

# Tree Growth Stage and Environment After Pathogen Inoculation Alters Susceptibility of Pear Trees to *Phytophthora* Canker

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**Abstract:** We investigated whether pear (*Pyrus communis* L. OHF97) tree growth stage, nitrogen (N) status, wound age, and infection environment influences susceptibility to *Phytophthora syringae* Klebahn. Actively growing trees were susceptible to the pathogen when inoculated before terminal budset and cooler temperatures increased disease severity. In the autumn, during early stages of dormancy development after budset there was no relationship between temperature after inoculation and susceptibility and as trees became more dormant a negative relationship developed between temperature and susceptibility. Altering tree N status in the autumn after budset by spraying leaves with urea had no influence on susceptibility, regardless of environmental conditions after inoculation. Lesions developed when fresh wounds (0 d to 1 d old) were inoculated after budset, but as wounds aged they were less susceptible to infection. These results suggest a combination of tree growth stage when inoculated with the pathogen and environmental conditions afterward play important roles in disease development. Differences in susceptibility may be a direct result of temperature on pathogen activity or an indirect effect of temperature on tree metabolic activity. Differences in tree susceptibility related to dormancy development may be related to stem N status or the ability of trees to produce a barrier to infection after stems are wounded. Surface wounds inflicted during handling after budset may serve as infection locations for *P. syringae*; however the length of time between wound formation and exposure to the pathogen dictates whether infection will occur when trees are in the early stages of dormancy development.

**Keywords:** Dormancy, *Phytophthora syringae*, *Pyrus communis*.

## INTRODUCTION

During the winter, *Phytophthora syringae* Klebahn can cause disease on bare-root deciduous nursery stock in the Pacific Northwest (PNW) region of the USA, especially on trees harvested and stored in coolers or in outdoor sawdust beds [1]. In the PNW, *Phytophthora* spp. found in stool beds caused stem and root rot disease of apple rootstocks and can potentially act as a primary source of inoculum after transplanting into orchards [1]. Infected rootstock of indigenous inoculum can cause *Phytophthora* diseases in nursery trees after transplanting can [2, 3].

*Phytophthora syringae* is most active during the cool, rainy months of the year (September to November in the northern hemisphere) [4, 5]. The reported temperature range for growth of *P. syringae* broad and the minimum temperature for growth is <5 °C, optimum 15 to 20 °C and the maximum is 23-25 °C [4]. Increased activity of the pathogen coincides with the dormancy development of deciduous

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

Numerous structural and chemical changes occur in trees during seasonal development and these changes can influence tree susceptibility to *Phytophthora* spp. Responses of trees to *Phytophthora* spp. can occur when trees are actively growing [6] or when trees are dormant [7]. Tree susceptibility to *P. syringae* has been shown to vary between actively growing and dormant trees, 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 (*Rhododendron* spp.) kept at 4 °C, while the attempts to infect branches and buds of actively growing plants with the pathogen failed [8]. 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 [8].

Climate influences the development of diseases caused by *Phytophthora* spp., and climate constraints on certain species determine the geographic distribution of diseases they cause [9]. Cold climate restricts pathogenic activities by

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*P. cinnamomi* Rands in soil but does not appear to restrict the activities of either *P. syringae*, *P. cactorum* (Lebert et Cohn) Schröter, 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. [9]. Nearly all cankers on almond [*Prunus dulcis* (Mill.) D. A. Webb] 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 [10]. Fresh wounds on almond trees were more susceptible to infection than aged wounds, and the development of resistance to infection on bark wounds was slowed when temperature during bark wounding were low [11].

Wound inoculation is generally used in experiments with *P. syringae*, because this pathogen is unsuccessful in causing infection through uninjured bark [8, 10, 12]. *Phomopsis amygdali* (Del.) J. J. Tuset & T. Portilla, causal agent of constriction cankers, initiates infections of peach [*Prunus persica* (L.) Batch.] twigs through fresh leaf scars in fall and bud scale scars and flowers in spring [13]. In artificial inoculations of *P. syringae* in rhododendrons, wounds and low temperatures were prerequisite for infection [8]. Bare-rooted deciduous nursery trees that are defoliated before harvesting generally survive the stresses associated with handling and storage better than trees with leaves. Surface wounds, inflicted when trees are harvested and leaf scars caused by artificial or natural defoliation and subsequent handling, serve as potential infection locations for *P. syringae* [5,10, 12].

