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ALTERNARIA PANAX CONIDIAL CONCENTRATIONS
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TOM-CAST FOR MANAGEMENT OF ALTERNARIA BLIGHT
ON AMERICAN GINSENG

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EPIDEMIOLOGY OF *ALTERNARIA PANAX* ON AMERICAN GINSENG AND EVALUATION OF A DISEASE FORECASTER

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ABSTRACT

EPIDEMIOLOGY OF *ALTERNARIA PANAX* ON AMERICAN GINSENG AND EVALUATION OF A DISEASE FORECASTER

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Alternaria panax Whetzel, an annual problem for ginseng growers, incites blighting of the foliage and drupes of cultivated American ginseng (*Panax quinquefolium* Linnaeus). An epidemiological study investigated the influence of air temperature, duration of leaf wetness, rain, and relative humidity on atmospheric conidial concentrations of *A. panax*. Commercial ginseng gardens were monitored in central Wisconsin from mid-May to September for four growing seasons. Hourly concentrations of airborne *A. panax* conidia were enumerated using a Burkard volumetric spore sampler. Fungicides were not applied. Numbers of infected plants were assessed in predetermined portions of the ginseng garden. Disease pressure in the plots was severe, and significant concentrations of *A. panax* conidia were detected in the atmosphere from late May through September yearly. Conidial concentrations were greatest following rain events and periods of decreasing relative humidity.

Disease forecasting systems are developed based on weather variables in conjunction with the epidemiology of the pathogen. TOM-CAST, originally developed to predict leaf blight caused by *A. solani* on tomato, was evaluated for management of *A. panax* in commercial gardens. For three years, fungicide sprays initiated by TOM-CAST

(using 10 and 15 disease severity value thresholds) were compared with sprays applied at 7- and 10-day intervals. Three fungicide programs were evaluated, and included chlorothalonil alone or in alternation with pyraclostrobin, and pyraclostrobin alternated with copper hydroxide. Although select TOM-CAST treatment programs were comparable to the 7-day schedule in limiting foliar disease, only the 7-day applications adequately protected seed yield and quality.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LITERATURE REVIEW:

CHAPTER ONE: Factors influencing atmospheric conidial concentrations of *Alternaria panax* in cultivated American ginseng gardens

LIST OF FIGURES

LITERATURE REVIEW

American ginseng (*Panax quinquefolium*)

Ginseng is a member of the family *Araliaceae* and placed in the genera *Panax.* Of the several species included in the *Panax* genus, Oriental ginseng (*Panax ginseng,* Meyer) and North American ginseng (*Panax quinquefolium,* Linnaeus) are most important for trade. Oriental ginseng refers to plants native to North Korea and China, while American ginseng is native to Canada and the eastern half of the United States. In the Midwest, the cultivation season for ginseng is late April through late October (25, 47, 48).

Ginseng is an erect perennial plant with a canopy that can reach 30 to 40 cm in height (49). The canopy of a mature ginseng plant consists of three or four compound leaves arranged in a whorl. Each leaf contains five ovate, saw-toothed leaflets with the upper three leaflets being larger than the lower two. An immature plant has one or two leaves and adds an additional leaf every year until maturity. Generally, first year seedlings have one leaf with three leaflets.

Ginseng has a single umbelliferous inflorescence that forms at the terminus of the stem (54). The umbel has <50 small, white, perfect flowers (54) that are self-fertile. Each flower is capable of producing a green kidney-shaped drupe after fertilization. The drupes ripen and turn crimson red in late summer (48). Each drupe contains two seeds that are approximately 0.5 cm in diameter and slightly longer than they are wide. Stratification of the seed is required and is generally 18 to 22 months in duration (18, 50). During stratification, the endocarp of the seed remains tightly closed, opening slightly along its suture a month or so before germination (54). The series of warm and cold

temperatures over the 18- to 22- month stratification period is necessary for the embryo to grow and mature (24, 51, 54).

Ginseng has a fibrous taproot (2). The outer skin of the root is generally light to golden brown and the interior is white to light yellow in color. The root is fleshy and wrinkled with a rhizome at the crown. It is on this rhizome that the bud for the next year's shoot develops during the spring with the bud expanding so that the stem, rather than the leaves, is seen first (54). During the winter months the bud is dormant (24). Root age can be determined by counting the rings (scars from winter dormancy and annual abscission of the foliage) on the crown of the root. Root length at maturity can range from 7 to 13 cm (2).

Cultivation and range for American ginseng

American ginseng is an understory plant, requiring only 30% of full sunlight (49), and grows within USDA plant hardiness zones 3 to 9. In northeastern North America, it is most often found growing under sugar maple while in the Southeast it is often found under tulip poplar or black walnut. In the Midwest it occurs beneath several different hardwood species including oak (3).

Ginseng can be grown as a woods- or field-cultivated crop (Figure 1). Woods-cultivated ginseng is grown in a hardwood forest with the area cleared to remove existing understory plants and leaf litter that would naturally compete with or hinder ginseng growth. Cultivated ginseng is grown under woven panels providing 80% shade that are suspended via 2.4- to 3.0- m posts in a cleared field. Under both canopy types, ginseng is sown into plant beds that are generally 1.5 m wide and 2.7- to 3.7- m high (26). Each

cultivation method requires intensive maintenance including mulch such as straw or hay, weeding, and fungicide use.

Figure 1. American ginseng cultivated on raised beds (A) under a wood (B) and shade cloth (C and D) canopies. (Images are presented in color).

Ginseng grows best in loamy, light textured, well-drained soil with a pH of 5.5 to 6.5 (48). The crop also requires 0.5 to 1.52 m of precipitation annually and a minimum cold period (<9° C) of sixty days to break dormancy each spring (48). Following seed planting, 15 cm of straw mulch is generally applied to ginseng beds. In winter, straw mulch can prevent temperatures from dropping below the freezing point for roots (around -10° C) and in summer keeps soil temperatures 5 to 10° C below that of an open grassed area (61). Mulch also eliminates the need for irrigation as it prevents excessive moisture loss from the soil throughout the season (61). In addition to straw, ginseng growers may use saw dust as mulch (26).

American ginseng has been cultivated in Wisconsin for more than 100 years. Currently, more than 95% of the cultivated commercial ginseng in the U.S. is grown in Wisconsin (1). Canada is also a significant producer of ginseng with approximately 8,000 acres grown in Alberta, British Columbia, and Ontario (2). Several states including West Virginia (62), North Carolina (18), New York (22), Washington (11), and Oregon (55) have small but thriving ginseng industries. Production for each of these areas is primarily under shade cloth. Most of Michigan's ginseng production is located in the Upper Peninsula. Michigan has approximately 135 acres of woods-grown ginseng and 15 acres of field-cultivated ginseng (25).

Cultivation costs and profits

Cultivation of ginseng requires that growers make a large initial investment in mechanized equipment, shade materials, land, labor and crop stock (44). Approximate input costs for a new half-acre woods-cultivated garden is $24,135 (44). Approximate

input costs for a new half-acre garden under shade cloth is $33,500 including seed and labor costs (44). Field cultivated ginseng is currently valued at $20/lb (26). Current prices for woods-cultivated ginseng range between $80 and $320/lb. In addition to root profits, ginseng seeds are also a source of revenue for ginseng growers. Current market prices for stratified seed are $55/lb. With an average seed yield of 336 kg/ha, each ha represents a value of $18,537 (26).

Pathogens affecting ginseng

Ginseng is susceptible to a number of pathogens that can cause foliar blights and root rots. Some of the most devastating foliar pathogens include *Alternaria panax* (Whetzel) and *Botrytis cinerea* (Pers: Fr.). Important root rot pathogens include *Fusarium* spp. (Booth), *Cylindrocarpon destructans* (Zinssmeister) Scholten, and *Phytophthora cactorum* (Leb. et Cohn) Schroet (26). American ginseng seeds are also susceptible to pathogens. Commercial seeds from Wisconsin and Canada have been found to be contaminated with spores of *Alternaria, Cylindrocarpon, Phytophthora,* and *Fusarium* spp. (27, 28), all of which are capable of reducing plant stands. *Mucor* and *Trichoderma* spp. have also been recovered from stratified ginseng seeds in the U.S. (67).

P. cactorum is favored by cool and wet weather (16, 19, 48, 53). Infected roots develop a brown discoloration (5) and a loss of turgidity gives the tissue a spongy texture. Foliar infections of *P. cactorum* may also occur and result in dark green, water-soaked lesions on the leaves (19). Disease can progress rapidly down the main stem (21, 24), resulting in plant collapse and death (25). If not controlled, this pathogen may destroy an entire ginseng garden in just a few weeks (2, 16, 25).

Rusty root of ginseng is a complex of pathogens and includes *Cylindrocarpon destructans, Rhexocercosporidium panacis* sp. *nov* (Reeleder) and *Fusarium* spp. *C. destructans* can cause damping-off, crown and root rot of ginseng (26, 59). Infection normally starts near the root tip, progressing upward, and often destroying the entire root. A classic symptom of *C. destructans* root rot is "disappearing root," whereby the root tissue disintegrates due to rot. Another symptom of *C. destructans* rot is a reddish to dark brown canker on the root. These cankers are often dry and cracked (29). *R. panacis* lesions are superficial, but the outer layer of root tissue ruptures and sloughs off, giving roots a scabbed appearance (59). Infection by *Fusarium* spp. can cause stem and crown infections, although root rots are most common, resulting in red streaking of the tissue. When *Fusarium* root rot is advanced, the foliage wilts and becomes reddened.

Botrytis cinerea is extremely common and can survive on virtually any dead plant material found in a ginseng garden (10). Traditionally, ginseng growers considered *B. cinerea* a pathogen of flowers and fruits only, resulting in reduced seed yields (26). Frequently, berries will not develop if the flower has been infected. Green berries turn brown after infection and a gray fuzzy mold may appear on mature or immature berries.

Typical foliar symptoms include water-soaked, tan lesions that often have concentric rings, giving them the appearance of a bull's eye. Lesions often start at the leaf tips and proceed back along the leaf midrib (11, 25). *B. cinerea* can infect stems late in the growing season and may form small sclerotia on affected tissues that allow the fungus to overwinter (26).

Alternaria panax

The most common foliar disease of ginseng is Alternaria blight incited by *Alternaria panax* Whetzel (43). This disease is a yearly problem for ginseng growers in Wisconsin (43) and Michigan (26). Alternaria blight also occurs in other ginseng-growing regions including Alberta, Canada (13,14), West Virginia (62), North Carolina (19), Oregon and Washington (55).

Typical foliar symptoms include necrotic lesions with dark brown margins and yellow-green halos (43). Brown lesions often develop on the stem just above the soil line and cause girdling (9). Infected drupes may develop a water-soaked appearance followed by the development of gray mycelium and pathogen sporulation. Infection of the root by *A. panax* is rare (43); however, root weight can be reduced when blighting of the leaves and stem causes premature defoliation.

Figure 2. Alternaria blight lesions caused by *Alternaria panax* on American ginseng stem (A), drupes (B and C), progression of foliar infection (D – F) and observation of foliar lesions in contact with straw mulch (G). (Images presented are in color).

Alternaria infections are especially devastating under favorable environmental conditions with blight development and conidial production occurring within 5 to7 days (64). If not managed, Alternaria blight can reach epidemic proportions within a month after plant emergence and destroy all of the foliage. Young plantings are especially susceptible to infection and entire gardens can be destroyed. For maturing crops, loss of foliage and repeated outbreaks in consecutive years could result in reduced root yields (9). Wisconsin growers have experienced yield loss 75 to 100% when Alternaria blight is uncontrolled (20).

