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COMBINING HOST PLANT RESISTANCE AND MANAGED FUNGICIDE APPLICATIONS FOR CONTROL OF LATE BLIGHT IN POTATOES

By

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ABSTRACT

COMBINING HOST PLANT RESISTANCE AND MANAGED FUNGICIDE APPLICATIONS FOR CONTROL OF LATE BLIGHT IN POTATOES

By

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Fluazinam, a new protectant fungicide with excellent residual activity and active in relatively small doses, was applied on potato foliage in combination with cultivar resistance. Three rates [33, 66 and 100% of the manufacturer's recommended application rate (MRAR)] of fluazinam were applied on 5, 7, 10 and 14-day application intervals in 2001; two rates [50% and 100% MRAR] on 5, 10 and 15-day application intervals in 2002 and examined for foliar late blight control. The results of this study showed that reduced amounts of fluazinam were either partially or fully effective at all application rates tested on all cultivars compared to the non-treated controls at P= 0.05. On susceptible cultivars, applications of fluazinam at 10-day spray intervals or above were partially effective for controlling late blight at the doses tested. All cultivars treated at 100% and 50% MRAR of fluazinam were effectively protected against late blight on a 5-day spray interval and partially protected at all other rates and application intervals. Application of fluazinam at reduced rates and frequencies could successfully be used into a control program using host resistance. The impact of plant age and leaf position on susceptibility to potato late blight infection was also assessed. Susceptibility to potato late blight infection decreased with plant age and leaf position; from young plants to the old ones and from leaf 5 to leaf 15 in the cultivars tested.

To my daughter Delitha G. Muhinyuza and mom Winifried Kangabe,

I dedicate this thesis

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Jean-Baptiste Muhinyuza

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GENERAL INTRODUCTION

Phytophthora infestans (Mont.) Anton de Bary (1876), an oomycete that causes late blight of potato (*Solanum tuberosum* L.), is the most important potato disease globally (16). *P.infestans* is thought to have originated in South America (1, 3). Worldwide spread of late blight is enhanced by global trade (3). The pathogen attacks potatoes both in the field and during storage (2). Late blight is also an important disease of tomato (*Lycopersicon esculentum* Mill.) (18) and is also found on other wild plant species, mainly Solanaceous hosts (14).

Potatoes are very important worldwide, the fourth most important food crop and are essential for providing nutrition in a growing world population (39). It produces much energy, protein and contains substantial amounts of vitamins and minerals (48). At the turn of 21st century world potato production was estimated at 135 million tons (24).

However, potato late blight is considered to be the major constraint to potato production all over the world. The economic impact of potato late blight is compounded by costly fungicides; their application costs and the limited acceptance of late blight resistant/tolerant varieties because they do not meet the qualities that growers and consumers expect such as high yield, multiple resistances and cooking qualities (47).

Unchecked, potato late blight spreads very quickly given the polycyclic production of secondary inoculum, which can result in great yield losses (51). In developing countries, yield loss due to late blight was estimated at US $2.75 billion each year (7). Late blight is mainly controlled by fungicide applications (37). For example, in the U.S. the annual total cost of fungicides to protect potato crops against late blight disease is estimated at $77.1 million (20) and $740 million in developing countries (7).

It was estimated that 25% of total fungicide usage worldwide was for the control of potato late blight (14).

Chemical control of potato late blight with fungicides is becoming more challenging due to the appearance of new more aggressive forms of *P. infestans* that are difficult to control because they are insensitive to commonly used fungicides (18, 40, 44). In addition, there is an increasing concern about the intensive use of pesticides. Therefore, control of potato late blight has to be compatible with the current trend to use an integrated approach to disease management. This approach incorporates legislative, cultural, and chemical intervention as well as host resistance and is intended to minimize pesticide usage to appropriate levels in order to avoid negative effects of pesticides on the environment and human health. To address these issues, modern fungicides need to be effective at a high level and long duration of efficacy, in addition to minimizing environmental impacts.

Breeding for resistance has resulted in varieties more tolerant to *P. infestans* but complete resistance has not yet been achieved to control the disease without additional protection with fungicides (5, 47). There are some varieties with partial resistance but fully resistant varieties with desirable agronomic qualities have not been developed (47). Following the occurrence of new and more virulent strains of *P. infestans*, Fry (15, 16), observed that the use of protective fungicides could complement cultivar resistance to reduce foliar potato late blight. Kirk et al. (26) stated that reduced application rates and frequencies of a protectant residual fungicide could be successfully incorporated into a control program using host resistance.

The objectives of this research were to determine effective reduced fungicide rates and increased application intervals to control potato late blight using potato germplasm with a range of susceptibility to late blight and to assess resistance to late blight in relation to foliage maturity such as plant age and leaf position in some resistant and susceptible potato cultivars grown both in controlled and field environment.

Although some resistant cultivars are available, they need to be protected from an early stage of development. It has been reported that leaves of even more resistant cultivars can be susceptible to late blight (10) and therefore the application of fungicides may be justified because their resistance is not complete. Similar work has been done with some European cultivars (10); however, it was necessary to complete a limited version of the European experiment with US cultivars and a local isolate of *P. infestans*.

The approach developed in the fungicide trials will potentially be applied later in Rwanda to evaluate the efficacy of fungicides on resistant/tolerant varieties under the conditions of Rwanda with the purpose to be incorporated later into an integrated potato late blight control program in Rwanda. Potatoes grow well in several parts of Rwanda and play an important role in the economy of Rwanda as a cash crop and the diet of Rwandan people (38). In most areas at least two major crops are planted each year but the production is severely limited by late blight, the major constraint to the potato crop production in the country (38).

CHAPTER ONE

LITERATURE REVIEW

POTATO LATE BLIGHT

IMPORTANCE

Potato late blight is best known historically as the cause of the great Irish potato famine during the 1840s (37). The disease devastated the potato crop, the main staple crop in Ireland at that time. Potato tubers rotted in the field and in storage and this led to starvation and death of about 10% of the population (1.3 million). Many emigrated from Ireland to the United States and elsewhere in North America (2, 37).

At that time, microorganisms were believed to be the result rather than the cause of diseases. The role of fungi as the causative agents of plant diseases was not fully understood. Following the Irish potato famine, Anton de Bary (1876) confirmed that the oomycete *Phytophthora infestans* was the primary cause of the disease, potato late blight (34, 37).

Despite many efforts made by plant pathologists since to control the disease, potato late blight is still a major challenge in potato production. This is thought to be due to the appearance of new genotypes of *P. infestans* that are difficult to control in potato plants (15, 16). The appearance of novel genotypes is thought to have occurred during the 1970's through the import of potatoes from Mexico [the origin of *P. infestans* (18)] to Europe and during the early 1990's to North America (11, 33).

Control strategies developed over the past 150 years have failed to eliminate potato late blight completely. Thus, control of potato late blight relies on well-timed application of protectant fungicides in susceptible cultivars and moderately resistant varieties to prevent great yield losses (37).

DISEASE DEVELOPMENT

Phytophthora infestans is a biotrophic pathogen and over-winters as mycelium in potato tubers in storage to be used for seed, tubers that are inadvertently not harvested and left in fields, and in discarded piles of culled potatoes (12). *P. infestans* can also survive in soil as a sexually originated oospore (12, 35). However due to the predominance of the A2 mating type in North America, this over-wintering source is thought to be of limited importance. The thick walled oospore is a sexual spore resulting from conjugation of two mating types normally designated A1 and A2 (2, 45). Mycelia within potato tubers and possibly but rarely oospores serve as the primary inoculum for seasonal epidemics of potato late blight. Both asexual and sexual stages produce sporangia. Sporangia produced on infected leaves are carried by wind or splashed by water to the foliage of new plants, which germinate and initiate an epidemic (2).

Potato late blight is a temporally sporadic disease that occurs only when microclimate conditions within the canopy are favorable and inoculum is present (28). Conducive environmental conditions include air temperatures between 7 and 27°C and relatively long periods (10 hours or more) of leaf wetness. Favorable conditions for infection and development coincide with periods of high moisture (RH>90%), prolonged periods of leaf wetness and moderate temperatures (15-25°C); (2, 22).

During these conditions, sporangia can cause new infections and lead to a polycyclic epidemic due to the rapid production of asexual generations of secondary inoculum of the pathogen (51). Crop loss occurs through destruction of foliage and consequent reduction of photosynthetic capacity and tuber infection by spores that are washed down from leaves into the soil to enter tubers through wounds, lenticels and directly through the periderm of the tuber.

CONTROL AND MANAGEMENT

INTEGRATED DISEASE MANAGEMENT

No single measure used alone can successfully control late blight in potatoes (37). Integrated strategies such as utilization of resistant and/or tolerant varieties; certified late blight free-seed, limitation and avoidance of conducive environments, climatic monitoring and disease prediction are some agronomic practices that can be utilized for control of potato late blight. The use of resistant or tolerant varieties combined with additional control strategies such as cultural practices and well-applied fungicides can together limit crop and yield losses and maintain potato late blight at acceptable economic threshold levels (17, 39).

Breeding for resistance to *P. infestans* has resulted in resistant varieties and new resistant cultivars are continuously being released (47) but varieties that have durable and consistent resistance has not fully succeeded.

P. infestans is a genetically versatile organism and has overcome both vertical and horizontal resistance in *Solanum tuberosum*. Therefore cultivars with intermediate resistance would need to be complemented with additional protection by fungicide treatment in an integrated manner to provide better control of potato late blight (47).

Cultural practices include destruction of crop residues, disposal of culled tubers, management practices to avoid leaving volunteer tubers in the field at harvest, and appropriate rotations. All these practices are intended to reduce potential sources of inoculum (17, 39). Additional cultural practices include irrigation management to minimize the duration of leaf wetness periods, hilling of rows to increase depth of tubers in the soil to provide a mechanical barrier to spore penetration, planting when environmental conditions are least conducive to late blight development from seed and harvesting techniques that reduce the risk of spore deposition on tubers.

The application of fungicides is also an integral component of late blight management and possibly key to the reduction of the effects of potato late blight and contribute to prevention of yield loss. Fungicides have to be applied preventatively to increase their effectiveness, but also judiciously to limit their impact on the environment. Thus, scouting and weather-based disease models such as BLIGHTCAST (29), a climate-based model that combines a system based on relative humidity and temperature with a system based on rainfall and temperature are very important in potato late blight management to reduce the misuse of fungicides that encourage application when needed (14, 17, 39).

Microclimate-based late blight models were established originally in the 1940's and fungicide spray programs and other management decisions were based on the accumulation of 'blight favorable days', or thresholds of the disease severity values (DSV) in the 1960's (4, 29, 49, 50). The 90% relative humidity criterion is used for potato late blight monitoring since early analysis of canopy based records of the physical environment in relation to disease development (49).

Average air temperatures and duration of wetting period are combined in the daily assignment of Wallin disease severity values, which have become standard in estimating potato late blight risk (29). BLIGHTCAST model recommends the initiation of fungicide applications after the accumulation of either ten consecutive favorable days or 18 DSV following emergence (29).

CHEMICAL CONTROL

Non-systemic fungicides

Non-systemic or protectant fungicides are chemical compounds that are not taken up by the plant and are subject to weathering-decrease. This necessitates frequent and regular application to maintain protection. Protective fungicides are only active on the plant surface after germination of spores but before the pathogen has entered the host tissue. Therefore these fungicides must be applied before infection to prevent disease (13). They are therefore less effective when the pathogen has already penetrated leaf tissue because they are out of contact with the fungus and unable to interact with the pathogen inside the host (13, 14).

Contact fungicides with residual properties are applied to the plant surface to protect plants before the arrival of inoculum of the pathogen and prevent infection from occurring (Table 1).

The first chemical compound used for crop protection was Bordeaux mixture, an inorganic fungicide consisting of copper sulfate and calcium hydroxide (2). Bordeaux mixture adheres well to potato leaves even after rainfall. However, like all other copper-based fungicides, Bordeaux mixture can be phytotoxic due to copper ions, which are potentially toxic to plants (13, 14).

Copper-based chemicals were widely applied on potato crops until recently when they have been substituted by modern organic protectant fungicides that are less toxic to the foliage (13).

The dithiocarbamates were the first group of organic fungicides used to control potato late blight. The dithiocarbamate anion interacts with thiol groups of enzymes in the pathogen, affecting both spore germination and mycelial growth and prevents penetration of the host (13, 39). The dithiocarbamates continue to be used in potato late blight disease control programs and include compounds such as mancozeb, maneb, metiram and zineb. Known as ethylenebisdithiocarbamates (EBDCs), they are extensively used in mixtures with systemic fungicides to supplement efficacy (13).

Following the discovery of the dithiocarbamates, several classes of other protectant fungicides including tin compounds and phthalonitriles were developed.

Among the organic tin compounds, the most important are fentin acetate and fentin hydroxide, triphenyltin is the active component.

They are considered effective as protectant fungicides and are somewhat curative against actively sporulating *P. infestans* on foliage and may therefore prevent tuber blight (13). They have antisporulant activity and provide tuber protection from sporangia spread but they can be phytotoxic when applied to developing foliage (13). Triphenyltins are mixed with the dithiocarbamates and applied late in the season when an active disease epidemic occurs during the late stages of potato crop development (13, 14).

