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Late blight, caused by *Phytophthora infestans*, is a devastating problem to potato production in many parts of the world. While the foliar phases of this disease are well characterized, tuber infection, leading to quality losses and disease perpetuation, is less well understood. Experiments were conducted during 1998 and 1999 in irrigated sandy loam soils to evaluate the relative importance of cultural treatments, i.e. mulches and hill sizes, as barriers to the movement of *P. infestans* inoculum from potato foliage to developing tubers. In the mulching experiment, five treatments were applied to field plots of cultivar 'Red LaSoda' in a randomized block design: 1) no mulch, 2) expandable polyurethane spray foam in the 8 cm-diameter immediately surrounding the potato stem, 3) black polyethylene film over the entire hill area except the 8 cm-diameter immediately surrounding the potato stem, 4) a combination of treatments 2 and 3, and 5) a copper hydroxide-treated textile applied over the same area as in treatment 3. When compared to the appropriate control, black polyethylene film reduced tuber blight incidence by 10 to 24% while polyurethane spray foam did not reduce ($P > 0.1$) tuber blight. Copper hydroxide-treated textile

also reduced ($P \leq 0.05$) tuber blight incidence by 10 to 33%. In the hill size experiment, conducted once in 1998 and twice in 1999, potato cultivars 'Russet Burbank', 'Red LaSoda' and 'Shepody', were planted as whole plots in a split-plot design with three hill size treatments. Hill size treatments were established by hilling with the planter only (small hill) and by supplementary hilling with tractor mounted disks once or twice after potato emergence (medium and large hills, respectively). In all three trials, consistent and significant differences ($P \leq 0.05$) in tuber blight incidence were observed among the cultivars with 'Red LaSoda' the most susceptible and 'Russet Burbank' the least susceptible to tuber blight. Hill size had a significant effect ($P \leq 0.05$) on tuber blight incidence only in 1998 when 40% of tubers in small hills and 30% tubers in medium and large sized hills were blighted. Comparison of tuber blight incidence examined by tuber depth in the hill, however, revealed few differences among hill size treatments, although, deep tubers had a lower incidence of tuber blight (5.5%) than tubers at shallow (34.1%) or intermediate (19.7%) depths. Because black polyethylene film and copper hydroxide-treated textile reduced the incidence of tuber blight, movement of *P. infestans* inoculum from potato foliage to tubers does not appear to be restricted to large channels in the soil such as those created by the stems. The difficulty of direct tuber blight suppression after establishment of a foliar epidemic suggests that prevention of tuber blight in a conducive environment may be inseparably linked to suppression of the foliar phase.

Management of tuber blight remains one of the least understood areas in the late blight disease cycle, in part because tuber blight is difficult to assess and quantify. To help overcome problems associated with tuber blight assessment, a *P. infestans*

(US-8) isolate was transformed with the reporter gene β- glucuronidase (GUS) for use in future epidemiological studies of the tuber stage of the late blight disease cycle.

Assessment of Potato Tuber Blight Caused by *Phytophthora infestans*

by

Jenny Rebecca Glass

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Jenny Rebecca Glass, Author

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CONTRIBUTION OF AUTHORS

Dr. Kenneth Johnson provided ideas for the experiment, and was instrumental with planning its design. He also helped with data analysis, the writing and editing of the thesis, and provided financial support for the work. Dr. Lynda Ciuffetti and Dr. Howard Judelson assisted with the *Phytophthora infestans* transformation. Dr. Mary Powelson provided ideas, advice, and interpretation for the project and assisted with the editing of the manuscript.

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INTRODUCTION

Disease Significance

Late blight, caused by the pathogen *Phytophthora infestans* (Mont.) de Bary (Kingdom: Chromista, Class: Oomycete), is an important disease of solanaceous plants including potato (*Solanum tuberosum* L.) and tomato (*Lycopersicon esculentum* Mill.). This disease damages foliage, fruit and tubers, reducing both crop quantity and quality. Many plant pathologists (Fry *et al.*, 1993, 1992; Goodwin *et al.*, 1995, 1994), but not all (Abad and Abad, 1997), believe that *P. infestans* evolved as a plant pathogen in central Mexico. The first reports of this pathogen outside of Mexico occurred in the 1840s when a new disease struck potatoes near Philadelphia. Late blight also developed in Europe during the 1840s, where combined with cool rainy weather and economic factors of the time, the disease led to severe famines including the infamous Great Famine of Ireland. Over the next 150 years, through the study of the disease and development of management tactics such as elimination of inoculum sources and the use of synthetic fungicides, control of late blight improved and significance of the disease diminished. In the last 15 years, however, late blight has reemerged as an important threat to potato and tomato production in many growing regions of the world (Fry and Goodwin, 1997). Reasons for this reemergence include:

1) migration of new clones of *P. infestans* from Mexico that are more aggressive on potatoes than previously established clones, 2) insufficient host resistance in most commercial potato cultivars, and 3) the occurrence of strains of *P. infestans* resistant to the fungicide, metalaxyl, which had become an integral part of foliar late blight suppression.

Pathogen Biology

Species of *Phytophthora* are members of the Oomycetes, a group known as the "water molds", which have a phylogenic relationship with brown algae but not with true fungi. Oomycetes are fundamentally different from fungi as their cell walls are made up of glucans and cellulose rather than the chitin found in fungi. In addition, Oomycetes have diploid nuclei and coenocytic hyphae while fungi have haploid nuclei and septate hyphae. Other characteristics of the Oomycetes include the formation of motile, biflagellate zoospores borne within sporangia, and formation of thick-walled oospores upon the union of sexual gametes termed antheridia and oogonia.

Phytophthora infestans is probably the best known of all oomycete pathogens. Species of *Phytophthora* are typically destructive parasites on higher plants, and are favored by the presence of free moisture in soil or on foliage. Most *Phytophthora* species, including *P. infestans*, have a high reproductive capacity and a short generation time, which leads to polycyclic disease progression as the initial infections rapidly reproduce to form secondary inoculum (Erwin and Ribeiro, 1996). Whereas most *Phytophthora* species are soil inhabitants that cause rots of seeds and roots, *P.*

infestans is primarily an aerial pathogen infecting host foliage (Goodwin, 1997). This pathogen, however, also can infect potato tubers.

Disease Cycle and Pathogen Dispersal

Phytophthora infestans has a host range limited to solanaceous plant species. Sporangia are produced by the mycelia on branched sporangiophores. Sporangia, once detached, can be carried in air or water, with aerial dispersal being the principal process by which late blight epidemics are spread. The thin-walled sporangia last only a few days before they succumb to desiccation (Minogue and Fry, 1981) or solar radiation (Mizubuti *et al.*, 2000). Lesions may be initiated on the leaves, petioles or stems and a single lesion is capable of producing as many as 300,000 sporangia per day (Legard *et al.*, 1995). Cool (7 to 23°C) weather with relative humidity near 100% promotes foliar blight (Harrison, 1992), but even if the climate is apparently unfavorable, irrigation practices can create environments within potato canopies conducive to disease development (Hirst and Stedman, 1960). At temperatures between 15°C to 23°C, sporangia germinate directly forming a germ tube with an appressorium capable of penetrating intact leaf cells or natural openings, such as stomata (Crosier, 1934; Hohl and Suter, 1976; Pristou and Gallegly, 1953). At cooler temperatures, sporangia in water germinate indirectly to produce 3 to 12 zoospores that are released upon rupture of the sporangial wall. The biflagellate zoospores typically swim for a short period of time before they shed their flagella and encyst. Encysted zoospores form a cell wall from which a germ tube emerges to infect the

host (Kramer *et al.*, 1997). Depending on the environment, a complete asexual generation of the pathogen- penetration, colonization, sporulation, and dispersal- can occur in as few as 5 days. Thus, many generations can occur in a single growing season.

Population Genetics of *P. infestans*

Phytophthora infestans is heterothallic pathogen requiring both A1 and A2 mating types for sexual recombination and the production of oospores. Oospores function as thick-walled overwintering structures that can survive months, if not years, in the soil in the absence of a host. In the Toluca Valley of Mexico, where *P. infestans* may have originated as a pathogen (Fry *et al.*, 1993, 1992; Goodwin *et al.*, 1995, 1994), A1 and A2 mating types of the pathogen are found in a 50:50 ratio and oospore production is common. Production of oospores in most other potato growing areas of the world has been rare (Cohen *et al.*, 1997; Deahl *et al.*, 1993; Drenth *et al.* 1995; Pittis and Shattock, 1994). In these areas where oospores are absent, *P. infestans,* is a poor soil saprophyte and generally must rely on the overwintering of mycelium within blighted tubers to perpetuate the life cycle into the next year (Melhus, 1915; Peterson, 1947).

In the absence of sexual reproduction, populations of *P. infestans* exist as clonal lineages, which are defined as asexual descendants of a single genotype that differ from their originator only by mutation or mitotic recombination (Goodwin, 1997). Currently, characterization of clonal lineages is based on mating type and

genetic variation found at two allozyme loci, peptidase and glucose-6-phosphate isomerase (Goodwin, Schneider and Fry, 1995). The lack of allozyme variation found in over 300 isolates of *P. infestans* collected from 20 countries and five continents suggests that a single clone, now known as US-1 (mating type A1), was responsible for nearly all *P. infestans* populations outside of Mexico prior to the 1970s (Goodwin, Cohen and Fry, 1994). During the late 1980s and 1990s, severe late blight epidemics occurred in both potato and tomato in North America and Europe (Chychoski and Punja, 1996; Deahl *et al.*, 1991; Fry and Goodwin 1997; Goodwin *et al.*, 1995; Inglis *et al.*, 1996; Miller *et al.*, 1997, 1998). Allozyme variation found in isolates from these epidemics indicated that new *P. infestans* clones, which had most likely migrated out of Mexico, were responsible for these epidemics (Goodwin *et al.*, 1993, 1995). In the United States and Europe, these new clones rapidly displaced the existing populations in the field leading to the hypothesis that they had a greater ability to survive, grow, and reproduce than the US-1 clone previously established (Bashan *et al.*, 1989; Tooley *et al.*, 1986). Several of the recently introduced clones, especially US-8, also were more aggressive on potato tubers than isolates growers had previously learned to manage (Lambert and Currier, 1997), and several clones, US-6 and US-7, demonstrated pathogenic specialization on tomato (Legard *et al.*, 1995). In addition, many of these new clones were resistant to the systemic fungicide metalaxyl (Deahl *et al.*, 1993; Goodwin *et al.*, 1996). This fungicide had become an integral part of foliar blight management because it was the only chemical that could halt an established late blight epidemic (Fry and Goodwin, 1997; Platt, 1994). The introduction of these clones to the potato growing areas of the world has created

management challenges, and raised new questions about disease epidemiology, including questions on tuber blight development.

Tuber Infection

Tuber blight has not been demonstrated to be the result of systemic plant infection. Field infection of potato tubers, in the absence of sexual oospores, is initiated most commonly by inoculum produced on the plant foliage during the season. Thus, developing tubers can become blighted shortly after the establishment of late blight lesions on leaves and stems (Hirst *et al.*, 1965). Once the inoculum produced on the foliage is deposited onto the surface of the potato hill, water from irrigation or rain can carry spores into the soil or down cracks and channels in the potato hill (Lacey, 1966, 1967; Large, 1953; Zan, 1962). Once infested, soils can harbor viable sporangia and encysted zoospores for as long as 15 to 77 days depending on a variety of soil and environmental factors including texture, temperature, moisture, pH, and the presence of certain compounds such as Al^{3+} (Andrivon, 1995). The same temperatures that favor zoospore production (< 18°C) are also the temperatures most favorable to tuber blight (Sato, 1979). Under natural conditions, therefore, tuber blight infections caused by zoospores are thought to be more important than tuber infections initiated by sporangia. Zoospores can initiate lesions at eyes, lenticels, and wounds on the tuber (Adams, 1975; Lacey, 1967; Walmsey-Woodward and Lewis, 1977) but not through intact periderm (Patak and Clarke, 1987).

Soil covering the tubers is believed to protect tubers from blight by reducing direct contact of inoculum on tubers and filtering spores out of the soil water suspension (Lacey, 1965; Rowe and Secor, 1993). Soil near the surface has been demonstrated to contain more viable inoculum than deeper soils (Dubey and Stevenson, 1996; Lacey, 1965) and deeply buried tubers are less likely to become blighted than shallow tubers (Lacey, 1965, 1966). In a laboratory experiment using soil columns, however, as little as 0.6 cm of water was shown to carry sporangia through loamy sand, silt loam and muck soils to a depth of 40 cm (Dubey and Stevenson, 1996). In addition, zoospores can swim short distances in soil water and are able to follow gradients of potato root exudates (Lacey, 1965), which increases their likelihood of contacting a tuber and reduces the ability of the soil to protect tubers.

