This is to certify that the
thesis entitled

Survival of mycelium of *Phytophthora infestans* after exposure to temperatures below 3 degrees Celcius

presented by

Samantha Irene Hollosy

has been accepted towards fulfillment
of the requirements for the

Master of Science degree in Plant Pathology

R Hammerschmidt
Major Professor's Signature

3 December 2004
Date

SURVIVAL OF MYCELIUM OF *PHYTOPHTHORA INFESTANS* AFTER EXPOSURE TO TEMPERATURES BELOW 3 DEGREES CELCIUS

By

Samantha Irene Hollosy

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTERS OF SCIENCE

Department of Plant Pathology

2004

ABSTRACT

SURVIVAL OF MYCELIUM OF *PHYTOPHTHORA INFESTANS* AFTER EXPOSURE TO TEMPERATURES BELOW 3 DEGREES CELCIUS

By

Samantha Irene Hollosy

Phytophthora infestans, the causal agent of potato late blight, is the most important pathogen of potato. *P. infestans* is known to overwinter as mycelium in infected potato tubers and the mycelium can survive in seed tubers in cold storage, in volunteer tubers in fields, and in cull piles. Over the past decade in North America, there has been a decrease in A1, metalaxyl sensitive isolates of *P. infestans*, e.g. US-1, in favor of A2 metalaxyl insensitive isolates, e.g. US-8. The objective of this study is to characterize the effect of cold temperatures on survival of mycelium from different genotypes of *P. infestans in vitro* and in potato tuber tissue. Results indicate variability between and among genotypes with respect to cold tolerance. Survival of mycelium was favored at 3° and 0°C compared to -3° and -5°C. Isolates 95-7 and CO37 vary in aggressiveness in tuber tissue depending on the cultivar infected and the temperature to which it was exposed. All tuber cultivars displayed greater disease severity when exposed to 3°C rather than 0°C.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important host for the Oomycete pathogen *Phytophthora infestans*, the cause of the late blight disease. Potato late blight is considered to be the most important potato disease because it quickly destroys entire fields and can cause post harvest tuber decay that results in tremendous losses (Fry and Goodwin 1997). Global crop losses due to late blight are approximately 10 – 15% annually. The worldwide economic loss caused by the disease is estimated at $3 billion annually (Schiermeier 2001). The importance of late blight is further illustrated by the fact that the potato is the fourth largest food crop worldwide (Graves 2001).

The Host

The potato is believed to have originated in the Andes mountains of Peru where it was first noted to be a major food source for the Incas around 400 BC (Schumann 1991). The early Andean people were the first to develop techniques that improved cultivation and storage of potatoes. The potato was brought to Europe in the 16th century by Spanish explorers who traveled to South America in search of treasure. On their return trip home they brought potatoes with them (Schumann 1991). The Europeans did not initially accept potatoes as a food source because they were considered to be filthy and evil since they are grown underground. The potato became a staple food crop in Europe during the 17th and 18th century because it was nutritious, easy to grow, and a large yield could be obtained from a relatively small plot of land. The potato was first introduced into North America in the 17th century when European settlers brought it back to the New World.

Due to the abundance of potatoes and their nutritional value, the population in Europe grew tremendously from the 1500s to the 1800s. In Ireland alone, the population increased from less than 3 million in the 1500s to 8 million people in the 1840s (Schumann 1991).

Potatoes were relied upon as a major food crop in Europe. Therefore any factor that may have damaged the crop could threaten the population. This is illustrated by the Irish potato famine, which occurred in the 19th century and decimated the population of Ireland. Potatoes (cv. Lumper) susceptible to late blight were grown in monoculture throughout Ireland, thus providing a large host population for the pathogen. In the years 1845 through 1848, Ireland experienced cool wet weather, which provided the ideal environment for *Phytophthora infestans*. Because peasant farmers relied solely on potatoes as their food source, many were left starving as a result of late blight. It is estimated that about one million Irish people died due to starvation and malnutrition and another million immigrated to other areas hoping to escape the devastation of the famine (Schumann 1991).

The Pathogen

Pathogen Biology

Oomycetes are classified with diatoms and brown algae (Förster et al. 1990). *Phytophthora* species are classified taxonomically in the kingdom Chromista, phylum Oomycota, order *Peronosporales*, family *Peronosporaceae* and genus *Phytophthora* (Birch 2001). They differ from fungi because their cell walls are composed of β– 1,3 glucan polymers and cellulose rather than mostly chitin. Their hyphae are coenocytic,

and they are diploid at each stage of their life cycle except for haploid nuclei in the gametangia (Judelson 1997). *Phytophthora* species use the polysaccharide mycolaminarin for energy storage, and they need to obtain sterol and thiamine from outside sources for sporulation and growth (Kamoun 2003). *Phytophthora infestans* is a hemibiotrophic pathogen that feeds biotrophically during early stages of development by forming biotrophic feeding structures called haustoria. Later, the host cells are killed, producing necrotic lesions, and the pathogen feeds saprophytically. *P. infestans* has a high degree of host specificity, being pathogenic only to potato and tomato (Judelson 1997).

Most *Phytophthora* species are heterothallic, having A1 or A2 mating types. The mating types are determined by heterozygosity (A1) or homozygosity (A2) at a single mating type locus (Fabritius et al. 2002). *P. infestans* can reproduce both asexually (through sporangia and zoospores) and sexually (through oospore production from an antheridium and oogonium). The range of growth temperatures that *P. infestans* can survive is from 4-26°C, with an optimum temperature of 20°C (Erwin and Ribeiro 1996). Disease development is greatest at temperatures from 16-20°C, and sporulation is greatest at 21°C (Erwin and Ribeiro 1996).

Distribution of *Phytophthora infestans* isolates

Phytophthora infestans originated in the highlands of central Mexico, and this area is widely believed to be the source of all migrations to other areas (Fry et al. 1993). The region is important for the study of potato late blight because populations of *P. infestans* in central Mexico are more diverse than other regions (Fry and Goodwin 1997).

Prior to 1980, both the A1 and A2 mating types were found together only in central Mexico (Niederhauser 1991). Sexual reproduction could not therefore have yet happened outside of Mexico. Until 1980, only the A1 mating type was found outside of Mexico (Hohl and Iselin 1984), and this single clonal lineage was termed US-1 (Goodwin et al. 1994). Fry and Goodwin (1997) speculated that the A1 mating type was transported from Mexico to other areas in the world via infected plant material or infested soil. They also suggested that the previous worldwide predominance of a single clonal lineage of *P. infestans* was caused by tight bottlenecks occurring during the transport of the pathogen from Mexico to other parts of the world (Fry and Goodwin 1997).

Transport of potatoes from Mexico in the late 1970s may have been the means of introduction of the A2 mating type to Europe (Niederhauser 1991). The mating type change hypothesis suggested by Ko (1994), which proposed that the origin of the A2 mating type outside of Mexico was the result of a mating type change induced by self-fertilization, was rejected by Goodwin and Drenth (1997). Results by Goodwin and Drenth (1997) have shown that isolates from new populations were significantly different from US-1 and that the new genotypes are likely due to sexual recombination in Mexico prior to migration. The migration hypothesis, which proposes that plant material infected with an A2 isolate was transported from Mexico to Europe and North America, is the commonly accepted explanation for the origin of the A2 mating type outside of Mexico (Goodwin and Drenth 1997). The A2 mating type was likely introduced to the United States and Canada in the early 1990s. The US-8 (A2) genotype is widespread in the United States (Fry 1997). It has been found to be resistant to the fungicide metalaxyl (active ingredient of Ridomil-based products) and is more aggressive in potato foliage

than its predecessor US-1 (A1) (Goodwin et al. 1996). Most likely because of the US-8 genotype's aggressive nature, it has all but wiped out the US-1 population in the US to become the most commonly found genotype. Today A1 and A2 isolates are rarely found together in the US and Canada, making the occurrence of sexual reproduction uncommon. No oospores have ever been found in Michigan potato fields (Kirk 2003, personal communication).

The Disease

Disease Cycle

Sporangia and zoospores are the means by which the annual epidemics are initiated. This happens in the spring when infected tubers sprout and sporangia are produced on the aerial parts of the plant. The source of primary inoculum has been widely debated. However, most studies show that cull piles are the major cause of primary infection (Bonde and Schultz 1943, Zwankhuizen et al. 1998, Kirk 2003a). Diseased seed tubers pose an unlikely threat when planted deep into soil because most diseased tubers will rot when buried (Bonde and Schultz 1943). Zwankhuizen et al. (1998) also showed that infected seed tubers and volunteer plants were minor sources of late blight inoculum. Sources of early infection in potato fields were traced to cull piles, and infested organic potato fields were found to be a source of mid-season inoculum in the Netherlands (Zwankhuizen et al. 1998).

Sporangia produced on potato plants can be dispersed over large distances by wind (Hirst and Stedman 1960). Under humid conditions, zoospores may be produced

within sporangia and upon emergence can swim in water to a new infection site. Zoospores can move from cull piles to field plants in dense fog, rain puddles, or wind-blown rain (Bonde and Stedman 1943).

In locations where both mating types are found, sexual reproduction can occur and produce thick-walled oospores capable of withstanding extreme environmental conditions (Schumann 1991). Oospores appear to be of little importance in North America due to the widespread occurrence of monomorphic (same genotype) *P. infestans* populations (Fry and Goodwin 1997).

Infection Process

Primary infection occurs after emergence of the pathogen in the spring. Sporangia formed on aerial portions of infected potato plants are then transferred to uninfected plants and may directly germinate to form a germ tube or form zoospores that swim to an infection site, encyst, and form a germ tube (Judelson 1997). The germ tube then differentiates into an appressorium that produces degrading enzymes allowing a narrow hypha (known as a penetration peg) to invade the epidermis of the plant. Once inside the plant cell, the penetration peg differentiates into primary infection hyphae, which then colonize host-plant tissue.

Compatible Reaction

When a compatible race of *P. infestans* infects a potato plant, no hypersensitive response occurs. Brownian motion of the plant's cytoplasm is delayed, beginning at 2 to 8 hours after penetration. The pathogen is able to develop within the plant tissue

unimpeded (Goodman and Novacky 1994). Hyphae of a compatible race of *P. infestans* exhibit normal growth within plant tissue, creating many branches and colonizing more cells (Kitazawa and Tomiyama 1969). After colonization of plant tissue, the pathogen will begin to feed biotrophically by the production of feeding structures known as haustoria. These structures can go undetected by the plant because they do not penetrate the plant's plasma membrane and therefore do not come into contact with the plant cell's cytoplasm (Szabo and Bushnell 2001).

Incompatible Reaction

Many studies have been conducted concerning the resistance response of the plant to infection by *P. infestans*. One of the first changes occurs in the plant cell's plasma membrane. At the time of penetration, the permeability of the plants cell membrane to ionic substances increased more when infected by an incompatible race of *P. infestans* as compared to a compatible race (Matsumoto et al. 1976). Ten to sixty minutes after penetration, cytoplasmic streaming in the plant is accelerated and granules begin moving with a Brownian motion (Goodman and Novacky 1994). Studies by Tomiyama et al. (1983) on the changes of potato membrane potential during infection have shown that infection of tuber tissue by an incompatible race of *P. infestans* caused a decrease in passive diffusion potential and to compensate, an increase in respiration dependent electrogenic potential. One to three hours after penetration the respiration dependent electrogenic potential decreased and the cell content became granulated. A compatible race had no effect on cell membrane potential (Tomiyama et al. 1983).

The hypersensitive response (HR) occurs in response to infection with an incompatible race of *P. infestans* (Furuichi et al. 1979). Cells from intact potato tubers and leaves have a low initial ability to react hypersensitively to infection. However, 16 to 20 hours after wounding, potato tuber tissue at the wound site acquired full ability to react hypersensitively (Furuichi et al. 1979). *De novo* protein synthesis is necessary for a hypersensitive response to occur and is likely responsible for enhanced hypersensitive response in wounded tubers (Furuichi et al. 1979). Infection by an incompatible race of *P. infestans* also causes the accumulation of phenolic compounds and phytoalexins (Hächler and Hohl 1984, Sakai et al. 1982, Sato and Tomiyama 1976). Hyphae of an incompatible race of *P. infestans* remain contained within the cell that was first penetrated by the appressorium. The hyphae have an abnormal appearance and do not branch much or at all (Kitazawa and Tomiyama 1969).

Infection During Storage

Storage can alter a potato tuber's ability to react to infection. Dowley and O'Sullivan (1991) found that healthy tubers became infected with *P. infestans* from sporangia after being stored with diseased tubers at 20°C for up to 35 days. In cut potato seed, mycelium from diseased pieces can infect adjoining cut surfaces within 8 hours (Lambert et al. 1998). Spread of disease between whole tubers in storage is insignificant because of the barrier of intact skin and because tuber surfaces are usually dry, inhibiting production of sporangia (Lambert et al. 1998). The sesquiterpenoid phytoalexins rishitin and lubimin accumulated in tubers stored at 4°C after being infected by an incompatible race of *P. infestans* (Bostock et al. 1983). Sesquiterpenoid phytoalexin accumulation in

unstored tubers did not differ between incompatible and compatible reactions (Bostock et al. 1983). Treatment with a high concentration abscisic acid (ABA), a growth regulator that inhibits rishitin and lubimin accumulation, enabled incompatible races of *P. infestans* to cause infection of tubers stored at 4°C. In tubers stored for 5 months at 4°C, treatment with ABA inhibited sesquiterpenoid stress metabolite accumulation inducing a compatible reaction (Bostock et al. 1983).

Variability Among Potato Cultivars and *P. infestans* Isolates

Infection of a potato plant is extremely variable and depends on the cultivar and the isolate of *P. infestans* involved. Research has shown that the newer US-8 genotype is more aggressive in potato tubers than the US-1 genotype, causing more surface necrosis and deeper lesions (Medina et al. 1999). Infection of whole tubers from 12 different potato cultivars by US-1 and US-8 genotypes of *P. infestans* was investigated. The experiments included the use of cultivar Atlantic, and found that 'Atlantic' tubers were penetrated equally by both US-1 and US-8 genotypes (Medina et al. 1999). A study by Lambert reinforced the results from Medina and showed that some US-6 and US-7 isolates of *P. infestans* also cause faster infection spread than US-1 (Lambert and Currier 1997).

Currently, the predominant isolates of *P. infestans* in North America are resistant to the widely used fungicide metalaxyl. In untreated tubers, metalaxyl-resistant isolates of *P. infestans* (example US-8) aggressively colonized tubers, while metalaxyl-sensitive isolates (example US-1) produced smaller shallower lesions (Kadish and Cohen 1992). A study using potato tubers of different ages that were stored for different durations at

20°C found that metalaxyl-sensitive and metalaxyl-resistant isolates did not differ in mean incidence of diseased tubers, but metalaxyl-resistant isolates created lesions of larger area and depth (Grinberger et al. 1995). The older and newer isolates of *P. infestans* have the same host range therefore do not differ in pathogenicity. However, the newer isolates may have increased virulence, growing more aggressively in plant tissue (Grinberger et al. 1995). When considering the fact that infected tubers must remain viable in order to cause primary infection the following spring, metalaxyl-sensitive isolates may have an evolutionary advantage. Metalaxyl-resistant isolates have been shown to infect more tubers, but tubers infected with metalaxyl-sensitive isolates were more likely to produce infected plants because they were not completely destroyed by the disease (Walker and Cooke 1990).

Resistance to *P. infestans*

Resistance of a potato plant to late blight may be horizontal, vertical, or both. Horizontal resistance is defined as resistance that is expressed uniformly against all races of a pathogen; vertical resistance is expressed differently against races of a pathogen (Van der Plank 1984). At least 11 R (resistance) genes in potato have been described that protect against *P. infestans* infection (Tyler 2002). R3, R6, and R7 are located in a cluster, while other R genes have unique locations. Avr genes in *P. infestans* that correspond to six R genes have been defined (Tyler 2002). Avr genes 3, 10 and 11 occur in a tight cluster, while the other avr genes have unique locations in the pathogen's genome.

When plant breeders place too much emphasis on breeding for vertical resistance, it may cause an erosion of horizontal resistance; this phenomenon is known as the vertifolia effect (Van der Plank 1984). Many genes are involved in horizontal resistance, the genes are dispersed when cultivars are crossed and the horizontal resistance is easily lost (Van der Plank 1963). The vertifolia effect arises because breeding for vertical resistance reduces selection pressure, which thereby reduces horizontal resistance of cultivars that survive the selection process (Van der Plank 1963).

A resistance response occurs when an incompatible race of *P. infestans* attempts to infect a potato plant containing appropriate R genes. Responses by the plant include the accumulation of phenolic compounds, phytoalexins, and the hypersensitive response (Hächler and Hohl 1984, Sakai et al. 1982, Sato and Tomiyama 1976). Hächler and Hohl (1984) noted that the browning of cell walls and intercellular areas by phenolic compounds was more pronounced in the resistant potato cultivar than in the susceptible cultivar. They also found a positive correlation between resistance and the abundance of papillae in potato tuber cells produced in response to infection. There were eight times more papillae formed in the resistant cultivar after infection than in the susceptible cultivar (Hächler and Hohl 1984). Phytoalexins, like rishitin, have been found to be synthesized in healthy tissue near the infection site and then are transported into necrotic tissue and accumulated there (Sakai et al. 1982). In highly resistant tuber tissue, rishitin accumulated the earliest and fastest, and reached a maximum amount as pathogen development stopped. In tissue with a low level of resistance, rishitin accumulation occurred late and slowly (Sato and Tomiyama 1976).

Induced resistance is defined as the activation of defense mechanisms in plants in the course of their development (Ozeretskovskaya 1995). Resistance in plants may be induced by inoculation with avirulent races, nonpathogenic or inactivated strains, or treatment with biological or abiotic elicitors. Work by Ozeretskovskaya (1995) found that elicitors (β-1,3-β-1,6 glucan from *P. infestans* cell walls and lipoglycoprotein complex [LGP complex]) suppressed disease development on tuber slices at two concentrations. High concentrations (100 μg LGP/ml) induced resistance of challenged plants 48 hours after treatment. The resistance was localized and short-term, and based on elicitor-induced necrosis on the surface of the tissue and the production of phytoalexins. Low concentrations (5-10 μg LGP/ml) induced resistance despite a lack of necrosis and phytoalexins. The resistance was systemic and long-term. Systemic induced resistance was also marked by a metabolic change in tuber cells; the parenchymal tuber cells acquire enhanced respiratory and synthetic capacities (Ozeretskovskaya 1995). There is also a promotion of wound repair and an increase in activities of peroxidase and lipoxygenase [(Chalova et al. 1985, Yurganova et al. 1989) cited in Ozeretskovskaya 1995].

Resistance in potato tubers is variable depending on the cultivar, age of tubers, and site of infection. Over time, late blight resistance declines in stored tubers. Potato tubers containing the late blight resistance gene R1 had weakened late blight resistance, with an increase in depth of penetration by the pathogen and an increase in the number of necrotized cells (Chalenko et al. 1980). Tubers have also shown a reduction in outer cortical resistance to *P. infestans* during storage (Pathak and Clarke 1987). Varietal resistance of potato cultivars to late blight differs with inoculation method and location

(Davila 1964). The outer living cells of the cortex of some potato cultivars are resistant to *P. infestans*. The reaction to infection in this area is similar to a hypersensitive response although it involves more cells. The resistance was based on preformed factors, rather than an energy requiring system that is needed for HR (Pathak and Clarke 1987). Defense responses of the periderm, cortical, and medulla tuber tissues varied depending on the isolate of *P. infestans* that caused the infection (Flier et al. 2001).

Pathogen response to resistance in tubers may be genotype specific (Kirk et al. 2001a). For example, observations by Flier et al. (2001) indicate that the gene-for-gene pathosystem in potato is not solely responsible for the differential interaction in tuber infection. Experiments using the US-8 genotype of *P. infestans* found that foliar and tuber susceptibility were not correlated (Kirk et al. 2001a, Dorrance and Inglis 1998), however, experiments using the US-1 genotype found a strong correlation (Platt and Tai 1998). Flier et al. (2003) noted the presence of a differential interaction that was independent of R-gene based resistance. The differential interaction indicated an adaptation by *P. infestans* to partial resistance, causing a decline in stability and durability of partial resistance to late blight (Flier et al. 2003).

Effects of Environment on the Disease

The effects of temperature, humidity, precipitation, wind and irradiation on the development of late blight in potato foliage were described by Harrison (1992). Temperature is an important environmental factor affecting development of both the host and pathogen. Environmental temperatures that are above an organism's optimum can cause reduced growth, protein misfolding, and enzyme denaturing (Ang et al. 1991).