A. panax historically was known to only infect members of the *Araliaceae*, specifically ginseng (15, 56), however infections of the Umbrella Tree *(Schefflera actinophylla,* and False Aralia *(Schefflera elegantissima)* have been reported in Hawaii (64). On ginseng, Alternaria blight is characterized by target spot lesions with dark brown margins and yellow halos. As the disease progresses, a dark mat of conidia develops within the lesions. Stem infection results in the production of elongated, reddish to dark brown lesions (9, 56). The potential for repeated, widespread and devastating epidemics is great because large numbers of conidia are produced on the surface of diseased leaves and stems. Outbreaks of *A. panax* in one season may greatly increase the potential for epidemics in subsequent seasons, since the fungus overwinters (26). *A. panax* can survive as conidia or as mycelium in infected plant residue, on straw mulch or in and on seeds (26, 34).

In the spring, overwintered conidia can spread to the newly emerging healthy plants by rain or splashing water and begin the disease cycle for the new growing season (26). In addition, overwintering conidia can be a source of stem infection as plants

emerge through the infested mulch in the spring (26). Currently, there are no practical methods to reduce overwintering inoculum levels on the mulch or on infected crop residue.

A. panax is noted to have a wide range of conidial morphology (15). Conidia are obclavate, and typically 150 to 160 by 12 to 20 μm (9) with 1 to 2 vertical and 9 to 11 transverse septa (52). *A. panax* conidia germinate within 1 to 2 h of contact with host tissue with 70% relative humidity (57) by producing several germ tubes. The formation of bulbous appressoria on the end of germ tubes was found to occur 6 h after conidial-host tissue contact (57). Conidia may penetrate through the leaf surface either over epidermal cells or at sites of cell junctions and indirectly through stomatal openings (57). Conidiophores are geniculate (9).

Conidia are introduced into gardens via air currents, resulting in spread of *A. panax* from a diseased garden to nearby healthy gardens. *Alternaria* can also be moved into gardens on equipment (23). High humidity and warm weather can influence disease severity and under favorable conditions, blight development and conidial production can occur in 5 to 7 days (64). Optimum conidial production occurs with temperatures ranging from 18 to 25° C (9, 64). Optimum mycelial growth occurs at 24 to 27° C (15, 17).

Many *Alternaria* spp. produce host-selective toxins that diffuse ahead of the fungus. These toxins are usually associated with the yellow halo that fades into the healthy tissue surrounding the lesion (34). When an *A. panax* isolate recovered from an Ontario ginseng garden was used to inoculate various hosts (members of *Cruciferae*, *Fabaceae*, *Solanaceae*, *Graminae* and *Araliaceae*); water-soaked, chlorotic lesions

followed by necrosis were only produced within *Araliaceae*, and only on ginseng (56). Analysis of the germination fluid showed that it contained a phytotoxic, 35- kDa monomer (56, 57). This monomer, named AP-toxin, was later shown to be produced by several *Alternaria* isolates from Ontario, British Columbia and Wisconsin (57).

The production of the AP-toxin by *A. panax*, (like Deoxyradicin produced by *A. helianthi* and AB-toxin produced by *A. brassicicola and A. brassicae*) is likely induced by host-related factors during the early host-pathogen interactions (40, 56). Despite the germination of conidia and detection of germination fluid on other hosts, AP-toxin is only produced by *A. panax* on ginseng. Temperature can affect the activity of the AP-toxin. If the toxin is held at 45° C for 15 minutes, activity is significantly reduced and is inactivated at 80° C. The toxin activity at 4, 22, 30 and 37° C was similar, and when tested for activity at the respective temperatures, lesions diameters ranged between 15 and 20 mm (56).

Disease Management of *Alternaria*

Alternaria blight is currently managed through cultural methods and fungicide use. Cultural practices such as avoiding dense plantings and garden sites with poor air circulation may help limit disease. The destruction and removal of infected plant debris and the replacement of straw mulch is also recommended to reduce subsequent epidemics (19). Unfortunately, these strategies are not practical and would not preclude the reintroduction of inoculum via air currents.

In the interest of adapting Integrated Pest Management (IPM) strategies for managing pests on ginseng, previous researchers have investigated use of biocontrols as alternatives to traditional fungicides. *Burkholderia cepacia* AMMD (*Pseudomonas*

cepacia strain AMMD) was found to effectively inhibit *Alternaria* leaf and stem blight under growth chamber conditions. However, the agent was not adequate in reducing disease under field conditions due to poor survival on leaf surfaces (32, 41, 42).

When disease pressure is high, fungicides are necessary to maintain plant health. Crop protection can be difficult as plants emerge through infested mulch. It has been estimated that Alternaria blight would infect 80 to 100% of ginseng plants without effective chemical control (42). Currently, azoxystrobin and pyraclostrobin are relied upon in disease management programs (25, 26). Azoxystrobin is classified by the Environmental Protection Agency as reduced-risk chemistry. Reduced-risk products have a low impact on human health, low toxicity to non target organisms (birds, fish, and plants), low potential for groundwater contamination, lower use rates, low pest resistance potential, and compatibility with IPM (65). These Quinone outside Inhibitors (QoI) fungicides function by inhibiting fungal respiration. Mobility differs for the two fungicides as azoxystrobin is xylem mobile, while pyraclostrobin is locally systemic.

There is a high resistance risk for strobilurin fungicides, and so they must be used in alternation with protectant fungicides (i.e., mancozeb and chlorothalonil) to delay the development of pathogen resistance (25). Chlorothalonil and mancozeb have been available to Wisconsin and Michigan ginseng growers through yearly Section 18 Emergency Exemption labels. Mancozeb is a broad-spectrum dithio-carbamate, fungicide. Chlorothalonil is a broad-spectrum organochlorine.

Iprodione, a dicarboximide, was very effective in controlling *Alternaria* on ginseng. However in mid season of 1987, after applications of iprodione in an experimental plot for two consecutive seasons, the fungicide was found to be ineffective

against *A. panax* (58). Laboratory tests (58) confirmed the existence of an *Alternaria* population which had become resistant to iprodione. Other *Alternaria* spp. with documented dicarboximide resistance include *A. alternata* pv. *citri* (63), *A. alternata* (30, 37), and *A. brassicicola* (31). Although iprodione has been used for many years to control various fungal pathogens causing plant diseases, the mode of action and the resistance mechanisms are not well known (35).

Resistance of *A. panax* to iprodione has not been documented since the first report. Few growers now use the product because of its expense, although recent studies have indicated that it is effective in controlling Alternaria blight (25). In 1988, copper hydroxide (inorganic chemistry) was available for a tank mix with iprodione; however, the mixture was found inadequate for disease control throughout the growing season, and the combination appeared to reduce the seed yield of treated plants (25).

Disease Forecasting

Since the mid 1900s, the concept of predicting disease occurrence has been explored (66). Disease predictive or forecasting systems are developed based on weather variables (66), in conjunction with the biology and epidemiology of the pathogen to predict infection or development periods for the disease on a particular host (33).

The benefits of utilizing disease forecasting systems are numerous. Primarily, forecasting systems are useful for economically important diseases, especially those which are variable between seasons and known to depend on specific weather factors (8). Secondly, disease forecasting systems are suited for IPM programs, as the timing of fungicide applications are tailored, which minimizes environmental, human and animal hazards. Forecasting systems also have a potential cost benefit to farmers who typically

apply fungicides based on a calendar program (usually every 7 to 14 days). By using a forecasting system to account for disease potential and environmental factors, farmers could reduce the total number of fungicide applications per season and lessen the chances for pathogens to develop fungicide resistance (4, 7, 8, 60).

In 1978, a disease forecasting system was developed to identify environmental conditions that would favor development of early blight on tomatoes and to enhance the efficiency of fungicide use (36). The forecasting system FAST (Forecaster of *Alternaria solani* on Tomato), uses disease severity values (DSVs) based on hours of leaf wetness and mean temperature during leaf wetness to determine the first fungicide application. Subsequent fungicide applications are then made based on daily values of leaf wetness, mean air temperatures, rainfall and hours of relative humidity > 90% (36). Field studies showed that the weather-prompted FAST program was as effective as calendar-based fungicide applications (36).

The FAST system was modified by Pitblado in 1988 (45). The resulting system TOM-CAST (Tomato disease forecasting), is a computer system that calculates DSVs based on hours of leaf wetness and temperature. Sprays are based on cumulative DSV thresholds. When a threshold is reached, a fungicide is applied and the DSVs reset to zero (46). TOM-CAST has been successfully implemented to manage early blight on tomatoes (12, 46), purple spot on asparagus (38), foliar blights on carrots (7), and studies have shown it to be useful on parsley (39) and celery (6).

Considering the crops where TOM-CAST based fungicide applications were found to be a practical and economical alternative to calendar-based applications, ginseng is most similar to celery. Like ginseng, celery is a high-value crop. In Michigan, the

value for processing and fresh market celery is $20,431/ha compared to $3,424 and $2,420 for carrot and asparagus, respectively (6). Late blight incited by *Septoria apiicola* must be managed as the marketable portion of the celery is affected and consumers demand a blemish-free product (6). In comparing TOM-CAST with other disease predictors, a 10-DSV program was found to provide control equivalent to traditional 7-day applications (6).

TOM-CAST has been commercially implemented in Michigan for carrot (7) and asparagus (38). Carrots, unlike celery, are able to tolerate specific levels of disease as the marketed portion of the crop is not infected. Yield loss resulting from leaf blight occurs when infected carrot petioles snap during mechanical harvest, leaving the roots in the ground (7). Also, the pathogens causing late blight of carrot, *Alternaria dauci* and *Cercospora carotae*, do not defoliate the crop in a relatively short time frame as *A. panax* does with ginseng. Purple spot of asparagus, caused by *Stemphylium vesicarium,* can result in premature browning and defoliation of the photosynthetic tissue. Since 2000, TOM-CAST has been used to manage purple spot on the fern after spears have been harvested. Following infection of the fern, there is decreased carbohydrate availability to the crown which negatively impacts spear production. Asparagus, like ginseng is a perennial crop. When foliar disease is not controlled on asparagus over consecutive years, subsequent reductions in spear yields may result (38). There is a similar concern for ginseng, as repeated epidemics of Alternaria blight can result in reduced root and seed yields (26). For both asparagus and carrots, TOM-CAST with a 15-DSV threshold was found to provide adequate disease control.

Research Priorities

Under current practices, ginseng growers apply fungicides for crop protection from mid-May to early June until the middle of September (26). This is a concern as the current chemical controls registered for Alternaria blight have limited seasonal application allowances to reduce pathogen resistance potential. The products also should not be applied sequentially.

Given the yearly potential crop losses resulting from Alternaria blight, ginseng growers are interested in improving disease management in a cost-effective manner. Specifically, growers are interested in learning (i) when is *A. panax* inoculum available, (ii) when should control measures be initiated each season, (iii) what weather factors influence conidial concentrations, and (iv) can TOM-CAST be used to prompt fungicide sprays? In an effort to retain effective products, a reduction in seasonal fungicide applications is sought based on inoculum detection and environmental factors. In hopes of addressing these concerns, research utilizing current management tools were tested. This paper details the summary of the results.

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CHAPTER ONE

Factors influencing atmospheric conidial concentrations of *Alternaria panax* in cultivated American ginseng gardens

ABSTRACT

Factors influencing atmospheric conidial concentrations of *Alternaria panax* in cultivated American ginseng gardens.