Using field grown pear trees and an *in vivo* infection assay for *P. syringae*, the specific objectives of this study were to determine whether tree susceptibility to infection by the pathogen is altered by: (1) stage of plant growth after terminal bud set; (2) environmental conditions (primarily temperature) after dormant trees are inoculated with the pathogen; and (3) environmental conditions (primarily temperature) after actively growing trees are inoculated with the pathogen. Additionally, we also evaluated whether the wound age or increased tree nitrogen status during the autumn altered susceptibility of dormant trees to *P. syringae* infection.

## MATERIALS AND METHODS

### Inoculum Production and Wound Inoculation

Stock cultures of *P. syringae* (isolated from *Kalmia latifolia* L. by Robert Linderman, USDA-ARS, Corvallis, OR) were maintained on V8 juice agar (V8 A) medium in the dark at 20 °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 to 10 d before inoculation by transferring 4 mm dia. agar plugs to plates containing V8A and incubated in the dark at 20 °C. Wounds on stems were inoculated using mycelial plugs (4 mm dia.) taken from the actively growing margin of colonies of *P. syringae* growing on V8A. Plugs with or without the pathogen were placed into a wound made

with a cork borer (4 mm dia.). Wounds were wrapped with Parafilm ® after inoculation.

### Experiment 1: Tree Susceptibility During Dormancy Development in Autumn

Pear OHF-97 rootstock 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 mg l<sup>-1</sup> N using Plantex® 20-20-20 with micronutrients (Plantex Corp., Ontario, Canada) once a week, from 6 June to 5 September 2003. After terminal buds had set (1 October 2003) 100 trees were selected for uniformity based on stem diameter (7-8 mm.), divided into two groups of 50 trees, and moved to either the lath house (LH) or greenhouse (GH) to produce trees in different stages of dormancy development. The LH trees were kept in natural conditions in a lath house with transparent plastic over the roof and the GH trees were exposed to 16h/8h light/dark and 21 °C/15 °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. Every two weeks ten trees from each location (LH or GH) were inoculated with *P. syringae* and moved to one of two locations after inoculation. After trees were inoculated five trees from the LH were maintained in the LH (LH/LH) and five trees were moved to the GH (LH/GH). Five trees from the GH were maintained in the GH after inoculation (GH/GH) and five trees were moved to the LH (LH/LH).

Stems were inoculated in wounds with *P. syringae* on 7 October 2003, 21 October 2003, 11 November 2003, 25 November 2003, and 9 December 2003. Inoculum production and wound inoculation was performed as described above. Three wounds were made on the stem of each tree: two wounds were inoculated with *P. syringae* grown on V8 A and one wound was inoculated with V8 A without the pathogen. Disease incidence (percentage of wounds with lesions) and disease severity (length of lesion) 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.

### Experiment 2: Tree Susceptibility and Infection Environment in Winter

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 mg l<sup>-1</sup> N using Plantex® 20-20-20 with micronutrients once a week, from 14 June to 18 August 2004. After terminal bud set (mid October) 30 trees were selected for uniformity based on stem diameter (7-8 mm), divided into two groups of 15 trees, and moved to either the LH or GH to produce trees in different stages of dormancy development. Conditions in the LH and GH were the same as described for Experiment 1, above. On 22 January 2005, after natural defoliation of trees in the LH, 15 plants from each location (LH or GH) were inoculated with

*P. syringae* and moved to one of three locations after inoculation (LH, GH, CR). After trees were inoculated five trees from the LH were maintained in the LH (LH/LH), five trees were moved to the GH (LH/GH), and five trees were moved to a cold room (CR; 4°C, dark and each tree was covered with polyethylene bag to prevent desiccation) (LH/CR); Five trees from the GH were maintained in the GH after inoculation (GH/GH), five trees were moved to the LH (GH/LH) or five trees were moved to the (GH/CR).

Stems were inoculated with *P. syringae* on 22 January 2005. Inoculum production and wound inoculation was performed as described above. Three wounds were made on the stem on each tree: two wounds were inoculated with *P. syringae* grown on V8A and one wound was inoculated with V8A without the pathogen. Disease incidence (percentage of wounds with lesions) and disease severity (length of lesion) was measured on trees 8 weeks after inoculation. Stems of trees inoculated on 22 January 2005 were assessed on 19 March 2005.