Among the phthalonitriles, chlorothalonil is the only commercial product available. Chlorothalonil is a protectant foliar fungicide that can be used in reduced rates in combination with a dithiocarbamate to provide effective control of foliar blight by preventing spore germination and penetration (13).

Fluazinam is the most commonly used fungicide in the recent group of pyridinamines (13). It is a protectant fungicide with an excellent residual activity (13). Fluazinam is used for control of a wide group of pathogenic fungi and water molds including white mold (*Sclerotinia minor*) on peanuts, white mold (*Sclerotinia sclerotiorum*) and late blight (*P. infestans*) in potatoes (46). It is active in relatively small doses and inhibits spore germination and infection (16).

Fluzinam is a broad spectrum fungicide with a multi-site mode of action. It is a powerful uncoupler in oxidative phosphorylation and inhibits proton transfer across the mitochondrial membrane (21). It may be used to prevent or delay resistance to single-site fungicides. It is also reported to have better persistence on the leaf surface compared to other contact fungicides (46). When applied to potato foliage in combination with cultivar resistance, fluazinam has been shown to be active at reduced rates and increased spray intervals to control potato late blight (13, 26).

The product was recently registered in the US and it is still being tested for its efficacy to control plant diseases including potato late blight.

Table 1. Recommended protectant fungicides for the management of potato late blight.

Chemical class	Mode of action
Coppers - copper oxychloride 50% WP - copper hydroxide 50% - copper oxide 50% WP	Nonspecific denaturation of proteins and enzymes. Inside the spore, the Cu^{2+} may link to different chemical groups such as imidazols, carboxyls, phosphates, sulphidryls, aminae or hydroxyls to produce a toxic effect that disturbs the normal cellular activities.
Dithiocarbamates - EBDCs (mancozeb, maneb, metiram ,zineb)	Inactivate –SH groups in amino acids, proteins and enzymes. The dithiocarbamate anion interacts with thiol groups of enzymes affecting both spore germination and mycelial growth.
Organic tins - Fentin acetate - Fentin hydroxide	Inhibition of oxidative phosphorylation in fungal cells.
Phthalonitriles - Chlorothalonil	Cell membrane toxicity
Pyridinamines Fluazinam	Multi-site mode of action. Uncoupler of oxidative phosphorylation and inhibitor of proton transfer across the mitochondrial membrane.

Systemic fungicides

A systemic fungicide is a chemical compound that can be absorbed by the plant tissue and moves within the plant either acropetally, basipetally or both (Table 2). Unlike protectants, systemic fungicides penetrate and act inside the plant to stop the progression of the disease and are not subjected to washing off by rain or irrigation (14). Inside the plant, systemic fungicides interfere with the pathogen, act against the mycelium of the fungus to stop further spread of the disease whereas protectants act upon surface inoculum against spore tissue penetration. Some systemic fungicides act as both protectant and curative. They are able to protect uninfected potato and cure pre-established infections (Table 2).

Systemic fungicides have a specific mode of action and act at a specific biochemical sites controlled by one or few genes. Thus simple changes in target enzyme gene sequence may result in the appearance of populations able to overcome the activity of the fungicide, known as fungicide resistance (43).

In the 1970's systemic fungicides such as the phenylamides for the control of oomycete-caused diseases were developed (14).

The following chemical groups, cyanoacetamide-oximes, phosphonates, carbamates and phenylamides were introduced for the control of oomycetes.

Carbamates such as propamocarb hydroxide are not well translocated and thus their systemic activity is limited (17, 36).

Phosphonate compounds such as fosetyl-Al and phosphorous acid are more effective on *Phytophthora* tree root diseases than on *P. infestans* (14).

Cymoxanil is an acetamide compound, trade name Curzate (® DuPont), and the only commercial fungicide available in the cyanoacetamide-oximes group. Cymoxanil is used to protect and to cure potato foliage from *P. infestans* infection. This foliar fungicide penetrates the potato leaf locally and is effective against *P. infestans* (14). Cymoxanil also acts as a protectant fungicide and inhibits zoospore release and formation of appressoria. Cymoxanil is moderately curative when it acts as a systemic fungicide and inhibits hyphal growth (25). The fungicide is quickly metabolized in plant tissue and it is used in mixtures with non-systemic fungicides to provide a synergistic effect (14). Cymoxanil controls potato late blight during its incubation period and limits potato late blight damage to the crop.

The phenylamide fungicides are highly and specifically active on the members of Peronosporales. They have been very efficient against *P. infestans* since their introduction in commercial use in 1978. Potato growers all over the world relied on them to control potato late blight. Metalaxyl is the most important phenylamide used to control potato late blight (14). When applied on roots or lower foliar portions of potato plant, the fungicide rapidly penetrates the plant tissue and it is acropetally translocated within the plant to protect all the foliage including new growth against the pathogen (9). Metalaxyl inhibits fungal growth and controls both previous and post infection in potato plants (6). Phenylamide sprays are recommended in the early season or during the period of active vegetative growth of the crop. Novel genotypes of *P. infestans* resistant to metalaxyl are now reported in the US and elsewhere in the world (18, 19, 23, 27, 31, 32, 41, 42).

Following the development of resistance to the phenylamides, dimethomorph, a new curative fungicide was introduced which is effective against some species of *Phytophthora* including *P. infestans* (14). The product is a morpholine fungicide known by the trade name Acrobat (® BASF). It has been reported to have excellent antisporulant activity and can prevent sporangia and oospore production (13). It is active against metalaxyl-resistant isolates of *P. infestans*. Dimethomorph acts on the biogenesis of the pathogen cell wall and has been shown to be most effective in mixture with a protectant fungicide such as chlorothalonil or mancozeb to control *P. infestans* (8, 14).

Table 2. Recommended systemic fungicides for the management of potato late blight.

Chemical class	Mode of action
Cyanoacetamide-oximes - Cymoxanil	Inhibition of multiple cellular processes leading to inhibition of zoospore release, formation of appressoria and hyphal growth.
Phenylamides - Metalaxyl	Inhibition of RNA synthesis.
Morpholine - Dimethomorph	Inhibition of sterol production; disruption of fungal cell wall formation.

CONCLUSION

Potato late blight remains the most important and challenging potato disease worldwide and was the cause of the Irish famine in the 1840's. From that time, many efforts have been made to contain the disease and this gradually reduced the impact of late blight in the world potato growing areas.

An integrated approach for control of potato late blight including application of fungicides, host resistance, cultural practices and climate-based prediction models have been developed over the past 150 years.

The emergence in the 1970-90's of new more aggressive strains of *P. infestans* able to overcome previously tolerant potato varieties and resistant to frequently used fungicides augmented efforts to search for better replacement products and develop new, agronomically acceptable, late blight-tolerant potato cultivars. Novel compounds effective against potato late blight such as fluazinam were released during the last decade in order to overcome the new genotypes of the pathogen.

Disease forecasting methods have improved both the timing of application and type of fungicide appropriate to maximize late blight control.

It is clear that no single approach to a universal control method for late blight will be successful, however an integrated approach based on all known and partially successful management techniques should result in a more stable production system.

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CHAPTER TWO

COMBINING HOST PLANT RESISTANCE WITH MANAGED FUNGICIDE APPLICATIONS FOR CONTROL OF POTATO LATE BLIGHT

INTRODUCTION

Potato late blight, caused by *Phytophthora infestans,* continues to cause losses in potato production areas throughout the world. This situation is in part due to the resistance developed by some strains of *P. infestans* to phenylamide fungicides that were previously efficient and widely applied to control the pathogen (15). The increased aggressiveness and phenylamide resistance of novel genotypes of *P. infestans* (22) resulted in an intensification of research on new control strategies based mainly on the development of host resistance combined with the managed application of foliar fungicides. The problem is compounded by global demand to decrease reliance on pesticides and their perceived impact on environmental quality. Moreover, the cost of fungicides used to protect potato crops against potato late blight is relatively high (5, 11, 19). Therefore, there is a need to find effective control methods to manage potato late blight in a safe, environmentally benign manner and at low cost.

It has already been observed that a combination of cultivar resistance and regular applications of protective fungicides based on forecasting techniques reduces foliar potato late blight and chemical input in agricultural systems (13, 14, 16, 21). However, the active ingredients of the commonly used fungicides, such as chlorothalonil and mancozeb, have recently been identified as B2 carcinogens and their use may be curtailed or discontinued in food crops in the future (18).

In response, the agrochemical industry in co-operation with legislative bodies within governments, such as the Environmental Protection Agency (EPA) in the US, have recently adopted an approach of developing crop protection products that are active at low rates, durable and have a broad spectrum of targets.

Fluazinam, is such a product and was registered in the US as a product likely to have less environmental impact than some older chemistries (26). In addition, the mode of action of fluazinam, which is an uncoupler of the oxidative phosphorylation in the respiration chain involving protonation/deprotonation has a minimal risk of resistance development and was internationally introduced for control of several plant diseases including potato late blight (*P. infestans*) and white mold (*Sclerotinia sclerotiorum*); (2, 25). Recently, fluazinam was compared to the commercial standard fungicide, chlorothalonil, applied at reduced rates and increased application intervals on selected potato cultivars and advanced breeding lines (ABL) from the Michigan State University (MSU) potato breeding program with a range of susceptibility to late blight. Chlorothalonil and fluazinam were not found to be significantly different with respect to control of late blight at comparative application rates and frequencies on any of the cultivars and advanced breeding lines (20).

Decreased emphasis on regionalization within the US has resulted in increased co-operation between potato research and extension programs with common geographic and climatic characteristics. Such a program has come to be known as the Quad State Program in which potato producing states within north central US share research findings and recently has resulted in testing of common germplasm across locations.

An example of this co-operation has resulted in the inclusion of potato cultivars and advanced breeding lines from four of the Land Grant University potato-breeding programs in the USA: Michigan, Minnesota, North Dakota and Wisconsin.

It was recommended by Kirk et al. (21) that prior to release of new potato varieties; trials should be conducted to determine appropriate crop protection recommendations, particularly for potato late blight.

The objective of this study was to determine the susceptibility of different cultivars and advanced breeding lines from the Quad State breeding programs and test the efficacy of reduced rates of fluazinam applied at different frequencies on the foliage of these cultivars and advanced breeding lines against the predominant genotype of the pathogen *P. infestans* in north central US [US8, A2 mating type, metalaxyl insensitive (17)].

MATERIALS AND METHODS

Field experiments were conducted at the Muck Soils Research Farm in Bath, Clinton County, Michigan in 2001 and 2002. After being plowed to 20 cm depth during October following harvest of preceding crops, soils were prepared for planting with a mechanical cultivator in early May and fertilizer applied during final bed preparation on the day of planting. Cultivars and advanced breeding lines were planted in June 2001 and 2002 in two-row by 8 m plots (0.9 m row spacing). Fertilizers were applied in accordance with results from soil testing carried out in the spring of each year and about 250 kg N. ha^{-1} (total N) was applied in two equal doses at planting and hilling. Additional micronutrients were applied according to petiole sampling recommendations in both years. Approximately 0.2, 0.3 and 0.2 kg. ha^{-1} boron, manganese and magnesium, respectively were applied as chelated formulations.

Cut and whole seed pieces (75-150g) of selected cultivars and advanced breeding lines were used in all experiments. When percent relative humidity (RH) fell below 80% (measured with RH sensors mounted within the canopy, described below), a mist irrigation system (described below) was turned on to maintain RH at > 95% within the plant canopy.

Plots were irrigated with turbine rotary garden sprinklers (Gilmour Group, Somerset, PA, U.S.A.) at 1055 l H_2O ha/hr and managed under standard potato agronomic practices as necessary to maintain canopy and soil moisture conditions conducive for development of foliar late blight (24).

Weeds were controlled by hilling and with metolachlor at 2.3 l.ha^{-1} 10 days after planting (DAP), bentazon salt at 2.3 l. ha^{-1}(20 and 40 DAP) and sethoxydim at 1.8 l.ha^{-1} (60 DAP). Insects were controlled with imidacloprid at 1.4 kg. ha^{-1}(at planting), carbaryl at 1.4 kg. ha^{-1} (31 and 55 DAP), endosulfan at 2.7 l. ha^{-1} (65 and 87 DAP) and permethrin at 0.56 kg. ha^{-1} (48 DAP).

PATHOGEN PREPARATION AND INOCULATION

A single isolate of *Phytophthora infestans* (Pi 95-7; US8 genotype, metalaxyl resistant; A2 mating type), the predominant biotype of the pathogen in North America (6) was grown on rye agar plates (7) for 14 days in the dark at 15°C.

Sporulation media were made of modified rye B agar (4) consisting of the filtrate of pre-rinsed rye (*Secale cereale* L.) seeds (100.0 g. l^{-1}) boiled for 1 hour, de-ionized (di) H_20 added to a final volume of 1.0 l, glucose (7.5 g. l^{-1}) , β-sitosterol (0.05 g. l^{-1}) and agar (15.0 g. l^{-1}).

The mycelial/sporangial mat was rinsed in cold (4°C) sterile, distilled water and scraped from the agar plate surface with a rubber policeman. Sporangia were counted with a hemacytometer and the final concentration was adjusted to 1x10^4 sporangia. ml^{-1}. Sporangial cultures were incubated for 2-3 hours at 4°C to stimulate zoospore release. Plots were inoculated by injecting a zoospore suspension of *P. infestans* into the irrigation water feed pipeline under 0.5kg. cm^{-2} CO_2 pressure and applied at a rate of about 150ml of inoculum solution. m^{-2} trial area.