Leaf blight, caused by *Alternaria panax*, is the most common disease of cultivated ginseng and is an annual threat. To determine the influence of weather parameters on atmospheric conidial concentrations, 3- and 4-year-old commercial American ginseng (*Panax quinquefolium* L.) gardens were monitored from mid-May to September for three growing seasons. Hourly concentrations of airborne *A. panax* conidia were enumerated using a Burkard volumetric spore sampler. The hourly averages of air temperature, rainfall, and relative humidity were also collected. Fungicides were not applied. The incidence of leaf blight was assessed in predetermined portions of each garden. In each year, atmospheric conidial concentrations of *A. panax* were detected beginning in late May and continued through the growing season. Daily conidial concentrations followed a diurnal pattern and were greatest during periods of rapidly decreasing relative humidity. A significant correlation was observed between hourly conidial concentrations and temperatures greater than 18°C. Moisture negatively impacted hourly conidial concentrations. Disease pressure from *A. panax* was high each year and many plants were dead by the end of the growing season.

INTRODUCTION

American ginseng (*Panax quinquefolium* L.) is a perennial herb grown primarily for the medicinal properties of its root (23). Currently, more than 95% of the cultivated commercial ginseng in the United States is grown in Wisconsin (1) with a revenue totaling $50 to $75 million annually (12). Cultivated ginseng is grown on a raised plant bed under a natural tree or artificial black woven polypropylene canopy (23). The shade required by the crop and the dense plant spacing create a microclimate with limited air movement and extended leaf wetness periods. The annual growing season for ginseng occurs over 5 to 6 months with plant emergence beginning in mid to late April.

Alternaria blight incited by *Alternaria panax* Whetzel is the most common disease of ginseng throughout the world (22). This disease is a yearly problem for ginseng growers in Wisconsin and can be especially destructive, causing rapid defoliation and plant death (11, 12, 13, 22). Alternaria blight also occurs in other ginseng-growing regions including Alberta, Canada (3, 4), West Virginia (30), North Carolina (7), Michigan (11, 12), Oregon and Washington (25).

Typical Alternaria blight symptoms include necrotic lesions on the foliage with dark brown margins and yellow-green halos (22). Brown lesions often develop on the stem just above the soil line and cause girdling (2). Infected drupes may develop a water-soaked appearance followed by the development of gray mycelium and pathogen sporulation (11, 12). Infection of the root by *A. panax* is rare (22); however, root weight can be reduced when blighting of the leaves and stem causes premature defoliation (2).

A. panax conidia are produced in short chains of 2 to 3, but are more commonly borne singly (2, 33). Although the morphology of *A. panax* conidia is variable (5),

conidia are typically obclavate, 150 to 160 by 12 to 20 μm (2) with 1 to 2 vertical and 9 to 11 transverse septa (24). *A. panax* can survive as conidia or as mycelium in infected plant residue, or in and on seeds (17). Lesion development and conidial production can occur in 5 to 7 days under favorable conditions (33). Optimum conidial production and mycelial growth occurs with temperatures ranging from 18 to 25° C and 24 and 27° C, respectively (2, 5, 6, 33). Specific leaf wetness parameters for *A. panax* are unknown.

A. panax conidia may overwinter in mulch and crop debris providing initial inoculum for emerging crops in the spring (12). Fifteen cm of straw mulch is generally applied to ginseng beds during crop establishment. In the winter, straw mulch can prevent temperatures from dropping below the freezing point of the roots (around -10° C) and during the summer keeps soil temperatures 5 to 10° C below that of an open-grassed area (29). Mulch also eliminates the need of irrigation as it prevents excessive moisture loss from the soil throughout the season (29). Despite these benefits, the use of straw mulch can also be problematic as *A. panax* mycelium can spread from plant to plant through the mulch; the pathogen may also spread through the soil as a saprophyte until it contacts the ginseng host (11, 15, 17).

In the absence of effective treatments for Alternaria blight, growers could potentially lose $13,930/ha (current market price for ginseng root) (12). Since *A. panax* also infect drupes, seed can also be threatened. With an average seed yield of 363 kg/hectare, growers could lose an additional $18,537/ha (current market value $55/lb) due to Alternaria blight (12).

Cultural strategies to manage Alternaria blight include increasing plant spacing to enhance air circulation, removing infected foliage and replacing straw mulch in affected areas (7). Unfortunately, these strategies are not practical and would not preclude the

reintroduction of inoculum via air currents. The use of biocontrols as alternatives to traditional fungicides has also been considered for Alternaria blight management. *Burkholderia cepacia* AMMD (*Pseudomonas cepacia* strain AMMD) was found to effectively inhibit Alternaria blight under growth chamber conditions. However, the biocontrol agent did not adequately reduce disease under field conditions due to poor survival on leaf surfaces (16, 20, 21).

Currently, growers rely on fungicides to protect their crop from Alternaria blight (11). Growers must apply fungicide sprays judiciously during the relatively long growing season of May through September to protect the crop and comply with the labeled seasonal application limits. To support the development of new management strategies that are less dependent on fungicides, additional epidemiological information for *A. panax* is needed. Ginseng growers would like to know when fungicide applications should be initiated in the spring and what specific weather parameters are associated with increased atmospheric conidial concentrations (ACCs). The objectives of this study were to determine (i) when inoculum is available each season, (ii) when control measures need to be applied and how long they should be maintained, and (iii) what weather factors influence conidial concentrations.

MATERIAL AND METHODS

Plot establishment. Field studies were conducted in commercial cultivated ginseng gardens located in Marathon County, WI. Gardens were established from stratified seed that was broadcast planted into 1.5 m-wide, raised plant beds that were 22 to 30 cm high. Ginseng seeds were not planted on the lower portion (20 cm) of the beds.

After seeding, the entire plant bed was covered with 15 cm of straw mulch. Each year, woven panels providing 80% shade were suspended via 2- to 3-m posts over the plant beds to mimic wood-lot conditions. Weeds were hand pulled. Each year, plant emergence began in March and plants were fully emerged by May 1.

Disease incidence. Garden sections for monitoring disease incidence were identified prior to the start of spore trapping. The number of plants with one or more lesions was determined in four 1.5 x 3-m sections located in the eastern half of each garden. Fungicides were applied according to commercial standards to the areas surrounding the monitored garden sections only. A 24-ft untreated buffer was maintained between the monitored garden sections (untreated) and remaining commercial garden treated according to industry practices.

Inoculum availability and atmospheric concentrations. Concentrations of airborne *A. panax* conidia were monitored each growing season from 2005 to 2007 using a 7-day volumetric spore sampler (Burkard Scientific, Uxbridge, UK) (Figure 1). Monitoring began in either May or June (prior to the development of Alternaria blight symptoms) and concluded in August or September (Table 1). Spore traps were located on the eastern half of each garden, within the disease incidence monitoring area. Spore traps were operated at a flow rate of 10 liters/min and the orifice was free to move with changing wind direction. Conidia were collected on melinex tape (Burkard Scientific, Uxbridge, UK) coated with an adhesive mixture of petroleum jelly and paraffin (9:1, wt/wt) heated to 60° C and cooled overnight before being dissolved with sufficient toluene to give a thick, liquid consistency. Prior to petroleum jelly and paraffin coating, the melinex tapes were coated with a polyvinol alcohol (Airvol/Gelvatol, grade 603,

Burkard Scientific, Uxbridge, UK) and phenol mixture (35 g polyvinol alcohol, 25 ml glycerol, 50 ml distilled water, heated to 40° C to combine, then cooled to 23° C before adding 2 g of phenol) and allowed to dry overnight at 23° C. Prepared tapes were maintained at 23° C until field use.

Tapes were removed from the spore trap weekly and cut into 48-mm lengths and scored at 2-mm intervals corresponding to a 24 h day using a razor blade. Tape sections were mounted onto 3x2 cm glass slides and stained with aniline blue (0.14 mg aniline blue, 20 ml distilled water, 15 ml glycerol, 10 ml of 85% lactic acid). Slides were covered with 22x50 mm coverslips and sealed using Cytoseal (Richard-Allan Scientific, Kalamazoo, MI). Mounted slides were allowed to dry at 23° C for at least 24-h before observation by light microscopy.

Hourly conidial counts were made using a compound light microscope. *A. panax* was identified based on morphological characters (2, 24). Occasionally, conidia of *A. alternata* (conidium 20-63 μm) and *A. solani* (conidia 150-300 μm) (27) were observed but were not included in hourly conidia counts. Hourly conidial counts were converted to numbers of conidia per cubic meter of air sampled per hour (conversion factor= x/ 0.6). PROC CORR (SAS Institute Inc., Cary, NC) was used to determine correlations among hourly atmospheric conidial concentrations, rainfall, leaf wetness, relative humidity and temperature for each growing season (10).

Weather monitoring. Hourly measurements of air temperature and relative humidity were recorded using a data recorder (Watchdog series 450, Spectrum Technologies, Inc., Plainfield, IL). An unpainted Watchdog leaf wetness sensor (Spectrum Technologies, Inc.) was placed at a 45° angle facing north within the upper

25% of the ginseng canopy. Data were downloaded weekly to a laptop computer using a computer program (Specware 6.01 and 7.01; Spectrum Technologies, Inc.). The program was set to record temperatures between 0° and 100° C. Rainfall was measured hourly using a tipping-bucket rain collector (series 3554WD, Spectrum Technologies, Inc.) (Figure 1).

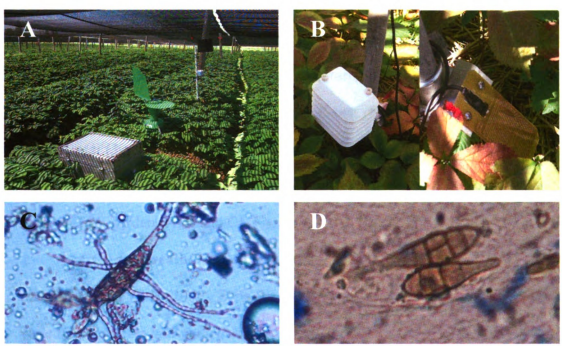


Figure 1. Burkard spore trap, tipping rain bucket (A), leaf wetness sensor and temperature/humidity sensors (B) used to monitor weather parameters and atmospheric concentrations of *Alternaria panax* conidia (C, D) within WI ginseng gardens. (Images are presented in color.)

RESULTS

Conidial availability, diurnal pattern and atmospheric concentrations. Each year, ACCs were detected at the onset of spore trapping and occurred daily throughout the monitoring period. In 2005, ACCs during the first week of monitoring were < 1000 conidia/m^3/h whereas in 2006 and 2007, the ACCs exceeded >1000 conidia/m^3/h. When hourly ACCs were averaged over the monitoring periods, peaks occurred at 1200 h in 2005 (53 conidia/m^3/h), 1100 h in 2006 (233 conidia/m^3/h), and 1300 h in 2007 (115 conidia/m^3/h). Relatively few conidia were trapped between 0100 h and 0600 h, with an average of 18, 49 and 29 conidia/m^3/h in 2005, 2006 and 2007, respectively, (Figure 2).

When a commercial garden was monitored over two growing seasons, greater hourly ACC totals were observed in the second year (4-year-old planting) compared with the first year (3-year-old planting). Across all gardens monitored, the highest daily ACCs were observed during the latter portion of each growing season (e.g. July 2005, garden B; August 2006, gardens A, B and C) (Figure 3).

