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MANAGING FOLIAR BLIGHTS ON SPECIALTY CROPS

By

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ABSTRACT

MANAGING FOLIAR BLIGHTS ON SPECIALTY CROPS

By

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Specialty crops contribute 40 billion (about 40%) of all annual agricultural sales in the U.S. *Botrytis cinerea* and *Alternaria panax* are common pathogens on specialty crops. Control of these foliar blights includes fungicides such as chlorothalonil, a B2 carcinogen and an industry standard. However, consumers are interested in products that are environmentally friendly and safe to humans. An *in vitro* bioassay was used to assess the ability of biopesticides/reduced risk fungicides to protect geraniums from *B. cinerea* infection. Polyoxin D zinc salt and azoxystrobin were effective in limiting germination to < 4.0 %, limiting appressorial development to < 3.0, and preventing germ tube elongation from reaching > 5.0 μm over five incubation intervals. The efficacy of biopesticides/reduced risk products for *B. cinerea* control was evaluated under greenhouse conditions on geranium and ginseng. Azoxystrobin and polyoxin D zinc salt significantly minimized disease severity on geraniums. Polyoxin D zinc salt, boscalid, fluazinam, and fenhexamid significantly limited number of lesions, disease progression, and disease severity on ginseng. The efficacy of biopesticides/reduced risk products was evaluated for *A. panax* control on a ginseng field plot. Polyoxin D zinc salt, fluazinam, and boscalid provided significant control against defoliation and disease severity. The fluazinam, boscalid, and polyoxin D zinc salt treatments provided significantly better yields of the dried, marketable product than the untreated.

DEDICATION

ég gaf ykkur von sem varð að vonbrigðum... þetta er ágætis byrjun...

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Introduction

Specialty crops (or minor crops), as defined by the Food Quality Protection Act, are those which are grown on ≤ 300,000 acres nationally, and include crops produced for niche markets such as some fruits, vegetables, nuts, herbs, and nursery plants (Pennsylvania State Univ., 2004). Together, these specialty crops generate more than $40 billion of all annual agricultural sales and account for over 50% of agricultural sales in 23 states, including Michigan (NASS, 2004).

Specialty crops include greenhouse-grown geraniums (*Pelargonium x hortorum*) and field-grown ginseng (*Panax quinqefolium*). Michigan is the third largest producer of floriculture crops in the nation with an estimated wholesale value of $342 million in 2004. Approximately 20% of the U.S. geranium market is supplied/produced by Michigan growers (NASS, 2004). Ginseng plants, grown and harvested for their roots, are a high value crop in Michigan and Wisconsin and have an estimated value of over $100 million. These two states provide approximately 90% of the cultivated ginseng in the U.S. and contribute 10% of the global supply. In the last decade, Michigan has developed a new ginseng industry with significant acreage located in the Upper Peninsula and represents a Michigan inventory of over $50 million. This ginseng is produced under a natural forest canopy and has a higher market value (up to 10 times) than that of cultivated ginseng in Wisconsin, which implements artificial shade cloths over agricultural soils (Hausbeck, 2004).

Both geranium and ginseng growers have reported leaf and flower blighting caused by *Botrytis cinerea*, commonly known as gray mold (Hausbeck, 2004). Dense

canopies, close plant spacing, and senescing flower petals provide an ideal environment for Botrytis blight in geranium production. For ginseng, cool, wet weather and cultivation under shade cloths promote infection of flowers, foliage, and berries. Botrytis blight hinders healthy plant growth, resulting in poor quality ginseng roots with reduced market value. Developing seed within the berries may also become infected, thereby hindering future seed production and seedling gardens (Hausbeck, 2004).

Botrytis cinerea

Disease Cycle. Affecting a wide variety of ornamentals, vegetables, fruits, and other crops, *Botrytis* species cause numerous diseases in the form of blights, cankers, leaf spots, damping-off, and rots of fruits, stems, corms, tubers, bulbs, and roots (Agrios, 1997). Some specialty crops, including greenhouse-grown geraniums and field-grown ginseng, are particularly susceptible to Botrytis blight because of propagation methods and production environment. Poor air circulation, close plant spacing, promotion of dense canopies, and high levels of relative humidity increase the crop's vulnerability to leaf blight. Blight on these leaves may provide secondary inoculum for subsequent flower and stem blight (Hausbeck & Moorman, 1996).

The blight or gray mold disease caused by *B. cinerea* becomes established under cool, humid conditions. Improper aeration, relative humidity $\geq$ 85% (DeLozier, 1980), and temperatures ranging from 15-23 °C provide the ideal conditions for infection, sporulation, and germination (Agrios, 1997; Jarvis, 1977; Salinas, 1995). Once established, *B. cinerea* produces a profusion of gray mycelium and long, branched conidiophores bearing clusters of one-celled, ovoid, asexual conidia, that resemble a cluster of grapes. Under humid conditions, conidia are readily released and transported

by air currents. Spore germination can occur on all parts of the host plant above the soil line. Irregularly shaped, flat, black sclerotia (masses of mycelia which serve as resting or overwintering structures) often develop and germinate by way of mycelial threads which can infect plant tissues (Coley-Smith *et al.*, 1980). On rare occasion, conidia and sclerotia yield a *Botryotinia* perfect stage in which sexual ascospores are produced in an apothecium. Germinating sexual and asexual spores invade tissues through wounds or by penetrating old flower petals or dying leaves. In field crops, *B. cinerea* overwinters in the soil as mycelium in decaying plant material or as sclerotia (Agrios, 1997).

Classification. The classification of *B. cinerea* has been difficult due to the inability of obtaining the perfect (sexual) state from the imperfect (asexual) state under controlled conditions (Coley-Smith *et al.*, 1980). Ascomycetes belonging to the subphylum Ascomycotina (class Hyphomycetes) are further categorized according to the morphology of their sexual (teleomorph) and asexual (anamorph) structures. Members of Sclerotiniaceae (order Leotiales) have distinctive anamorphs, while the teleomorphs are relatively uniform. Despite classification based on the teleomorph form, genera have been distinguished by characteristics of their anamorphs, as well as named after them. Thus, the sexual form, *Botryotinia fuckeliana* (de Bary) Whetz., produces the anamorph *Botrytis cinerea* Pers.:Fr. (Coley-Smith *et al.*, 1980; Farr, 1989; Kohn, 1979). To further describe the holomorph, Morgan (1971) (as cited by Jarvis, 1977), was able to recognize two forms of *B. cinerea* in a numeric analysis of twelve *Botrytis* taxa; Type A was characterized by gray or dark brown colonies, luxuriant spore production, and few to no sclerotia, and Type B colonies were cream or white, with sclerotial formation preferred at the expense of conidial proliferation. Munoz *et al.* (2002) confirmed that *B. cinerea* is

made up of two groups, where both have been genetically isolated and could be considered two distinct species.

Pathogen Life Cycle. *Botrytis cinerea* has four important infective propagules: conidia, mycelia, ascospores, and sclerotia (Coley-Smith *et al.*, 1980). The asexual stage (producing conidia and sclerotia) occurs through the spring and summer and is the primary inoculum source. On extremely rare occasions, the sexual stage (ascospore development) may take place with the onset of cold weather in the fall.

The conidiophores arise from mycelia as straight, cylindrical columns, which develop terminal branches of conidiogenous cells. At maturity, some branches inflate into spherical ampullae, with 8x15 μm, smooth, elliptical conidia attached by a fine denticle. Gray-brown conidia appear as a grape-like cluster under microscopic examination. Dry and hydrophobic upon release, one to five germ tubes may germinate from each conidium in the presence of free water (Coley-Smith *et al.*, 1980).

Botryotinia ascospores are produced within apothecia. The *Botryotinia* apothecia are open, disk- or cup-shaped structures, 1-5 mm in diameter, which may or may not be stalked, and have the asci borne in a palisade layer within the cup. Eight ovoid ascospores develop within the apothecia and are discharged under conditions of high relative humidity (Coley-Smith *et al.*, 1980). It should be noted that these sexual structures are not typically found in nature, but have been seen and documented on onion and bean crops (Ellerbrock & Lorbeer, 1977; Polach & Abawi, 1975).

Botrytis cinerea frequently produces a dense, compact mass of mycelia to create flat, blackened, 1-2 mm, irregularly-shaped sclerotia which are firmly attached onto or just below the host cuticle. Sclerotia may germinate for direct infection of tissue during

the growing season or overwinter in the soil. Although it appears that seeds are not typically susceptible to infection by *B. cinerea*, seed lots may become contaminated by sclerotial bodies that are of the same size and dimension of seeds of ornamental crops (Agrios, 1997; Coley-Smith *et al.*, 1980).

Infection Process. Sclerotia germinate and produce filamentous mycelial growth, which yields conidia-producing sporocarps. Conidia, mycelia, and ascospores all have the capacity to initiate the infection process by invading senescent leaves and flowers through stomata or wounded tissues in the presence of free water. Although conidia, mycelial fragments, sclerotia and ascospores are all considered inocula, the conidia of *B. cinerea* act as the primary infection propagules during the repeated asexual life cycle throughout the host plant's growing season. The conidial infection process involves several distinct phases: (i) attachment to the host surface, (ii) germination of the spore and penetration of the plant surface, and (iii) colonization of the plant tissue (Agrios, 1997).

Proper and secure attachment to the host surface by conidia is critical for subsequent infection. Doss *et al.* (1995) categorized two phases, in which immediate adhesion takes place once the conidia comes in contact with the surface tissue. Immediate attachment occurs in the presence of free water and hydration of the spore(s). Delayed adhesion takes place several hours after the initial inoculation and increases the strength of adhesive forces. These adhesive forces are carried out via a secreted mucilaginous material consisting of glycoproteins and/or enzymes (Nicholson, 1996).

Attachment is followed by conidial germination, during which one or more germ tubes develop along the tissue surface. The length of the developing germ tube and the

formation of an appressorium, or penetration structure, are contingent on the availability of nutrients in the surrounding microenvironment. It has been observed that in the presence of nutrients, the length of germ tubes are much longer than those that germinate in the absence of exogenous nutrients (Clark and Lorbeer, 1976; Cole *et al.*, 1996; Salinas and Verhoeff, 1995; Van den Heuvel and Waterreus, 1983).

Immediate penetration and delayed penetration are two mechanisms by which *Botrytis* spp. invade the host tissue. *Botrytis cinerea* commonly displays delayed penetration with extensive hyphal growth along the host surface prior to appressorial development. The hyphal tips may differentiate into appressoria over the top of epidermal cells or anticlinal wall junctures and affix to the infection surface in a similar method as the germ tube (Clark and Lorbeer, 1976). Doss *et al.* (1995) reported that germ tubes are encased in a sheath that is involved in adhesion and cannot be removed with proteases or carbohydrate-degrading enzymes. Moreover, Cole *et al.* (1996) revealed that a mucilaginous substance secreted from appressoria formed a pad of matrix material that assists in attachment to the penetration site. They also noted that spores germinating under wet conditions became adhered with a fibrillar-like matrix, while dry conditions cause the spores to produce a condensed, amorphous matrix adhesion pad.