The amount and rate of inoculum applied was estimated from prior calibration of the overhead irrigation system and was intended to expose all potato foliage to inoculum of *P. infestans*.

PLANT MATERIAL

Ten potato cultivars advanced breeding lines, Jacqueline Lee, Snowden, Dakota Pearl, Dakota Rose, Torridon, MN 19350, MN 1915, MNA 157-4, W 1355-1, and W 1386, previously identified with different responses to foliar late blight, were evaluated in this study. Cultivars Jacqueline Lee and Snowden were used, respectively, as the resistant and susceptible standards following five years of testing at Michigan State University (7, 8, 9, 10).

FUNGICIDE APPLICATIONS

Fluazinam 5SC (non-commercial formulation, ISK Biosciences Corporation, 5966 Heisley Road, PO Box 8000, Mentor, OH 44061-8000) a residual contact fungicide was used for this experiment. The manufacturer's recommended application rate [(MRAR) 100%] was 0.15kg ai.ha^{-1}. $application^{-1}$ (29).

Fungicide treatments were applied with an ATV rear-mounted spray boom (R&D Sprayers, Opelousas, LA, U.S.A.) that traveled at 1 m/s, delivering 230 L H_2O. ha^{-1} (3.5 kg. cm^{-2} pressure) with three XR11003VS nozzles per row positioned 30 cm apart and 45 cm above the canopy.

The first application of fungicide was made when plants were about 5 cm tall, about 30 days after planting. Potato plants were treated with fungicide until complete defoliation in non-treated plots of susceptible controls.

The fungicide was applied at a 5, 7, 10 and 14-day spray intervals at 33, 66 and 100% MRAR in 2001 and at a 5, 10 and 15-day spray intervals at 50 and 100% MRAR in 2002. The rate was adjusted to 50% MRAR after the fungicide omega (® Syngenta) was introduced commercially in 2002. The 33% rate was below the legal recommended rate of application.

EXPERIMENTAL DESIGN, DISEASE EVALUATION AND DATA ANALYSIS

The field trials were planted as described above into a completely randomized plot design into plots with three replicates due to limitations of seed supply.

Late blight symptoms were detected about 7 days after inoculation. Each plant within each plot was visually rated at 3-5 day intervals for percent leaf and stem area with late blight lesions. The mean percent blighted foliar area per treatment was calculated. Evaluations continued until untreated plots of susceptible cultivars were 100% blighted.

For all plots and assessment dates, the area under the disease progress curve AUDPC (3) was calculated and the relative area under the disease progress curve [RAUDPC (1)] was used to estimate the relative severity of foliar late blight using the following formula:

$$RAUDPC = \frac{\sum (T_{i+1} - T_i) * \left(\frac{D_{i+1} + D_i}{2} \right)}{T_{Total} * 100}$$

Where T_i was the i^{th} day after inoculation when an estimation of percent foliar late blight was made, D_i was the estimated percentage of area with blighted foliage at Ti and T_{Total} was the number of days after inoculation at which the final foliar assessment was recorded.

Data were analyzed by ANOVA and all pair-wise comparison using Fisher's least significant differences (LSD) at $\alpha = 0.05$ were calculated (SAS-Institute 2003. Statistical Analysis Software v.8.2, Cary, NC.). Cultivars or advanced breeding lines with foliar late blight severity not significantly higher than that of treated Jacqueline Lee were classified as late blight resistant (R); susceptible (S) if the severity was significantly higher or not significantly different from that of non-treated Snowden; and moderately resistant (M) when foliar late blight severity was significantly higher than that of Jacqueline Lee but significantly lower than that of non-treated Snowden.

A fungicide treatment on a cultivar that resulted in a RAUDPC value not significantly higher than that of treated Snowden with a full rate of fluazinam at a 5-day spray interval (2001 and 2002) was classified as effective late blight control (E).

Any fungicide treatment and cultivar combination in which the RAUDPC was significantly higher than that of treated Snowden and significantly less than that of non-treated Snowden was classified as a partially effective treatment (PE). If a fungicide treatment on a cultivar resulted in a RAUDPC not significantly different from that of non-treated Snowden it was classified as a non-effective treatment (NE).

MICROCLIMATE MEASUREMENTS

Climatic variables were measured with a Davis Weather Station (Spectrum Groweather ET Station, Spectrum Technologies, Inc., 2389 W. Andrew Road, Plainfield, IL 60544) equipped with air temperature and humidity sensors located within the potato canopy on site. Microclimate within the potato canopy was monitored beginning when 50% of the potato plants had emerged and ending when canopies of healthy plants reached 100% senescence. The Wallin Late Blight Prediction Model (28) was developed in the Eastern United States under conditions similar to those in Michigan and was adapted to local conditions (1). Late blight disease severity values (DSV) were estimated from the Wallin Late Blight Prediction Model and accumulated from inoculation to final evaluation to estimate the conduciveness of the environment for late blight development.

RESULTS

2001 EXPERIMENT

Table 3. Environmental conditions for late blight development at Muck Soils Research Farm in 2001.

Climatic variables	Month			
	June	July	August	September
Air temperature (°C)				
- Maximum	32.4	35.1	30.0	30.7
- Minimum	17.4	23.1	16.2	15.6
Soil temperature (°C)				
- Maximum	29.8	29.6	26.5	24.7
- Minimum	22.6	24.1	21.6	17.8
Precipitation (mm)	27.90	14.73	6.35	3.05

From 50% emergence to harvest, 81 late blight disease severity values were accumulated (base 80% ambient relative humidity). These conditions were conducive for development of late blight.

Cultivars and advanced breeding lines are ranked in order of increasing RAUDPC in untreated plots (Table 4). Application of fluazinam at full rate at a 5 or 7-day spray interval resulted in an effective control in almost all cultivars and advanced breeding lines (Table 4). The mean RAUDPC value for non-treated Jacqueline Lee and Torridon was about 0.2, which were classified as resistant (Figure 1).

The thresholds used to determine the efficacy of the fungicide and variety combination programs was a RAUDPC value of 2.7 (Snowden MRAR, 5 day interval) and RAUDPC of 37.2 (Snowden, non-treated control); (Figure 1 and Table 4). There was no significant effect of fungicide treatment on resistant cultivars Jacqueline Lee and Torridon (Table 4).

Therefore fungicide treatment and variety combinations with an RAUDPC $\leq$ 6.8 (not significantly different from Snowden, 100% MRAR, 5-day application interval) were defined as effective (E); combinations not significantly different from the non-treated Snowden control (RAUDPC $\geq$ 33.1) were defined as non-effective (NE); and combinations of RAUDPC values significantly different from both standards were defined as partially effective (PE).

W 1355-1 was effectively protected by application of fluazinam on 5-day spray interval at all rates, on 7-day spray intervals at 66% and 100% MRAR fluazinam and on 10-day and 14-day spray intervals at 100% MRAR fluazinam (Figure 2). The disease was partially effectively controlled with 33% MRAR fluazinam applied at 7-day spray interval and at 66% MRAR fluazinam applied at a 10 and 14-day spray intervals in the foliage of this cultivar (Figure 2).

Snowden was effectively protected at 100% MRAR fluazinam applied at 5 and 7-day spray intervals; late blight was partially effectively controlled at all other rates and application frequencies in this cultivar (Figure 3).

MNA 157-4 was effectively protected by the fungicide at 100% MRAR fluazinam at a 5-day spray interval; the disease was partially effectively controlled at all other rates and application frequencies in this advanced breeding line except at 100% MRAR fluazinam on 14-day spray interval where MNA 157-4 was not effectively protected (Figure 4).

Dakota Pearl was partially protected by the fungicide at all rates and application frequencies except at 33% MRAR fluazinam on 7, 10 and 14-day spray intervals. At 66% of the MRAR on 10 and 14-day spray intervals and at 100% MRAR fluazinam on 14-day spray interval, fluazinam did not provide sufficient protection in this cultivar (Figure 5).

Dakota Rose was effectively protected by the fungicide applied at 100% MRAR fluazinam on 5-day spray interval; late blight was partially effectively protected at all other rates and application frequencies in this cultivar (Figure 6).

MN 19350 was effectively protected at 100% MRAR fluazinam applied at a 5-day spray interval and was partially protected at all other rates and application frequencies except at 33% and 66% MRAR on 10 and 14-days application intervals and at 100% MRAR fluazinam on 14-day spray interval (Figure 7).

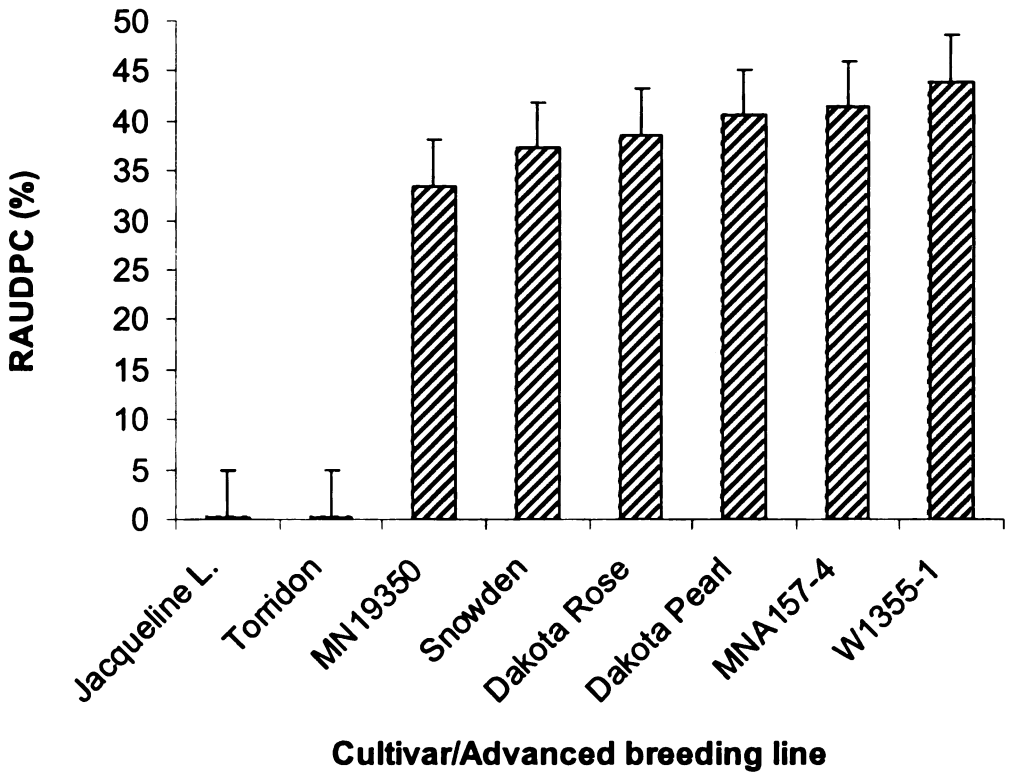

Figure 1. Susceptibility expressed in terms of relative area under the disease progress curve values of different cultivars and advanced breeding lines (untreated) to *P. infestans* in 2001 growing season. Bars represent $LSD_{p=0.05} = 4.60$.

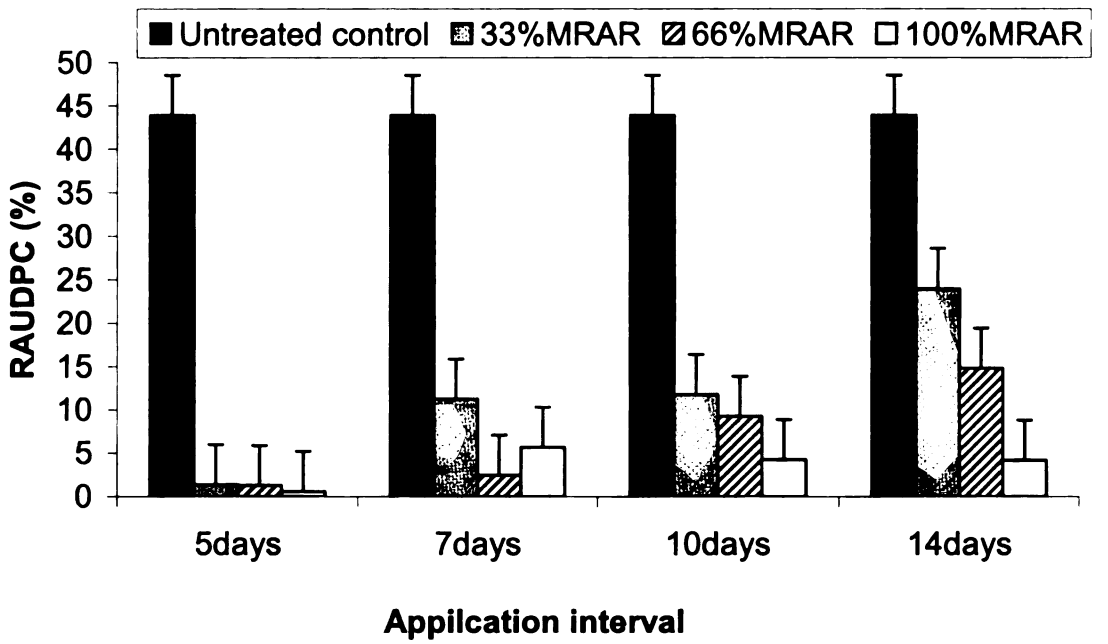

Figure 2. Range of RAUDPC values for W 1355-1 treated with different rates of fluazinam in 2001 growing season. Bars represent $LSD_{p=0.05} = 4.60$.