ACCs and weather effects. Weekly rainfall averaged 12.9, 9.9, 111.0, and 7.8 mm for gardens A, B (2005), B (2006) and C, respectively. Rain (%) /leaf wetness (%) varied across years and gardens occurring 4.2/15.2 (A), 3.0/21.3 (B-2005), 6.2/27.3 (B-2006) and 3.8/24.9% (C) of the monitoring periods. Each year, hourly rainfall and leaf wetness were negatively correlated to ACCs (Table 2). In all monitored gardens, averages of 15≤112 conidia/ m3/h were detected when rain was absent. Whereas 7≤40 conidia m^3/h (average) were present in the atmosphere during rain events. Despite a negative correlation between hourly rainfall and ACCs; daily totals for ACCs following rain events (1 to 3 days) were at least 50% greater than ACCs on the day of the rain event

(Figure 4). A positive correlation for ACCs occurring 16 h or more after a rain event was detected ($p \geq 0.05$). In all gardens, the average hourly ACCs during no leaf wetness were similar to periods without rainfall. Similar to the periods with rainfall, an average of ≤ 40 conidia on average were present in the atmosphere during hours when leaves were wet.

Within each 24-h period, ACCs peaked during periods of decreasing (20 to 80%) relative humidity (RH) for all monitored periods (Figure 4). RH exceeding 80% was negatively correlated to hourly ACCs for all gardens (Table 2). Hourly ACCs were positively correlated to RH between 20 and 49%. For all monitoring years, ACCs were greatest when RH was low (i.e. 20%) (Figure 5).

During 2005 and 2006, temperatures between 18 and 25° C were most common. In 2007, overall temperatures were lower and were commonly within the range of 10 to 17° C (48.4 h/monitoring week). Temperatures rarely fell below 10° C, with fewer than 10 h/week on average recorded. Temperatures exceeded 25° C each year, with an average of 19 to 24 h/week observed. The highest hourly temperature recorded in each garden was 34.5 (A), 36.6 (B-2005), 44.4 (B-2006) and 34.0° C (C).

Temperatures of 18 to 25° C were positively correlated to hourly ACCs (Table 2). In garden A, when hourly temperatures were predominantly between 18 and 25° C, an average of 50 conidia//m^3/h were observed. Similarly for garden C, when hourly temperatures were predominantly between 18 and 25° C, an average of 69 conidia//m^3/h were observed. In garden B, ACCs averaged 16 and 113, in 2005 and 2006, respectively. A significant correlation ($p \leq 0.05$) was also present for temperatures > 25° C. When hourly temperatures were predominantly >25° C in each 24-h period, average hourly ACCs were greater than 50 for all gardens except B in 2005 when the average hourly

ACC was 22. In garden B during 2005, the spore trap was inoperable for 3 weeks in August.

Temperatures less than 18° C were negatively correlated with hourly ACC. When hourly temperatures were predominantly <10° C within each 24-h period, the hourly ACC averages were ≤ 25 for all gardens. When hourly temperatures were between 10° and 17° C, average ACCs were 29 and 40 for gardens A and C, respectively. ACC averages of 9 and 58 were collected in garden B for 2005 and 2006, respectively.

Disease incidence. For all years, disease increased throughout the growing season resulting in significant levels of foliar blighting and death (Figure 6). The first Alternaria blight lesions were observed on the foliage on 24 June, 20 June, and 27 June of 2005, 2006, and 2007, respectively. A significant correlation (P=0.0048) between weekly conidial concentrations and plant disease incidence was observed. Commonly, high levels (85%) of crop defoliation were observed in early August (Figure 3). By the end of the monitoring period, many of the garden sections were completely defoliated.

Table 1. Atmospheric monitoring dates for Alternaria blight caused by *Alternaria panax* in commercial Wisconsin ginseng gardens 2005 through 2007.

Year	Garden[z]	Plant age (Year)	Monitoring Period
2005	A	3	5/24 – 8/22
	B	3	5/26 – 9/9*
2006	B	4	5/25 – 8/30
2007	C	4	6/1 – 9/20

[z]Garden size = 1.6 ha. * Spore trap malfunction 8/3 – 8/16 and 8/24-30

Table 2. Pearson correlation coefficients testing the relationship between hourly *Alternaria panax* conidial concentration, rainfall, leaf wetness, relative humidity and temperature in commercial Wisconsin ginseng gardens from 2005 through 2007

	Pearson correlation coefficients, *r* (probability, P)[z]								
	Temperature				**Moisture**		**Relative humidity**		
Garden/ Year	< 10° C	10-17° C	18-25° C	>25° C	Rainfall	Leaf wetness	20-49%	50-79%	80-100%
A 2005	-0.04397	-0.06599	0.04532	0.03841	-0.00665	-0.02379	0.06727	0.01257	-0.06535
	(0.0472)	(0.0029)	(0.0408)	(0.0830)	(0.7642)	(0.2832)	(0.0024)	(0.5707)	(0.0032)
B 2005	-0.10960	-0.12947	0.04305	0.15944	-0.02566	-0.13613	0.11471	0.12199	-0.20092
	(<.0001)	(<.0001)	(0.0463)	(<.0001)	(0.2351)	(<.0001)	(<.0001)	(<.0001)	(<.0001)
B 2006	-0.10960	-0.12947	0.00210	0.16692	-0.02962	-0.04841	0.08511	0.04049	-0.10787
	(<.0001)	(<.0001)	(0.9269)	(<.0001)	(0.1947)	(0.0340)	(0.0002)	(0.0763)	(<.0001)
C 2007	-0.09562	-0.13171	0.07825	0.13858	-0.00311	-0.11664	0.21875	0.09780	-0.12690
	(<.0001)	(<.0001)	(0.0002)	(<.0001)	(0.8816)	(<.0001)	(<.0001)	(<.0001)	(<.0001)

[z]Probability of a greater absolute value of *r* between hourly *Alternaria panax* conidia counts and the remaining factors.

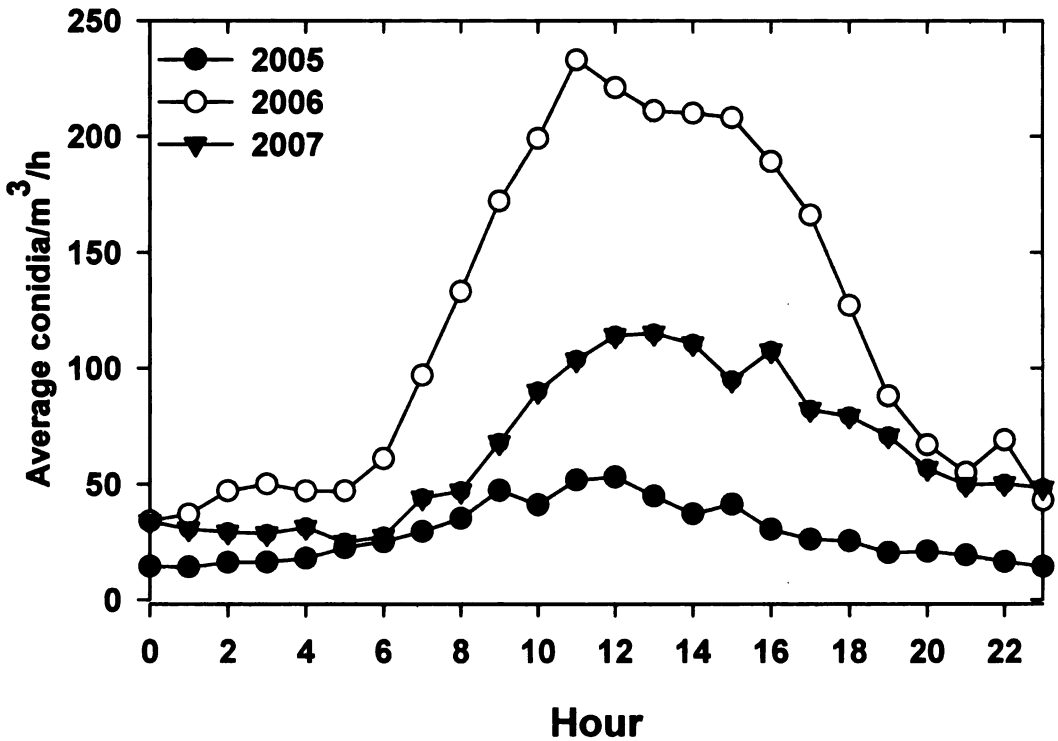
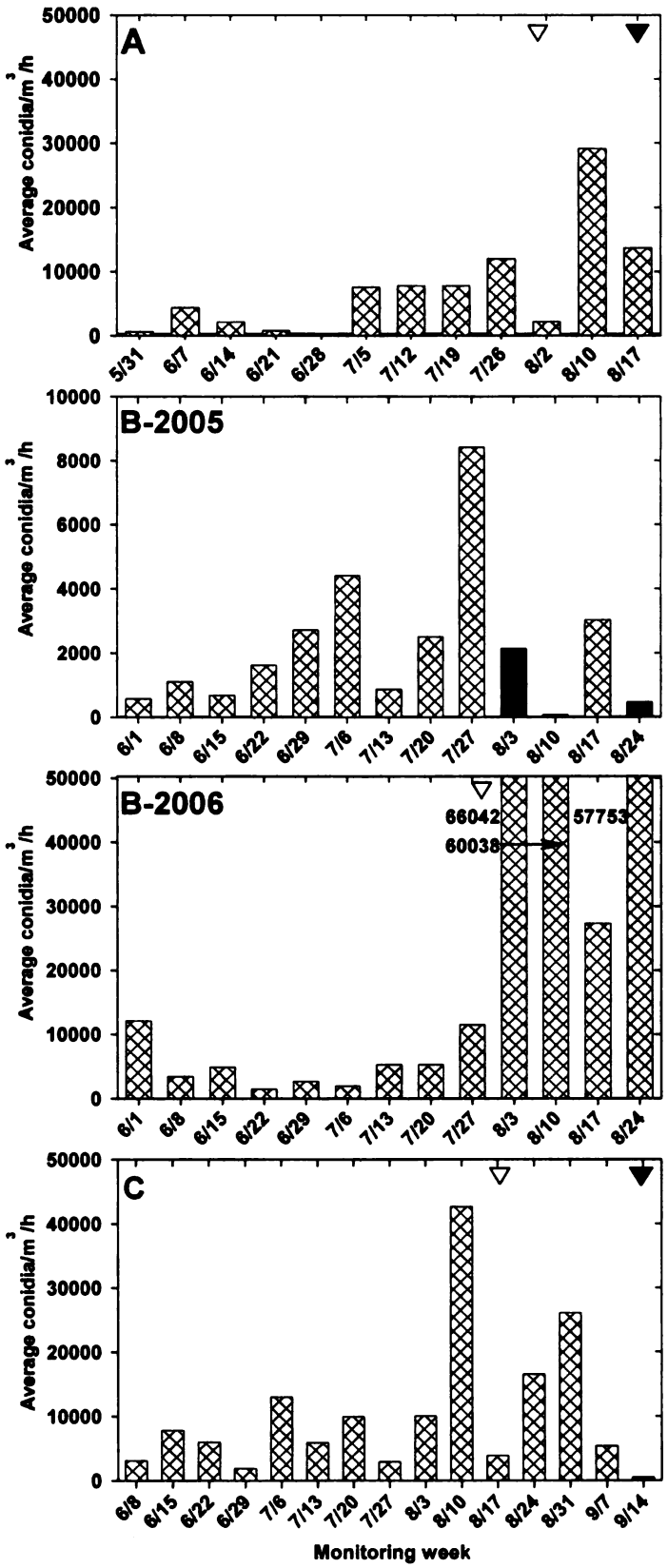


Figure 2. Hourly average concentration of airborne *Alternaria panax* conidia in Wisconsin ginseng gardens A (91 monitoring days (md)), B-2005 (107 md), B-2006 (98 md) and 2007 (112 md).