Tuber blight infection is not limited to movement of sporangia and zoospores from foliage into the hill. Infection of tubers via sporangia produced by overwintering oospores in soil is a second pathway that may become more important as A1 and A2 mating types of *P. infestans* spread around the world. Third, and also common, tubers blight can occur during harvest if viable inoculum produced on the foliage is redistributed onto tubers during the lifting operation. For this reason, most potato vines are treated with chemical herbicides 2 to 3 weeks before harvest to kill vines and promote maturation of tuber periderm.

Late Blight Management

Management of potato late blight relies on cultural strategies combined with protectant fungicide use, particularly since the advent of *P. infestans* clones resistant to the systemic fungicide metalaxyl (Erwin and Ribeiro, 1996; Stevenson 1993). Because most potato cultivars possess only limited resistance to *P. infestans*, care must be taken to avoid disease establishment. Sanitation measures, such as using seed from fields that did not have late blight in the previous season, and destroying cull piles, aim to eliminate primary inoculum, mycelium overwintering in tubers. After plant establishment, hilling is recommended to cover tubers and shield them from inoculum (Rowe and Secor, 1993). During the growing season, irrigation management to limit long periods of leaf wetness is recommended though often impractical to apply (Rotem *et al.*, 1970). In environments conducive for late blight development, routine applications of protectant fungicides, such as chlorothalonil, are used to prevent the development of foliar blight epidemics. Chemical control programs can be fine-tuned by field scouting and use of disease warning models (Johnson *et al.*, 1996, 1998; Raposo *et al.*, 1993). Currently, no chemical control methods are employed that directly protect tubers from blight. Prior to harvest, potato vines are sprayed with chemical herbicides and allowed to die to reduce the possibility of contaminating tubers with inoculum of *P. infestans* during the harvest operation.

β-glucuronidase (*GUS*) Reporter Gene.

Management of tuber blight remains one of the least understood areas in the late blight disease cycle, in part because tuber blight is difficult to assess and quantify. Blighted tubers can be difficult to assess for several reasons: 1) lesions may be small, and nearly undetectable at the time of assessment, 2) secondary soft rots are often present, and 3) lesions can be mistaken for other diseases. A method to enhance the visualization of *P. infestans,* such as tagging the pathogen with a reporter gene, is needed to aid in the evaluation of tuber blight. Reporter genes have easily detectable traits or products, such as antibiotic resistance or pigment production, that can be observed and used to infer location or behavior of tagged genes or organisms (Gallagher, 1992). Tagging *P. infestans* with a reporter gene could potentially eliminate some of the difficulties associated with tuber blight assessment and facilitate the study of the tuber blight stage of the disease.

The β-glucuronidase (GUS) expression system from *Echerichia coli* developed by Jefferson *et al.* (1987) is a good candidate for such work. The usefulness of *GUS* as a genetic marker is based on the fact that endogenous GUS activity is not expressed in most pathogens, plants, or environments (Gallagher, 1992; Jefferson *et al.*, 1987). The *GUS* gene product (β-glucuronidase) when mixed with certain glucuronide substrates produces indigo-colored or fluorescent compounds that can be easily visualized. Indigo-colored compounds show the presence and location of the tagged organism, while the activity of the fluorescent compounds can be measured fluorimetrically to quantify the organism's biomass (Gallagher, 1992).

GUS reporter gene technology has been adapted for use in several studies involving etiology and detection of fungal plant pathogens. GUS expression allowed researchers to overcome visual bias in a study of *Bipolaris sorokiniana* root colonization when fungal mycelia, as measured by fluorimetic GUS activity, was shown to grow at faster rate on wheat and triticale than on barley despite lesions appearing smaller on these two species than on barley (Liljeroth *et al.*, 1996). By tagging *Fusarium subglutinans* with *GUS*, this pathogen could be distinguished from other potential causes of the mango malformation disease (Freeman *et al.*, 1999). After inoculating healthy mango shoots with a GUS-expressing transformant, indigo colored mycelia, revealed after staining symptomatic lesions, was used as evidence to establish that *F. subglutinans* was the causal agent of the mango malformation disease. GUS-expressing transformants of *Pseudocercosporella herpotrichoides* were used to compare resistance levels in six wheat genotypes (de la Pena and Murray, 1994). Fluorimetric measurement of GUS activity in wheat tissue following inoculation enabled differentiation among highly resistant, resistant, and susceptible wheat genotypes while an enzyme-linked immunosorbent assay (ELISA) was unable to differentiate the genotypes. In this system, GUS detection was less subjective and less labor intensive than visual assessment, and was not limited to an arbitrary rating scale as imposed by visual assessment.

Judelson transformed *P. infestans* with *GUS* (Judelson and Michelmore, 1991; Judelson *et al.*, 1991) and suggested that GUS-expressing *P. infestans* isolates may be used to study epidemiology of the late blight disease cycle. Histochemical staining to form an indigo-colored compound could potentially overcome difficulties involved

with tuber lesion assessment. Similarly, quantification of pathogen biomass within a blighted tuber may be achieved more accurately by measuring the activity produced by a fluorimetric compound than by visual assessing the extent of lesion development. Investigations of many aspects of tuber blight, including resistance to infection as influenced by tuber maturity and placement in the hill, may be improved through the use of GUS-expressing *P. infestans* transformants.

Objectives

The goal of this study was to increase the understanding of the factors leading to infection of potato tubers by *P. infestans*. The first objective was to investigate the relative importance of barriers (mulches and soil) to the movement of *P. infestans* inoculum from potato foliage to developing tubers. The second objective of this research was to achieve a GUS-expressing *P. infestans* transformant that could be used in future studies to enhance the detection and quantification of tuber blight infection.

CHAPTER 1

ASSESSMENT OF BARRIERS TO INOCULUM MOVEMENT OF *PHYTOPHTHORA INFESTANS* FROM DISEASED POTATO FOLIAGE TO TUBERS

Jenny Rebecca Glass and Kenneth B. Johnson

Abstract

Experiments were conducted during 1998 and 1999 in irrigated sandy loam soils to evaluate the relative importance of cultural treatments, mulches and hill sizes, as barriers to the movement of *Phytophthora infestans* inoculum from potato foliage to developing tubers. In the mulching experiment, five treatments were applied to field plots of cultivar 'Red LaSoda' in a randomized block design: 1) no mulch, 2) expandable polyurethane spray foam in the 8 cm-diameter immediately surrounding the potato stem, 3) black polyethylene film over the entire hill area except the 8 cm-diameter immediately surrounding the potato stem, 4) a combination of treatments 2 and 3, and 5) a copper hydroxide-treated non-woven textile applied over the same area as in treatment 3. For treatments 1 to 4, a 15 cm segment of 8 cm-diameter PVC pipe positioned vertically around each potato stem separated the area immediate to the stem from the remaining hill area. When compared to the appropriate control, black polyethylene film reduced tuber blight incidence by 10 to 24% while polyurethane spray foam did not reduce ($P > 0.1$) tuber blight. Copper hydroxide-treated textile also reduced ($P \leq 0.05$) tuber blight incidence by 10 to 33%. Most lesions were apparently initiated at eyes on the tubers but the polyethylene film treatment shifted some of the distribution from eyes toward the stolons of the tubers. In the hill size experiment, conducted once in 1998 and twice in 1999, potato cultivars 'Russet Burbank', 'Red LaSoda' and 'Shepody', were planted as whole plots in a split-plot design with three hill size subplot treatments. Hill size treatments were established by hilling with the planter only (small hill) and by supplementary hilling with tractor-

mounted disks once or twice after potato emergence (medium and large hills, respectively). Cross-sectional areas of small, medium and large hills (measured mid season) averaged 263, 494, 662 cm^2 in 1998, and 325, 585, 961 cm^2, and 213, 626, and 1055 cm^2, respectively, in the first and second 1999 trial. In all three trials, consistent and significant differences ($P \leq 0.05$) in tuber blight incidence were observed among the cultivars with 'Red LaSoda' the most susceptible and 'Russet Burbank' the least susceptible to tuber blight. Hill size only had a significant effect ($P \leq 0.05$) on tuber blight incidence in 1998 when 40% of tubers in small hills and 31% tubers in medium and large sized hills were blighted. Comparison of tuber blight incidence examined by tuber depth in the hill, however, revealed few differences among hill size treatments, although, deep tubers had a lower incidence of tuber blight (5.5%) than tubers at shallow (34.1%) or intermediate (19.7%) depths. Because black polyethylene film and copper hydroxide-treated textile reduced the incidence of tuber blight, movement of *P. infestans* inoculum from potato foliage to tubers does not appear to be restricted to large channels in the soil such as those created by potato stems. The difficulty of direct tuber blight suppression after establishment of a foliar epidemic suggests that prevention of tuber blight in a conducive environment may be inseparably linked to suppression of the foliar phase.

Introduction

Late blight, caused by *Phytophthora infestans* (Mont.) de Bary, is a devastating disease of potato foliage and tubers, reducing crop quantity and quality. Additional

costs are incurred when money is spent on management practices to suppress the disease. During the late 1980s and 1990s, introduction of new clonal lineages of *P. infestans* to potato growing areas of the world led to severe late blight outbreaks (Fry and Goodwin, 1997; Inglis *et al.*, 1996). These new clonal lineages created management challenges because many were resistant to the fungicide, metalaxyl, which had become an integral tool for foliar late blight suppression. These lineages quickly displaced the older, previously established clones (Bashan *et al.*, 1989; Tooley *et al.*, 1986). In addition, new clonal lineages were more aggressive on potato tubers than the older *P. infestans* clones that growers had learned to manage (Lambert and Currier, 1997). The increased incidence of tuber blight has led to a new emphasis on management of tuber infection, which remains the least understood part of the late blight disease cycle.

Because soilborne oospores of *P. infestans* are rare in most potato production systems, infection of potato tubers in the field is most commonly initiated by inoculum, sporangia and zoospores, produced on the plant foliage. Thus, developing tubers can become blighted shortly after late blight is established on potato foliage (Hirst et al, 1965). Once the inoculum produced on the foliage is deposited onto the surface of the potato hill, water from irrigation or rain can carry it into the soil (Lacey 1966, 1967; Large, 1953; Zan 1962). Lacey (1967) attributed high incidences of tuber blight in tubers clustered around the potato stem to inoculum moving in water channeling down cracks in the soil created by the emergence of the potato stem. He did not, however, quantify the relative importance of this route of inoculum movement to the overall incidence of tuber blight. In contrast, Dubey and Stevenson (1996)

demonstrated that as little as 0.6 cm of water when applied to soil columns containing sandy loam soil could carry sporangia to a depth of 40 cm. Under natural conditions, tuber blight is most often initiated at eyes, lenticels, and wounds (Adams, 1975; Lacey, 1967; Walmsey-Woodward and Lewis, 1977). In this regard, use of large potato hills is commonly recommended as a cultural practice that can protect tubers from blight by filtering spores out of the soil water suspension and reducing direct contact of inoculum and tubers (Lacey, 1965; Rowe and Secor, 1993). Data supporting this recommendation, however, are limited, particularly with respect to the new lineages of *P. infestans*, the amount of soil required to protect tubers, and the relative value of this practice to other late blight suppression tactics. In addition, judging from the lack of published information, the potential for suppression of tuber blight through application of barriers other than soil has received little research effort.

The purpose of this study was to examine the relative importance of different barriers to the movement of *P. infestans* inoculum from foliage to tuber. This objective was accomplished through the strategic placement of mulches on the potato hill or the addition of soil to the hill profile followed by a determination of the effect of these treatments on the incidence of tuber blight. Data collected may indicate where inoculum is travelling within the potato hill and provide insight into potential cultural management of this phase of the late blight disease cycle.

Materials and Methods

Mulching Experiment

Field plot preparation and experimental design. Field plots were planted in sandy loam soils 9 June 1998 and 24 May 1999 at the Department of Botany and Plant Pathology Field Laboratory near Corvallis, OR. Each experimental plot contained two rows of 15 plants of cultivar 'Red LaSoda' spaced 46 cm within rows and 86 cm between rows. Thirty five (1998) or 40 plots (1999) were established, each separated by a meter of bare soil. Mulching treatments were assigned to plots in a randomized complete block design with seven (1998) or eight (1999) replicates. Potato plants were thinned to a single stem before treatment establishment.