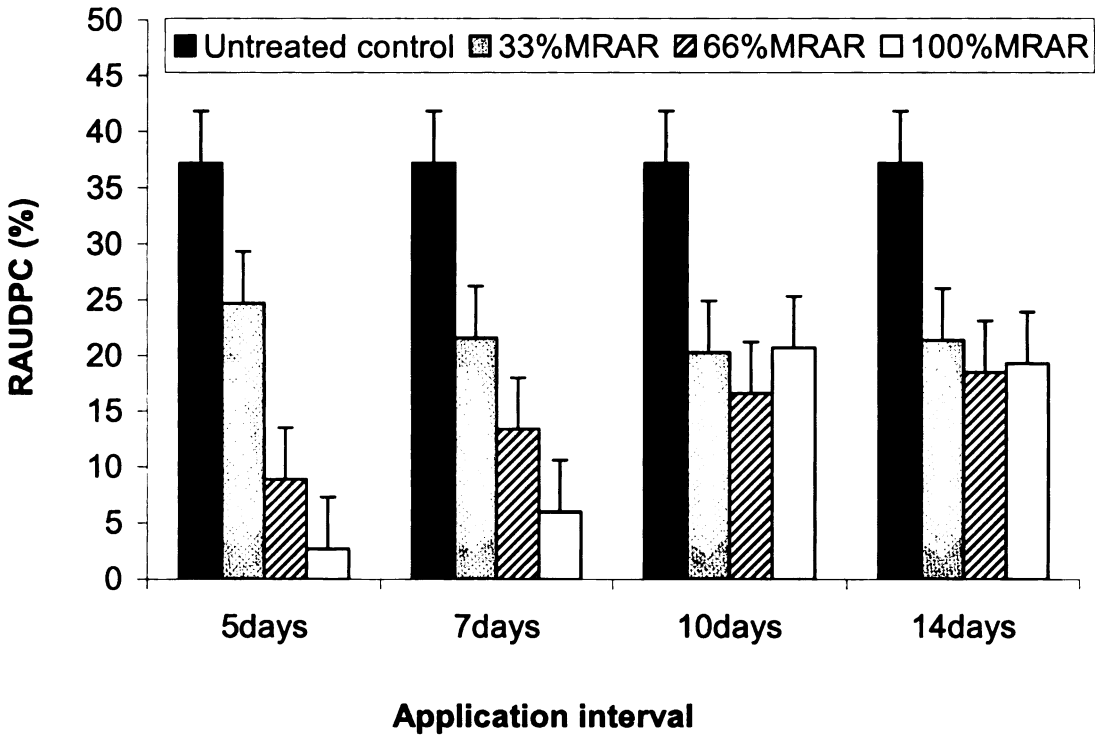

Figure 3. Range of RAUDPC values for cultivar Snowden treated with different rates of fluazinam in 2001 growing season. Bars represent $LSD_{p=0.05} = 4.60$.

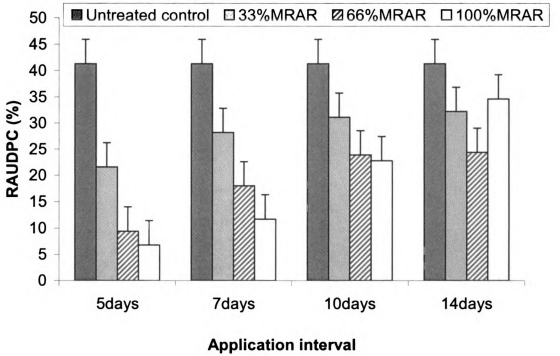

Figure 4. Range of RAUDPC values for MNA 157-4 treated with different rates of fluazinam in 2001 growing season. Bars represent $LSD_{p=0.05} = 4.60$.

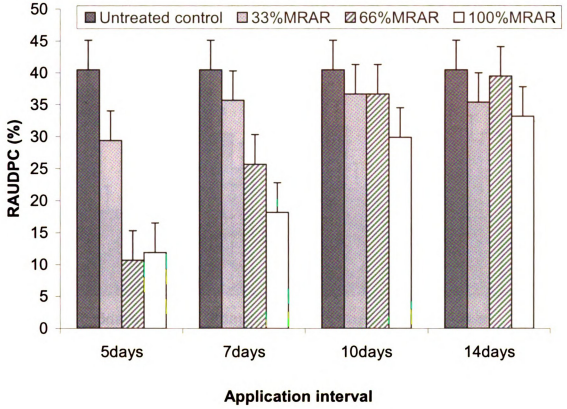

Figure 5. Range of RAUDPC values of cultivar Dakota Pearl treated with different rates of fluazinam in 2001 growing season. Bars represent $LSD_{p=0.05} = 4.60$.

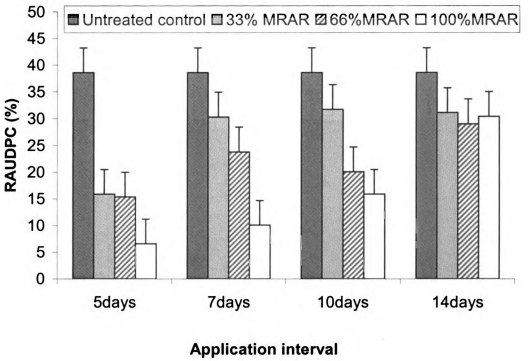

Figure 6. Range of RAUDPC values for cultivar Dakota Rose treated with different rates of fluazinam in 2001growing season. Bars represent $LSD_{p=0.05} = 4.60$.

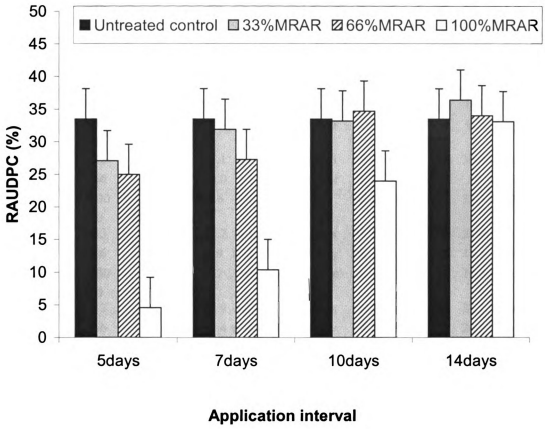

Figure 7. Range of RAUDPC values for MN 19350 treated with different rates of fluazinam in 2001 growing season. Bars represent $LSD_{p=0.05} = 4.60$.

Table 4. Summary of efficacy results: fluazinam applied at reduced rates and frequencies on potato cultivars and advanced breeding lines from North Central US potato breeding programs, MSU 2001.

Cultivar/ABL	Rate of fluazinam[1]	0			5			7			10			14		
		RAUDPC[2]														
Jacqueline Lee	0	0.2	op[3]	R[4]												
	33				0.3	o'p'	E[5]	0.1	o'p'	E	0.06	p'	E	0.06	P'	E
	66				0.2	o'p'	E	0.2	o'p'	E	0.06	P'	E	0.2	o'p'	E
	100				0.06	p'	E	0.1	o'p'	E	0.06	P'	E	0.06	P'	E
Torridon	0	0.2	o'p'	R												
	33				0.2	o'p'	E	0.1	p'	E	0.1	p'	E	0.3	o'p'	E
	66				0.2	o'p'	E	0.2	o'p'	E	0.1	o'p'	E	0.2	o'p'	E
	100				0.2	o'p'	E	0.1	o'p'	E	0.0	p'	E	0.3	o'p'	E
MN19350	0	33.5	f-j	S												
	33				27.1	n-r	PE	31.9	g-l	PE	33.2	f-k	NE	36.4	c-f	NE
	66				25.0	p-t	PE	27.3	m-r	PE	34.7	e-h	NE	34	f-i	NE
	100				4.6	l'-n'	E	10.4	g'-i'	PE	24.0	q-v	PE	33.1	f-k	NE
Snowden	0	37.2	b-f	S												
	33				24.7	q-t	PE	21.6	s-x	PE	20.3	u-y	PE	21.4	t-x	PE
	66				8.9	h'-k'	PE	13.4	c'-g'	PE	16.6	y-c'	PE	18.5	x-b'	PE
	100				2.7	l'-p'	E	6.0	j'-m'	E	20.7	t-y	PE	19.3	w-a'	PE
Dakota Rose	0	38.6	b-e	S												
	33				15.9	z-d'	PE	30.3	i-n	PE	31.7	g-m	PE	31.1	h-n	PE
	66				15.4	a'-e'	PE	23.8	r-v	PE	20.1	v-z	PE	29.0	k-p	PE
	100				6.6	i'-m'	E	10.1	g'-j'	PE	15.9	z-d'	PE	30.4	i-n	PE
Dakota Pearl	0	40.5	a-c	S												
	33				29.4	j-o	PE	35.7	d-g	NE	36.7	c-f	NE	35.4	d-g	NE
	66				10.7	f'-i'	PE	25.7	o-s	PE	36.7	c-f	NE	39.5	b-d	NE
	100				11.9	d'-h'	PE	18.2	x-b'	PE	29.9	i-n	PE	33.2	f-i	NE
MNA157-4	0	41.3	a-b	S												
	33				21.6	s-x	PE	28.2	l-q	PE	31.1	h-n	PE	32.2	g-l	PE
	66				9.4	g'-k'	PE	18.0	x-b'	PE	23.9	q-v	PE	24.4	q-u	PE
	100				6.8	i'-l'	E	11.7	e'-h'	PE	22.8	s-w	PE	34.6	e-h	NE
W1355-1	0	43.9	a	S												
	33				1.4	n'-p'	E	11.3	e'-h'	PE	11.8	d'-h'	PE	24.0	q-v	PE
	66				1.3	n'-p'	E	2.5	m'-p'	E	9.3	g'-k'	PE	14.8	b'-f'	PE
	100				0.6	n'-p'	E	5.7	k'-m'	E	4.3	l'-o'	E	4.2	l'-p'	E

1: Application rate of fluazinam as percent of manufacturer's recommended rate **2**: Relative area under the disease progress curve from inoculation to 100% late blight in susceptible control (Snowden); max = 100.
3: Means followed by the same letter were not significantly different at p = 0.05; comparison between all combinations of fungicide application rate and frequency of application in all cultivars/ABL.
4: Susceptibility of non-treated control to late blight; R = Resistant, not significantly different from Jacqueline Lee (non-treated); S = Susceptible, not significantly different from Snowden (non-treated); M= Moderately resistant, significantly different from both Jacqueline Lee and Snowden (non-treated).
5: Effectiveness of fungicide treatment in comparison to Snowden treated with a full application rate of fluazinam at a 5-day interval or with non-treated Snowden control; E = RAUDPC not significantly different from treated Snowden control; PE significantly different from treated Snowden control and non-treated control; NE = not significantly different from Snowden non-treated control at p = 0.05.

Table 5. Environmental conditions for late blight development at Muck Soils Research Farm in 2002.

Climatic variables	Month			
	June	July	August	September
Air temperature (°C)				
- Maximum	33.4	33.6	31.5	32.9
- Minimum	18	22.5	20.3	18.2
Soil temperature (°C)				
- Maximum	27.8	29.2	29.1	27.9
- Minimum	21.6	23.4	23.4	20.7
Precipitation (mm)	8.13	28.96	10.42	0.0

From 50% emergence to harvest, 100 late blight disease severity values were accumulated (base 80% ambient relative humidity). These conditions were conducive for development of late blight.

Cultivars and advanced breeding lines were ranked in order of increasing RAUDPC in untreated plots (Figure 8 and Table 6). There was no significant effect with fungicide treatment on Jacqueline Lee and Torridon that were classified as late blight resistant (R) with mean RAUDPC value = 0.2 (Table 6). Dakota Pearl, W 1335-1, Dakota Rose and MN 19350 whose mean RAUDPC values were significantly higher than that of Jacqueline Lee and not significantly lower than that of Snowden (non-treated) were classified as moderately resistant (M); (Table 6). The mean RAUDPC values of cultivars W 1386, MN 1915 and Snowden were higher than that of Snowden and were classified as susceptible (S); (Table 6).

The thresholds used to determine the efficacy of the fungicide and cultivar combination were RAUDPC = 1.7 and 34.4. Therefore, any fungicide treatment and cultivar combination with RAUDPC value $\leq$ 5.1 (not significantly different from Snowden with a full application rate of fluazinam at a 5-day spray interval) were defined as effective (E); any combinations with RAUDPC $\geq$ 29.6 (not significantly different from the non-treated Snowden) were defined as non-effective (NE); and any combinations with RAUDPC values significantly different from both standards were defined as partially effective (PE).

All cultivars were effectively protected against late blight by full and half rate applications of fluazinam on 5-day spray interval and partially protected at all other rates and application intervals except Dakota Pearl, which was only effectively protected at full rate of fluazinam on 5-day spray interval (Figures 9-15). Advanced breeding lines W 13551-1 and W1386 were effectively protected by the fungicide application at both full and half rate on 5-day spray interval and also at full rate on 10-day spray interval (Figures 10, 13).