Figure 3. Atmospheric *Alternaria panax* conidial concentrations occurring in commercial Wisconsin ginseng gardens with 3-yr-old (A and B-2005) and 4-yr-old plants (B-2006) and (C) and occurrence of 85% (▼) and 100% (▼) crop defoliation. Black-filled bars represent weeks where spore traps malfunctioned.


A
B-2005
B-2006
C
Average conidia/m3/h
Monitoring week
66042
60038
57753
5/31
6/7
6/14
6/21
6/28
7/5
7/12
7/19
7/26
8/2
8/10
8/17
6/1
6/8
6/15
6/22
6/29
7/6
7/13
7/20
7/27
8/3
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8/10
8/17
8/24
8/31
9/7
9/14

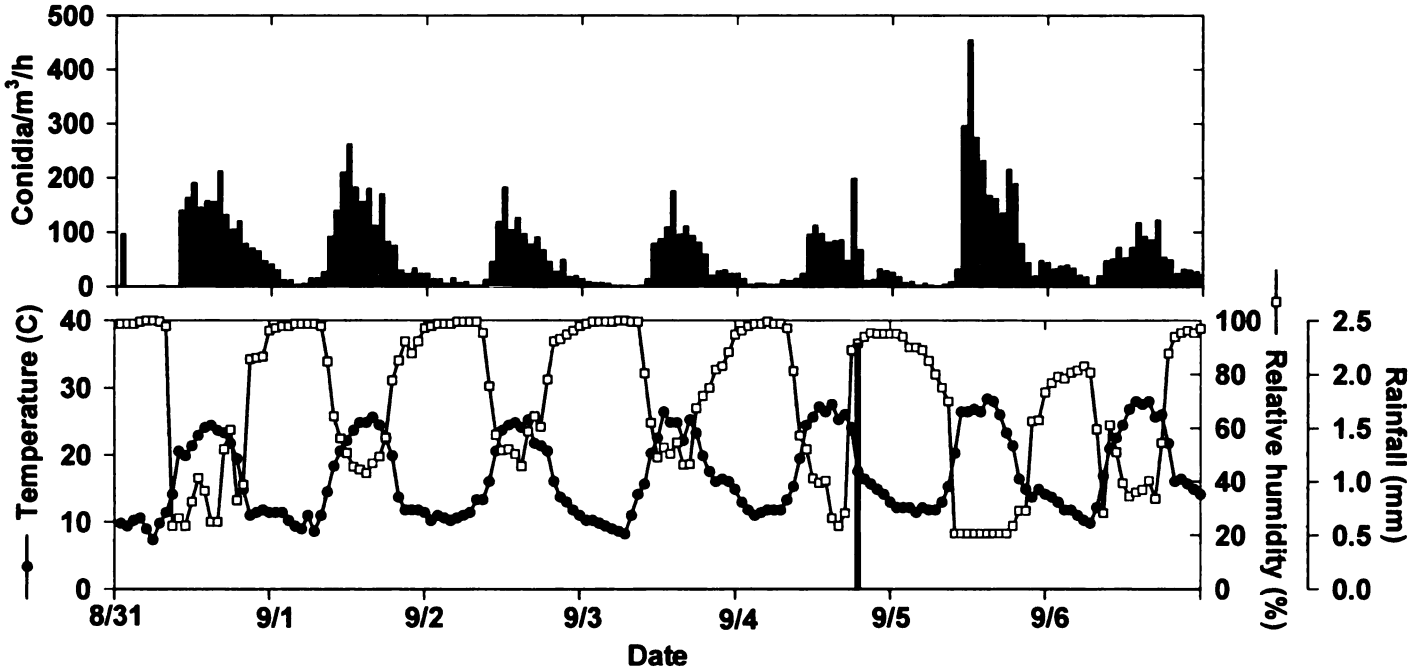

Figure 4. Typical association of atmospheric *Alternaria panax* conidia, temperature, relative humidity and rainfall in commercial ginsengs garden.

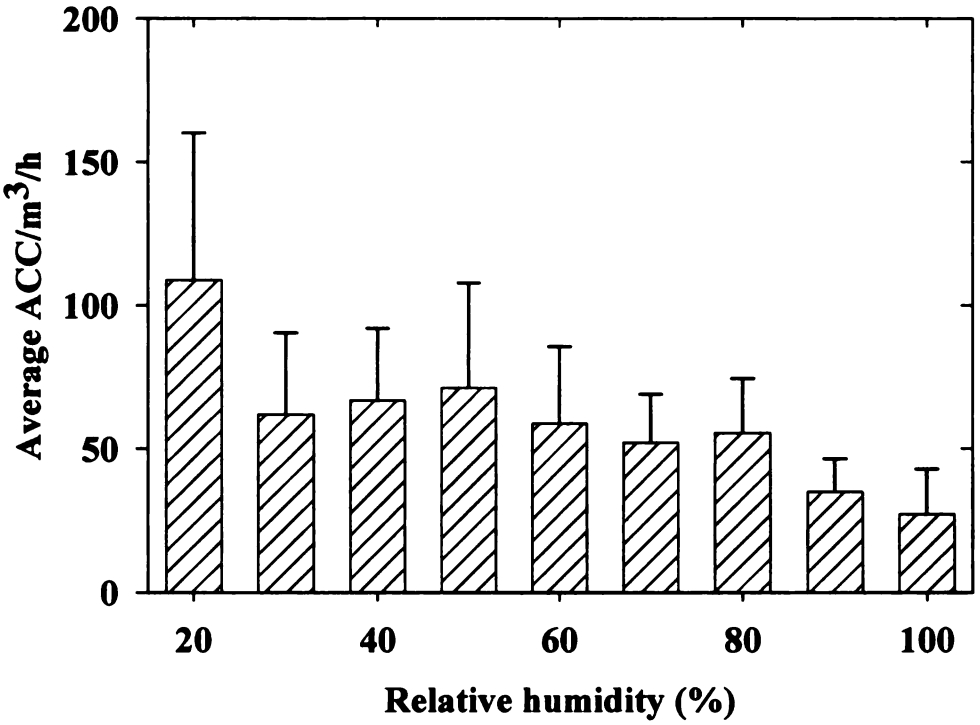

Figure 5. Relationship between atmospheric conidia of *Alternaria panax* and relative humidity averaged for monitoring periods in 2005 - 2007. Vertical bars represent the standard errors of means.

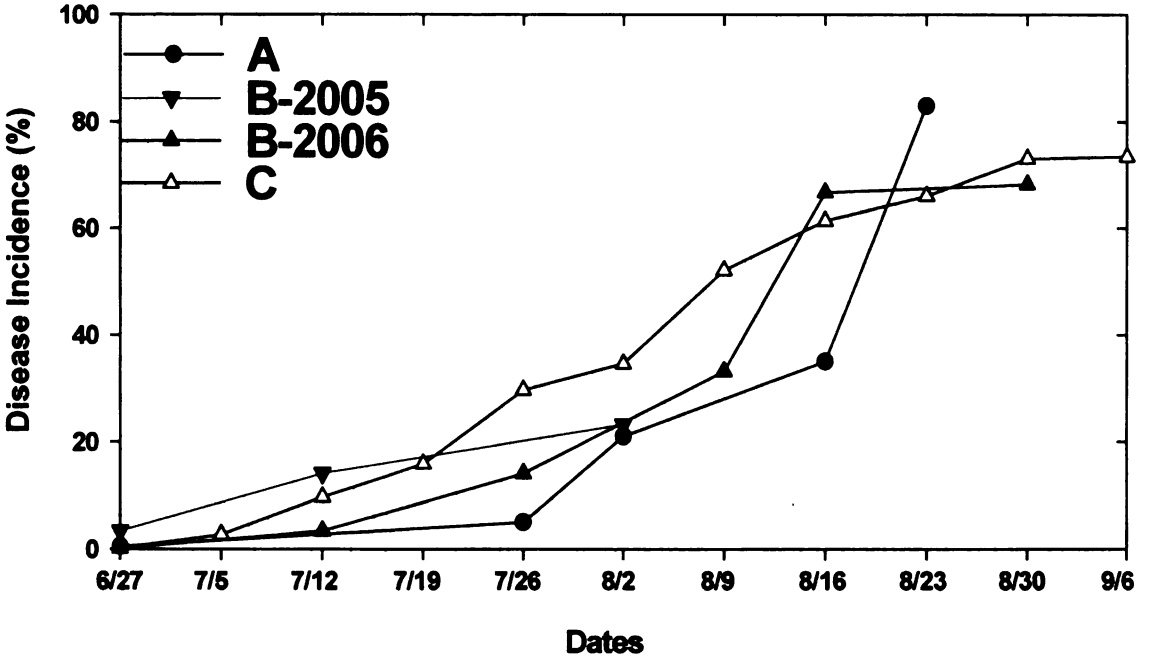

Figure 6. Disease progression resulting from infection by *Alternaria panax* in Wisconsin ginseng gardens during the 2005 - 2007 growing seasons.

DISCUSSION

Traditionally, ginseng growers apply fungicides for crop protection beginning with plant emergence (approximately mid-May) until the middle of September (12). In our study, *A. panax* conidia were detected at the onset of the growing season, prior to the development of lesions, suggesting that growers should initiate fungicide sprays immediately upon plant emergence as currently practiced. The detection of conidia throughout the growing season into late August and early September indicates that growers should continue fungicide sprays through September into mid October depending on weather conditions and harvest date.

Atmospheric concentrations of *A. panax* conidia in ginseng gardens were correlated with specific weather factors. ACCs were greatest during periods of decreasing relative humidity. While conidia were detected during periods of high relative humidity, concentrations were low and a significant negative correlation was found. This suggests that conidia of *A. panax* are not actively released under high humidity conditions. Despite a negative correlation between hourly rainfall and ACCs, a positive correlation was detected for ACCs following 16 h or more after rainfall. The rainfall events may temporarily remove conidia from the air, but the coincident leaf wetness period provides favorable conditions for sporulation (14). The reduced ACCs during rain events is likely attributed to rain scrubbing from the atmosphere (9). Optimum conidial production of *A. panax* occurs with temperatures ranging from 18 to 25° C (2, 33). In our study, significant ACCs were observed within that range and ≥25° C.

Similar correlations between ACCs and weather parameters reported in our study have been reported for other *Alternaria* spp. *A. solani* conidia in controlled

environmental chambers were released with abrupt increases or decreases in relative humidity, vibration, and rain events (8). Conidia of *A. tenuis* on banana are released during a shift from wet to dry. During surface drying air bubbles are formed on conidial chains of *A. tenuis* causing the conidial chains to twist and resulting in the release of conidia (19). Conidia of *A. tenuis* are also released following both an increase and decrease in relative humidity (18). Conidia of *A. alternata* on tangerine similarly are released following sudden increases or decreases in relative humidity and with simulated rainfall events (32). While moisture is important for sporulation of *Alternaria*, most *Alternaria* species including *A. panax,* are able to survive longer and reproduce faster, and cause greater infection in drier (16 to 20° C) conditions (28, 32, 33).

Uncontrolled outbreaks of *A. panax* in one season may increase the potential for epidemics in subsequent seasons, since the fungus overwinters in infested plant debris and mulch. Each year, as ginseng prepares for dormancy, the stem collapses at the rhizome. Premature defoliation as a result of foliar disease may also occur. Ginseng growers practice a no-till cultivation system and mulch and plant debris are not removed during the life of the crop (≥3 years). Seasonally infected crop material can become integrated into the plant beds.