In both years, 15-15-15 fertilizer (84 kg N, P_2O_5, K_2O /ha) was banded into rows at planting and followed 4 weeks after planting by a topdressing of 15-15-15 (16.8 kg N, P_2O_5, K_2O /ha) in 1998 or 45-0-0 urea (25.2 kg/ha) in 1999. Plots were irrigated by overhead sprinklers that applied 4 cm of water twice a week throughout the season. Hand hoeing supplemented with spot treatments of glyphosate (RoundUp Ultra, 1.5%, Monsanto, St. Louis, MO) on 12 July and 3 August 1998 and 1 July and 18 August 1999 suppressed weeds. On 22 June 1999, s-ethyl dipropylthiocarbamate (Eptam 7-E, 3.5 L/ha, Zeneca Ag Products, Wilmington, DE) was also sprayed between the hills to control the emergence of purple nutsedge, *Cyperus rotundus*, and other weeds. To prevent the potential early onset of foliar late blight, chlorothalonil (Bravo Weather Stik, Zeneca Ag Products, Wilmington, DE) was applied in 1998 at

rates of 0.7 L/ha on 17 July, 1.5 L/ha on 27 July, and 2.2 L/ha on 5 and 12 August, and in 1999, at the rate of 0.9 L/ha on 22 June and 2 July.

Texture of the soil in the study area was determined by the Soil Physical Characterization Laboratory, Department of Crop and Soil Science, Oregon State University. Air temperatures were recorded at weather stations located at the Field Laboratory. Near the end of the 1999 growing season, a CR21X data micrologger (Campbell Scientific, Logan UT) with eight temperature probes monitored soil temperatures in two plots of the each of four treatments. Temperature probes were buried in soil at approximately 10 cm below the crest of the hill, i.e. where the majority of the tubers grew. Relative soil moisture tensions (Pascals) were monitored between 1 September and 4 October 1999 using tensiometers (Irrometer Company Inc., Riverside CA) placed at both the crest and the base of the hills in two plots of all five mulching treatments.

Treatment establishment. Mulching treatments were designed to block the movement of *P. infestans* inoculum traveling from foliage to tubers in two regions of the potato hill: the area immediately surrounding the potato stem, and the remainder of the hill surface. The mulching treatments were: 1) no mulch, 2) expandable polyurethane spray foam in the 8 cm-diameter immediately surrounding the potato stem, 3) black polyethylene film over the entire hill surface except the 8 cm-diameter immediately surrounding the potato stem, 4) a combination of treatments 2 and 3, and 5) a copper hydroxide-treated ($6g/m^2$), non-woven textile, (Tex-R-Pro, Texel, Beauce-Nord, Quebec, Canada), applied over the same area as in treatment 3. The objective of

spraying expandable polyurethane spray foam into the area immediately surrounding the plant stem was to block the passage of inoculum-laden water down the channels in the soil created by the emergence of the potato stem and prevent inoculum infiltrating this area. Black polyethylene film along the surface of the potato hill prevented water and inoculum penetration on the hill surface, while the copper hydroxide-treated textile did not prevent water penetration on the hill. Treatments, with the exception of the spray foam mulch, were established 5 weeks after planting. For all treatments except the copper hydroxide-treated textile, the area immediately surrounding the potato stem was separated from the rest of the hill by vertically positioning a 15 cm segment of 8 cm-diameter PVC pipe around the stem of each potato plant. Black polyethylene film cut to 0.8 x 9.6m (152 μm thick) was placed over each row of the film plots with cuts made to accommodate the PVC pipes and potato stems. Copper hydroxide-treated textile was cut into 1 x 9.6 m strips and positioned similarly. Spray foam treatments were established in mid August of both years, prior to inoculation with *P. infestans*. Polyurethane spray foam (Great Stuff, Flexible Products Co., Joliet IL) was sprayed into the area within the PVC pipes and allowed to cure to form a tight seal between the potato stem and PVC pipe. These seals were inspected routinely, and if gaps appeared, spray foam was reapplied.

Inoculation and epidemic development. In 1998, inoculum of *P. infestans* (US-8) was obtained by collecting leaves with sporulating late blight lesions from a nearby potato field. Sporangia were washed into distilled water and sprayed onto the plots at dusk with a backpack sprayer equipped with a hand wand. Dates of inoculation were

27 August, and 10 and 17 September. In 1999, inoculum was obtained from laboratory-grown cultures of a *P. infestans* isolate (US-8) maintained on rye and pea agar at 18°C in the dark. Sporangia were suspended in distilled water at a concentration of 10^4 sporangia/ml and were sprayed onto spreader row plants in early evening on 27 and 31 August. In both years, plots were irrigated at dusk for 15 to 30 minutes daily through September to promote an environment conducive to the development of foliar late blight. As the foliar blight epidemics developed, visual assessments of the percentage of foliar disease were recorded every 4 days.

Treatment evaluation. In each season, tuber harvest began when 75 to 100% of the potato foliage had become blighted and continued over the next 6 to 10 days. Six potato plants were randomly selected from each plot for hand harvest. During harvest, haulms were removed and tubers were carefully hand dug and divided into three hill-placement classifications based on the location of the tuber within the hill: 1) shallow or exposed tubers that were not completely covered with soil and grew at the surface of the potato hill; 2) intermediate tubers that were completely buried by soil and grew within the first 15 cm of the hill surface; and 3) deep tubers that were covered with more than 15 cm of soil. Tubers from each hill and classification were stored separately in plastic bags in an unheated area until assessment.

All tubers were washed, weighed, and assessed for blight within 2 weeks of harvest. The presence or absence of late blight lesions and the percentage of tuber surface blighted were recorded. The incidence of tuber blight was calculated as the percent of total tubers visibly infected by late blight. As part of the assessment,

lesions were sliced to confirm the presence of brown discoloration in the tuber beneath the lesion, a symptom typical of late blight infection. In 1999, symptoms of late blight lesion in tubers were further confirmed by sporangial production by placing a sample of tubers with lesions into crispers containing moist paper towels. When possible to distinguish, the morphological feature of the tuber near the center of the lesion, typically an eye or stolon, was recorded.

Apparently healthy tubers also were evaluated for non-symptomatic infections by storing a sample in the dark at 2.2°C for a month. Estimates of the incidence of non-symptomatic tuber infection were made from 728 tubers in 1998 and 1832 tubers in 1999; these samples represented one quarter (1998) to one third (1999) of the non-symptomatic tubers harvested in each experiment.

Data Analysis. Analysis of variance (PROC MIXED) and multivariate analysis of variances (PROC GLM) (Statistical Analysis Software System, release 6.12 for Windows, SAS Institute, Inc., Cary, NC, 1996) were used to analyze the incidence of tuber blight, the sample yield, and the average number of tubers per hill. Tuber blight incidence was transformed to stabilize variation using the arcsine square root transformation. No transformation was needed for sample yield and number. In one model, the effects of block, black polyethylene film, polyurethane spray foam, and an interaction between black polyethylene film and spray foam on the measured responses were compared. In another model, effects of no mulch, copper hydroxide-treated textile, and black polyethylene film treatments were compared. P-values $\leq$ 0.05 were considered significant. If a significant F value was found ($P \leq 0.05$),

treatment means were then separated by Fisher's protected least significant difference (LSD). Daily mean soil temperatures recorded for the four mulch treatments were compared to the no mulch control using a paired t-test. Frequency distributions of lesions located at the eye and stolon of the tuber were calculated for the five mulching treatments. In a chi square test with one degree of freedom, the lesion location distributions of black polyethylene film, polyurethane spray foam, combination of film and spray foam, and copper hydroxide-treated textile were compared to the no mulch control.

Hill Size Experiment

Field plot preparation and experimental design. Hill size experiments were planted next to the mulching experiment in both years. On 9 June 1998, and 24 May 1999, seed of three cultivars 'Russet Burbank', 'Red LaSoda', and 'Shepody' were planted into plots containing three rows of 20 seed tubers each; plant spacing was 46 cm within row and 86 cm between rows. In 1999, a second trial was planted on 10 June with similar plant spacing but only 15 seed tubers per row. Cultivar plots were planted side by side in 1998, while in 1999 a spacer row of bare soil was added to separate plots of different cultivars. Each cultivar plot was replicated six times for a total of 18 whole plots established per trial; these plots were divided into three hill size treatments for a total of 54 subplots per trial.

Field maintenance was identical to that described for the mulching experiment except that the second 1999 hill size trial received a topdressing of 15-15-15 (16.8 kg N, P_2O_5, K_2O/ha) on 8 July.

Treatment Establishment. Hill size treatments were assigned randomly to each row of the cultivar whole plot. The smaller hill size was established by using the planter hill only. The medium and large hill sizes were established by running tractor-mounted disks over the plots once or twice, respectively. Dates of supplementary hilling were 10 July 1998, and 21 June and 9 July 1999. In 1999, additional soil was shoveled onto the large hill treatment shortly after the tractor operation.

Cross-sectional areas of hills were measured on six small, medium and large hill sizes in each experiment. The hill profile was drawn by placing a sheet of metal cross-sectionally through the hill in 1998, and by stretching a piece of wire taut over the surface of the hill in 1999. Hill profiles were transferred to paper, cut out, and weighed. Areas of the hills were calculated by dividing the weight of the paper hill profile by the weight of 1 cm^2 of the same paper. In 1998, cross-sectional areas of the small, medium, and large hills averaged 263 ± 23 (s.e.), 494 ± 19, and 662 ± 33 cm^2 of soil, respectively. The 1999 small, medium, and large hill sizes were measured to contain 325 ± 40, 585 ± 22, and 961 ± 34 cm^2 of soil, respectively, in the first trial, and 213 ± 31, 626 ± 37, and 1055 ± 56 cm^2 of soil, respectively, in the second trial.

Inoculation and treatment evaluation. Methods of inoculation, plot irrigation, measurement of foliar epidemic, potato harvest, and tuber blight assessment were as described in the mulching experiment. In 1999, the 'Red LaSoda' and 'Shepody' plots

from trial one and two were harvested first as the 'Russet Burbank' plots had a slower rate of foliar epidemic progress.

Data Analysis. Data analysis was conducted as described for the mulching experiment. In the models used to describe the tuber blight incidence, sample yield, and tuber number, the effects of block, cultivar, hill size, the interaction of block and cultivar, and the interaction of cultivar and hill size on the measured responses were compared.

Results

Foliar Epidemic and Environmental Conditions.

Foliar epidemic. Each season, inoculation of potato foliage with *P. infestans* led to the establishment of a foliar late blight epidemic in the experimental plots. Date of epidemic onset was approximately 25 September in 1998 and 5 September in 1999 (Figure 1.1). After establishment, epidemics progressed rapidly with over 80% of the foliage blighted within 3 weeks. Potato stems as well as leaves were blighted. Mulching treatments on plots of 'Red LaSoda' had no apparent effect on foliar disease progress; within the hill size trial, however, the cultivar treatment resulted in some variation in foliar disease progress as 'Russet Burbank' showed a slightly reduced rate of disease progress compared to 'Shepody' or 'Red LaSoda'.

Environment. During the period of foliar epidemic, maximum daily temperatures ranged from 12 to 27°C (1998) and 19 to 35°C (1999) (Figure 1.2) while minimum daily temperatures ranged from 1 to 11°C (1998) and 2 to 16°C (1999). Soil temperatures measured in 1999 were intermediate to daily minimum and maximum air temperatures, with daily means in the range of 20 to 24°C during August then declining to 13 to 16°C in September. Compared to the no mulch control, mulch treatments had a significant effect (paired t-test $P \leq 0.0005$ with 35 df) on measured soil temperatures, through differences were not large. Relative to no mulch treatments, black polyethylene film raised the temperature of the soil in the hill by 0.5°C, and black film combined with polyurethane spray foam raised the soil temperature by 1°C; the copper hydroxide-treated textile lowered the soil temperature by 0.4°C. Relative measurements of soil moisture tension, recorded in 1999, also revealed differences among the five mulching treatments. Soils under the black polyethylene film (1000 Pa) and the polyethylene film and spray foam (1200 Pa) were drier than soils in the no mulch treatment (850 Pa). Soils in the polyurethane spray foam (460 Pa) and the copper hydroxide-treated textile (540 Pa) treatments were moister than soils in the no mulch treatment. These soils were characterized in 1998 as 49.1% sand, 36.6% silt, and 14.3% clay, and in 1999 as 72% sand, 18.9% silt, and 9.1% clay.