Once an appressorium is attached, turgor pressure increases within the rounded structure from imbibing external water by osmosis. This forces a hyphal peg out from the bottom of the appressorium to penetrate the host tissue (Agrios, 1997). Most penetration may be aided by the release of fungal cutinases and cell wall-degrading enzymes (Struck *et al.*, 1998). Following penetration of the cuticle, the penetration peg differentiates into swollen infection hyphae within the epidermis. Infection hyphae grow into adjacent cells

and cause further degradation. As infection progresses, the epidermis swells and lesions become evident on plant surfaces. Advanced lesions eventually sink and form cavities from the loss of structural integrity of the epidermal and mesophyll cells (Pie and De Leeuw, 1991). Clark and Lorbeer (1976) pointed out that this swelling of the epidermal wall (up to 2-3 times its normal thickness) and ensuing cavity formation may occur in advance of internal hyphal development. The expression of swelling and cavity formation on host tissue is dependent upon the pathogen's ability to release pectic and cell wall-degrading enzymes or toxins (Coley-Smith *et al.*, 1980). Once the structural integrity and physiological processes of the host tissue are altered at the invasion site, necrotic lesions develop and may coalesce on leaf and flower tissue. Fruit, tuber, corm, bulb, and root tissues may become soft while woody stems may develop cankers (Agrios, 1997).

Management Strategies. Manipulating environmental parameters is important for the successful production of crops under greenhouse or greenhouse-like conditions. Densely planted ginseng plots and closely spaced geranium potted plants are methods which maximize production space. However, these characteristics create a favorable environment for colonization by *B. cinerea* due to a lack of airflow within ginseng canopies and extensive dieback of the lower leaves in geranium crops. Colonized, senescent leaves produce sufficient sporulation to successfully inoculate healthy plants that are in direct contact. Conidia of *B. cinerea* are released and carried on air currents and/or from vibrations received by the host plant, as Hausbeck and Pennypacker (1991b) illustrated in a study on the influence of grower activity during the production of geranium propagation. Under conditions suitable for disease incidence, the environment

surrounding newly harvested cuttings was analyzed for peak conidial concentrations (PCCs) throughout the propagation process. Irrigation, shipment, sanitation, and cutting placement all provided opportunities for the increased occurrence of PCCs. Thus, it is recommended that minimal activity take place in the propagation area and that different stages of the process be segregated into distinct greenhouses. Hausbeck and Pennypacker (1991a) suggest that plant spacing be increased to encourage better air circulation and light penetration to reduce senescence of the lower leaves and create an unsuitable microenvironment for sporulation and germination of the pathogen. Increased plant spacing also allows for better fungicide coverage.

Sirjusingh and Sutton (1996) looked at wetness duration and temperature in relation to flower and leaf infection by *B. cinerea.* Since it is common for older, infected flower petals to fall onto lower leaves and facilitate new infections under free water conditions, Sirjusingh and Sutton (1996) quantified the relationship of wetness duration and temperature during the wetness period to infection. Sporulation of *B. cinerea* on geraniums was higher when wetness on flowers and leaves persisted for more than 4 to 6 h at 21 to 30°C, or greater than 6 to 8 h at 15°C. The authors suggest that periods of wetness greater than 4 h and overhead irrigation be avoided, and that controlling humidity with proper heating, ventilation, and air circulation be used to prevent infection on geranium flowers and leaves.

Hausbeck *et al.* (1996) investigated the effect of low humidity on disease by testing the benefits of forced heated air, along with plastic mulch on greenhouse-grown geranium stock plants. Individual treatments of heated air and plastic mulch were not as effective in disease management as the two in combination. When used alone, the plastic

mulch was not cost effective and was impractical unless the plants remained in one greenhouse for the duration of the growing season. Forced heated air seemed beneficial in systems where plants were moved around within one growing season. Combining both strategies may reduce initial infection prior to the onset of stem blight and subsequent disease progression.

Chemical control. Despite significant research on managing *B. cinerea*, controlling this pathogen has been difficult because it can infect host crops at almost any stage of growth, and can infect all plant parts. Treatments which are designed and implemented for protection of a specific plant part (i.e., flowers) are usually insufficient for providing protection to all other susceptible parts (i.e., stems, leaves) (Coley-Smith *et al.*, 1980). Despite the inability of targeting Botrytis management on whole plants, fungicides have been relied upon for management on field and greenhouse crops for several decades. Due to extensive use of fungicides, *B. cinerea* has evolved resistance to some of the traditional chemicals, eliminating the efficacy of two entire fungicidal classes altogether.

Vali and Moorman (1992) showed that frequent and consistent use of a dicarboximide, such as vinclozolin, produced increased proportions of resistant strains of the pathogen due to *B. cinerea*'s adaptive capabilities. As a result, management goals have focused on reducing spray frequencies per year, combining fungicides or alternating fungicides that provide different modes of action, and timing applications with flowering or fruiting periods.

Two classes of fungicides that have become ineffective for Botrytis management include: the benzimidazoles (inhibits tubulin formation) and the dicarboximides (affects cell division, DNA and RNA synthesis and metabolism). Resistance to dicarboximides

(vinclozolin, iprodione and procymidone) has been reported in greenhouses and vineyards in the U. S. (Moorman & Lease, 1992b; Vali & Moorman, 1992), Canada (Northover & Matteoni, 1986), the U. K. (Locke & Fletcher, 1988), Italy (Gullino *et al.*, 1982), Israel (Katan & Ovadia, 1985), and Greece (Panayotakou & Malathrakis, 1983). Following the discovery of such extensive Botrytis resistance to benzimidazoles in Pennsylvania greenhouses, it was determined that benzimidazoles alone could no longer be recommended for Botrytis blight management.

Since the early 1990s, experiments have been conducted to test the effectiveness of applying a mixture of different pesticides with separate and distinct modes of action. In addition, tests were run with biological control agents and with chemicals with multi-site activity to address concerns of pathogen resistance. Although results varied, they did suggest that pathogen resistance was likely when the same treatment(s) were used consistently over time (Elad *et al.*, 1992; Faretra & Pollastro, 1993; Li & Leifert, 1994; Moorman & Lease, 1992b, 1995; Pollastro *et al*, 1996; Vali & Moorman, 1992).

The difficulty for many ornamental producers in avoiding the repeated exposure of plants to chemicals of the same modes of action has been with the production process itself. Ornamentals are commonly grown in more than one greenhouse throughout their production cycle. Each greenhouse facility exercises its own fungicide regime and does not pass along application records as the crop is moved from one phase of production to the next (Moorman & Lease, 1992a). For ginseng production, polyoxin D zinc salt recently became registered to manage *B. cinerea*; some additional control can be achieved with products registered for *Alternaria*. Crisis exemptions or yearly Specific Exemptions to Section 18 of FIFRA have enabled ginseng growers from Michigan and

Wisconsin to use fungicides effective in controlling foliar blight outbreaks (Hausbeck, 2004).

Alternaria panax

Alternaria panax is a fungal pathogen that causes blights on shoots, stems, and leaves of both American and Asian ginseng, as well as multiple members of the Araliaceae family that are indigenous to Hawaii and southern Florida (Garibaldi *et al.*, 2004; Hausbeck, 2004; Uchida, 2003). Of the few foliar pathogens on American ginseng, *A. panax* can cause severe losses for ginseng growers in the U.S. by infecting the foliage, stems, and berries. If not controlled, the pathogen can reach epidemic proportions within a month of hot and humid conditions (Hausbeck, 2004). Alternaria blight first appears as water-soaked lesions or spots on plant foliage. As the disease progresses, these lesions turn tan-colored with dark brown margins and are sometimes accompanied by yellow, chlorotic haloes surrounding the lesion. The tissue in the center of the lesion may dry up and fall out, leaving a "shot-hole" appearance. Stems and petioles may also become infected and collapse. Diseased leaves and stems may develop dark brown, fuzzy patches of conidia which are released by wind and rain to spread the epidemic to healthy plants (Hausbeck, 2004; Uchida, 2003).

Disease cycle. *Alternaria panax* overwinters in plant debris from the previous growing season. In the spring, the asexual spores become active and can infect newly emerging plants by wind and rain-splash. These asexual spores are born on conidiophores and released for dissemination. Each spore is multicellular and resembles a drumstick in appearance. Once the spores become adhered to host tissue, it will germinate in the presence of moisture/free water via germ tubes. These germ tubes will penetrate the

tissue surface and invade leaf cells. As the fungus develops within the leaf tissue, conidiophores arise and produce new conidia to infect healthy plants and start the disease cycle again (Agrios, 1997; Hausbeck, 2004; Uchida, 2003).

Left uncontrolled, Alternaria blight may develop into a significant epidemic in less than 30 days. If most or all of the foliage is destroyed, there is decreased root development and growth which results in a yield reduction at harvest. Subsequent outbreaks in the following harvest years can reduce future yields drastically. Reports of yield losses from growers range from 50-100% if the epidemic is left unmanaged. In addition, Alternaria blight can ruin seed production on mature plants, thus reducing healthy seed supplies for future growing seasons (Hausbeck, 2004).

Control. Cultural control is a good way to prevent ideal conditions for the pathogen to thrive. Increasing the spacing between plants and avoiding weeds and shrubs around the garden perimeter improves airflow throughout crop canopies. Drip irrigation is also encouraged to maintain dry foliage. Sanitation of diseased leaves can prevent the spread of a sudden outbreak. Chemical control is a necessary means to prevent infections for ginseng growers. Fungicides such as mancozeb and chlorothalonil are used frequently for the duration of the growing season for adequate protection. In order to delay the development of pathogen resistance to a particular chemical, it is recommended that the chemical fungicides mentioned above be alternated with differing modes of action (Hausbeck, 2004; Uchida, 2003).

Fungicide alternatives. Recently, reduced-risk fungicides have/will become available for use against foliar pathogens. The strobilurins are an example of a group of fungicides that offer some degree of control for ornamental growers while reducing safety concerns

for applicators, non-target organisms, and the environment (Hausbeck *et al.*, 2003). In addition to the toxicity risks associated with industry standard chemicals (i.e. chlorothalonil and thiophanate-methyl), the production of these chemicals is costly, time consuming (up to seven years for testing and approval), and involves extensive research and development. This risky process can cost an agrichemical company an estimated $40 to $60 million in production costs before it's reviewed for its first registration. It is estimated that only one in twenty thousand new chemicals actually survive the screening process for new registration (Bischoff, 1993). Alternatively, reduced-risk fungicide registration is expedited by the EPA because of the lower toxicity to human health and natural resources.

Another class of products that poses lower risks is that of biopesticides or biofungicides. These are naturally-based microbial or biochemical products which are effective in reduced concentrations and decompose quickly. Alternating standard chemicals with differing modes of action and reduced-risk fungicides may provide effective management of foliar pathogens while reducing the risk of pathogen resistance (Environmental Protection Agency, 2004).

In the following pages, several reduced-risk and biopesticide products are tested against foliar infection at different stages of development. This has been carried out by: i) *in vitro* bioassay to observe conidial germination and the early initiation of the infection process on treated leaf tissue, ii) greenhouse trials on specialty crops geranium and ginseng to measure efficacy of products under a *Botrytis cinerea* epidemic in a controlled environment, and iii) a field plot trial on ginseng to measure efficacy of products under an *Alternaria panax* epidemic in a Wisconsin research garden.

