AN ABSTRACT OF THE DISSERTATION OF

Srisangwan Laywisadkul for the degree of Doctor of Philosophy in Plant Physiology presented on December 3, 2007.

Title: Factors Affecting the Incidence and Severity of *Phytophthora syringae* Cankers in Pear (*Pyrus communis*) Trees

Abstract approved:

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Leslie H. Fuchigami

Container grown pear trees were used to determine the effects of the following factors on the susceptibility to *Phytophthora syringae*: 1) tree nitrogen (N) status and foliar sprays of urea and copper-EDTA (CuEDTA); 2) dormancy and environmental conditions; 3) wound and wound age; and 4) chemical and biological control measures. Lesion size and disease incidence from a stem assay test were used to evaluate tree susceptibility to the pathogen.

Increasing rate of N fertigation during the growing season and spraying trees with urea increased the concentration of N and amino acids in stems. CuEDTA had no

influence on stem N concentration. Urea had no influence on tree susceptibility while the effect of CuEDTA varied between experiments (years) however the combined application of urea and CuEDTA had no influence on disease severity. Increasing plant N concentration increased susceptibility of plants to *P. syringae.*

Lesions caused by *P. syringae* were greater in November than in October, due to the colder and wetter conditions that favor disease development. Decreasing temperatures after inoculating pear trees with *P. syringae* increased disease severity. Trees incubated in the greenhouse showed smaller lesions than those in the lath house. The lesion size increased in the lath house from October through December. The progression of the disease increased due to the cooler and wet conditions that favored the development of *P. syringae*. Both actively growing and dormant trees were susceptible to *P. syringae* when trees were exposed to low temperatures after inoculation. Wound was important for *P. syringae* infection and disease incidence and lesion size decreased with increasing wound age.

The application of either Aliette (6 g/l) or PhytoFOS (1%) was effective in preventing disease when applied as a foliar treatment 1 week or 2 days respectively before inoculation under all treatment conditions.

Factors Affecting the Incidence and Severity of *Phytophthora syringae* Cankers in Pear (*Pyrus communis*) Trees

by
Srisangwan Laywisadkul

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APPROVED:

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Srisangwan Laywisadkul, Author

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CONTRIBUTION OF AUTHORS

Dr. Leslie H. Fuchigami was involved in overseeing this work, discussion of experiments and editing all chapters. Dr. Carolyn F. Scagel assisted with statistical analysis, writing and editing all chapters. Dr. Robert G.Linderman was involved in discussion of experiments. Minggang Cui assisted in statistical analysis.

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1 GENERAL INTRODUCTION

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Nursery production of bareroot deciduous trees is one of the largest segments of the nursery industry in the Pacific Northwest (PNW). The long daylengths and bright sunny days and cool nights during summer and the relatively mild climate of the Northwest are ideal for production of many deciduous plant species. Diseases caused by species in the genus *Phytophthora* are responsible for significant economic losses on a wide range of host plants commonly grown in PNW nurseries (Erwin and Ribeiro, 1996; Harris, 1979; Wormald, 1919). *Phytophthora syringae* occurs as a winter disease of nursery stock in the PNW, especially on trees that are harvested and stored in coolers or in outdoor sawdust beds (Tidball and Linderman, 1990; Young and Milbrath, 1959).

Efficient defoliation of bareroot deciduous nursery plants prior to harvest and storage before the rainy season is essential to increase efficiency of harvesting and storage. Foliar application of urea and CuEDTA in autumn is a practice currently used by growers of deciduous nursery trees to cause faster defoliation and increase plant nitrogen (N) storage (Bi, 2004; Guak et al., 2001). However, recent studies have shown that OHF97 pear plants sprayed with urea and chemical defoliants suffer severe stem dieback caused by *P. syringae* (personal communication, Shufu Dong). It was hypothesized that the combination of urea and defoliant treatment predisposes the plants to *P. syringae* infection by either inflicting injury to the pear tissue or making the plants more susceptible to infection due to increase N content of the pear plant. The physiological or physical condition of the plant at the time of harvesting and subsequent handling and storage may also play a role in the disease severity in storage because plants are harvested before they are physiologically mature (dormant).

A majority of plant species treated with urea and defoliants are not affected by *P. syringae*, and nurserymen report that the early defoliation of plants may actually reduce the incidence of diseases (personal communication, Lance Lyon, Femrite Nursery). This may

be the result of more rapid healing of leaf scars when conditions favor wound healing, prior to the cold wet conditions, or by enabling the early harvest and storage of plants prior to the onset of conditions that promote the activity and subsequent plant infection by *P. syringae*. The effect of urea and defoliants on disease incidence caused by *P. syringae* appears to be species-specific and may be related to the timing of application.

The relationship between plant N status, use of chemical defoliants, and susceptibility of pear nursery stock to *P. syringae* has not been investigated. Understanding how nursery production practices influence tree susceptibility to this pathogen is important in developing control measures as part of an integrated disease management program.

1.1 Nitrogen and Plant Growth

In deciduous trees, nutrients stored in the tree are important to the growth of the tree in the subsequent year. The amount of N stored in trees (reserves) is especially important because of its positive relationship to spring growth. A positive relationship between N reserves and spring growth was found in apple (Cheng et al., 2002; Oland, 1959), peach (Taylor and May, 1967), almond (Bi, 2004) and pear (Cheng et al., 2001; Taylor and Ende, 1975). Early growth in several deciduous species is mainly supported by N mobilization from storage tissues before any significant root uptake of N occurs in the spring (Tagliavini et al., 1998; Titus and Kang, 1982). Nitrogen uptake from the soil occurred 3 weeks after bud break in apple (Dong et al., 2001b) and 2 weeks after transplanting in almond (Bi, 2004).

Nitrogen from senescing leaves of deciduous trees is translocated to storage tissues in the autumn (Titus and Kang, 1982) and is an important component of total N in trees

(Chapin and Kedrowki, 1983; Taylor and May, 1967). Over the winter, N is stored in deciduous trees' roots as amino acids or proteins (Millard and Proe, 1991; Tromp, 1983) and in bark as proteins (Titus and Kang, 1982). During the summer, N is stored in leaves primarily as ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) in C3 plants (Kawashima and Wildman, 1970). In apple, about 30-50% of total N is present in the foliage in late summer and early autumn, and 50-70% of it can be mobilized back to storage tissues during senescence.

1.2 Use of Defoliants and Urea Sprays in Nursery Production

Most pear varieties in the Pacific Nortwest are propagated by grafting on selected rootstock. All pear trees and rootstocks are defoliated before harvesting and storage like many other deciduous nursery trees (Frecon, 1982). Methods used for defoliation include: manual or mechanical removal of leaves, and application of chemicals that result in early abscission of leaves, usually while still green. Early chemically induced defoliation of deciduous plants decreases the amount of N remobilization from leaves to stems and roots in the autumn (Bi, 2004; Guak et al., 2001), thus reducing nitrogen reserves required for growth the following spring (Guak et al., 2001). Application of copper chelate (CuEDTA), a chemical defoliant commonly used in nursery production of deciduous trees, results in inefficient N mobilization from leaves in deciduous fruit nursery plants (Guak et al., 2001). Early defoliation before terminal budset can also inhibit the development of dormancy and cold hardiness (Fuchigami, 1970). In order to survive in cold storage and grow well during the following growing season, plants must have enough reserve nutrients and develop dormancy.

Foliar application of urea in autumn is a practice currently used by growers of deciduous nursery trees to increase N storage. The amount of N used for new shoot and leaf growth of young Fuji/M.26 apple trees is positively correlated with total amount of N accumulated in the plant during the previous year (Bi, 2004; Dong et al., 2004). Foliar application of urea in the autumn to pear nursery trees, prior to cold storage, can increase the total amount of N in plants (Dong et al., 2001a) by rapid conversion to amino acids and transport to the stems and roots. The combination of foliar applications of urea and copper chelate (CuEDTA) can be used to obtain efficient early defoliation and promote N storage without reducing plant growth performance the following year (Bi, 2004; Guak et al., 2001).

It is important that the combination of urea and CuEDTA is applied after terminal budset in order to avoid damage to plants. According to a degree growth stage (^{o}GS) model (Fuchigami et al., 1982), it is proper to apply the treatment after 180 ^{o}GS. At 180 ^{o}GS, growth stops and leaf removal does not stimulate dormant buds to resume growth, suggesting the end of correlative inhibition phase or onset of rest. Plants defoliated after this point survive prolonged storage and acclimate to freezing temperature (Fuchigami et al, 1982). The model divided the annual growth cycle into 360 ^{o}GS: 0 ^{o}GS (spring budbreak), 90 ^{o}GS (maturity induction point), 180 ^{o}GS (vegetative maturity or onset of rest), 270 ^{o}GS (maximum rest), 315 ^{o}GS (end of rest) and 0 ^{o}GS end of quiescence or onset of spring budbreak). The first and last stages are identical. The sine function from 0 to 180 ^{o}GS described the growth period, and the function from 180 to 360 ^{o}GS described the dormant period.

1.3 *Phytophthora syringae*

Phytophthora species are members of the kingdom Protista. They are diploid and different from the "true fungi" (Erwin and Ribeiro, 1996). They exist as a variety of structures that are able to change freely from one to another in response to external conditions, although not all possible transitions have been observed in all species-mycelium, sexual organs (produce oospores), chlamydospores, and sporangia (capable of germinating as mycelia or emitting zoospores) (Erwin and Ribeiro, 1996).

Phytophthora species have a short regeneration time because of the rapid production of flagellate zoospores; thus, the inoculum from an initial infection can be amplified rapidly (Erwin and Ribeiro, 1996). Most *Phytophthora* species attack only healthy, intact plant tissue or freshly made wounds and do not invade plant tissue previously invaded by other microorganisms; that is, they are primary, not secondary, invaders (Erwin and Ribeiro, 1996).

Diseases caused by *Phytophthora* spp. are influenced by climate. The seasonal activities of host and the influence of physical factors of the environment determine the time course of epidemics caused by *Phytophthora* spp. (Duniway, 1983). *Phytophthora syringae* is a homothallic, relatively low-temperature fungus whose activity is not restricted by cold climate (Erwin and Ribeiro, 1996). *Phytophthora syringae* spreads via splash dispersal of inoculum from soil to leaves, stems, and fruit (Upstone, 1978; Upstone and Gunn, 1978). Rapid rates of disease increase will occur if splash dispersal of inoculum from soil to aerial parts of plants occurs with rainfall or overhead irrigation events (Ristaino and Gumpertz, 2000). The primary sources of *P. syringae* inoculum in nursery production of pear trees are in outdoor sawdust beds and on infected rootstocks placed into cold storage (Pscheidt and

Ocamb, 2002; Young and Milbrath, 1959). In nursery stock, dark, slightly sunken cankers occur on stems and in severe cases the stem is girdled (Erwin and Ribeiro, 1996; Young and Milbrath, 1959). Moreover, oospores produced in infected tissue are capable of surviving for long periods. Oospores of *P. syringae* can survive in apples leaves in soil in orchards for 2.5 years (Harris, 1985).

1.4 Nutrient Management Practices and Disease Susceptibility

Nutrient management practices during nursery production can potentially increase susceptibility of pear nursery trees to *P. syringae.* Nitrogen abundance results in the production of young, succulent growth, a prolonged vegetative period, and delayed plant maturity. These effects make the plant more susceptible to pathogens that normally attack such tissues (Agrios, 1997). There are several reports of high N fertization rates increasing plant susceptibility to pathogen infection, including high rates of N fertilizer increasing disease severity caused by *P. infestans* on potato (Herlihy, 1970), black shank caused by *P. parasitica* on tobacco (Apple, 1961), fire blight caused by *Erwinia amylovora* (Frecon, 1982), and *Septoria tritici* (Simon et al., 2003) and *Fusarium* head blight caused by *F. graminearum* and *F. culmorum* (Lemmens et al., 2004) on wheat.

To increase tree nutrient status, some growers spray pear trees with urea in the autumn. It is unknown whether nutrient management practices, such foliar sprays with urea, increases the susceptibility of pear trees to *P. syringae.*

1.5 Nursery Handling Practices and Disease Susceptibility

Wounds to tree stems caused prior to or during harvesting have the potential to increase susceptibility of nursery pear trees to *P. syringae*. *Phytophthora syringae* is unsuccessful in causing infection to uninjured bark (Bostock and Doster, 1985; De Bruyn, 1924; Linderman, 1986). Surface wounds on stems, inflicted during harvest and leaf scars caused by artificial or natural defoliation and subsequent handling, serve as the infection openings for *P. syringae* (Bostock and Doster, 1985; De Bruyn, 1924; Pscheidt and Ocamb, 2002). In experiments using artificial inoculations with *P. syringae* on rhododendrons, wounds and low temperatures were prerequisite for infection (Linderman, 1986). Nearly all cankers on almond trees caused by *P. syringae* were associated with pruning wounds or injuries created during pruning in late autumn and winter, and infection was unsuccessful on uninjured bark (Bostock and Doster, 1985).

Wound age can also play a role in plant susceptibility to *P. syringae*. Fresh wounds on almond were more susceptible to infection than aged wounds, and the development of resistance to infection on bark wounds was slowed when temperatures were low (Doster and Bostock, 1988b). The development of wound resistance is related to the formation of wound periderm or the infusion of lignin, suberin, waxes, and/or wound gums in the layers of cells immediately subtending the wound surface and the resistance was increased as wounds aged (Biggs, 1986; Bostock and Middleton, 1987; Bostock and Stermer, 1989; Doster and Bostock, 1988c). The rate of deposition of lignin and suberin in the wound depended on the time of the year (Bostock and Stermer, 1989) and temperature (Doster and Bostock, 1988b) when wounds were inflicted.

Bare-rooted nursery pear trees are defoliated in the autumn prior to harvest for best handling and storage. It is unknown whether defoliation practices used during nursery production of pear trees increases the susceptibility of trees to *P. syringae* or what role wound age may play in tree response to the pathogen.

1.6 Plant Growth Stage and Disease Susceptibility

Plant growth or developmental stage has the potential to alter susceptibility of pear nursery trees to *P. syringae*. Optimal environmental conditions of activity of the pathogen coincide with the dormancy period of deciduous plants. Thus some researchers and growers speculate that the incidence of the disease may be related to the dormancy status of the plant (De Bruyn, 1924), while others hypothesize that increased disease during the dormant period is due to the wet-cold environment and not the dormancy status of the plant (Bostock and Doster, 1985). The virulence of *P. syringae* in branch segments of almond was ranged from 2 to 20 ^{o}C (Bostock and Doster, 1985) and the development of resistance to infection on bark wounds is slowed when temperatures are low (Doster and Bostock, 1988b). *Phytophthora syringae* activity is most active during the cool, rainy periods in the PNW (Pscheidt and Ocamb, 2002) which coincide with dormancy in pear trees. The combination of tree growth status and climatic conditions favorable to pathogen infection may make pear trees particularly susceptible to this pathogen in the PNW.

The combination of host predisposition and favorable, cold-wet conditions in cold room or outdoors for pathogen activity increases the potential for severe damage to occur. These favorable conditions for disease development may also be influenced by the dormancy status of the plant. It is unknown whether the disease caused by *P. syringae* is a result of

only the cold and wet environment or also related to the dormancy status of the deciduous plant.

1.7 Disease Control Measures

The disease caused by *P. syringae*, can be controlled using a combination of good nursery sanitation practices and chemical applications. Phosphonate-containing fungicides, including fosetyl-Al (Aliette) and its breakdown product, phosphorous acid (also referred to as phosphite) are commonly used to control *Phytophthora* species. (Doster and Bostock, 1988a; Erwin and Ribeiro, 1996). It is applied to plants as either sprays, drenches, dips, or by injection (Erwin and Ribeiro, 1996; Pegg et al., 1987; Quimete and Coffey, 1989; Tidball and Linderman, 1990). Fosetyl-Al translocates both upward and downward (in the transpiration stream and in the phloem sap) and may have direct toxicity against *Phytophthora* species (Erwin and Ribeiro, 1996). Other than fosetyl-Al, dimethomorph was reported as an effective fungicide in controlling disease caused by oomycetes (Albert et al., 1988; Cohen et al., 1995). Application of dimethomorph to potato leaf discs 24 or 46 hours before inoculation with *P. infestans* completely inhibits symptom development, while application after inoculation generally had little effect (Stein and Kirk, 2003). Dimethomorph-mancozeb suppressed late blight, caused by *P. infestan*s, in petunias and tomatoes (Becktell et al., 2005).

Due to questions about the potential carcinogenicity and costs of fungicides, world trends are moving toward reduced pesticide use. In response, several biological materials have been evaluated as safer alternatives to the use of synthetic, chemical fungicides. Bacterial antagonists are common components of biological disease control strategies.

Various microbial antagonists have been shown to interfere with the growth of plant pathogens, but only some of these microorganisms have been developed into commercial products, such as bacteria: *Agrobacterium radibacter* K-84, *Pseudomonas fluorescens* and *Bacillus subtilis* (Agrios, 1997). *Bacillus subtilis* Cot1 prevented *Phytophthora* and *Pythium* damping-off of *Astilbe*, *Photinia*, and *Hemerocallis* microplants and conventional *Brassica* seedlings under high humidity conditions in fogging glasshouses(Berger et al., 1996). Strains EBW-4, AB8, BACTX, NZB1, AB7, AB8, and BACT2 of *Bacillus subtilis* significantly reduced the number of apple trees infected by *P. cactorum* (Utkhede et al., 2001; Utkhede and Smith, 1991).

Phosphonate-containing fungicides, dimethomorph, and *Bacillus subtilis* have been used against *Phytophthora* species. It is unknown whether a single spray of those agents is effective in controlling disease caused by *P. syringae* in pear nursery stock when applied after terminal bud set.

1.8 Summary

While a great deal is already known about factors influencing the susceptibility of plants to diseases caused by *Phytophthora* spp., the specific relationships between nursery production practices and susceptibility of *Pyrus communis* nusery trees to *P. syringae* has not been fully investigated. The experiments described in this thesis address (1) whether tree N status, growth stage, or wound age alters tree susceptibility the pathogen; (2) how environmental conditions after exposure to the pathogen alters tree susceptibility; (3) whether use of foliar sprays of urea and defoliants in the autumn increase tree susceptibility

to the pathogen; and (4) how application of chemicals before and after exposure to the pathogen alters tree susceptibility.

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2 PLANT N STATUS, FUNGICIDE APPLICATION, AND TIME OF YEAR ALTER SUSCEPTIBILITY OF *PYRUS COMMUNIS* TO *PHYTOPHTHORA SYRINGAE*

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2.1 Abstract

The influence of plant nitrogen (N) status and timing of foliar sprays of urea, CuEDTA, and chemical control measures on plant susceptibility to *Phytophthora syringae* was studied in three experiments using OHF97 pear rootstocks. In Experiment 1, different rates of soil-applied N during the growing season and foliar application of urea and CuEDTA in October and November were used to alter tree N status and stems were inoculated with the pathogen before and after foliar sprays in the autumn. In Experiment 2, trees were sprayed with a factorial combination of urea, CuEDTA, and Aliette in either October or November, prior to inoculation with the pathogen. In Experiment 3, trees were inoculated with the pathogen before or after being sprayed with a factorial combination of urea plus CuEDTA and PhytoFos in either October or November. Lesion size and disease incidence were used to evaluate tree susceptibility to the pathogen and the relationship between tree N status and susceptibility was determined by measuring stem N and amino acid concentrations. Increasing rate of N fertigation during the growing season increased the concentrations of N and amino acids in stems and susceptibility to the pathogen. Trees sprayed with urea in the autumn had higher N and amino acid concentrations in stems; however, in all experiments, urea sprays had no influence on tree susceptibility to *P. syringae*. Spraying trees with CuEDTA had no influence on stem N concentration, slightly increased tree susceptibility to the pathogen in experiment 1, but had no influence on tree susceptibility in experiment 2. In general, lesions that developed when inoculations were made in October were smaller than when inoculations were made in November, regardless of urea and CuEDTA application. *Phytophthora syringae* caused no lesions on trees treated with a single foliar application of Aliette (6 g/l) or PhytoFOS (1%) 1 wk or 2 d respectively before inoculation. The

application of either Aliette, PhytoFOS alone or combined with urea+CuEDTA had a similar effect in controlling disease when applied before inoculation. These results indicate that the practice of using foliar sprays of urea does not increase pear tree susceptibility to *P. syringae*; however application of CuEDTA alone has the potential to increase tree susceptibility to the pathogen when used without chemical control measures.