### Experiment 3: Susceptibility of Actively Growing Trees

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 mg l<sup>-1</sup> N using Plantex® 20-20-20 with micronutrients once a week, from 14 June to 18 August 2004. During the actively growing period (before terminal bud 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 either the LH, GH, or CR. Conditions in the LH, GH, and CR were the same as described above.

Stems were inoculated with *P. syringae* on 13 September 2004. Inoculum production and wound inoculation was performed as described 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 without the pathogen. Disease incidence (percentage of wounds with lesions) and disease severity (length of lesion) was measured on trees 4 weeks after inoculation. Stem of trees inoculated on 13 September 2004 were assessed on 11 October 2004.

### Experiment 4: Relationships Between Susceptibility, Tree N Status, and Wound Age

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 1 June 2004. The trees were grown in a lath house (LH) at OSU, Corvallis, OR, USA, trained to a single stem, and fertigated with 200 mg l<sup>-1</sup> N using Plantex® 20-20-20 with micronutrients once per week from 14 June to 18 August 2004.

After terminal bud 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 Cu-EDTA (1% solution, Ciba® Librel® Cu (Cu-EDTA) 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. 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. On 26 November 2004 all wounds were inoculated with the pathogen, trees were divided into two groups and moved to either the LH or CR. Conditions in the LH and CR were the same as described above.

Trees were inoculated with the pathogen on 26 November 2004. Inoculum production and wound inoculation was performed as described above. Seven wounds were inoculated on the stem of each tree: six wounds of different ages were inoculated with *P. syringae* and one of the two wounds made on 25 November 2005 was inoculated with V8A without the pathogen. Disease incidence (percentage of wounds with lesions) and disease severity (length of lesion) was measured on trees on 21 January 2005 (8 weeks after inoculation).

### Experimental Designs and Statistical Analyses

Experiment 1 was a completely randomized design with 3 factors: Growing location before inoculation (LH, GH), time of inoculation (1, 3, 5, 7, 9 weeks after terminal buds had set), and incubation location after inoculation (LH, GH). Each treatment had 5 replications. Each tree had 2 pathogen-inoculated wounds on the same stem. Lesions did not occur on wounds inoculated with on V8A and were therefore not included in analyses. Data were analyzed using Kruskal-Wallis ANOVA to determine whether treatments influenced lesion size and treatment comparisons were made at P<0.05 (K-W<sub>0.05</sub>).

Experiment 2 was a completely randomized design with two factors: Growing location before inoculation (LH, GH), and incubation location after inoculation (LH, GH, CR). Each treatment had 5 replications. Each tree had 2 pathogen-inoculated wounds on the same stem. Lesions did not occur on wounds inoculated with on V8A and were therefore not included in analyses. Data were analyzed using analysis of variance (ANOVA) to determine whether treatments influenced lesion size and where indicated by ANOVA, means were separated using Tukey's Honestly Significant Difference at P<0.05 (THSD<sub>0.05</sub>).

Experiment 3 was a completely randomized design with the treatments (LH, GH, CR). Each treatment had 4 replications. Each tree had 2 pathogen-inoculated wounds on the same stem. Lesions did not occur on wounds inoculated with on V-8 agar and were therefore not included in analyses. Data were analyzed using Kruskal-Wallis ANOVA to determine whether lesion size was influenced by the incubation condition and treatment comparisons were made at P<0.05 (K-W<sub>0.05</sub>).

Experiment 4 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). No lesions occurred on wounds inoculated with only V8A; therefore, only data from wounds inoculated with the pathogen were included in the analyses. Each treatment had 8 replications. Data were analyzed using Kruskal-

Wallis ANOVA to determine whether treatments influenced lesion size and treatment comparisons were made at  $P < 0.05$  ( $K-W_{0.05}$ ).

All data were tested for homogeneity of variance using Levene's test and for normality using the Kolmogorov-Smirnov test. When transformation could not be used to correct for lack of homogeneity of variance or normality, non-parametric analyses were applied to the data to test specific hypotheses. The relationships between the lesion size and temperatures were analyzed using Spearman's Rank correlation coefficient (R). All statistical analyses were performed with S-PLUS (MathSoft, Inc, Seattle, WA, USA) and Statistica® (Statsoft, Inc., Tulsa, Okla., USA, 1996).