INTRODUCTION

Specialty crops include greenhouse-grown geraniums (*Pelargonium x hortorum*) and field-grown ginseng (*Panax quinqefolium*). Michigan is the third largest producer of floriculture crops in the nation and supplies approximately 20% of the U.S. geranium market (NASS, 2004). Ginseng plants are a high value crop, grown and harvested for their roots. Total crop value in Michigan and Wisconsin combined is estimated at over $100 million. These two states provide approximately 90% of the cultivated ginseng in the U.S. and contribute 10% of the global supply (Hausbeck, 2004).

Botrytis cinerea causes leaf and flower blight on these two specialty crops during their production. Dense canopies, close plant spacing, and senescing flower petals provide *B. cinerea* with an ideal environment for infection on geraniums (Hausbeck and Pennypacker, 1991a). Cool, wet weather and cultivation under shade cloths promote infection of flowers, foliage, and berries of ginseng plants (Hausbeck, 2004). In response to *B. cinerea* infection, growers implement fungicidal applications into their production schedule. Among the available products, chemical fungicides provide adequate control as long as *B. cinerea* hasn't developed resistance to the product's single mode of action. In the past, *B. cinerea* has developed resistance to two fungicide classes: dicarboximides and benzimidazoles (Gullino *et al.*, 1982; Katan & Ovadia, 1985; Locke & Fletcher, 1988; Northover & Matteoni, 1986; Panayotakou & Malathrakis, 1983). The use of products from either category is therefore not recommended (Moorman & Lease, 1992b; Vali & Moorman, 1992). In addition to resistance issues, chemical fungicides pose risks to the non-targeted environment and health risks to the applicators (Hausbeck *et al.*, 2003). Reduced-risk fungicides and biopesticides are becoming increasingly available

and are registered more quickly than chemical products because of the lower toxicity to human health and natural resources (Environmental Protection Agency, 2004)). Alternating standard chemicals with differing modes of action and reduced-risk fungicides may provide effective management of *B. cinerea* and *A. panax* while reducing and/or prolonging the risk of pathogen resistance.

To assess the ability of fungicides to protect geraniums from *Botrytis cinerea*, a leaf bioassay was developed for *Pelargonium x hortorum* 'Orbit White' plants (Byrne, 1996; Dwyer, 2004). Treatments included an untreated control, a biopesticide, a biocontrol agent, a reduced-risk fungicide, and an industry chemical standard. By quantifying spore germination (%), appressoria formation, and average length of germ tubes, the bioassay may reveal how and when these products impact the infection process on the 'Orbit White' cultivar. Using similar products, a greenhouse trial was conducted to observe the efficacy of biopesticides and reduced-risk fungicides in a controlled environment similar to geranium production conditions. Data collected determined disease severity and was assessed for AUDPC values and lesion counts on 'Orbit White' geraniums.

American ginseng (*Panax quinquefolium*) is grown in shaded gardens which create greenhouse-like conditions that are suitable for Botrytis blight (Hausbeck, 2004). Because of this moderated microenvironment surrounding ginseng canopies, a second trial was carried out in a greenhouse to mimic these controlled conditions. The trial consisted of products from similar categories as stated above to observe efficacy against *B. cinerea* infection. Data collected was a measure to determine disease severity and was assessed for AUDPC values and lesion counts on two-year ginseng plants.

Alternaria panax is a common pathogen of ginseng, causing foliar and stem blights to crops. If left untreated, rapid dissemination of asexual spores is possible under ideal conditions and may incur losses ranging from 50 to 100% of production yield (Hausbeck, 2004). Very few fungicides are approved for use on ginseng against *A. panax*, which increases the risk of the pathogen developing resistance to the few approved products. A field trial was conducted on a research plot in Wausau, WI to test the efficacy of biopesticides and reduced-risk products against *A. panax*. An identical protocol of fungicides used for the ginseng greenhouse trial described above was also used for this field trial. Data collected determined disease severity and was assessed for lesion counts and defoliated/dead plants on two- to three-year ginseng plants.

MATERIALS AND METHODS

In vitro bioassay on 'Orbit White' geranium:

Inoculum preparation and procedure. *Botrytis cinerea* was isolated from zonal (*Pelargonium x hororum*) and ivy (*Pelargonium peltatum*) geraniums obtained from the Michigan State University Demonstration Gardens. Cultures of *B. cinerea* were grown on potato dextrose agar (Difco Laboratories, Detroit, MI) + Streptomycin in 100 x 15 mm Petri dishes under cool white fluorescent light. Spore suspensions were made by flooding cultures with 10 mL of sterile distilled water (dH_2O) and gently agitated with a brush to release spores. The suspension was filtered through one layer of cheesecloth to remove mycelial fragments. The concentration was adjusted to 1 x 10^6 conidia/mL using a hemacytometer (Buck, 2002).

Treatment application and inoculation. Leaves at approximately the second to third node were tagged on mature, seed-propagated geraniums (cv. Orbit White) that were maintained in a research greenhouse at Michigan State University. Five treatments (Table 1) were applied to respective plants: a biopesticide (Endorse), a biocontrol agent (*Bacillus subtilis*), a reduced-risk fungicide (azoxystrobin), a standard chemical protectant (chlorothalonil), and an untreated control. Twenty-four hours after treatment, tagged leaves were removed and 12 mm discs were excised with a sterilized cork borer and placed into corresponding Petri dishes. All dishes contained a plastic mesh grid (sterilized in a 10% bleach solution) that separated leaf discs from filter paper that was saturated with sterile distilled water (dH_2O). Leaf discs were placed adaxial surface up and inoculated with 20 µl of spore suspension with 0.2% of Tween 20 in the center of each of six discs per plate. Petri dishes were wrapped in Parafilm, to maintain humidity and avoid evaporation, and incubated in a diurnal growth chamber for 3, 6, 12,

Table 1. Products tested *in vitro* for efficacy on excised 'Orbit White' geranium discs against *Botrytis cinerea* infection.

Product	Active Ingredient	Manufacturer	Formulation Used (per 100 gallons)	Classification[z]
Daconil Weather Stik 6F	chlorothalonil	Syngenta Crop Proc. Greensboro, NC	22 fl oz (650.6 ml)	B2 carcinogen
Endorse 2.5WP	polyoxin D zinc salt	Arysta LifeScience Corp. San Francisco, CA	2.2 lb (997.9 g)	Biopesticide
Heritage 50WG	azoxystrobin	Syngenta Crop Proc. Greensboro, NC	8.0 oz (226.8 g)	Reduced risk
Rhapsody 1.34%AS	*Bacillus subtilis*	AgraQuest Inc. Davis, CA	8.0 qt (7,570 ml)	Biopesticide

[z] Human risk assessment: B2 carcinogen = likely human carcinogen; Reduced risk = reduced pesticide risk to human health, non-target organisms, and environmental resources; Biopesticide = naturally based microbial or biochemical product with low toxicity.

24, and 36 h at 16 °C under continuous illumination. A separate preliminary test was conducted twice to determine the length of time (hours) for conidial germination and to monitor the effects of using a biocontrol agent under procedural conditions. Similar methods as mentioned above were used with the exception of longer incubation time intervals.

Observations: Conidial observations were made with a compound light microscope at 400x (Olympus America Inc., Melville, NY). Thirteen conidia were randomly selected from one of four quadrants on each disc to determine percentage of germination and subsequent appressorial development. Individual conidia were considered germinated if the germ tube length measured at least half the width of the conidium (Lacy, 1994). The average length of germ tube (μm) was determined by randomly selecting five germinated conidia from each disc. Observations for the parameters listed above were only made on single conidia having one germ tube; conidia in pairs or clusters were not considered.

Experimental design and statistical analysis: This experiment was arranged in a randomized block design with two separate trials carried out. Percent germination and appressorial development were analyzed using the same statistical model, though the residuals for appressorial development were not grouped by treatment because of the low number of observations. The trial by treatment interaction was a random effect due to high variability of treatments for each of the two runs. The time by treatment interactions were fixed effects. Analysis of variance (ANOVA) was conducted using the MIXED procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Differences were determined by pairwise comparisons within interaction terms using Least Square means ($P > 0.05$). Square root transformations were made on percent germination and

appressorial development data; results were back-transformed. A separate model was used for germ tube length, with each trial treated as a random effect. Data were averaged across each disc and $\log_{x+1}$ transformations were used because of the high quantity of zeros. All data were back-transformed for reporting analysis of variance and pairwise comparisons using Least Square means ($P > 0.05$). Lower and upper confidence intervals were used instead of standard errors on back-transformed data.

Fungicide greenhouse trial on 'Orbit White' geranium:

Plant material. A 288-cell flat was seeded with 'Orbit White' geraniums (*Pelargonium x hortorum*) in September, 2004. Six weeks after seeding, seedlings were potted in 6-inch plastic pots containing Baccto high porosity professional planting mix (Michigan Peat Company, Houston, TX) and maintained in a research glass greenhouse at Michigan State University. Plants were fertilized with 250 ppm Peter's 20N-20P-20K liquid feed fertilizer (The Scotts Company, Marysville, OH) three times a week, and fertilized with acid treatment (1100 ml diluted phosphoric acid) once a week. Glasshouses were maintained at 24 °C for both day and night. No supplemental lighting was used.

Inoculum preparation and procedure. *Botrytis cinerea* was isolated from zonal (*Pelargonium x hororum*) and ivy (*Pelargonium peltatum*) geraniums obtained from the Michigan State University Demonstration Gardens. Cultures were grown on potato dextrose agar (Difco Laboratories, Detroit, MI) amended with 5.0 g Streptomycin sulfate (Sigma Chemical Co., St. Louis, MO)/ L of PDA solution in 100 x 15 mm Petri dishes under cool white fluorescent light. Spore suspensions were made by flooding cultures with 10 mL of sterile distilled water (dH_2O) and gently agitated with a brush to release spores. The suspension was filtered through one layer of cheesecloth to remove mycelial fragments. The concentration was adjusted to 1 x 10^6 conidia/mL using a hemacytometer (Buck, 2002).

The trial consisted of nine treatments (Table 2) including an untreated control. Eight fungicide treatments were applied using a hand-pressurized spray bottle to runoff. Plants were placed in individual humidity chambers immediately after fungicidal treatment and allowed to dry. Four hours after treatment, plants were inoculated with the

Table 2. Products tested for efficacy on 'Orbit White' geraniums against *Botrytis cinerea* infection.

Product	Active Ingredient	Manufacturer	Formulation Used (per 100 gallons)	Classification[z]
Daconil Weather Stik 6F	chlorothalonil	Syngenta Crop Proc. Greensboro, NC	22 fl oz (650.6 ml)	B2 carcinogen
Endorse 2.5WP	polyoxin D zinc salt	Arysta LifeScience Corp. San Francisco, CA	2.2 lb (997.9 g)	Biopesticide
Heritage 50WG	azoxystrobin	Syngenta Crop Proc. Greensboro, NC	8.0 oz (226.8 g)	Reduced risk
Primastop 1%WP	*Gliocladium catenulatum*	Kemira Agro Oy Helsinki, FIN	100.0 oz (2,835 g)	Biopesticide
Rhapsody 1.34%AS	*Bacillus subtilis*	AgraQuest Inc. Davis, CA	8.0 qt (7,570 ml)	Biopesticide
Citrex 100L	ascorbic acid	Citrex, Inc. Miami, FL	12.9 fl oz (381.5 ml)	-
Scala 400SC	pyrimethanil	Bayer Crop Science Research Triangle, NC	1.6 pt (757.1 ml)	Reduced risk
Triact 70EC	neem oil	Olympic Hort. Prod. Bradenton, FL	1 gal (3,790 ml)	Biopesticide
Messenger 3WDG	harpin protein	EDEN Bioscience Bothell, WA	9.0 oz (255.1 g)	Biopesticide

[z] Human risk assessment: B2 carcinogen = likely human carcinogen; Reduced risk = reduced pesticide risk to human health, non-target organisms, and environmental resources; Biopesticide = naturally based microbial or biochemical product with low toxicity.