2.2 Introduction

Diseases caused by species in the genus *Phytophthora* are responsible for significant economic losses on a wide range of host plants, including pear, *Pyrus communis* (Erwin and Ribeiro, 1996; Harris, 1979; Wormald, 1919). *P. syringae* occurs as a winter disease of nursery stock in the Pacific Northwest, especially on trees that are harvested and stored in coolers or in outdoor sawdust beds (Erwin and Ribeiro, 1996; Pscheidt and Ocamb, 2002; Tidball and Linderman, 1990).

Phytophthora syringae infects wounds on stems caused by handling or pruning or through leaves and leaf scars (Bostock and Doster, 1985; De Bruyn, 1924). The fungus spreads via splash dispersal of inoculum from soil to leaves, stems, and fruit (Upstone, 1978). Rapid rates of disease increase will occur if splash dispersal of inoculum from soil to aerial parts of plants occurs with rainfall or overhead irrigation events (Ristaino and Gumpertz, 2000). The primary sources of *P. syringae* inoculum in nursery production of pear trees are in outdoor sawdust beds and on infected rootstocks placed into cold storage (Pscheidt and Ocamb, 2002). In nursery stock, dark, slightly sunken cankers occur on stems, and in severe cases the stem is girdled (Erwin and Ribeiro, 1996; Young and Milbrath, 1959).

The disease caused by *P. syringae*, can be controlled using a combination of good nursery sanitation practices and chemical applications. Phosphonate-containing fungicides, including fosetyl-Al (Aliette) and its breakdown product, phosphorous acid (also referred to as phosphite) are commonly used to control *Phytophthora* species (Doster and Bostock, 1988; Erwin and Ribeiro, 1996). It is applied to plants as either sprays, drenches, dips, or by injection (Erwin and Ribeiro, 1996; Pegg et al., 1987; Quimete and Coffey, 1989; Tidball and Linderman, 1990). Fosetyl-Al translocates both upward and downward (in the transpiration stream and in the phloem sap) and may have direct fungal toxicity against *Phytophthora* species (Erwin and Ribeiro, 1996).

Most pear varieties in the Pacific Northwest are propagated by grafting on selected rootstock. All pear trees and rootstocks are defoliated before harvesting and storage like many other deciduous nursery trees (Frecon, 1982). Methods used for defoliation include: manual or mechanical removal of leaves, and application of chemicals that result in early abscission of leaves, usually while still green. Early chemically-induced defoliation of deciduous plants decreases the amount of N mobilization from leaves to stems and roots in the autumn (Bi, 2004; Guak et al., 2001), thus it can reduce nitrogen reserves required for growth the following spring (Guak et al., 2001). Copper chelate (CuEDTA), a chemical defoliant commonly used in nursery production of deciduous trees, results in inefficient N mobilization from leaves in deciduous fruit nursery plants (Guak et al., 2001). The combination of foliar applications of urea and copper chelate (CuEDTA) can be used to obtain efficient early defoliation and promote N storage without reducing plant growth performance the following year (Bi, 2004; Guak et al., 2001). Early defoliation before terminal buds set can also inhibit the development of dormancy and cold hardiness

(Fuchigami, 1970). In order to survive in cold storage and grow well during the following growing season, plants must have enough reserve nutrients and develop dormancy.

Recent studies have shown that OHF97 pear plants sprayed with urea and chemical defoliants suffer severe stem lesions caused by *P. syringae* (personal communication, Shufu Dong). It was hypothesized that the combination of urea and defoliant treatment predisposes the plants to *P. syringae* by either inflicting injury to the pear tissue or making the plants more susceptible to infection by increasing the N content of the pear plant. The effect of abundant N can make plants more susceptible to pathogens and susceptible for a longer time (Frecon, 1982; Simon et al., 2003).

A majority of plant species treated with urea and defoliants are not affected by *P. syringae*, and some nurserymen report that the early defoliation of plants treated with the combination of urea and defoliant may actually reduce the incidence of diseases (personal communication, Lance Lyon, Femrite Nursery). This may be the result of more rapid healing of leaf scars prior to the cold wet conditions due to the early defoliation when conditions favor wound healing or by enabling the early harvest and storage of plants prior to the onset of conditions that promote the activity and subsequent plant infection by *P. syringae*. The effect of urea and defoliants on disease incidence caused by *P. syringae* appears to be species-specific and may be related to the timing of application.

Phytophthora syringae is a serious problem in Pacific Northwest deciduous plant nurseries. With an increased use of urea and defoliants in nursery production of deciduous trees a better understanding of the relationship between urea and defoliants and the susceptibility of plants to *P. syringae* is needed. Using container grown pear trees and an *in vivo* infection assay for *P. syringae*, the specific objectives of this study were to determine

whether: (1) tree N status (from urea or soil N application) alters susceptibility; (2) spraying trees with the defoliant CuEDTA alters susceptibility; (3) whether the timing of urea and defoliant applications influences susceptibility; and (4) the effects of fosetyl-Al and PhytoFOS can protect the pear plants from infection.

2.3 Materials and Methods

2.3.1 Experiment 1 (2002)

The influence of tree N status on susceptibility of pear (*Pyrus communis*) to *P. syringae* was evaluated by using different rates of soil N applications during the growing season and foliar application of urea and CuEDTA in the autumn to alter tree N status. To determine whether time of inoculation influences tree response to the pathogen, an inoculation test using stems was used to evaluate plant susceptibility to the pathogen before or after application of urea or CuEDTA in autumn.

2.3.1.1 Plant culture

Pear (*Pyrus communis*) OHF-97 rootstocks were planted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) in late March (26 March 2002). The trees were grown in a lath house at OSU (44^{o} 30' N, 123^{o} 17' W), USA, trained to a single stem, and fertigated with 150 ppm N using Plantex® 20-20-20 with micronutrients (Plantex Corp., Ontario, Canada) once per week from 18 June to 30 July 2002.

2.3.1.2 Fertilizer and defoliant treatments

In August 2002, 144 trees were selected for uniformity based on stem diameter (7-8 mm) and fertigated twice a week with one of three nitrogen concentrations (0, 10 and 20 mM N from NH_4NO_3) by applying 400 ml of a complete nutrient solution to each pot. The nutrient solution was modified from Hoagland's solution number 2 (Hoagland and Arnon, 1950).

Trees from each fertigation treatment were divided into four groups (Table 2-1) in September 2002. Leaves on trees were sprayed with one of four different foliar treatments. Twelve trees in each fertigation treatment were sprayed with either water, urea (3% solution, urea 46-0-0), CuEDTA (1% solution, Ciba® Librel® Cu (CuEDTA) Ciba), or urea followed by urea plus CuEDTA 2 weeks after the first application. Trees were sprayed until run-off with foliar treatments on 26 October 2002 and 9 November 2002 (two weeks between applications).

2.3.1.3 *Phytophthora syringae* inoculum production

Stock cultures of *P. syringae* (isolated from *Kalmia latifolia* by Robert Linderman, USDA-ARS) were maintained on V8 juice agar medium in the dark at 20 ^{o}C. To prepare the medium, 4 g calcium carbonate added to 340 ml of V8 juice was heated and then filtered through cheese cloth. Then 100 ml of the filtered juice was added to 17g of agar and distilled water added to 1 liter before autoclaving (121 °C, 20 min). Fresh cultures were prepared 7-10 d before inoculation by transferring 4 mm agar plugs to plates containing V8A medium and incubated in the dark at 20 ^{o}C.

2.3.1.4 Wound inoculation test

Stems on 6 trees in each foliar and N fertigation treatment were inoculated with the pathogen at one of two inoculation times: 1 week before foliar application (19 October 2002) or 1 week after foliar treatments (16 November 2002) (Table 2-1). Mycelial plugs (4 mm dia.) were taken from the actively growing margin of colonies of *P. syringae* (7-10 d) growing on V8A medium. Plugs containing either the pathogen or without the pathogen were placed into a wound made with a cork borer (4 mm dia.). Wounds were wrapped with paraffin after inoculation.

2.3.1.5 Data Collection

Disease development (length of lesion, cm) was measured on plants after natural defoliation occurred in the control groups (21 December 2002). All stems were removed from trees using pruning shears and analyzed for concentration of N and amino acids. All samples were placed into a -80^{o}C freezer, freeze-dried, then ground with a Wiley mill (20 mesh) and reground with a cyclone mill (60 mesh). The N concentration (mg g^{-1}) of ground samples was determined using the Kjeldahl method (Horneck et al., 1989). Concentration of free amino acids was determined by the ninhydrin assay (Yemm and Cocking, 1955).

2.3.1.6 Statistical Analysis

The experiment was a completely randomized design with four factors: Nitrogen fertigation rate (0, 10, 20 mM), urea treatment (water, urea), defoliant treatment (water, CuEDTA), and inoculation time (before or after foliar treatments). Treatment combinations are shown in (Table 2-1). No lesions occurred on wounds inoculated with only V-8 agar; therefore only data from wounds inoculated with the pathogen were included in the

analyses. Each treatment had 6 replications. Data were analyzed using analysis of variance (ANOVA) to determine whether treatments influenced: (1) the nitrogen (N) and amino acid concentrations in stems, and (2) lesion size on stems. Where indicated by ANOVA, means were separated using Tukey's Honestly Significant Difference at $P<0.05$ ($THSD_{0.05}$) and polynomial contrasts were used to evaluate the influence of N fertigation rate on response variables. Binomial analyses were used to determine treatment effects on disease incidence. The relationships between the lesion size and stem N and total amino acid concentrations were analyzed using Pearson's correlation coefficient (R). All statistical analyses were performed with S-PLUS (MathSoft, Inc., Seattle, WA., USA).

Table 2-1. Experimental treatments for Experiment 1 (2002)

Inoculation Time [z]	**Foliar Treatments** [y]		**N Fertigation**
	Urea	**CuEDTA**	**Rate (mM N)** [x]
October	Water	Water	0
			10
			20
		CuEDTA	0
			10
			20
	Urea	Water	0
			10
			20
		CuEDTA	0
			10
			20
November	Water	Water	0
			10
			20
		CuEDTA	0
			10
			20
	Urea	Water	0
			10
			20
		CuEDTA	0
			10
			20

[z]October = Stems inoculated on 19 October 2002 one week before the foliar spray treatment**;** November = Stems inoculated on 16 November 2002 one week after the foliar spray treatment

[y] Trees were sprayed on 2 times (26 October 2002 and 9 November 2002) with Urea (3% urea solution), CuEDTA (1% copper EDTA) or Water.

[x]Rate of N application during August 2002.

2.3.2 Experiment 2 (2003)

The effect of urea, CuEDTA and Aliette applied as foliar spray in October or November prior to inoculation with *P. syringae* on stem N status and susceptibility of pear (*P. communis*) to the pathogen was evaluated. The stem inoculation test was used to evaluate tree susceptibility to the pathogen after foliar application.

2.3.2.1 Plant culture

Pear OHF-97 rootstocks were planted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) on 21 May 2003. The trees were grown in a lath house at OSU, trained to a single stem, and fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients once a week, from 6 June to 5 September 2003.

2.3.2.2 Fertilizer, CuEDTA, and Aliette treatments

In October 2003, 80 trees were selected for uniformity based on stem diameter (7-8 mm.) and divided into two groups of 40 trees and sprayed with foliar treatments on either 5 October 2003 or 13 November 2003. Leaves on 5 trees were sprayed once with one of eight foliar treatments (Table 2-2) after natural cessation of plant growth.

Trees were sprayed with combinations of water, urea (3% solution, urea 46-0-0), CuEDTA (1% solution, Ciba® Librel® Cu (CuEDTA) Ciba), or Alliette (6 g/l, Bayer). Chemicals in each treatment were sprayed together until run-off.

Table 2-2. Experimental treatments for Experiment 2 (2003)

Foliar Treatment Time [z]	Foliar Treatments [y]		
	Urea	**CuEDTA**	**Alliette**
October	Water	Water	Water
			Alliette
		CuEDTA	Water
			Alliette
	Urea	Water	Water
			Alliette
		CuEDTA	Water
			Alliette
November	Water	Water	Water
			Alliette
		CuEDTA	Water
			Alliette
	Urea	Water	Water
			Alliette
		CuEDTA	Water
			Alliette

[z]October = Foliar treatments applied on 5 October 2003 and trees inoculated on 12 October 2003; November = Foliar treatments applied on 13 November 2003 and trees inoculated on 20 November 2003.
[y]Water = plants spayed with water at same time as other foliar treatments. Urea = 3% urea solution. CuEDTA = 1% copper EDTA solution. Aliette = Aliette (6g/l).

2.3.2.3 *Phytophthora syringae* inoculum production

Inoculum of *P. syringae* was produced as described in 2.3.1.3, above.

2.3.2.4 Wound inoculation test

Stems on five trees in each foliar application treatment were inoculated with *P. syringae* 1 week after foliar treatments (12 October 2002 and 20 November 2002). Wound inoculation was performed as described in 2.3.1.4, above. Three wounds were made on the stem of each tree: two wounds were inoculated with *P. syringae* grown on V8A medium and one wound was inoculated with V8 agar to serve as a control.

2.3.2.5 Data Collection

Disease development (length of lesion, cm) was measured on trees 8 weeks after inoculation. Stem of trees inoculated on 12 October 2003 and 20 November 2003 were assessed on 7 December 2003 and 15 January 2004, respectively. After each assessment, stems were removed from trees using pruning shears and N concentration of stems was determined as described in 2.3.1.5, above.

2.3.2.6 Statistical Analysis

The experiment was completely randomized design with 4 factors: Time of foliar treatment (October and November), urea treatment (water, urea), defoliant treatment (water, CuEDTA), and Aliette (water, Aliette). Treatment combinations are shown in (Table 2-2). No lesions occurred on wounds inoculated with only V-8 agar; therefore only data from wounds inoculated with the pathogen were included in the analyses. Each treatment had 5 replications. Data were analyzed using analysis of variance (ANOVA) to determine whether treatments influenced (1) the N concentrations in stems, and (2) lesion size on stems. Where indicated by ANOVA, means were separated using Tukey's Honestly Significant Difference at $P<0.05$ ($THSD_{0.05}$). The relationships between the lesion size and stem N concentrations were analyzed using Pearsons correlation coefficient. All statistical analyses were performed with S-PLUS.

2.3.3 Experiment 3 (2004)

The effect of urea, CuEDTA and PhytoFOS applied as a foliar spray in October or November before and after inoculation with *P. syringae* on stem N status and susceptibility

of pear (*Pyrus communis*) to the pathogen was evaluated. The stem inoculation test was used to evaluate tree susceptibility to the pathogen after foliar application.

2.3.3.1 Plant culture

Pear OHF-97 rootstocks were planted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) in 1 June 2004. The trees were grown in a lath house at OSU, trained to a single stem and fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients once a week, from 14 June to 18 August 2004.

2.3.3.2 Fertilizer, CuEDTA, and PhytoFOS treatments

In October 2004, 128 trees were selected for uniformity based on diameter (8-9 mm) and divided into two groups of 64 trees and sprayed with foliar treatments on either 22 October 2004 or 24 November 2004. Leaves on 16 trees were sprayed with one of four foliar treatments (Table 2-3) after natural cessation of plant growth.

Trees were sprayed with combinations of water, urea (3% solution, urea 46-0-0) plus CuEDTA (1% solution, Ciba® Librel® Cu (CuEDTA) Ciba), or PhytoFOS (1%– SIPCAM AGRO USA, Inc.). Chemicals in each treatment were sprayed together until run-off.

Table 2-3. Experimental treatments for Experiment 3 (2004)

Time of Foliar Treatment[x]	Foliar Treatments[y]		Inoculation Times (d from foliar treatment)[z]
	Urea	PhytoFOS	
October[z]	Water	Water	-7d, 2d, 7d, 21d
		Phosphite	-7d, 2d, 7d, 21d
	Urea+CuEDTA	Water	-7d, 2d, 7d, 21d
		Phosphite	-7d, 2d, 7d, 21d
November	Water	Water	-7d, 2d, 7d, 21d
		Phosphite	-7d, 2d, 7d, 21d
	Urea+CuEDTA	Water	-7d, 2d, 7d, 21d
		Phosphite	-7d, 2d, 7d, 21d

[x]October = Foliar treatments applied on 22 October 2004. November = Foliar treatments applied on 24 November 2004.
[y]Water = plants spayed with water at same time as other foliar treatments. Urea+CuEDTA = a mixture of 3% urea solution and 1% CuEDTA solution. Phosphite = PhytoFOS (1%).
[z]Stems were inoculated 7d (-7d) before foliar treatment or 2, 7, or 21 d after foliar treatment.

2.3.3.3 *Phytophthora syringae* inoculum production

Inoculum of *P. syringae* was produced as described in 2.3.1.3, above.

2.3.3.4 Inoculation tests

Stems on 4 trees in each foliar application treatment were inoculated with *P. syringae* at one of four inoculation times: 1 week before foliar treatments (-7 d, either 15 October 2004 or 17 November 2004), and 2 days (2 d, either 24 October 2004 or 26 November 2004), 1 week (7 d, either 29 October 2004 or 1 December 2005), and 3 weeks (21 d, either 12 November 2004 or 15 December 2004) after foliar application treatments (total of 32 treatments, Table 2-3).

Wound inoculation tests were performed as described in 2.3.1.4, above. Three wounds were made on one stem on each tree: two wounds were inoculated with *P. syringae* grown on V8 agar and one wound was inoculated with V8 agar to serve as a control. Three

inoculations were also made on the same stem of each tree without artificially wounding the stem: two locations were inoculated with the pathogen and one location was inoculated with V8 agar to serve as control.

2.3.3.5 Data Collection

Disease development (length of lesion, cm) was measured on plants 8 weeks after inoculation. Stem of trees inoculated at -7d, 2d, 7d, and 21d in the October foliar treatments were assessed on 10 December 2004, 19 December 2004, 24 December 2004, and 7 January 2005, respectively. Stem of trees inoculated at -7d, 2d, 7d, and 21d in the November foliar treatment were assessed on 12 January 2005, 21 January 2005, 26 January 2005, and 9 February 2005, respectively. All stems were harvested using pruning shears and N concentrations of stems were determined as described in 2.3.1.5, above. The same measurements were used to measure disease development on control trees without artificial wounding.