In the spring, overwintered inoculum from previously infected tissues spreads to the newly emerging healthy plants via rain or splashing water and initiate the disease cycle for the new growing season (12). In addition, overwintering conidia and/or mycelium can be a source of stem infection as plants emerge through the infested mulch in the spring (26). The overwintering of conidia on crop residue which serve as primary inoculum in the spring, has similarly been observed for *A. alternata* (27, 34). Currently, there are no

practical methods to reduce overwintering inoculum levels on the mulch or on infected crop residue (17).

Leaf wetness is a common and important component of disease models and forecasters (31). The leaf wetness sensor in our study was placed within the top 25% of the canopy, 25 cm from the bed gutter. As plants located on top of the bed were likely exposed to increased air movement, the detection of leaf wetness could have been compromised. Future studies should incorporate multiple leaf wetness sensors to detect leaf wetness on the sides of the ginseng beds, where the plant canopy tends to remain wet longer due to limited air movement. Additional studies including in vivo analysis of conidial response to weather parameters should be conducted. In particular, determining leaf wetness parameters for *A. panax* infection, and sporulation, and confirming the temperature ranges for conidial sporulation and survival is important. Assessing the effect of crop age on sporulation may also be helpful.

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CHAPTER TWO

Evaluation of TOM-CAST in timing fungicide sprays for management of Alternaria blight on American ginseng

NOTICE

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ABSTRACT

Evaluation of TOM-CAST in timing fungicide sprays for management of Alternaria blight on American ginseng.

By

Shaunta Nichelle Hill

Alternaria panax incites blighting of the foliage, peduncles, and drupes of cultivated American ginseng (*Panax quinquefolium*). A disease forecaster (TOM-CAST), originally developed to predict leaf blight caused by *A. solani* on tomato, was evaluated for management of *A. panax* in commercial ginseng gardens. For three years, fungicide sprays initiated by TOM-CAST (using 10- and 15-disease severity value thresholds) were compared with sprays applied at 7- and 10-day intervals. Three fungicide programs were evaluated: (i) chlorothalonil alone, (ii) chlorothalonil alternated with pyraclostrobin, and (iii) copper hydroxide alternated with pyraclostrobin. As many as 10 fewer fungicide applications were made when using TOM-CAST or the 10-day programs. Although select TOM-CAST treatment programs were comparable to the 7-day schedule in limiting foliar disease, only the 7-day applications adequately protected drupe and seed yield. Both *A. panax* and *alternata* were recovered from drupe tissues and seed coats. Only *A. alternata* was recovered from endosperm halves. Ginseng seed yield and quality is an important consideration when assessing fungicide programs.

INTRODUCTION

American ginseng (*Panax quinquefolium*) is a perennial herb grown primarily for the medicinal properties of its root (24). Currently, more than 95% of the cultivated commercial ginseng in the United States is grown in Wisconsin (1), totaling $50 to $75 million annually (11). Ginseng is grown on a raised plant bed under a natural tree or artificial black woven polypropylene canopy (24). The shade required by the crop and the dense plant spacing create a microclimate with limited air movement, increased temperatures, and extended leaf wetness periods that are favorable for foliar pathogens.

The most common foliar disease of ginseng is Alternaria blight, caused by *Alternaria panax* Whetzel (22). This disease is a yearly problem for ginseng growers in Wisconsin (22) and Michigan (11). Alternaria blight also occurs in other ginseng growing regions, including Alberta, Canada (7, 8), West Virginia (28), North Carolina (9), Oregon, and Washington (25). Typical foliar symptoms include necrotic lesions with dark-brown margins and yellow-green halos (22). Brown lesions often develop on the stem just above the soil line and cause girdling (5). Infected drupes may develop a water-soaked appearance followed by the development of brown mycelium and pathogen sporulation. Infection of the root by *A. panax* is rare (22); however, root weight can be reduced when blighting of the leaves and stem causes premature defoliation.

A. panax can survive as conidia or mycelia in infected plant residue, on straw mulch, or in and on seed (16). In the spring, overwintered conidia may spread to and infect emerging healthy plants via rain splashing or air currents. Under favorable conditions, blight development and conidial production can occur in 5 to 7 days (30). Optimum conidial production occurs with temperatures ranging from 18 to 25°C (5, 30).

Leaf wetness parameters for *A. panax* are unknown. However, researchers have shown that conidia can germinate on leaf tissue within 1 to 2 h when incubated at 25°C and 70% relative humidity (26).

Cultural strategies to manage Alternaria blight include increasing plant spacing to enhance air circulation, removing infected foliage, and replacing mulch in the affected area (9). However, these strategies are not practical and would not preclude the reintroduction of inoculum via air currents. Previous researchers have investigated the use of biocontrols as alternatives to traditional fungicides. *Burkholderia cepacia* AMMD (*Pseudomonas cepacia* strain AMMD) was found to effectively inhibit Alternaria leaf and stem blight under growth-chamber conditions. However, the biocontrol agent did not adequately reduce disease under field conditions due to poor survival on leaf surfaces (14, 21).

Currently, the strobilurin fungicides azoxystrobin and pyraclostrobin are used in alternation with either chlorothalonil or mancozeb (11). Chlorothalonil and mancozeb have been available to Wisconsin and Michigan ginseng growers through yearly Section 18 Emergency Exemption labels. Iprodione is also registered for the control of Alternaria blight on ginseng and can be effective. However, following control failure in 1987, an iprodione-resistant *Alternaria* population was identified in Wisconsin (27) and use of this product declined.

Managing Alternaria blight is a priority of the ginseng industry in Michigan and Wisconsin (11). When Alternaria blight is not controlled, complete defoliation of ginseng can occur (12, 31). Using a forecasting system to assess disease potential associated with environmental conditions may reduce the number of fungicide

applications needed per season (3, 4), lessen the chances for pathogens to develop fungicide resistance, and reduce grower costs. Disease-forecasting systems use weather variables (32) in conjunction with information about the biology and epidemiology of the pathogen to predict when conditions favor infection or disease development (15). TOM-CAST (developed for tomato disease forecasting) is a disease forecasting system that calculates daily disease severity values (DSVs) based on cumulative hours of leaf wetness and temperature. When the predetermined DSV threshold is reached, a fungicide is applied and the DSV is reset to zero (23). TOM-CAST has been successfully implemented to manage early blight on tomato (6, 23), purple spot on asparagus (18), and foliar blights on carrot (3), and studies have shown it to be useful on crops such as parsley (19) and celery (2).

Growers have expressed interest in identifying the environmental conditions that favor blight development and testing the TOM-CAST forecasting system as a tool to assist in timing fungicide applications (10). The objective of this study was to compared the TOM-CAST disease forecasting system with a calendar-based spray schedule using various fungicide programs for Alternaria blight control. The impact of these treatment programs on seed yield and health was also of interest.

MATERIAL AND METHODS

TOM-CAST plot establishment. Field studies were conducted in a 3-year-old (2005) or 4-year-old (2006 and 2007) commercial ginseng garden located in Marathon County, WI. Each research site was 1.6 ha in area and consisted of a silt loam soil. Ginseng gardens were established from seed planted into 1.5-m-wide raised beds that

were 22 to 30 cm high. After seeding, beds were covered with 15 cm of straw mulch. Each year, woven polyethylene or polypropylene cloth panels providing 80% shade were suspended via 2- to 3-m posts over the plant beds to mimic woodlot conditions. Weeds were hand pulled. Treatment plots consisted of 1.5- by 3-m raised beds, with 0.60-m buffers on each end. Treatments were replicated four times in a randomized complete block design. Fungicides were applied with a CO2 backpack boom sprayer equipped with four 8006 nozzles spaced 45.7 cm apart, operating at 275.8 kPa, and delivering 467.7 liter/ha.

Weather monitoring and DSV calculation. Hourly measurements of air temperature and relative humidity were recorded using a WatchDog data recorder (series 450; Spectrum Technologies, Inc., Plainfield, IL). An unpainted WatchDog leaf wetness sensor (Spectrum Technologies, Inc.) was placed at a 45° angle facing north within the upper 25% of the ginseng canopy. Data were downloaded weekly to a laptop computer using a computer program (Specware 6.01 and 7.01; Spectrum Technologies, Inc.). The program was set to record temperatures between 0 and 100°C and to detect leaf wetness whenever moisture was present on the grid. Rainfall was measured hourly using a tipping bucket rain collector (series 3554WD, Spectrum Technologies, Inc.).

For the TOM-CAST treatments, the hours of leaf wetness and the average air temperature during the periods of leaf wetness were used to determine a daily DSV ranging from 0 to 4, corresponding to environmental conditions unfavorable to highly favorable for disease development, respectively (23). To accurately reflect the duration of a morning dew period, a 24-h monitoring period of 1100 h to 1100 h was used. Daily DSVs were accumulated until a threshold of 10 or 15 was reached, thus prompting a

fungicide spray. After each fungicide application, the DSV was reset to zero for both of the TOM-CAST treatment programs.

Fungicide programs and application schedules. Fungicide programs included chlorothalonil (Bravo Weather Stik 6SC, 0.68 kg of active ingredient [a.i.]/ha; Syngenta Crop Protection, Inc., Greensboro, NC), chlorothalonil (0.68 kg a.i./ha) alternated with pyraclostrobin (Cabrio 20EG, 0.07 kg a.i./ha; BASF Ag Products Research Triangle Park, NC), and copper hydroxide (Kocide 2000 54DF, 0.73 kg a.i./ha; DuPont Crop Protection, Wilmington, DE) alternated with pyraclostrobin (0.07 kg a.i./ha). Fungicide programs were initiated on 26 May 2005, 26 May 2006, and 5 June 2007. Subsequent applications were scheduled according to a calendar based interval of every 7 or 10 days or according to the TOM-CAST disease forecaster using 10- or 15-DSVs. Fungicide programs were terminated on 9 September 2005, 13 September 2006, and 30 August 2007.

Disease assessment. Disease was assessed by counting the number of *A. panax*-infected plants in a 3-m section of the bed on 31 August and 14 September 2005; 20 July, 3, 16, and 24 August 2006; and weekly from 3 July to 4 September 2007. Data were subjected to analysis of variance (ANOVA) and a Student-Newman-Keuls least significant difference test ($P = 0.05$) was used to compare treatment efficacy (Statistical Analysis Software [SAS] 9.1.3; SAS Institute, Inc., Cary, NC) (20). The area under the disease progress curve (AUDPC) was calculated according to the methods of Shaner and Finney (29) to express the yearly cumulative numbers of infected plants throughout the seasons. The cumulative AUDPC values for each fungicide program and application interval were subjected to ANOVA and a Student- Newman-Keuls significant difference

test ($P = 0.05$; SAS 9.1.3) was used to compare treatment efficacy. Treatments were also assessed for phytotoxicity.

At the completion of the 2007 TOMCAST trial, drupes within the treatment and control plots were harvested. After recording the fresh weights for each plot, 20 drupes per replicate of each treatment and control were selected for fungal assessment. The remaining drupes were placed in 3.8-liter plastic zip bags and stored at 37°C for 2 weeks. Selected drupes were soaked in a 70% ethanol solution for 1 min and allowed to air dry in a sterile laminar flow hood. Portions of drupe tissue were aseptically excised and embedded in water agar (16 g agar/liter) plates (100 by 15 mm) amended with ampicillin (2 ml/liter) and incubated at 25°C under fluorescent lighting.