B. cinerea conidial suspension using a spray bottle to runoff. The plastic bags were immediately sealed and reopened only for subsequent treatment and inoculum application. After each treatment, plants were reinoculated four hours later using the method previously described. Six replicates per treatment were arranged in a completely randomized design in a research glass greenhouse at Michigan State University. This experiment was conducted twice; Messenger was substituted for Primastop in the second trial of the experiment.

Disease assessment and statistical analysis. Disease ratings were taken weekly (28 Dec, 4, 11, 18, 25 Jan, and 1 Feb: trial 1; 17, 24, 31 Mar, 7, 14, 21, and 28 Apr: trial 2). This included disease severity, percentage of infected leaves (number of leaves with Botrytis blight divided by total number of leaves for each plant), number of lesions and number of lesions with sporulating *B. cinerea*. Plant disease severity was visibly assessed using a scale: 1 = healthy plant; 2 = 5% infection; 3 = 15%; 4 = 25%; 5 = 40%; 6 = 60%; 7 = 75%; 8 = > 75% + partial leaf abscission; 9 = > 75% + leaf abscission; 10 = plant death. The area under the disease progress curve (AUDPC) was calculated to express the progression of lesion incidence on plant foliage using the method of Shaner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2][X_{i+1} - X_i]$$

where Y_i = number of lesions per plant at the ith observation, X_i = time (days) at the ith observation, and n = total number of observations. Data for all disease assessments were analyzed with analysis of variance (ANOVA) using the GLM procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Differences among treatment means were determined by pairwise comparisons using Fisher's protected LSD ($P > 0.05$).

Fungicide greenhouse trial on American ginseng:

Plant material. Two-year old, field-grown ginseng (*Panax quinquefolium*) roots were dug from commercial Wisconsin fields in the fall of 2003 and 2004 and stored at approximately 3 °C for 100 days in silica sand for each respective year. Roots were transplanted into 1 gallon pots containing Baccto high porosity professional planting mix (Michigan Peat Company, Houston, TX) and placed under 63% shade (to mimic production systems) in a research glass greenhouse at Michigan State University. Plants were fertilized with 250 ppm Peter's 20N-20P-20K liquid feed fertilizer (The Scotts Company, Marysville, OH) three times a week, and fertilized with an acid treatment (1100 ml diluted phosphoric acid) once a week. Glasshouses were maintained at 24 °C for both day and night. No supplemental lighting was used.

Inoculum preparation and procedure. *Botrytis cinerea* was isolated from infected ginseng (*Panax quinquefolium*) plants from a Michigan State University research greenhouse. Cultures were grown on potato dextrose agar (Difco Laboratories, Detroit, MI) amended with 5.0 g Streptomycin sulfate (Sigma Chemical Co., St. Louis, MO) /L of PDA solution in 100 x 15 mm Petri dishes under cool white fluorescent light. Spore suspensions were made by flooding cultures with 10 mL of sterile distilled water (dH_2O) and gently agitated with a brush to release spores. The suspension was filtered through one layer of cheesecloth to remove mycelial fragments. The concentration was adjusted to 1×10^6 conidia/mL using a hemacytometer (Buck, 2002).

The trial consisted of eleven treatments (Table 3) including an untreated control. Ten fungicide treatments were applied using a hand-pressurized spray bottle to runoff. Plants were placed in individual humidity chambers immediately after fungicidal treatment and allowed to dry. Four hours after treatment, plants were inoculated with the

Table 3. Products tested for efficacy on American ginseng against *Botrytis cinerea* infection.

Product	Active Ingredient	Manufacturer	Formulation Used (per 100 gallons)	Classification[z]
Bravo Weather Stik 6SC	chlorothalonil	Syngenta Crop Proc. Greensboro, NC	2.0 pt (946.4 ml)	B2 carcinogen
Endorse 2.5WP	polyoxin D zinc salt	Arysta LifeScience Corp. San Francisco, CA	2.2 lb (997.9 g)	Biopesticide
Elevate 50WDG	fenhexamid	Arysta LifeScience Corp. San Francisco, CA	1.5 lb (680.4 g)	Reduced risk
Topsin 4.5L	thiophanate-methyl	Cerexagri, Inc. King of Prussia, PA	21.8 fl oz (644.7 ml)	B2 carcinogen
Serenade 10%WP	*Bacillus subtilis*	AgraQuest Inc. Davis, CA	10.0 lb (4,536 g)	Biopesticide
Actigard 50WDG	acibenzolar	Syngenta Crop Proc. Greensboro, NC	0.75 oz (21.3 g)	Plant activator
Endura 70WG	boscalid	BASF Corp. Research Triangle, NC	6.8 oz (192.8 g)	Reduced risk
PlantShield 1%WP	*Trichoderma harzianum*	BioWorks, Inc. Fairport, NY	100.0 oz (2,835 g)	Biopesticide
Messenger 3WDG	harpin protein	EDEN Bioscience Bothell, WA	9.0 oz (255.1 g)	Biopesticide
Omega 500F	fluazinam	Syngenta Crop Proc. Greensboro, NC	1.0 pt (473.2 ml)	Reduced risk

[z] Human risk assessment: B2 carcinogen = likely human carcinogen; Reduced risk = reduced pesticide risk to human health, non-target organisms, and environmental resources; Biopesticide = naturally based microbial or biochemical product with low toxicity; Plant activator = activates plant's defense mechanisms against diseases/pests.

B. cinerea conidial suspension using a spray bottle to runoff. The plastic bags were immediately sealed and reopened only for subsequent treatment and inoculum application. After each treatment, plants were reinoculated four hours later using the method previously described. Six replicates per treatment were arranged in a completely randomized design in a research glass greenhouse at Michigan State University. This experiment was repeated two times.

Disease assessment and statistical analysis. Plant ratings were taken weekly (19, 26 May and 2, 9, 16, 23, 30 Jun: trial 1 in 2004; 12, 19, 26 May and 2 Jun: trial 2 in 2005) and assessed for disease severity, percentage of infected leaves (number of leaves with Botrytis blight divided by total number of leaves for each plant), number of lesions and number of lesions with sporulating *B. cinerea*. Plant disease severity was visibly assessed using a scale where 1 = healthy plant; 2 = 5% infection; 3 = 15%; 4 = 25%; 5 = 40%; 6 = 60%; 7 = 75%; 8 = > 75% + partial leaf abscission; 9 = > 75% + leaf abscission; 10 = plant death. The area under the disease progress curve (AUDPC) was calculated to express the progression of lesion incidence on plant foliage using the method of Shaner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2][X_{i+1}-X_i]$$

where Y_i = number of lesions per plant at the ith observation, X_i = time (days) at the ith observation, and n = total number of observations. Data for all disease assessments were analyzed with analysis of variance (ANOVA) using the GLM procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Differences among treatment means were determined by pairwise comparisons using Fisher's protected LSD ($P > 0.05$).

Fungicide field trial on American ginseng:

Plant material. Field-grown ginseng (*Panax quinquefolium*) roots were planted in the fall of 2002 and grown for two seasons in a research garden in Marathon County, WI under wooden laths. Weed control and fertilization were done according to commercial production standards. Inoculation of plant material was not necessary due to *Alternaria panax* occurring naturally in the field. Ginseng roots were harvested on 14 and 28 Sep, 2005, had foliage removed, and were lightly rinsed with distilled water to remove excess soil. Fresh weights from each treatment block were recorded immediately following harvest. Roots were then laid out to dry for 14 days on 4 x 10 ft screens and subjected to forced heated air to mimic standard harvesting/drying procedures by ginseng growers (Table 4). Weights were recorded on dried roots, as this is the marketable product.

Experimental design and statistical analysis. The trial consisted of eleven treatments (Table 3) including an untreated control. All plants were grown on 4-ft wide, raised beds with 1 ft in between bed rows. Fungicide treatments were replicated four times and arranged in a randomized complete block design. Treatment blocks were 10-ft sections of bed rows with a 2 ft buffer on each end. Ten fungicide treatments were applied using a CO_2 backpack sprayer with a 4 ft boom equipped with four 8006 nozzles spaced 18 in apart, operating at 40 psi, and delivering 100 gal/A. Treatments were applied at 7-day intervals from 1 Jun through 4 Aug. Plants were assessed for disease severity, the number of plants infected and the number of plants with partial to complete defoliation from *Alternaria panax* on 28 Jun, 11 Jul, and 1, 10 Aug. The final two ratings are being reported from 1 and 10 Aug. Plant disease severity was visibly assessed using a scale where 1 = no visible lesions; 2 = 25% infected; 3 = 50% infected; 4 = 75% infected; 5 = 100% infected + some defoliation; 6 = 25% defoliation; 7 = 50%

Table 4. Environmental conditions for post harvest drying procedure on American ginseng.

	Temperature		Relative Humidity	
Day	High	Low	High	Low
1	69.0	49.6	98	47
2	67.0	48.1	100	57
3	68.4	62.2	99	55
4	67.7	64.2	100	87
5	86.6	60.1	92	53
6	90.3	86.6	59	43
7	96.4	89.6	55	39
8	102.7	94.8	51	34
9	103.5	95.6	39	21
10	102.7	95.6	22	21
11	103.5	95.6	27	21
12	102.7	94.8	28	21
13	100.3	93.3	26	21
14	115.6	87.4	21	21
15	117.4	63.5	68	21
16	68.4	67.7	36	27

defoliation; 8 = 75% defoliation; 9 = 95% defoliation; 10 = complete defoliation and plant death. Harvested roots were weighed for yield data. Fresh weights were taken on the harvest date, and dried root yields were recorded two weeks later, as this is the marketable product. Data for all disease assessments were analyzed with analysis of variance (ANOVA) using the GLM procedure of the Statistical Analysis System (SAS Institute Inc., Cary, N.C.). Differences among treatment means were determined by pairwise comparisons using Fisher's protected LSD ($P > 0.05$).

RESULTS

***In vitro* bioassay on 'Orbit White' geranium:**

Germination of conidia: The effects of time and treatment on conidial germination were significant at $P < 0.05$ (Table 5). Conidial germination occurred between three and six hours at 16 °C (Fig. 2). Germination on untreated leaf discs increased dramatically and reached 16.2 % after six hours of incubation, but then decreased after 12 hours. Germination rose again to 14.3 % at the 24 h interval and slowly increased to a peak of 16.4 % at the 36 h incubation time.