Temperatures below an organism's growth optimum will also cause reduced growth and temperatures near 0°C can induce ice nucleation or cellular dehydration causing stress or death (Pearce 2001). Humidity affects the development of the pathogen and the infection process (Harrison 1992). High humidity and leaf wetness are essential for the germination of *P. infestans* spores and infection. Precipitation, wind, and irradiation are environmental factors that have an effect on leaf wetness and therefore effect germination and infection (Harrison 1992). This project focuses on effect of cold temperature on mycelium survival in order to better understand by what means the pathogen is overwintering and to determine if different isolates of *P. infestans* vary in ability to survive freezing temperatures. The effect of warm temperatures on development of *P. infestans* mycelia was not examined in this project, however warm temperature (above 35°C) experiments will be carried out by W.W. Kirk's lab (personal communication).

Cold Tolerance of Organisms

During cold adaptation, the structural integrity of proteins, ribosomes and membranes of the organism must be maintained. There have been many unsuccessful attempts to isolate cold adapted mutants of mesophillic organisms. A high number of mutations would be required and their occurrence in a single cell is unlikely [(Russel 1990) cited in Berry and Foegeding 1997]. Microorganisms adjust the fatty acid composition of membrane phospholipids in response to changes in temperature. As temperatures decrease, the fatty acid chains undergo a change in state from fluid to a more ordered crystalline array of fatty acid chains. A cold adapted organism responds to freezing temperatures by incorporating fatty acids with lower melting points into their

membranes. This action lowers the temperature of lipid phase transition, thus maintaining membrane fluidity and cell function. Cold tolerant organisms generally have higher proportions of unsaturated fatty acids in their membrane phospholipids, and there is a correlation of minimum growth temperature and fatty acid composition (Berry and Foegeding 1997). Plants that undergo cold acclimation experience changes in membrane lipid composition and accumulation of solutes such as sugars, proline, betaines, and proteins called dehydrins. Solutes and dehydrins have been proposed to stabilize membranes by interaction with membrane surfaces or by interaction with surrounding water (Pearce 2001).

Effects of Cold Temperature on Plants

Plants freeze when they can neither avoid ice nucleation nor prevent growth of ice (Pearce 2001). Ice may be formed in plants either by nucleation within plant tissue or by nucleation on a leaf surface where the ice then enters a plant through stomata or hydathodes. Experiments investigating the growth of ice in plants have shown that in leaf tissue there is an initial rapid growth of ice that causes a small proportion of leaf water to freeze, then there is a second more intense freezing event in which water is drawn out of cells and freezes extracellularly (Pearce 2001). Damage due to low temperatures is usually caused by freezing induced dehydration; other less common factors include large ice masses affecting tissue or organ structure, embolisms in the xylem, and pathogens that may enter through lesions (Pearce 2001). Cell membranes are damaged when freezing induced dehydration exceeded the cells' tolerance for

dehydration. The result is a loss of compartmentation that is visualized as leakage of electrolytes and other solutes.

In potatoes, storage at low temperatures causes a damaging effect on the glycolytic metabolism by tubers (Viola and Davies 1994). Potato cultivars reacted differently to cold storage in terms of partitioning carbon into either starch or sucrose. For example, after storage at 3°C for 8 weeks, potato cultivar Record exhibited approximately equal carbon partitioning into both starch and sucrose, however cultivar Brodick partitioned a greater amount of carbon into starch than sucrose (Viola and Davies 1994). Viola and Davies' study (1994) illustrated that low temperatures lead to reduced glycolytic flux that increases the availability of hexose phosphates for the synthesis of sucrose or starch. The partitioning of hexose phosphates into either sucrose or starch is determined by competition between the two synthetic pathways (Viola and Davies 1994). A four year study evaluating carbohydrate metabolism in potato tubers was conducted using cultivars that were known to be either cold tolerant (low sugar accumulating) or cold sensitive (high sugar accumulating) (Blenkinsop et al. 2003). Tubers in low temperature storage are expected to undergo a shift in metabolism from aerobic to anaerobic respiration as a means to compensate for impaired mitochondrial function during cold storage (Blenkinsop et al. 2003). Cold tolerant tubers are proposed to undergo a greater shift in metabolism, diverting more carbon away from the accumulation of reducing sugars (Blenkinsop et al. 2003).

Winter wheat undergoes a hardening process, which is induced by decreasing temperatures during the fall and early winter. Cold hardening is essential for development of snow mold resistance and freezing tolerance (Gaudet et al. 2000). The

hardening process includes reduced growth rate, change in growth habit, reduced tissue water content, an increase in abscisic acid, and changes in membrane lipid composition. Plants that did not completely undergo hardening were more susceptible to snow mold (*Microdochium nivale*) infection. Cold hardening also caused increased resistance to other fungal diseases, and it appears to be a non-specific form of disease resistance (Gaudet et al. 2000).

Potato tubers for seed or table stock are typically stored at 3°C, and processing tubers at 7 to 10°C (Kirk et al. 2001b). Tubers stored at 10°C are at greater risk for development of late blight because *P. infestans* has been shown to develop maximally at this temperature rather than at 3 or 7°C (Kirk et al. 2001b). This work illustrates the importance of understanding the interaction of *P. infestans* and tubers at cold temperatures.

Overwintering of *P. infestans*

An ongoing question concerning the epidemiology of potato late blight is how the pathogen overwinters. It is possible that *P. infestans* may overwinter as spores (de Bruyn 1926), mycelium (Shattock 1976, Bonde 1943), or both. Another uncertainty is the temperature threshold for *P. infestans* survival. In order to understand how *P. infestans* overwinters, knowledge of winter weather trends is very helpful. Weather conditions such as temperature and precipitation will affect the survival of *P. infestans* during winter months and can potentially affect disease development in the summer (Easton 1982). Figures 1 and 2 illustrate the temperature and precipitation trends during winter months in Michigan over the past one hundred years (Lawrimore 2004). Average

temperatures during winter are below freezing for December through March, while the temperature trends have increased slightly over the past hundred years. Average precipitation is greatest during the early and late winter months, and the precipitation trends over the past hundred years have increased slightly with the exception of February, which has shown a slight decrease. As a result of the greenhouse effect, it has been estimated that winters in North America will become 1 - 6°C warmer than at present. Climate data illustrate that, in Michigan, warmer winters are accompanied by lower winter precipitation (Isard and Schaetzl 1998). There is generally a greater incidence of soil freezing in Michigan during warm dry winters due to recurring freeze-thaw episodes (Isard and Schaetzl 1998). It seems that global warming may hinder the winter survival of *P. infestans* found in volunteer potato tubers.

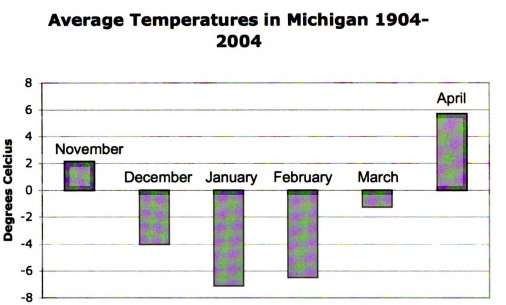


Figure 1: Average monthly temperatures in Michigan during winters 1904-2004.

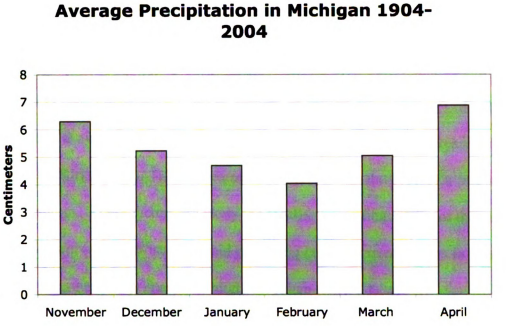


Figure 2: Average monthly precipitation in Michigan during winters 1904-2004.

As stated earlier, a major source of early infection in commercial potato fields are cull piles. Infected seed tubers and volunteer plants are of minor importance for late blight inoculum. Infested organic potato fields were a source of mid-season late blight inoculum (Zwankhuizen et al. 1998). A study of the thermal properties of cull piles has shown that the average temperatures at the center and base of a pile increase with the size of the pile (Kirk 2003a). Tubers located at the center and the base of a pile were more likely to sprout. These factors are likely the result of insulating effects of the surrounding tubers. Growers were advised to avoid producing cull piles over one ton in mass in order to reduce the source of primary late blight inoculum (Kirk 2003a).

Isolates of *P. infestans* vary in their ability to survive winter freezing (Kadish and Cohen 1992, Kirk 2003b, Shattock 1976). More aggressive isolates of *P. infestans* are able to colonize potato tissue faster and pose a larger threat during the growing season than their less aggressive counterparts; however, the aggressive isolates may be at a selective disadvantage when it comes to overwintering (Shattock 1976). The more aggressive isolates of *P. infestans* were found to completely destroy host tubers during the winter so that there were few tubers left to sprout and cause disease in the spring. It has been suggested that the pathogen population and variability is severely reduced during winter, but the variability is regenerated each year within the expanding population (Shattock 1976).

Cold Temperature Effects on Pathogenicity

The effect of temperature on the development of *P. infestans* in potato foliage is well documented. There is a wide variation in the temperature effect on different life

stages of *P. infestans* (Harrison 1992). The optimal temperature for growth of hyphae in foliage is 15-20°C (Harrison 1992). Kadish and Cohen (1992) found that the rate of sporangial development decreased at temperatures below 10°C. Tubers are usually held at 10°C for up to 6 months, therefore colonization of individual tubers poses a greater risk than spread of disease in storage by sporangia (Kirk et al. 2001b). Mizubuti and Fry (1998) studied the effect of temperature on sporangial germination from US-1, -7, -8 isolates of *P. infestans*. Their results indicate that US-1 isolates germinate better at higher temperatures (15-25°C) than US-7 or US-8, which germinate better at 10°C (Mizubuti and Fry 1998).

The effect of temperature on the development of zoospores has been widely studied. For example, it has been demonstrated that cooler temperatures cause zoospores to be released more slowly and allow them to swim for longer durations [(Melhus 1915a, 1915b, Crosier 1934) cited in Harrison 1992]. Zoospores were found to be motile for 22 hours at 5-6°C, while at 24-25°C zoospores were motile for only 19 minutes [(Melhus 1915a) cited in Harrison 1992]. Work by Crosier [(1934) cited in Harrison 1992] supported the results of Melhus [(1915a,b) cited in Harrison 1992], showing that zoospores swam for 24 hours at 1°C and for only 15 minutes at 24°C.

The survival of mycelium of different genotypes of *P. infestans* was recently studied and measured by the average reflective intensity (ARI) of the cultures (Kirk 2003b). Results indicated that exposure of mycelium of genotypes US-1, -8, -11, -14 on rye agar plates to –20°C and -10°C for durations greater than one hour proved lethal to the cultures. Mycelium survived exposure to -3°C for up to 3 days but could not survive exposure to -5°C for even one day (Kirk 2003b).

Phytophthora infestans has been shown to be capable of enduring -17°C for durations of up to 10 days (deBruyn 1926). After 10 days there was a reduction in survival of the cultures. Growth of the pathogen was better after a sudden change in temperature (from cold to warm) than after a gradual change. Results also have shown that *P. infestans* survived best when grown on manure because it encouraged good growth and strong resistance to cold (deBruyn 1926). Sato has considered the effect of soil temperatures on field infection of potato tubers. Results indicate that cooler soil temperatures (<17°C) favored tuber infection as water in the cooler soil assists release and motility of zoospores that cause the primary infection (Sato 1979). In storage, disease was not transmitted between tubers when healthy and infected whole or cut tubers were mixed and stored at 1°C, however, disease was transmitted very well among cut tubers stored at 17°C in either humid or dry conditions [(Löhnis 1922) cited in Lambert and Currier 1998].

Metalaxyl-resistant and metalaxyl-susceptible isolates of *P. infestans* exhibit differing abilities to grow at and survive cold temperatures. Metalaxyl-resistant isolates were shown to grow faster on tuber slices incubated at 5°C than metalaxyl-sensitive isolates (Walker and Cooke 1990). The capacity of metalaxyl-resistant isolates (e.g. US-8) of *P. infestans* to overwinter in tubers is inferior to that of metalaxyl-susceptible isolates (e.g. US-1) (Kadish and Cohen 1992, Walker and Cooke 1988, 1990). Thus metalaxyl-susceptible isolates are more likely to survive the winter in tuber tissue because they do not completely colonize the tubers predisposing them to secondary rots.

Temperature Effects on Other Fungi

Many studies have been carried out concerning the ability of other fungi to grow at cold temperatures. Wong et al. (1996) studied the biocontrol properties of cold tolerant *Gaeumannomyces graminis* var. *graminis* (*G.g.g.*) and *Phialophora* species against wheat take-all. They concluded that cold tolerance in *G.g.g.* is important for biocontrol of take-all because soil temperatures in the root zone are between 5°C and 15°C during the early growth phase. Growth rates of *G.g.g.* and *Phialophora* at 5°C was equal to or greater than that of the antagonist species *G.g.* var. *tritici*, indicating that they can compete against the take-all fungus for colonization of wheat roots (Wong et al. 1996). *Typhula* species are known for causing Typhula blight of turfgrass and grey and speckled snow mold of forage grasses and winter cereals. *Typhula* species overwinter as sclerotia in soil, crop debris, or turfgrass thatch (Smith 1987). Sclerotia of *Typhula* species may germinate myceliogenically under the snow serving as the primary inoculum (Burpee 1994).

Ice nucleation is the initiation of the phase transition of water from the liquid to solid state (Lundheim 2002). Ice nucleators can protect freeze-tolerant microorganisms while causing injuries to plants that the microorganisms feed upon. Five species of *Fusarium* (*F. acuminatum, F. avenaceum, F. moniliforme, F. oxysporum*, and *F. tricinctum*) have been reported to exhibit ice nucleation activity (Humphreys et al. 2001). It has been suggested that the ice nucleation activity in *Fusarium* is a trait in pathogenic species that allows a competitive advantage over non ice nucleating species because freezing enhances infection of roots by *Fusarium* species (Richard et al. 1996). A study involving ice nucleation activity of *F. acuminatum* SRSF 616 found that it produced ice

nuclei independent of nutrient availability (Humphreys et al. 2001). The author notes that further investigations would be needed in order to fully understand the ecological significance of ice nucleation in *Fusarium* species (Humphreys et al. 2001).

Plasmopara viticola, the causal agent of grape downy mildew, is capable of surviving and reproducing at low temperatures (13 to 18°C) [(Lafon and Bulit 1982) cited in Bakshi et al. 2001]. A biocontrol agent (*Fusarium proliferatum*) was isolated and found to be antagonistic against *P. viticola* at low temperatures (Bakshi et al. 2001). *F. proliferatum* was isolated from atypical grape downy mildew lesions and was found to parasitize hyphae and sporangia of *P. viticola* (Bakshi et al. 2001). These two fungi may have coevolved together on grape plants, which could explain why both species are capable of surviving low temperatures.

The effect of temperature on the growth and infectivity of *Phytophthora cinnamomi* on its avocado host was studied by Zentmeyer (1981). The growth curves with respect to temperature (9 to 27°C) of *P. cinnamomi* and avocado were similar except at 33°C where the pathogen stopped growing and the host grew well (Zentmeyer 1981). This data represents another scenario where the pathogen and host coevolved in the same environment leading to similar growth curves with respect to temperature.

It is widely known that potato cull piles are the major source of primary inoculum for late blight in the spring (Bonde 1943, Zwankhuizen et al. 1998, Kirk 2003a). The surface layer of cull piles is exposed to winter temperatures fluctuating between 10°C and -10°C (Kirk 2003a); tubers can withstand temperatures as low as -3°C before being broken down (Seymour 2002). It is probable that infected tubers located on the surface

layer would not survive winter. Tubers located at the middle and base of cull piles however are exposed to a temperature range of 10° to 0°C (Kirk 2003a). My project aims to determine if *P. infestans* is capable of surviving temperatures typical to those found in the center of a cull pile (3 to -5°C).

Research Objectives

The objectives of this research are to determine how different genotypes of *P. infestans* respond to storage at temperatures around 0°C both *in vitro* and in tuber tissue. Results are recorded based on average reflective intensity of mycelium grown on Petri plates and of sections from inoculated tubers after being stored at temperatures near freezing. Another objective of my study was to have a cytological assessment of the development of *P. infestans* in tuber tissue slices after exposure to cold temperatures. A qualitative estimate of the degree by which *P. infestans* was capable of colonizing the tuber slices was made.

CHAPTER 2: SURVIVAL OF MYCELIUM OF *PHYTOPHTHORA INFESTANS* AT COLD TEMPERATURES *IN VITRO*

Introduction

Understanding how plant pathogens survive freezing temperatures is important for the control of disease in temperate climates. This is because knowledge of a pathogen's low temperature survival mechanisms and the lethal temperature threshold can help shape a grower's cultural practices toward prevention of pathogen survival over winter.

Many studies have been conducted concerning the temperature ranges suitable for growth of *Phytophthora infestans*. Researchers have used *in vitro* and soil experiments to investigate optimal and high lethal temperatures for hyphae and sporangia of *P. infestans* (Juarez-Palacios et al. 1991). The different life stages of *P. infestans* exhibit a wide range of optimal temperatures (Harrison 1992). The optimal temperature for growth of hyphae in foliage is 15-20°C (Harrison 1992). Most work has concentrated on survival of sporangia of *P. infestans* at various temperatures (Mizubuti and Fry 1998, Kadish and Cohen 1992, Fay and Fry 1997). *P. infestans* has been shown to be capable of enduring -17°C for a duration of up to 10 days (de Bruyn 1926). Exposure of *P. infestans* mycelium of genotypes US-1, US-8, US-11, and US-14 on rye agar to -20°C and -10°C for durations greater than one hour proved lethal to the cultures (Kirk 2003b). *P. infestans* mycelium survived exposure to -3°C for up to 3 days but could not survive exposure to -5°C for even one day (Kirk 2003b). Using methodology developed by Kirk (2003b), an

in vitro assay was devised to determine the ability of mycelium of various genotypes of *P. infestans* to survive freezing temperatures.

The objectives of this research were to determine potential lethal temperature thresholds for various isolates of *P. infestans* and to determine which *P. infestans* isolates were the most cold tolerant and cold sensitive. Based on results from Kirk (2003b), we hypothesized that the -5°C temperature treatment would be lethal for cultures exposed for durations of 3 to 5 days, and that *P. infestans* isolates of the US-8 genotype, and isolate CO37 from Mexico would be the most cold tolerant based on the knowledge of their aggressive growth in culture and in potato tissue.

Materials and Methods

Maintenance of *Phytophthora infestans* Cultures

Isolates of *Phytophthora infestans* used for this project are listed in Table 1. Cultures of *P. infestans* were maintained on rye A agar (Caten and Jinks 1968) at 25°C. Isolates were incubated on rye agar at room temperature for approximately one month before subcultures were made. In order to preserve the virulence of the isolates, only four subsequent subcultures were made of each isolate after which it was transferred to slices of potato tuber tissue, which were stored at room temperature. Seven to ten days after transfer to tuber tissue, the mycelium of the isolate was harvested and placed onto a rye agar plate. This culture was labeled '1' for the first subculture from plant tissue.

Inoculation of Petri Plates

Sterol-free rye A agar [10.0 ml; (Caten and Jinks 1968)] were added to polystyrene Petri dishes (60 x 15 mm) with a sterile pipette. The dishes containing rye agar were inoculated by placing 5 mm diameter plugs of *P. infestans* (grown on rye agar for 3 to 4 weeks) onto the center of the Petri dish. Five replicates were prepared for each temperature treatment. Inoculated dishes were sealed with Parafilm and labeled with the appropriate treatment name. Negative control samples were non-inoculated Petri dishes containing 10.0 ml rye agar. Positive control samples were inoculated with 5 mm diameter plugs of *P. infestans* and stored at 25°C for the duration of the experiment.

The Petri dishes were stored in PTC-1 Peltier effect temperature chambers controlled by PELT-3 Peltier effect temperature controllers (Sable Systems International, Las Vegas, NV 89119) set to 3°C for 2 days prior to exposure to the treatments in order to reduce sporulation. The temperatures in the Peltier chambers were adjusted to the experimental temperatures (3, 0, -3, and -5°C). The Petri dishes were stored in the Peltier chambers set to experimental temperatures for durations of 1, 2, 3, 4, or 5 days. After removal from the Peltier chambers, the dishes were stored at 25°C with a light/dark cycle of 9/15 hours for 28 days in order to allow for the culture to attempt to recover and grow onto the agar plate.