2.3.3.6 Statistical Analysis

The experiment was completely randomized design with 4 factors: Time of foliar application (October and November), urea treatment (water and urea), PhytoFOS (water and PhytoFOS), and time of inoculation (-7, 2d, 7d, and 21d). Treatment combinations are shown in (Table 2-3). No lesions occurred on wounds inoculated with only V8 agar and no lesions occurred on stems inoculated without wounds; therefore only data from wounds and stems inoculated with the pathogen were included in the analyses. Each treatment had 4 replications. Data were analyzed using analysis of variance (ANOVA) to determine whether treatments influenced (1) the N concentrations in stems, and (2) lesion size on stems. Where

indicated by ANOVA, means were separated using Tukey's Honestly Significant Difference at $P<0.05$ ($THSD_{0.05}$) and polynomial contrasts were used to evaluate the influence of time of inoculation on response variables. The relationships between the lesion size and stem N concentrations were analyzed using Pearsons correlation coefficient. All statistical analyses were performed with S-PLUS.

2.4 Results

2.4.1 Experiment 1 (2002)

2.4.1.1 Stem Nitrogen Concentration

N concentrations in stems were similar in October and November (Figure 2- 1). Increasing N fertilizer application rate in August, 2002, increased N concentrations in stems (linear contrast, $P<0.0001$). Spraying trees with urea in the autumn of 2002 increased N concentrations in stems. Spraying trees with CuEDTA in the autumn of 2002 had no influence on N concentrations in stems (data not shown).

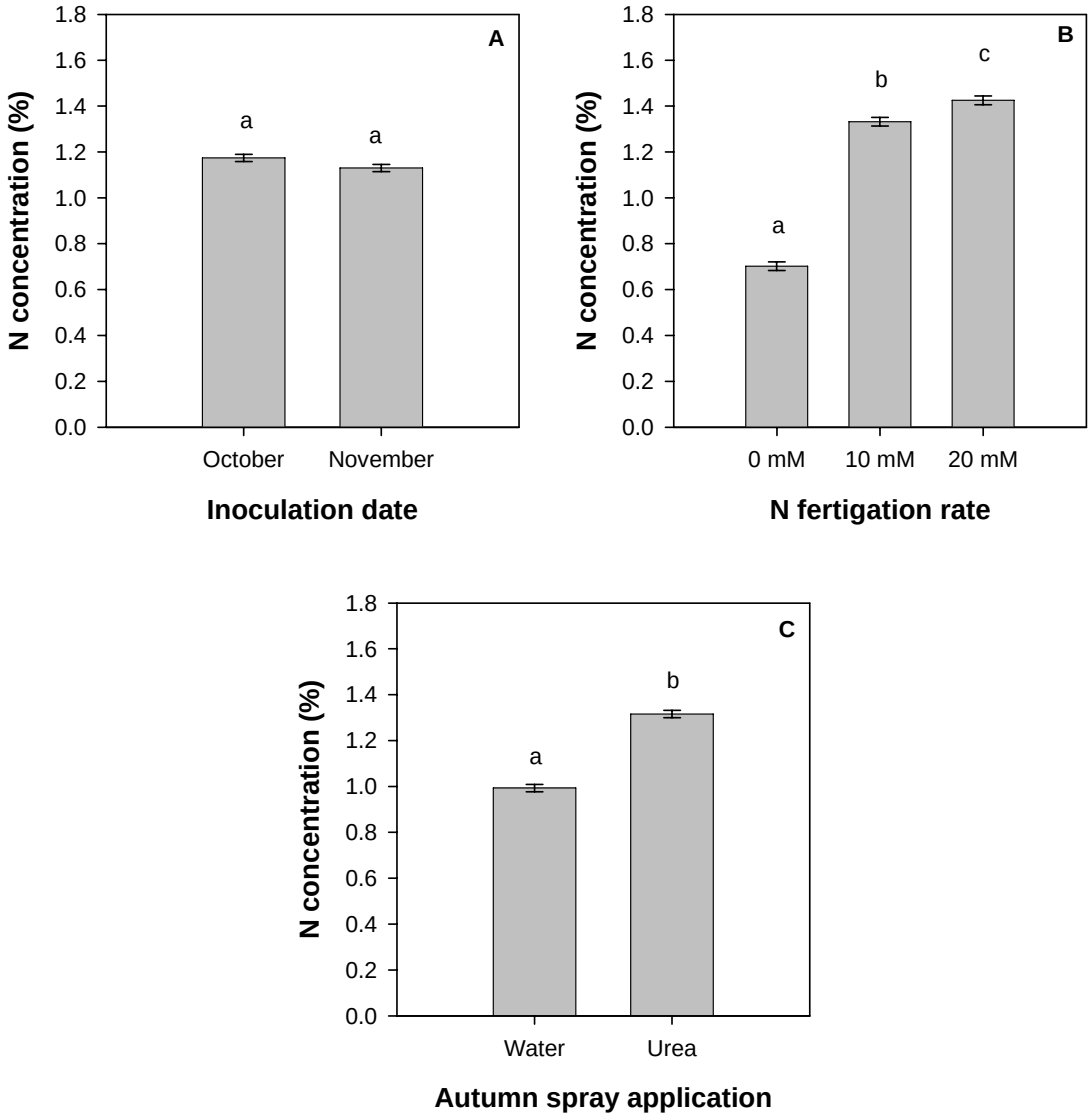

Figure 2- 1. Influence of (A) inoculation time, (B) nitrogen fertigation rate during August 2002, and (C) urea sprays in the autumn on nitrogen (N) concentration (%) in stems of pear (*Pyrus communis*) inoculated with *Phytophthora syringae*. (A) Trees inoculated one week before (October) or one week after (November) spray treatments. (B) Trees fertigated with 400 ml of 0, 10, or 20 mM N. (C) Trees sprayed with water or 3% urea solution (Urea) on 26 October and 9 November 2002. Columns represent means (A, C, n=72; B, n=48) and error bars are standard errors. Column denoted by the same letter within a graph are not significantly different (P>0.05, $THSD_{0.05}$).

2.4.1.2 Stem Amino Acid Concentration

Increasing rate of N fertilization in August 2002 and spraying trees with urea increased the concentration of amino acids in stems (Figure 2- 2). In general CuEDTA had no influence on amino acid concentration in stems. The concentration of amino acids in stems was positively correlated with N concentrations in stems (R = 0.865, P<0.0001).

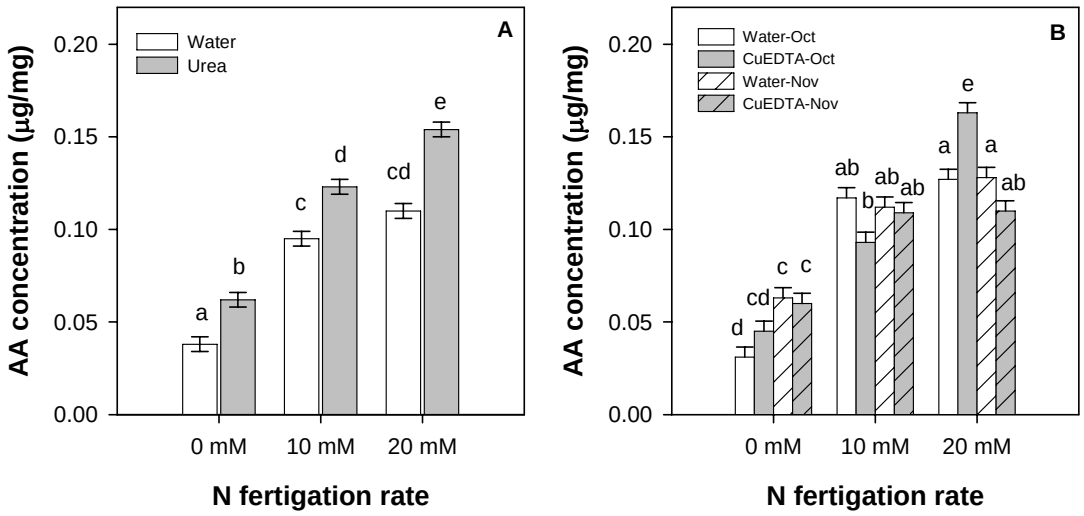

Figure 2- 2. Influence of (A) urea and (B) copper EDTA (CuEDTA) sprays in the autumn on the concentration of amino acids (AA) in stems of pear (*Pyrus communis*) fertigated with 400 ml of 0, 10, or 20 mM N during August 2002 and inoculated with *Phytophthora syringae*. (A) Trees sprayed with water or 3% urea solution (Urea) on 26 October and 9 November 2002. (B) Trees sprayed with water or 1% CuEDTA solution and inoculated one week before (Oct) or one week after (Nov) spray treatments. Columns represent means (A, n=24; B, n=12) and error bars are standard errors. Columns denoted by the same letter within a graph are not significantly different (P>0.05, $THSD_{0.05}$).

2.4.1.3 Size of Stem Lesions and Disease Incidence

Stems inoculated with *P. syringae* in November developed larger lesions compared to stems inoculated in October, and disease incidence (% of stems with lesions was greater on trees inoculated in November compared to trees inoculated in October (Figure 2-3). Spraying trees with CuEDTA in the autumn of 2002 increased lesion size but had no effect on disease incidence. Increasing rate of N fertigation in August 2002 increased lesion size (linear contrast, $P<0.001$) but had no influence on disease incidence. Spraying trees with urea had no influence on lesion size or disease incidence compared to the control treatment (water).

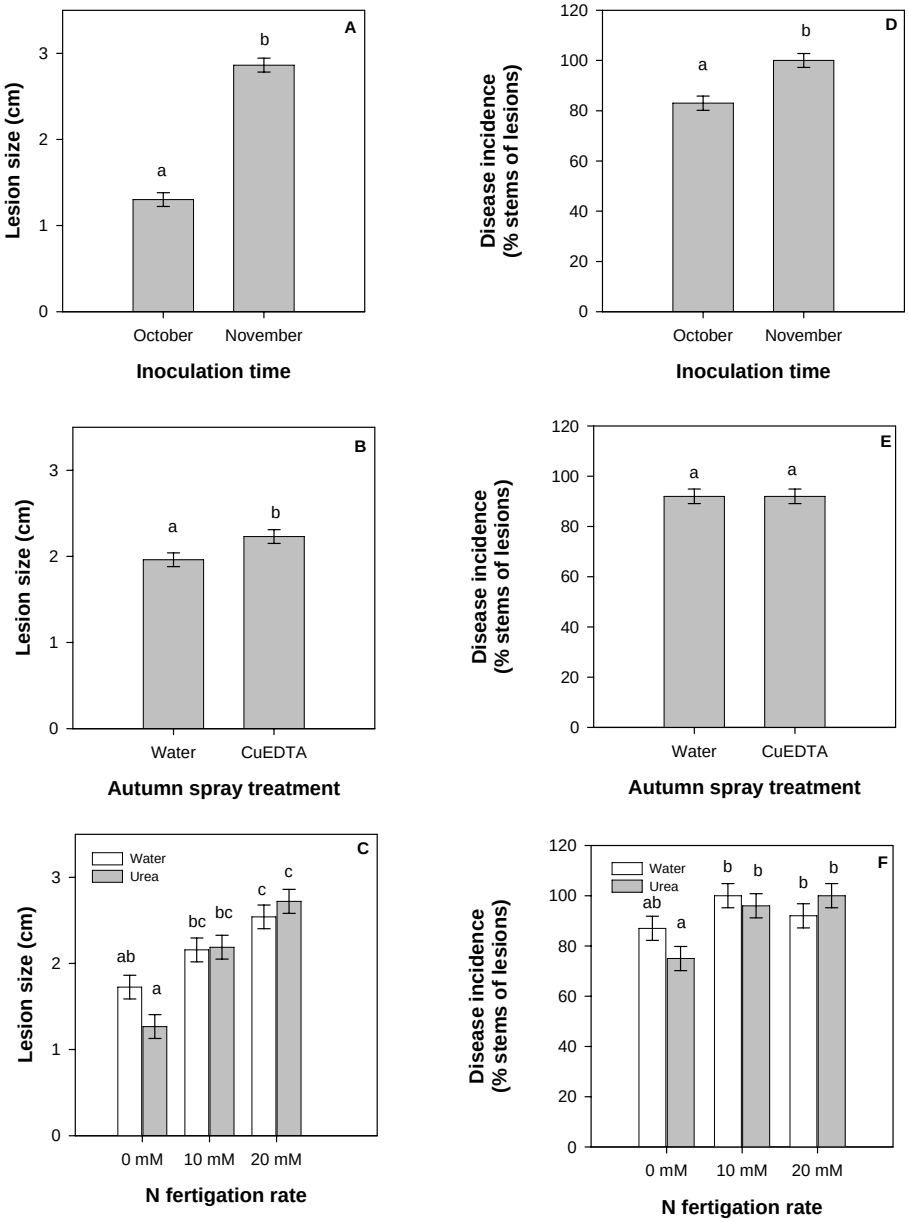

Figure 2- 3. Size of stem lesions (A-C) and disease incidence (D-F) caused by inoculating wounded pear (*Pyrus communis*) stems with *Phytophthora syringae*. (A, D) Trees inoculated one week before (October) or 1 week after (November) spray treatments. (B, E) Trees sprayed with water or 1% CuEDTA solution on 26 October and 9 November 2002. (C, F) Trees fertigated with 400 ml of 0, 10, or 20 mM N during August 2002 and sprayed with water or 3% urea solution (Urea) on 26 October and 9 November 2002. Columns represent means (A, B, D, E, n=72; C, F, n=24) and error bars are standard errors. Columns denoted by the same letter within a graph are not significantly different (P>0.05; lesion size, $THSD_{0.05}$ and disease incidence, $K\text{-}W_{0.05}$).

2.4.1.4 Relationships between N and Lesion Size

Increasing N concentrations in tree stems was positively correlated with lesion size (R=0.3440, $P<0.001$). Increasing amino acid concentrations in tree stems was positively correlated with lesion size (R=0.321, $P<0.001$).

2.4.2 Experiment 2 (2003)

2.4.2.1 Stem N Concentration

Spraying trees with urea in the autumn of 2003 increased N concentrations in stems (Figure 2- 4). The influence of urea on N concentration was greater when urea was applied in October than in November. Spraying trees with CuEDTA, water, or Alliette without urea had no influence on N concentrations in stems.

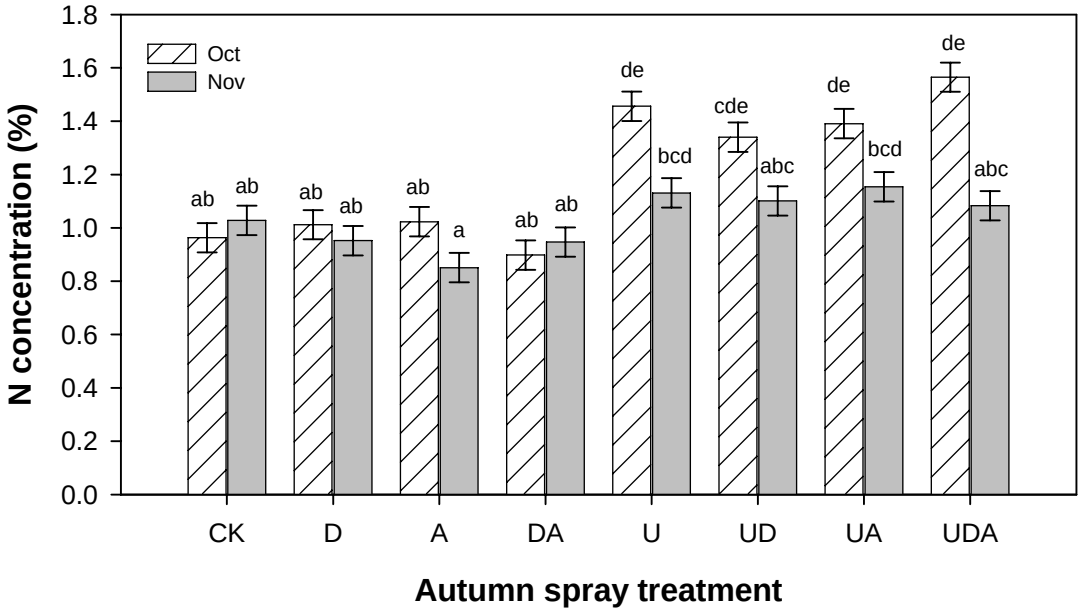

Figure 2- 4. Influence of spraying trees with urea, copper EDTA (CuEDTA), or Alliette in the autumn on the nitrogen (N) concentration (%) in stems of pear (*Pyrus communis*) inoculated with *Phytophthora syringae*. Trees were sprayed on 5 October or 13 November 2003 and stems were inoculated one week after spray treatments on 12 October (Oct) or 20 November (Nov) 2003. CK = trees sprayed with water at same time as other treatments. D = trees sprayed with 1% CuEDTA solution. A = trees sprayed with 6 g/l Alliette. U = trees sprayed with 3% urea solution. Columns represent means (n=5) and error bars are standard errors. Columns denoted by the same letter are not significantly different (P>0.05, $THSD_{0.05}$).

2.4.2.2 Size of Stem Lesions and Disease Incidence

All treatments inoculated with the pathogen caused disease, except all treatments that include Alliette (disease incidence data not shown). Stems from plants treated with foliar sprays in October had smaller lesions than stems from trees treated with foliar sprays in November (Figure 2- 5). Spraying trees with urea in the autumn of 2003 had no influence on lesion size. Alliette decreased lesion size when applied in either October or November. Spraying trees with CuEDTA had no influence on lesion size (data not shown). All stems from trees that were inoculated with *P. syringae* and not treated with Alliette showed signs of disease and only 1 stem from trees that were inoculated with *P. syringae* and treated with Alliette showed signs of disease (disease incidence data not shown).

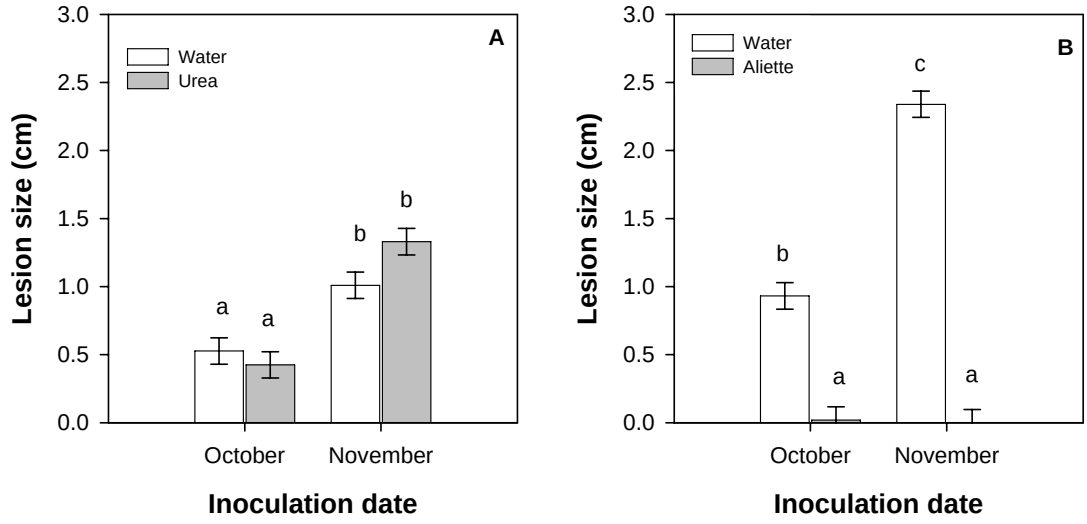

Figure 2- 5. Influence of spraying trees with (A) urea and (B) Alliette in the autumn on the size of stem lesions caused by inoculating wounded pear (*Pyrus communis*) stems with *Phytophthora syringae*. Spray treatments applied on 5 October or 13 November 2003 and stems were inoculated one week after spray treatments on 12 October (October) or 20 November (November) 2003. (A) Trees sprayed with water or 3% urea solution (Urea). (B) Trees sprayed with water or 6 g/l Alliette solution. Columns represent means (n=20) and error bars are tandard errors. Columns denoted by the same letter within a graph are not significantly different (P>0.05, $THSD_{0.05}$).