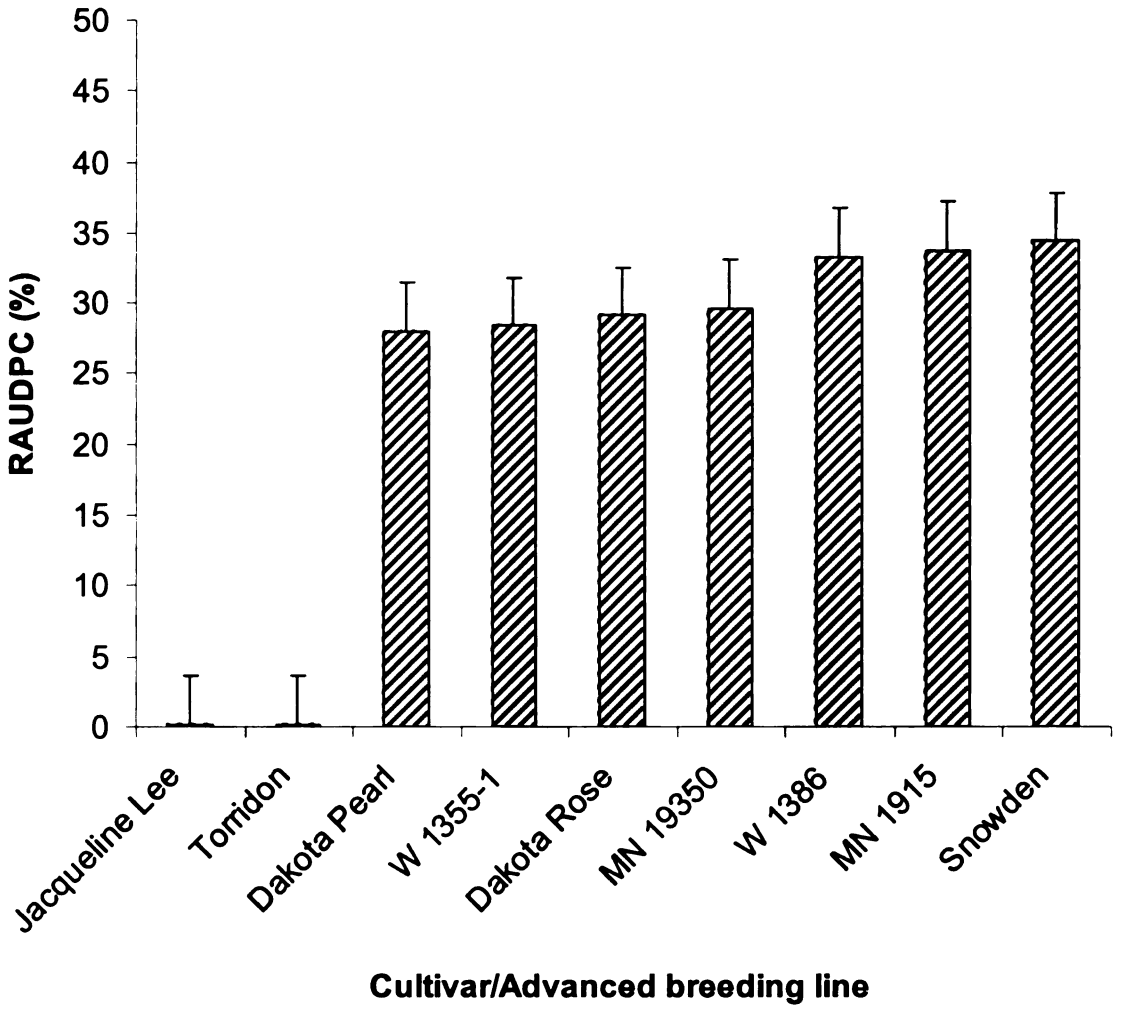

Figure 8. Susceptibility expressed in terms of relative area under the disease progress curve values of different cultivars and advanced breeding lines (untreated) to *P. infestans* in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

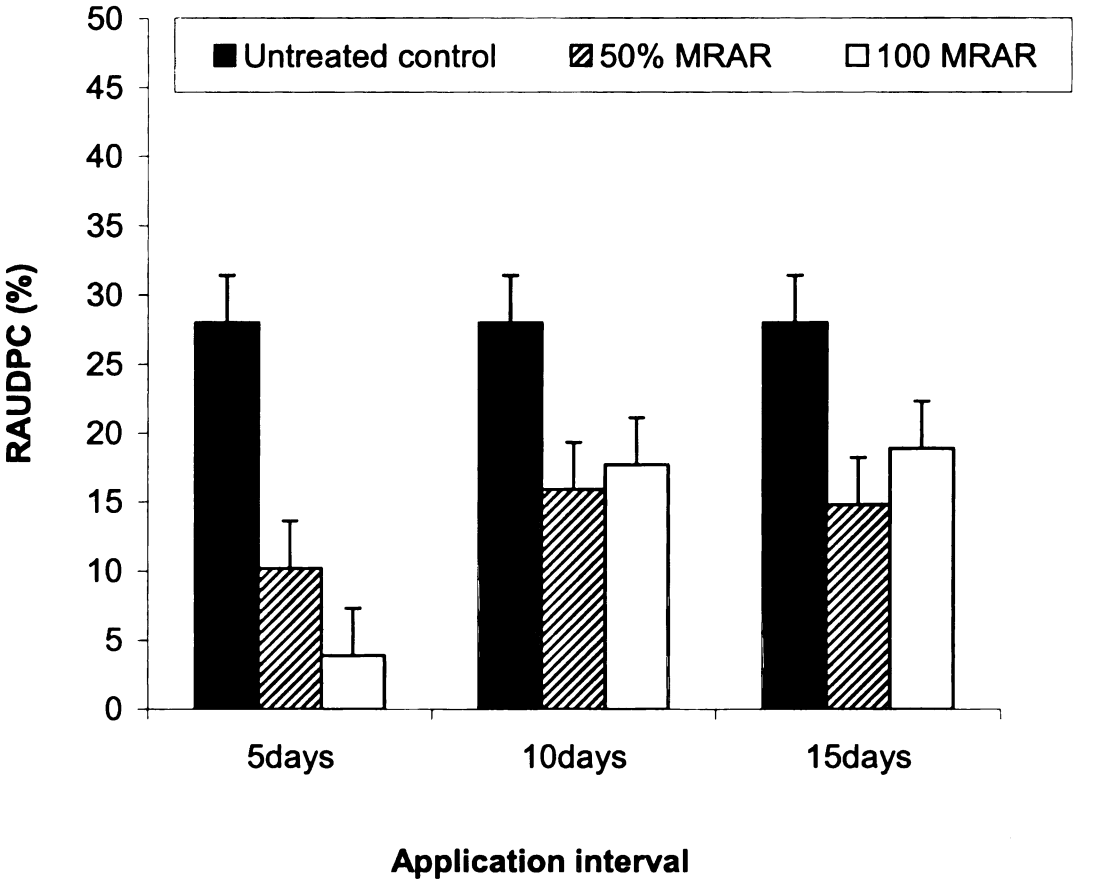

Figure 9. Range of RAUDPC values for cultivar Dakota Pearl treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

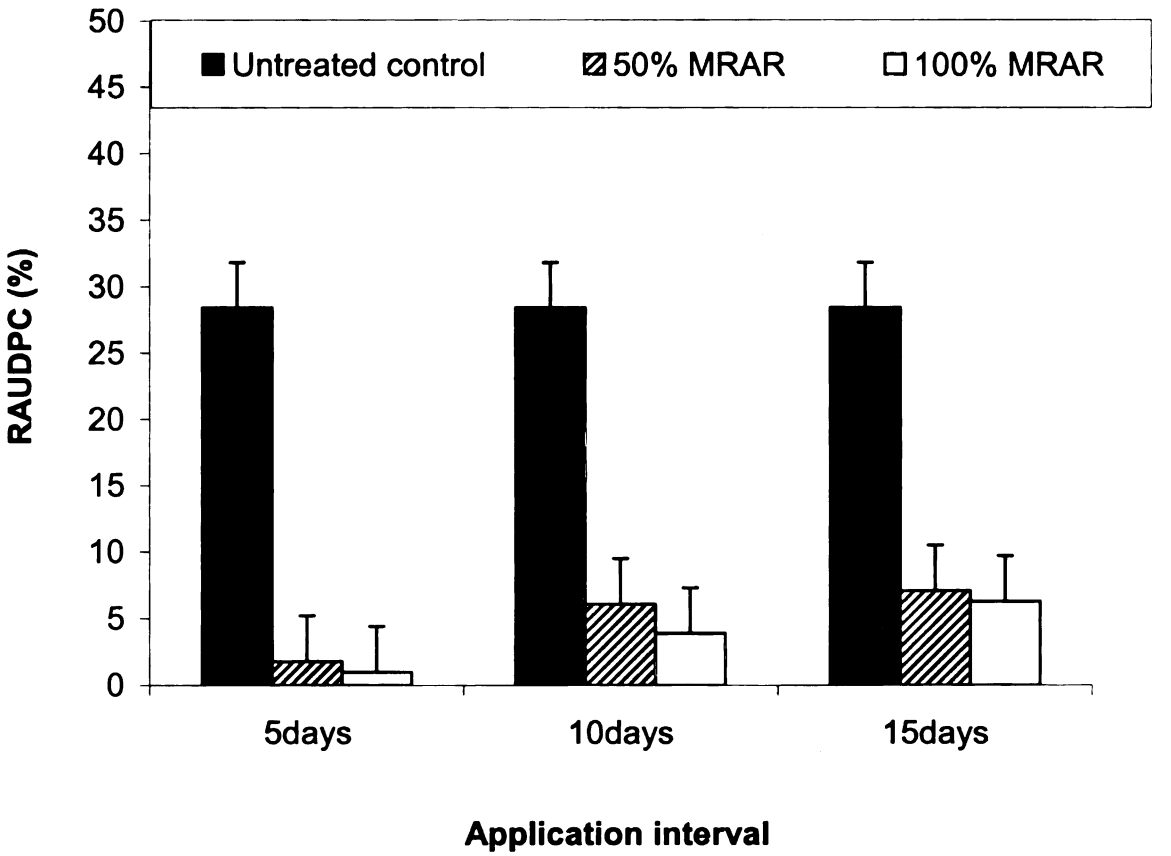

Figure 10. Range of RAUDPC values for W 1355-1 treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

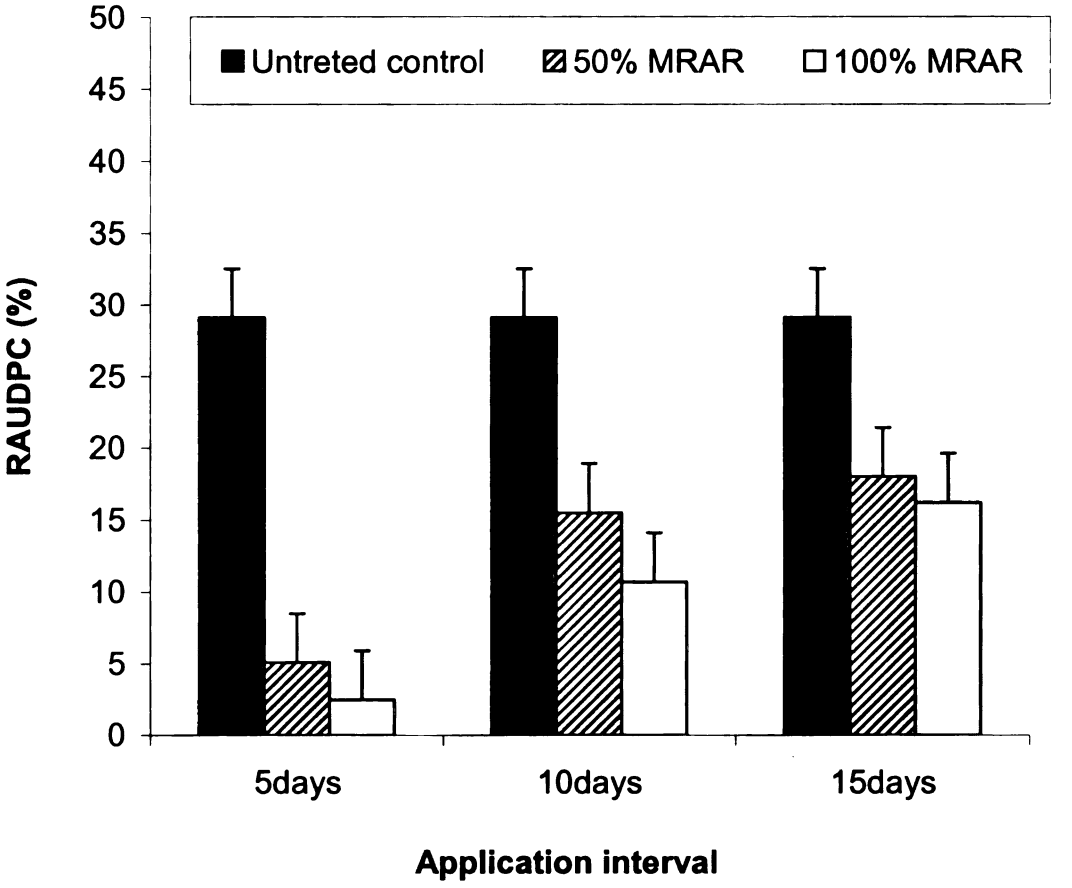

Figure 11. Range of RAUDPC values for cultivar Dakota Rose treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