Table 1. *Phytophthora infestans* isolates used for the *in vitro* temperature experiment, and their corresponding genotype and mating type.

P. infestans Isolate[1]	Genotype	Mating Type
671 (WA)	US14	A2
95-3 (MI)	US1	A1
94-1 (MI)	US17	A1
98-1 (MI)	US14	A2
CO37 (Toluca, MX)	US6	A1
3222 (WA)	US8	A2
95-7 (MI)	US8	A2
458 (ND)	US8	A2
364 (WA)	US17	A1

[1] Isolate identification listed as laboratory identification code followed by location of collection.

Digital Image Analysis

The dishes were prepared for image analysis after 28 days of incubation. The lids were removed from the Petri dishes, and the dishes were placed with the open side down onto a glass plate large enough to lie over the bed of the scanner. The glass plate containing the Petri dishes was placed over the scanner bed (Epson Perfection 2400 Scanner, Epson America Inc., Long Beach, CA 90806). Using computer software provided with the scanner, color images (70.9 pixels per cm^2 resolution) of the dishes were made. The images of the dishes were analyzed using digital image analysis software (Sigma Scan Pro 5, SPSS Inc., Chicago, IL 60606) in order to determine the average reflective intensity (ARI) of the mycelium (Kirk 2003b). Average reflective intensity in light intensity units (LIU) was used to measure the growth of mycelium after exposure to cold temperature. ARI is a measure of the intensity of light reflection off of the scanned surface in light intensity units (LIU) where LIU = 0 is black and 255 is white. The manual threshold level of the Sigma Scan program was adjusted in order to set an intensity range for an image. A manual threshold level of 40 enabled the software to differentiate between the sample and the background within an image.

This experiment was repeated three times for each isolate of *P. infestans* used. Because different scanners and scanner settings were used over the course of the experiment, the brightness/contrast levels of images of Petri dishes were adjusted in Adobe Photoshop (Adobe Photoshop version 5.0, Adobe Systems International, San Jose, CA 95110) in order to obtain consistent ARI values for each experiment. A color image of Petri plates of isolate 95-7 grown on rye agar and exposed to -3°C for durations of 1 to 5 days is shown in Figure 3. Images in this thesis are presented in color.

Data Analysis

Interactions among *P. infestans* isolate, temperature, and duration of exposure for all trials of the experiment combined were determined by three-way analysis of variance (ANOVA), (SAS version 8.1, SAS Institute, Cary, NC 27513). Two-way ANOVA was used to determine if recovery of cultures of *P. infestans* occurred from exposure periods at each temperature within individual isolates. The analysis was done by comparing the ARI of the treated samples with the ARI of the negative control that underwent the same temperature exposure, at $p=0.05$. Cultures that were not significantly different from the negative control (did not recover from temperature treatment, therefore considered cold sensitive) were given a survival index value of 0, while those that were significantly different from the negative control (recovered from temperature treatment, therefore considered cold tolerant) received an index value of 2, and cultures that were neither significantly different from the negative control nor the positive control (some samples recovered from temperature treatment and some did not recover) were assigned an index value of 1. Index values for each temperature treatment were averaged over corresponding trials and the mean index values calculated.

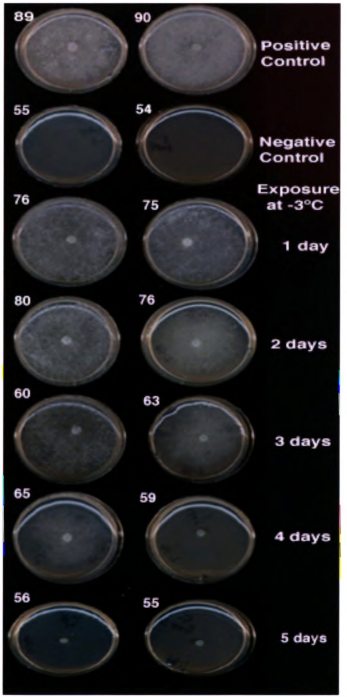


Figure 3. Images of *Phytophthora infestans* genotype US-8 on sterol-free rye agar plates exposed to -3°C for durations of 1, 2, 3, 4, and 5 days, then incubated at 25°C for 28 days. The positive control plates were exposed to 25°C for 5 days and incubated as treatments. The negative control plates were left non-inoculated and exposed to -3°C for 5 days and incubated as treatments. The numerical values are the average reflective intensities (ARI) in light intensity units as measured with Sigma Scan.

Results

The effects of *Phytophthora infestans* isolate, temperature, and exposure period on hyphal growth and the interactions of these variables on ARI are shown in Table 2.

Table 2. Sources of variation and their statistical significance (LSD, p=0.05) for the Petri plate experiments, which determine the recovery of cultures of *Phytophthora infestans* after exposure to cold temperatures for different durations.

Source of Variation	**DF**	**F value**	**p value**
Isolate	8	60.67	<0.0001
Temperature	3	14.19	<0.0001
Duration	4	8.69	<0.0001
Isolate*Temperature	24	2.33	0.0003
Isolate*Duration	32	2.93	<0.0001
Iso*Temp*Dur	108	1.81	<0.0001
Total	179	5.28	<0.0001

Statistical analysis showed that the effects of isolate, temperature and exposure period were all significant. The average reflective intensity values for each isolate are listed in Table 3 in the Appendix. Isolate 93-3 (US-1 genotype) survived exposure to 3°, 0°, and -3°C for durations of 1 to 5 days, and -5°C for 1 to 4 days (Table 3a). Isolates 94-1 and 364 (US-17 genotype) survived exposure to 3°, 0°, and -3°C very well, and survived moderately well at -5°C (Tables 3b and 3c). CO37 (US-6 genotype) was able to survive all temperature treatments (Table 3d). 98-1 (US-14 genotype) displayed a moderate ability to survive 3°, 0°, and -3°C, and was less able to survive -5°C (Table 3e) but survived the colder temperatures (-3 and -5°C) for 2 or 3 days. 671 (US-14 genotype) survival varied between trials. However, data from trial 3 indicated significant survival

of isolate 671 at all temperature treatments (Table 3f). US-8 genotype isolates 95-7 and 458 were moderately capable of surviving the temperature treatments with results varying between trials (Tables 3g and 3h). Isolate 3222, also US-8, displayed a very strong ability to survive all temperature treatments (Table 3i).

The response of three isolates of *P. infestans* to exposure to temperatures from -5° to 3°C for durations of 1 to 5 days is shown in Figures 4a and 4b. The various points on the graphs represent the mean response of five replicates. The bars at each point represent the standard deviation. *Phytophthora infestans* isolate 95-7 (US-8) was selected because it showed a response representative of the moderately cold tolerant isolates studied, isolate 458 (US-8) was chosen as representative of cold sensitive isolates, and isolate CO37 (US-6) represents the cold tolerant isolates studied. No *P. infestans* isolates were found to survive the -5°C temperature treatment for durations longer than 3 days, however isolate CO37 (US-6) survived -5°C for 1 and 2 day exposures. Isolate CO37 (US-6) survived -3°C for all exposure durations and 0°C for durations of 1 to 4 days. Isolate 458 (US-8) was unable to survive any of the cold temperature treatments. Isolate 95-7 (US-8) was shown to survive -3°C for 1 to 4 days, 0°C for 1 to 4 days, and 3°C for 1 and 3 days.

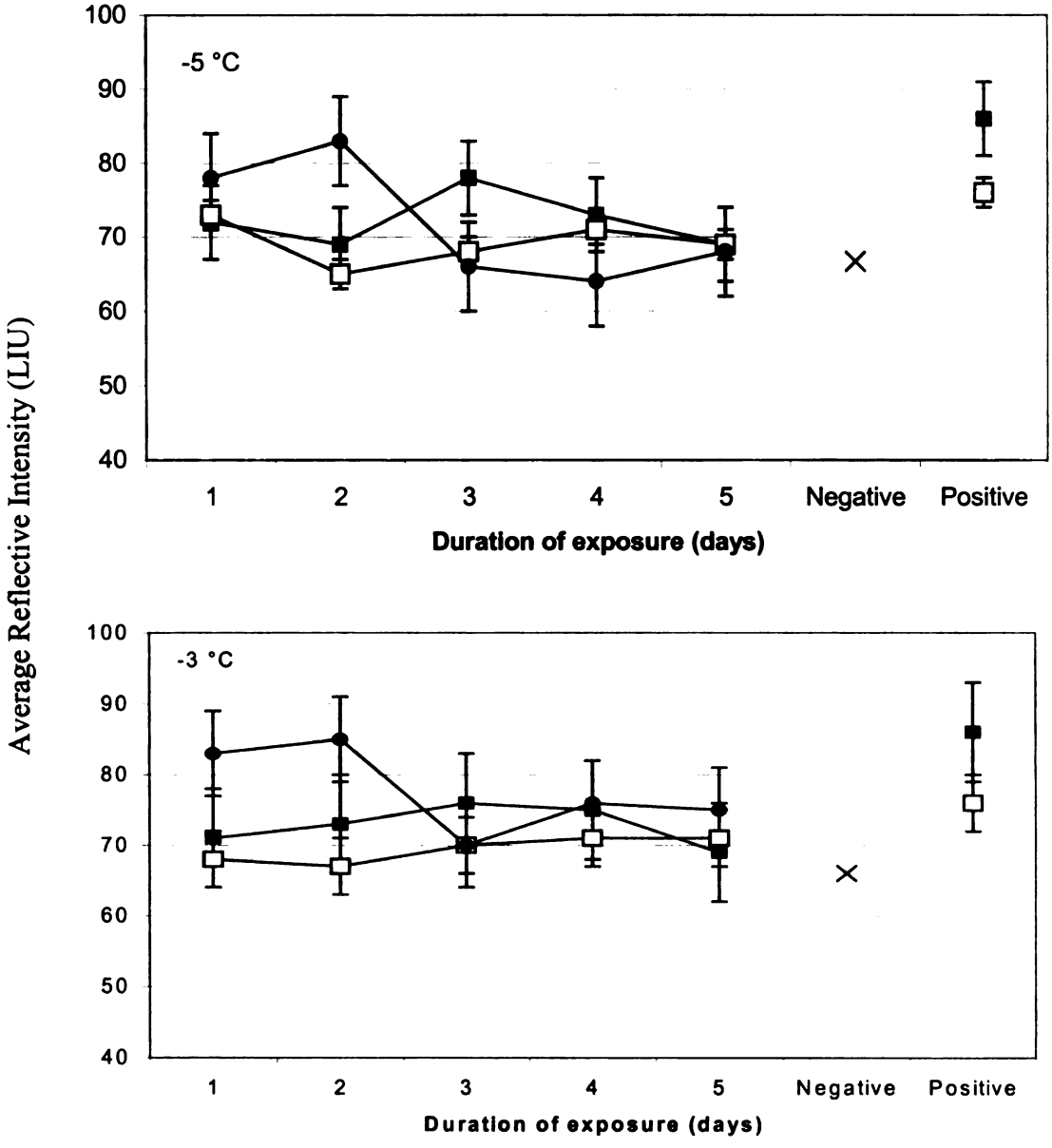


Figure 4a. Example of survival response (average reflective intensity) of different isolates of *Phytophthora infestans* exposed to temperatures of -5 and -3°C for durations from 1 to 5 days and then incubated at 25°C for 4 weeks. ■ = 95-7 (US-8); □ = 458 (US-8); ● = CO37 (US-6); x = Negative Control (sterile rye agar).

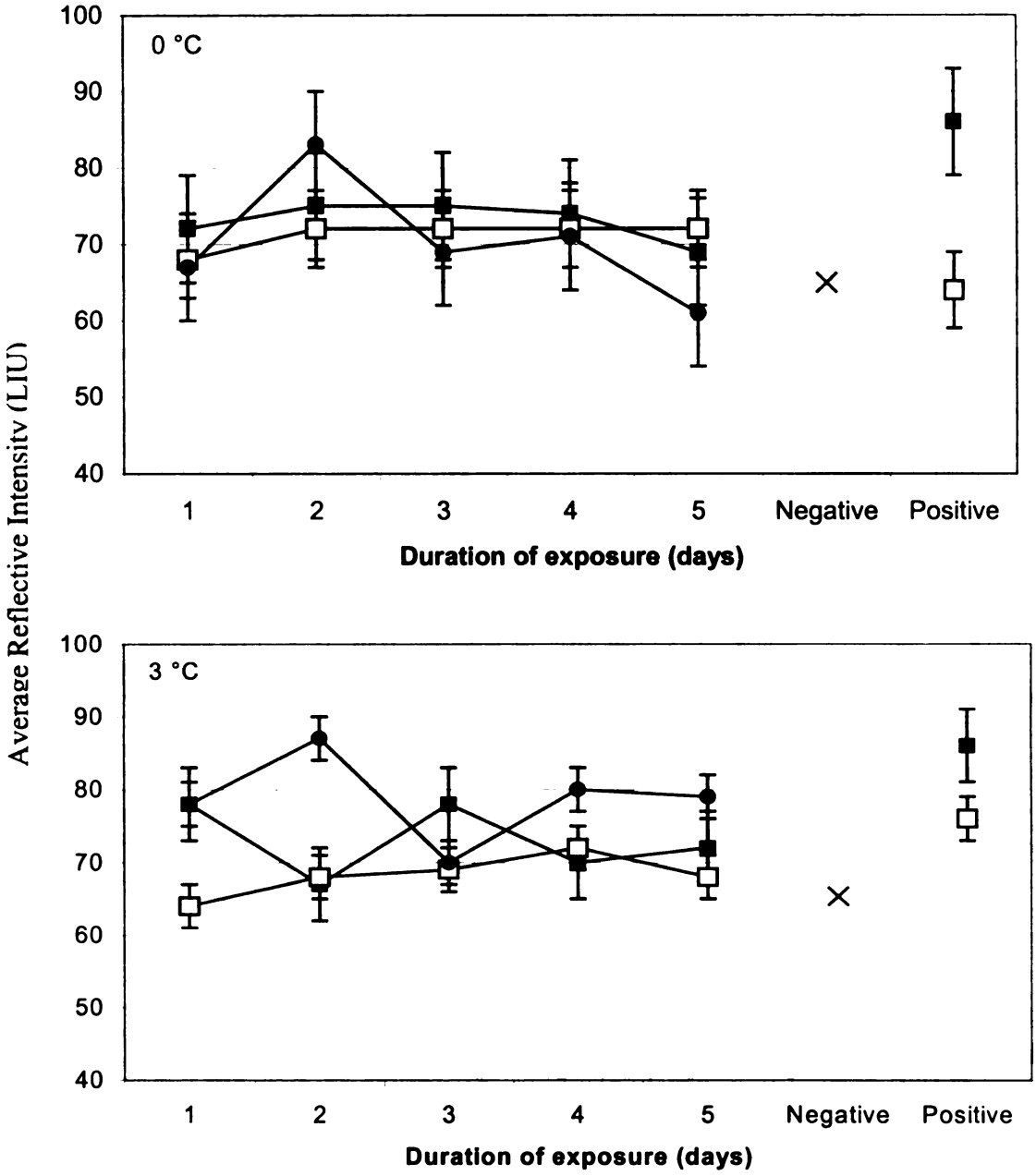


Figure 4b. Example of survival response (average reflective intensity) of different isolates of *Phytophthora infestans* exposed to temperatures of 0 and 3°C for durations from 1 to 5 days and then incubated at 25°C for 4 weeks. ■ = 95-7 (US-8); □ = 458 (US-8); ● = CO37 (US-6); x = Negative Control (sterile rye agar).

The *P. infestans* isolates were classified as being either cold sensitive (Table 4), moderately cold tolerant (Table 5), or cold tolerant (Table 6). Survival responses are illustrated in Tables 4 – 6 by the letters **a** (ARI not significantly different from negative control), **b** (ARI significantly different from negative control), and **ab** (neither significantly different from negative control ARI nor positive control ARI, indicates intermediate survival). A survival response of the letter **a** was given a numerical value of zero, a response that was designated with **ab** was given a value of one, and a response that was designated with **b** was given a value of two. The index of survival rating (I) is the mean survival number for all trials of a particular treatment. An index of survival rating of zero indicates that the isolate of *P. infestans* was not statistically different from the negative control. An index of survival rating of two indicates that the survival of the isolate of *P. infestans* exposed to cold temperature was significantly different from that of the negative control. Intermediate index of survival ratings indicate that the isolate of *P. infestans* exhibited an intermediate survival response to cold temperature.

Survival of *P. infestans* isolates was favored at 3° and 0°C (Tables 4 – 6). There was a large difference between the number of cultures, which survived -3° and -5°C temperature treatments, with greater survival in cultures undergoing -3°C temperature treatments. When examining the ability of different *P. infestans* isolates to survive -3° and -5°C for different durations it is clear that the isolates were better able to survive shorter exposure durations to freezing temperature than longer durations of exposure. Isolates 95-3, CO37, and 3222 displayed the greatest ability to survive -5°C treatments. Isolates 98-1, 671 and 95-7 were the most sensitive to freezing.

Table 4. Responses of mycelium of cold sensitive isolates of *Phytophthora infestans* to cold temperature exposure for durations of 1 to 5 days.

	Exposure	Exposure Temperature °C																	
	Duration	3					0					-3					-5				
Isolate	(days)	1[1]	2[1]	3[1]	4[1]	I[2]	1	2	3	4	I[2]	1	2	3	4	I[2]	1	2	3	4	I[2]
98-1[4]	1	_[3]	b	b		2	b	b	-		2	b	b	b		2	-	b	b		2
US-14	2	-	ab	b		1.5	a	ab	-		0.5	b	b	b		2	a	ab	b		1
A2	3	-	b	b		2	b	a	-		1	a	b	b		1.6	a	ab	a		0.3
	4	-	ab	b		1.5	b	b	-		2	a	b	a		0.7	a	ab	a		0.3
	5	-	b	b		2	b	b	-		2	a	b	b		1.6	-	b	a		1
671[5]	1	-	a	b		1	b	a	b		1.3	b	a	b		1.3	ab	ab	b		1.3
US-14	2	-	a	b		1	b	a	b		1.3	b	a	b		1.3	a	a	b		0.7
A2	3	-	a	b		1	b	a	b		1.3	a	a	b		0.7	a	a	b		0.7
	4	-	a	b		1	b	-	b		2	a	a	b		0.7	b	a	b		1.3
	5	-	a	b		1	a	a	b		0.7	a	a	b		0.7	b	a	b		1.3
95-7[6]	1	-	b	b	-	2	b	b	a	b	1.5	a	b	ab	b	1.3	a	a	ab	b	0.8
US-8	2	-	a	a	b	0.7	b	a	ab	b	1.3	a	a	b	b	1	a	a	a	b	0.5
A2	3	-	a	b	b	1.3	b	a	a	b	1	ab	a	a	b	0.8	ab	b	b	b	1.8
	4	-	ab	-	b	1.5	b	a	a	b	1	a	a	a	b	0.5	a	ab	a	b	0.8
	5	-	a	a	b	0.7	ab	a	a	b	0.8	-	a	a	b	0.7	a	a	a	b	0.5
Negative Control		a	a	a	a	0	a	a	a	a	0	a	a	a	a	0	a	a	a	a	0
Positive Control		b	b	b	b	2	b	b	b	b	2	b	b	b	b	2	b	b	b	b	2

[1] Numbers refer to different trials of the experiment. Treatments sharing a common letter had mean average reflective intensity not significantly different at p=0.05 (LSD).
[2] Values are index numbers that indicate degree of survival. Index value of 0 indicates no samples recovered, 1 indicates half of the samples survived, 2 indicates all of the samples survived temperature treatment. Index numbers for each treatment were calculated by taking the mean of survival numbers for each trial.
[3] - denotes data missing.
[4] Average reflective intensity values for the various repetitions of culture 98-1 are shown in Table 3e in the Appendix.
[5] Average reflective intensity values for the various repetitions of culture 671 are shown in Table 3f in the Appendix.
[6] Average reflective intensity values for the various repetitions of culture 95-7 are shown in Table 3g in the Appendix.

Table 5. Responses of mycelium of moderately cold tolerant isolates of *Phytophthora infestans* to cold temperature exposure for durations of 1 to 5 days.