Alternatively, conidia exposed to chlorothalonil had a slight increase in germination at 6 h, but did not germinate at any of the other time intervals and was statistically better than the untreated control at 3, 6, 24, and 36 h intervals. The azoxystrobin treatment limited germination almost as effectively as chlorothalonil and was significantly better than the untreated control at time intervals: 3, 6, 24, and 36. Conidia treated with *B. subtilis* rose to 1.8 % germination at the 12 and 24 h intervals, but decreased by the 36 h interval to differ significantly from the untreated control. Germination was limited to < 1 % by Polyoxin D zinc salt treatment except for at the 24 h incubation interval where it reached approximately 3 %. However, this treatment decreased by 2.2 % at 36 h to be significantly better than the untreated control. Each of the four fungicides tested were statistically better than the untreated control at the 36 h incubation interval ($P = 0.05$).

Appressorial development: The effects of time and treatment on appressorial development were significant at $P < 0.05$ (Table 6). Appressorial structures began

Table 5. Analysis of variance for percent germination of *Botrytis cinerea* conidia on 'Orbit White' geranium leaf discs.

Effect	Num DF	Den DF	F Value	Prob>F
TRT	4	5	7.67	0.0232
Time	4	270	8.49	<.0001
TRT*Time	16	270	2.85	0.0003

Table 6. Analysis of variance for appressorial development of *Botrytis cinerea* conidial germ tubes on 'Orbit White' geranium leaf discs.

Effect	Num DF	Den DF	F Value	Prob>F
TRT	4	5	3.99	0.0808
Time	4	270	5.41	0.0003
TRT*Time	16	270	2.57	0.0010

Table 7. Analysis of variance for mean length of *Botrytis cinerea* conidial germ tubes on 'Orbit White' geranium leaf discs.

Effect	Num DF	Den DF	F Value	Prob>F
TRT	4	24	33.70	< 0.0001
Time	4	24	7.58	0.0004
TRT*Time	16	24	4.11	0.0009

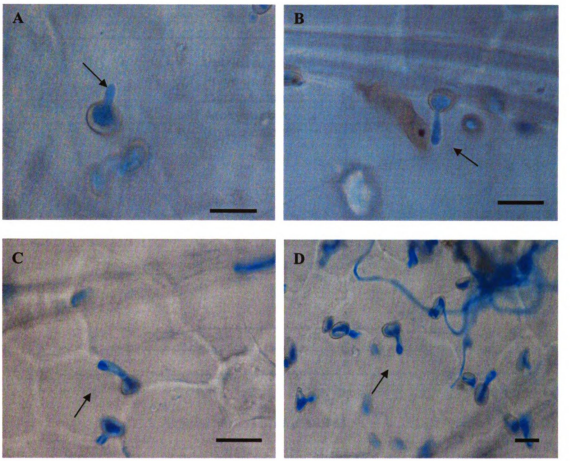

Figure 1. Conidial germination stages of *Botrytis cinerea*. (A,B) Emergence of a germ tube from *B. cinerea* conidium at 6 and 12 h, respectively. (C,D) Enlarged, rounded ends of germ tubes visible on developing appressoria from *B. cinerea*. Black scale bars = 20 μm. Images in this thesis are presented in color.

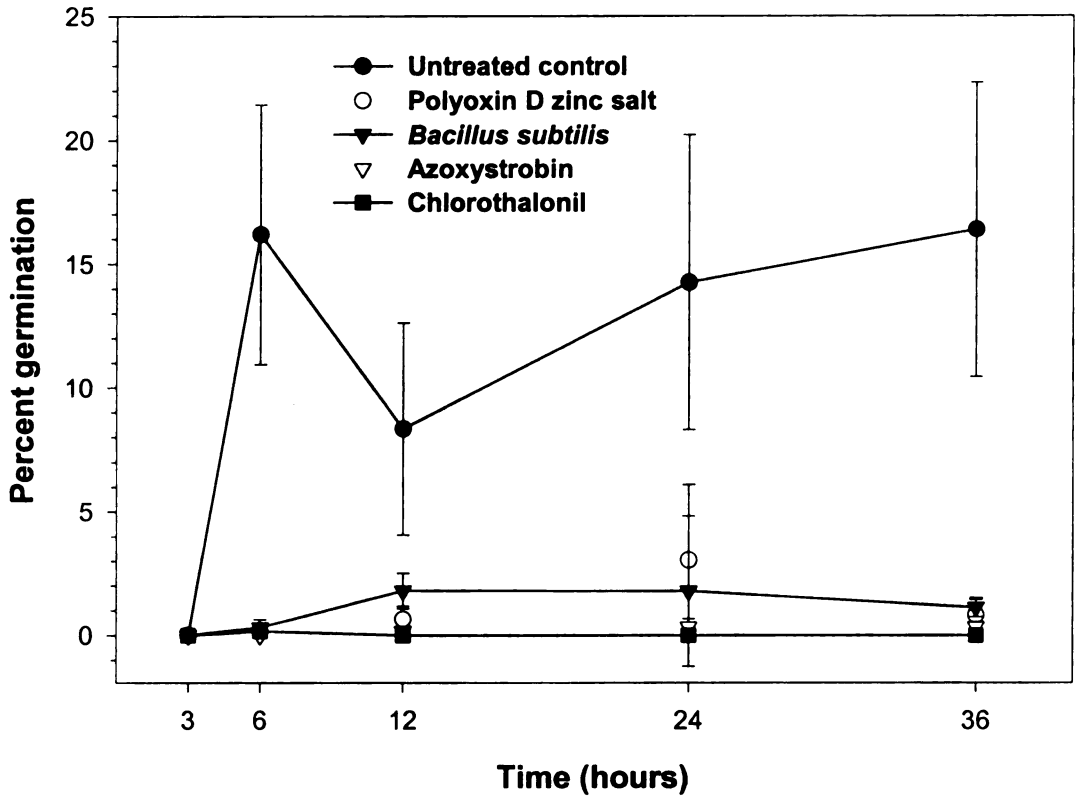

Figure 2. Mean percent of *Botrytis cinerea* conidia germinated at five incubation intervals and treated with one of four fungicides. Bars indicate standard error of the means.

developing from germ tubes between 3 and 6 h (Fig. 3). Untreated conidial germ tubes had a rapid increase in appressorial development between 3 and 6 h, and then rose steadily to the 24 h time interval. Development of appressoria had a slowed increase from 24 to 36 h. Although leaf discs treated with either chlorothalonil or azoxystrobin limited appressoria from developing to < 0.2 % at all incubation intervals, the differences were not statistically significant from the untreated control until the 24 and 36 h intervals. The polyoxin D zinc salt treatment allowed some penetration structures to develop with a slow increase that peaked at 24 h, but then limited development to < 0.2 % at 36 h. *Bacillus subtilis* controlled appressorial development at 3 h, but saw a rise in development between 6 and 12 h, followed by a slow decline from 12 to 36 h intervals. Polyoxin D zinc salt and *B. subitlis* did not differ significantly from the untreated control until the 36 h incubation interval. All fungicide treatments were statistically better and had fewer appressoria developed than the untreated control at 36 h ($P = 0.05$).

Mean germ tube length: The effects of time and treatment on mean germ tube length were significant at $P < 0.05$ (Table 7). Due to the lack of germination and subsequent growth of infection germ tubes at the 3 h time interval, significant elongation of germ tubes was not seen until the 6 h interval (Fig. 4). The mean length of *B. cinerea* germ tubes on untreated geranium discs slightly increased between 3 and 6 h, and remained constant from 6 to 12 h. An increase in average germ tube length took place between the 12 and 24 incubation intervals and then rapidly increased to 55.25 μm in mean length at the 36 h interval. Minimal elongation took place on discs treated with polyoxin D zinc salt, azoxystrobin, and *Bacillus subtilis*. Only discs treated with chlorothalonil were significantly different from the untreated control. At 12 h, *B. subtilis* and polyoxin D

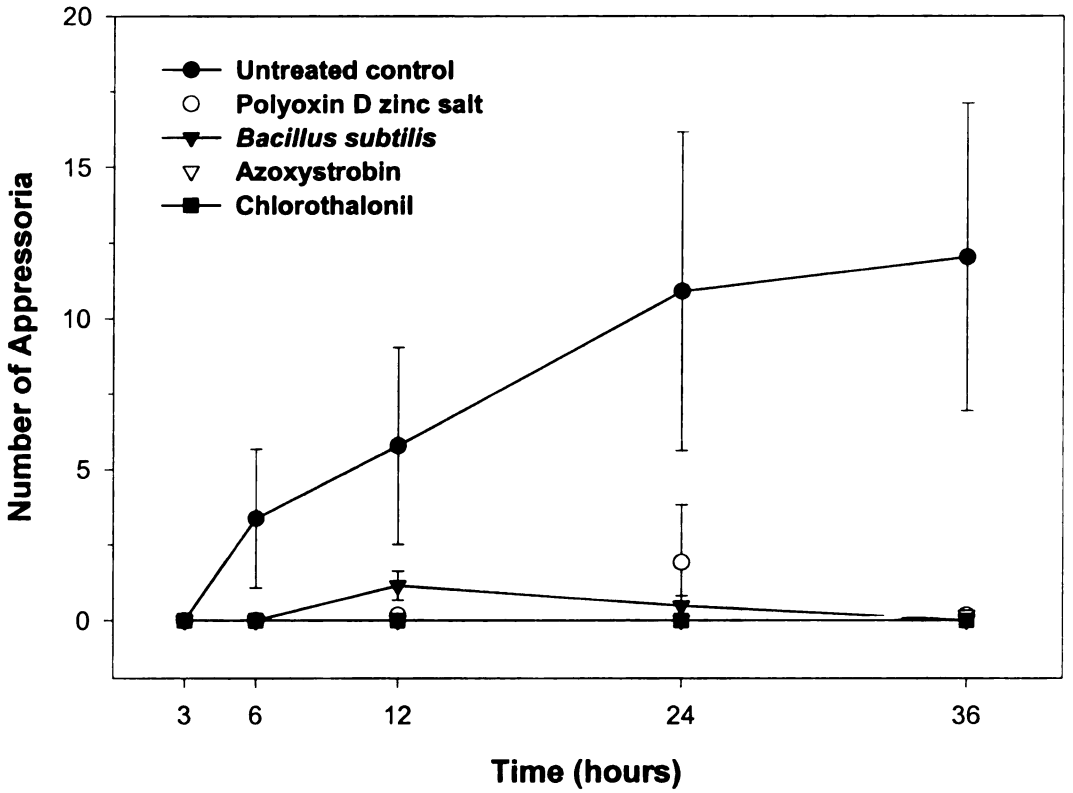

Figure 3. Mean number of *Botrytis cinerea* germ tubes per disc that developed appressoria at five incubation intervals and treated with one of four fungicides. Bars indicate standard errors of the means.