Figure 1.1. Foliar disease progress curves for late blight epidemics in potato plots located near Corvallis, OR, during 1998 (**A** and **B**) and 1999 (**C, D,** and **E**). Panels **A** and **B** represent disease severity ratings (percent of plot infected) for cultivar 'Red LaSoda' that received one of the following mulch treatments: no mulch (+), polyurethane spray foam (□), black polyethylene film (O), polyurethane spray foam and black polyethylene film (△), and copper hydroxide-treated textile (∗). Panels **C**, **D**, and **E**, are data for cultivar whole plots of 'Red LaSoda' (●), 'Shepody' (▲), and 'Russet Burbank' (■), which were split to establish the hill size treatments.

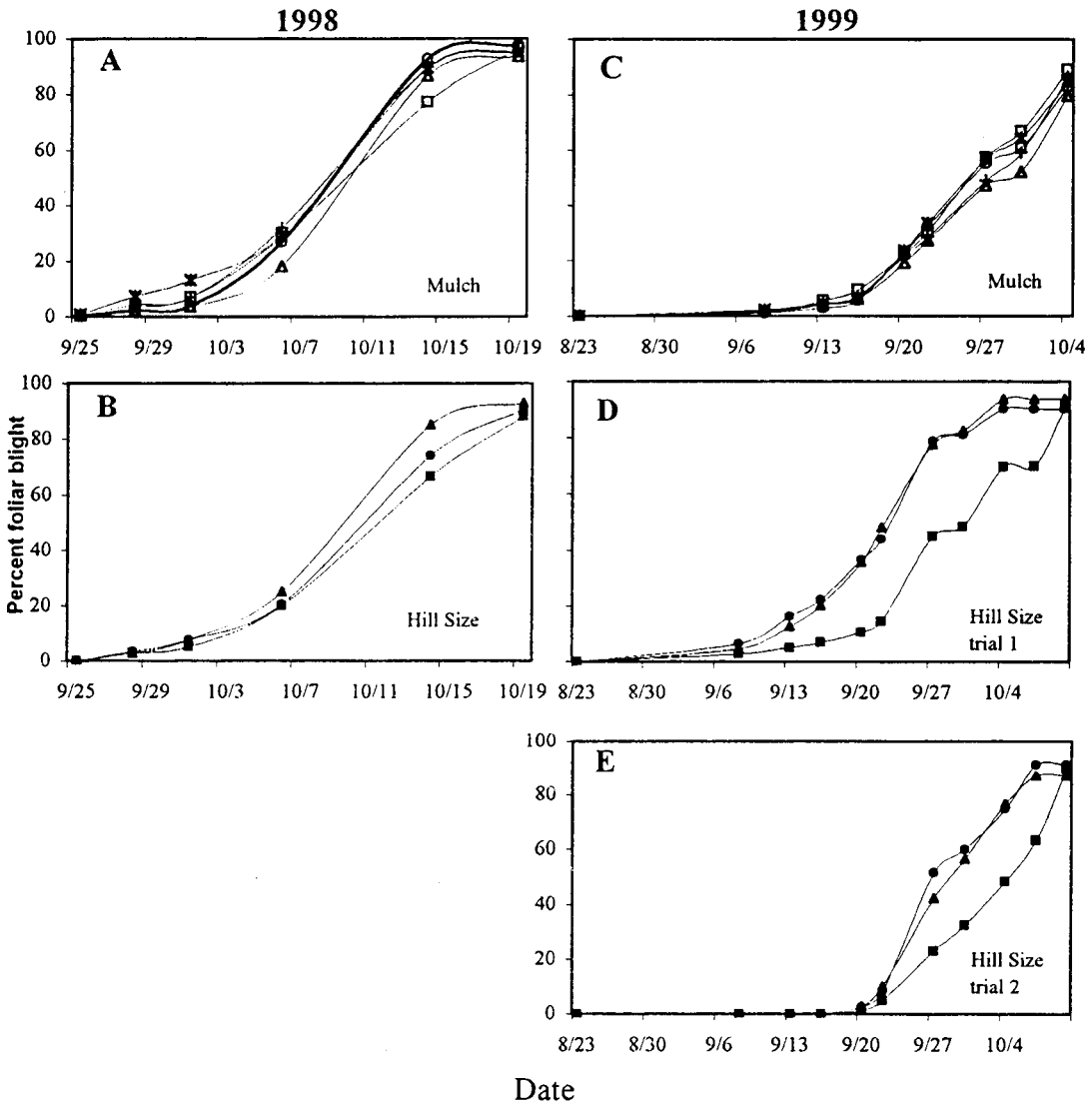
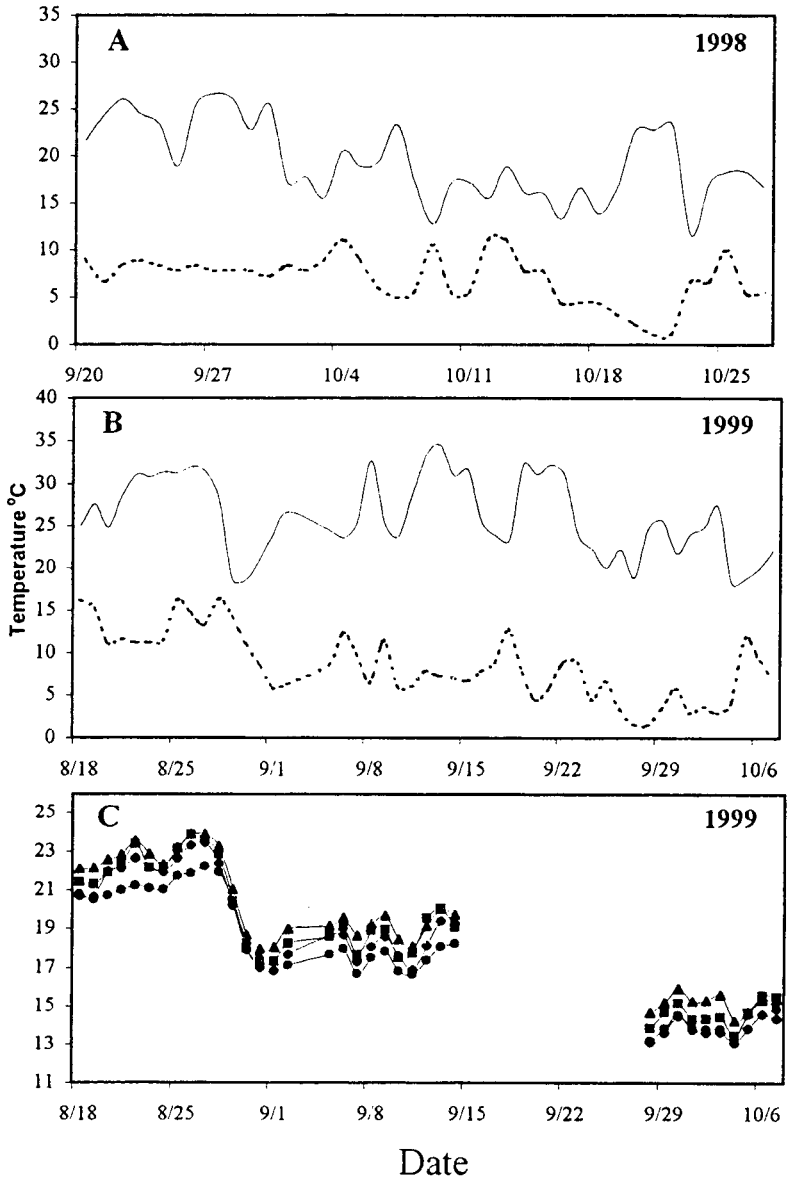


Figure 1.2. Daily minimum (dotted line) and maximum (solid line) air temperatures during foliar late blight epidemics in potato plots located near Corvallis, OR, in 1998 (**A**) and 1999 (**B**). Daily mean soil temperatures (**C**) at 10 cm below the crest of the hill in the 1999 mulching trial. Soil temperatures graphed are the average of measurements made for potato hills that received the following mulching treatments: no mulch (◆), black polyethylene film (■), polyurethane spray foam and black polyethylene film (▲), and copper hydroxide-treated textile (●).



Plastic Mulching Trial

Tuber number and yield. Number of tubers of cultivar 'Red LaSoda' was not affected in either year by any of the mulching treatments ($P \geq 0.1$); the majority of the tubers grew in the intermediate region in the hill. The number of tubers found at shallow, intermediate, and deep locations within the potato hill averaged 0.98 ± 0.2 (s.e.), 4.7 ± 0.5, 2.6 ± 0.4, respectively, in 1998, and 1.1 ± 0.2, 5 ± 0.5, 0.2 ± 0.1, respectively, in 1999. Polyurethane spray foam and black polyethylene film treatments did not significantly ($P \geq 0.11$) affect sample yield per hill in either year. Sample yield per hill averaged 2.7 ± 0.04 kg in 1998, and 2.4 ± 0.08 kg in 1999. Copper hydroxide-treated textile only significantly ($P = 0.006$) affected yield in 1998 when the average yield per hill in this treatment was 3.4 ± 0.2 kg compared to 2.6 ± 0.2 kg for the no mulch and black polyethylene film treatments.

Incidence of tuber blight. Tuber blight developed in all treatments but was more severe in 1998, when overall tuber blight incidence averaged 45%, than in 1999, when overall blight averaged 14%. In both trials (Figure 1.3), shallow to intermediate tubers showed higher incidences of tuber blight than did deeply buried tubers. Black polyethylene film was the only main effect that was significant ($P \leq 0.009$) in this experiment in both 1998 and 1999; no evidence ($P \geq 0.1$) was obtained to suggest that an interaction of polyurethane spray foam and black film, or that spray foam alone had an effect on the incidence of blighted tubers. The average incidence of blighted tubers in the plots covered with black polyethylene film was reduced by 24.3 ± 3.6 (s.e.)% in

1998, and by 10.8 ± 3.4% in 1999, respectively, as compared to treatments without this polyethylene film. When examined as a function of tuber depth, the black polyethylene film had stronger effects on shallow tubers (1998 $P = 0.0004$; 1999 $P = 0.0895$) and intermediate tubers (1998 $P = 0.0001$; 1999 $P = 0.03$) than on deeper tubers (1998 $P = 0.54$). For example in 1998 (Figure 1.3 A), black polyethylene film reduced the incidence of tuber blight relative to treatments without this mulch by 47.5 ± 7% for shallow tubers while the reduction for deep tubers averaged only 2.9 ± 7%.

ANOVA comparison of the copper hydroxide-treated textile, black polyethylene film and the no mulch treatments revealed a significant treatment effect ($P \leq 0.012$) on the incidence of blighted tubers in both years (Figure 1.4). In 1998, no mulch plots averaged tuber blight incidences of 52.9 ± 4.3%. Placing black polyethylene film to block water from penetrating the hill surface reduced tuber blight incidence to 27.8 ± 4.3% while treatment with the copper hydroxide-treated textile reduced the blight incidence to 20.4 ± 4.3%. In 1999, 17.6 ± 2.8% of tubers grown in the no mulch treatment were blighted as compared to a tuber blight incidence of 6.2 ± 2.8% in both the black polyethylene film and the copper hydroxide-treated textile treatments.

Figure 1.3. Incidence of late blight on potato tubers at three depths in the potato hill for the four mulch treatments: no mulch (□), polyurethane spray foam (▩), black polyethylene film (■), and spray foam and black polyethylene film (▨) during 1998 (**A**) and 1999 (**B**). Shallow tubers were defined as partially exposed near the crest of the potato hill, intermediate tubers as completely covered with soil but less than 15 cm deep, and deep tubers as greater than 15 cm below crest of hill. The bars represent the average of six potato hills harvested for each of seven (1998) or eight (1999) treatment replicates. Lines positioned on the top of the bars represent one standard error of the mean. Black polyethylene film had a significant effect ($P \leq 0.009$) on overall incidence of tuber blight in both seasons.