## RESULTS AND DISCUSSION

### Experiment 1: Tree Susceptibility During Dormancy Development in Autumn

The influence of tree growth stage in the autumn and infection environment on susceptibility of pear OHF97 rootstock to *P. syringae* was evaluated by inoculating trees at different stages of dormancy development in the autumn after terminal buds set (October to December) and maintaining trees under different environmental conditions after inoculation. Susceptibility of pear trees in the autumn to infection by *P. syringae* was influenced by environmental factors after inoculation with the pathogen. Stems from trees maintained in the LH after inoculation had larger lesions than stems on trees maintained in the GH after inoculation (Table 1). The environmental conditions before inoculation and when trees were inoculated also influenced lesion size (significant interaction between location before inoculation

and time of inoculation). This suggests tree development (as a function of environmental conditions before and after inoculation) as well as environmental effects on the pathogen influences disease development. Lesions on trees grown in the GH were similar in size regardless of when stems were inoculated after terminal buds had set indicating trees at a similar stage of early dormancy development were equally susceptible to the pathogen regardless of the environmental conditions after inoculation. Lesions on trees grown in the lath house increased in size with increasing time after terminal buds had set (contrast  $P < 0.00001$ ) suggesting as trees became more susceptible as they became more dormant.

The maximum, minimum, and mean daily temperatures from October to January in the LH decreased gradually (Fig. 1A). The increase in the size of lesions on trees in the LH as they became more dormant corresponded with the decrease in temperature. The cooler environment of the LH after inoculation favored disease development over the warmer conditions in the GH. When trees were maintained in the GH after inoculation, temperatures from October to December were relatively stable and susceptibility to the pathogen did not change during this time. When trees were in the LH before and after exposure to the pathogen (LH/LH) there were negative relationships between lesion size and maximum temperature ( $R = -0.5482$ ,  $P < 0.0001$ ), minimum temperature ( $R = -0.5486$ ;  $P < 0.0001$ ), and average temperature ( $R = -0.5609$ ,  $P < 0.0001$ ) after inoculation. This negative relationship between temperature and disease severity (size of lesion) when trees were in the LH suggests the decreasing temperatures from October to December after inoculation increases tree susceptibility. In comparison, when trees from the GH and were moved to the LH after inocula-

**Table 1. Influence of Different Environmental Conditions Prior to Inoculation and After Inoculation (After Terminal Budset) on the Size of Lesions Caused by Inoculating Wounded Pear (*Pyrus communis*) OHF97 Rootstock Stems with *Phytophthora syringae***