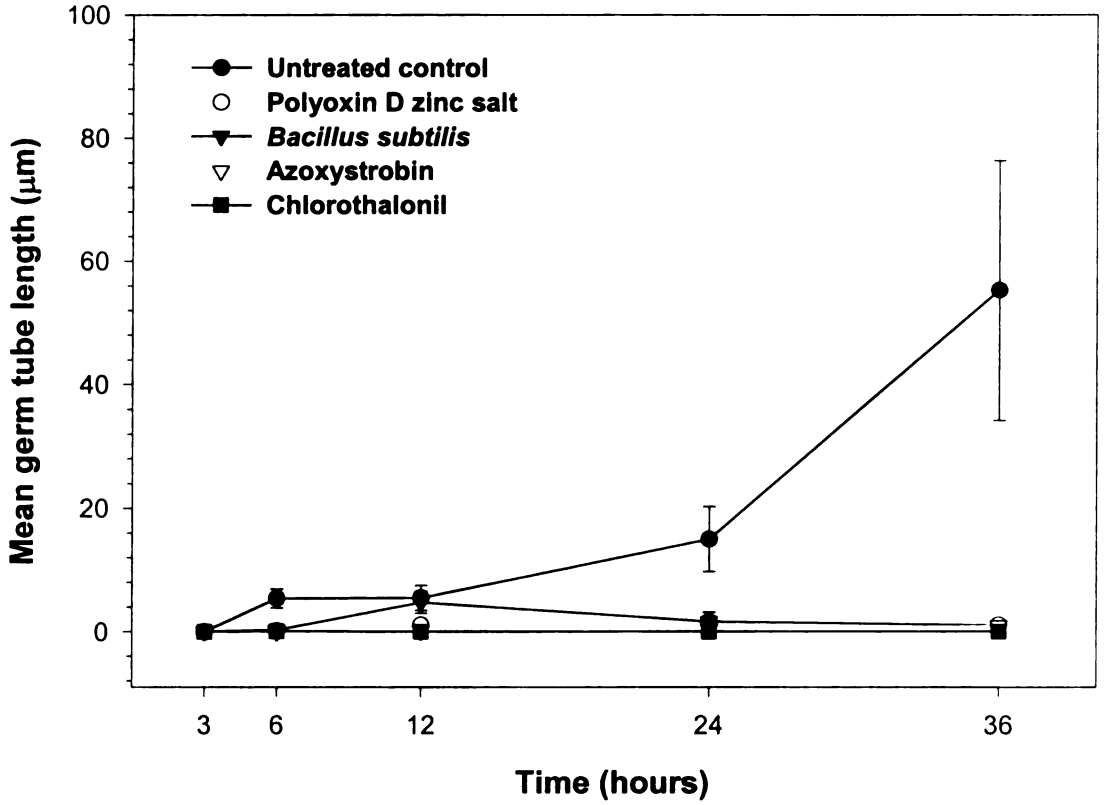

Figure 4. Mean germ tube length (µm) of *Botrytis cinerea* per disc recorded at five incubation intervals and treated with one of four fungicides. Bars indicate standard error of the means.

zinc salt increased in mean germ tube length; no treatments were significantly different from one another. The 24 h incubation interval saw a decline in mean length for discs treated with *B. subtilis*. Chlorothalonil and azoxystrobin were statistically better than the untreated control. At 36 h, mean length of germ tubes slightly rose for those treated with azoxystrobin. Discs treated with one of the four fungicide treatments were all significantly better with shorter germ tubes than the untreated control.

Fungicide greenhouse trial on 'Orbit White' geranium: In 2004, untreated plants showed symptoms of Botrytis blight and infection with 5.8 lesions per plant at the last rating date (Table 8). Treatment with chlorothalonil limited lesion development to 1.3 per plant, but was not significantly better than the untreated control. Plants treated with polyoxin D zinc salt, a biopesticide, and azoxystrobin, a reduced risk fungicide, had fewer lesions than the untreated control. However, the reduction was not significantly different. The two biocontrol agents, *Bacillus subtilis* and *Gliocladium catenulatum*, did not limit disease and plants treated with these products had significantly more lesions than the untreated control. According to AUDPC values, chlorothalonil, azoxystrobin, and polyoxin D zinc salt resulted in less disease than the control plants, but the differences were not significant (Table 8). However, all other treatments resulted in AUDPC values significantly greater than the untreated control. At the final rating date, untreated control plants received a disease severity value of 4.7. Chlorothalonil, polyoxin D zinc salt, and azoxystrobin had lower severity ratings which were statistically better than the untreated control. All other products had severity values > 4.7. Pyrimethanil and neem oil were statistically worse than the untreated control.

In 2005, heavy disease pressure was evident with 16.0 mean lesions per plant (Table 8). Chlorothalonil was significantly better than all other treatments in limiting disease at the last assessment date. Plants treated with Polyoxin D zinc salt, ascorbic acid, and azoxystrobin were not statistically better than the untreated control plants. The biocontrol agent, *Bacillus subtilis*, did not provide effective control and was significantly worse than the untreated control. AUDPC calculations show that chlorothalonil and

Table 8. The effect of foliar fungicides applied to 'Orbit White' geraniums on number of lesions/plant, area under the disease progress curve (AUDPC), and on disease severity caused by *Botrytis cinerea*. Values are according to last rating dates for trials (1 Feb, 2004 and 28 Apr, 2005, respectively).

Treatment and rate/100 gal; applied at 7-day intervals	Number of Lesions/plant		AUDPC		Severity[z]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Untreated control	5.8 ab[y]	16.0 bc	89 a	319 cd	4.7 b	5.5 cd
Daconil WeatherStik 6F 22 fl oz	1.3 a	4.2 a	36 a	50 a	1.8 a	2.7 a
Endorse 2.5WP 2.2 lb	3.0 a	13.3 b	55 a	199 bc	2.0 a	4.3 b
Heritage 50WG 8.0 oz	3.0 a	17.7 bcd	40 a	182 b	2.2 a	4.5 bc
Primastop 1%WP 100.0 oz	11.2 cd	[x]	181 bc	[x]	5.2 b	[x]
Rhapsody 1.34%AS 8 qt	10.7 cd	25.0 e	210 bc	485 e	5.2 b	6.5 de
Citrex 100L 12.9 fl oz	9.2 bc	14.8 b	180 b	244 bc	5.3 b	4.7 bc
Scala 400SC 1.6 pt[w]	14.7 de	22.5 cde	268 c	412 de	7.5 c	7.3 e
Triact 70EC 1 gal[w]	18.0 e	24.0 de	502 d	459 e	7.8 c	6.5 de
Messenger 3WDG 9.0 oz	[x]	22.8 de	[x]	419 de	[x]	5.8 d

[z] Severity was rated on a scale of 1 to 10; where 1=healthy, 2 to 8=varying degrees of blighting, and 10=dead.
[y] Column means followed by the same letter are not significantly different (Fisher's Protected LSD; P=0.05).
[x] Messenger 3WDG was substituted for Primastop 1%WP on second trial.
[w] Plants experienced phytotoxic response. Symptoms included brown spots, chlorosis, leaf curl, and some premature leaf abscission.

Table 9. Analysis of variance for average number of lesions/plant caused by *Botrytis cinerea* on 'Orbit White' geranium.

Source	DF	SS	MS	Prob>F
Total for Experiment 1	53	2299.43		
Total for Treatment	8	1556.93	194.62	<.0001
Error	45	742.50	16.50	
Total for Experiment 2	53	3614.15		
Total for Treatment	8	2133.48	266.69	<.0001
Error	45	1480.67	32.90	

Table 10. Analysis of variance for area under the diseased progress curve (AUDPC) caused by *Botrytis cinerea* on 'Orbit White' geranium.

Source	DF	SS	MS	Prob>F
Total for Experiment 1	53	1319855.83		
Total for Treatment	8	1059690.33	132461.28	<.0001
Error	45	260165.50	5781.46	
Total for Experiment 2	53	1595980.13		
Total for Treatment	8	1056202.26	132025.30	<.0001
Error	45	539777.88	11995.06	

Table 11. Analysis of variance for disease severity caused by *Botrytis cinerea* on 'Orbit White' geranium.

Source	DF	SS	MS	Prob>F
Total for Experiment 1	53	294.59		
Total for Treatment	8	242.26	30.28	<.0001
Error	45	52.33	1.16	
Total for Experiment 2	53	133.64		
Total for Treatment	8	97.48	12.19	<.0001
Error	45	36.17	0.80	

azoxystrobin provided adequate control of Botrytis blight with values that were statistically better than the untreated control. Polyoxin D zinc salt and ascorbic acid had relatively low AUDPC values, but did not significantly differ from the untreated control. AUDPC values of *Bacillus subtilis* and neem oil were significantly higher than that of the untreated control and several other treatments. Disease severity values for untreated plants were given a 5.5 at the final rating date. Plants treated with chlorothalonil or polyoxin D zinc salt received low disease values that were statistically better than the untreated control. Azoxystrobin and ascorbic acid received rating values that were less than the untreated control. However, their rating values were not significantly different. Pyrimethanil was significantly worse than the untreated control.

In both trials, phytotoxicity was observed on all plants treated with pyrimethanil and neem oil extract. Brown spots, chlorosis, leaf curl, and subsequent partial abscission of leaves was observed on treated foliage (Fig. 5). Dessicating leaf margins and an overall decline in plant vigor provided an opportunistic environment for *B. cinerea* to infect and cause a greater degree of disease progression than the untreated control.

Figure 5: Evaluation of biopesticides/reduced risk fungicides on 'Orbit White' geranium against *Botrytis cinerea*. (A) Untreated control; (B) Chlorothalonil; (C) Polyoxin D zinc salt; (D) Azoxystrobin. Phytotoxic response observed on: (E) Pyrimethanil and (F) neem oil extract. Images in this thesis are presented in color.

Fungicide greenhouse trial on American ginseng: In 2004, untreated plants had a mean of 5.0 foliar lesions per plant at the last rating date (Table 12). No treatment in this trial was statistically better than the untreated control. However, chlorothalonil and boscalid limited lesion development to fewer than 0.3 lesions/plant. The biocontrol agent (*Trichoderma harzianum*) and the reduced risk fungicides (fenhexamid, fluazinam, and polyoxin D zinc salt) resulted in $\leq$ 1.5 lesions/plant. Similar results were observed according to AUDPC values (Table 12), where chlorothalonil and the reduced risk products (fenhexamid, fluazinam, and polyoxin D zinc salt) had lower values than the untreated control plants. In terms of disease severity, fenhexamid, chlorothalonil, fluazinam, and boscalid received values that were all significantly better than the untreated control rating (5.8). All other treatments received disease severity ratings < 5.8, but were not statistically different.

In the 2005 trial, heavy disease pressure was observed on untreated plants (8.6 lesions/plant). Fluazinam, a reduced risk fungicide, differed significantly from the untreated control and prevented disease development. Plants treated with chlorothalonil, boscalid, fenhexamid, and polyoxin D zinc salt had statistically fewer lesions than the untreated control. AUDPC values for treatments reflect similar results. A disease severity rating of 6.4 was received by the untreated control group in this trial. All other treatments had disease severity ratings < 6.4, but only fenhexamid, chlorothalonil, fluazinam, polyoxin D zinc salt, boscalid, and *B. subtilis* were significantly better than the untreated control. No phytotoxicity was observed on any plants (Figure 6).

Table 12. The effect of foliar fungicides applied to American ginseng on number of lesions/plant, area under the disease progress curve (AUDPC), and on disease severity caused by *Botrytis cinerea*. Values are according to the last rating dates for trials (30 Jun, 2004 and 2 Jun, 2005, respectively).