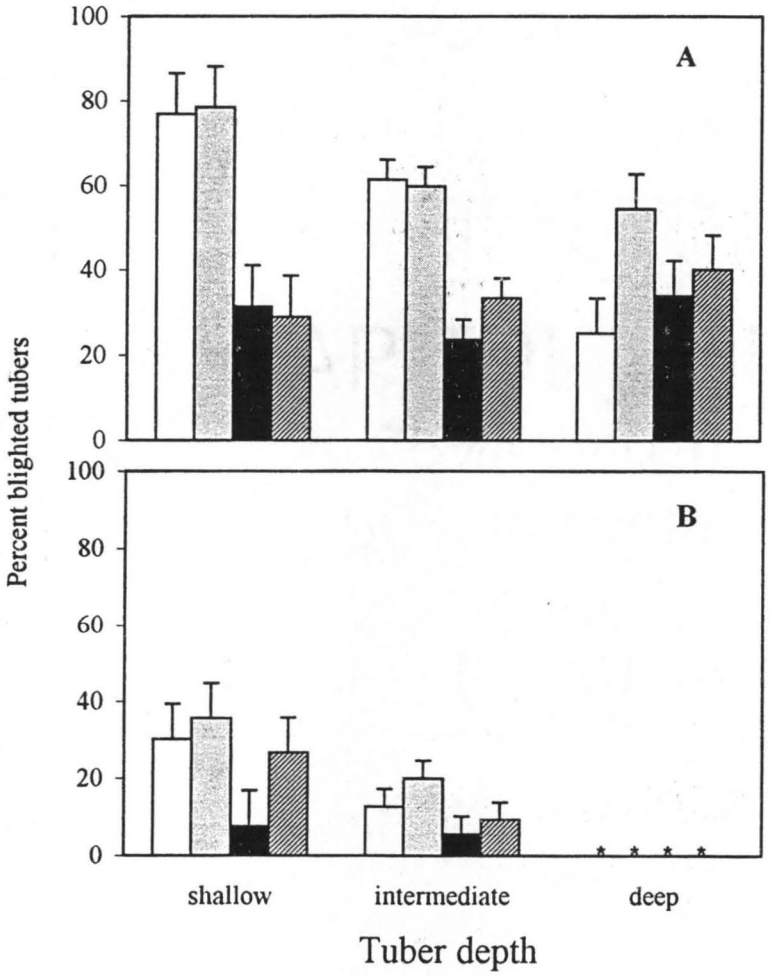
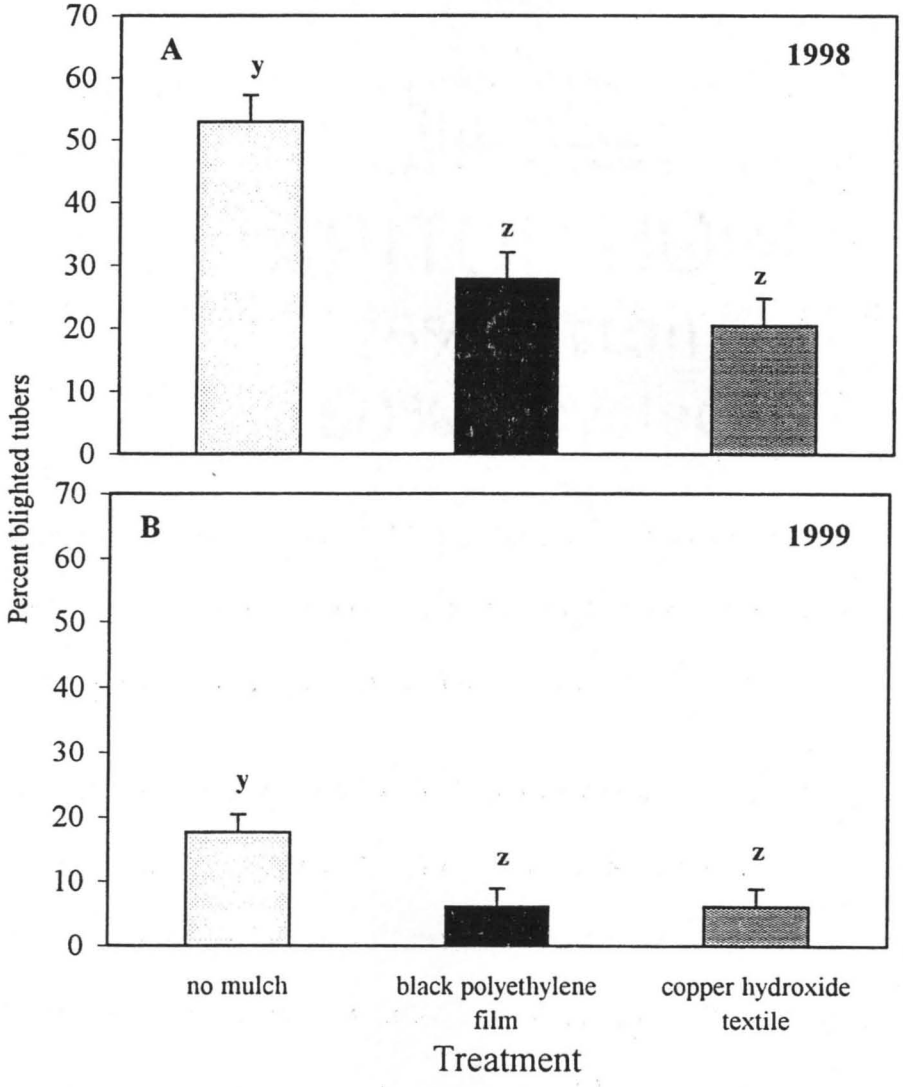


Figure 1.4. Incidence of tuber blight of potato cultivar 'Red LaSoda' to which no mulch (▨), a black polyethylene film (■), or a copper hydroxide-treated textile (▩) was applied. Experiments were conducted in 1998 (**A**) and 1999 (**B**). The bars represent the average of six potato hills harvested for each of seven (1998) or eight (1999) treatment replicates. Lines positioned on the top of the bars represent one standard error of the mean. Within each panel, lower-case letters indicate significant differences ($P \leq 0.05$) as based on Fisher's protected least significant difference test.



Lesion location on tubers. When the morphological feature of the tuber at the center of the lesion could be identified, the majority of lesions were associated with eyes on the tuber or with the point of stolon attachment. Averaged over both years, frequency distributions for lesion location on tubers grown under the black polyethylene film and the copper hydroxide-treated textile differed significantly (λ^2 test with 1 df $P < 0.001$) from the distribution on tubers in the no mulch treatment. The frequency of lesions on tubers in the no mulch control was 72.3% at eyes and 27.7% at the stolon in 1998 and 76.6% at eyes and 23.4% at the stolon in 1999. Relative to the no mulch control, black polyethylene film shifted 10.5% and 29.2% of the lesion distribution, in 1998 and 1999, respectively, from an eye on the tuber to the point of stolon attachment. No clear pattern emerged for tubers in the copper hydroxide-treated textile treatment. In 1998, the number of lesions associated with eyes was 6.9% greater for tubers grown under the copper hydroxide-treated textile than for tubers in the no mulch control; in 1999, however, 48.8% of the lesion distribution relative to the no mulch control was shifted from the eye to the point of stolon attachment.

Non-symptomatic lesions at harvest. Non-symptomatic tuber infection was present in both years; 16% (1998) and 7% (1999) of the tubers sampled from the mulching experiment showed symptoms of late blight infection after a month of cold storage. In 1998, 16% of the no mulch, 14% of the spray foam, 23% of the polyethylene film, 13% of the combined spray foam and polyethylene film, and 15% of the copper hydroxide-treated textile treatments, respectively, were blighted. In 1999, blighted tubers averaged 17% of the no mulch, 10% of the spray foam, 1% of the polyethylene

film, 6% of the combined spray foam and film, and 2% of the copper hydroxide-treated textile samples.

Hill Size Experiment

Tuber number and yield. Number of tubers per hill was affected by cultivar grown in the 1998 trial ($P = 0.02$) but not in the 1999 trials ($P \geq 0.05$). In both years, hill size did not affect ($P \geq 0.3$) the number of tubers per hill but did have a significant effect ($P \leq 0.0002$) on how many tubers were classified as shallow or deep (Figure 1.5). In 1998, and the first and second trials of 1999, number of tubers per hill averaged 10.2 ± 0.1 (s.e.), 8 ± 0.1, and 8.3 ± 0.2 tubers, respectively. The cultivar grown had a significant effect ($P = 0.03$) on sample yield per hill in 1998 and the first trial of 1999, but not ($P = 0.91$) in the second trial of 1999. Hill size ($P \geq 0.07$) did not significantly affect yield in any trial. Yield per hill averaged 2.9 ± 0.2, 2.3 ± 0.3, and 2.4 ± 0.2 kg in 1998, and the first and second 1999 trials, respectively.

Incidence of tuber blight. As in the mulching experiment, tuber blight developed in all plots in both years, and shallow and intermediate tubers showed higher incidences of blight, on average 34.1 ± 6.6 (s.e.)% and 19.7 ± 3.4%, respectively, than did the deep tubers, 5.5 ± 2.2%. The main effect of cultivar on the incidence of tuber blight was significant ($P \leq 0.05$) in all three trials while hill size was significant only in the 1998 experiment ($P = 0.0006$). No evidence ($P \geq 0.1$) was obtained to indicate that an interaction between cultivar and size of the hill affected the incidence of blighted

tubers. In each trial, tubers of 'Red LaSoda' were the most diseased, followed by 'Shepody' and then 'Russet Burbank' (Figure 1.6). In the 1998 trial, tuber blight averaged 40.3 ± 2.6% in the small hills, 30.1 ± 2.6% in the medium hills, and 31.0 ± 2.6% in the large hills. Analysis of tuber blight incidence examined as a function of tuber depth (Figure 1.5 B, D, F), however, showed that size of the hill had very little effect on the incidence of blighted tubers particularly for tubers at intermediate depths, which represented the majority of tubers.

Lesion location on tubers. When the morphological feature of the tuber at the center of the lesion could be identified, the majority of lesions were associated with eyes on the tuber and with the point of stolon attachment. Averaged over the three trials, for lesions on 'Russet Burbank' tubers, 63% were associated with eyes and 19% with the stolon, for lesions on 'Shepody' tubers, 61% were at eyes and 24% at the stolon, and for lesions on 'Red LaSoda' tubers, 66% were located at eyes and 12% at the stolon.

Non-symptomatic lesions at harvest. Non-symptomatic tuber infection was present in 7% of 1998 hill size experiment sample, and 5% and 12% of the sample for the first and second 1999 hill size experiment trials, respectively. In 1998, 12% of the 'Russet Burbank', 5% of the 'Shepody', and 5% of the 'Red LaSoda' tubers, respectively, were blighted after a month of cold storage. In the first trial of 1999, blighted tubers averaged 2% of the 'Russet Burbank', 1% of the 'Shepody', and 12% of the 'Red LaSoda' samples, respectively, while in the second trial, 1% of the 'Russet Burbank', 11% of the 'Shepody', and 23% of the 'Red LaSoda' tubers were blighted.

Figure 1.5. Average number of tubers per hill (**A**, **C**, and **E**) and the incidence of tuber blight (**B**, **D**, and **F**) at three tuber depths in the hill in small, medium and large hill sizes in an experiment conducted in a single trial in 1998 (**A** and **B**) and two trials in 1999 (**C-F**). Tuber number and blight incidence are averaged over the three potato cultivars, 'Red LaSoda', 'Russet Burbank', and 'Shepody' ' for tubers grown in small hills (□), medium hills (▨), and large hills (■). Shallow tubers were defined as partially exposed near the crest of the potato hill, intermediate tubers as covered with soil but less than 15 cm deep, and deep tubers as greater than 15 cm below crest of hill. The bars represent six potato hills harvested for each of six treatment replicates. Lines positioned on the top of the bars represent one standard error of the mean.