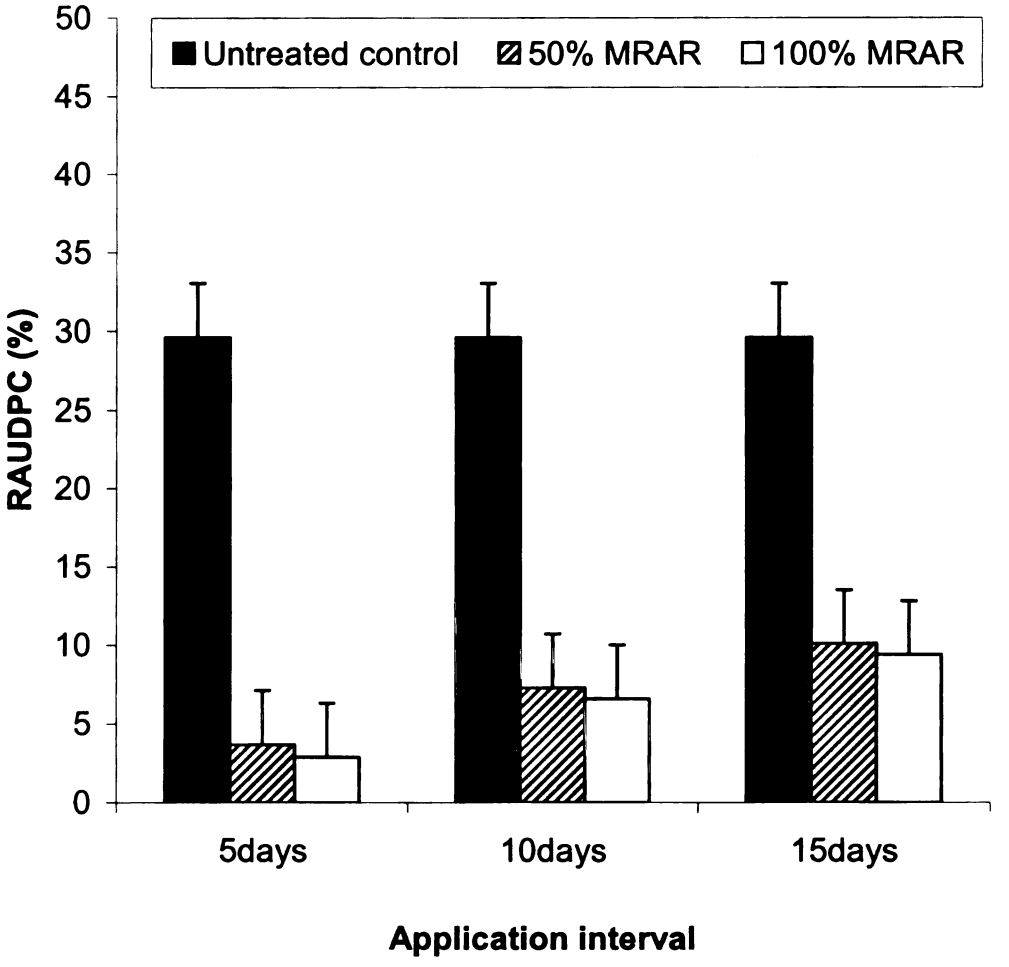

Figure 12. Range of RAUDPC values for MN 19350 treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

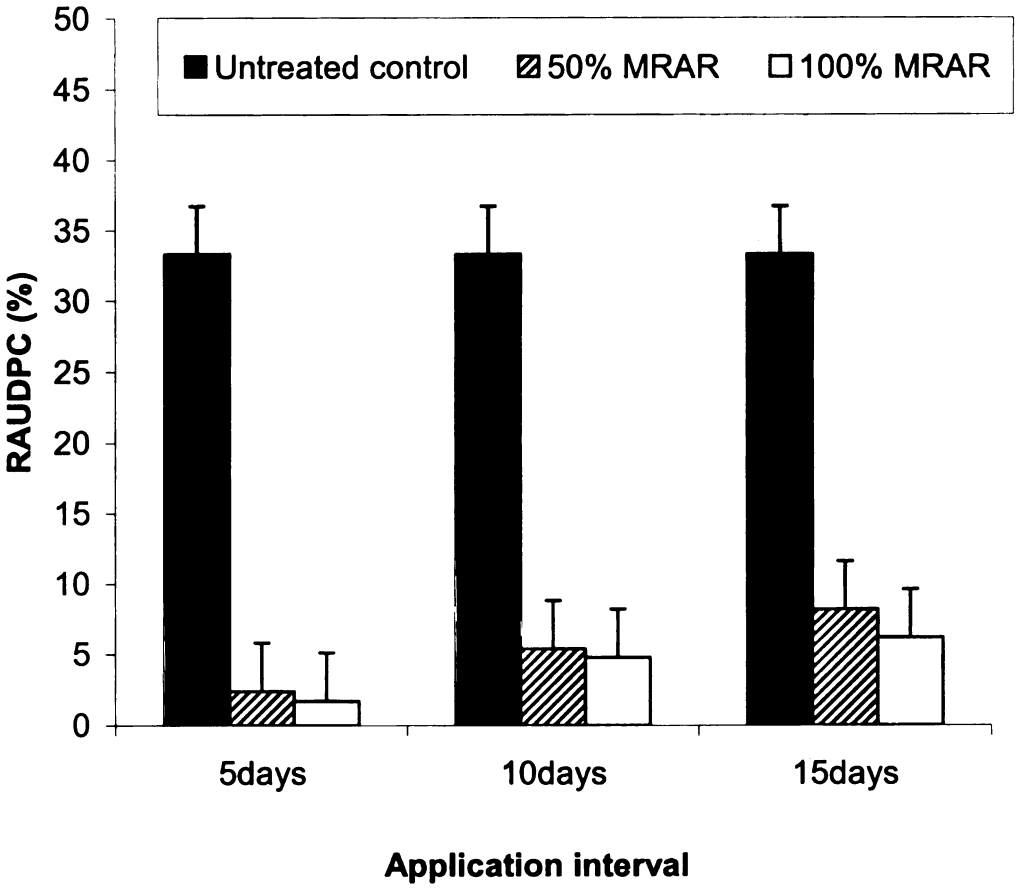

Figure 13. Range of RAUDPC values for W 1386 treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

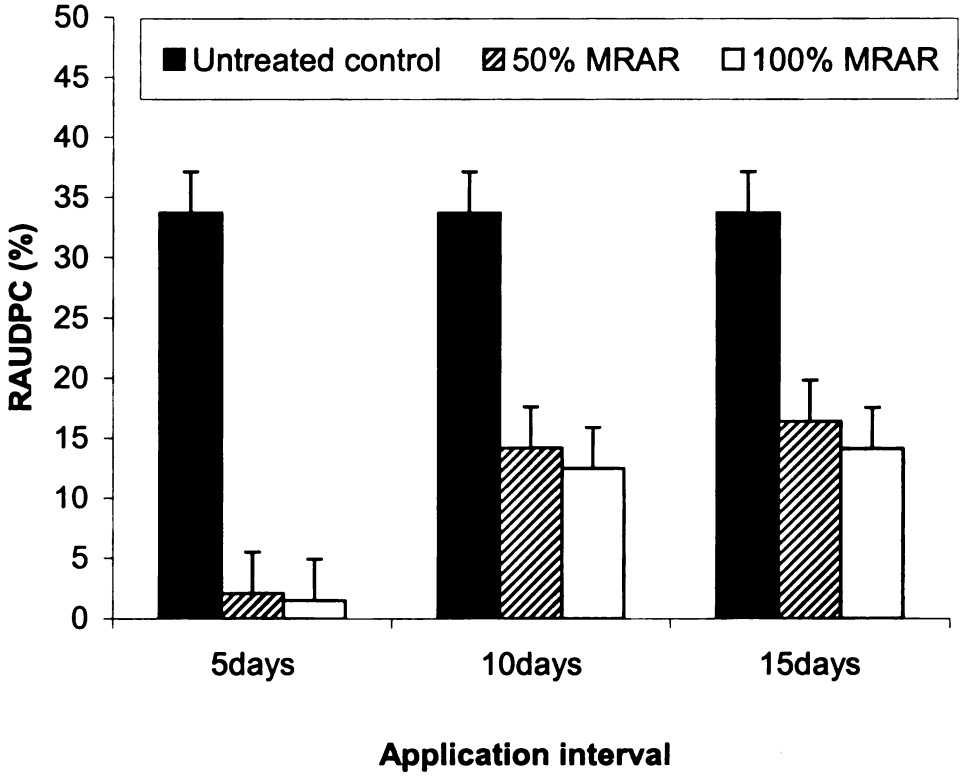

Figure 14. Range of RAUDPC values for MN 1915 treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

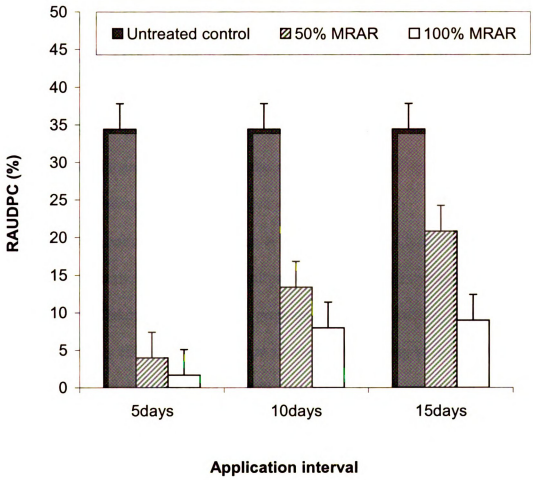

Figure 15. Range of RAUDPC values for cultivar Snowden treated with different rates of fluazinam in 2002 growing season. Bars represent $LSD_{p=0.05} = 3.42$.

All cultivars and advanced breeding lines tested in this study ranged from resistant to susceptible to *P. infestans* but most were susceptible. Jacqueline Lee and Torridon were classified as late blight resistant (R) with mean RAUDPC value = 0.2. Dakota Pearl, W 1355-1, Dakota rose and MN 19350 were classified as moderately resistant (M). W 1386, MN 1915, MNA 157-4 and cultivar Snowden were classified as susceptible (S). W 1355-1, W 1386 and cultivar MN 19350 were very responsive to fungicide applications. W 1355-1 was the most responsive of the susceptible cultivars and advanced breeding lines during the two years of testing.

In 2001, application of fluazinam at 100% MRAR at a 5 or 7-day spray interval resulted in an effective control in most cultivars and advanced breeding lines except those from the North Dakota and Minnesota programs. All cultivars and advanced breeding lines were partially effectively protected at all other rates and application intervals.

In 2002, all cultivars were at 50% and 100% MRAR of fluazinam effectively protected against late blight on a 5-day spray interval and partially effectively protected at all other rates and application intervals.

Table 6. Summary of efficacy results: fluazinam applied at reduced rates and frequencies on potato cultivars and advanced breeding lines from North Central US potato breeding programs, MSU 2002.

Cultivar/ABL	Rate of fluazinam[1]				Application frequency (days)								
					RAUDPC[2]								
					5			10			15		
Jacqueline Lee	0	0.2	y[3]	R[4]									
	50				0.06	y	E[5]	0.13	y	E	0.13	y	E
	100				0.13	y	E	0.06	y	E	0.06	y	E
Torridon	0	0.2	y	R									
	50				0.3	y	E	0.2	y	E	0.1	y	E
	100				0.2	y	E	0.1	y	E	0.1	y	E
Dakota Pearl	0	28.0	b	M									
	50				10.2	i-l	PE	15.9	d-f	PE	14.8	e-g	PE
	100				3.9	p-w	E	17.7	c-e	PE	18.9	cd	PE
W 1355-1	0	28.4	b	M									
	50				1.8	u-y	E	6.1	m-s	PE	7.1	l-q	PE
	100				1.0	w-y	E	3.9	q-w	E	6.3	m-s	PE
Dakota Rose	0	29.1	b	M									
	50				5.1	n-u	E	15.5	d-g	PE	18	c-e	PE
	100				2.5	t-y	E	10.7	h-k	PE	16.2	d-f	PE
MN19350	0	29.6	b	M									
	50				3.7	r-x	E	7.3	l-p	PE	10.1	i-l	PE
	100				2.9	s-y	E	6.6	m-r	PE	9.4	j-m	PE
W 1386	0	33.3	a	S									
	50				2.4	t-y	E	5.4	n-t	PE	8.2	k-n	PE
	100				1.7	v-y	E	4.8	o-v	E	6.2	m-s	PE
MN 1915	0	33.7	a	S									
	50				2.1	t-y	E	14.2	fg	PE	16.4	d-f	PE
	100				1.5	v-y	E	12.5	g-j	PE	14.1	f-h	PE
Snowden	0	34.4	a	S									
	50				4.0	p-w	E	13.4	f-i	PE	20.7	c	PE
	100				1.7	u-y	E	8.0	k-o	PE	9.0	k-m	PE

1: Application rate of fluazinam as percent of manufacturer's recommended rate.
2: Relative area under the disease progress curve from inoculation to 100% late blight in susceptible control (Snowden); max = 100.
3: Means followed by the same letter were not significantly different at p = 0.05; comparison between all combinations of fungicide application rate and frequency of application in all cultivars/ABL.
4: Susceptibility of non-treated control to late blight; R = Resistant, not significantly different from Jacqueline Lee (non-treated); S = Susceptible, not significantly different from Snowden (non-treated); M= Moderately resistant, significantly different from both Jacqueline Lee and Snowden (non-treated).
5: Effectiveness of fungicide treatment in comparison to Snowden treated with a full application rate of fluazinam at a 5-day interval or with non-treated Snowden control; E = RAUDPC not significantly different from treated Snowden control; PE significantly different from treated Snowden control and non-treated control; NE = not significantly different from Snowden non-treated control at p = 0.05.