2.4.2.3 Relationships between Stem N and Lesion Size

When trees were not treated with Alliette, lesion size was positively correlated with the N concentrations in stems in November (R=0.5412, P<0.0137) but not October (R=0.3074, P=0.1874).

2.4.3 Experiment 3 (2004)

2.4.3.1 Stem N Concentration

N concentrations in stems increased during each inoculation period (e.g. from 7d before to 14 d after inoculation) (linear contrast, P<0.001) (Figure 2- 6). Spraying trees with urea increased stem N concentrations in November but not in October. In October, phosphite

sprays increased stem N concentrations in trees sprayed with urea and CuEDTA, but phosphite sprays had no influence on stem N concentrations in other treatments or at other times.

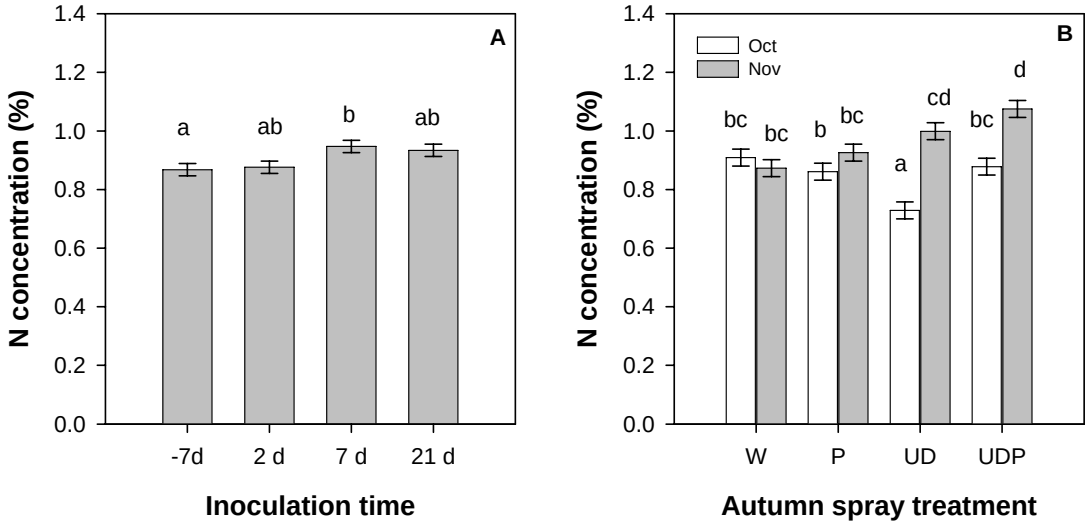

Figure 2- 6. Influemce of (A) inoculation time and (B) spray treatments in the autumn on nitrogen (N) concentration (%) in stems of pear (*Pyrus communis*) inoculated with *Phytophthora syringae*. (A) Trees inoculated 7d before (-7d), 2, 7, or 21 d after spray treatments on 22 October or 24 November 2004. (B) Trees sprayed with W, P, UD, or UDP on 22 October (Oct) or 24 November (Nov), 2004. W = trees sprayed with water at same time as other treatments. P = trees sprayed with 1% PhytoFOS solution. UD = trees sprayed a mixture of 3% urea solution and 1% CuEDTA. UDP = trees sprayed with a mixture of 3% urea solution, 1% CuEDTA solution and 1% PhytoFOS (1%). Columns represent means (A, n=32; B, n=16) and error bars are standard errors. Columns denoted by the same letter are not significantly different (P>0.05, $THSD_{0.05}$).

2.4.3.2 Size of Stem Lesions and Disease Incidence

In 2004, the same set of trees was used in both wound and non-wound inoculation with *P. syringae*. Trees inoculated with pathogen on non-wounded stems showed no symptoms of disease (data not showed). All artificial wounds inoculated with the pathogen, except

those treated with PhytoFOS before inoculation, showed signs of disease (disease incidence data not shown).

When trees were inoculated in October, time of inoculation (e.g. before or after foliar spray treatments) had no influence on lesion size (Figure 2- 7). Stem lesions were larger when trees were inoculated in November compared to October. When trees were inoculated in November, stem lesions were largest when trees were inoculated before foliar treatments. Spraying trees with urea+CuEDTA or phosphite had no influence on lesion size when stems were inoculated in October. When stems were inoculated in November, trees sprayed with urea+CuEDTA and no phosphite had smaller lesions compared to trees that were only sprayed with water. Phosphite application decreased lesion size in November but not in October.

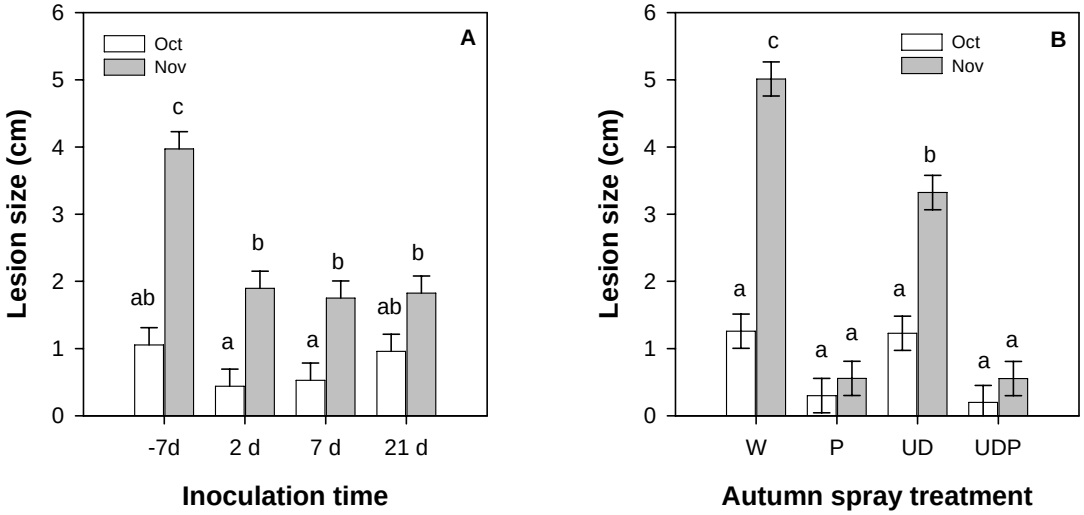

Figure 2- 7. Influence of (A) inoculation time and (B) spray treatments in the autumn on the size of stem lesions caused by inoculating wounded pear (*Pyrus communis*) stems with *Phytophthora syringae*. (A) Trees inoculated 7d before (-7d), 2, 7, or 21 d after spray treatments on 22 October (Oct) or 24 November (Nov), 2004. (B) Trees sprayed with W, P, UD, or UDP on 22 October (Oct) or 24 November (Nov), 2004. W = trees sprayed with water at same time as other treatments. P = trees sprayed with 1% PhytoFOS solution. UD = trees sprayed a mixture of 3% urea solution and 1% CuEDTA. UDP = trees sprayed with a mixture of 3% urea solution, 1% CuEDTA solution and 1% PhytoFOS (1%). Columns represent means (A, B; n=16) and error bars are standard errors. Columns denoted by the same letter are not significantly different (P>0.05, $THSD_{0.05}$).

2.4.3.3 Relationships between Stem N and Lesion Size

When trees were not treated with phosphite, there was a positive relationship between stem N concentration and lesion size but only when stems were inoculated 7 d before foliar treatments in October (R=0.7537, P<0.0210). There was no relationship between stem N concentrations and lesion size when stems were inoculated after foliar treatments or in November.

2.5 Discussion

In contrast to wounded stems, there was no sign of disease when inoculation of *P. syringae* was made on non-wound stems. Our results suggest that wounding may be essential for *P. syringae* infection of pear stems. Others have reported that *P. syringae* was unsuccessful in causing infection to uninjured bark of almond (Bostock and Doster, 1985; De Bruyn, 1924; Linderman, 1986), and *P. syringae* can infect wounds caused by handling, pruning or through leaves and leaf scars (Bostock and Doster, 1985; Pscheidt and Ocamb, 2002). In artificial inoculations of *P syringae* in rhododendrons, wounds and low temperatures were prerequisites for infection (Linderman, 1986).

Nitrogen fertigation during the growing season and spraying trees with urea in the autumn significantly influenced N concentration and total amino acids in stems of pear trees. Increasing N supply from fertigation and spraying trees with urea caused similar increases in stem N concentration in bench-grafted Fuji/M26 apple (Cheng and Fuchigami, 2002) and June-budded 'Nonpareil' on 'Nemaguard' rootstocks almond (Bi, 2004). However, the effect of CuEDTA in our experiments was not consistent; CuEDTA did not cause early defoliation in 2002 and 2003 while it effectively caused early defoliation in 2004. The efficiency of CuEDTA as a defoliant was supported by its lack of influence on stem N concentrations in 2002 and 2003. Trees sprayed with CuEDTA and trees that underwent natural defoliation (control, water application) had similar stem N concentrations in 2002 and 2003. This indicates that the defoliant did not cause premature defoliation prior to natural N translocation from leaves into tree stems.

Spraying Fuji/M26 apple trees with CuEDTA can cause >80% defoliation within 6 d of application and results in low N recovery from leaves compared to natural defoliation

(Guak et al. 2001). Similar effects of CuEDTA on defoliation and loss of N have been reported for almond nursery trees (Bi, 2004)) Two weeks after spraying almond trees with CuEDTA, trees sprayed with CuEDTA lost more leaves compared to control trees (natural defoliation) and had reduced N concentrations in roots, stems, and branches. Defoliation after spraying container-grown pear trees with CuEDTA took longer than defoliation in the experiments described above with apple and almond. With container-grown pear trees, it is possible that this longer time for defoliation resulted in mobilization of N back to stem and the other parts of the trees. Also, only N concentrations in pear stems were analyzed, it is possible that N concentrations in roots were significantly decreased by spraying trees with CuEDTA similar to the results described by Guak et al. (2001).

In 2004, the N concentrations in pear stems increased between each inoculation date and stem N concentrations in November were higher compared to October. Compared to the natural defoliation, trees sprayed with urea+CuEDTA in October had lower stem N concentrations, while trees sprayed with urea+CuEDTA in November had similar stem N concentrations as naturally defoliated trees. Spraying trees with urea+CuEDTA in October defoliated leaves before trees were sprayed with urea+CuEDTA in November. The time between October and November spray treatments may have allowed N mobilization to occur from leaves to stems, and with the additional urea from the urea+CuEDTA spray treatment resulting in higher N concentrations in stems of trees receiving urea+CuEDTA in November than October.

The influence of spraying pear trees with urea+CuEDTA on stem N concentrations in October was different than the effects of urea+CuEDTA reported for almond (Bi, 2004), and apple (Guak et al., 2001). The concentration and application differences between our

Urea+CuEDTA treatment and those of Bi (2004) and Guak et al. (2001) may account for the different responses. In our experiment, single foliar application of 3 %urea+1% CuEDTA was used. Apple trees were sprayed two times with 3% urea followed by one spray treatment of 1% CuEDTA by Guak et al. (2001) and almond trees were sprayed with 3% urea followed by a combined spray treatment of 3% urea + 1% CuEDTA by Bi (2004).

The timing of phosphite application when applied with urea+CuEDTA influenced stem N status. Application of phosphite with urea+CuEDTA increased stem N concentration when applied in October but had no influence on stem N concentrations in November. Phosphite has been reported as both a nutritional and fungicidal material (Guest and Grant, 1991; Rickard, 2000; Varadarajan et al., 2002). Phosphite is not used as a substrate for phosphate-dependant enzymes because it is not converted to phosphate in plants (Carswell et al., 1996). However, it is not known how phosphite may alter N status when applied with urea+CuEDTA. Phosphite application to citrus can cause similar increases in growth and fruit set (Lovatt, 1998) as foliar applications of urea. It is possible that the October application of phosphite may have had a growth stimulating influence on trees resulting in increased stem N concentrations. Although phosphite has been described as having low toxicity to plants (Guest and Grant, 1991), there are a number of reports of the development of phytotoxicity symptoms after foliar application of phosphite, including leaf burn on almond, and cherry trees (Wicks and Magarey, 1990; Wicks et al., 1991). It is possible that the October application of phosphite may have had a phytotoxic effect on pears, causing premature leaf senescence and early mobilization of N from leaves back into stems.

Increasing stem N concentration from fertigation increased lesion size on pear stems and there was a positive correlation between N concentration and lesion size in 2002. This

indicates that increasing N concentration in stems by increasing fertigation concentration may increase plant susceptibility to infection. There are several reports for high N fertilization rates increasing plant susceptibility to pathogen infection, including, high rates of N fertilizer increasing disease severity caused by *P. infestans* on potato (Herlihy, 1970), black shank caused by *P. parasitica* on tobacco (Apple, 1961), fire blight caused by *Erwinia amylovora* (Frecon, 1982), and *Septoria tritici* (Simon et al., 2003) and *Fusarium* head blight caused by *F. graminearum* and *F. culmorum* (Lemmens et al., 2004) on wheat.

The relationships between stem N concentration and lesion size on pear stems were not consistent between years. In 2004, there was no relationship between N concentration and lesion size. Trees sprayed with urea+CuEDTA had smaller lesions than those treated with water and similar N concentrations. Our data from 2002 and 2003 suggest that N concentration has an influence on tree susceptibility to the pathogen. It is possible that these differences between years were related to the range of N concentrations in the different experiments. For example, N concentration in 2004 varied by only 0.3% (~0.7-1.0%) while the N concentration varied by over 1.0% in 2002 (0.6-1.7%). The larger range in N concentration in pear stems in 2002 maybe the reason for the positive relationship between N concentration and disease susceptibility. It is also possible that there is a critical N concentration above which the relationship between N concentration and susceptibility does not change. For example, in both 2002 and 2003, there was little variation in lesion size when stem N concentration was greater than 1.0%. Other environmental conditions and physiology of plants at the time of inoculation may change the relationship between stem N concentration and tree susceptibility to the pathogen. Experiments in all years were done

under natural environmental conditions and differences in climate and physiology of plants between years may have caused differences in susceptibility associated with tree N status.

Interestingly, even though spraying trees with urea increased N concentrations in stems of container-grown pear in all years, lesions on trees that received urea sprays were similar in size or smaller to those on trees that were sprayed with water. This indicates that although stem N concentrations are correlated with plant susceptibility, other factors also alter plant susceptibility to *P. syringae*.

Stem lesions that developed in October were smaller compared to lesions that developed in November across all N and CuEDTA treatments. This suggests that the level of pathogen activity or host susceptibility is related to temperature. The increased activity of the pathogen coincides with the climatic conditions during the dormancy period of deciduous plants. *P. syringae* activity is restricted to cold climates (Duniway, 1983; Erwin and Ribeiro, 1996). Apple (Sewell and Wilson, 1973) and lilac (De Bruyn, 1924) were more susceptible to *P. syringae* infection during dormant period than actively growing period. The pathogen activity in apple (Sewell and Wilson, 1973) and almond (Bostock and Doster, 1985) was higher in cooler months or low temperature. This is also true for the activity of pathogen in orchard soil in south-east England which the pathogen was quiescent during warmer months and was active in cooler months (Harris, 1979). The harvesting of trees in bareroot nurseries coincides with the greatest activity of the pathogen. If infection occurs during this time, and appropriate conditions follow, the risk of infection could be high, especially during cold storage because the fungus can actively grow at low temperatures.

Alliette and PhytoFOS (phosphorous acid neutralized with potassium hydroxide) effectively controlled the infection caused by *P. syringae* when applied as a foliar spray 7 days and 2 days before inoculation occurred. The result is consistent with those reported that fosetyl-Al is a systemic fungicide with good activity against *P. cactorum* on apple (Orlikowski et al., 1986), cherry (Bielenin and Jones, 1988), peach (Thomidis and Elena, 2001) and strawberry (Ellis et al., 1998), *P.cinnamomi* on avocado (Coffey et al., 1984; Darvas et al., 1984; Pegg et al., 1987) and walnut (Matheron and Mircetich, 1985) , *P. citrophthora* on walnut (Matheron and Mircetich, 1985) ,citrus (Farih et al., 1981), and *P. syringae* in almond (Doster and Bostock, 1988). The effect of phosphorous acid against *P. cambivora* in cherry (Wicks and Hall, 1988), *P. cinnamomi* on pineapple (Rohrbach and Schenck, 1985), eucalyptus (Pilbeam et al., 2000; Wilkinson et al., 2001) and avocado (Pegg et al., 1987), *P. citrophthora* in citrus (Afek and Sztejnberg, 1989), and *P. palmivora* in cocoa (Holderness, 1990).

2.6 Conclusions

Stems inoculated in October developed smaller lesions compared to those inoculated in November regardless of foliar treatments in all 3 years of experiments. The application of urea and CuEDTA for early defoliation of pear in October may be better than in November because of the lower pathogen activity and favorable conditions for wound healing. However, trees had lower N reserves when sprayed with urea+CuEDTA in October compared to trees sprayed in November or natural defoliation; therefore spraying trees in October may have a greater influence on growth performance in the following year. Splitting two urea applications, as described for almond (Bi, 2004), with a pretreatment of

3% urea followed by the combination of urea+CuEDTA may be a more useful strategy to improve N status in October compared to a single application of urea+CuEDTA. Additionally, spraying trees with urea+CuEDTA and either Aliette or PhytoFOS in early autumn (October) can be of benefit for both early harvesting and preventing the contamination and/or infection of *P.syringae* in the field or storage.

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3 PLANT GROWTH STAGE AND ENVIRONMENT ALTER SUCEPTIBILITY OF *PYRUS COMMUNIS* TO *PHYTOPHTHORA SYRINGAE*

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3.1 Abstract

The effect of plant growth status on susceptibility of OHF97 pear rootstocks to *Phytophthora syringae* under different environmental conditions was assessed using a stem inoculation assay. In experiment 1, trees were maintained in the lath house (LH) until terminal bud set and either continued in the lath house (LH) or transferred to a greenhouse (GH) after inoculation with the pathogen at different times. The susceptibility of the plants in the GH and LH was related to temperature. Regardless of growth status the plants exposed to lower temperatures were more susceptible to *P. syringae*. Similar results were found in experiment 2 and 3 when dormant or actively growing plants were inoculated and incubated in either the LH, GH, or a 4 °C cold room (CR). Susceptibility of the trees was greatest in the CR followed by the LH and the GH. Our results indicate that disease severity was related to temperature. Dormancy of trees is not required for disease development as actively growing trees are susceptible when they are exposed to low temperatures after inoculation.

3.2 Introduction

During the winter, *Phytophthora syringae* can cause disease on bare-root deciduous nursery stock in the Pacific Northwest, especially on trees that are harvested and stored in coolers or in outdoor sawdust beds (Tidball and Linderman, 1990). *Phytophthora syringae* is most active during the cool, rainy months of the year (September to November in the northern hemisphere) (Erwin and Ribeiro, 1996; Pscheidt and Ocamb, 2002). The increased activity of the pathogen coincides with the dormancy period of deciduous plants. Thus

some researchers and growers speculate that the incidence of the disease may be related to the dormancy status of the plant, while others hypothesize that the reason for increased disease during the dormant period is due to the wet-cold environment and not the dormancy status of the plant per se.