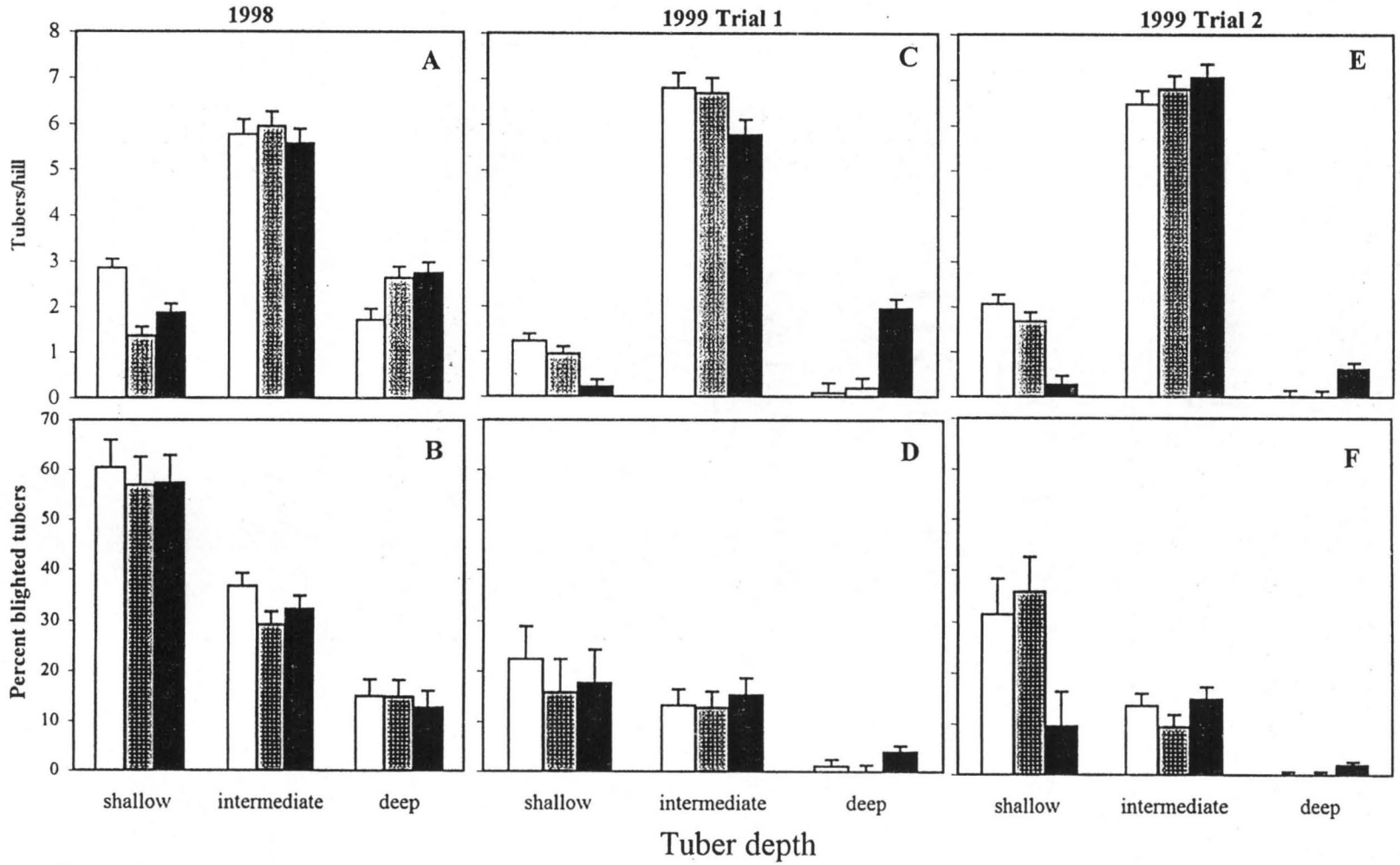
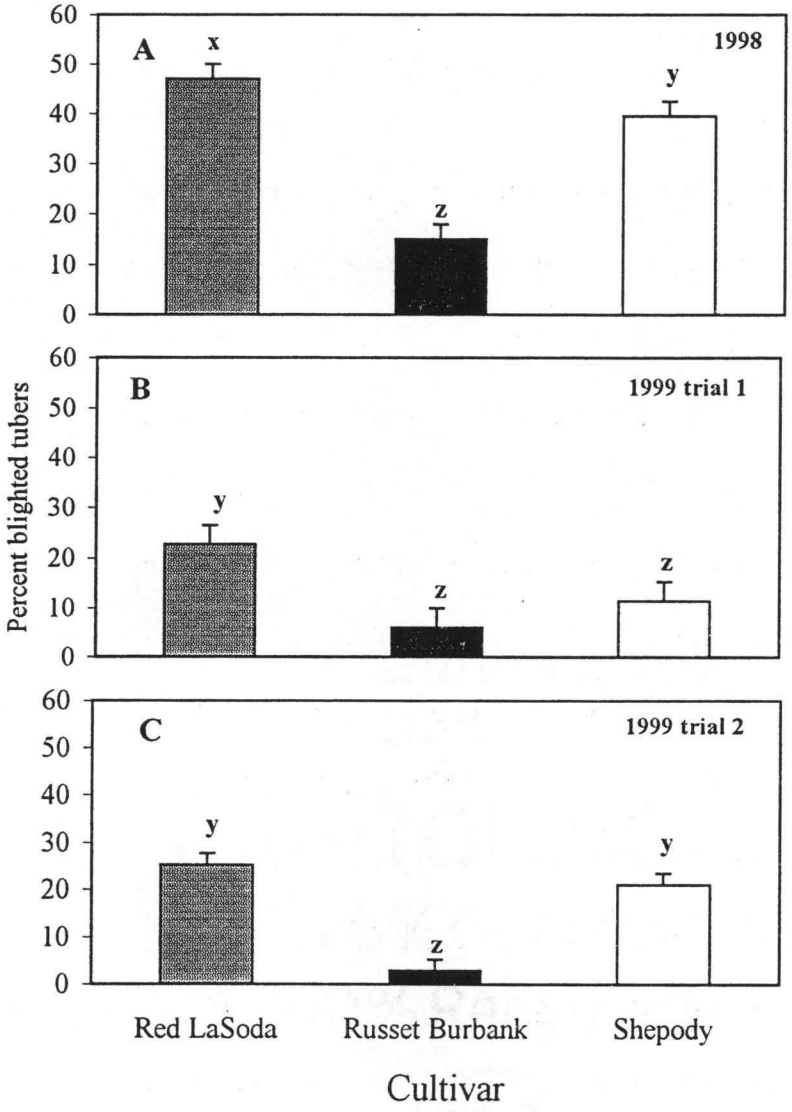

1998
1999 Trial 1
1999 Trial 2
A
B
C
D
E
F
Tubers/hill
Percent blighted tubers
shallow
intermediate
deep
Tuber depth

Figure 1.5

Figure 1.6. Incidence of tuber blight caused by *Phytophthora infestans* for the three potato cultivars, 'Red LaSoda' (▒), 'Russet Burbank' (▮), and 'Shepody' (□), grown under three hill sizes (small, medium and large) in an experiment conducted in a single trial in 1998 (**A**) and two trials in 1999 (**B** and **C**). The hill size treatments were established by hilling with the planter only (small hill) and by supplementary hilling once or twice with tractor-mounted disks after potato emergence (medium and large hills, respectively). The bars represent the six potato hills harvested for each of six treatment replicates. Lines positioned on the top of the bars represent one standard error of the mean. Within each panel, lower-case letters indicate significant differences ($P \leq 0.05$) as based on Fisher's protected least significant difference test.



Characterization of Blighted Tubers

Sporangia production confirmation. Ninety seven percent of tubers incubated in 1999 to confirm diagnosis of late blight symptoms produced sporangia within 5 to 7 days after being placed in a moist chamber. In addition to late blight, all tuber samples showed signs of additional rots including bacterial soft rot and rots caused by *Fusarium* species.

Discussion

Experimental conditions in these experiments were highly conducive to the development of tuber blight by *P. infestans*, which allowed us to make relative comparisons of various barriers to movement of inoculum of *P. infestans* from potato foliage to developing tubers. Two barriers that covered the potato hills, the black polyethylene film and copper hydroxide-treated textile, reduced the incidence of tuber blight relative to appropriate controls while the other barriers investigated, polyurethane spray foam applied in the area of the hill immediate to the potato stem and additional soil in the hill profile, had little effect on tuber blight incidence. In spite of some success in preventing infection with the two hill coverings, however, tuber blight was never reduced to a level that would meet industrial standards. Consequently, in addition to providing insight on how inoculum of *P. infestans* moves from foliage to tubers, we believe the data also highlight the difficulty of direct suppression of tuber blight in a field once the foliar stage of the disease has been allowed to develop.

Black polyethylene film was one of the two effective barriers for the suppression of tuber blight. The effectiveness of this barrier as a deterrent to tuber blight may be attributable to two reasons: the blocking of water, and thus inoculum, from infiltrating the potato hill, and alteration of the soil environment within the hill. Hills covered with black plastic were drier and slightly warmer than uncovered hills thus potentially affecting zoospore movement and infection capacity. The copper hydroxide-treated textile, unlike the polyethylene film, allowed water to penetrate the potato hill, resulting in a soil water content that was similar to the no mulch control. In addition, the textile covering the hill slightly lowered soil temperatures relative to the no mulch control. Thus, given little effect of the textile on soil temperature and moisture within the hill, we attribute the effectiveness of this mulch to the coating of copper hydroxide ($6g/m^2$) applied to the textile. Copper hydroxide is a chemical that has long been known to be an effective fungicide for late blight control and has been shown to kill both sporangia and zoospores when applied to soil columns in a laboratory experiment (Dubey and Stevenson, 1997, 1998, 1999).

With regard to mechanism or paths of inoculum movement, the success of the black polyethylene film and copper hydroxide-treated textile treatments for reduction of tuber blight suggests that a significant proportion of the inoculum moves through the soil at the hill surface, and is not restricted solely to movement in large channels in the soil such as those created by emergence of the plant stem as observed by Lacey (1965, 1966). While channels in the soil undoubtedly allow inoculum to penetrate into the soil (Lacey 1965; Zan 1962), they do not appear to be an essential factor leading to tuber blight infection. In fact, our attempt to alter the channeling of water down plant

stems, through blockage of the potato stem area with polyurethane spray foam, had no effect on reducing tuber blight incidence. One reason for why we did not observe an effect of the spray foam is stated above, i.e. a significant proportion of the inoculum moves through the soil at the hill surface rather than being restricted to cracks in the soil including ones made by the emerging stem.

For several reasons, however, the spray foam mulch applied in the stem area was not an ideal treatment in this study. First, the polyurethane spray foam treatments were not completely successful at sealing the stem channel as an avenue to inoculum travel. To examine how tightly the spray foam sealed the area, during harvest we examined the moisture patterns under the combined treatment of black polyethylene film and polyurethane spray foam and noted that the soil near the potato stem was typically more damp than soil further from the stem. Thus, the spray foam plug was only partially effective in preventing the infiltration of inoculum-laden water from the area immediate to the potato stem. Second, the PVC pipe, which we used to separate the stem area from the rest of the hill, may have created artificial, albeit shallow, channels in which the water and inoculum could travel to infect tubers. Third, 'Red LaSoda', the potato cultivar grown in the mulching experiment, grew top heavy with sprawling vines by the time of the epidemic. Lacey (1967) when investigating water deposition on the hill and how it related to inoculum reaching tubers often associated water channeling down stems with potato cultivars that had upright growth. Perhaps, 'Red LaSoda' was not the most appropriate cultivar to use to investigate the importance of inoculum movement down the stem.

Interestingly, the frequency distribution of likely sites of infection on tubers suggests that both avenues, movement through the soil at the surface of the hill and movement down the stem channels may be involved. For example, when black polyethylene film was used alone, the number of lesions located at eyes decreased while the number associated with the stolon increased, relative to the no mulch distribution, suggesting that this treatment shifted inoculum concentrations to higher levels in the region immediate to the potato stem. This finding agrees with Lacey (1966) who noted in a severe late blight infection in 1960 that lesions on tubers close to the surface of the potato hill were most often associated with eyes while many of the lesions on deeper blighted tubers appeared to be initiated near the stolon.

The higher incidence of blight in tubers close to the surface of the potato hill than in deeply buried tubers has been noted in other studies (Lacey 1965, 1967; Dubey and Stevenson, 1996) and has been usually attributed to ability of inoculum to reach the tubers. Perhaps most surprising in this experiment, however, were the relatively high incidences of tuber blight on tubers that were deep in the hill profile during 1998, and that none of the barriers affected the incidence of blight in these tubers. These findings suggest that the inoculum is capable of penetrating some distance in the soil although the source and pathway of this inoculum movement is unclear. Other possible explanations for the high incidence of blight in the deep tubers are secondary tuber to tuber infections initiated either in the field (Lacey, 1967) or during the storage period before assessment (Dowley and O'Sullivan, 1991), or tuber blight infection initiated during the harvest procedure. We discount both these mechanisms, however,

because many lesions on deep tubers were of a similar severity to lesions on shallower tubers.

The soil temperatures measured during 1999 suggest that soil conditions were conducive for the indirect germination of sporangia to zoospores. Consequently, zoospores may have been the principal type of inoculum initiating tuber blight in these experiments, though we never directly identified the form of inoculum. On a relative scale, tuber blight initiated by zoospores is thought to be more important than tuber blight initiated by sporangia since zoospores are motile and are also able to follow chemical gradients of potato root exudates, which increases their likelihood of contacting tubers (Lacey, 1965, Sato 1979, Zan 1962).