DISCUSSION

Weather conditions were conducive for the development of late blight in both 2001 and 2002 at the Muck Soils Research Farm. The difference between 2001 and 2002 in terms of accumulation of late blight disease severity values (DSV) was minimal. Late blight is predicted to occur when inoculum is present, the host is in a susceptible state and when climatic conditions are favorable for infection to occur; the Wallin model (28) and the adapted Blightcast model (23) predict that infection will occur after the accumulation of 18 DSV. The accumulated DSV used in these studies was adapted to a 80% RH threshold, as recommended by Baker et al. (1). The traditional threshold of 90% RH resulted in accumulations of 38 and 32 DSV in 2001 and 2002 over the same period, respectively. Baker et al. (1) reported that 80% RH measured 1.0 meter above the canopy was equivalent to 90% RH within a mature potato canopy.

The results of this study were consistent with previous studies (12, 13, 21, 27) and indicate that a combination of cultivar and advanced breeding line resistance and managed application of protective fungicides will reduce foliar late blight to acceptable levels in most situations. However, when environmental conditions were extremely favorable for the development of late blight, lower application rates (33 and 66% MRAR) provided unsatisfactory control for some moderately resistant and susceptible cultivars and advanced breeding lines. In some cultivars and advanced breeding lines, 33% of the MRAR of the fungicide was sufficient to achieve acceptable control, whereas other cultivars and advanced breeding lines required 66% MRAR of fluazinam to control late blight.

However, there was rarely a further reduction in disease in any cultivar and advanced breeding lines when fluazinam was applied at the 100% MRAR of the fungicide.

On late blight susceptible cultivars, applications of fluazinam at either a 10-day application interval or above were either partially or non- effective for controlling late blight at any dose tested. In the resistant cultivars Torridon and Jacqueline Lee, the fungicide did not reduce the RAUDPC in comparison with untreated plots of these cultivars.

The opportunity to manage late blight by applying reduced rates of fungicides at increased spray intervals to cultivars less susceptible to late blight was demonstrated in this study. However, more critical dose response studies will be required before effective rates of application can be established for new fungicides. In addition, the efficacy of reduced rates and increased application intervals of fungicides against other potato pathogens such as early blight has not been established and may prove to be a major constraint in the adoption of managed fungicide applications.

The application rates and application frequencies of fungicides used in this study were selected to cover the range of responses likely to be exhibited by the range of cultivars and advanced breeding lines used over the period of the study. In addition, as microclimatic conditions at the experimental site were likely to differ over the study period it was important to have high and low level fungicide input treatments. The study largely supported the dose rates recommended by the manufacturer but also showed that less susceptible potato cultivars required lower levels of input for effective late blight control.

As new cultivars with enhanced late blight resistance are developed and released it will be important to provide growers with recommendations for the most effective and economical chemical control of late blight in these new cultivars.

In the future, the type of information gathered in this study will be used to develop models, based on cultivar resistance and response to fungicide application, to advise and guide growers as to which fungicide, rate and frequency of application is required to provide protection against late blight. Climatic conditions within the canopy will also impact choice of fungicide and rate and frequency of application (1). Therefore, new cultivars will need to be carefully screened in the manner described in this study, over several seasons in order to develop accurate models for fungicide application.

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CHAPTER THREE

IMPACT OF PLANT AGE AND LEAF POSITION ON SUSCEPTIBILITY TO LATE BLIGHT

INTRODUCTION

Breeding for late blight resistance in potatoes was initiated then intensified in Europe and North America following serious epidemics that occurred in 1845-1847 (1, 12). Initially breeding for resistance was focused on horizontal resistance (12). Later when race-specific resistance in the wild potato *Solanum demissum* was detected, breeders turned their efforts to breed for vertical resistance by transferring major R-genes from *S. demissum* into the domesticated potato (*S. tuberosum*); (12, 22). Since then, vertical resistance was identified as being more effective than horizontal resistance, resulting in very rapid selection and production of varieties resistant to potato late blight. A broad spectrum of acceptable qualities from different *Solanum* spp. are necessary when selecting for horizontal resistance in order to incorporate as many traits as possible from different species to achieve durable resistance to late blight in potatoes. Thus, this kind of resistance becomes difficult to handle, labor intensive and time consuming.

Breeding for vertical resistance in potato plants resulted in the production of many resistant cultivars until the late 1960's when *P. infestans* overcame vertical resistance (22). These varieties were no longer resistant and vertical resistance proved unreliable. Currently, efforts are being redirected towards breeding for horizontal resistance.

Breeding for horizontal resistance has already resulted in the development of some cultivars with intermediate resistance, known as partial resistance. However, fully resistant varieties that can provide adequate protection without using fungicides have not yet been achieved (23).

Results from some studies on factors related to resistance of potato foliage to late blight such as plant age and leaf position have been published (2, 3, 4, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21). Horizontal resistance to late blight is often observed in late maturing cultivars rather than in early maturing ones (22). This has resulted in efforts to search for factors affecting differences among these cultivars in terms of resistance. Horizontal resistance is under polygenic control and expression of resistance is quantitative resulting from an additive contribution of several genes. Different genes may be expressed during the potato season. This was achieved in late maturing cultivars and made early season screening difficult for horizontal resistance (22).

Results from some studies (3, 4, 18, 21) on the change in resistance to *P. infestans* in potatoes allow some general conclusions to be drawn on late blight resistance in potato foliage. In susceptible cultivars young plants are highly susceptible to infection; this susceptibility decreases in resistant cultivars prior to flowering and may increase during senescence (3, 18). At senescence, resistant cultivars become more resistant whereas some other susceptible cultivars become more susceptible at equivalent maturities (3). Apical leaves have been reported to be more resistant to potato late blight than the basal leaves (3, 4, 21). The objective of this study was to determine the impact of plant age and leaf position on susceptibility to late blight in some susceptible and resistant potato cultivars.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN AND PLANT MATERIAL

Three experiments, two in 2002 and one in 2003 were conducted. Three cultivars were tested in each experiment except in one, experiment in 2003 where only two cultivars were used. All cultivars tested were, in general, susceptible to potato late blight except the cultivar Jacqueline Lee, which was previously classified as resistant (5, 6, 7, 8). In each of the three experiments, leaf positions 5, 10 and 15, counted from the base of the main stem of the potato plant above the soil, were randomly harvested and tested for susceptibility to *P. infestans*.

Experiment 1

Twenty potato tubers per cultivar of Russet Norkotah, Dakota Pearl and Jacqueline Lee were grown at random in three field plots at MSU plant pathology farm and used in experiment 1. Leaves to be inoculated with *P. infestans* were harvested from each cultivar at plant age of 24, 39, 46, 59 and 78 days after emergence during this experiment. Soil preparation, fertilizer application, insects and weed control were as described above in chapter two.

Experiment 2

Cultivars FL1879, Dakota Rose and Jacqueline Lee were grown in a growth chamber and were used to test for resistance to late blight. Ten potato plants per cultivar were planted into individual pots (one tuber per pot) filled with potting soil. A total of 30 pots containing 30 potato tubers were placed in a climatic growth chamber.

The relative humidity was 80% in the climatic chamber and the illumination was provided by 500 W Philips-HPIT lamps. Potato plants were grown under a constant temperature of 24°C and a photoperiod of 16 hours day length. Leaves were harvested at plant ages of 20, 33, 46, 60 and 72 days after emergence and inoculated with *P. infestans* (described below).

Experiment 3

In the third experiment cultivars Snowden and Jacqueline Lee were used. In order to minimize variation due to inoculation procedures, tubers were planted at intervals. The experiment was conducted in similar conditions as in experiment 2. But three growth chambers each containing ten pots per cultivar were used in this experiment at each of three different planting times. Leaves to be inoculated with *P. infestans* were harvested from the same position at plant age of 20, 32, 46, 60 and 74 days after planting. Leaves harvested from the same position differed in age by seven days.

PATHOGEN PREPARATION AND INOCULATION

Inoculum was prepared from isolate Pi 95-7 of *P. infestans* (US-8 genotype; A2 mating type obtained from Dr William W. Kirk's collection at MSU) and produced on rye agar as described in Chapter Two. Sporangia were harvested from agar plates on the day of inoculation. The suspension of sporangia was adjusted to a final concentration of 5 X 10^4 sporangia ml^{-1} and chilled at 4°C for 4 hours prior to inoculation to enhance the release of zoospores.

Prior to inoculation, leaf discs were cut with a cork borer (12mm) from the primary potato leaflet on each side of the main vein, about 1 cm from the point of attachment to the petiole. Five leaf discs were placed on rye B agar amended with antibiotics (rifamycin 75 mg. l^{-1}, ampicillin 20mg. l^{-1} and nystatin 75 mg. l^{-1} dissolved in 1.0 ml DMSO) to reduce contamination with bacteria.

A 50µl droplet of the sporangia/zoospore suspension was applied to each of five leaf discs per plate. A total of 10 leaf discs, per cultivar per leaf position per plant age were inoculated. Inoculated leaf discs were incubated at 21°C light/18 °C dark (12 hours cycle) and were examined with a dissecting microscope 96 hours post-inoculation. Symptoms and signs of infection by *P. infestans*, such as sporulation were noted. Pathogen identity was confirmed with a compound microscope.

DISEASE EVALUATION AND DATA ANALYSIS

Late blight infection was estimated on the fifth day after inoculation by counting sporangia harvested from each leaf disc with late blight lesions. The leaf discs were placed into a 1.5 ml micro-centrifuge tube with 1.0 ml of sterile de-ionized H_2O (diH_2O) and agitated to dislodge the sporangia. Samples were taken from each sporangium suspension to count the final number of sporangia using a hemacytometer for a total of 6 counts per leaf disc. The number of sporangia produced per leaf disc per cultivar was used to estimate susceptibility or resistance in different cultivars. Any plant age per leaf position per cultivar treatment in which the average number of sporangia produced per leaf disc was significantly different at $p = 0.05$ from that of the overall mean number of sporangia produced per leaf disc for the resistant cultivar Jacqueline Lee was classified as susceptible (S) to blight infection and resistant (R) to infection when the mean number of sporangia per leaf disc was not significantly different from that of the cultivar Jacqueline Lee.

Data were analyzed by ANOVA and all pair-wise comparison calculated using Fisher's least significant differences (LSD) at $\alpha=0.05$ (SAS-Institute 2003. Statistical Analysis Software v.8.2, Cary, NC.).

RESULTS

EXPERIMENT 1

The average number of sporangia produced per inoculated leaf disc in the cultivar Jacqueline Lee was 60. Any plant age or leaf position on a cultivar that resulted in an average number of sporangia of *P. infestans* produced per leaf disc ≤ 60 (not significantly different from that of the cultivar Jacqueline Lee) was defined as resistant to infection, susceptible when the average number of sporangia produced per leaf disc was > 60 (significantly different from that of the cultivar Jacqueline Lee). The average number of sporangia of *P. infestans* produced per leaf disc for all cultivars tested was significantly different from that of the cultivar Jacqueline Lee. The mean numbers of sporangia produced per leaf disc for the susceptible cultivars Russet Norkotah and Dakota Pearl were not significantly different from each other. The average number of sporangia produced per leaf disc for the resistant cultivar Jacqueline Lee was significantly different from both Russet Norkotah and Dakota Pearl (Table 7).

The analysis of variance indicated a significant effect of plant age on the average number of sporangia of *P. infestans* produced per leaf disc ($P<0.0001$) at $\alpha=0.05$ for all cultivars. The effect of leaf position on the number of sporangia of *P. infestans* produced was also significant for all cultivars tested.

The numbers of sporangia of *P infestans* produced per leaf disc for the resistant cultivar Jacqueline Lee were significantly different at plant ages of 22 and 39 days after emergence (Table 7) where this cultivar was classified as susceptible to late blight infection. Mean number of sporangia produced per leaf disc was not significantly different at all leaf positions tested for this cultivar (Table 7). All leaf positions tested for this cultivar were resistant to late blight infection, but resistance was not significantly different from each other among these leaf positions (Table 7).