	Exposure	Exposure Temperature °C											
	Duration	3				0				-3				-5			
Isolate	(days)	1[1]	2[1]	3[1]	I[2]	1	2	3	I[2]	1	2	3	I[2]	1	2	3	I[2]
364[4]	1	b	/[3]	b	2	b	b	b	2	b	b	b	2	b	b	b	2
US-17	2	b	b	b	2	b	b	/	2	b	b	b	2	b	b	b	2
A1	3	b	b	b	2	b	b	/	2	b	b	b	2	b	b	a	1.3
	4	b	b	b	2	b	a	/	1	b	b	b	2	a	a	a	0
	5	b	b	b	2	b	b	/	2	b	b	b	2	a	a	a	0
3222[5]	1	b	b	b	2	b	b	b	2	b	b	b	2	b	b	b	2
US-8	2	b	b	b	2	b	b	b	2	b	b	b	2	a	b	b	1.3
A2	3	b	b	b	2	b	b	b	2	b	b	b	2	b	b	b	2
	4	a	b	b	1.3	b	b	b	2	b	b	b	2	a	b	b	1.3
	5	b	b	b	2	b	b	b	2	a	b	b	1.3	a	b	b	1.3
458[6]	1	/	a	b	1	b	a	b	1.3	b	a	b	1.3	b	ab	b	1.7
US-8	2	/	a	b	1	b	a	b	1.3	b	a	b	1.3	b	a	b	1.3
A2	3	/	a	b	1	b	a	b	1.3	b	a	b	1.3	b	a	b	1.3
	4	/	a	b	1	b	a	b	1.3	b	a	b	1.3	b	a	b	1.3
	5	/	a	b	1	b	a	b	1.3	b	a	b	1.3	b	a	a	0.7
Negative Control		a	a	a	0	a	a	a	0	a	a	a	0	a	a	a	0
Positive Control		b	b	b	2	b	b	b	2	b	b	b	2	b	b	b	2

[1] Numbers refer to different trials of the experiment. Treatments sharing a common letter had mean average reflective intensity not significantly different at p=0.05 (LSD).
[2] Values are index numbers that indicate degree of survival. Index value of 0 indicates no samples recovered, 1 indicates half of the samples survived, 2 indicates all of the samples survived temperature treatment. Index numbers for each treatment were calculated by taking the mean of survival numbers for each trial.
[3] / denotes data missing.
[4] Average reflective intensity values for the various repetitions of isolate 364 are shown in Table 3c in the Appendix.
[5] Average reflective intensity values for the various repetitions of isolate 3222 are shown in Table 3i in the Appendix.
[6] Average reflective intensity values for the various repetitions of isolate 458 are shown in Table 3h in the Appendix.

Table 6. Responses of mycelium of cold tolerant isolates of *Phytophthora infestans* to cold temperature exposure for durations of 1 to 5 days.

	Exposure	Exposure Temperature °C															
	Duration	3				0				-3				-5			
Isolate	(days)	1[1]	2[1]	3[1]	I[2]	1	2	3	I[2]	1	2	3	I[2]	1	2	3	I[2]
95-3[4]	1	_[3]	b	-	2	b	b	-	2	b	b	-	2	b	b	-	2
US-1	2	b	b	-	2	b	-	-	2	b	b	-	2	b	b	-	2
A1	3	b	b	-	2	b	-	-	2	b	b	-	2	b	b	-	2
	4	b	b	-	2	b	-	-	2	b	b	-	2	b	b	-	2
	5	b	b	-	2	b	-	-	2	b	b	-	2	b	a	-	1
94-1[5]	1	b	b	b	2	b	-	b	2	b	b	b	2	b	b	b	2
US-17	2	b	b	b	2	b	-	b	2	b	b	b	2	b	b	b	2
A1	3	b	a	b	1.3	b	-	b	2	b	b	b	2	b	b	b	2
	4	b	b	b	2	-	-	b	2	b	b	b	2	a	b	b	1.3
	5	b	b	b	2	b	-	b	2	a	b	b	1.3	a	b	ab	1
CO37[6]	1	b	b	b	2	b	b	-	2	b	b	b	2	b	b	b	2
US-6	2	b	b	b	2	b	b	-	2	b	b	b	2	b	b	b	2
A1	3	b	b	a	1.3	b	b	-	2	b	b	b	2	b	b	b	2
	4	b	b	b	2	b	b	-	2	b	b	b	2	a	b	b	1.3
	5	b	b	b	2	a	b	-	1	b	b	b	2	b	b	a	1.3
Negative Control		a	a	a	0	a	a	a	0	a	a	a	0	a	a	a	0
Positive Control		b	b	b	2	b	b	b	2	b	b	b	2	b	b	b	2

[1] Numbers refer to different trials of the experiment. Treatments sharing a common letter had mean average reflective intensity not significantly different at p=0.05 (LSD).
[2] Values are index numbers that indicate degree of survival. Index value of 0 indicates no samples recovered, 1 indicates half of the samples survived, 2 indicates all of the samples survived temperature treatment. Index numbers for each treatment were calculated by taking the mean of survival numbers for each trial.
[3] / denotes data missing.
[4] Average reflective intensity values for the various repetitions of isolate 95-3 are shown in Table 3a in the Appendix.
[5] Average reflective intensity values for the various repetitions of isolate 94-1 are shown in Table 3b in the Appendix.
[6] Average reflective intensity values for the various repetitions of isolate CO37 are shown in Table 3d in the Appendix.

Discussion

The temperature trends in the Great Lakes region of the United States have displayed an increase in the annual mean temperature for the area (Baker et al. 2002). The warmer climate has resulted in an increased potential for the survival of tubers left in fields and cull piles, which may lead to a greater capability of *P. infestans* inoculum to survive the winter. Cultivated soils in the Great Lakes region have been shown to freeze to a depth of 5 cm (Isard and Schaetzl 1998, Schaetzl and Tomczak 2001). Potato tubers left in the field are often buried deeper than 5 cm indicating they may be protected from frost. A previous study found that buried tubers are not often exposed to soil temperatures (about -3°C) that normally cause tissue decomposition (Kirk unpublished data). It can be concluded that potato tubers infected with *P. infestans*, if buried at a depth greater than 5 cm, would likely survive winter without rotting and therefore harbor inoculum for the following growing season.

Temperatures at the center and base of cull piles found in Michigan range from 10° to 0°C during the winter months (Kirk 2003a). This work has shown that the isolates of *P. infestans* tested would be capable of surviving a Michigan winter within a cull pile. Temperature data gathered from the surfaces of cull piles in Michigan ranged from 10° to -10°C during winter months. The isolates of *P. infestans* tested in this study are unlikely to survive exposure to such temperatures at the surfaces of cull piles. Knowledge of the ability of *P. infestans* to survive cold temperatures is therefore important in understanding the possible source of primary inoculum.

The results of this study illustrate great variability among genotypes in their ability to tolerate cold temperatures. The US-8 isolates tested in this study all displayed varying tolerance to cold temperature. Among the US-8 isolates, 3222 had the greatest survival index, 458 was moderately able to survive and 95-7 had the least ability to survive freezing temperatures. Peters et al. (1999) also found variation within the US-8 genotype with regard to aggressiveness of growth. These results imply that cold tolerance may not be associated with the genotype of *P. infestans*. The US-14 isolates 98-1 and 671 were concluded to be sensitive to freezing temperatures. These results conflict with findings from Kirk (2003b) in that isolates 671 and 95-7 had increased cold tolerance. The variability between studies may be due to the age of the cultures tested and therefore in future work cultures of the same age should be used consistently throughout the experiment. More tests should therefore be conducted to fully examine the cold tolerance of these isolates.

Isolate CO37 from Mexico exhibited great ability to survive freezing temperatures. Such results may be related to the general vigor of this isolate, which grew rapidly in culture and was highly virulent in tests conducted by Rubio-Covarrubias et al. (2004a, b). The US-1 isolate 95-3 had very aggressive culture re-growth compared to other isolates tested, and was concluded to be cold tolerant. Cold temperature experiments using a different US-1 isolate (95-6) found the isolate to be less cold tolerant. The inconsistency in these results may be attributed to a wider than expected range in cold tolerance variability within *P. infestans* genotypes.

Evaluating survival of *P. infestans* cultures based on digital image analysis is an important development by Kirk (2003b). The scanned images of cultures can be stored

indefinitely allowing for comparisons between different trials of an experiment. Average reflective intensity (ARI) was used to rate survival of *P. infestans* cultures, and it is based on light reflectance from mycelium grown on agar plates. A previous study established that ARI is an effective estimation of survival of *P. infestans* cultures because there was a direct relationship between ARI and mycelial weight (Kirk 2003b). Results from both Kirk's study (2003b) and this experiment illustrate that ARI provides a good estimate of mycelial biomass whereas radial growth measurements provide an estimate on the rate of hyphal extension. When determining a culture's ability to survive cold temperature, the biomass of a culture is more helpful than the rate of hyphal expansion. ARI is considered to be a better estimate of culture survival than radial growth measurements because the latter may overestimate survival potential of cultures. For example, the growth pattern of a marginally surviving culture would be less dense. However, it may spread to the edge of a Petri plate; the growth pattern of a cold tolerant culture would exhibit dense, fluffy mycelium. However, it may not spread to the edge of a Petri plate. In this example, a radial growth measurement would overestimate the recovery of the marginally surviving culture, whereas an ARI measurement would provide a more reasonable estimate of survival of both cultures.

CHAPTER 3: COLONIZATION OF TUBER TISSUE BY *PHYTOPHTHORA INFESTANS* AT FREEZING TEMPERATURES

Introduction

Temperature studies involving growth and pathogenesis of *Phytophthora infestans* have mainly centered on development on and within potato foliage (Harrison and Lowe 1989, Harrison 1992). Optimal temperatures for growth of *P. infestans* in potato foliage were reported to range from 10° to 17°C depending on the potato cultivar (Harrison 1992). Harrison (1992) inferred from his results that polygenic resistance in certain potato cultivars might be broken down at higher temperatures allowing for greater proliferation of the pathogen. A lethal cold temperature for *P. infestans* hyphae in foliar tissue has not been reported.

Potato tubers colonized by *P. infestans* are typically the means by which the pathogen overwinters. The ability of *P. infestans* to survive the winter in potato tuber tissue depends on the degree of colonization of the tissue. For example if an aggressive isolate of *P. infestans* invaded tuber tissue causing massive rotting, it would be less likely to cause disease the following spring because it had destroyed its host. Previous studies have shown that different genotypes of *P. infestans* vary in aggressiveness in potato tubers (Lambert and Currier 1997, Medina et al. 1999, 2000). It has been suggested that the newer more aggressive isolates such as US-8, which are often metalaxyl resistant, colonize tuber tissue more quickly making tubers more susceptible to secondary rots and therefore unable to produce viable plants (Walker and Cooke 1988, 1990). Studies

involving *P. infestans* isolates from Israel and the UK have shown that the optimal temperature for hyphal growth in tuber tissue is 10°C (Kadish and Cohen 1992, Walker and Cooke 1988, 1990).

The effect of cold temperatures on development of *P. infestans* in potato tuber tissue has been measured using digital image analysis (Kirk et al. 2001b) and was used to examine the effect of freezing temperatures on *P. infestans* growth in tuber tissue in the current study. The hypotheses tested in this study were that *P. infestans* would survive better when exposed to 3°C rather than 0° or -3°C, and that isolate CO37, which displayed cold tolerance, would cause more infection than isolate 95-7 which was more cold sensitive.

Another objective of this study was to determine the development of *P. infestans* hyphae in tuber tissue slices after exposure to cold temperatures by microscopy. The purpose of the cytological study was to visualize how *P. infestans* hyphae, after exposure to cold temperatures, developed within tuber tissue after exposure to cold temperatures. The hypothesis was that hyphae would colonize tissue best after exposure to 3°C rather than after exposure to colder temperatures such as 0°C.

Materials and Methods

Whole Tuber Experiments

Tubers of cultivars Jacqueline Lee and Atlantic, and advanced breeding line MSJ461-1 were used for this experiment. The tubers were washed in water, then surface sterilized in 10% bleach for 15 minutes, rinsed in sterile water and allowed to air dry. The disinfected tubers were stored in a growth chamber set at 10°C until use. *P.*

infestans isolates 95-7 and CO37 were grown on rye A agar (Caten and Jinks 1968) for 14 days prior to temperature treatment exposures for this experiment.

Inoculation of tubers

Tubers were inoculated using plugs of mycelium taken from rye agar. To inoculate tubers, a 6.0 mm diameter plug of tuber tissue was removed from an area about 1.0 cm from the stem end of the tuber. A 5.0 mm diameter plug of *P. infestans* mycelium grown on rye agar for 2 to 3 weeks was inserted into the hole in the tuber. The 6.0 mm plug of tuber tissue was replaced on top of the agar plug in the tuber and the plug was sealed with petroleum jelly. Negative control samples were treated as above except 5.0 mm plugs of sterile rye agar were used. Positive control samples were inoculated with 5.0 mm plugs of *P. infestans* and were stored in paper bags at 10°C, 90% relative humidity (RH) for the duration of the experiment (Medina et al. 1999). Each treatment had a sample size of four tubers. The experiment was executed twice.

The inoculated tubers and the negative control tubers were stored in Peltier effect temperature control chambers (Sable Systems International, Las Vegas, NV 89119) set to treatment temperatures of 3, 0, or -3°C for durations of 1, 2, 3, 4, or 5 days. After exposure of tubers to their appropriate treatments, they were removed from the Peltier chambers. Tubers from each replication of each treatment were placed into 10 kg capacity paper bags (Medina et al. 1999). The paper bags were stored in a chamber set at 10°C and 90% RH until tuber blight assessment. Tubers from trial 1 of the experiment were stored in a 10°C 95% RH chamber for 49 days before disease assessment. Tubers from trial 2 were stored at 10°C, 95% RH for 18 days before disease assessment. The

tubers in trial 2 were incubated at 10°C, 95% RH for a shorter duration than trial 1 because of excessive rotting that occurred during prolonged storage during trial 1.

Digital image analysis for disease assessment

Images of the tubers were made as described by Kirk et al. (2001b) using an Epson scanner (Epson Perfection 2400 Scanner, Epson America Inc., Long Beach, CA 90806). The tubers for each treatment were cut into three sections [apical (1.0 cm from apical end of tuber), middle (equidistant from apical and basal ends of the tuber), basal (1.0 cm from basal end of tuber)]. The cut tuber sections were placed cut face down onto a glass plate large enough to lie on top of the scanner bed. The glass plate was placed onto the scanner bed and images were made using the computer software provided with the scanner. Color images with 120.6 pixels per cm^2 resolution were created. The images of tuber sections were analyzed using digital image analysis software (Sigma Scan Pro 5, SPSS Inc., Chicago, IL 60606) to determine the average reflective intensity (ARI). ARI is a measure of the intensity of light reflection off of the scanned surface in light intensity units (LIU) where LIU = 0 is black and 255 is white. The color of the sections from the tubers is related to the amount of infection in the tissue. Healthy tissue is lighter in color and will exhibit a higher ARI value. Diseased tuber tissue that displays browning symptoms will have a lower ARI value than the negative control. The manual threshold level of the Sigma Scan program was adjusted in order to set an intensity range for an image. A manual threshold level of 35 enabled the software to differentiate between the sample and the background within an image. Images in this thesis are presented in color.

Data analysis

The significance of the effect of *P. infestans* isolate, potato cultivar, temperature, and duration of temperature exposure were determined using analysis of variance (SAS version 8.1, SAS Institute, Cary, NC 27513). Two-way ANOVA was used to determine if *P. infestans* was able to cause tuber blight in potato tubers after exposure for different periods to a range of temperature treatments close to 0°C. The analysis was done by comparing ARI of the treated samples with the ARI of the negative control that underwent the same temperature exposure and the ARI of the positive control at p=0.05.

Cytological Assessment of *Phytophthora infestans* Development

Preparation and inoculation of tuber slices

Potato tubers of cultivars Jacqueline Lee and Atlantic, and advanced breeding line MSJ461-1 were used for this experiment. Jacqueline Lee and MSJ461-1 have exhibited foliar resistance to late blight in the field (Kirk 2003, personal communication); they were used in this experiment to determine if they also exhibit tuber resistance. Atlantic has been shown to be susceptible to late blight in both foliage and tubers, it was chosen for this experiment because it is a known susceptible variety. Washed tubers were surface sterilized by soaking in a 10% bleach solution for 3 minutes. Tubers were rinsed twice in sterile distilled water and allowed to air dry in a laminar flow hood. Once tubers were dry, aseptic procedure was used to prepare tuber tissue discs. A knife was used to cut away the apical and basal ends of the tuber exposing the internal tuber tissue. Using a core borer (20.0 mm diameter), a central core of the tuber was taken. Using a razor blade, the tuber core was cut into 5.0 mm thick slices. The slices were stored in a sterile

glass beaker containing enough sterile distilled water to cover the slices. The slices were rinsed twice in sterile distilled water. Using forceps the slices were transferred to sterile glass Petri plates (15.0 cm diameter) containing sterile filter paper that was moistened with 2.0 ml sterile distilled water. Three slices were used for each sample.

Isolates 95-7 (US-8) and CO37 (US-6) of *P. infestans* were used for this experiment. The tuber slices were inoculated by placing 5.0 mm agar plugs of two week old *P. infestans* mycelium onto the top center of the slice. The glass Petri plates were then sealed with Parafilm. The Petri plates were stored in Peltier chambers set at 3° or 0°C for durations of 1, 2, 3, or 4 days. After removal from the Peltier chambers, the plates containing slices were incubated at 25°C (9/15 hours light/dark cycle) for 3 days.

Microscopical analysis

Sections were then taken from the tuber slices for microscopic analysis. A thin section (less than 1.0 mm thick) across the top of a slice and a thin section (less than 1.0 mm thick) through the middle of the slice was prepared with a razor blade. The sections were stored in glass vials containing 95% ethanol until examination. The sections taken from storage in 95% ethanol were mounted onto glass microscope slides, rinsed with distilled water, and stained with lactophenol cotton blue stain (Clark 1983). The stained sections were covered with 1 or 2 drops of glycerol and then a cover slip was placed over the section. Sections were viewed using a light microscope (Leica DMLB, Leica Microsystems, Wetzlar 35578 Germany). Photographs of the samples were taken with Sony digital still camera (Model DKC-CM30, Sony Corporation of America, New York, NY 10022) and MGI PhotoSuite SE photo editing software (Roxio Inc, Santa Clara, CA 95050). Images in this thesis are presented in color.

Results

Whole Tuber Experiments

The effect and statistical significance levels of the variables *P. infestans* isolate, potato cultivar, temperature, and exposure duration for experiments 1 and 2 are shown in Table 7. The effects of *P. infestans* isolate and temperature were significant for samples from experiment 1, which remained in the 10°C, 95%RH chamber for 49 days. For experiment 2, in which the tubers remained in the 10°C, 95%RH chamber for 18 days, the effect of *P. infestans* isolate, potato cultivar, and temperature were significant.

The ARI values of tuber slices from the whole tuber inoculation experiment are shown in Figures 5 - 7. Atlantic tubers inoculated with *P. infestans* displayed darker tissue (lower ARI value) than tubers of the negative control (Figure 5). Isolate CO37 caused darker tissue in the positive control tubers than isolate 95-7. The tubers that underwent the 3°C temperature treatments exhibited darker tissue than samples that underwent the 0°C temperature treatment. Samples that were subjected to the –3°C treatment had the darkest tissue.

MSJ461-1 tubers inoculated with isolate 95-7 showed darker tissue than tubers inoculated with isolate CO37 or the negative control (Figures 6, 8 - 13). Samples that underwent the -3°C temperature treatment displayed the darkest tissue (Figure 6).

Tubers of cultivar Jacqueline Lee inoculated with *P. infestans* exhibited slightly darker tissue than the negative control (Figure 7). Tubers inoculated with isolate CO37 that were stored at 10°C, 95%RH for 49 days had higher ARI values (lighter tissue) than those stored at 10°C, 95% for 18 days. Tubers inoculated with isolate 95-7 that were

stored at 10°C, 95% RH for 18 days had higher ARI values (lighter tissue) than those stored at 10°C, 95% RH for 49 days. Samples that underwent the -3°C temperature treatment had the darkest tissue.

Overall, the positive control samples inoculated with isolate 95-7 and stored at 10°C 95% RH for 18 days had darker tissue than those stored at 10°C, 95% RH for 49 days (Figures 5 - 8, 10). Positive control samples inoculated with CO37 and stored at 10°C, 95% RH for 49 days had darker tissue than those stored at 10°C, 95% RH for 18 days (Figures 9 and 11).