When assessing resistance to tuber blight in potato cultivars, Dorrance and Inglis (1998) noted that pre- and post-storage evaluations of tuber blight were necessary to achieve reliable results. In the mulching and hills experiments after a month of cold storage, tuber blight was present in tubers that were non-symptomatic at harvest. Incidence of tuber blight after storage was quite variable and did not provide any additional information on assessing the treatments as barriers to tuber blight infection.

From a practical perspective, hilling is the most common recommendation given for direct suppression of tuber infection by *P. infestans*. While we did find that deeper tubers were less likely to become infected than shallow to intermediate tubers, it was somewhat unexpected that the practice of hilling had almost no value in terms of tuber blight suppression, at least for the hill sizes and the loam soils used in this study. This result was consistent over the three cultivars studied, 'Red LaSoda',

'Shepody', and 'Russet Burbank'. For the majority of the tubers, i.e. those growing in intermediate regions of the hill, no difference in the incidence of tuber blight could be attributed to the amount of soil covering the tubers. This result is in direct contrast to Lacey's (1966) conclusions from a distribution study of blighted and healthy tubers within potato hills where he found more infections on shallow tubers and stressed the importance of "good ridging" to reduce the potential for blighted tubers. In the hill size experiment, the intermediate and large hill sizes were typical of hill sizes used in many irrigated commercial fields in the United States. Unfortunately, hills of this size, while useful in terms of prevention of tuber sunscald and weed suppression, are apparently not large enough to prevent *P. infestans* inoculum from reaching tubers and initiating blight.

From a more positive perspective, the hill size experiment did demonstrate the value of host resistance. The three cultivars chosen are susceptible to *P. infestans* but 'Russet Burbank' is ranked only moderately susceptible, as shown in this experiment by a delayed foliar epidemic development and a lower incidence of tuber blight. The relative frequencies of tuber blight found for 'Shepody' and 'Russet Burbank' in the hill size experiment agree with the field ranking found in a previous study of several potato cultivars and the US-11 genotype of the pathogen (Inglis *et al.*, 1996). While 'Red LaSoda' had a low incidence of tuber blight the one year it was included in that cultivar trial, the high tuber blight incidence in 'Red LaSoda' from our study reflect its known susceptibility to late blight and were consistent over both years. Heightened concern about late blight requires more emphasis to be placed on using resistance as a tool in late blight management strategies.

Clearly, tuber blight is difficult to control in an environment conducive to disease establishment. These experiments demonstrate how effectively *P. infestans* inoculum moves from foliage to tuber and highlight the difficulties associated with achieving direct tuber blight control through the prevention of inoculum reaching the tubers. The inability to reduce tuber blight to a suitable level of control by any of the treatments suggests that control of tuber blight may be better achieved by prevention of foliar epidemics or through host resistance, than by any manipulations to the potato hill involving mulches and soil, or even chemical controls applied to the soil.

CHAPTER 2

TRANSFORMATION OF A US-8 ISOLATE OF *PHYTOPHTHORA INFESTANS* TO EXPRESS β-GLUCURONIDASE (GUS) AND RESISTANCE TO THE ANTIBIOTIC G418 FOR USE IN FUTURE EPIDEMIOLOGY STUDIES

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Abstract

A β-glucuronidase (GUS)-expressing transformant *Phytophthora infestans* was achieved using lipofectin-mediated protoplast transformation of an US-8 isolate of the pathogen. The transformant also expresses resistance to the antibiotic G418. The transformant is mitotically stable, able to successfully invade potato leaf and tuber tissue, and grows more rapidly *in vitro* than the parental isolate. GUS expression is revealed through the formation of an indigo-colored compound when the mycelium is stained with 5-bromo-4-chloro-3-indoyl β-D glucuronide (X-GlcA). The use of this transformant should enhance the ability to detect and measure potato tuber blight in future epidemiological studies.

Introduction

During the late 1980s and 1990s, migration of clones of *Phytophthora infestans* out of Mexico led to severe outbreaks of the late blight disease on potato and tomato (Fry and Goodwin, 1997; Inglis *et al.*, 1996). Study of the tuber cycle of the disease has gained increased interest in the recent years since these *P. infestans* clones, particularly the US-8 clone, are more aggressive on potato tubers than clones that growers had previously learned to manage (Lambert and Currier, 1997). Management of tuber blight remains one of the least understood areas in the late blight disease cycle, in part because tuber blight is difficult to assess and quantify. Blighted tubers can be difficult to assess for several reasons: 1) lesions may be small and nearly undetectable at the time of assessment, 2) secondary rots are often present, and 3)

lesions can be mistaken for other diseases. A method to enhance the visualization of *P. infestans,* such as tagging the pathogen with a reporter gene, could potentially aid in the evaluation of tuber blight. Reporter genes have easily detectable traits or products, such as antibiotic resistance or pigment production, that can be observed and used to infer location or behavior of tagged genes or organisms (Gallagher, 1992). Tagging *P. infestans* with a reporter gene could eliminate some of the difficulties associated with tuber assessment and facilitate the study of the tuber blight stage of the disease.

The β-glucuronidase (GUS) expression system from *Echerichia coli* developed by Jefferson *et al.* (1987) is a good candidate for such work. The *GUS* gene product when mixed with certain glucuronide substrates produces indigo-colored or fluorescent compounds that are capable of being visualized or measured. The *GUS* reporter gene has been adapted for use with viruses, bacteria, fungi, and oomycetes (Ashby and Johnstone, 1993; Judelson and Michelmore, 1991; Liljeroth *et al.*, 1996; Lojkowska *et al.*, 1993; Oliver et *al.*, 1993). Fungal pathogens transformed with GUS to enhance detection include: *Pseudocercosporella herpotrichoides* (de la Pena and Murray, 1994), *Bipolaris sorokiniana* (Liljeroth *et al.*, 1996), *Pyrenopezia brassicae*, (Ashby and Johnstone, 1993), and *Fusarium subglutinans* (Freeman *et al.*, 1999). The practical objectives of studies employing GUS-expressing transformants include assessing plant resistance to pathogens, investigating pathogen development, and determining disease etiology. Judelson *et al.* (1991) transformed *P. infestans* to express GUS and suggested the utility of using transformants in epidemiology and pathogenicity studies of *P. infestans.*

GUS is a useful tool to enhance visualization of the pathogen in studies of plant disease epidemiology because endogenous GUS activity is not found in most pathogens, environments, or plants (Jefferson *et al.*, 1987; Lojkowska *et al.*, 1993). The use of GUS expression to enhance visualization has one major limitation, however, since destructive sampling of the pathogen and host must be done before staining. When stained with substrates, such as 5-bromo-4-chloro-3-indoyl β-D glucuronide (X-GlcA), which produce indigo-colored compounds, the presence and location of the transformant within infected tissue can be visualized due to the blue pigment. For example, the presence of blue GUS-stained mycelia, found within lesions that formed after inoculation of healthy mango shoots with a GUS-expressing transformant of *Fusarium subglutinans*, was used as evidence to establish that *F. subglutinans* was the causal agent of the mango malformation disease (Freeman *et al.*, 1999). When stained with substrates, such as 4-methylumbelliferyl glucuronide (MUG), which produce fluorescent compounds, the growth and biomass of the transformant can be quantified through measurement of the fluorimetric activity (Gallagher, 1992). For example, fluorimetric measurements of the activity of *GUS*-transformed *Pseudocercosporella herpotrichoides* isolates were used to compare resistance levels of wheat and enabled differentiation among highly resistant, resistant, and susceptible wheat genotypes while an enzyme-linked immunosorbent assay (ELISA) failed to differentiate among the genotypes. In this system when compared to visual assessment, GUS detection was less subjective and less labor intensive, and was not limited to an arbitrary rating scale as imposed by visual assessment.

Many aspects of tuber blight, including resistance in relationship to tuber maturity and placement in the hill, could be studied through the use of GUS-expressing *P. infestans* transformants. Histochemical staining to form indigo-colored compounds should overcome difficulties involved with tuber lesion assessment, and the extent of lesion development may be more accurately quantified through measurement of activity of fluorescent compounds than by visual observation. Achieving a *GUS*-transformed isolate, however, can be difficult, particularly if transformation efficiency, as in the case of *P. infestans*, is low. The objective of this project was to create a stable GUS-expressing *P. infestans* isolate that could be easily identified within potato tubers for use in future investigations of the tuber blight stage of the late blight disease cycle.

Materials and Methods

Growth of *P. infestans* and isolation of protoplasts. The *P. infestans* isolate 97-368-1 (US-8) from blighted potato fields in the Columbia Basin (cultured by Joy Jaeger, Hermiston, OR) was maintained on rye and pea agar amended to 50 ppm ampicillin (RAP) in the dark at 18°C. To maintain pathogenicity and increase sporulation, the isolate was inoculated onto a potato field trial in 1999 and reisolated from the new lesions. Adding ground potato leaf to RAP agar also helped maintain the vitality of the culture and increased sporulation. Judelson and Michelmore (1991) developed the protoplasting method used for this experiment. Clean sporangial solutions of the isolate were streaked onto RAP media and grown for 10 to 15 days at

18°C in the dark. Sporangia were harvested by pouring 5 to 10 ml of water onto each plate and gently rubbing a sterile glass rod over the culture to liberate sporangia. The liquid was decanted from the plate and strained through a 50 μm mesh to remove mycelial and agar fragments. In 1000 ml flasks, the sporangial culture was adjusted to 2.5×10^5 sporangia/ml using half strength lima bean broth (Bruck *et al.*, 1980) and amended to 50 μg/ml ampicillin and 4 ml/L nystatin (Sigma, St. Louis, MO). The flasks were incubated stationary at 18°C in the dark for 48 hours. The young *P. infestans* mycelium were harvested by capture onto a 50 μm mesh, transferred to a centrifuge tube, and resuspended in osmoticum KC (0.64 M KCl, 0.2 M $CaCl_2$) containing 5 mg/ml Novozyme 234 (Novo Bio Labs, Lot 2145) and 2.5 mg/ml cellulase (Sigma, St. Louis, MO). The mixture was shaken gently at room temperature; after 30 to 45 minutes, most of the mycelia had converted to protoplasts. The mixture was spun in a swinging bucket rotor (Sorvall HB-4, Dupont Medical Products, Newtown CT) for 4 minutes at 700g. Supernatant was removed and the protoplasts were resuspended in a 30 ml solution containing equal volumes of KC and MT (1 M mannitol, 10 mM Tris pH 7.5). The mixture was respun, supernatant discarded, the protoplasts were resuspended in 30 ml MT, and spun once more before discarding most of the MT supernatant. Protoplasts were counted and resuspended so that there were 10^7 protoplasts/ml MT.

Transformation. Transformation method was followed as previously described in Judelson and Michelmore (1991). Plasmid DNA (pTH209/35G; kindly provided by H. Judelson, University of California, Riverside, CA) contained *GUS* (β-

glucuronidase) fused to the *ham34* promoter of *Bremia lactucae,* and *npt* (neomycin phosphotransferase), which confers resistance to the antibiotic G418, fused to the 5'*hsp*70 promoter of *B. lactucae*. Both *GUS* and *npt* genes were fused to the 3'*ham*34 terminator of *B. lactucae*. Six tubes were made up that contained 0, 10, 15, 20, 25, 30 µg of DNA, respectively, mixed with water so that the total volume of each tube was 40 µl. To each tube, 60 µl of Lipofectin (BRL, Gaithersburg, MD) was added. After the DNA-lipofectin mixtures sat at room temperature for 15 minutes, 10^7 protoplasts were added to each tube and left to rest for 5 minutes. After this time, 1 ml of 50% polyethylene glycol (3350 PEG; Sigma, St. Louis, MO) containing 10 mM $CaCl_2$ and 10 mM Tris pH 7.5 was slowly added to each tube. This mixture was allowed to rest at room temperature for an additional 5 minutes before adding rye broth amended to 1 M mannitol, 50 µg/ml ampicillin, and 4 ml/L nystatin. The protoplasts were incubated in 20 ml of rye mannitol broth for 16 hours at 18°C. The young germlings were recovered by centrifugation and spread onto selection rye plates containing 6 µg/ml G418 (Geneticin, Sigma, St. Louis, MO) and 50 µg/ml ampicillin. Mycelia growing on the selection rye were transferred to fresh selection rye and assayed for GUS activity.