Seed of the respective drupe samples were also soaked in a 70% ethanol solution for 1 min and allowed to air dry in a sterile laminar flow hood. Following transverse sectioning, seed coat and endosperm halves were embedded in water agar and incubated as described for drupe tissues. After 48 h, all cultures were examined microscopically (×200) for identification of fungi. *Alternaria* spp. were identified as *A. alternata* according to Lagopodi and Thanassoulopoulos (17) (average conidium size, 30.4 by 11.1 μm) or as *A. panax* according to Brammall (5) (average conidium size, 150-160 by 12-20 μm). The percentage of *Alternaria* spp. isolated from both drupe and seed tissues were subjected to ANOVA and a Student-Newman-Keuls significant difference test as previously described. Following cold storage, the remaining drupes were macerated by hand and the seeds removed. Seeds were soaked for 5 min in a 10% bleach solution, triple rinsed with distilled water, and allowed to air dry before weighing. Seed and drupe weights were also statistically analyzed.

RESULTS

Evaluation of fungicide programs and spray schedules with TOM-CAST. The 7-day schedule resulted in 16 sprays in 2005 and 2006 and 12 sprays in 2007. The 10-day schedule resulted in 10 (2005), 12 (2006), and 8 (2007) applications. The TOM-CAST 10-DSV schedule prompted 10, 7, and 4 applications and the 15-DSV schedule prompted 6, 5, and 3 fungicide applications in 2005 (89 total DSVs), 2006 (83 total DSVs), and 2007 (57 total DSVs), respectively.

In each year of the study, Alternaria blight resulted in premature defoliation and death of untreated plants. In 2005 and 2006, all fungicide programs were similar and limited foliar blight compared with the untreated control (Figure 1; Table 1). Similar results were seen for 2005 and 2006 AUDPC data (Table 1). In 2007, disease Pressure was greater. The 7-day schedule, regardless of fungicide program, significantly limited numbers of infected plants compared to the untreated control (Figure 1; Table 1). Across all years tested, the following treatment programs were similar to the 7-day treatments in numbers of plants infected: chlorothalonil applied every 10-days, chlorothalonil alternated with pyraclostrobin applied every 10-days and according to TOM-CAST 10-DSV and 15-DSV schedules, and copper hydroxide alternated with pyraclostrobin applied according to TOM-CAST 10-DSV (Table 1). However, according to the AUDPC data, only chlorothalonil alternated with pyraclostrobin applied every 10 days or according to TOM-CAST 10- and 15-DSV schedules and copper hydroxide alternated with pyraclostrobin applied according to TOM-CAST 10-DSV provided consistent season-long protection similar to the 7-day schedules (Table 1). Phytotoxicity was not observed with any of the treatments.

Regardless of the fungicide program, the fresh weights of the drupes were significantly higher for treatments sprayed every 7 days compared with all other treatments and the untreated control (Table 2). The seed weight was significantly greater for the treatments applied every 7 days, regardless of fungicide program, when compared with the untreated control. The weight of the seed from plots treated with chlorothalonil alternated with pyraclostrobin every 10 days or according to TOM-CAST 10- and 15-DSV schedules was similar to that resulting from the 7-day schedule (Table 2) but was not significantly different from the untreated control. Similarly, copper hydroxide alternated with pyraclostrobin applied every 10 days or according to TOM-CAST 10-DSV resulted in seed weight similar to the 7-day schedule but was not significantly different than the untreated control.

After the drupe tissue was incubated for 48 h, *A. alternata* and *A. panax* were isolated from all treatments (Table 3). Only copper hydroxide alternated with pyraclostrobin applied to plants every 10 days significantly reduced the incidence of *A. panax* on drupes and seed coats compared with the untreated control. *A. panax* was not detected on any of the seed endosperms, regardless of treatment (*data not shown*). *A. alternata* was commonly found on the drupe, seed coat, and endosperm tissue. Only a 7-day treatment of chlorothalonil alternated with pyraclostrobin significantly reduced the occurrence of *A. alternata* on the drupe and seed coat compared with the untreated and all other treatments.

Table 1. Effect of fungicide programs and application schedules on the number of infected plants and the area under disease progress curve (AUDPC) values for management of *Alternaria panax* on ginseng during the 2005 to 2007 growing seasons

Treatments[w]	Number of applications			Number of infected plants[xz]			AUDPC infected plants[yz]		
	2005	2006	2007	2005	2006	2007	2005	2006	2007
Untreated	0	0	0	60.3 b	133.5 b	183.8 c	36.0 b	55.8 b	21.3 e
7-day schedule	16	16	12						
Chlorothalonil				0.0 a	7.8 a	33.0 a	0.0 a	1.7 a	2.1 ab
Chlorothalonil alt. pyraclostrobin				0.0 a	1.8 a	11.5 a	0.0 a	0.4 a	1.1 a
Copper hydroxide alt. pyraclostrobin				0.0 a	0.8 a	19.8 a	0.0 a	0.2 a	1.6 a
10-day schedule	10	12	8						
Chlorothalonil				0.0 a	9.0 a	134.3 ab	0.0 a	2.3 a	8.8 abcd
Chlorothalonil alt. pyraclostrobin				0.0 a	2.3 a	120.0 ab	0.0 a	0.5 a	6.7 abc
Copper hydroxide alt. pyraclostrobin				0.5 a	7.0 a	191.5 c	0.6 a	2.0 a	9.9 bcd
TOM-CAST 10 DSV schedule	10	7	4						
Chlorothalonil				0.0 a	42.3 a	170.5 c	0.0 a	11.5 a	13.7 cd
Chlorothalonil alt. pyraclostrobin				0.0 a	6.5 a	42.5 a	0.0 a	1.6 a	4.1 ab
Copper hydroxide alt. pyraclostrobin				0.0 a	10.5 a	79.3 ab	0.0 a	2.8 a	7.0 abc
TOM-CAST 15 DSV schedule	6	5	3						
Chlorothalonil				0.0 a	26.5 a	175.0 c	0.0 a	9.2 a	13.6 cd
Chlorothalonil alt. pyraclostrobin				0.0 a	21.0 a	80.3 ab	0.0 a	5.1 a	6.4 abc
Copper hydroxide alt. pyraclostrobin				0.0 a	38.5 a	184.8 c	0.0 a	10.5 a	15.4 d

[w] Chlorothalonil was applied at 0.68 kg a.i./ha, pyraclostrobin at 0.07 kg a.i./ha, and copper hydroxide at 0.73 kg a.i./ha.

[x] Mean number of *A. panax*-infected plants per 3 m of treated garden section on the final rating date.

[y] Mean AUDPC values per 3 m of treated garden section on the final rating date.

[z] Column means followed by the same letter are not significantly different (Student-Newman-Keuls, P=0.05).

Table 2. Effects of fungicide programs and application schedule in management of *Alternaria panax* on drupe and seed weight in 2007

Treatments[y]	Fresh weight (g)[z]	
	Drupe	Seed
Untreated	25.7 b	11.1 b
7-day schedule		
Chlorothalonil	515.4 a	118.7 a
Chlorothalonil alt. pyraclostrobin	618.6 a	143.7 a
Copper hydroxide alt. pyraclostrobin	602.9 a	142.9 a
10-day schedule		
Chlorothalonil	67.1 b	21.1 b
Chlorothalonil alt. pyraclostrobin	177.1 b	53.9 ab
Copper hydroxide alt. pyraclostrobin	176.7 b	53.8 ab
TOM-CAST 10 DSV schedule		
Chlorothalonil	47.9 b	20.4 b
Chlorothalonil alt. pyraclostrobin	243.1 b	62.3 ab
Copper hydroxide alt. pyraclostrobin	188.6 b	54.9 ab
TOM-CAST 15 DSV schedule		
Chlorothalonil	44.2 b	16.4 b
Chlorothalonil alt. pyraclostrobin	272.2 b	75.6 ab
Copper hydroxide/ alt. pyraclostrobin	33.1 b	13.9 b

[y] Number of fungicide applications scheduled according to 7-day: 16 (2005 and 2006) and 12 (2007), 10-day: 10 (2005), 12 (2006) and 8 (2007), 10 DSV: 10 (2005), 7 (2006) and 4 (2007) and 15 DSV: 6 (2005), 5 (2006) and 3 (2007) timing intervals. Chlorothalonil was applied at 0.68 kg a.i./ha, pyraclostrobin at 0.07 kg a.i./ha, and copper hydroxide at 0.73 kg a.i./ha.

[z] Column means followed by the same letter are not significantly different (Student-Newman-Keuls, P=0.05).

Table 3. Effect of fungicide programs and application schedules on recovery of *Alternaria* spp. from drupe, seed coat and endosperm tissues in 2007

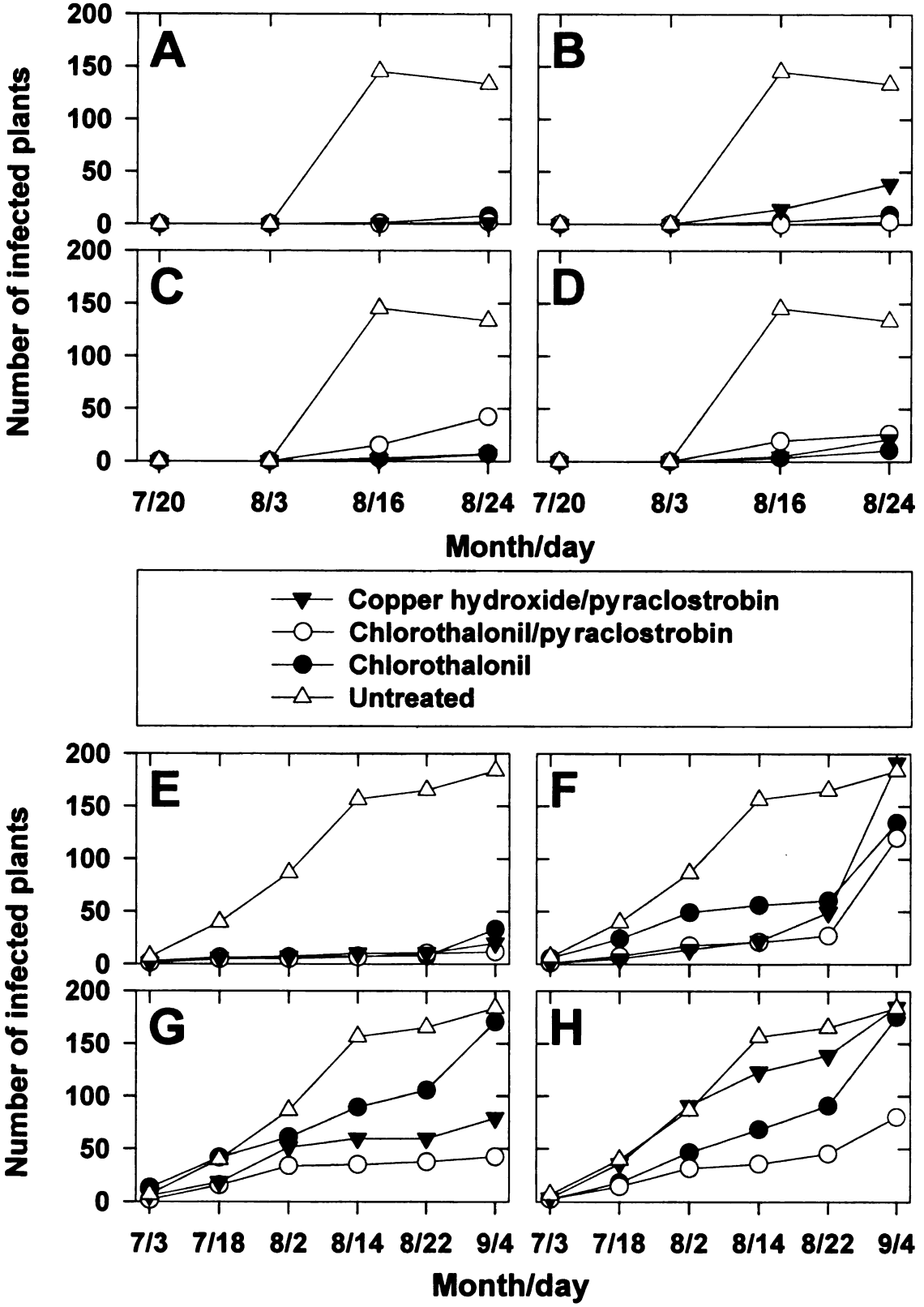
	Alternaria spp. recovered[xy]				
	A. panax		*A. alternata*		
Treatments[z]	**Drupe**	**Coat**	**Drupe**	**Coat**	**Endosperm**
Untreated	100.0 b	100.0 b	100.0 b	100.0 d	67.0 a
7-day schedule					
Chlorothalonil	60.0 ab	60.0 ab	18.0 b	17.5 bcd	11.0 ab
Chlorothalonil alt. pyraclostrobin	45.0 ab	45.0 ab	7.8 a	3.0 a	5.0 ab
Copper hydroxide alt. pyraclostrobin	55.0 ab	55.0 ab	14.0 b	10.0 bc	14.0 ab
10-day schedule					
Chlorothalonil	70.0 ab	70.0 ab	80.0 b	45.0 b	65.0 ab
Chlorothalonil alt. pyraclostrobin	75.0 ab	75.0 ab	70.0 b	87.5 bcd	50.0 ab
Copper hydroxide alt. pyraclostrobin	40.0 a	40.0 a	100.0 b	70.0 bcd	39.0 ab
TOM-CAST 10 DSV schedule					
Chlorothalonil	70.0 ab	70.0 ab	84.0 b	65.0 bcd	37.5 ab
Chlorothalonil alt. pyraclostrobin	97.5 b	97.5 b	100.0 b	100.0 d	85.0 b
Copper hydroxide alt. pyraclostrobin	52.5 ab	52.5 ab	100.0 b	100.0 d	67.5 ab
TOM-CAST 15 DSV schedule					
Chlorothalonil	100.0 b	100.0 b	100.0 b	94.0 cd	37.5 ab
Chlorothalonil alt. pyraclostrobin	100.0 b	100.0 b	100.0 b	84.0 bcd	41.3 ab
Copper hydroxide alt. pyraclostrobin	100.0 b	100.0 b	100.0 b	100.0 d	40.0 ab