Table 7. Sources of variation and their statistical significance (LSD, p=0.05) for the whole tuber experiments, which determine the effect of cold temperature on the development of *Phytophthora infestans* in potato tuber tissue.

Experiment 1			
Source of Variation	DF	F value	p value
P. infestans isolate	2	78.72	<0.0001
Potato cultivar	2	12.33	<0.0001
Temperature	3	22.98	<0.0001
Duration	5	7.27	<0.0001
Isolate*Temperature	4	13.35	<0.0001
Isolate*Cultivar	4	17.3	<0.0001
Isolate*Duration	8	19.27	<0.0001
Cultivar*Temperature	4	15.98	<0.0001
Cultivar*Duration	8	21.19	<0.0001
Iso*Cv*Temp	6	11.63	<0.0001
Iso*Cv*Duration	16	13.05	<0.0001
Iso*Cv*Temp*Dur	31	6.26	<0.0001
Total	98	14.66	<0.0001
Experiment 2			
Source of Variation	DF	F value	p value
P. infestans isolate	2	574.45	<0.0001
Potato cultivar	2	108.48	<0.0001
Temperature	1	23.65	<0.0001
Duration	4	2.13	0.075
Isolate*Temperature	2	9.8	<0.0001
Isolate*Cultivar	4	128.51	<0.0001
Isolate*Duration	8	4.12	<0.0001
Cultivar*Temperature	2	15.54	<0.0001
Cultivar*Duration	8	2.32	0.018
Iso*Cv*Temp	4	2.98	0.018
Iso*Cv*Duration	16	1.55	0.075
Iso*Cv*Temp*Dur	36	1.11	0.297
Total	95	28.97	<0.0001

Figure 5. Average tuber tissue infection of cultivar Atlantic caused by different genotypes of *Phytophthora infestans* stored at different temperatures and measured as average reflective intensity of cut tuber slices. ■ =3°C treatment exposure, incubated at 10°C for 49 days; □ =0°C, incubated at 10°C for 49 days; ▲=-3°C, incubated at 10°C for 49 days; ■ = positive control 10°C for 49 days; ● =3°C, incubated at 10°C for 18 days; ○ =0°C, incubated at 10°C for 18 days; ● = positive control 10°C for 18 days.

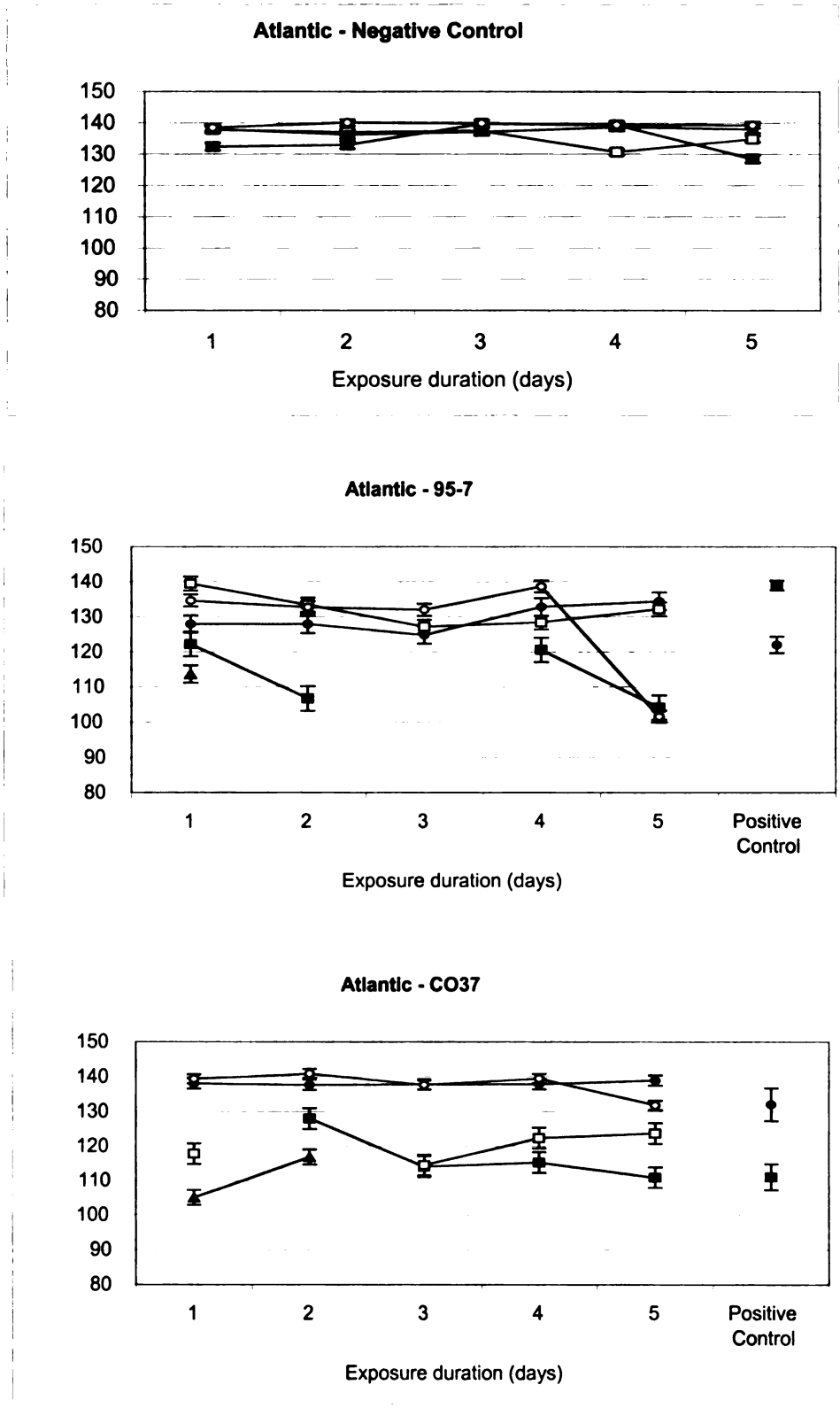
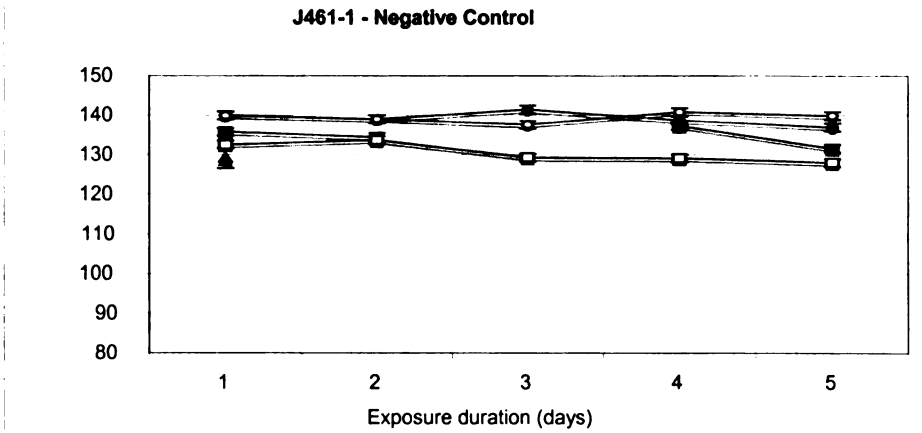
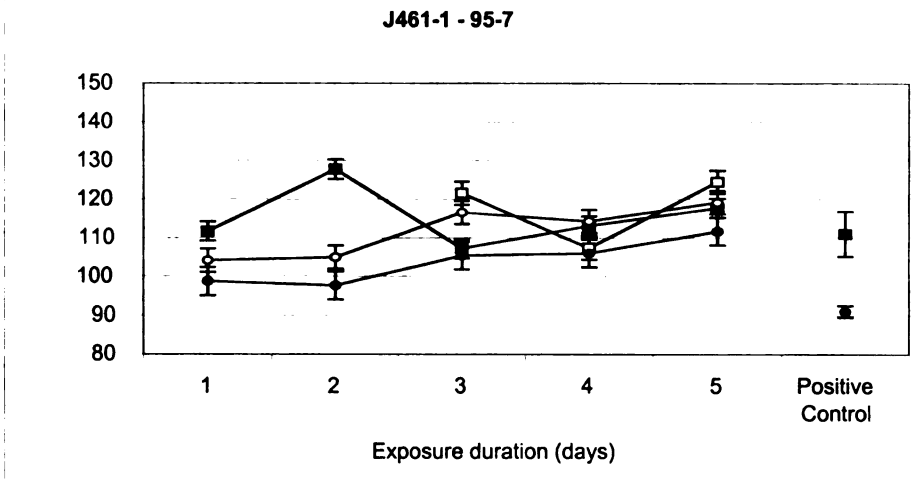

Atlantic - Negative Control
150
140
130
120
110
100
90
80
1
2
3
4
5
Exposure duration (days)
Atlantic - 95-7
150
140
130
120
110
100
90
80
1
2
3
4
5
Positive Control
Exposure duration (days)
Atlantic - CO37
150
140
130
120
110
100
90
80
1
2
3
4
5
Positive Control
Exposure duration (days)

Figure 5.

Figure 6. Average tuber tissue infection of breeding line MSJ461-1 caused by different genotypes of *Phytophthora infestans* stored at different temperatures and measured as average reflective intensity of cut tuber slices. ■ =3°C treatment exposure, incubated at 10°C for 49 days; □ =0°C, incubated at 10°C for 49 days; ▲=-3°C, incubated at 10°C for 49 days; ■ = positive control 10°C for 49 days; ● =3°C, incubated at 10°C for 18 days; ○ =0°C, incubated at 10°C for 18 days; ● = positive control 10°C for 18 days.


J461-1 - Negative Control
150
140
130
120
110
100
90
80
1
2
3
4
5
Exposure duration (days)


J461-1 - 95-7
150
140
130
120
110
100
90
80
1
2
3
4
5
Positive Control
Exposure duration (days)

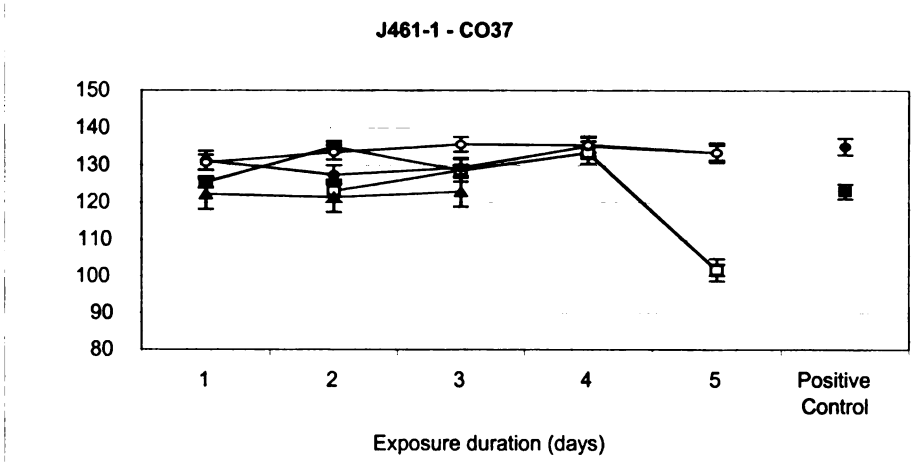
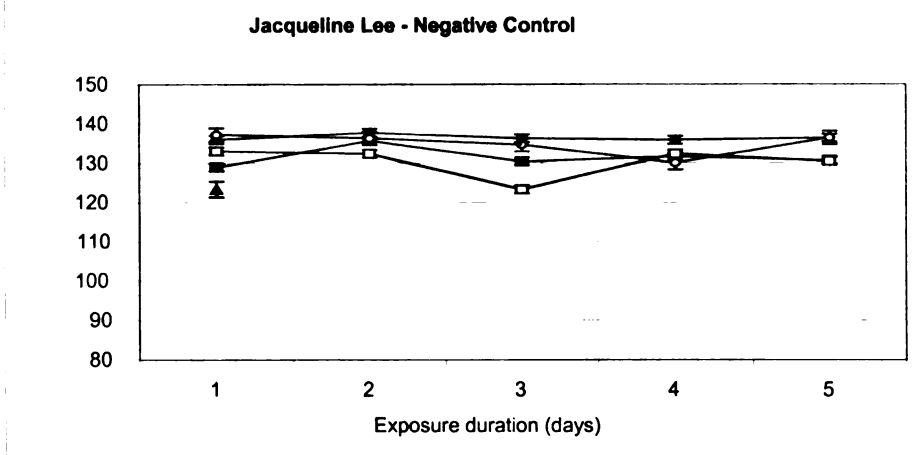
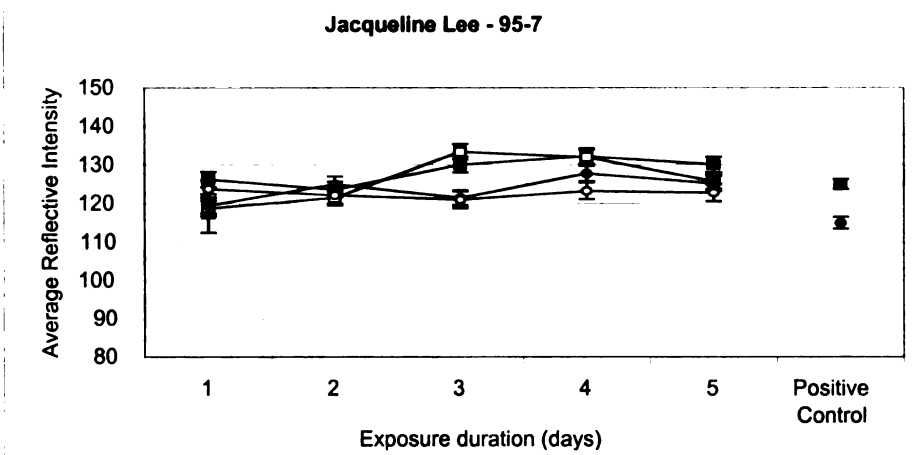

J461-1 - CO37
150
140
130
120
110
100
90
80
1
2
3
4
5
Positive Control
Exposure duration (days)

Figure 6.

Figure 7. Average tuber tissue infection of cultivar Jacqueline Lee caused by different genotypes of *Phytophthora infestans* stored at different temperatures and measured as average reflective intensity of cut tuber slices. ■ =3°C treatment exposure, incubated at 10°C for 49 days; □ =0°C, incubated at 10°C for 49 days; ▲=-3°C, incubated at 10°C for 49 days; ■ = positive control 10°C for 49 days; ● =3°C, incubated at 10°C for 18 days; ○ =0°C, incubated at 10°C for 18 days; ● = positive control 10°C for 18 days.


Jacqueline Lee - Negative Control
150
140
130
120
110
100
90
80
1
2
3
4
5
Exposure duration (days)


Jacqueline Lee - 95-7
Average Reflective Intensity
150
140
130
120
110
100
90
80
1
2
3
4
5
Positive Control
Exposure duration (days)

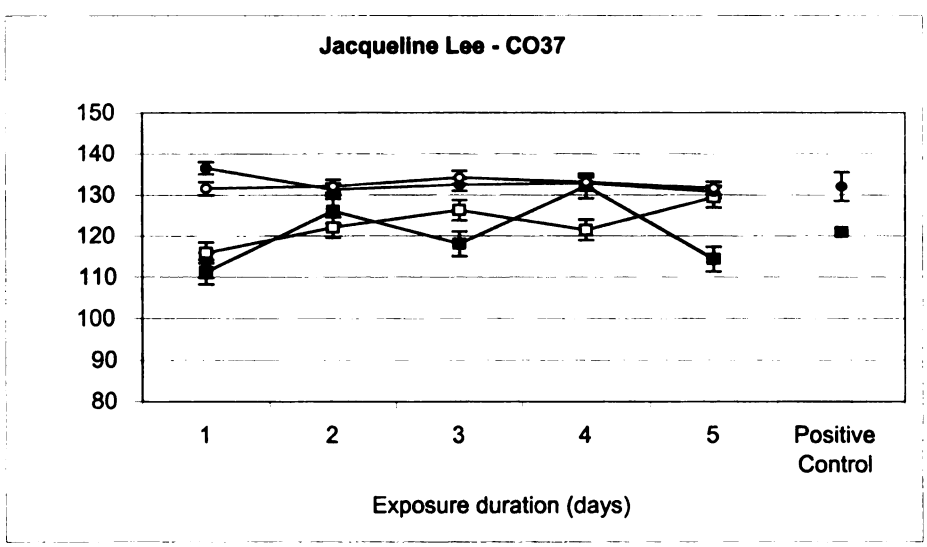

Jacqueline Lee - CO37
150
140
130
120
110
100
90
80
1
2
3
4
5
Positive Control
Exposure duration (days)

Figure 7.

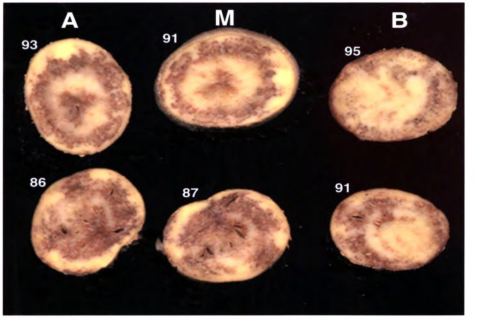


Figure 8. Slices of potato tubers of breeding line MSJ461-1 inoculated with 95-7 (US-8) and stored at 10°C 95% RH for 18 days (positive control). Numbers refer to the average reflective intensity of each section in light intensity units. A – apical section, M – middle section, B – basal section.

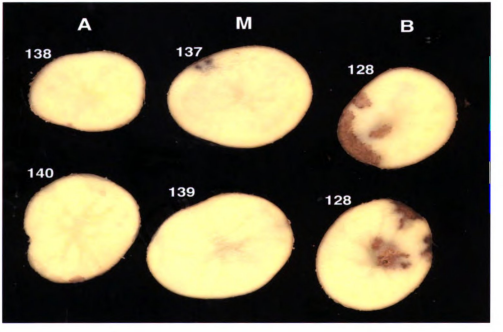


Figure 9. Slices of potato breeding line MSJ461-1 inoculated with CO37 (US-6) and stored at 10°C 95% RH for 18 days (positive control). Numbers refer to the average reflective intensity of each section in light intensity units. A – apical section, M – middle section, B – basal section.

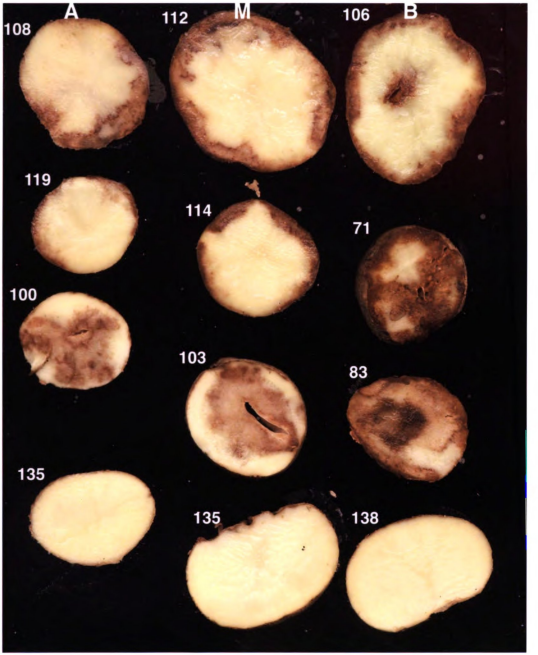


Figure 10. Slices of potato tuber breeding line MSJ461-1 inoculated with 95-7 (US-8) and stored at 10°C 95% RH for 49 days (positive control). Numbers refer to the average reflective intensity of each section in light intensity units. A – apical section, M – middle section, B – basal section.