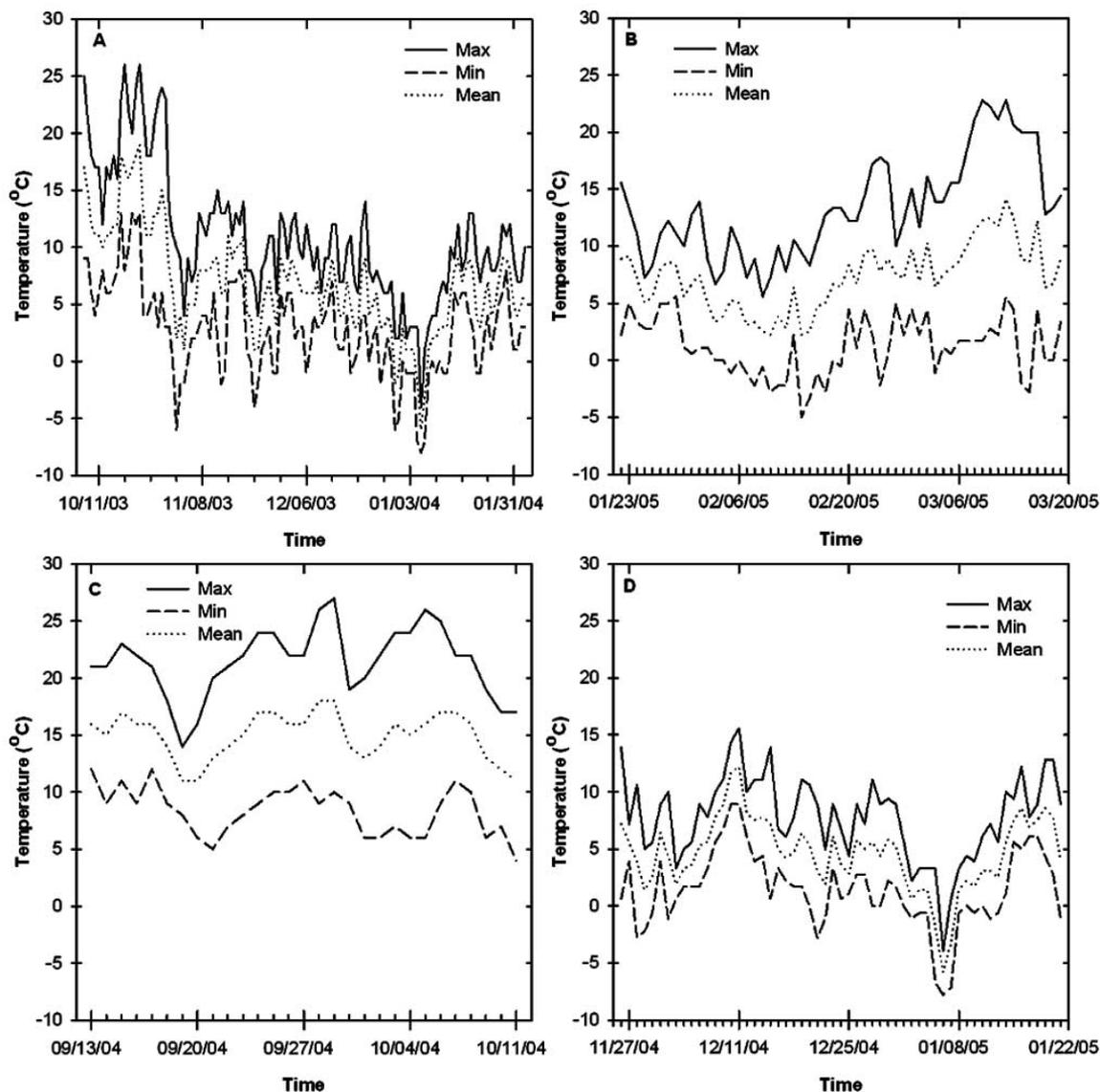
Factors <sup>z</sup>	Treatments <sup>y</sup>	Lesion Size (cm)				
		Time (d) <sup>x</sup>				
		7	21	42	56	70
<b>Growing Location x Time</b>						
	LH	0.435 aA <sup>w</sup>	1.155 bA	0.940 bB	1.515 cB	1.720 cB
	GH	0.485 aA	1.240 cA	0.460 aA	0.695 abA	0.915 bcA
<b>Incubation Location x Time</b>						
	LH	0.920 aB	1.425 bB	0.695 aA	1.550 bB	1.580 bB
	GH	0.000 aA	0.970 bcA	0.705 bA	0.660 bA	1.055 cA
<b>Growing Location x Incubation Location</b>						
	LH/LH			1.502 C		
	LH/GH			0.804 B		
	GH/LH			0.966 B		
	GH/GH			0.552 A		

<sup>z</sup>Specific treatment comparisons evaluated using Kruskal-Wallis test for interactions between Growing Location x Time (n=20), Incubation Location and Time (n=20), and Growing Location x Incubation Location (n=50).

<sup>y</sup>Trees grown in lath house (LH) and incubated in LH after inoculation (LH/LH), trees grown in greenhouse (GH) and incubated in GH after inoculation (GH/GH), trees grown in GH and incubated in LH after inoculation (GH/LH), and trees grown in LH and incubated in GH after inoculation (LH/GH).

<sup>x</sup>Time after terminal budset (1 October 2003). Trees inoculated 7 October, 21 October, 11 November, 25 November, and 9 December 2003. Stems collected for analyses 8 weeks after inoculation.

<sup>w</sup>Means followed by the same lower case letter within a row and factor are not significantly different ( $K-W_{0.05}$ ). Means followed by the same upper case letter within a column and factor are not significantly different ( $K-W_{0.05}$ ).



**Fig. (1).** Daily maximum (max), minimum (min), and mean temperature in Corvallis, OR between (A) 7 October 2003 and 3 February 2004, (B) 22 January 2005 and 19 March 2005, (C) 13 September 2004 and 11 October 2004, and (D) 26 November 2004 to 21 January 2005. Source: <http://www.ocs.orst.edu> (Zone 2 - Climate Data).

tion (GH/LH) there was no relationship between disease severity and temperature ( $R < 0.24$ ,  $P > 0.05$ ), suggesting the growth stage of the tree also played a role in disease development when temperatures were conducive to disease.