The numerous structural and chemical changes that occur in plants during development can influence plant susceptibility to *Phytopthora* spp. Responses of plants to *Phytopthora* spp. can occur when plants are actively growing (Awan and Struchtemeyer, 1957) or when plants are dormant (Choi et al., 1992). Plant susceptibility to *P. syringae* has been shown to vary between actively growing and dormant plants, and also vary with plant tissue used for testing. For example, wound inoculation was successfully used to demonstrate *P. syringae* pathogenicity on branches and buds of dormant rhododendrons kept at 4 ^{o}C, while the attempts to infect branches and buds of actively growing plants with the pathogen failed (Linderman, 1986). Although branches and buds of actively growing plants could not be infected with the pathogen, inoculation of detached leaves from actively growing plants can cause lesions (Linderman, 1986).

Climate influences the development of diseases caused by *Phytophthora* spp. and climate constraints on certain species determine the geographic distribution of diseases they cause. The cold climates that restrict pathogenic activities by *P. cinnamomi* in soil may not restrict the activities of either *P. syringae, P. cactorum*, or several other species. The seasonal activities of host plants and the influence of physical factors of the environment determine the time course of epidemics caused by *Phytophthora* spp. (Duniway, 1983). Nearly all cankers on almond trees caused by *P. syringae* were associated with pruning wounds or injuries created during pruning in late autumn and winter, and infection was

unsuccessful on uninjured bark. The virulence of the pathogen in branch segments was ranged from 2 to 20 ^{o}C (Bostock and Doster, 1985). Fresh wounds on almond were more susceptible to infection than aged wounds, and the development of resistance to infection on bark wounds was slowed when temperatures during bark wounding were low (Doster and Bostock, 1988a).

Bare-rooted nursery plants that are defoliated prior to harvest handle and store best. Surface wounds, inflicted during harvest and leaf scars caused by artificial or natural defoliation and subsequent handling, serve as the infection openings for *P. syringae* (Bostock and Doster, 1985; De Bruyn, 1924; Pscheidt and Ocamb, 2002). Wound inoculation was used in this experiment since *P. syringae* was unsuccessful in causing infection of uninjured bark (Bostock and Doster, 1985; De Bruyn, 1924; Linderman, 1986). *Phomopsis amygdali*, causal agent of constriction cankers, initiates infections of peach twigs through fresh leaf scars in fall and bud scale scars and flowers in spring (Lalancette and Robison, 2002). In artificial inoculations of *P. syringae* in rhododendrons, wounds and low temperatures were prerequisite for infection (Linderman, 1986).

Using container grown pear trees and an *in vivo* infection assay for *P. syringae*, the specific objectives of this study were to determine whether: (1) temperature after inoculation in dormant trees altered tree susceptibility to infection and (2) temperature after inoculation in actively growing trees altered tree susceptibility to infection.

3.3 Materials and Methods

3.3.1 Experiment 1 (2003)

The influence of environment on susceptibility of pear (*Pyrus communis*) trees after terminal bud set to *Phytophthora syringae* was evaluated. To determine whether environmental conditions after inoculation influenced tree response to the pathogen in late autumn, a stem inoculation test was used to evaluate tree susceptibility to the pathogen at different times from October to December.

3.3.1.1 Plant culture

Pear OHF-97 rootstocks were planted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) on 21 May 2003. The trees were grown in a lath house at Oregon State University, Corvallis, OR (44° 30' N, 123° 17' W) and trained to a single stem. Trees were fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients (Plantex Corp., Ontario, Canada) once a week, from 6 June to 5 September 2003.

3.3.1.2 Environmental treatments after inoculation

After terminal buds had set (1 October 2003) 50 trees were selected for uniformity based on stem diameter (7-8 mm.). Ten trees were inoculated with *P. syringae* 1, 3, 5, 7, and 9 weeks after terminal buds had set and moved to one of two incubation locations (Table 3-1). Five trees were maintained at the lath house (LH, natural condition) covered with transparent plastic over the roof and the other group was moved to greenhouse (GH, 16h/8h light/dark, 21^{o}C/15 ^{o}C day/night). The light in the greenhouse was extended from

6:00 a.m. to 10:00 pm with (1000 watt) sodium vapor lamps placed 2 meters above the top of the benches.

3.3.1.3 *Phytophthora syringae* inoculum production

Stock cultures of *P. syringae* (isolated from *Kalmia latifolia* by Robert Linderman, USDA-ARS) were maintained on V8 juice agar medium in the dark at 20 ^{o}C. To prepare the medium, 4 g calcium carbonate added to 340 ml of V8 juice was heated and then filtered through cheese cloth. Then 100 ml of the filtered juice was added to 17 g of agar and distilled water added to 1 liter before autoclaving (121 °C, 20 min). Fresh cultures were prepared 7-10 d before inoculation by transferring 4 mm agar plugs to plates containing V8A medium and incubated in the dark at 20 ^{o}C.

3.3.1.4 Wound inoculation test

Stems on ten trees were inoculated in wounds (see below) with *P. syringae* on 7 October 2003, 21 October 2003, 11 November 2003, 25 November 2003, and 9 December 2003. Mycelial plugs (4mm dia.) were taken from the actively growing margin of colonies of *P. syringae* (7-10 d) growing on V8A medium. Plugs containing either the pathogen or without the pathogen were placed into a wound made with a cork borer (4 mm dia.) by removing the bark. Wounds were wrapped with paraffin after inoculation. Three wounds were made on the stem of each tree: two wounds were inoculated with *P. syringae* grown on V8 agar and one wound was inoculated with V8 agar to serve as a control.

3.3.1.5 Data Collection

Disease development (length of lesion, cm) was measured on trees 8 weeks after inoculation. Stems of trees inoculated on 7 October 2003, 21 October 2003, 11 November 2003, 25 November 2003, and 9 December 2003 were assessed on 2 December 2003, 16 December 2003, 6 January 2004, 20 January 2004, and 3 February 2004, respectively.

3.3.1.6 Statistical Analysis

The experiment was a completely randomized design with 2 factors: time of inoculation (5 dates total - 1, 3, 5, 7, 9 weeks after terminal buds had set), and incubation location after inoculation (LH, GH), Treatment combinations are shown in Table 3-1. Each treatment had 5 trees. Each tree had 2 pathogen-inoculated wounds on the same stem. Lesions did not occur on control wounds and were therefore not included in analyses. Data were analyzed using factorial regression to determine whether treatments influenced lesion size. Where indicated by ANOVA, means were separated using Tukey's Honestly Significant Difference at $P<0.05$ ($THSD_{0.05}$). Differences in lesion size over time were evaluated using linear regression method at $P<0.05$. All statistical analyses were performed with S-PLUS (MathSoft, Inc, Seattle, WA).

Table 3-1. Experimental treatments for Experiment 1 (2003)

Inoculation time [x]	Incubation location [y]
7 October 2003	GH
	LH
21 October 2003	GH
	LH
11 November 2003	GH
	LH
25 November 2003	GH
	LH
9 December 2003	GH
	LH

[x]Inoculation time = trees inoculated on different dates.
[y]Incubation condition = plants kept in LH or GH after inoculation.

3.3.2 Experiment 2 (2004)

The influence of infection environment on susceptibility of pear trees to *P. syringae* was evaluated using trees after natural defoliation maintained under different environmental conditions after inoculation. To determine whether environmental conditions after inoculation influence tree response to the pathogen in winter, a stem inoculation test was used to evaluate tree susceptibility to the pathogen in January.

3.3.2.1 Plant culture

Pear OHF-97 rootstocks were transplanted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) in 1 June 2004. The trees were grown in a lath house at OSU and trained to a single stem. Trees were fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients once a week, from 14 June to 18 August 2004.

3.3.2.2 Environmental treatments after inoculation

After terminal buds set had set (mid October) 15 trees were selected for uniformity based on stem diameter (7-8 mm) and kept in the lath house (LH, natural condition) until natural defoliation. On 22 January 2005, trees were inoculated with *P. syringae*. Five trees were either incubated at the LH, moved to the greenhouse (GH, 16h/8h light/dark, 21 ^{o}C/ 15 ^{o}C day/night, and similar conditions as described above in 3.3.1.2), or moved to a cold room (CR; 4°C, dark and each tree was covered with polyethylene bag to prevent desiccation).

3.3.2.3 *Phytophthora syringae* inoculum production

Inoculum of *P. syringae* was produced as described in 3.3.1.3, above.

3.3.2.4 Wound inoculation test

Stems on 15 trees were inoculated with *P. syringae* on 22 January 2005. Wound inoculation tests were performed as described in 3.3.1.4, above. Three wounds were made on the stem on each tree: two wounds were inoculated with *P. syringae* grown on V8 agar and one wound was inoculated with V8 agar to serve as a control.

3.3.2.5 Data Collection

Disease development (length of lesion, cm) was measured on trees 8 weeks after inoculation. Stem of trees inoculated on 22 January 2005 were assessed on 19 March 2005.

3.3.2.6 Statistical Analysis

The experiment was a completely randomized design with 3 treatments (LH, GH, and CR). Each treatment had 5 trees. Each tree had 2 pathogen-inoculated wounds on the same

stem. Lesions did not occur on control wounds and were therefore not included in analyses. Data were analyzed using analysis of variance (ANOVA) to determine whether treatments influenced lesion size. Where indicated by ANOVA means were separated using Tukey's Honestly Significant Difference at $P<0.05$ ($THSD_{0.05}$). All statistical analyses were performed with S-PLUS.

3.3.3 Experiment 3 (2004)

The influence of infection environment on susceptibility of pear trees to *P. syringae* was evaluated using actively growing trees maintained under different environmental conditions after inoculation. To determine whether environmental conditions after inoculation influence tree response to the pathogen, a stem inoculation test was used to evaluate tree susceptibility to the pathogen in September before terminal buds had set.

3.3.3.1 Plant culture

Pear OHF-97 rootstocks were transplanted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) in 1 June 2004. The trees were grown in a lath house at OSU and trained to a single stem. Trees were fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients once a week, from 14 June to 18 August 2004.

3.3.3.2 Environmental treatments after inoculation

During the actively growing period (before terminal buds had set, 13 September 2004) 12 trees were selected for uniformity based on stem diameter (6-7 mm.), inoculated with *P. syringae*, divided into three groups of 4 trees, and moved to one of three locations (LH, GH, or CR). One group was maintained in the lath house (LH, natural condition), one group

was moved to a greenhouse (GH; 16h/8h light/dark, 21^{o}C/15 ^{o}C day/night, and similar conditions as described above in 3.3.1.2), and the remaining group was moved to a cold room (CR; 4°C, dark, and each tree was covered with polyethylene bag to prevent desiccation).

3.3.3.3 *Phytophthora syringae* Inoculum Production

Inoculum of *P. syringae* was produced as described in 3.3.1.3, above.

3.3.3.4 Wound Inoculation Tests

Stems on 12 trees were inoculated with *P. syringae* on 13 September 2004. Wound inoculation tests were performed as described in 3.3.1.4, above. Three wounds were made on one stem on each tree: two wounds were inoculated with *P. syringae* grown on V8 agar and one wound was inoculated with V8 agar to serve as a control.

3.3.3.5 Data Collection

Disease development (length of lesion, cm) was measured on trees 4 weeks after inoculation. Stem of trees inoculated on 13 September 2004 were assessed on 11 October 2004.

3.3.3.6 Statistical Analysis

The experiment was a completely randomized design with 3 treatments (LH, GH, CR). Each treatment had 4 trees. Each tree had 2 pathogen-inoculated wounds on the same stem. Lesions did not occur on control wounds and were therefore not included in analyses. Data were analyzed using analysis of variance (ANOVA) to determine whether lesion size was influenced by the incubation condition. Where indicated by ANOVA, means were separated

using Tukey's Honestly Significant Difference at $P<0.05$ ($THSD_{0.05}$). All statistical analyses were performed with S-PLUS.

3.4 Results

3.4.1 Experiment 1 (2003)

3.4.1.1 Size of Stem Lesions

Stems from plants incubated in the LH after inoculation had larger lesions than stems incubated in the GH (Figure 3-1). Lesions on trees incubated in the LH increased in size with increasing time after terminal buds had set ($P<0.0001$). Stems of control (inoculated with V-8 agar) trees had no lesions (data not shown).

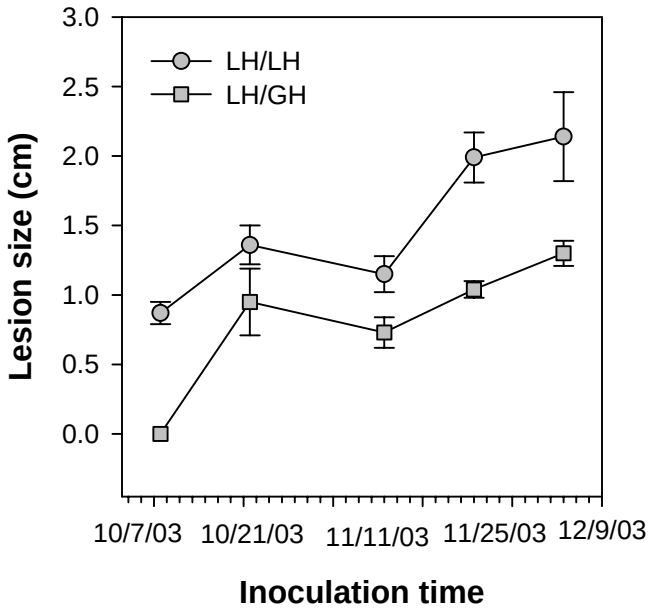

Figure 3- 1. Influence of incubation location and time of inoculation (after terminal buds had set) on the size of stem lesions caused by inoculating wounded pear (*Pyrus communis*) stems with *Phytophthora syringae*. Trees growing in lath house (LH) and incubated in greenhouse (LH/GH) and lath house (LH/LH) after inoculation. Data points represent means (n=10) and error bars are standard errors.

The maximum, minimum, and mean daily temperatures from October to January decreased gradually (Figure 3-2). The increase in lesion size on trees incubated in the LH corresponded with the decrease in temperature. There was a negative relationship between maximum, minimum, and mean temperature over an 8-wk period of incubation and lesion size (R=-0.629, R=-0.619, and R=-0.627, $P<.0001$, respectively) when trees were incubated in the LH (Figure 3- 3). There was no relationship between maximum, minimum, and mean temperature over an 8-wk period of inoculation (from 7 October 2003 to 3 February 2004) and lesion size when trees were incubated in GH after inoculation.

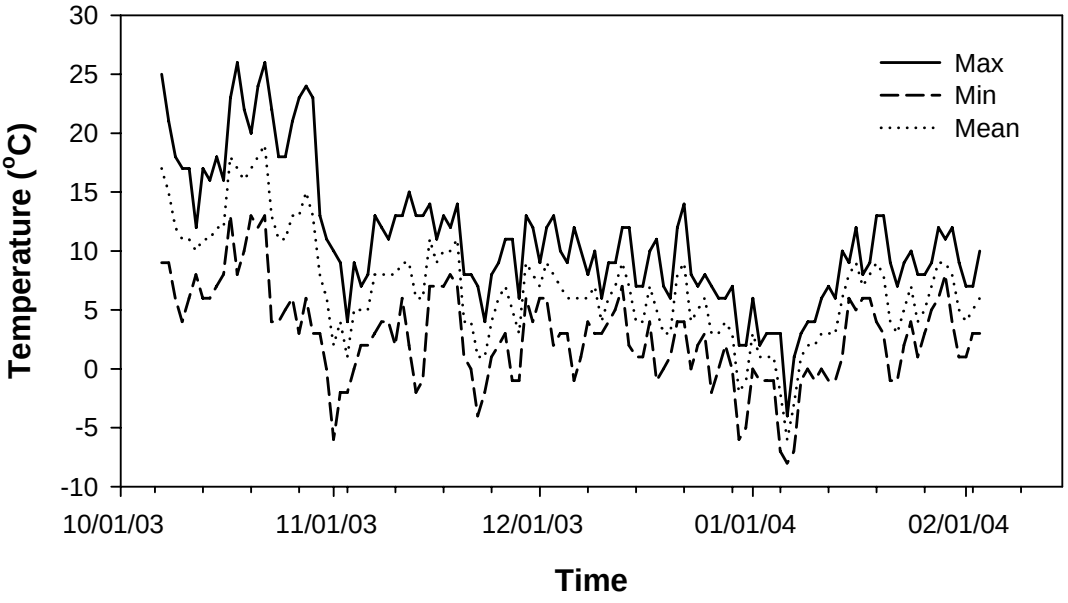

Figure 3- 2. Daily maximum (max), minimum (min), and mean temperature (°C) from the first inoculation date (7 October 2003) to the assessment date of the last inoculation (3 February 2004) under natural conditions at Corvallis, OR. Source: http://www.ocs.orst.edu (Zone 2 - Climate Data Archives, Hyslop Exp. Stn., Corvallis).

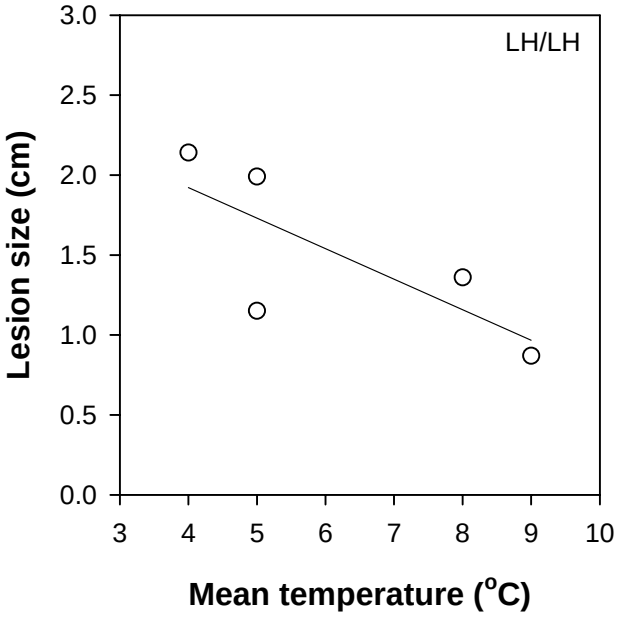

Figure 3- 3. Relationship between average daily mean temperature during incubation in lath house (LH) and lesion size (n=50) on pear (*Pyrus communis*) stems inoculated with *Phytopthora syringae*. Average maximum temperatures for each incubation time were between 7 October 2003 and 3 February 2004. Each point represents the average data of 10 lesions. Correlation between mean temperature and lesion size for LH, R=-0.627, $P<0.0001$.

3.4.2 Experiment 2 (2004)

3.4.2.1 Size of Stem Lesions

Lesion size was greatest when trees were placed in the CR for incubation and lesions were smallest on trees incubated in the GH (Figure 3- 4). Stems of control trees (inoculated with V8 agar) controls had no lesions (data not shown).