Treatment and rate/100 gal; applied at 7-day intervals	Number of Lesions/plant		AUDPC		Severity[z]	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Untreated control	5.0 ab[y]	8.6 c	86 ab	115 cd	5.8 c	6.4 e
Elevate 50WDG 1.5 lb	0.7 ab	2.0 ab	4 a	25 ab	1.8 ab	2.8 abc
Bravo Weather Stik 6SC 2 pt	0.3 a	0.8 a	1 a	9 ab	1.3 a	2.0 ab
Omega 500F 1 pt	1.0 ab	0.0 a	6 a	0 a	1.8 a	1.4 a
Endorse 2.5WP 2.2 lb	1.5 ab	1.5 a	6 a	25 ab	2.5 abc	2.5 abc
Endura 70WG 6.8 oz	0.3 a	0.5 a	7 a	22 ab	2.2 ab	2.3 ab
PlantShield 1%WP 100 oz	0.6 ab	4.7 abc	10 a	81 bcd	3.3 abc	4.8 cde
Serenade 10%WP 10 lb	4.3 ab	2.6 ab	20 a	47 abc	5.2 bc	3.6 abcd
Actigard 50WDG 0.75 oz	5.4 ab	3.6 ab	36 a	53 abc	5.6 c	4.0 bcde
Topsin 4.5L 21.8 fl oz	2.0 ab	6.8 bc	52 ab	106 cd	3.2 abc	5.3 de
Messenger 3WDG 9 oz	7.0 b	8.5 c	140 b	139 d	3.6 abc	5.7 de

[z] Severity was rated on a scale of 1 to 10; where 1=healthy, 2 to 8=varying degrees of blighting, and 10=dead.
[y] Column means followed by the same letter are not significantly different (Fisher's Protected LSD; P=0.05)

Table 13. Analysis of variance for average number of lesions/plant caused by *Botrytis cinerea* on American ginseng.

Source	DF	SS	MS	Prob>F
Total for Experiment 1	59	1716.733		
Total for Treatment	10	306.08	30.61	0.4078
Error	49	1410.65	28.79	
Total for Experiment 2	54	1125.75		
Total for Treatment	10	499.26	49.93	0.0018
Error	44	626.48	14.24	

Table 14. Analysis of variance for area under the disease progress curve (AUDPC) caused by *Botrytis cinerea* on American ginseng.

Source	DF	SS	MS	Prob>F
Total for Experiment 1	61	392263.97		
Total for Treatment	10	102672.33	10267.23	0.0828
Error	51	289591.63	5678.28	
Total for Experiment 2	58	302385.23		
Total for Treatment	10	121972.74	12197.27	0.0029
Error	48	180412.49	3758.59	

Table 15. Analysis of variance for disease severity caused by *Botrytis cinerea* on American ginseng.

Source	DF	SS	MS	Prob>F
Total for Experiment 1	59	492.18		
Total for Treatment	10	133.32	13.33	0.0816
Error	49	358.87	7.32	
Total for Experiment 2	54	302.44		
Total for Treatment	10	138.92	13.89	0.0011
Error	44	163.52	3.72	

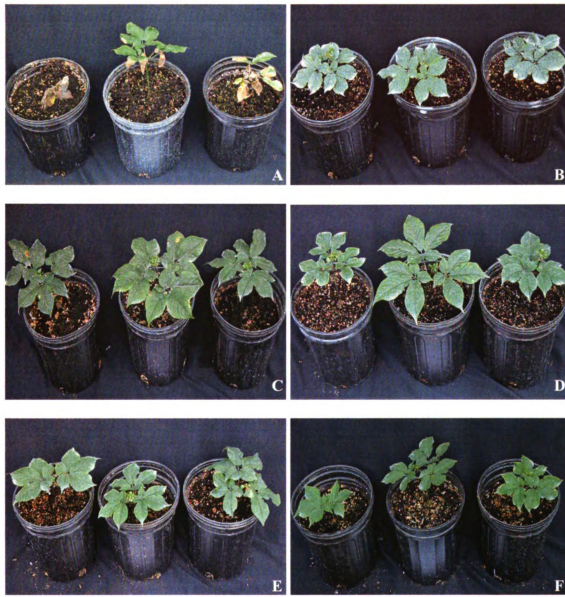

Figure 6: Evaluation of biopesticides/reduced risk fungicides on American ginseng against *Botrytis cinerea*. (A) Untreated control; (B) Chlorothalonil; (C) Polyoxin D zinc salt; (D) Fenhexamid; (E) Boscalid; (F) Fluazinam. Images in this thesis are presented in color.

Fungicide field trial on American ginseng: Substantial infection by *Alternaria panax* was observed on ginseng plants due to above average hot and humid conditions in Marathon County, WI in 2005.

Untreated plots had an average of 212.5 infected plants (approximate mean number of plants/plot = 235) on 1 Aug rating (Table 16). Plots treated with fluazinam and boscalid had the lowest mean of infected plants and had statistically fewer diseased plants than the untreated control. Polyoxin D zinc salt and chlorothalonil were also significantly different from untreated control plots. Fenhexamid and thiophanate methyl had fewer infected plants than the untreated control plots. However, the differences were not statistically different. Plants that became defoliated due to *A. panax* infection averaged 191.0 defoliated plants/control plot on the 1 Aug rating. Plots treated with fluazinam, boscalid, polyoxin D zinc salt, and chlorothalonil had the lowest means, respectively, and were significantly better than the untreated control. Fenhexamid, thiophanate methyl, and acibenzolar treated blocks had fewer defoliated plants, but were not significantly different from the untreated. Fluazinam boscalid, polyoxin D zinc salt, chlorothalonil, and fenhexamid received the lowest disease severity ratings, respectively, which were statistically lower than the untreated control.

Disease pressure had increased at the 10 Aug rating date. Untreated blocks had an average of 216.0 infected plants/plot (Table 16). Plots treated with boscalid and fluazinam had the lowest number of infected plants and were significantly better than the untreated control. No other treatments were statistically better. Of those infected with *A. panax*, plants that became defoliated averaged 215.8 in the control plots. Those treated with fluazinam, boscalid, polyoxin D zinc salt, and chlorothalonil had significantly less

Table 16. The effect of foliar fungicides on number of American ginseng plants infected, number defoliated, and disease severity caused by *Alternaria panax*.

Treatment and rate/A[z]	Number of plants infected with *Alternaria* per 10 ft[y]		Number of plants defoliated per 10 ft		Severity per 10'	
	Aug 1	Aug 10	Aug 1	Aug 10	Aug 1	Aug 10
Untreated control	212.5[x] c[w]	216.0 bc	191.0 de	215.8 c	9.3[v] e	10.0 c
Omega 500F 1 pt	13.5 a	82.8 a	2.0 a	6.8 a	1.8 a	3.0 a
Endura 70WG 6.8 oz	27.5 a	78.3 a	23.3 ab	55.5 a	2.5 ab	4.0 ab
Endorse 2.5WP 2.2 lb	125.3 b	226.8 c	34.0 abc	61.3 a	4.8 bc	6.3 b
Bravo Weather Stik 6SC 2 pt	113.0 b	135.3 ab	70.8 abc	96.3 ab	5.0 c	5.5 b
Elevate 50WDG 1.5 lb	192.8 bc	219.8 bc	114.3 bcd	213.5 c	7.0 cd	9.5 c
Serenade 10%WP 10.0 lb	251.5 c	251.5 c	233.0 e	249.8 c	9.5 e	9.8 c
Actigard 50WDG 0.75 oz	222.3 c	222.3 bc	135.7 cde	221.3 c	8.3 de	9.7 c
PlantShield 1%WP 100.0 oz	228.3 c	228.3 c	206.8 de	221.3 c	9.5 e	9.5 c
Topsin 4.5L 21.8 fl oz	193.8 bc	203.8 bc	117.0 bcd	194.3 bc	7.5 de	9.3 c
Messenger 3WDG 9 oz	261.0 c	261.0 c	243.3 e	258.0 c	9.0 de	9.5 c

[z] The treatments were applied on seven-day intervals from 1 Jun through 4 Aug.
[y] Includes plants with visible lesions and partial/complete defoliation from *A. panax* infection.
[x] Approximate mean number of plants per 10' block = 235.
[w] Column means followed by the same letter are not significantly different (Fisher's Protected LSD; P=0.05).
[v] Severity was a rated on a scale of 1 to 10; where 1=healthy, 2 to 8=varying degrees of blighting and defoliation, and 10=dead.

Table 17. Analysis of variance for number of American ginseng plants infected by *Alternaria panax.*

Source	DF	SS	MS	F Value	Prob>F
Total for 1 Aug	42	389106.42			
Model	13	295932.81	22764.06	7.09	< 0.0001
Error	29	93173.62	3212.88		
Total for 10 Aug	42	268836.60			
Model	13	165815.35	12755.03	3.59	0.0020
Error	29	103021.25	3552.46		

Table 18. Analysis of variance for number of American ginseng plants defoliated from infection by *Alternaria panax.*

Source	DF	SS	MS	F Value	Prob>F
Total for 1 Aug	41	468065.90			
Model	13	312549.40	24042.26	4.33	0.0006
Error	28	155516.51	5554.16		
Total for 10 Aug	42	456615.07			
Model	13	321306.93	24715.92	5.30	< 0.0001
Error	29	135308.14	4665.80		

Table 19. Analysis of variance for disease severity on American ginseng plants caused by *Alternaria panax.*

Source	DF	SS	MS	F Value	Prob>F
Total for 1 Aug	42	409.07			
Model	13	335.34	25.87	10.32	< 0.0001
Error	29	72.73	2.51		
Total for 10 Aug	42	361.67			
Model	13	278.84	21.45	7.51	< 0.0001
Error	29	82.83	2.86		

Table 20. The effect of foliar fungicides against *Alternaria panax* on yield of American ginseng roots.

Treatment and rate/A[z]	Root yield (lbs)	
	Fresh	Dried[y]
Untreated control	4.1 c[x]	0.9 e
Omega 500F 1 pt	10.8 a	2.3 ab
Endura 70WG 6.8 oz	10.4 a	2.6 a
Endorse 2.5WP 2.2 lb	7.0 b	2.2 abc
Bravo Weather Stik 6SC 2 pt	6.0 bc	1.4 bcde
Elevate 50WDG 1.5 lb	4.8 bc	1.1 de
Serenade 10%WP 10.0 lb	4.9 bc	1.1 de
Actigard 50WDG 0.75 oz	5.6 bc	1.3 bcde
PlantShield 1%WP 100.0 oz	5.3 bc	1.2 cde
Topsin 4.5L 21.8 fl oz	7.2 b	2.0 abcd
Messenger 3WDG 9 oz	6.3 bc	1.4 bcde

[z] The treatments were applied on seven-day intervals from 1 Jun through 4 Aug.
[y] Dried root yield taken two weeks following harvest.
[x] Column means followed by the same letter are not significantly different (Fisher's Protected LSD; P=0.05).

Table 21. Analysis of variance for American ginseng root yield infected by *Alternaria panax*.