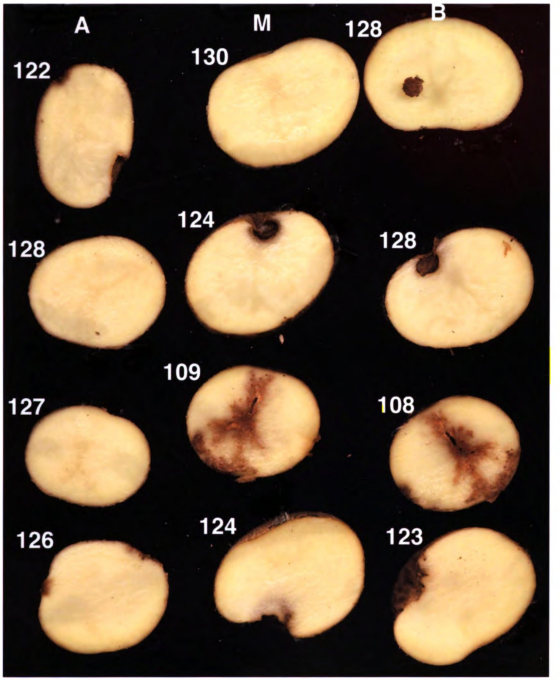


Figure 11. Slices of potato tuber breeding line MSJ461-1 inoculated with CO37 (US-6) and stored at 10°C 95% RH for 49 days (positive control). Numbers refer to the average reflective intensity of each section in light intensity units. A – apical section, M – middle section, B – basal section.

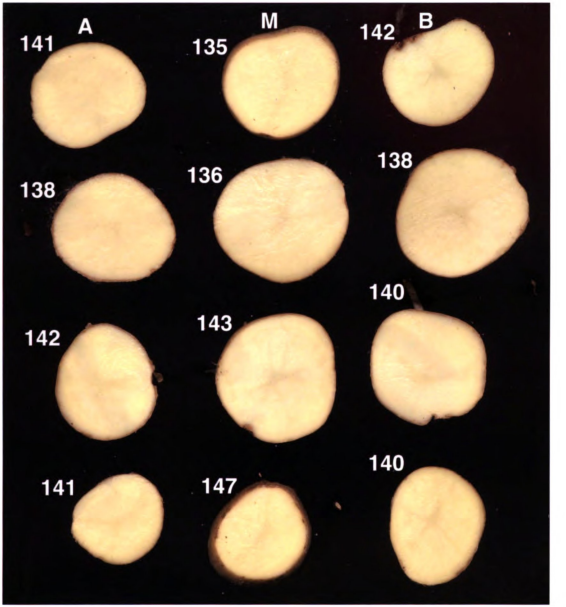


Figure 12. Slices of potato tuber breeding line MSJ461-1 inoculated with sterile rye agar (negative control) and incubated at 3°C for one day, followed by incubation at 10°C 95% RH for 18 days. Numbers refer to the average reflective intensity of each section in light intensity units. A – apical section, M – middle section, B – basal section.

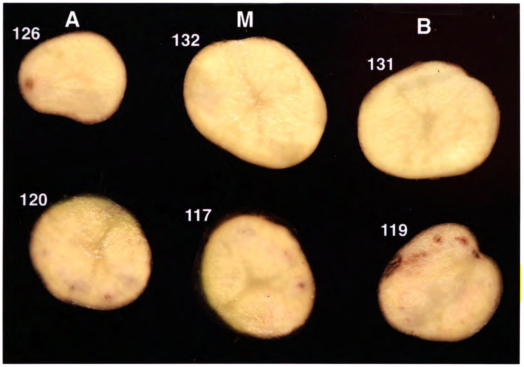


Figure 13. Slices of potato tubers of breeding line MSJ461-1 inoculated with sterile rye agar (negative control) and incubated at -3°C for one day, followed by storage at 10°C 95% RH for 49 days. Numbers refer to the average reflective intensity of each section in light intensity units. A – apical section, M – middle section, B – basal section.

Cytological Assessment of *Phytophthora infestans* Development

P. infestans isolate 95-7 was able to colonize the surface of slices of all three potato cultivars after exposure to 3°C (Table 8a). The duration of exposure to 3°C did not appear to affect the ability of isolate 95-7 to colonize potato tuber tissue. Breeding line MSJ461-1 inoculated with 95-7 and exposed to 3°C for 3 days showed surface coverage of about 10% by mycelium and sporangia were also found on the surface of the slice (Figures 14 and 15). Fifteen to 25% of the surface of cultivar Jacqueline Lee tuber slices inoculated with 95-7 and stored at 3°C for 1 day were covered by mycelium (Figures 16 and 17). Fifty percent of the surface area of slices of cultivar Atlantic were covered by mycelium (Figure 18). During storage at 3°C, isolate 95-7 was able to penetrate three cell layers of potato tissue intercellularly (Figures 19 and 20). Tuber slices inoculated with 95-7 and exposed to 0°C displayed less colonization of the tissue as compared with the 3°C exposure temperature. The surface of slices of cultivar Atlantic was found to be colonized 80% by 95-7 after storage at 0°C for 1 day (Figure 21). After storage at 0°C for 4 days, 95-7 was able to penetrate 2 cell layers intercellularly in cultivar Atlantic (Figure 22).

P. infestans isolate CO37 was also able to colonize the surface of slices of all three potato cultivars after exposure to 3°C (Table 8b). Again, the duration of exposure to 3°C did not affect the ability of isolate CO37 to colonize tuber slices. A slice of cultivar Atlantic was colonized 85% by mycelium of CO37 after exposure to 3°C for 2 days (Figure 23). Slices of breeding line MSJ461-1 that were inoculated with CO37 and exposed to 0°C displayed greater colonization of tissue than cultivars Atlantic and Jacqueline Lee. The duration of exposure of MSJ461-1 to 0°C did effect colonization of

tissue by CO37, longer durations at 0°C led to less colonization of hyphae. Slices of MSJ461-1 inoculated with CO37 and exposed to 0°C for 2 and 3 days exhibited mycelium coverage of 10% and 20% respectively (Figures 24 and 25). However, when the slice was exposed to 0°C for 4 days the mycelium coverage was reduced to 2% (Figure 26). Cultivars Atlantic and Jacqueline Lee displayed less colonization by CO37 after exposure to 0°C than compared to 3°C exposure.

Table 8a. Cytological assessment of colonization by *Phytophthora infestans* isolate 95-7 (US-8) on potato cultivar Atlantic, breeding line MSJ461-1, and cultivar Jacqueline Lee after exposure to cold temperature for durations of 1, 2, 3, or 4 days.

Cultivar	Time (days)	3 °C	0 °C
Atlantic	1	50 - 85% surface coverage. Penetrated 2 cell layers.	80% surface coverage. Large mass of hyphae in center of slice. Penetrated 1 - 2 cell layers.
	2	53% surface coverage. Sporangia found on surface. Hyphae not found to penetrate tissue	5% surface coverage. Few hyphae near center of slice. Hyphae not found to penetrate tissue.
	3	5% surface coverage. Hyphae penetrated 4 cell layers along edge of slice.	Hyphae not found on tissue.
	4	25% surface coverage. Sporangia found on center surface of slice. Hyphae not found to penetrate tissue.	No hyphae found on surface. Penetrated 1-2 cell layers.
MSJ461-1	1	10% surface coverage. Penetrated 3 cell layers.	2% surface coverage. Hyphae not found to penetrate tissue.
	2	2% surface coverage. Hyphae not found to penetrate tissue.	5% surface coverage. Hyphae penetrated 1 cell layer.
	3	10% surface coverage. Sporangia and mycelium dense in center of slice. Hyphae penetrated 3 cell layers.	No hyphae found.
	4	75% surface coverage. Sporangia and mycelium dense near center of slice. Hyphae not found to penetrate tissue.	2% surface coverage, hyphae found near edge of slice. Hyphae not found to penetrate tissue.
Jacqueline Lee	1	34% surface coverage. Hyphae not found to penetrate tissue.	2% surface coverage. Two hypha near center of slice.
	2	8% surface coverage. Mycelium near center of slice. Penetrated 3 cell layers.	None
	3	40% surface coverage. Mycelium dense at center of slice. Hyphae penetrated 2 cell layers.	Single hypha near center of slice.
	4	30% surface coverage. Sporangia near center, mycelium found all over slice. Hyphae not found to penetrate tissue.	Hyphae penetrated 2 cell layers.

Table 8b. Cytological assessment of colonization by *Phytophthora infestans* isolate CO37 (US-6) on potato cultivar Atlantic, breeding line MSJ461-1, and cultivar Jacqueline Lee after exposure to cold temperature for durations of 1, 2, 3, or 4 days.

Cultivar	Time (days)	3 °C	0 °C
Atlantic	1	5% surface coverage. Hyphae not found to penetrate tissue	No hyphae found.
	2	85% surface coverage. Sporangia found, mycelium dense near center of slice. Hyphae penetrated 2 cell layers	5% surface coverage. Few hyphae near center. Hyphae not found to penetrate tissue.
	3	5% surface coverage. Hyphae not found to penetrate tissue	2% surface coverage. Single hypha near center.
	4	75% surface coverage. Dense growth of hyphae at edge. Hyphae penetrated 3 cell layers.	No hyphae found.
MSJ461-1	1	No hyphae found.	30% surface coverage. Hyphae penetrated 1 cell layer.
	2	5% surface coverage. Hyphae not found to penetrate tissue	10% surface coverage. Hyphae penetrated 2 cell layers.
	3	70% surface coverage. Hyphae not found to penetrate tissue	20% surface coverage. Hyphae penetrated 1 cell layer.
	4	25% surface coverage. Sporangia found. Hyphae not found to penetrate tissue	5% surface coverage. Hyphae not found to penetrate tissue.
Jacqueline Lee	1	25% surface coverage. Mycelium dense in areas. Hyphae penetrated 3 cell layers.	25% Surface coverage. Hyphae penetrated 2 cell layers.
	2	5% surface coverage. Hyphae not found to penetrate tissue	No hyphae found.
	3	10% surface coverage. Hyphae penetrated 4 cell layers.	2% surface coverage. Single hypha near center.
	4	85% surface coverage. Mycelium dense near center. Hyphae not found to penetrate tissue.	No hyphae found.

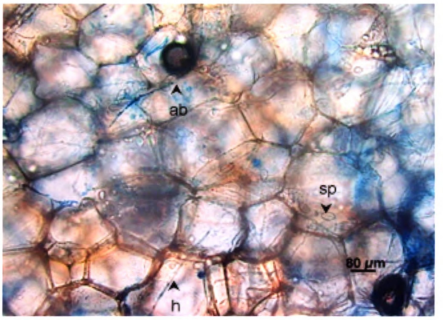


Figure 14. Surface section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 3°C for 3 days. 100X magnification. ab, air bubble; sp, sporangium; h, hypha.

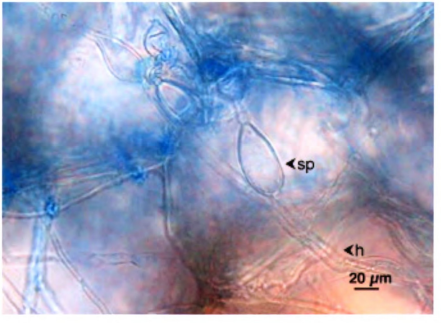


Figure 15. Surface section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 3°C for 3 days. 400X magnification. sp, sporangium; h, hypha.

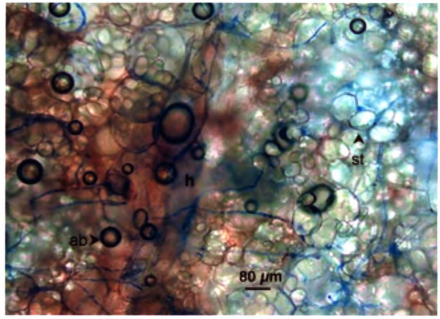


Figure 16. Surface section of a slice of potato tuber cultivar Jacqueline Lee inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 3°C for 1 day. 100X magnification. ab, air bubble; h, hypha; st, starch granule.

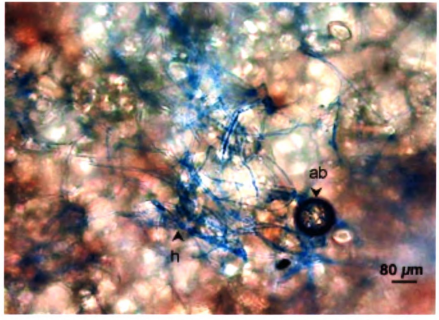


Figure 17. Surface section of a slice of potato tuber cultivar Jacqueline Lee inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 3°C for 1 day. 100X magnification. h, hyphae; ab, air bubble.

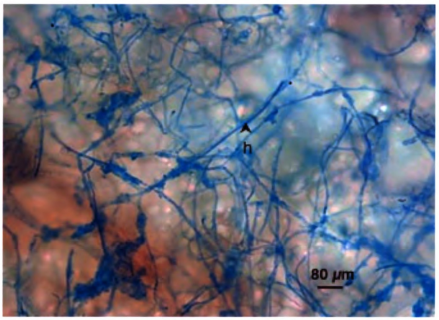


Figure 18. Surface section of a slice of potato tuber cultivar Atlantic inoculated with ***Phytophthora*** *infestans* isolate 95-7 (US-8) and incubated at 3°C for 1 day. 100X magnification. h, hypha.

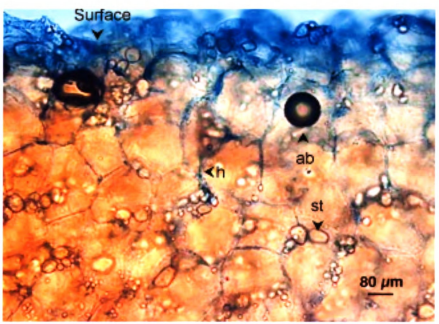


Figure 19. Cross section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 3°C for 1 day. 100X magnification. h, hypha; ab, air bubble; st, starch granule.

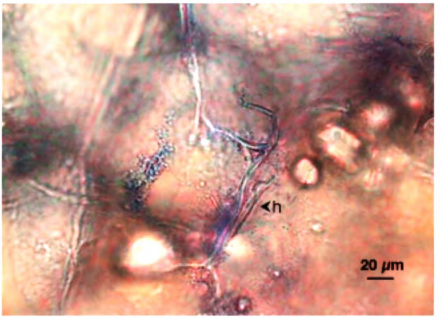


Figure 20. Cross section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 3°C for 1 day. 400X magnification. h, hypha.

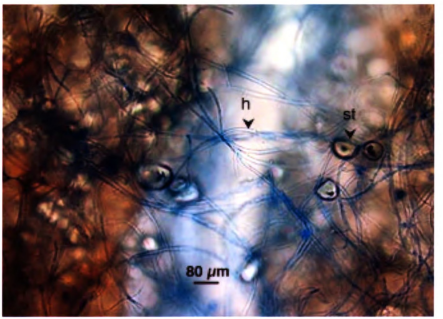


Figure 21. Surface section of a slice of potato tuber cultivar Atlantic inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 0°C for 1 day. 100X magnification. h, hyphae; st, starch granule.

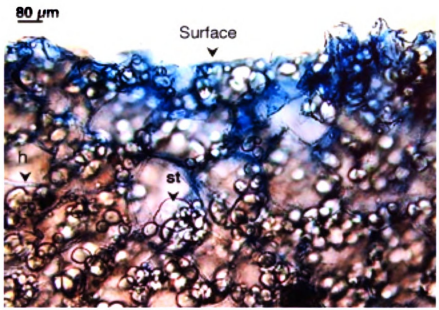


Figure 22. Cross section of a slice of potato tuber cultivar Atlantic inoculated with *Phytophthora infestans* isolate 95-7 (US-8) and incubated at 0°C for 4 days. 100X magnification. h, hypha; st, starch granule.

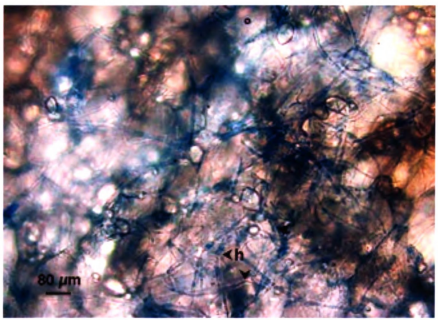


Figure 23. Surface section of a slice of potato tuber cultivar Atlantic inoculated with *Phytophthora infestans* isolate CO37 (US-6) and incubated at 3°C for 2 days. 100X magnification. h, hyphae.

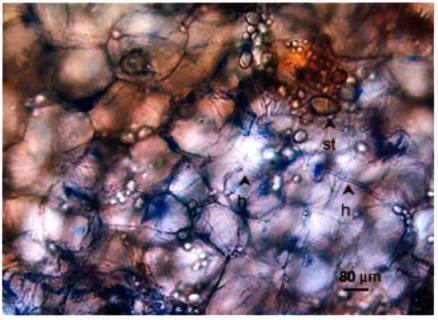


Figure 24. Surface section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate CO37 (US-6) and incubated at 0°C for 2 days. 100X magnification. h, hypha; st, starch granule.

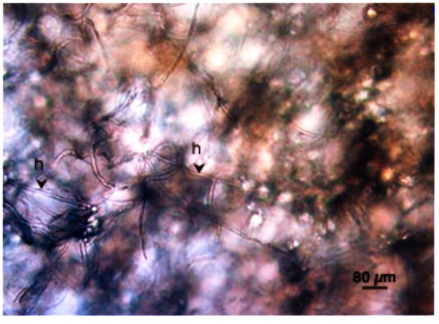


Figure 25. Surface section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate CO37 (US-6) and incubated at 0°C for 3 days. 100X magnification. h, hypha.

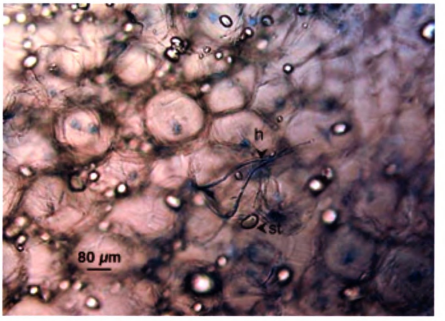


Figure 26. Surface section of a slice of potato tuber breeding line MSJ461-1 inoculated with *Phytophthora infestans* isolate CO37 (US-6) and incubated at 0°C for 4 days. 100X magnification. h, hypha; st, starch granule.

Discussion

As described in Chapter 2, the annual mean temperature in the Great Lakes region has increased. This may lead to an increased potential for the survival of tubers left in cull piles and therefore also for *P. infestans* inoculum harbored within tubers for surviving the winter. The experiments described in this chapter were aimed at determining if tubers infected with *P. infestans* could be successfully colonized or infected after being exposed to freezing temperatures.

Results from the whole tuber experiments indicated that tubers exposed to -3°C had darker tissue than tubers exposed to 0° and 3°C (Figures 6 - 8). Most samples of tubers exposed to -3°C for two days or longer completely rotted and were unable to be scanned for analysis. Other studies have shown that tuber tissue is killed and begins to break down at temperatures around -3°C (Seymour and Boydston 2002, Kirk unpublished data). The darkening of tissue from tubers exposed to -3°C was likely not due to infection by *P. infestans*; instead, it was caused by breakdown of tissue from exposure to cold temperature and secondary rots.

Tubers of cultivar Atlantic displayed a greater severity of disease when exposed to 3°C as compared to 0°C. Atlantic tubers inoculated with isolate CO37 and stored at 10°C, 95% RH for 49 days after receiving the cold temperature treatment exhibited greater disease development than tubers that were stored at 10°C, 95% RH for only 18 days. This may indicate that CO37 needs more than 18 days to fully develop within Atlantic tubers.

Tubers of breeding line MSJ461-1 also had greater tuber late blight when exposed to 3°C as compared to 0°C. MSJ461-1 tubers inoculated with isolate 95-7 had a lower range of ARI values than tubers inoculated with isolate CO37. The results indicate that 95-7 is more aggressive than CO37 in MSJ461-1 tubers.

Tubers of cultivar Jacqueline Lee that were inoculated with CO37 and stored at 10°C, 95% RH for 49 days displayed more disease than those stored at 10°C, 95% RH for 18 days. This result is similar to that of CO37 in Atlantic tubers where CO37 developed more severe tuber blight symptoms after 49 days incubation. However, Jacqueline Lee tubers that were inoculated with 95-7 and stored at 10°C, 95% RH for 18 days displayed more disease than those stored at 10°C, 95% RH for 49 days.

Positive control samples of all three cultivars inoculated with 95-7 displayed more disease symptoms when stored at 10°C, 95% RH for 18 days as opposed to 49 days. The difference in ARI values for the 95-7 positive controls does not appear to be due to the different incubation periods; instead it is likely due to a failed inoculation. A closer look at CO37 positive control samples revealed that after 18 days of storage at 10°C, 95% RH infection was present only in the basal region of tuber tissue near the site of infection. After 49 days of storage at 10°C, 95% RH, infection by CO37 had spread to the apical region of tubers. Because positive control samples inoculated with 95-7 and stored at 10°C, 95% RH for 18 days had lower ARI values than the respective CO37 positive control samples, it can be concluded that 95-7 spreads faster within tuber tissue than isolate CO37. This observation corresponds with previous findings that the US-8 genotype of *P. infestans* is more aggressive in tuber tissue (Kadish and Cohen 1992, Grinberger et al. 1995, Medina et al. 1999).