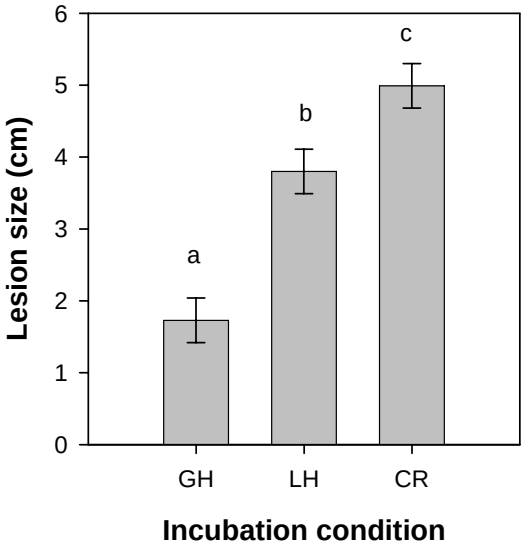

Figure 3- 4. Influence of incubation conditions on the size of stem lesions caused by inoculating wounded pear (*Pyrus communis*) stems with *Phytophthora syringae*. Trees incubated in greenhouse (GH), lath house (LH), and cold room (CR) after inoculation. Columns and data points represent means (n=10) and error bars are standard errors. Means within a graph denoted by the same letter are not significantly different (P>0.05, $THSD_{0.05}$).

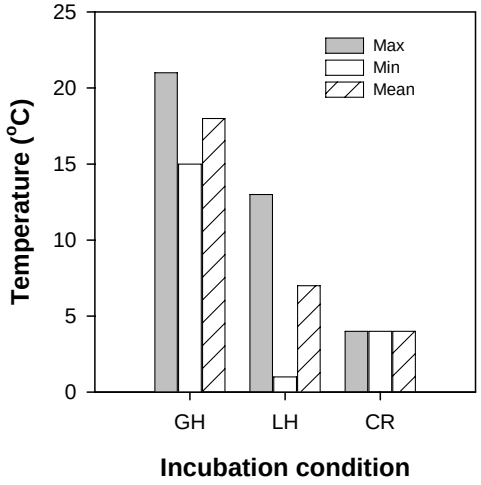

Figure 3- 5 Average daily maximum (Max), minimum (Min), and mean temperature (^{o}C) during incubation in the greenhouse (GH), lath house (LH), and cold room (CR) after inoculation with pathogen. Average temperatures between 22 January 2005 to 19 March 2005.

The temperature in the greenhouse and cold room varied less than 5 ^{o}C during incubation while in the lath house the minimum, maximum, and average daily temperature varied by almost 15 ^{o}C (Figure 3- 5) and gradually increased throughout the incubation (Figure 3- 6).

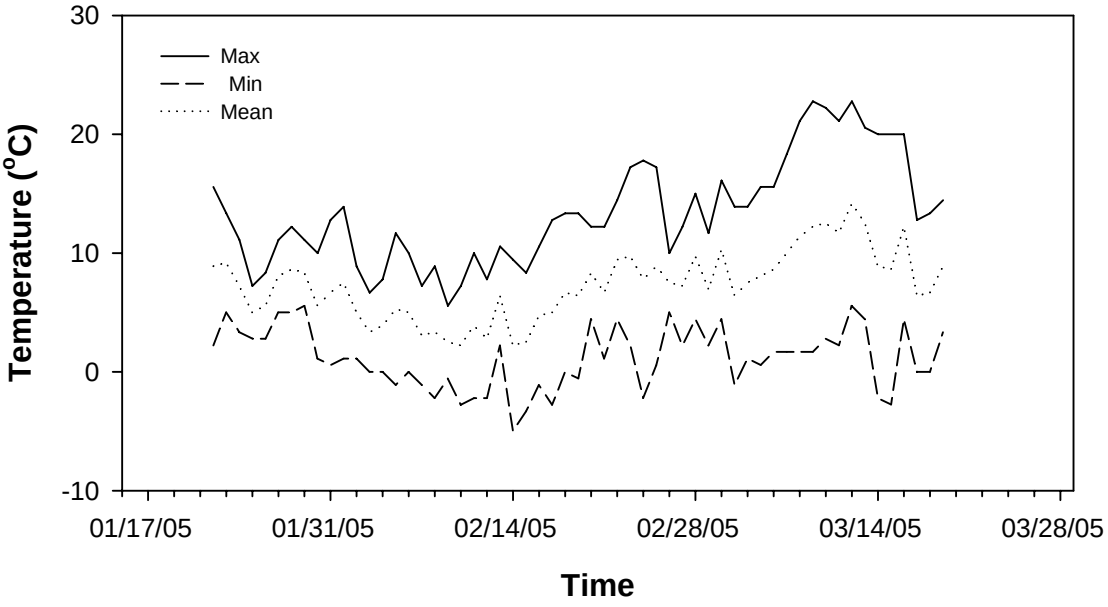

Figure 3- 6. Daily maximum (max), minimum (min), and mean temperature (°C) from the inoculation date to the assessment date (22 January 2005 to 19 March 2005) under natural conditions at Corvallis, OR. Source: http://www.ocs.orst.edu (Zone 2 - Climate Data Archives, Hyslop Exp. Stn., Corvallis)

3.4.3 Experiment 3 (2004)

3.4.3.1 Size of Stem Lesions

Lesion size was greatest when trees were incubated in the CR compared to trees incubated in either the LH or the GH (Figure 3- 7). Lesions on trees incubated in the LH

were similar in size to lesions on trees incubated in the GH. All plants inoculated with V-8 agar controls showed no infection (data not shown).

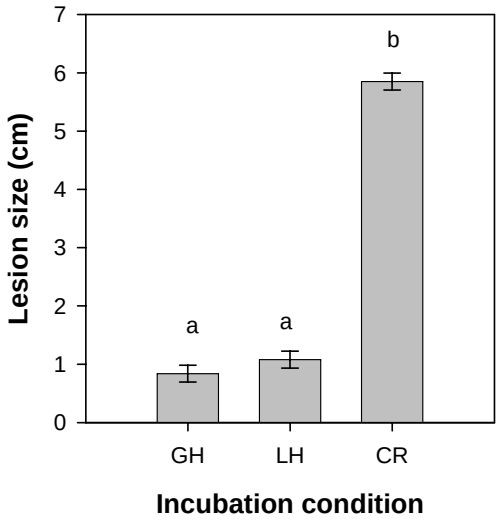

Figure 3- 7. Size of stem lesions caused by inoculating wounded pear (*Pyrus communis*) stems with *Phytophthora syringae* and incubating trees in the greenhouse (GH), lath house (LH), or cold room (CR) after inoculation. Columns and data points represent means (A, n=4) and error bars are standard errors. (A) Means denoted by the same letter are not significantly different (P>0.05, $THSD_{0.05}$).

The temperature in the CR and GH were 4 ^{o}C and 21 ^{o}C/15 ^{o}C (day/night), respectively, and the difference between minimum and maximum temperatures was less than 5 ^{o}C during incubation (Figure 3- 8). The minimum, maximum, and average daily temperature in the LH after inoculation was 21, 12, and 16 ^{o}C, respectively and the temperature remained relatively the constant during incubation (mean, maximum, and average temperatures varied by less than 5 ^{o}C) (Figure 3- 9).

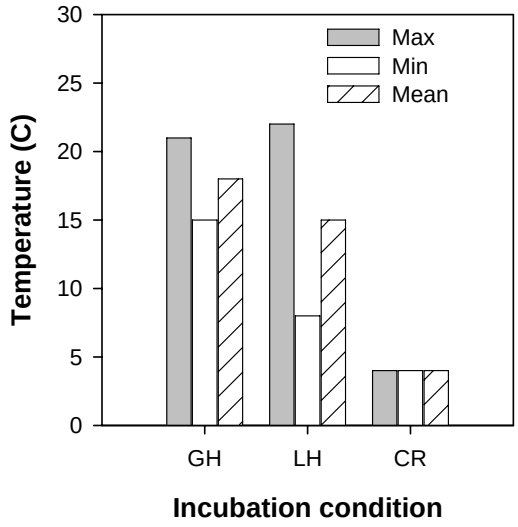

Figure 3- 8 Average of daily maximum (Max), minimum (Min), and mean temperature (^{o}C) during incubation in the greenhouse (GH), lath house (LH), and cold room (CR) after inoculation with pathogen. Average temperatures between 13 September 2004 and 11 October 2004.

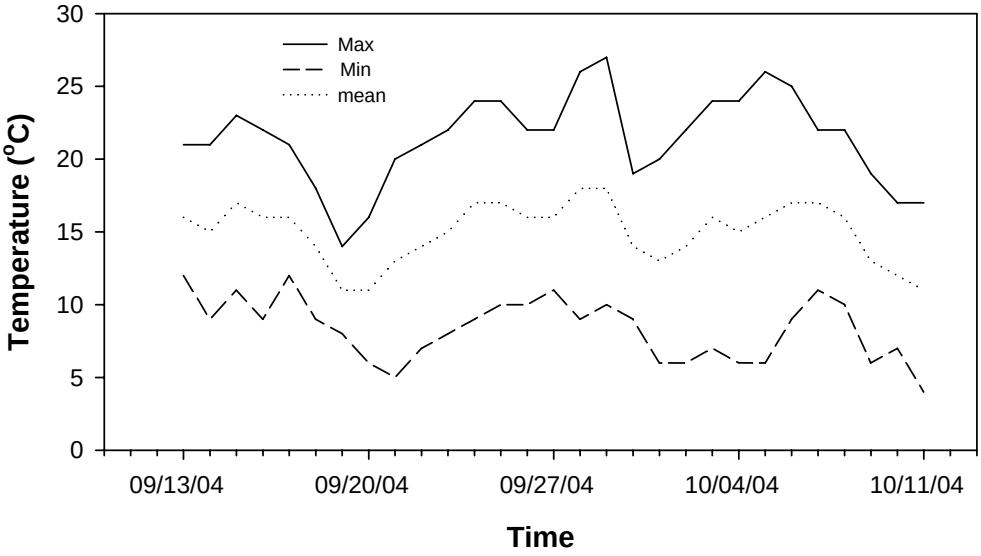

Figure 3- 9. Daily maximum (max), minimum (min), and mean temperature (°C) from the time of inoculation (13 September 2004) to the lesion assessment (11 October 2004) under natural conditions at Corvallis, OR. Source: http://www.ocs.orst.edu (Zone 2 - Climate Data Archives, Hyslop Exp. Stn., Corvallis).

3.5 Discussion

3.5.1 Effect of environment on disease caused by *P. syringae*

Susceptibility of pear trees in the autumn to infection by *P. syringae* was influenced by environmental differences after inoculation with the pathogen. For example, in experiment 1, the colder incubation conditions in the lath house favored disease development over the warmer incubation conditions in the greenhouse. The negative correlation between temperature and lesion size when trees were incubated in the lath house also suggests that the decreasing temperatures from October to December after exposure to the pathogen increases tree susceptibility. In contrast plants incubated in the warm greenhouse after inoculation developed significantly less disease throughout the treatment period. Our results suggest *P. syringae* activity and/or plant susceptibility increases with decreasing temperature. Our results also indicate that the negative relationship between temperature and disease exists regardless of plant growth status, since both actively growing trees (experiment 3) and dormant trees (experiment 2) showed similar relationships between lesion size and temperature after exposure to the pathogen.

The influence of temperature on plant disease development has been reported in *Phytophthora* species. *Phytophthora syringae* can actively grow under cold and wet conditions (Pscheidt and Ocamb, 2002). Cankers on almond caused by *P. syringae* are observed soon after pruning in the autumn and winter when temperatures are relative low (mean daily temperature usually 5-15 ^{o}C (Doster and Bostock, 1988a). Excised branch pieces of almond inoculated with *P. syringae* developed cankers at temperatures between 2 ^{o}C and 20 ^{o}C but not at 27 ^{o}C. Isolation of the pathogen during late spring and summer from stems of almond was not successful (Bostock and Doster, 1985). Suppression of *P.*

cinnamomi on avocado seedlings has been correlated with temperature influences on mycelium growth (Zentmyer, 1980). In experiment 2, lesion size on trees incubated under the cooler conditions in the lath house (max, min, and mean temperature = 13 ^{o}C, 1 ^{o}C, and 7 ^{o}C, respectively) was greater compared to trees incubated in warmer conditions in the greenhouse (max, min, and mean temperature = 21 ^{o}C, 15 ^{o}C, and 18 ^{o}C, respectively). In experiment 3, temperatures during incubation and lesion size were similar between trees incubated in the lath house (max, min, and mean temprature = 22 ^{o}C, 8 ^{o}C, and 15 ^{o}C, respectively) and trees incubated in the greenhouse (max, min, and mean temperature = 21 ^{o}C, 15 ^{o}C, and 18 ^{o}C, respectively). Different temperatures during incubation conditions may have resulted in the differences in lesion size. It is possible that the higher temperatures in the greenhouse in experiment 2 and in the greenhouse and lath house in experiment 3 were not favorable for the pathogen (Bostock and Doster, 1985).

Higher temperatures in the greenhouse in experiment 2 and in the greenhouse and lath house in experiment 3 may also have promoted the stem wound to heal thus decreasing the ability of the pathogen to penetrate the stem. The development of wound resistance is related to the formation of wound periderm or the infusion of lignin, suberin, waxes, and/or wound gums in the layers of cells immediately subtending the wound surface. Resistance of wounds to fungal infection increases as wounds age (Biggs, 1986; Bostock and Middleton, 1987; Bostock and Stermer, 1989; Doster and Bostock, 1988b) and the rate of deposition of lignin and suberin in the wound depends on the time of the year (Bostock and Stermer, 1989), and temperature (Doster and Bostock, 1988a) when wounds are inflicted. Wound cork layers on apple bark form more quickly in summer compared to winter (Tamura and

Saito, 1982) and low temperature slows down lignin and suberin formation in almond stem wounds, resulting in low disease resistance (Doster and Bostock, 1988a).

The results from Experiment 1, 2, and 3 clearly indicated that *P. syringae* causes more disease on pear trees under lower temperatures compared to higher temperatures and confirms the findings of others who have reported a relationship between low temperature and infection by *P. syringae* in the field (Bostock and Doster, 1985; Doster and Bostock, 1988a; Edney, 1978; Pscheidt and Ocamb, 2002).

3.5.2 Effect of growth status on disease caused by *P. syringae*

Both actively growing (Experiment 3) and dormant (Experiment 2) pear trees were susceptible to *P. syringae* when incubated in CR condition. The data suggested that under low temperature that favorable for the activity of the pathogen all trees were susceptible to infection.

During production, it is probably impossible to separate the effects of temperature on plant growth status and tree susceptibility to *P. syringae*. Trees may be exposed to the pathogen when they are actively growing (as in experiment 3), or during dormancy (as in experiment 1 and 2). In all cases the temperatures after exposure to the pathogen appear to be extremely important for disease development; however, our results indicate the magnitude of the response of trees to the pathogen in low temperature varies depending upon the growth status of the tree.

Our experiments support previous reports of *P. syringae* actively causing disease in cold conditions (Bostock and Doster, 1985; Doster and Bostock, 1988a; Edney, 1978; Pscheidt and Ocamb, 2002). With pear trees, we also determined that the pathogen can cause disease in both actively growing and dormant plants if the temperatures after

inoculation are conducive to pathogen activity and when temperatures increase tree susceptibility. The seasonal activities of the host and the influence of physical factors of the environment determine the time course of epidemics caused by *Phytophthora* spp. (Duniway, 1983). In rhododendrons (Linderman, 1986), infection by *P. syringae* only occurred when dormant portions of the plants were inoculated and infection failed to occur when actively growing portions of the plants were inoculated during, even under low temperature. *Phytophthora syringae* was not able to inflict lilac (De Bruyn, 1924), apple (Sewell and Wilson, 1973; Sewell et al., 1974), and almond (Bostock and Doster, 1985) during summer. Dormant lilac plants were more susceptible to *P. syringae* than actively growing plants (De Bruyn, 1924; Sewell and Wilson, 1973). Under natural condition, the lack of disease caused by *P. syringae* in summer and susceptibility of dormant plant tissue may be due to the coincidence of high temperature in promoting plant growth and suppressing pathogen activity and the coincidence of low temperature in promoting dormancy and increased pathogen activity. Both actively growing and dormant plants were susceptible to *P. syringae* infection when they were incubated in the cold room in our experiment. These data suggest that temperature during incubation was more important than plant growth status.

3.6 Conclusions

Susceptibility of pear trees to *P. syringae* varies between actively growing and dormant trees, and incubation conditions, particularly temperature, after exposure of trees to the pathogen is important for disease development regardless of plant growth stage. Under

pathogen-favorable conditions, *P. syringae* can infect stems of both actively growing and dormant pear trees. This information can be useful in nursery management. In areas infested with *P.syringae* or during cold storage when the potential for exposure to the pathogen is high, both dormant plants and actively growing plants should be inspected to prevent and/or reduce the build up of inoculum to avoid epidemics

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4 THE EFFECT OF CHEMICAL AND BIOLOGICAL CONTROL AGENTS ON *PHYTOPHTHORA SYRINGAE* INFECTION

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4.1 Abstract

The influence of chemical and biological control treatments on susceptibility of pear (*Pyrus communis*) OHF-97 rootstocks to *Phytophthora syringae* was assessed using a stem assay. Plants were treated with a single foliar application of the phosphonate-containing fungicides -Aliette (6g/l), Biophos (3%) and PhytoFOS (1%), Dimethomorph -Ardent™ 50 WP (0.5g/l), or biological agents-Biocure (3g/l), Biocoat (3g/l), and Companion (0.3%) in the autumn after terminal buds had set. Two weeks after disease control treatments were applied, stems were inoculated with *P. syringae*. Eight weeks after inoculation disease incidence and lesion size were measured. Aliette, Biophos, and PhytoFOS effectively prevented infection by *P. syringae*. Dimethomorph, Biocure, Biocoat, and Companion had no effect in preventing disease. The application of Aliette, Biophos, and PhytoFOS before harvesting deciduous nursery stock could prevent *P. syringae* infection through the wounds inflicted during defoliation, harvesting and handling procedures.

4.2 Introduction

Diseases caused by species in the genus *Phytophthora* are responsible for significant economic losses on a wide range of host plants, including pear, *Pyrus communis* (Erwin and Ribeiro, 1996; Wormald, 1919). *P. syringae* occurs as a winter disease of nursery stock in the Pacific Northwest, especially on deciduous trees that are harvested and stored in coolers or in outdoor sawdust beds (Tidball and Linderman, 1990).

The disease caused by *P. syringae*, can be controlled using a combination of good nursery sanitation practices and chemical applications. Phosphonate-containing fungicides,

including fosetyl-Al (Aliette and its breakdown product, phosphorous acid (also referred to as phosphite) are commonly used to control *Phytophthora* species. (Doster and Bostock, 1988a; Erwin and Ribeiro, 1996). It is applied to plants as either sprays, drenches, dips, or by injection (Erwin and Ribeiro, 1996; Pegg et al., 1987; Quimete and Coffey, 1989; Tidball and Linderman, 1990). Fosetyl-Al translocates both upward and downward (in the transpiration stream and in the phloem sap) and may have direct toxicity against *Phytophthora* species (Erwin and Ribeiro, 1996).

The chemical dimethomorph has also been report(Cohen et al., 1995)ed as an effective fungicide in control disease caused by oomycetes (Albert et al., 1988; Cohen et al., 1995). *In vitro* activity of dimethomorph prevents sporangium formation, reduces the duration of zoospore motility, and germination of encysted zoospores of *P. capsici*, *P. citrophthora*, and *P. parasitica* (Matheron and Porchas, 2000). *In vivo*, dimethomorph has been reported to suppress canker development on citrus bark after inoculation with *P. citrophthora* or *P. nicotianae* (Matheron, 2002). The combination of dimethomorph-mancozeb can suppress late blight, caused by *P. infestans*, in petunias and tomatoes (Becktell et al., 2005). However, while application of dimethomorph to potato leaf discs 24 or 46 h before inoculation with *P. infestans* completely inhibits symptom incidence, application after inoculation generally has no influence on disease (Stein and Kirk, 2003).