Source	DF	SS	MS	F Value	Prob>F
Total for fresh yield	32	265.76			
Model	12	205.47	17.12	5.68	0.0003
Error	20	60.29	3.01		
Total for dried yield	32	19.71			
Model	12	11.78	0.98	2.48	0.0353
Error	20	7.93	0.40		

defoliation than the untreated control at the final rating date. Fenhexamid and thiophanate methyl had fewer plants defoliated than the untreated control, but did not differ statistically. A disease severity rating of 10.0 was given to the untreated control plots at the final rating date. Again, fluazinam, boscalid, polyoxin D zinc salt, and chlorothalonil had the lowest severity ratings which were significantly less than the untreated control. No other treatments were statistically different from the untreated control at the final rating date.

Root yield of American ginseng was assessed after the final rating date. Fresh weight was taken at time of harvest, and dried weight was recorded two weeks post-harvest (Table 20). Fresh and dried root yield on the untreated control plants were the lowest of all treatment plots, with 4.05 and 0.9 lb, respectively. Root yield for plants treated with fluazinam, boscalid, polyoxin D zinc salt, and thiophanate methyl had the highest recorded fresh and dried weights and were statistically higher than the untreated control plots. No other treatment plots yielded significant differences from the untreated control.

DISCUSSION

In addition to cultural methods, fungicides are a primary method for controlling foliar blights caused by *Botrytis cinerea*, and are necessary to minimize infection and prevent an epidemic during production. Chlorothalonil is the current industry standard and continues to show strong efficacy in controlling foliar blights by using a multi-site mode of action. However, this active ingredient is considered a B2 carcinogen and poses high toxicity risks. Due to the increasing desire to register less toxic fungicides for specialty crop use, other products offering different modes of action may show similar efficacy to this chemical standard.

Azoxystrobin is a reduced risk fungicide currently registered for use on turf grass and vegetable crops to control numerous pathogens including *B. cinerea*. It's efficacy on 'Orbit White' geraniums in the greenhouse trial in 2004 was better than most products evaluated aside from chlorothalonil. Though not statistically different from the control in terms of lesion counts per plant, the active ingredient slowed disease progession and significantly minimized disease severity compared to untreated plants. Similar efficacy by azoxystrobin has been demonstrated in recent years on other geranium cultivars (Hausbeck *et al.*, 2000a; Hausbeck *et al.*, 2000b), as well as poinsettia (Benson & Parker, 1999), by consistently limiting leaf lesions and subsequent sporulation caused by Botrytis blight. In controlling *B. cinerea* outbreaks, geranium growers currently have a reduced risk product to incorporate into their IPM programs.

Polyoxin D zinc salt is a biopesticide that was originally registered for use on multiple turf grass pathogens. Its control against *B. cinerea* on 'Orbit White' geraniums in 2005 limited number of lesions to even less than azoxystrobin and significantly

minimized disease severity in 2004 and 2005 compared to the untreated plants. Another study showed similar effectiveness in controlling Botrytis blight on 'Red II' geraniums in terms of minimizing diseased foliage and leaves with sporulation from *B. cinerea* (Hausbeck *et al.*, 2002). In addition to geranium, American ginseng is a host plant for *B. cinerea* infection and foliar blighting is a common issue for ginseng growers in Wisconsin and Michigan (Hausbeck, 2004). Polyoxin D zinc salt has recently become registered for use on these specialty crops. The greenhouse trial conducted here shows that this biopesticide offers good efficacy in controlling foliar blighting by significantly limiting number of lesions, disease progression, and disease severity caused by *B. cinerea* in 2005. Similar results were observed in a field experiment using identical formulations (Hausbeck & Harlan, 2004a). In addition to this product, other reduced risk fungicides were tested for efficacy against *B. cinerea* on ginseng due to the lack of numerous products registered for use on this specialty crop. The greenhouse trial in 2005 shows significant control of disease severity, progression and lesion development by boscalid, fluazinam, and fenhexamid. Similarly, significant results were observed in controlling Botrytis blight when these three products were tested by Hausbeck & Harlan (2004c).

In 2005, a field study on American ginseng was carried out to test the efficacy of these biopesticide/reduced risk products in the presence of another foliar pathogen: *Alternaria panax*. As a result of the unseasonably hot temperatures, Alternaria blight caused heavy disease pressure on the untreated plants. Visible efficacy of the tested products was dramatic and differences were seen as early as July. By the last rating date in August, polyoxin D zinc salt, fluazinam, and boscalid provided significant control against defoliation and disease severity caused by *A. panax*. Fluazinam and boscalid

showed better efficacy than chlorothalonil in this experiment, and limited foliar infection significantly compared to the untreated control. Moreover, harvested ginseng roots from the fluazinam, boscalid, and polyoxin D zinc salt treatments provided significantly better yields of the dried, marketable product than the untreated. Identical differences were observed on ginseng plants treated with fluazinam and boscalid against Alternaria blight in the previous growing season (Hausbeck & Harlan, 2004b).

A simultaneous *in vitro* assay was carried out on geranium foliage to evaluate the various modes of action on the infection process of *B. cinerea*, as has been done in the past (Buck, 2002). Overall, conidial germination, appressorial development, and elongation of germ tubes were less than what was observed in the preliminary assay trials. It should be noted that high variability such as this may be contingent on the presence or absence of exogenous nutrients (Clark & Lorbeer, 1976), attributed to a latent infection by *B. cinerea* (Coley-Smith *et al.*, 1980), or affected by the selection of *B. cinerea* isolates used (Buck, 2004).

Since chlorothalonil is the current standard, its efficacy was included for comparisons to the other products evaluated in this leaf disc assay. Germination was limited by the chlorothalonil treatment, as was expected. When absorbed into the fungal body, this chemical disrupts energy production by inhibiting enzymes at several sites (Syngenta Crop Protection, 2005). Another multi-site fungicide is a biocontrol agent that uses a patented strain (QST 713) of *Bacillus subtilis*. The bacterium contains lipopeptides that puncture pathogen cell membranes, causing conidia and mycelia to collapse and die (Agraquest fact sheet, 2005). In this experiment, *B. subtilis* did not completely limit (< 3%) germination or appressorial development between the 6 and 12 h

incubation interval. However, after 12 h, there was a gradual decline in germination and appressorial development until the last incubation interval. This delay may be indicative of the antimicrobial activity taking effect and becoming a limiting factor to fungal development. This treatment was inconsistent in limiting disease by *B. cinerea* and *A. panax* in the greenhouse and field trials.

The biopesticide polyoxin D zinc salt, a by-product of a bacterium found in soil, was also evaluated. The fungicide has an active ingredient that inhibits the development of chitin in the cell walls of the fungus (Environmental Protection Agency, 2001). A fungus lacking chitin is unable to continue growing and infecting plant cells. This experiment shows that polyoxin D zinc salt limited conidial germination to < 2.0 % except at the 24 h interval, where germination reached 3.0 %. Overall, this mode of action was effective in limiting germination to < 4.0 %, limiting appressorial development to < 3.0, and preventing germ tube elongation from reaching > 5.0 μm over all incubation intervals.

Azoxystrobin was tested as the reduced risk fungicide. This product prevents energy production in the fungus by inhibiting respiration of the mitochondria that's found inside the nucleus of fungal cells (Syngenta Crop Protection, 2005). In this experiment, azoxystrobin limited infection by < 1.0 % for all three parameters under the five incubation intervals observed. This product provided similar efficacy to chlorothalonil but has a lower toxicity rating and is non-carcinogenic. Moreover, the three non-carcinogenic products evaluated here provided significant control at various time intervals observed.

Future use of these reduced risk and biopesticide products on geranium cultivars and American ginseng could provide growers with effective control strategies in managing epidemics caused by *B. cinerea* and *A. panax* without causing toxicity to non-target organisms and the applicators.

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APPENDIX A

REDUCED RISK AND BIOPESTICIDE STUDIES IN 2004

GERANIUM (*Pelargonium xdomesticum* 'Emperor')
Botrytis blight; *Botrytis cinerea*

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Evaluation of reduced risk fungicides and biopesticides for control of Botrytis blight of geranium, 2004.

Geranium cuttings were taken from stock plants on 27 Feb and placed into Oasis strips for rooting. Rooted cuttings were transplanted into 4-in. plastic pots containing a soilless medium (Baccto Professional Planting Mix, Michigan Peat Company, Houston, TX) on 19 Mar. Plants were fertilized three times weekly with 250 ppm Peter's 20-20-20 liquid feed (The Scotts Company, Marysville, OH). Six replicates per treatment were arranged in a completely randomized design. *Botrytis cinerea* inoculum was grown on potato dextrose agar for three weeks under fluorescent light. Plates were flooded with distilled water and gently agitated with a plastic-bristled brush to dislodge conidia. Liquid from the plates was strained through cheesecloth, and adjusted to 2.9×10^7 conidia/fl oz. Fungicide treatments were applied with a hand-pressurized sprayer to runoff. Plants were placed into clear plastic bags supported by wire meshing immediately after treatment. Four hours after fungicide treatment, plants were inoculated with the *B. cinerea* conidial suspension using a janitorial spray bottle to runoff. The plastic bags were immediately sealed and reopened only for subsequent treatment and inoculum application. Treatments were applied on 24, 31 Aug and 7, 14 Sep. After each fungicide treatment, plants were re-inoculated four hours later using the method previously described. Lesions were counted and disease severity ratings were taken on 21 Sep.

Disease pressure was moderate in this trial. All products significantly limited disease development compared to the inoculated control. Significant differences among treatments were not observed. Several products limited the number of lesions and disease severity to 3.0 or less and included the following: Endorse 2.5WP, Rhapsody 1.34AS, Daconil Weather Stik 6F, and Decree 50WDG.

Table 22. The effect of foliar fungicides applied to 'Emperor' geraniums on number of lesions, number of lesions with sporulation, and on disease severity caused by *Botrytis cinerea*. Values are according to last rating date for trial.

Treatment and rate/100 gal; applied at 7-day intervals	Number of Lesions	Number of Sporulating Lesions	Disease Severity[z]
Untreated control	21.5 b[y]	19.0 b	6.3 b
Endorse 2.5WP 2.2 lb	1.5 a	1.2 a	1.7 a
Daconil Weather Stik 6F 22 fl oz	2.2 a	1.7 a	1.7 a
Decree 50WDG 24 oz	2.2 a	2.0 a	2.0 a
Insignia 20WDG 8 oz	3.8 a	2.5 a	2.3 a
Rhapsody 1.34%AS 8 qt	3.0 a	2.3 a	2.2 a
Endorse 2.5WP 2.2 lb alt.[x] Rhapsody 1.34%AS 8 qt	4.8 a	3.2 a	2.7 a
Heritage 50WG 8 oz	5.3 a	3.3 a	2.7 a
Endorse 2.5WP 2.2 lb alt. Heritage 50WG 8 oz	6.8 a	3.5 a	3.0 a
BAS 510 70WG 4.5 oz	7.0 a	4.7 a	3.0 a
Endorse 2.5WP 2.2 lb alt. BAS 510 70WG 4.5 oz	7.5 a	4.8 a	3.2 a

[z] Severity was rated on a scale of 1 to 10; where 1=healthy, 2 to 8=varying degrees of blighting, and 10=dead.
[y] Column means followed by the same letter are not significantly different (Tukey's Studentized Range Test, $P \leq 0.5$).
[x] Alt. = fungicides were alternated weekly.