[x] Percentage of infected seeds per 3 m of treated garden section.

[y] Column means followed by the same letter are not significantly different (Student-Newman-Keuls, P=0.05).

[z] Number of fungicide applications scheduled according to 7-day: 16 (2005 and 2006) and 12 (2007), 10-day: 10 (2005), 12 (2006) and 8 (2007), 10 DSV: 10 (2005), 7 (2006) and 4 (2007) and 15 DSV: 6 (2005), 5 (2006) and 3 (2007) timing intervals. Chlorothalonil was applied at 0.68 kg a.i./ha, pyraclostrobin at 0.07 kg a.i./ha, and copper hydroxide at 0.73 kg a.i./ha.

Figure. 1. Disease progress curves for *Alternaria panax* infection of 4-year-old ginseng plants not treated or treated with the fungicide chlorothalonil (0.68 kg a.i./ha), chlorothalonil (0.68 kg a.i./ha) alternated with pyraclostrobin (0.07 kg a.i./ha), or copper hydroxide (0.73 kg a.i./ha) alternated with pyraclostrobin (0.07 kg a.i./ha). Fungicide programs were scheduled during the 2006 growing season every, **A,** 7 days, **B,** 10 days, or according to the TOM-CAST disease forecaster using, **C,** 10 DSV or **D,** 15 DSV. Fungicide programs were scheduled during the 2007 growing season every, **E,** 7 days, **F,** 10 days, or according to the TOM-CAST disease forecaster using, **G,** 10 DSV or **H,** 15 DSV.


A
B
C
D
E
F
G
H
200
150
100
50
0
Number of infected plants
7/20
8/3
8/16
8/24
7/3
7/18
8/2
8/14
8/22
9/4
Month/day
Copper hydroxide/pyraclostrobin
Chlorothalonil/pyraclostrobin
Chlorothalonil
Untreated

DISCUSSION

Alternaria leaf blight on ginseng is especially destructive, causing rapid defoliation and plant death when left untreated. Without effective control measures, growers risk reduced root yields or losing entire plantings (5). Wisconsin and Michigan Ginseng growers currently rely on fungicides to manage Alternaria blight during the relatively long growing season of May through September. Ginseng growers must apply fungicide sprays judiciously to protect the crop while complying with the labeled seasonal application limits.

Optimizing fungicide applications by using a disease forecaster with reduced-risk fungicides may offer an integrated pest management (IPM) strategy suitable for ginseng production. A conservative threshold (10-DSV) of TOM-CAST used with an alternating program of chlorothalonil and pyraclostrobin provided control equivalent to 7-day applications. Under moderate disease pressure, fungicides applied according to TOM-CAST 10- or 15-DSVs adequately protected the crop foliage. In 2007, DSVs did not accumulate quickly and fewer fungicide treatments were prompted. Some of the TOM-CAST programs that included pyraclostrobin provided control similar to the 7-day programs. Initial inoculum levels in 2007 may have been higher than in 2005 and 2006, increasing disease pressure.

Although TOM-CAST 15-DSV is a practical and economical alternative to calendar-based applications in asparagus (18), carrot (3), and tomato (6), high-value crops such as celery and ginseng pose unique challenges. Carrot plants can tolerate some Alternaria leaf blight and Cercospora leaf spot (caused by *A. dauci* and *Cercospora carotae*, respectively) as long as they can withstand the mechanical harvesting which

pulls the roots from the ground (3). Also, these foliar pathogens do not typically defoliate the crop as rapidly as observed with *A. panax* on ginseng. Similarly, purple spot of asparagus (incited by *Stemphylium vesicarium*) causes premature browning and defoliation of the photosynthetic tissue but consecutive years of premature defoliation is required before yield is reduced (18). Carrot and asparagus are valued at $3,424 and $2,420/ha, respectively (2), whereas celery and ginseng root average $20,431/ha (2) and $13,930/ha (11), respectively. There is no tolerance for late blight on celery incited by *S. apiicola* because the marketable portion of the celery is affected and consumers demand unblemished product (2). Similarly, *A. panax* cannot be tolerated in a ginseng crop because of its ability to rapidly blight and defoliate the crop, negatively affecting root and seed yield.

Ginseng seed yield and quality is an important consideration when assessing fungicide programs. Current market prices for stratified seed are $55/kg. With an average seed yield of 336.3 kg/ha, each hectare represents a value of $18,537. Even when fungicide programs were adequate, seed yield and quality was not always acceptable. Sprays according to TOM-CAST 10-or 15-DSV or every 10 days resulted in reduced seed weights compared with spraying every 7 days. Future research will test a hybrid program whereby TOMCAST 10 DSV with chlorothalonil and pyraclostrobin is used from plant emergence to flowering (mid- to late June), after which fungicides are applied every 7 days to protect developing seed from becoming infected by *A. panax* inoculum. As ginseng growers harvest their own seed for planting and to sell to other growers, therefore, the potential seed dissemination of *Alternaria* is an important component of overall disease management and must be addressed for an IPM program to

be successful. In our studies, *A. panax* was prevalent on the ginseng drupe and seed coat. Although *A. panax* was not isolated from the endosperm in this study, previous seed samples from ginseng growers submitted for diagnosis have often yielded this pathogen from the endosperm tissue (13). In addition to *A. panax*, *A. alternata* was recovered from ginseng seed. *A. alternata* is often encountered on other ginseng tissue, including stem and leaves submitted for diagnostic evaluation. The impact of *A. alternata* associated with seed tissues on viability and crop health is unknown.

Considering the chemical control measures needed to maintain commercial ginseng gardens, continued efforts to explore methods to reduce sprays without risking crop health are desirable. Alternating applications of fungicides with different modes of action is critical due to restrictions on numbers of applications or amounts of fungicides per season and to delay development of resistance in the pathogen. In our study, alternating chlorothalonil with pyraclostrobin provided protection of the foliage and seed. Because all fungicide programs tested in our project exceeded seasonal label allowances, growers must incorporate additional labeled fungicides (i.e., mancozeb or boscalid) into their management programs. Although these products were not included in this study, field studies have been conducted that tested these fungicides against *Alternaria* and indicated that mancozeb and boscalid would be helpful (12).

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PROPOSAL

FOR FUTURE WORK

Alternaria blight is a significant threat to ginseng (2, 3). If not controlled, Alternaria blight can reach epidemic proportions within a month. The destructiveness of *A. panax* is exacerbated by the cultivation practices for ginseng. To utilize limited land resources and maximize profits, gardens are often densely planted. Dense crop planting, coupled with the use of artificial shade creates a unique microclimate within the plant canopy which can be beneficial to *A. panax* and other foliar pathogens. The primary objectives of this research were to determine (i) *A. panax* inoculum availability and what weather factors influence conidial concentrations, and (ii) if TOM-CAST could be helpful in timing fungicide applications. While summarizing these objectives, other research foci were identified to address garden monitoring, mulch contamination, and additional forecasting systems.

As *A. panax* can overwinter in crop debris and mulch, there is a potential for epidemics in subsequent seasons. Overwintering conidia can also serve as the first inoculum source as plants emerge through infested mulch. In this research, ACCs were detected throughout the monitoring periods. However, conidia may have been present in the atmosphere earlier than May. In the future, spore trapping should include an extended monitoring period to assess inoculum levels prior to crop emergence. It would also be helpful to determine ACCs after natural crop defoliation.

Ginseng cultivation is a no-till system. Currently, there are no practical methods to reduce overwintering inoculum levels on the mulch. A common recommendation is to remove straw mulch in infected areas yearly (1) however, most growers do not find it practical when entire gardens are infected. A modification to this recommendation would be to consider the complete mulch removal in the fall following the second or third

growing season. By moving the straw just once during the cultivation process, growers may benefit from the reduction in overwintered inoculum and the financial costs of replacing straw once as opposed to three or more times would be reduced.

The correlation between weather variables and ACCs is critical to the development of disease models and disease management. Temperatures considered in TOM-CAST range from 13 to 29°C and are based on conidiophore and conidium formation of *A. solani* (4). With the use of a black polypropylene canopy, temperatures within the ginseng gardens occasionally exceeded 29°C. TOM-CAST may require modification to include higher temperatures as observed in commercial ginseng gardens. In addition, placement of the leaf wetness sensor should be evaluated to adequately assess moisture within the ginseng gardens. Prior to modification of TOM-CAST, temperature and moisture requirements for *A. panax* germination and infection of ginseng should be assessed. The infection process and potential treatments for *A. panax* on ginseng seed would also be helpful.

As an alterative to TOM-CAST, the forecasting system Alter-Rater could be tested. Alter-Rater uses a daily point value ranging from 0 to 11 depending on rainfall +/- 2.5 mm, leaf wetness +/- 10 h, and average temperatures <20, 20-28, and >28°C (5). Fungicide applications are then made once a threshold value has been reached. Alter-Rater may be effective in ginseng gardens as it allows for a daily point accumulation with an average day temperature between 20 and 28°C, rain < 2.5 mm and leaf wetness < 10 h, whereas with TOM-CAST, leaf wetness must be present for a DSV calculation.

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