The potential carcinogenicity and high costs of fungicides have resulted in trends towards production practices that reduce pesticide use. In response to an increased demand for alternatives to chemical control measures, several biological agents have been evaluated as safer alternatives to the use of synthetic, chemical fungicides. Bacterial antagonists are common components of biological disease control strategies. Various microbial antagonists

have been shown to interfere with the growth of plant pathogens, but only some of these microorganisms have been developed into commercial products, such as isolates of the bacteria: *Agrobacterium radibacter* K-84, *Pseudomonas fluorescens* and *Bacillus subtilis* (Agrios, 1997). *Bacillus subtilis* Cot1 has been reported to prevent *Phytophthora* and *Pythium* damping-off of *Astilbe*, *Photinia*, and *Hemerocallis* microplants and conventional *Brassica* seedlings under high humidity conditions in fogging glasshouses (Berger et al., 1996). Strains EBW-4, AB8, BACTX, NZB1, AB7, AB8, and BACT2 of *B. subtilis* can significantly reduce the number of apple trees infected by *P. cactorum* (Utkhede et al., 2001; Utkhede and Smith, 1991).

The primary sources of *P. syringae* innoculum in nursery production of pear trees are on infected rootstocks placed either in outdoor sawdust beds or into cold storage (Pscheidt and Ocamb, 2002). Growers commonly apply fungicides during the autumn to prevent infection by *P. syringae*. Using an *in vivo* infection assay, the objective of this study was to compare the efficacy of chemicals and biocontrol agents in controlling infection by *P. syringae* on pear trees.

4.3 Materials and Methods

The ability of several chemical and biological agents to prevent infection of *Phytophthora syringae* was evaluated on pear trees after terminal buds had set using a stem inoculation assay to assess plant susceptibility to the pathogen after application of chemical or biological control products.

4.3.1 Plant culture

Pear (*Pyrus communis*) OHF-97 rootstocks were planted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) in late March (26 March 2002). Trees were grown in a lath house at OSU (44^{o} 30' N, 123 ^{o} 17' W), USA, trained to a single stem and fertigated with 150 ppm N using Plantex® 20-20-20 with micronutrients (Plantex Corp., Ontario, Canada) once per week from 18 June 2002 to 10 September 2002.

4.3.2 Disease control treatments

In October 2002, 80 trees were selected for uniformity based on stem diameter (7-8 mm) and were randomly divided into eight groups. On 15 October, 2002 after natural cessation of plant growth (terminal buds had set), ten trees were sprayed with one of seven chemical or biological control products or water (Table 4- 1) until the chemicals ran off the foliage. All products were applied at manufacturers' suggested rates.

4.3.3 *Phytophthora syringae* inoculum production

Stock cultures of *P. syringae* (isolated from *Kalmia latifolia* by Robert Linderman, USDA-ARS) were maintained on V8 juice agar medium in the dark at 20 ^{o}C. To prepare the medium, 4 g calcium carbonate added to 340 ml of V8 juice was heated and then filtered through cheese cloth. Then 100 ml of the filtered juice was added to 17g of agar and distilled water added to 1 liter before autoclaving (121 °C, 20 min). Fresh cultures were prepared 7-10 d before inoculation by transferring 4 mm agar plugs to plates containing V8A medium and incubated in the dark at 20 ^{o}C.

4.3.4 Wound inoculation test

Stems on 10 trees in each treatment were inoculated with the pathogen on 29 October 2002 (2 weeks after spray treatments). Mycelial plugs (4 mm dia.) were taken from the actively growing margin of colonies of *P. syringae* (7-10 d) growing on V8A medium. Plugs containing either the pathogen or without the pathogen were placed into a wound made with a cork borer (4 mm dia.) by removing the bark. Wounds were wrapped with paraffin after inoculation. Two wounds were made on the stem of each tree: one wound was inoculated with *P. syringae* grown on V8 agar and the other wound was inoculated with V8 agar to serve as a control.

4.3.5 Data Collection and Statistical Analyses

Disease incidence (% of stems with lesions) and disease development (length of lesion, cm) were measured on trees on 24 December, 2002 (8 weeks after inoculation).

The experiment was a completely randomized design with 8 treatments (Table 4- 1) and 10 replications (trees) per treatment. Lesions did not occur on control wounds (wounds inoculated with agar plugs without the pathogen) and therefore data from these wounds were not included in analyses. Data were analyzed using analysis of Kruskal-Wallis ANOVA by Ranks to determine whether lesion size was influenced by chemical or biological control products and treatment comparisons were made at $P<0.05$ ($K\text{-}W_{0.05}$). All statistical analyses were performed with S-PLUS (MathSoft, Inc, Seattle, WA).

Table 4- 1. Disease control products and application rates

Product	Type	Detail and Source	Rate
Water	Control	-	-
Ardent™ 50 WP	Chemical	Dimethomorph. Commerically available from SePRO Corporation.	0.5 g /l
Biophos	Chemical	Dipotassium phosphonate 15.1%; Inert ingredient 69.8%; Experimental product from ECO-RIGHT, LLC.	3% by vol.
Phyto FOS	Chemical	Phosphorous acid neutralized with potassium hydroxide. Soluble Potash (K_2O) 18% derived from Potassium phosphite. Commercially available from SIPCAM AGRO USA, Inc.	1% by vol.
Aliette WDG	Chemical	Fosetyl-Al. Commercially available from Bayer	6 g /l
Companion	Biological	*Bacillus subtilis*. Commerically available from Growth Product, Ltd.	0.3% by vol.
Biocure	Chemical + Biological	Protected composition. Experimental product from Michael Wisnieski	3 g /l
Biocoat	Chemical + Biological	Protected composition. Experimental product from Michael Wisnieski	3 g /l

4.4 Results

Stems inoculated with *P. syringae* showed no sign of disease when trees were treated with Alliette WDG, Biophos, or Phyto FOS (Table 4- 2). In contrast, the size of lesions on trees treated with Biocure, Biocoat, Ardent™ 50 WP and Companion were similar to lesions on trees sprayed with water. Plants inoculated with V-8 agar plugs without the pathogen showed no infection (data not shown).

Table 4- 2. Size of stem lesions on pear trees sprayed with disease control products and inoculated with *Phytophthora syringae*.

Disease Control Product[z]	Lesion size (cm)[y]	
Control	2.0±0.2	b
Alliette	0.0	a
Biophos	0.0	a
PhytoFOS	0.0	a
Biocure	1.4±0.2	b
Biocoat	1.7±0.2	b
Ardent™ 50 WP (Dimethomorph)	2.4±0.5	b
Companion	3.0±0.7	b

[z]Single spray application of products on 15 October 15 2002. Stems were inoculated on 29 October 2002. Lesion size was evaluated on 24 December, 2002.
[y]Means followed by the same letter are not significantly different ($K\text{-}W_{0.05}$)
[x]Standard error (n=10).

4.5 Discussion

Alliette, Biophos and PhytoFOS effectively controlled infection of *P. syringae* when applied 2 weeks before inoculation. The disease control we obtained using these products confirms the findings of others who have reported that phosphonate-containing fungicides and their breakdown product, phosphorous acid, can control *Phytophthora* species (Cohen and Coffey, 1986; Doster and Bostock, 1988b; Erwin and Ribeiro, 1996) when applied to plants as either sprays, drenches, dips, or through injection systems (Erwin and Ribeiro, 1996; Pegg et al., 1987; Quimete and Coffey, 1989; Tidball and Linderman, 1990). Fosetyl-Al is a systemic fungicide with good activity against *P. cactorum* on apple, cherry, peach, and strawberry (Ellis et al., 1998), *P.cinnamomi* on avocado and walnut (Matheron and Mircetich, 1985), *P. citrophthora* on walnut and citrus (Farih et al., 1981), and *P. syringae* on almond (Doster and Bostock, 1988a). Phosphorous acid has also been reported to be effective against *Phytophthora cambivora* on cherry (Wicks and Hall, 1988), *P.*

cinnamomi on pineapple, eucalyptus, and avocado (Pegg et al., 1987), and *P. palmivora*.in cocao (Holderness, 1990). Surface wounds inflicted during harvest and leaf scars caused by artificial or natural defoliation and subsequent handling serve as the infection openings for *P. syringae* (Bostock and Doster, 1985; De Bruyn, 1924; Pscheidt and Ocamb, 2002). Application of Alliette, Biophos, PhytoFOS, or similar products prior to defoliation and harvesting may help decrease infection by *P. syringae* on pear trees.

In our assay, spraying the pear trees with Ardent was not effective in controlling infection of *P. syringae* on pear stems. Others have reported that dimethomorph, as a foliar spray, is ineffective in controlling *P. cinnamomi* stem infection of *Rhododendron ponticum*, *Eucalyptus sieberi*, and *Leucodendron salignum x L. leureolum*, while application of dimethomorph using soil drenches can effectively control infection (Marks and Smith, 1990). However, dimethomorph applied as a trunk paint or soil drench was not effective for the control of *P. cactorum* in peach tree (Thomidis and Elena, 2000). However the *in vitro* activity of dimethomorph prevents sporangium formation, reduces the duration of zoospore motility, and germination of encysted zoospores of *P. capsici*, *P. citrophthora*, and *P. parasitica* (Cohen et al., 1995; Matheron, 2002; Matheron and Porchas, 2000). Dimethomorph failed to control *P. syringae* infection of pear stems in our study, possibly because dimethomorph may not have translocated from one leaf to another in either acropetal or basipetal direction (Cohen et al., 1995) and/or we did not apply dimethomorph at a concentration effective at inhibiting *P. syringae* as mycelium.

None of the biological agents evaluated in our study effectively controlled infection of pear stems by *P. syringae*. Others have reported that certain biological agents, such as *Bacillus subtilis*, are not effective in controlling *P. infestans* in petunia and tomato under

greenhouse conditions (Becktell et al., 2005), *Phytophthora* spp. in *Daphne blayana* (Li et al., 1998), and *P. cactorum* in micropropagated strawberry (Vestberg et al., 2004). However several biological agents are reported to be effective against diseases caused by *Phytopthora* spp. (Berger et al., 1996), High level of *B. subtilis* Cot1 was required to suppress *Phytophthora* and *Pythium* in *Aster* and *Hemerocallis* (Li et al., 1998). Environmental conditions influenced *B. subtilis* survival (Reddy and Rahe, 1989), reproduction, and antifungal production (Gupta and Utkhede, 1986). The degree of growth inhibition of *B. subtilis* depended on the challenge pathogens (Kudryashova et al., 2005). The isolation and/or formulation of commercial products of *B. subtilis* varied in efficacy in controlling *P. infestans* (Stephan et al., 2005). The inability of biological agents to control *P. syringae* infection of pear stems in our study may be a result of low populations of *B. subtilis* on stems. Low populations of the biological agent could be caused by (1) using too low of an application rate, (2) not enough time between application of the biological agent and exposure to the pathogen, (3) unfavorable conditions for survival, reproduction, or antifungal production by *B. subtilis*, (4) incompatibility for the specific isolate(s) in the products to inhibit the growth of specific isolate of *P. syringae*.

4.6 Conclusions

During nursery production, bareroot pear trees and rootstocks are defoliated before harvesting and storage (Frecon, 1982). The application of Aliette, Biophos or PhytoFOS after terminal buds have set but before defoliation may prevent *P. syringae* infection through surface wounds inflicted during defoliation, harvesting and grading operations.

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5 WOUND AGE ALTERS SUSCEPTIBILITY OF *PYRUS COMMUNIS* TO *PHYTOPHTHORA SYRINGAE*

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5.1 Abstract

The effect of spraying trees with combination of urea and copper-EDTA (Urea+CuEDTA) in the autumn and wound age on susceptibility of pear (*Pyrus communis*) OHF-97 rootstocks to *Phytophthora syringae* under different environmental conditions was assessed using a stem assay. In the autumn, after terminal buds had set, trees were sprayed with water or Urea+CuEDTA. Starting 1 week after spray treatments, wounds were made on stems every 7 d for 5 weeks. Seven weeks after spray treatments, stem wounds were inoculated with *P. syringae* and trees were kept under natural conditions or moved to a cold room. Eight weeks after inoculation, disease incidence and lesion size were measured. Disease incidence and lesion size decreased with increasing wound age. Stems on trees sprayed with Urea+CuEDTA had similar disease incidence and lesion size compared to trees sprayed with water regardless of where trees were incubated after inoculation. Disease incidence and lesion size were similar on trees kept under natural conditions or moved to a cold room. In general, inoculation of 0- and 1-day-old wounds resulted in higher disease incidence and larger stem lesions compared to inoculation of older wounds. Surface wounds inflicted during harvest and leaf scars caused by chemical defoliation or natural defoliation may serve as the infection openings for *P. syringae*; however our data indicate the length of time between wound formation and exposure to the pathogen dictates whether infection will occur.

5.2 Introduction

Diseases caused by species in the genus *Phytophthora* are responsible for significant economic losses on a wide range of host plants, including pear, *Pyrus communis* (Erwin and Ribeiro, 1996; Harris, 1979; Wormald, 1919). *Phytophthora syringae* activity is restricted to cold climates (Erwin and Ribeiro, 1996; Pscheidt and Ocamb, 2002) and occurs as a winter disease of nursery stock in the Pacific Northwest, especially on trees that are harvested and stored in coolers or in outdoor sawdust beds (Tidball and Linderman, 1990; Young and Milbrath, 1959).

Bare-rooted nursery plants are defoliated prior to harvest for best handling and storage. Most pear varieties in the Pacific Nortwest are propagated on selected rootstock. All pear trees and rootstocks are defoliated before harvesting and storage like many other deciduous nursery trees (Frecon, 1982). Surface wounds inflicted during harvest and leaf scars caused by artificial or natural defoliation and subsequent handling serve as the infection openings for *P. syringae* (Bostock and Doster, 1985; De Bruyn, 1924; Pscheidt and Ocamb, 2002). *Phytophthora syringae* can infect wounds caused by handling or pruning or through leaves and leaf scars (Pscheidt and Ocamb, 2002) but is unsuccessful in causing infection of uninjured bark (Bostock and Doster, 1985; De Bruyn, 1924; Linderman, 1986). In artificial inoculations of rhododendron with *P. syringae*, wounds and low temperatures are prerequisite for infection (Linderman, 1986). The combination of host predisposition and favorable, cold-wet conditions in cold room or outdoors for pathogen activity increases the potential for severe damage to occur. The primary sources of *P. syringae* inoculum in nursery production of pear trees are in outdoor sawdust beds and on infected rootstocks placed into cold storage (Pscheidt and Ocamb, 2002). In nursery stock, dark, slightly

sunken cankers occur on stems and in severe cases the stem is girdled (Erwin and Ribeiro, 1996; Young and Milbrath, 1959).

Methods used for defoliation include: manual or mechanical removal of leaves, and application of chemicals that result in early abscission of leaves. Early chemical induced defoliation of deciduous plants decreases the amount of N mobilization from leaves to stems and roots in the autumn (Bi, 2004; Guak et al., 2001), thus can reduce nitrogen reserves required for growth the following spring (Guak et al., 2001). Copper chelate (CuEDTA), a chemical defoliant commonly used in nursery production of deciduous trees, results in inefficient N mobilization from leaves in deciduous fruit nursery plants (Guak et al., 2001). Spraying trees with urea in autumn is a practice currently used by growers of deciduous nursery trees to increase N storage. The amount of N used for new shoot and leaf growth of young Fuji/M.26 apple trees is positively correlated with total amount of N accumulated in the plant during the previous year (Bi, 2004; Dong et al., 2004). Spraying pear nursery trees with urea in the autumn before cold storage can increase the total amount of N in plants (Dong et al., 2001) by rapid conversion to amino acids and transport to the stems and roots. Spraying trees with a combination of urea and CuEDTA in the autumn can be used to obtain efficient early defoliation and promote N storage without reducing plant growth performance the following year (Bi, 2004; Guak et al., 2001).

Recent studies have shown that OHF97 pear plants sprayed with urea and chemical defoliants suffer severe stem lesions caused by *P. syringae* (personal communication, Shufu Dong). In this case, it is possible that the combination of urea and defoliant treatment predisposes the plants to *P. syringae*. The effect of abundant N can make plants more susceptible to pathogens and susceptible for a longer time (Frecon, 1982; Simon et al.,

2003). Although spraying pear nursery plants with urea may improve plant N status, the resulting increase in N may make plants more susceptible to *P. syringae.* This increased susceptibility to the pathogen could be a result of a combination of factors including wounding tissues prone to infection (e.g. stems, leaves, or abscission zone) and/ or increasing the N status of the plants.

Most plant species sprayed with urea and defoliants are not affected by *P. syringae* and nurserymen report that the early defoliation of plants treated with the combination of urea and defoliant may actually benefit from the treatment and reduce the incidence of diseases (personal communication, Lance Lyon, Femrite Nursery). This may be the result of more rapid healing of leaf scars prior to the cold wet conditions due to the early defoliation when conditions favor wound healing or by enabling the early harvest and storage of plants prior to the onset of conditions that promote the activity and subsequent plant infection by *P. syringae*. The effect of urea and defoliants on disease incidence caused by *P. syringae* appears to be species-specific and may be related to the timing of application.

Phytophthora syringae infection of bare-rooted nursery plants during cold storage can result from subjecting plants to environmental conditions that favor the growth of the pathogen and/or are unfavorable to maintaining optimal physiological condition of the plant. In addition, the occurrence of *P. syringae* infection might be favored by the physiological or physical condition of the plant at the time of harvesting and subsequent handling and storage. In the first instance, plants that are harvested before they are physiologically mature (dormant) may be more susceptible to *P. syringae*. In the latter case, surface wounds inflicted by either the urea and defoliant treatment directly or indirectly through the leaf scars caused by the defoliation and the damage caused by the

harvesting and handling procedures of the nurserymen may serve as the infection openings for *P. syringae*.

Phytophthora syringae is a serious problem in Pacific Northwest deciduous plant nurseries. With an increased use of urea and defoliants in nursery production of deciduous trees, a better understanding of the relationship between urea and defoliants and the susceptibility of plants to *P. syringae* is needed. Using container grown pear trees and an *in vivo* infection assay for *P. syringae*, the specific objectives of this study were to determine whether susceptibility of pear stems to the pathogen is altered by: (1) spraying trees with urea and CuEDTA; (2) incubation conditions after exposure to the pathogen; or (3) wound age.

5.3 Materials and Methods

The influence of combination of urea and CuEDTA (Urea+CuEDTA) and wound age on susceptibility of pear trees to *P. syringae* was evaluated by spraying trees with Urea+CuEDTA in the autumn and wounding the stems at different times before inoculation. An inoculation test using stems was used to evaluate plant suceptibility to the pathogen.

5.3.1 Plant culture

Pear (*Pyrus communis*) OHF-97 rootstocks were planted into 3.8 L containers containing a mixture of Douglas-fir bark, peat moss, and pumice (1:1:1) on 1 June 2004. The trees were grown in a lathhouse (LH) at OSU (44^{o} 30' N, 123 ^{o} 17' W), USA, trained to a single stem, and fertigated with 200 ppm N using Plantex® 20-20-20 with micronutrients once a week, from 14 June to 18 August 2004.