For the susceptible cultivar Russet Norkotah, the average numbers of sporangia of *P. infestans* produced per leaf disc at all plant ages tested were significantly different from that of the cultivar Jacqueline Lee except at the ages of 39 post-planting where the mean number of sporangia of *P. infestans* produced was not significantly different from that of the cultivar Jacqueline Lee (Table 7). The average numbers of sporangia produced per leaf disc were also significantly different from that of the cultivar Jacqueline Lee at all leaf positions tested for this cultivar (Table 7). All leaf positions tested for this cultivar were susceptible to late blight infection, but susceptibility was significantly higher in leaf position 5.

The numbers of sporangia of *P. infestans* produced per leaf disc for the cultivar Dakota Pearl were significantly different from that of the cultivar Jacqueline Lee at all plant ages tested except at age 24 post-emergence where the mean number of sporangia of *P. infestans* was not significantly different from the number harvested from the cultivar Jacqueline Lee (Table 7). Dakota Pearl was defined as resistant at plant age of 24 days after emergence and susceptible at all other plant ages tested.

Mean numbers of sporangia of *P infestans* produced per leaf disc were significantly different from that of the cultivar Jacqueline Lee at all leaf positions tested for this cultivar (Table 7). Dakota Pearl was also susceptible to infection at all leaf positions, but susceptibility was significantly higher in leaf positions 5 and 10 for this cultivar.

Experiment 2

The average number of sporangia produced per inoculated leaf disc in the cultivar Jacqueline Lee was 70. Any plant age or leaf position on a cultivar that resulted in a number of sporangia of *P. infestans* produced per leaf disc $\leq$ 70 (not significantly different from that of the cultivar Jacqueline Lee) was defined as resistant to blight infection, susceptible when the mean number of sporangia produced per leaf disc was > 70 (significantly different from that of the cultivar Jacqueline Lee). The average number of sporangia of *P. infestans* produced per leaf disc for all cultivars tested was significantly different from that of Jacqueline Lee. The average numbers of sporangia produced per leaf disc for the susceptible cultivars FL1879 and Dakota Rose were not significantly different from each other. The average number of sporangia produced per leaf disc for the resistant cultivar Jacqueline Lee was significantly different from both FL 1879 and Dakota Rose (Table 7).

The analysis of variance showed a significant effect of plant age on the numbers of sporangia of *P. infestans* produced ($P<0.0001$) at $\alpha=0.05$ for all cultivars. There was no significant effect of leaf position on susceptibility to blight infection in all cultivars tested in experiment 2. For the susceptible cultivar FL1879, the average numbers of sporangia produced per leaf disc were significantly different from that of the cultivar Jacqueline Lee at plant ages of 20 and 60 days after emergence. Mean numbers of sporangia produced per leaf disc were not significantly different from that of the cultivar Jacqueline Lee at plant ages of 33, 46 and 72 days post-emergence (Table 7). FL1879 was classified as resistant to infection at plant ages of 33, 46 and 72 days and susceptible at plant ages of 20 and 60 days after emergence

The average numbers of sporangia produced per leaf disc for the other susceptible cultivar Dakota Rose were significantly different from that of the cultivar Jacqueline Lee at all plant ages tested except at plant age of 46 after emergence where the average number of sporangia produced was not significantly different from that of the cultivar Jacqueline Lee (Table 7). Cultivar Dakota Rose was defined as susceptible to infection at plant ages of 20, 33, 60 and 72 days and resistant at plant age of 46 days post-emergence.

For the resistant cultivar Jacqueline Lee, the average numbers of sporangia produced per leaf disc were significantly different at plant ages of 20 and 33 days after emergence (Table 7) where Jacqueline Lee was defined as susceptible to late blight infection.

Experiment 3

The average number of sporangia produced per inoculated leaf disc in the cultivar Jacqueline Lee was 59. Any plant age or leaf position on a cultivar that resulted in a number of sporangia of *P. infestans* produced per leaf disc $\leq$ 59 (not significantly different from that of the cultivar Jacqueline Lee) was defined as resistant to infection. And treatments that resulted in the numbers of sporangia produced per leaf disc > 59 (significantly different from that of the cultivar Jacqueline Lee) were defined as susceptible to blight infection. The mean number of sporangia for the susceptible cultivar Snowden was significantly different from that of the cultivar Jacqueline Lee (Table 7).

The analysis of variance indicated a significant effect of plant age on the number of sporangia of *P. infestans* produced per leaf disc at α=0.05. The effect of leaf position on the number of sporangia of *P. infestans* produced was also significant.

For the susceptible cultivar Snowden, the average numbers of sporangia produced per leaf disc were significantly different from that of the cultivar Jacqueline Lee at all plant ages tested (Table 7). Mean numbers of sporangia produced were also significantly different from that of the cultivar Jacqueline Lee for all leaf positions tested for this cultivar (Table 7). All leaf positions tested for cultivar Snowden were susceptible to late blight infection, but susceptibility was not significantly different from each other among these leaf positions.

The average number of sporangia of *P. infestans* produced per leaf disc for the resistant cultivar Jacqueline Lee was significantly different at plant ages of 20 and 32 days post-emergence (Table 7) where Jacqueline Lee was classified as susceptible to blight infection. The average numbers of sporangia of *P. infestans* produced per leaf disc were significantly different at leaf position 5 (Table 7) where cultivar Jacqueline Lee was classified as susceptible to infection.

Table 7. Summary of susceptibility results: average number of sporangia of *P. infestans* produced per leaf disc per plant age per cultivar (all leaves together) and per leaf position (all plant ages together) for the six cultivars tested in the three experiments

Plant age	*Number of sporangia per leaf disc*					
Experiment 1	Russet Norkotah	Dakota Pearl	Jacqueline Lee	FL 1879	Dakota Rose	Snowden
Plant age						
- 24 days	220a[b] S[c]	40c R	110a S	-	-	-
- 39 days	40b R	70bc S	80ab S	-	-	-
- 46 days	190a S	250a S	50bc R	-	-	-
- 59 days	250a S	150b S	20c R	-	-	-
- 78 days	120ab S	70bc S	20c R	-	-	-
- $LSD_{\alpha=0.05}$[a]	140	90	50	-		
Leaf position						
- Leaf 5	250a S	190a S	60a R	-	-	-
- Leaf 10	130b S	150a S	50a R	-	-	-
- Leaf 15	110b S	90b S	50a R	-	-	-
- $LSD_{\alpha=0.05}$	90	60	30	-	-	-
Average for the cultivar	160 S	140 S	**60 R**	-	-	-
Experiment 2						
Plant age						
- 20 days	-	-	250a S	130a S	310b S	-
- 33 days	-	-	90b S	60b R	80bc S	-
- 46 days	-	-	40b R	60b R	20c R	-
- 60 days	-	-	40b R	190a S	990a S	-
- 72 days	-	-	20 R	60b R	280bc S	-
- $LSD_{\alpha=0.05}$	-	-	80	80	250	-
Average for the cultivar	-	-	**70 R**	90b S	310a S	-
Experiment 3						
Plant age						
- 20 days	-	-	118 a S	-	-	520a S
- 32 days	-	-	86 ab S	-	-	210bc S
- 46 days	-	-	42 bc R	-	-	200bc S
- 60 days	-	-	38 c R	-	-	360ab S
- 74 days	-	-	39 c R	-	-	140c S
- $LSD_{\alpha=0.05}$			45			190
Leaf position						
- Leaf 5	-	-	72 a S	-	-	220a S
- Leaf 10	-	-	56 a R	-	-	270a S
- Leaf 15	-	-	48 a R	-	-	300a S
- $LSD_{\alpha=0.05}$	-	-	31	-	-	130
Average for the cultivar	-	-	**59 R**	-	-	268 S

a: LSD= least significant difference
b: Means followed by the same letter within a column in each experiment are not significantly different
c: S: Susceptible to infection, significantly different from the cultivar Jacqueline Lee; R = Resistant, not significantly different from the cultivar Jacqueline Lee.

DISCUSSION

The effects of plant age and leaf position on susceptibility to potato late blight infection were investigated in three different experiments conducted both in the field and controlled environment using six different potato cultivars with a range on susceptibility to late blight. The results of this study supported previous reports (5, 6, 7, 8) that Jacqueline Lee is resistant to potato late blight.

Susceptibility to infection due to plant age in susceptible cultivars was variable depending on cultivar tested. Resistant cultivar Jacqueline Lee was susceptible at young plant ages in all experiments.

All leaf positions tested were susceptible to infection for all susceptible cultivars in all experiments and resistant for the resistant cultivar Jacqueline Lee. Leaf position has effect on susceptibility to infection for all cultivars tested in all experiments and leaf position 5 was the most susceptible.

Results from this research demonstrated the increase of resistance to blight infection from leaf position 5 to leaf position 15 and supported those from previous studies (2, 3, 11, 14, 16, 21) that there is gradual increase to blight susceptibility due to *P. infestans* from apical leaves to basal ones and confirmed that apical leaves are the most resistant to potato late blight infection.

Resistant cultivar Jacqueline Lee was susceptible for short periods during the early stages of leaf development and then became resistant at later stages. Plant age has less effect on susceptible cultivars tested, which were susceptible at any stage of leaf development.

This was in accordance with Carnegie and Calhoun (3) that young plants are very susceptible to infection in the resistant cultivars, whereas susceptible cultivars are less affected by plant age.

Plant age and leaf position were important factors that were affecting susceptibility to blight infection in all cultivars tested but leaf position was the most important factor during this study. I would therefore suggest breeders to consider both plant age and leaf position in different tests and evaluations for resistance to potato late blight.

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CHAPTER FOUR

CONCLUSIONS AND SUMMARY

Plants need to be protected against diseases in order to reduce or avoid serious epidemics, which can lead to severe famines such as the Irish potato famine that occurred in the 1840's. The aim of controlling plant diseases is to reduce food losses and at the same time to improve food quality (1).

Current strategies to protect potatoes against potato late blight disease include the use disease free seed, growing resistant varieties, inoculum-suppressing cultural practices, crop scouting, weather-based disease modeling and chemical control programs (7).

Breeding for resistance has resulted in development of resistant varieties but high levels of stable resistance have not been fully successful (2). Even resistant cultivars are susceptible when immature and need to be protected with fungicide applications especially at early stages of crop and canopy development to reduce the potential risk of an epidemic to occur when inoculum becomes present.

In temperate regions such as Michigan, potatoes may be exposed to late blight inoculum produced on infected volunteer potato plants and discarded piles of culled potatoes or the disease may originate from infected seed tubers. Favorable conditions for disease development will also favor disease spread throughout the canopy.

In warm regions such as the tropical highlands of Rwanda, inoculum is almost continuously present due to continuous cropping and conditions for late blight occurrence and spread. In most potato growing areas in Rwanda, average annual precipitation ranges from 1200 mm to over 1600 mm. In general, rainfall is bimodal with a minor peak occurring in October and a major peak in March/April. High elevations and low latitudes combine to form an isothermal temperature regime with an average annual temperature of about 16°C (3, 4). Moreover farmers do not have the financial capabilities to spray fungicides regularly and they do not have enough knowledge either to manage the disease well. In addition they use low quality seed due to inadequate seed selection and storage from their harvested crop (farmers generally replant seed from their own fields).

Cultural management of the potato crop includes removal of cull piles and volunteer potatoes to minimize the source of inoculum of *P. infestans*, irrigation management and hilling of rows to reduce tuber contact with free water, planting and harvesting time to reduce tuber damage and desiccation of potato foliage prior to harvest to eliminate foliar-borne inoculum (7).

The basis of this thesis was to demonstrate the potential for using managed fungicide application in combination with varieties ranging from marginal to full tolerance of potato late blight. The reason for using potato varieties with a range of tolerance to potato late blight was to determine if different amounts and frequencies of application of contact/protectant fungicide are required for control of late blight in individual varieties under late blight conducive conditions. Overall, the use of resistant varieties would potentially reduce losses due to late blight and reduce the cost of crop protection in potato production.

However, it could be argued that the major benefit would be the removal of a negative impact on the environment and consequent positive impact on the safety and quality of a globally important food source. Moreover, plant age has proved to be an important factor when testing and evaluating potato late blight resistance.

The use of the modern fungicide, fluazinam in combination with cultivar resistance was evaluated to be incorporated later into an integrated potato late blight control program both in Michigan and Rwanda. Potatoes play an important role in the Rwandan economy as a cash crop [estimates of Rwandan marketed potato production range between 35% and 50% (6)] and the diet of Rwandan people. The country is able to increase potato production especially in the northern parts (highland areas) of Rwanda if late blight, the major constraint to crop production is better controlled. There is also a potential potato market in the neighboring countries in the Democratic Republic of Congo, Burundi, Uganda and Kenya that can be developed so that the grower can generate more income and the country earn more foreign currencies (5).

The problem of potato late blight in Rwanda is associated with serious crop and yield losses, costly fungicides for the Rwandan farmer with limited resources and few resistant varieties (4). Thus the approach developed in this research would be applied in Rwanda to reduce the cost of production and losses due to potato late blight. The fungicide trials described here will be applied under the conditions of Rwanda on some late blight tolerant/resistant varieties with improved potato yield that are available in Rwanda.

The information gathered based on cultivar resistance and response to fungicide application, will be used to advise and guide Rwandan growers as to the rate and frequency of the fungicide application required to provide better protection against late blight. Rwandan farmers with limited resources will be able to reduce fungicides input on potato production, increase yield, generate more income and improve their life in a safer environment.

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