Field trials have shown that MSJ461-1 and Jacqueline Lee possess foliar resistance to late blight (Douches et al. 2001). However, not much is known about the response of tubers of these cultivars to *P. infestans* infection. Atlantic tubers and foliage are much more susceptible to late blight than MSJ461-1 and Jacqueline Lee. Results asserted in this chapter demonstrated that *P. infestans* isolate 95-7 (US-8) was able to develop within all three potato cultivars used at the various treatment temperatures. *P. infestans* isolate CO37 (US-6) was able to develop within potato cultivar Atlantic, but less so in MSJ461-1 and Jacqueline Lee. An earlier study found that *P. infestans* mycelium develops best within susceptible tubers; however, once a tuber is wounded, the pathogen can thrive even in resistant cultivars (Toxopeus 1958). Wounding will likely bypass a tuber's natural resistance mechanisms obscuring the differences in aggressiveness between *P. infestans* isolates (Peters et al. 1999).

There are a number of factors that have contributed to the loss of samples or lack of infection of samples in the whole tuber experiment. One factor leading to the loss of samples was the storage of tubers in paper bags after exposure to the temperature treatments. Better ventilation of tubers would have been achieved in mesh bags, which may have reduced the occurrence of secondary rots. The site of tuber inoculation was a factor that may have caused a reduction in the number of infected tubers. Most experiments involving inoculation of potato tubers perform inoculations at the apical end of tubers. A second round of these experiments could be conducted in which the apical ends of tubers are inoculated in order to determine if the site of inoculation is an important factor concerning disease development.

The cytological study illustrated that a temperature of 3°C did not hinder hyphal development of either *P. infestans* isolate on any of the cultivars used. The duration of exposure to 3°C did not have a significant effect on the development of either *P. infestans* isolate. The 0°C temperature treatment caused a reduction of *P. infestans* development in most samples. Breeding line MSJ461-1 inoculated with CO37 was the only sample where there was little difference between the 3°C and 0°C temperature treatments. The duration of exposure to 0°C had an effect on the surface growth and tissue penetration of both *P. infestans* isolates; longer durations of exposure caused less growth of hyphae.

In the cytological study, the small sample size may have affected the results of the experiments. For future work at least 4 slices for each sample should be used (2 for transverse sections, 2 for cross sections) in order to make a better estimate of mycelium development.

This work illustrated that potato tubers can become infected with *P. infestans* isolates 95-7 and CO37 at freezing temperatures. The wintertime temperature within Michigan cull piles rarely reaches below freezing especially in larger piles (Kirk 2003a). The results of this study indicate that *P. infestans* isolates 95-7 and CO37 would not survive a Michigan winter on the surface of cull piles of any size. However, infected tubers located in the middle or at the base of cull piles have the potential of overwintering. Cull piles should either not be left or be kept to a size of one ton or less in order to reduce the number of infected tubers that survive the winter. A better waste potato management strategy would be to spread unused tubers in a thin layer over the field so that they are less insulated from winter temperatures.

CONCLUSIONS AND FUTURE RESEARCH

Variability was exhibited between and among *Phytophthora infestans* genotypes with respect to cold tolerance. From this study, I concluded that cold tolerance is not associated with *P. infestans* genotype. Survival of mycelium on rye agar was favored at 3° and 0°C as compared to -3° and -5°C. Tubers left in the field are typically buried at depths deeper than 5 cm. Because freezing does not occur at such depths, it can be concluded that infected tubers would likely survive winter without rotting. Thus, these tubers could serve as a source of inoculum in spring.

Exposure of tubers to -3°C lead to discoloration of tuber tissue from cold stress and secondary rots. Isolates 95-7 and CO37 vary in aggressiveness in tuber tissue depending on the cultivar infected and the temperature to which it was exposed. All tuber cultivars displayed greater disease severity when exposed to 3°C as compared to 0°C. Isolate 95-7 caused infection of tuber tissue faster than isolate CO37. Cytological observations showed that tubers exposed to 3°C were colonized more than tubers treated at other temperatures. The duration of exposure to 3°C did not affect the degree of colonization. Tuber slices exposed to 0°C displayed less colonization by hyphae, and the degree of colonization was reduced as the exposure at 0°C increased.

Based on these results, I propose the following experiments:

1. Genetic analysis of cold sensitivity by crossing a cold sensitive isolate with a cold tolerant isolate and observing the F1.

2. Conduct mycelium survival experiments in Petri plates exposing the cultures to warm temperatures (around 35°C) for durations of 1 to 5 days, in order to determine an upper temperature threshold.

3. Conduct a cytological study exposing inoculated tuber slices to warm temperatures.

4. Analysis of cell membrane composition of cold sensitive and cold tolerant isolates of *P. infestans*.

APPENDIX

Survival of various isolates of *Phytophthora infestans* grown on rye agar after exposure to temperature treatments of 3°, 0°, -3°, or -5°C for durations of 1 to 5 days. Survival is quantified by the average reflective intensity (ARI) of a culture's mycelium. A culture is considered to have survived the temperature treatment if it's ARI value is statistically different from the ARI of the negative control.

Table 3a. Survival of *Phytophthora infestans* isolate 95-3 (US-1) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration[1]								
95-3 Trial 1	1	79.27[2]		78.16[2]	*[3]	79.26[2]	*[3]	80.36[2]	*[3]
	2	79.75	*	79.91	*	80.56	*	78.72	*
	3	83.69	*	79.69	*	85.58	*	86.1	*
	4	85.15	*	81.93	*	86.71	*	83.79	*
	5	81.09	*	79.31	*	82.27	*	74.02	*
Negative Control	1	-[4]		63.56		63.8		63.62	
	2	63.66		63.49		63.6		62.75	
	3	66.92		68.87		66.94		66.79	
	4	68.27		67.37		65.87		66.04	
	5	69.29		67.97		78.05		66	
95-3 Trial 2	1	83.97	*	84.86	*	85.56	*	88.28	*
	2	84.74	*	-		86.25	*	84.19	*
	3	81.49	*	-		82.31	*	84.53	*
	4	85.27	*	-		83.79	*	83.33	*
	5	83.7	*	-		83.57	*	73.73	
Negative Control	1	69.94		60.7		70.47		70.78	
	2	71.34		-		70.66		70.17	
	3	71.41		-		71.22		70.88	
	4	70.96		-		71.67		71	
	5	67.5		-		68.69		69.84	

[1] Duration of temperature exposure in days.
[2] Mean ARI of five replicate samples in light intensity units.
[3] * denotes significant difference from negative control ARI.
[4] - denotes data missing.

Table 3b. Survival of *Phytophthora infestans* isolate 94-1 (US-17) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
94-1 Trial									
1	1	79.77	*	77.66	*	75.38	*	77.31	*
	2	77.92	*	79.68	*	81.32	*	80.46	*
	3	82.35	*	83.91	*	79.59	*	84.73	*
	4	77.73	*	-		83.21	*	73.37	
	5	79.33	*	80	*	74.79		75.63	
Negative									
Control	1	67.66		66.98		66.96		66.94	
	2	68		68.7		67		67.69	
	3	69.89		72.67		73.26		71.28	
	4	68.75		68.98		70.8		72.49	
	5	70.08		68.98		71.26		71.26	
94-1 Trial									
2	1	79.17	*	-		75	*	73.91	*
	2	70.49	*	-		70.77	*	71.77	*
	3	69.88		-		69.9	*	71.07	*
	4	72.69	*	-		71.63	*	70.37	*
	5	72.88	*	-		71.23	*	68.64	*
Negative									
Control	1	71.32		-		69.67		67.01	
	2	67.43		-		67.45		67.29	
	3	70.11		-		65.39		65.73	
	4	67.41		-		65.55		65.97	
	5	67.04		-		65.91		65.83	
94-1 Trial									
3	1	133.95	*	133.67	*	132.21	*	131.33	*
	2	86.09	*	85.15	*	84.41	*	82.85	*
	3	72.87	*	71.22	*	70.98	*	71.87	*
	4	85.54	*	84.19	*	83.17	*	80.09	*
	5	84.84	*	83.84	*	84.62	*	72.24	
Negative									
Control	1	119.74		122.57		119.87		120.59	
	2	73.31		71.66		72.49		72.06	
	3	63.43		62.24		61.93		62.35	
	4	72.83		71.95		69.9		72.14	
	5	71.95		71.99		71.47		79.38	

Table 3c. Survival of *Phytophthora infestans* isolate 364 (US-17) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
364 Trial 1	1	134.81	*	139.46	*	132.77	*	135.09	*
	2	89.85	*	84.7	*	81.98	*	84.7	*
	3	72.12	*	74.79	*	70.29	*	74.79	*
	4	83.16	*	83.81	*	81.72	*	83.81	
	5	84.22	*	83.09	*	82.94	*	84.22	
Negative Control	1	119.74		122.57		119.87		120.59	
	2	73.31		71.66		72.49		72.06	
	3	63.43		62.24		61.93		62.35	
	4	72.83		71.95		69.9		72.14	
	5	71.95		71.99		71.47		79.38	
364 Trial 2	1	72.45		72.47	*	72.27	*	73.22	*
	2	72.55	*	71.49	*	71.85	*	71.9	*
	3	77.59	*	77.1	*	77.83	*	84.1	*
	4	75.25	*	67.37		74.81	*	67.99	
	5	75	*	72.74	*	72.19	*	69.19	
Negative Control	1	-		63.56		63.8		63.62	
	2	63.66		63.49		63.6		62.75	
	3	66.92		68.87		66.94		66.79	
	4	68.27		67.37		65.87		66.04	
	5	69.29		67.97		78.05		66	
364 Trial 3	1	79.78	*	79.72	*	80.87	*	80.62	*
	2	80.07	*	-		80.25	*	79.18	*
	3	80.54	*	-		78.47	*	73.53	
	4	78.04	*	-		78.47	*	71.65	
	5	75.5	*	-		75.45	*	71.31	
Negative Control	1	69.94		60.7		70.47		70.78	
	2	71.34		-		70.66		70.17	
	3	71.41		-		71.22		70.88	
	4	70.96		-		71.67		71	
	5	67.5		-		68.69		69.84	

Table 3d. Survival of *Phytophthora infestans* isolate CO37 (US-6) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
CO37 Trial 1	1	78.35	*	67.09	*	82.83	*	77.61	*
	2	87.02	*	82.72	*	84.78	*	83.48	*
	3	69.83	*	68.99	*	69.99	*	66.02	*
	4	79.96	*	70.96	*	75.66	*	64.09	
	5	79.12	*	61.16		75.32	*	68.3	*
Negative Control	1	60.67		58.66		62.36		60.62	
	2	68.21		68.31		67.91		65.47	
	3	53.82		53.82		53.35		59.2	
	4	56.75		58.05		57.07		64.52	
	5	61.14		60.25		59.09		57.45	
CO37 Trial 2	1	75.61	*	74.37	*	69.69	*	70.95	*
	2	73.53	*	69.89	*	74.87	*	75.19	*
	3	73.06	*	73.72	*	72.09	*	73.15	*
	4	83.35	*	73.17	*	74.27	*	72.32	*
	5	71.13	*	72.09	*	72.67	*	69.97	*
Negative Control	1	61.95		58.95		55.87		56.43	
	2	63.19		63.11		59.52		58.35	
	3	63.1		61.87		63.03		62.76	
	4	65.65		59.71		56.29		54.51	
	5	61.52		61.47		62.89		62.54	
CO37 Trial 3	1	74.13	*	-		73.21	*	73.13	*
	2	73.52	*	-		71.35	*	71.13	*
	3	69.61		-		72.39	*	71.29	*
	4	69.25	*	-		71.77	*	70.51	*
	5	70.24	*	-		70.73	*	67.34	
Negative Control	1	71.32		-		69.67		67.01	
	2	67.43		-		67.45		67.29	
	3	70.11		-		65.39		65.73	
	4	67.41		-		65.55		65.97	
	5	67.04		-		65.91		65.83	

Table 3e. Survival of *Phytophthora infestans* isolate 98-1 (US-14) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
98-1									
Trial 1	1	-		73.33	*	77	*	-	
	2	-		68.47		81.38	*	62.83	
	3	-		85.21	*	64.71		59.89	
	4	-		82.25	*	57.48		56.42	
	5	-		85.24	*	65.87		-	
Negative									
Control	1	-		61.5		54.51		53.18	
	2	-		63.4		64		64.05	
	3	-		64.47		66.88		65.57	
	4	-		62.45		60.48		60.48	
	5	-		67.5		66.19		65.18	
98-1									
Trial 2	1	69.58	*	67.82	*	66.96	*	68.58	*
	2	70.33		62.8		67.29	*	62.13	
	3	71.14	*	60.89		63.91	*	61.79	
	4	67.46		63.86	*	103.74	*	94.45	
	5	72.04	*	68.57	*	60.77	*	56.57	*
Negative									
Control	1	58.28		59.19		55.16		51.82	
	2	62.27		56.11		53.11		55.07	
	3	59.36		55.74		52.87		54.67	
	4	61.99		51.23		54.24		86.69	
	5	63.19		54.49		51.08		68.56	
98-1									
Trial 3	1	75.06	*	-		74.11	*	72.18	*
	2	73.15	*	-		71.48	*	70.89	*
	3	73.99	*	-		68.32	*	64.62	
	4	72.82	*	-		67.22		66.19	
	5	72.54	*	-		57.56	*	66.96	
Negative									
Control	1	71.32		-		69.67		67.01	
	2	67.43		-		67.45		67.29	
	3	70.11		-		65.39		65.73	
	4	67.41		-		65.55		65.97	
	5	67.04		-		65.91		65.83	

Table 3f. Survival of *Phytophthora infestans* isolate 671 (US-14) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
671 Trial 1	1	-		75.37	*	70.03	*	60.18	
	2	-		73.56	*	76.51	*	66.9	
	3	-		77.26	*	65.92		66.63	
	4	-		75.71	*	58.38		49.56	*
	5	-		66.27		64.45		56.25	*
Negative Control	1	-		61.5		54.51		53.18	
	2	-		63.4		64		64.05	
	3	-		64.47		66.88		65.57	
	4	-		62.45		60.48		60.48	
	5	-		67.5		66.19		65.18	
671 Trial 2	1	69.18		68.99		68.47		71.41	
	2	68.32		69.29		69.57		68.15	
	3	69.71		72.18		73.97		71.8	
	4	69.47		-		70.97		69.67	
	5	70.33		73.74		67.16		70.23	
Negative Control	1	67.66		66.98		66.96		66.94	
	2	68		68.7		67		67.69	
	3	69.89		72.67		73.26		71.28	
	4	68.75		68.98		70.8		72.49	
	5	70.08		68.98		71.26		71.26	
671 Trial 3	1	78.74	*	76.12	*	74.74	*	73.63	*
	2	77.79	*	77.76	*	79.83	*	74.49	*
	3	80.26	*	81.12	*	77.15	*	77.29	*
	4	91.06	*	79.27	*	78	*	80.72	*
	5	78.66	*	79.58	*	77.78	*	70.29	*
Negative Control	1	61.95		58.95		55.87		56.43	
	2	63.19		63.11		59.52		58.35	
	3	63.1		61.87		63.03		62.76	
	4	65.65		59.71		56.29		54.51	
	5	61.52		61.47		62.89		62.54	

Table 3g. Survival of *Phytophthora infestans* isolate 95-7 (US-8) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
95-7 Trial 1	1	-		70.26	*	52.89		58.53	
	2	-		72.61	*	60.23		67.05	
	3	-		79.89	*	72.79		71.82	
	4	-		53.18	*	63.85		63.95	
	5	-		72.91		-		69.16	
Negative Control	1	-		61.5		54.51		53.18	
	2	-		63.4		64		64.05	
	3	-		64.47		66.88		65.57	
	4	-		62.45		60.48		60.48	
	5	-		67.5		66.19		65.18	
95-7 Trial 2	1	68.45	*	73.72	*	76.39	*	59.23	
	2	65.38		70.48		73.81		68.84	
	3	55.03		54.48		59.61		51.49	*
	4	62.87		61.98		61.24		59.64	
	5	63.1		60.89		53.08		54.09	
Negative Control	1	60.67		58.66		62.36		60.62	
	2	68.21		68.31		67.91		65.47	
	3	53.82		53.82		53.35		59.2	
	4	56.75		58.05		57.07		64.52	
	5	61.14		60.25		59.09		57.45	
95-7 Trial 3	1	78.42	*	71.54		71.07		71.67	
	2	67.14		75		72.85	*	69.06	
	3	78.12	*	74.84		76.37		77.94	*
	4	-		74.08		75.31		72.99	
	5	72.22		68.95		69.09		69.29	
Negative Control	1	67.66		66.98		66.96		66.94	
	2	68		68.7		67		67.69	
	3	69.89		72.67		73.26		71.28	
	4	68.75		68.98		70.8		72.49	
	5	70.08		68.98		71.26		71.26	
95-7 Trial 4	1	72.37		73.97	*	72.64	*	73.13	*
	2	74.23	*	71.39	*	73.55	*	73.98	*
	3	78.91	*	74.32	*	77.73	*	84.7	*
	4	77.46	*	76.41	*	76.48	*	71.37	*
	5	76.02	*	75.98	*	78.05	*	69.16	*
Negative Control	1	-		63.56		63.8		63.62	
	2	63.66		63.49		63.6		62.75	
	3	66.92		68.87		66.94		66.79	
	4	68.27		67.37		65.87		66.04	
	5	69.29		67.97		78.05		66	

Table 3h. Survival of *Phytophthora infestans* isolate 458 (US-8) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
458 Trial 1	1	-		89.39	*	79.66	*	80.18	*
	2	-		83.39	*	80.84	*	82.49	*
	3	-		85.51	*	88.44	*	85.33	*
	4	-		84.73	*	88.92	*	83.74	*
	5	-		87.06	*	89.61	*	86.81	*
Negative Control	1	-		61.5		54.51		53.18	
	2	-		63.4		64		64.05	
	3	-		64.47		66.88		65.57	
	4	-		62.45		60.48		60.48	
	5	-		67.5		66.19		65.18	
458 Trial 2	1	64.19		64.02		68.34		72.56	
	2	68.21		67.75		66.57		65.35	
	3	68.61		72.18		70		68.34	
	4	71.52		72.44		71		70.62	
	5	68.13		71.99		71.14		69.09	
Negative Control	1	67.66		66.98		66.96		66.94	
	2	68		68.7		67		67.69	
	3	69.89		72.67		73.26		71.28	
	4	68.75		68.98		70.8		72.49	
	5	70.08		68.98		71.26		71.26	
458 Trial 3	1	78.73	*	75.34	*	69.85	*	72.26	*
	2	74.54	*	72.06	*	74.04	*	73.35	*
	3	72.62	*	72.52	*	77.04	*	71.04	*
	4	85.64	*	72.41	*	63.14	*	64.92	*
	5	73	*	74.37	*	68.37	*	63.27	
Negative Control	1	61.95		58.95		55.87		56.43	
	2	63.19		63.11		59.52		58.35	
	3	63.1		61.87		63.03		62.76	
	4	65.65		59.71		56.29		54.51	
	5	61.52		61.47		62.89		62.54	

Table 3i. Survival of *Phytophthora infestans* isolate 3222 (US-8) exposed to temperatures from 3° to -5°C for durations up to 5 days and then incubated at 25°C for 28 days after exposure.