5.3.2 Foliar application and wound treatments

After terminal buds had set (mid October), 32 trees were selected for uniformity based on diameter (8-9 mm) and divided into two groups of 16 trees. Leaves on 16 trees were sprayed until run-off with either water or a combination of urea (3% solution, urea 46-0-0) and CuEDTA (1% solution, Ciba® Librel® Cu (CuEDTA) Ciba) (Urea+CuEDTA) on October 22, 2004. Starting 1 week after spray treatments, stems on 16 trees in each spray treatment were wounded every 7 d for 5 weeks. A total of 7 wounds were randomly made on each stem. Five wounds were made on each stem on 29 October 2004, 5 November 5 2004, 12 November 2004, 19 November 2004, and 25 November 2004. Two wounds were made on 26 November 2004. Wounds were made by removing a 4 mm diameter circle of the bark tissue from stems using a cork borer. All wounds were inoculated on the same date on 26 November 2004.

5.3.3 Environmental treatments after inoculation

After inoculation, 8 of the 16 trees were moved to a cold room (CR; 4^{o}C, dark, each tree was covered with a polyethylene bag to prevent desiccation) while the remaining 8 trees were kept outside in the lath house (LH, natural conditions) (Table 5-1).

5.3.4 *Phytophthora syringae* inoculum production

Stock cultures of *P. syringae* (isolated from *Kalmia latifolia* by Robert Linderman, USDA-ARS) were maintained on V8 juice agar medium in the dark at 20 ^{o}C. To prepare the medium, 4 g calcium carbonate added to 340 ml of V8 juice was heated and then filtered through cheese cloth. Then 100 ml of the filtered juice was added to 17g of agar and distilled water added to 1 liter before autoclaving (121 °C, 20 min). Fresh cultures were

prepared 7-10 d before inoculation by transferring 4 mm agar plugs to plates containing V8A medium and incubated in the dark at 20 ^{o}C.

5.3.5 Wound inoculation test

Thirty two plants from all treatments (2 spray treatments and 2 incubation locations) were inoculated with the pathogen on 26 November 2004. Mycelial plugs (4mm dia.) were taken from the actively growing margin of colonies of *P. syringae* (7-10 d) growing on V8A medium. Plugs containing either the pathogen or without the pathogen were placed into one of the wounds on the stem. Seven wounds were inoculated on the stem of each tree: six wounds of different ages were inoculated with *P. syringae* and one wound made on the inoculation date (0d) was inoculated with V8 agar to serve as a control.

5.3.6 Data Collection and Statistical Analyses

Disease incidence (% of stems with lesions) and disease development (length of lesion, cm) were measured on on 21 January 2005 (8 weeks after inoculation).

The experiment was a completely randomized design with 3 factors: foliar treatments (water and Urea+CuEDTA), incubation location (LH and CR), and wound age (0, 1, 7, 14, 21, and 28 d) (Table 5-1). No lesions occurred on wounds inoculated with only V-8 agar; therefore only data from wounds inoculated with the pathogen were included in the analyses. Each treatment had 8 replications (trees). Data were analyzed using Kruskal-Wallis ANOVA by Ranks to determine whether treatments influenced lesion size and treatment comparisons were made at $P<0.05$ ($K\text{-}W_{0.05}$). All statistical analyses were performed with S-PLUS (MathSoft, Inc., Seattle, WA).

Table 5- 1. Experimental treatments used to assess the influence of spraying trees with urea and copper-EDTA (Urea+CuEDTA), wound age, and environmental conditions after inoculation (Incubation location) on susceptibility of pear stems to *Phytopthora syringae*.

Foliar treatment[z]	**Incubation location[y]**	**Wound Time (date)**	**Wound age (d)**
Water	LH	26 November 2004	0
		25 November 2004	1
		19 November 2004	7
		12 November 2004	14
		5 November 2004	21
		29 October 2004	28
	CR	26 November 2004	0
		25 November 2004	1
		19 November 2004	7
		12 November 2004	14
		5 November 2004	21
		29 October 2004	28
Urea+CuEDTA	LH	26 November 2004	0
		25 November 2004	1
		19 November 2004	7
		12 November 2004	14
		5 November 2004	21
		29 October 2004	28
	CR	26 November 2004	0
		25 November 2004	1
		19 November 2004	7
		12 November 2004	14
		5 November 2004	21
		29 October 2004	28

[z]Foliar treatment = trees spraying with water and a combination of urea+CuEDTA.

[y]Incubation location = trees kept in lathhouse (LH; natural condition) or cold room (CR; 4^{o}C, dark) after inoculation. All wounds were inoculated on November 26, 2004.

5.4 Results

5.4.1 Size of Stem Lesions and Disease Incidence

Inoculation of fresh wounds (0d) resulted in 100% disease incidence (% of stems with lesions) and disease incidence was similar on 7 to 28 day-old wounds (Figure 5-1 A, C). In general, inoculation of 0- and 1-day-old wounds resulted in larger stem lesions compared to inoculation of older wounds (Figure 5-1 B, D). Stems on trees sprayed with Urea+CuEDTA had similar disease incidence and lesion size compared to trees sprayed with water (Figure 5-1 A, B). Stems on trees kept outside in the lath house had similar disease incidence and lesion size compared to trees moved into a cold room (Figure 5-1 C, D). The stems of control (inoculated with V-8 agar) trees had no lesions (data not shown).

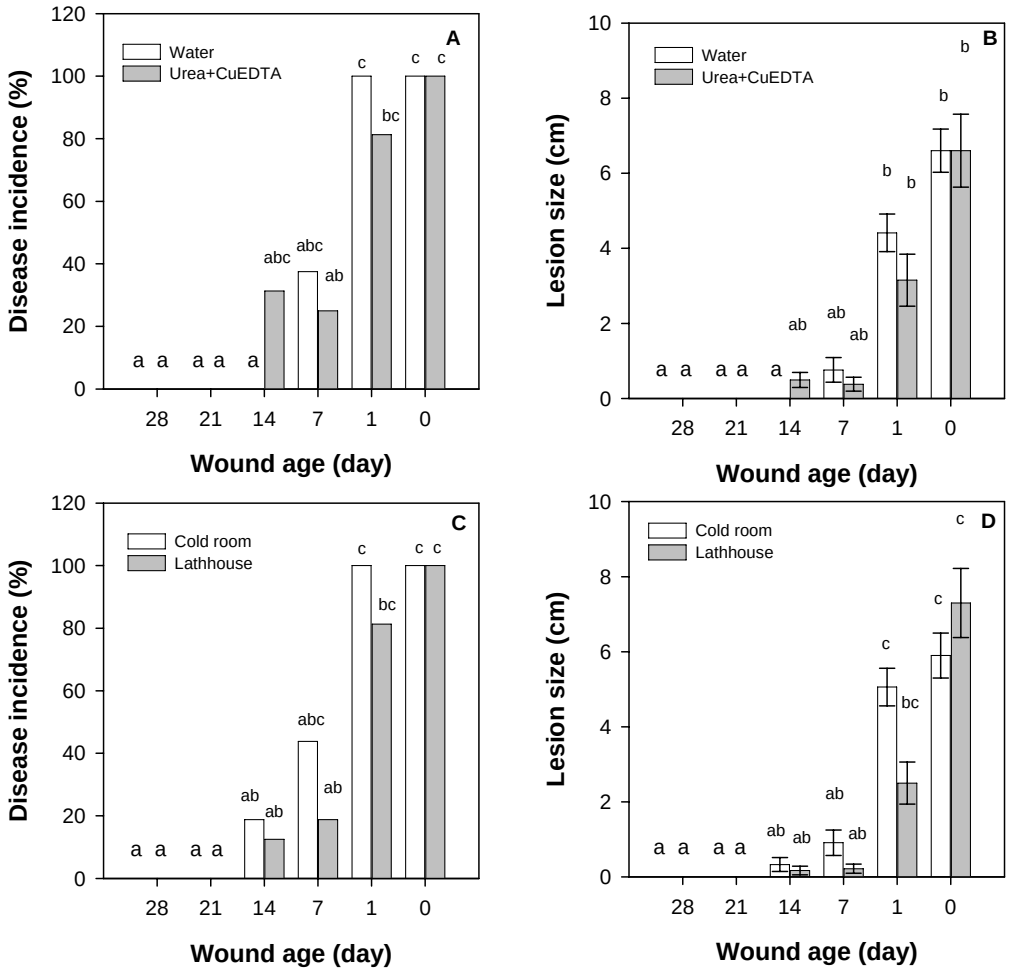

Figure 5- 1. Influence of (A, B) spray treatments and wound age, and (C, D) incubation condition and wound age on disease incidence and lesion size (cm), in stems of pear (*Pyrus communis*) inoculated with *Phytophthora syringae*. Trees sprayed with water or a combination of 3% urea and 1% CuEDTA (Urea+CuEDTA) on 22 October 2004 and wounded on 29 October 2004, 5 November 2004, 12 November 2004, 19 November 2004, 25 November 2004, and 26 November 2004, representing wound age 28, 21, 14, 7, 1, and 0 day, respectively. All wounds were inoculated on 26 November 2004 and trees were incubated in cold room or lath house after inoculation. Columns represent means (A, B, C and D n=16) and error bars are standard errors. Column denoted by the same letter within a graph are not significantly different ($K\text{-}W_{0.05}$).

5.4.2 Environmental conditions

The temperature in cold room was stable during the experiment (4°C). In the lath house the daily minimum, maximum, and mean temperature from the time of inoculation to the end of experiment varied (Figure 5-2). The temperature range in the lath house was small (~6 ^{o}C) after inoculation. The average minimum, maximum, and average daily temperature during the 8 weeks after inoculation was 7.8, 1.4, and 4.6 ^{o}C, respectively.

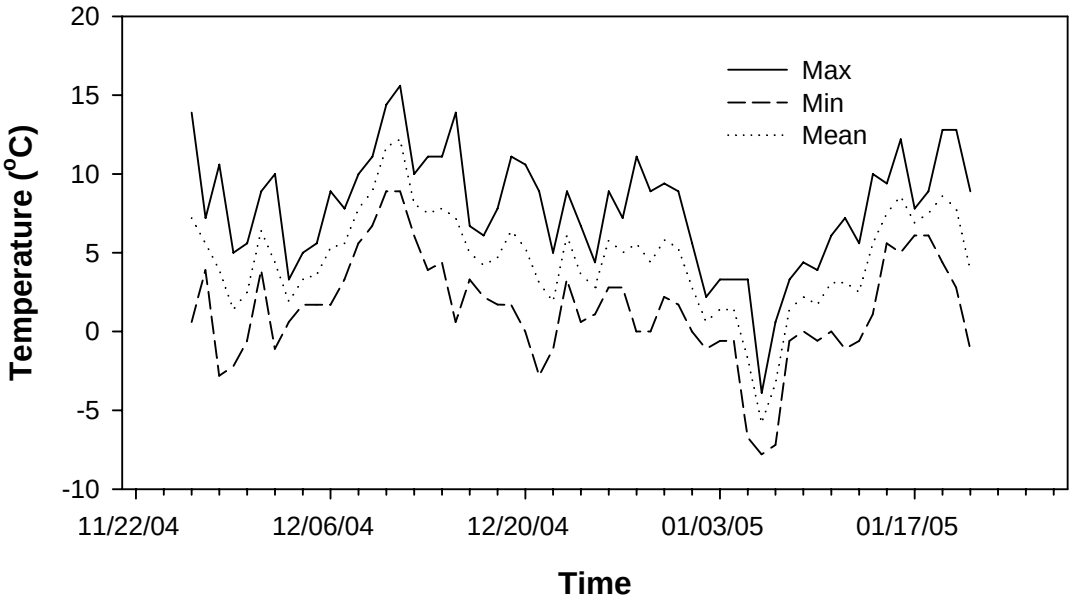

Figure 5- 2. Daily maximum (max), minimum (min), and mean temperature (°C) from the inoculation date to the assessment date (26 November 26 2004 to 21 January 2005) under natural conditions at Corvallis, OR. Source: http://www.ocs.orst.edu (Zone 2 - Climate Data Archives, Hyslop Exp. Stn., Corvallis)

5.5 Discussion

Wound age had a significant influence on the susceptibility of pear stems to infection by *P. syringae*. Fresh wounds inoculated with *P. syringae* developed lesions, and as wounds became older they became less susceptible to infection. Surface wounds inflicted during harvest and leaf scars caused by chemical defoliation or natural defoliation may serve as the infection openings for *P. syringae* (De Bruyn, 1924; Pscheidt and Ocamb, 2002); however our data indicate the length of time between wound formation and exposure to the pathogen dictates whether infection will occur.

Others have reported that nearly all cankers on almond trees caused by *P. syringae* are associated with pruning wounds or injuries created during pruning in late autumn and winter and infection is unsuccessful on uninjured bark (Bostock and Doster, 1985). Fresh wounds on almond have also been reported to be more susceptible to infection by *P. syringae* compared to aged wounds (Doster and Bostock, 1988a). The development of wound resistance is related to the formation of wound periderm or the infusion of lignin, suberin, waxes, and/or wound gums in the layers of cells immediately subtending the wound surface and resistance to pathogen infection generally increases as wounds age (Biggs, 1986; Bostock and Middleton, 1987; Bostock and Stermer, 1989; Doster and Bostock, 1988b). The rate deposition of lignin and suberin in the wound depended on the time of the year (Bostock and Stermer, 1989) and temperature (Doster and Bostock, 1988a) when wounds are inflicted. Wound cork layers on apple bark formed more quickly in summer than winter (Tamura and Saito, 1982). Low temperature slowed down lignin and suberin formation in almond wounds, resulting in low disease resistance (Doster and Bostock, 1988a).

Our data indicate that the influence of wound age on susceptibility of pear stems to *P. syringae* was not influenced by the specific environmental conditions after inoculation in our study. It is possible that the relatively low temperatures during incubation in both lathhouse (max, min, and mean temprature = 7.8 ^{o}C, 1.4 ^{o}C, and 4.6 ^{o}C, respectively) and cold room (4 ^{o}C) were favorable for the pathogen. *Phytophthora syringae* activity is restricted to cold climates and it is most active during the cool, rainy months of the year (September to November in the northern hemisphere) (Erwin and Ribeiro, 1996; Pscheidt and Ocamb, 2002). *Phytophthora syringae* can cause cankers on almond branch segments at temperatures between 2 to 20 ^{o}C but not at 27 ^{o}C (Bostock and Doster, 1985). In our study the average daily temperature in the cold room and the lathhouse after inoculation were similar (<1 ^{o}C difference) and differences between the lathhouse and the cold room after inoculation were less than 4 ^{o}C. The development of resistance to infection by *P. syringae* on bark wounds of almond is slowed when temperatures are low (Doster and Bostock, 1988a). This similarity in temperature between the two environmental conditions after inoculation could account for the similarity in disease incidence and lesion size on stems in the two locations.

Our data indicate that the influence of wound age on susceptibility of pear stems to *P. syringae* was not influenced by spraying trees with Urea+CuEDTA. Across all wound ages, stems on trees sprayed with Urea+CuEDTA or water had similar disease incidence and lesion size suggesting that Urea+CuEDTA did not physiologically alter pear tree susceptibility to *P. syringae* infection. Others have reported that the immature leaf scars remaining after early defoliation could served as primary avenue for infection by *P. syringae* (Bostock and Doster, 1985; De Bruyn, 1924; Pscheidt and Ocamb, 2002). In

our study, only wounds on pear stems were inoculated with the pathogen and not leaf scars; therefore our results do not address whether the physical changes due to Urea-CuEDTA influence trees' susceptibility to *P. syringae*.

5.6 Conclusions

Spraying trees with a combination of urea and CuEDTA after terminal buds have set in early autumn prior to harvest and storage can be of benefit to the nurserymen because the pathogen is less active in warm dry environments and the trees may be better able to heal wounds caused by defoliation or the chemical treatments.

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6 SUMMARY AND CONCLUSIONS

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Spraying trees with urea and defoliants, such as copper chelate (CuEDTA), is a practice currently used by growers of deciduous nursery trees for the purpose of early defoliation and to increase N storage. Trees are generally sprayed after the onset of vegetative maturity when the terminal buds have stopped growing. There have been reports that OHF97 pear trees sprayed with the urea and defoliants suffer stem lesions caused by *Phytophthora syringae*. The working hypothesis for this research was that spraying trees with a combination of urea and CuEDTA (Urea+CuEDTA) predisposed the plants to *P. syringae*. The primary goal of this research was to understand how tree N status and the timing of Urea and CuEDTA sprays influence susceptibility of pear to *P. syringae*. Additionally, because of the variation in the timing and types of nursery production practices for deciduous bareroot trees (e.g. cold storage, disease control measures, pruning), it was important to determine how plant growth stage, environmental conditions, and wounding might contribute to the susceptibility of pear trees to *P. syringae* infection.

Six main conclusions can be drawn from the results presented in this thesis:

1. Spraying pear trees with 3% urea and 1% CuEDTA (UD) was phytotoxic to leaves but not the stems and will increase greater stem N concentrations more in November than in October. A positive relationship exists between tree susceptibility to *P. syringae* and stem N concentrations resulting from fertigation during the growing season; but increased N concentrations in stems resulting from urea sprays does not increase tree susceptibility to *P syringae.* The combination of urea and defoliant sprays did not increase pear tree susceptibility to *P. syringae* infection, and because disease development is slower in October than November, early autumn spray treatments may be the most effective in promoting defoliation and preventing *P. syringae* infection.

2. Inoculation of non-wounded pear stems with *P. syringae* does not cause pathogen infection. Fresh wounds are more susceptible to infection than older wounds. Therefore, wounds from harvesting or pruning are probably required for *P. syringae* infection of pear stems and control measures prior to handling and environmental conditions conducive to wound healing may decrease disease severity.

3. Spraying trees with only CuEDTA has variable effects on tree N status and may increase tree susceptibility to *P. syringae*. Therefore, it is possible that not only may the physical damage (leaf scars) caused by defoliants increase susceptibility of pear trees to *P. syringae*, but physiological changes imposed by use of defoliant may also contribute.

4. *Phytophthora syringae* can infect both actively growing and dormant pear trees; however the environmental conditions after exposure to the pathogen alters the magnitude of the response. Therefore, even though most field reports of *P. syringae* on pear occur in the autumn and winter, it is possible that if the appropriate environmental conditions occur in late summer, infection may occur if the pathogen is present.

5. Aliette (6g/l), Biophos (3%), and PhytoFOS (1%) effectively prevented infection of wounded pear stems by *P. syringae*. Dimethomorph, Biocure, Biocoat, and Companion had no effect in preventing infection on wounded pear stems using the specific application rates and methods in this study.

6. The mixture of either Aliette or PhytoFOS with urea and CuEDTA treatments had similar effect in controlling the disease during October and November compared to fungicide alone applied before inoculation.

In summary, data from this research suggests that the application of urea and CuEDTA for early defoliation of pear in October may be better than November because of the lower

activity of the pathogen and favorable conditions for wound healing. Splitting two urea applications, as described for almond (Bi, 2004), with a pretreatment of 3% urea followed by the combination of urea+CuEDTA may be a more useful strategy to improve N status in October compared to a single application of urea+CuEDTA. Additionally, application of either Aliette or PhytoFOS in early autumn (September-October) as a spray treatment to the leaves can prevent the infection of *P.syringae* in the field or cold storage conditions. The application of fosetyl-Al during the growing season had a long-term residual effect in reducing disease development in apple rootstock even after a 9-month cold storage (Tidball and Linderman, 1990).

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