GUS activity. GUS activity was observed through examination of histochemically stained mycelium according the procedure recommended by Judelson (1993). The staining solution was made of 50 mM $NaPO_4$, pH 7.0, containing 1% 5-bromo-4-chloro-3-indoyl β-D glucuronide dissolved in dimethyl formamide (X-GlcA Sigma, St. Louis, MO), 1% Triton X-100, and amended with 5 mM potassium ferricyanide

($K_3Fe(CN)_6$) and 5 mM potassium ferrocyanide ($K_4Fe(CN)_6$) to increase sensitivity. Mycelial fragments and leaf pieces, including controls with non-transformed *P. infestans*, were placed into the staining solution and incubated at 37°C for 1 hour.

Growth and stability studies. Rates of *in vitro* growth were measured by comparing the growth of the *GUS* transformant to the parental 97-368-1 isolate. Circular agar plugs (4 mm diameter) were cut from the actively growing edges of 10-day-old cultures grown on RAP agar. Plugs were placed near the edge of plates containing non-selection RAP agar. Ten (RAP) and 5 (RAP with ground potato leaf) plates per isolate were incubated at 18°C in the dark. This growth curve experiment was repeated once. Every two days, the radial growth of the cultures was measured. After measuring growth, an agar plug was excised from an actively growing edge of the transformant and placed onto selection RAP media amended with 6 μg/ml G418 to assay for mitotic stability of antibiotic selection in the transformant. Mycelia that grew on the selection plate were then stained with X-GlcA to confirm the stability of the GUS reporter gene. Growth measurements were averaged and standard errors of the two isolates were calculated. Average growth of the two isolates was compared using a two-sample t-test.

Pathogenicity on potato. An aqueous sporangial solution (2.5×10^5 sporangia/ml) of the GUS-expressing transformant was used to inoculate potato leaf pieces, foliage on whole plants, tuber slices, and whole tubers. Potato leaf pieces were disinfested in 10% bleach solution for 5 minutes while tubers were disinfested with 20% bleach solution for 30 to 45 minutes prior to slicing or whole tuber inoculation. Leaf pieces

and tuber slices were placed into petri dishes containing moist paper filters; the whole tubers were placed into crispers containing moist paper towels. After inoculation, the material was incubated at 18°C in the dark. Small potato plants were placed into plastic bags containing moist paper towels, inoculated, and incubated at 15°C under 12 hour photoperiod.

Results

Transformant. Transformation efficiency was extremely low for the *P. infestans* isolate used in this experiment. Six transformation attempts resulted in no transformants and a single transformant was recovered during the final attempt. This transformant was recovered from a plate inoculated with protoplasts mixed with 15 µg DNA and identified by both growth on media amended with 6 µg/ml G418 and the indigo-colored compound that formed upon mixing with mycelium with the X-GlcA substrate. This transformant grew more rapidly than the parental isolate on non-selection RAP agar (Figure 2.1). Transformant growth on RAP was significantly different ($P \leq 0.006$) than the parental strain after 4 days; while on RAP amended with potato leaves, both isolates grew slower and 8 days passed before the transformant had consistently faster ($P \leq 0.01$) growth than the parental isolate. After 2 weeks of mitotic divisions while growing on non-selective media, the transformant could still grow after being transferred to media containing 6 µg/ml G418 and GUS activity remained stable. The parental isolate was unable to grow on media containing 6 µg/ml G418 (Figure 2.2) and did not form any indigo color upon incubation in the X-

GlcA substrate. When used as inoculum, the transformed pathogen was able to successfully infect detached potato leaves, the foliage on whole plants, and tuber slices, and the plant material could be stained with X-GlcA to reveal an indigo-colored compound showing the location of the transformed pathogen (Figure 2.3).

Figure 2.1 Average radial growth of GUS-expressing transformant of *Phytophthora infestans* (solid line) and the parental isolate 97-368-1 (dotted line) grown on non selection rye and pea (RAP) media (**A**) or RAP amended with ground leaf pieces (**B**).

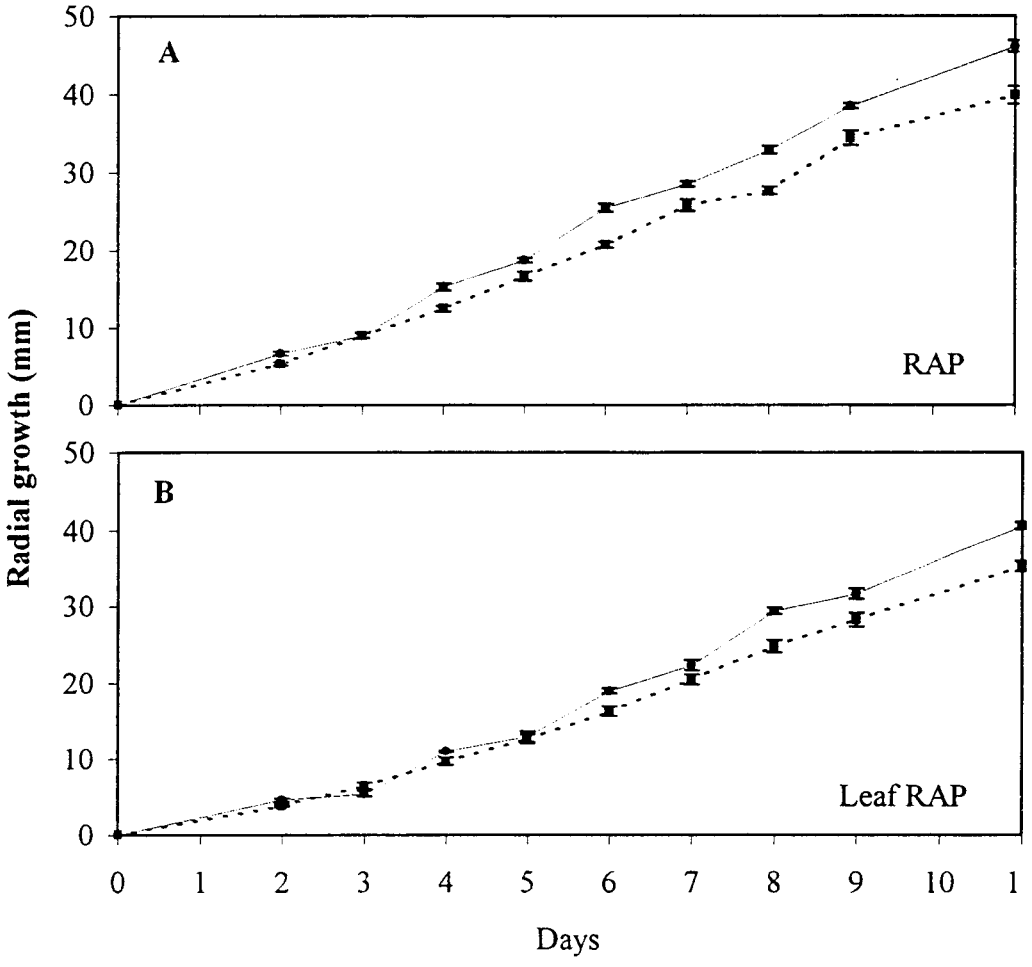


Figure 2.2. Cultures of GUS-expressing transformant of *Phytophthora infestans* (**A** and **B**) and the parental isolate 97-368-1 (**C** and **D**). The media is rye and pea agar amended to 6 µg/ml G418 (**A** and **C**) and non-selection rye and pea agar (**B** and **D**).

A **C**

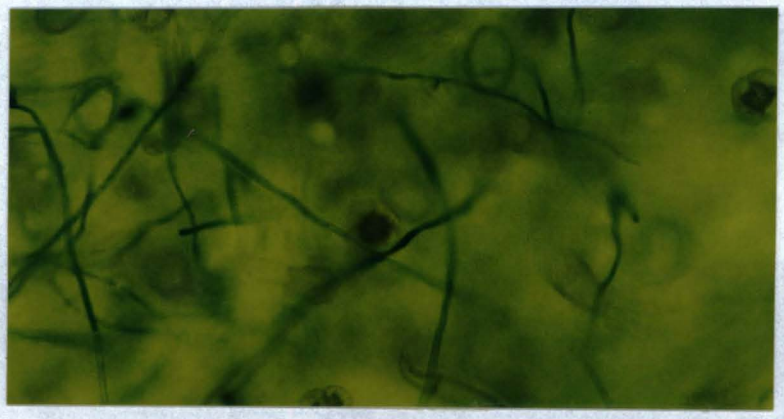
B **D**

Figure 2.2. Transformant of *Phytophthora infestans* stained with X-GlcA substrate. **A**. Mycelium and sporangia grown on rye and pea agar. **B**. Transformant growing on potato tuber tissue. **C**. Transformant growing on potato leaf tissue.

A.

B.

C.



Discussion

The β-glucuronidase-expressing *P. infestans* transformant achieved in this project could be useful in future epidemiological studies investigating the tuber blight stage of the late blight disease cycle. Since glucuronide staining for the transformed pathogen within a lesion is more objective than visual assessment, and can even be quantified, use of the transformant should help overcome problems associated with uncertainty due to similar symptoms created by other pathogens and with complications due to secondary soft rots.

The transformant created in this experiment has been stable with respect to *GUS* and *npt* antibiotic resistance activity, and growth and pathogenicity of the isolate was not disrupted by insertion of the DNA. Combining GUS and X-GlcA (5-bromo-4-chloro-3-indoyl β-D glucuronide) yields an indigo-colored compound useful for identifying the location of *P. infestans* within potato tissue. To measure the amount of the pathogen within potato tissue, methods for staining with the substrate MUG (4-methylumbelliferyl glucuronide) and measuring fluorescent activity will have to be developed. At this time, a few limitations need to be overcome to increase the power and practicality of this reporter gene tool in future tuber blight studies. Although *P. infestans* was a difficult pathogen to transform, additional US-8 isolates should be transformed so future studies can involve a number of isolates. If comparison to other *P. infestans* clones is desired, the other clones will have to be transformed to express GUS as well.

Currently work with the transformed *P. infestans* isolate must be confined to the laboratory or greenhouse as governmental regulations prevent the use of transformed pathogens in the field. Fortunately, greenhouse and laboratory studies, such as whole tuber assays to assess tuber resistance to *P. infestans* (Dorrance and Inglis, 1997, 1998), should lend themselves to use with GUS-expressing transformants. These studies can begin to address a host of questions that remain unanswered about the tuber blight stage of the late blight disease cycle.

SUMMARY

Late blight, caused by *Phytophthora infestans*, is a devastating problem to potato production in many parts of the world. While the foliar phases of this disease are well characterized, tuber infection, leading to quality losses and disease perpetuation, is less well understood. Field infection of potato tubers is initiated most commonly by inoculum, sporangia and zoospores, produced on the plant foliage. Experiments were conducted during 1998 and 1999 in irrigated sandy loam soils to evaluate the relative importance of cultural treatments, i.e. mulches and hill sizes, as barriers to the movement of *P. infestans* inoculum from potato foliage to developing tubers. Two barriers that covered the potato hills, black polyethylene film and copper hydroxide-treated textile, reduced the incidence of tuber blight by 10 to 33%, relative to appropriate controls. The other barriers investigated, polyurethane spray foam in the area of the hill immediate to the potato stem and additional soil in the hill profile, had little effect on tuber blight incidence. Tuber blight incidence, however, was never reduced to a level that would meet industrial standards. The efficacy of black polyethylene film and copper hydroxide-treated textile at reducing the incidence of tuber blight indicates that *P. infestans* inoculum on its journey from foliage to tubers may move through the greater soil surface and is not restricted to large channels in the soil such as those created by the stems. For this reason, the practice of hilling, at least for the hill sizes and the loam soils of this study, had almost no value in terms of tuber blight suppression. The data also highlight the difficulty of direct suppression of tuber blight in a field once the foliar stage of the disease has been allowed to develop

suggesting that prevention of tuber blight in a conducive environment may be inseparably linked to suppression of the foliar phase.

Management of tuber blight remains one of the least understood areas in the late blight disease cycle, in part because tuber blight is difficult to assess and quantify. To help overcome problems associated with tuber blight assessment, a β-glucuronidase (GUS)-expressing transformant *P. infestans* was achieved using lipofectin-mediated protoplast transformation of an US-8 isolate of the pathogen. The transformant is mitotically stable and able to successfully invade potato leaf and tuber tissue and grows more rapidly *in vitro* than the parental isolate. GUS expression is revealed through the formation of an indigo-colored compound when the mycelium is stained with 5-bromo-4-chloro-3-indoyl β-D glucuronide (X-GlcA). The use of this transformant in future epidemiological studies, such as laboratory whole tuber assays on tuber resistance, should enhance detection and measurement of potato tuber blight which may help to study a number of questions about the tuber blight that remain unanswered.

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