		Exposure Temperature °C							
		3		**0**		**-3**		**-5**	
	Duration								
3222 Trial 1	1	81.62	*	84.54	*	79.98	*	75.73	*
	2	82.51	*	82	*	79	*	71.85	
	3	79.26	*	86.09	*	82.56	*	74.3	*
	4	70.75		79.63	*	77.83	*	66.84	
	5	81.72	*	81.52	*	74.19		68.29	
Negative Control	1	70.15		68.21		67.45		66.39	
	2	71.09		70.33		67.79		71.72	
	3	69.86		68.58		72.02		67.45	
	4	66.66		67.81		67.87		67.02	
	5	66.51		68.66		70.75		72.32	
3222 Trial 2	1	81.64	*	75.72	*	74.29	*	73.28	*
	2	83.49	*	76.06	*	77.21	*	75.1	*
	3	77.48	*	76.93	*	77.99	*	70.66	*
	4	77.62	*	77.91	*	117.63	*	124.03	*
	5	83.27	*	79.98	*	75.95	*	59	*
Negative Control	1	58.28		59.19		55.16		51.82	
	2	62.27		56.11		53.11		55.07	
	3	59.36		55.74		52.87		54.67	
	4	61.99		51.23		54.24		86.69	
	5	63.19		54.49		51.08		68.56	
3222 Trial 3	1	82.67	*	80.07	*	77.11	*	74.99	*
	2	79.46	*	79.2	*	79.58	*	77.69	*
	3	81.25	*	78.72	*	81.06	*	76.11	*
	4	89.27	*	77	*	78.89	*	79.03	*
	5	78.19	*	75.99	*	76.75	*	72.44	*
Negative Control	1	61.95		58.95		55.87		56.43	
	2	63.19		63.11		59.52		58.35	
	3	63.1		61.87		63.03		62.76	
	4	65.65		59.71		56.29		54.51	
	5	61.52		61.47		62.89		62.54	

LITERATURE CITED

Ang, D., Liberek, K., Skowyra, D., Zylicz, M., Georgopoulos, C. 1991. Biological role and regulation of the universally conserved heat shock proteins. The Journal of Biological Chemistry 266: 24233-24236.

Baker, K.M., Kirk, W.W., Andresen, J.A., Stein, J.M. 2002. A problem case study: Influence of climatic trends on late blight epidemiology in potatoes. Acta Horticulturae 638: 37-42.

Bakshi, S., Sztejnberg, A., Yarden, O. 2001. Isolation and characterization of a cold-tolerant strain of *Fusarium proliferatum*, a biocontrol agent of grape downy mildew. Phytopathology 91: 1062-1068.

Berry, E. D., Foegeding, P. M. 1997. Cold temperature adaptation and growth of microorganisms. Journal of Food Protection 60: 1583-1594.

Birch, P.R.J., Whisson, S.C. 2001. *Phytophthora infestans* enters the genomics era. Molecular Plant Pathology 2: 257-263.

Blenkinsop, R.W., Copp, L.J., Yada, R.Y., Maragoni, A.G. 2003. A proposed role for the anaerobic pathway during low-temperature sweetening in tubers of *Solanum tuberosum*. Physiologia Plantarum 118: 206-212.

Bonde, R., Schultz, E.S. 1943. Potato refuse piles as a factor in the dissemination of late blight. Maine Agricultural Experimental Station Bulletin 416: 229-246.

Bostock, R.M., Nuckles, E., Henfling, J.W.D., Kuc, J.A. 1983. Effects of potato tuber age and storage on sesquiterpenoid stress metabolite accumulation, steroid glycoalkaloid accumulation, and response to abscisic and arachidonic acids. Phytopathology 73: 435-438.

Burpee, L.L. 1994. Interactions among low-temperature-tolerant fungi: Prelude to biological control. Canadian Journal of Plant Pathology 16: 247-250.

Caten, C. E., Jinks, J.L. 1968. Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. Canadian Journal of Botany 46: 329-34

Chalenko, G.I., Leont'eva, G.V., Yurganova, L.A., Karavaeva, K.A., Ozeretskovskaya, O.L. 1980. Resistance of potato tubers to *Phytophthora infestans* during storage. Applied Biochemistry and Microbiology 16: 194-199.

Chalova, L.I., Nogaideli, D.E., Karavaeva, K.A., Ozeretskovskaya, O.L. 1985. Activities of peroxidase and polyphenoloxidase as markers of systemic sensibilization in potato tubers. Mikologiya I Fitopatologiya 19: 495-498.

Clark, E.M., White, J.F., Patterson, R.M. 1983. Improved histochemical techniques for the detection of *Acremonium coenophialum* in tall fescue and methods of in vitro culture of the fungus. Journal of Microbiological Methods 1: 149-155.

Crosier, W. 1934. Studies in the biology of *Phytophthora infestans* (Mont.) De Bary. Cornell University Experimental Station Memorandum 155.

Davila, E. 1964. Late blight infection of potato tubers. American Potato Journal 41: 103-112.

de Bruyn, H.L.G. 1926. The overwintering of *Phytophthora infestans* (Mont.) de By. Phytopathology 16: 121-140.

Dixon, W.L., ap Rees, T. 1980. Carbohydrate metabolism during cold-induced sweetening of potato tubers. Phytochemistry 19: 1653-1656.

Dorrance, A.E., Inglis, D.A. 1998. Assessment of laboratory methods for evaluating potato tubers for resistance to late blight. Plant Disease 82: 442-446.

Douches, D.S., Jastrzebski, K., Coombs, J., Kirk, W.W., Felcher, K.J., Hammerschmidt, R., Chase R.W. 2001. Jacqueline Lee: A late-blight-resistant tablestock variety. American Journal of Potato Research 78: 413-419.

Dowley, L.J., O'Sullivan, E. 1991. Sporulation of *Phytophthora infestans* (Mont.) de Bary on the surface of diseased potatoes and tuber to tuber spread of infection during handling. Potato Research 34: 295-296.

Easton, G.D. 1982. Late blight of potatoes and prediction of epidemics in arid central Washington State. Plant Disease 66: 452-455.

Erwin, D.C., Ribeiro, O.K. 1996. *Phytophthora* Diseases Worldwide. St Paul: American Phytopathological Society Press.

Fabritius, A., Cvitanich, C., Judelson, H.S. 2002. Stage specific gene expression during sexual development in *Phytophthora infestans*. Molecular Biology 45: 1057-1066.

Fay, J.C., Fry, W.E. 1997. Effects of hot and cold temperatures on the survival of oospores produced by United States strains of *Phytophthora infestans*. American Potato Journal 74: 315-323.

Flier, W.G., Turkensteen, L.J., van den Bosch, G.B.M., Vereijken, P.F.G., Mulder, A. 2001. Differential interaction of *Phytophthora infestans* on tubers of potato cultivars with different levels of blight resistance. Plant Pathology 50: 292-301.

Flier, W.G., van den Bosch, G.B.M., Turkensteen, L.J. 2003. Stability of partial resistance in potato cultivars exposed to aggressive strains of *Phytophthora infestans*. Plant Pathology 52: 326-337.

Förster, H., Coffey, M.D., Elwood, H., Sogin, M.L. 1990. Sequence analysis of the small subunit ribosomal RNAs of three zoosporic fungi and implications for fungal evolution. Mycologia 82: 306-312.

Fry, W.E., Goodwin, S.B., Dyer, A.T., Matuszak, J.M., Drenth, A., Tooley, P.W., Sujkowski, L.S., Koh, Y.J., Cohen, B.A., Spielman, L.J., Deahl, K.L., Inglis, D.A., Sandlan, K.P. 1993. Historical and Recent Migrations of *Phytophthora infestans*: Chronology, Pathways, and Implications. Plant Disease 77: 653-661.

Fry, W.E., Goodwin, S.B. 1997. Re-emergence of potato and tomato late blight in the United States. Plant Disease 81: 1349-1357.

Furuichi, N., Tomiyama, K., Doke, N. 1979. Hypersensitive reactivity in potato: transition from inactive to active state induced by infection with an incompatible race of *Phytophthora infestans*. Phytopathology 69: 734-736.

Gaudet, D.A., Laroche, A., Frick, M., Davoren, J., Puchalski, B., Ergon, A. 2000. Expression of plant defence-related (PR-protein) transcripts during hardening and dehardening of winter wheat. Physiological and Molecular Plant Pathology 57: 15-24.

Goodman, R.N., Novacky, A.J. 1994. The Hypersensitive Reaction in Plants to Pathogens, A Resistance Phenomenon. St. Paul: American Phytopathological Society Press.

Goodwin, S.B., Cohen, B.A., Deahl, K.L., Fry, W.E. 1994. Migration from northern Mexico as the probable cause of recent genetic changes in populations of *Phytophthora infestans* in the United States and Canada. Phytopathology 84: 553-558.

Goodwin, S.B., Sujkowski, L.S., Fry. W.E. 1996. Widespread distribution and probable origin of resistance to Metalaxyl in clonal genotypes of *Phytophthora infestans* in the United States and western Canada. Phytopathology 86: 793-800.

Goodwin, S.B., Drenth, A. 1997. Origin of the A2 mating type of *Phytophthora infestans* outside Mexico. Phytopathology 87: 992-999.

Graves, C. 2001. The Potato, Treasure of the Andes From Agriculture to Culture. Lima, Peru: International Potato Center (CIP).

Grinberger, M., Kadish, D., Cohen, Y. 1995. Infectivity of Metalaxyl-sensitive and – resistant isolates of *Phytophthora infestans* to whole potato tubers as affected by tuber aging and storage. Phytoparasitica 23:165-175.

Hächler, H., Hohl, H.R. 1984. Temporal and spatial distribution patterns of collar and papillae wall appositions in resistant and susceptible tuber tissue of *Solanum tuberosum* infected by *Phytophthora infestans*. Physiological Plant Pathology 24: 107-118.

Harrison, J.G., Lowe, R. 1989. Effects of humidity and air speed on sporulation of *Phytophthora infestans* on potato leavcs. Plant Pathology 38: 585-591.

Harrison, J.G. 1992. Effects of the aerial environment on late blight of potato foliage – a review. Plant Pathology 41: 384-416.

Hirst, J.M., Stedman, J. 1960. The epidemiology of *Phytophthora infestans* II. The source of inoculum. Annals of Applied Biology 48: 489-517.

Hohl, H.R., Iselin, K. 1984. Strains of *Phytophthora infestans* with A2 mating type behaviour. Transcripts of the British Mycological Society 83: 529-530.

Humphreys, T.L., Castrillo, L.A., Lee, M.R. 2001. Sensitivity of partially purified ice nucleation activity of *Fusarium acuminatum* SRSF 616. Current Microbiology 42: 330-338.

Isard, S.A., Schaetzl, R.J. 1998. Effects of winter weather conditions on soil freezing in southern Michigan. Physical Geography 19: 71-94.

Juarez-Palacios, C., Felix-Gastelum, R., Wakeman, R.J., Paplomatas, E.J., DeVay, J.E. 1991. Thermal sensitivity of three species of *Phytophthora* and the effect of soil solarization on their survival. Plant Disease 75: 1160-1164.

Judelson, H.S. 1997. The genetics and biology of *Phytophthora infestans*: modern approaches to a historical challenge. Fungal Genetics and Biology 22: 65-76.

Kadish, D., Cohen, Y. 1992. Over seasoning of metalaxyl-sensitive and metalaxyl-resistant isolates of *Phytophthora infestans* in potato tubers. Plant Disease 82: 887-889.

Kamoun, S. 2003. Molecular genetics of pathogenic oomycetes. Eukaryotic Cell 2: 191-199.

Kirk, W.W., Felcher, K.J., Douches, D.S., Niemira, B.A., Hammerschmidt, R. 2001(a). Susceptibility of potato foliage and tubers to US-8 genotype of *Phytophthora infestans*. American Journal of Potato Research 78: 319-322.

Kirk, W.W., Niemira, B.A., Stein, J.M. 2001(b). Influence of temperature on the rate of potato tuber tissue infection caused by *Phytophthora infestans* (Mont.) de Bary estimated by digital image analysis. Potato Research 44: 86-96.

Kirk, W.W. 2003(a). Thermal properties of overwintered piles of cull potatoes. American Journal of Potato Research 80: 145-149.

Kirk, W.W. 2003(b). Tolerance of mycelium of different genotypes of *Phytophthora infestans* to freezing temperatures for extended periods. Phytopathology 93: 1400-1406.

Kitazawa, K., Tomiyama, K. 1969. Microscopic observations of infection of potato cells by compatible and incompatible races of *Phytophthora infestans*. Phytopathologische Zeitschrift 66: 317-324.

Ko, W.H. 1994. An alternative possible origin of the A2 mating type of *Phytophthora infestans* outside Mexico. Phytopathology 84: 1224-1227.

Lafon, R., Bulit, J. 1981. Downy mildew of the vine. In The Downy Mildews. Edited by D.M. Spencer. London: Academic Press.

Lambert, D.H., Currier, A.I. 1997. Differences in tuber rot development for North American clones of *Phytophthora infestans*. American Potato Journal 74: 39-43.

Lambert, D.H., Currier, A.I., Olanya, M.O. 1998. Transmission of *Phytophthora infestans* in cut potato seed. American Journal of Potato Research 75: 257-263.

Lawrimore, J. "Michigan Climate Summary". 16 March 2004. National Climatic Data Center. <http://lwf.ncdc.noaa.gov/oa/climate/research/cag3/MI.html>

Löhnis, M.P. 1922. Onderzoek over *Phytophthora infestans* (Mont.) de By. op de aardappelplant. Ph.D. Dissertation, Univ. Ultrecht.

Lundheim, R. 2002. Physiological and ecological significance of biological ice nucleators. Philosophical Transactions of the Royal Society of London Series B – Biological Sciences 357: 937-943.

Matsumoto, N., Tomiyama, K., Doke, N. 1976. Alteration of membrane permeability of potato tuber tissue infected by incompatible and compatible races of *Phytophthora infestans* during the initial phase of infection. Annals of the Phytopathological Society of Japan 42: 279-286.

Medina, M.V., Platt, H.W., Peters, R.D. 1999. Severity of late blight tuber infection caused by US-1 and US-8 genotypes of *Phytophthora infestans* in 12 potato cultivars. Canadian Journal of Plant Pathology 21: 388-390

Medina, M.V., Platt, H.W., Peters, R.D. 2000. Response of five potato cultivars to co-inoculation with US-1 and US-8 genotypes of *Phytophthora infestans*. Potato Research 43: 153-161.

Melhus, I.E. 1915a. Hibernation of *Phytophthora infestans* in the Irish potato. Journal of Agricultural Research 5: 71-102.

Melhus, I.E. 1915b. Germination and infection with the fungus of the late blight of potato (*Phytophthora infestans*). Wisconsin Agricultural Experimental Station Research Bulletin 37: 1-64.

Mizubuti, E.S.G., Fry, W.E. 1998. Temperature effects on developmental stages of isolates from three clonal lineages of *Phytophthora infestans*. Phytopathology 88: 837-843.

Niederhauser, J.S. 1991. *Phytophthora infestans*: The Mexican connection. Pages 25-45 In *Phytophthora*, edited by J.A. Lucas, R.C. Shattock, D.S. Shaw, and L.R. Cooke. Cambridge: Cambridge University Press.

Ozeretskovskaya, O.L. 1995. Induced resistance in the Solanaceae. Pages 31-62 In Induced Resistance to Disease in Plants, edited by R. Hammerschmidt and J. Kuc. Boston: Kluwer Academic Publishers.

Pathak, N., Clarke, D.D. 1987. Studies on the resistance of the outer cortical tissues of the tubers of some potato cultivars to *Phytophthora infestans*. Physiological and Molecular Plant Pathology 31: 123-132.

Pearce, R.S. 2001. Plant freezing and damage. Annals of Botany 87: 417-424.

Peters, R.D., Platt, H.W., Hall, R., Medina, M. 1999. Variation in aggressiveness of Canadian isolates of *Phytophthora infestans* as indicated by their relative abilities to cause potato tuber rot. Plant Disease 83: 652-661.

Platt, H.W., Tai, G. 1998. Relationship between resistance to late blight in potato foliage and tubers of cultivars and breeding selections with different resistance levels. American Journal of Potato Research 75: 173-178.

Richard, C., Martin, J., Pouleur, S. 1996. Ice nucleation activity identified in some phytopathogenic *Fusarium* species. Phytoprotection 77: 83-92.

Rubio-Covarrubias, O.A., Douches, D., Hammerschmidt, R., da Rocha, A., Kirk, W. 2004a. Effect of temperature and photoperiod on resistance against *Phytophthora infestans* in susceptible and resistant potato cultivars: 1) Effect on deposition of structural phenolics on the cell wall and resistance against penetration. American Journal of Potato Research (In press).

Rubio-Covarrubias, O.A., Douches, D., Hammerschmidt, R., da Rocha, A., Kirk, W. 2004b. Effect of temperature and photoperiod on resistance against *Phytophthora infestans* in susceptible and resistant potato cultivars: 2) Effect on symptoms associated with resistance after penetration. American Journal of Potato Research (In press).

Russel, N.J. 1990. Cold adaptation of microorganisms. Philosophical Transactions of the Royal Society of London, Series B 326: 595-611.

Sakai, S., Doke, N, Tomiyama, K. 1982. Relation between necrosis and rishitin accumulation in potato tuber slices treated with hyphal wall components of *Phytophthora infestans*. Annals of the Phytopathological Society of Japan 48: 238-240.

Sato, N., Tomiyama, K. 1976. Relation between rishitin accumulation and degree of resistance of potato-tuber tissue to infection by an incompatible race of *Phytophthora infestans*. Annals of the Phytopathological Society of Japan 42: 431-435.

Sato, N. 1979. Effect of soil temperature on the field infection of potato tubers by *Phytophthora infestans*. Phytopathology 69: 989-993.

Schaetzl, R.J., Tomczak, D.M. 2001. Wintertime temperatures in the fine-textured soils of Saginaw Valley, Michigan. The Great Lakes Geographer 8: 87-99.

Schiermeier, Q. 2001. Russia needs help to fend off potato famine, researchers warn. Nature 410: 1011.

Schumann, G.L. 1991. Plant Diseases: Their Biology and Social Importance. St Paul: American Phytopathological Society Press.

Seymour, M., Boydston, R. 2002. Volunteer potato update for the Columbia Basin 2002. Potato Progress, Research and Extension for Washington's Potato Industry 2: 1-2.

Shattock, R.C. 1976. Winter survival of field isolates of *Phytophthora infestans* in seed tubers and development of primarily infected plants. Annals of Applied Biology 84: 273-274.

Smith, J.D. 1987. Winter-hardiness and overwintering diseases of amenity turfgrasses with special reference to the Canadian prairies. Technical Bulletin. 1987-12E. Research Branch, Agriculture Canada.

Szabo, L.J., Bushnell, W.R. 2001. Hidden robbers: The role of fungal haustoria in parasitism of plants. Proceedings of the National Academy of Sciences of the US 98: 7654-7655.

Tomiyama, K., Okamoto, H., Katou, K. 1983. Effect of infection by *Phytophthora infestans* on the membrane potential of potato cells. Physiological Plant Pathology 22: 233-243.

Toxopeus, H.J. 1958. Some notes on the relations between field resistance to *Phytophthora infestans* in leaves and tubers and ripening time in *Solanum tuberosum* subsp. *tuberosum*. Euphytica 7: 123-130.

Tyler, B.M. 2002. Molecular basis of recognition between *Phytophthora* pathogens and their hosts. Annual Review of Phytopathology 40: 137-167.

Van der Plank, J.E. 1963. Plant Diseases: Epidemics and Control. New York: Academic Press. 349 pages.

Van der Plank, J.E. 1984. Disease Resistance in Plants. 2nd ed. New York: Academic Press. 194 pages.

Viola, R., Davies, H.V. 1994. Effect of temperature on pathways of carbohydrate metabolism in tubers of potato (*Solanum tuberosum* L.). Plant Science 103: 135-143.

Walker, A.S.L., Cooke, L.R. 1988. The survival of phenylamide-resistant strains of *Phytophthora infestans* in potato tubers. Brighton Crop Protection Conference – Pests and Diseases 1988 4C-1. 353-358.

Walker, A.S.L., Cooke, L.R. 1990. The survival of *Phytophthora infestans* in potato tubers – the influence of phenylamide resistance. Brighton Crop Protection Conference – Pests and Diseases 1990 9C-1. 1109-1114.

Wong, P.T.W, Mead, J.A., Holley, M.P. 1996. Enhanced field control of wheat take-all using cold tolerant isolates of *Gaeumannomyces graminis* var. *graminis* and *Phialophora* sp. (lobed hyphopodia). Plant Pathology 45: 285-293.

Yurganova, L.A., Nogaideli, D.E., Chalova, L.I., Chalenko, G.I., Ozeretskovskaya, O.L. 1989. Activity of lipoxygenase in potato tubers after immunization. Mikologiya I Fitopatologiya 23: 73-79.

Zentmeyer, G.A. 1981. The effect of temperature on growth and pathogenesis of *Phytophthora cinnamomi* and on growth of its avocado host. Phytopathology 71: 925-928.

Zwankhuizen, M.J., Govers, F., Zadoks, J.C. 1998. Development of potato late blight epidemics: Disease foci, disease gradients, and infection sources. Phytopathology 88: 754-763.