-Alanyl-Glycine	100	100	100	100	100	100	100	100	100	100	100	100	63	100	100
L-Asparagine	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Aspartic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Glutamic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Glycyl-L-Glutamic Acid	100	80	100	100	100	100	100	90	90	100	80	90	25	100	100
L-Ornithine	20	30	90	13	0	100	10	10	20	10	100	70	63	100	88
L-Phenylalanine	30	50	100	75	63	90	60	50	40	50	80	100	50	100	100
L-Proline	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
L-Pyroglutamic Acid	100	100	100	100	100	100	100	100	100	100	100	100	100	33	100
L-Serine	100	100	100	100	100	100	100	100	100	100	100	100	50	100	100
L-Threonine	100	100	100	100	100	60	100	100	100	100	100	100	50	100	100
2-Aminoethanol	10	20	100	13	0	100	10	30	10	10	100	100	88	83	0
Putrescine	50	50	90	50	50	10	50	60	40	50	100	80	0	100	0
Adenosine	50	70	100	25	38	100	50	70	40	50	100	100	38	67	100
Uridine	0	0	0	0	0	30	0	0	0	0	80	10	38	17	0
Adenosine-5'-Monophosphate	40	50	80	13	13	80	20	60	40	40	100	100	13	67	100

Appendix 10: Vita

Josh K. Marvel

Josh Kendall Marvel was born on August 31, 1976 to Kendall Richard Marvel and Linda Marie Marvel in Lewes, Delaware. He graduated from Sussex Technical High School in Georgetown, Delaware in 1994. Josh received a Bachelors of Agriculture degree from the University of Delaware as a Plant Science major with a concentration in Plant Pathology and Biology and Entomology minors in 1999. While at the University of Delaware, Josh enrolled in an introductory Plant Pathology class taught by Dr. Robert B. Carroll. During the summer of 1997, Josh took part in a horticulture internship for the University of Delaware Botanical Gardens. This further developed his interest in plants and plant disease. The next summer, he took part in an internship for Dupont agrochemicals. In 1998 and 1999, Josh worked on two independent studies with Dr. Thomas A. Evans focused on Severe Soybean Stunt Virus and multimedia based teaching aids. He worked for one year as a biological technician with the USDA focused on insect parasites, and left this position to pursue a Master's degree in plant pathology.

Josh joined the Eastern Shore Agriculture Research and Extension (AREC) Plant Pathology under the direction of Dr. Sam A. Alexander as a Master's candidate in the summer of 2001. The field of vegetable pathology was ideal for Josh, because it incorporated many aspects of Plant Pathology. In addition to his thesis work, Josh worked in other plant pathology projects as the Eastern Shore AREC and presented talks at regional professional meetings. Josh is a member of the American Phytopathological Society.