The influence of temperature on plant disease development has been reported for *P. syringae* as well as other *Phytophthora* species. *Phytophthora syringae* can actively grow in cold and wet conditions [5], similar to those frequently occurring in the autumn in the PNW. In California, cankers on almond caused by *P. syringae* are observed soon after pruning in the autumn and winter when temperatures are relatively low (mean daily temperature 5 °C to 15 °C) [14]. Excised branch pieces of almond inoculated with *P. syringae* developed cankers at temperatures between 2 °C and 20 °C but not at 27 °C. Suppression of *P. cinnamomi* on avocado (*Persea americana* L.) seedlings has been correlated with temperature influencing mycelial growth [15].

Our results suggest both temperature and tree growth stage play a role in susceptibility of pear trees to *P. syringae* in the autumn after terminal bud set. Leaves on trees maintained in the GH before and after exposure to the pathogen (GH/GH) did not senesce, whereas leaves on trees grown in the GH before inoculation and moved to the LH after inoculation (GH/LH) abscised in late-December and leaves on trees maintained in the LH before and after inoculation (LH/LH) abscised from mid- to late-November. Leaves on trees maintained in the GH did not senesce because leaf senescence and abscission in pear trees occurs in response to low temperature and progresses with development of dormancy [16]. These observations on differences in leaf abscission between trees in the different treatments suggest trees in the different treatments were at different stages in dormancy development when inoculated and when assessed for disease development. For example, trees grown in the LH before inoculation may have been in later stages of dor-

mancy development than trees grown in the GH before inoculation. Similarly, trees in the GH before inoculation and maintained in the LH after inoculation may have been in later stages of dormancy development after inoculation than trees grown in the GH and moved to the LH after inoculation.

Dormancy development involves a multitude of plant anatomical and biochemical changes in response to ontogeny and environmental stimuli. Even though the terminal buds had set on trees in the GH and the LH, physiological differences between the trees, resulting from differences in dormancy development, may have made trees in the LH/LH treatment more susceptible to *P. syringae* than trees in the GH/LH treatment. The leaves on trees in the LH/LH treatment senesced before leaves on trees in the GH/LH treatment, suggesting trees in the LH/LH treatment may have developed dormancy earlier than trees in the GH/LH treatments. Differences in the N concentration in stems may be one possible explanation for differences in susceptibility between trees in the LH/LH and GH/LH treatments. Mobilization of leaf N to the stems and roots of trees occurs during leaf senescence [17, 18], resulting in increased N content of stems. Earlier leaf abscission on trees in the LH/LH treatment may have resulted in higher N concentrations in inoculated stems than stems on trees in the GH/LH treatment. Our previous research indicated the N status of pear trees stems is positively related with disease development by *P. syringae* [19]. Larger lesions on trees in the LH/LH treatment than trees in the GH/LH treatment may be due to higher N status in stems from earlier N mobilization from leaves.

### Experiment 2: Tree Susceptibility and Infection Environment in Winter

The influence of tree growth stage and infection environment on susceptibility of pear trees to *P. syringae* was evaluated using trees maintained under different environmental conditions during the autumn after terminal bud set. Tree stems were inoculated in January to determine whether tree growth stage or environmental conditions after inoculation influenced tree response to the pathogen in winter. Stems from trees grown in the GH before inoculation had smaller lesions compared to stems on trees grown in the LH (Table 2). Trees moved in the CR after inoculation developed larger lesions than trees maintained in the LH or GH. When trees were grown in GH before inoculation, there was

no difference in lesion size when trees were maintained in the LH (GH/LH) or GH (GH/GH) after inoculation. When trees were grown in the LH before inoculation, lesions were larger on trees maintained in the LH after inoculation (LH/LH) than lesions on trees maintained in the GH (LH/GH). The temperature in the GH and CR varied less than 5 °C after trees were inoculated while in the LH the minimum, maximum, and average daily temperature varied by almost 15 °C (Table 2) and gradually increased after trees were inoculated (22 January to 19 March 2005) (Fig. 1B).

These results support the results from Experiment 1, indicating a negative relationship between temperature and disease activity by *P. syringae* on pear trees exists when trees are dormant. Lesions on stems from trees maintained in the cooler conditions in the LH after inoculation (max., min., and mean temperatures of 13 °C, 1 °C, and 7 °C, respectively) were larger than lesions on trees maintained in warmer conditions in the GH (max, min, and mean temperature of 21 °C, 15 °C, and 18 °C, respectively). These results confirm the findings of others who have reported a relationship between low temperature and infection by *P. syringae* in the field [5, 10, 11, 20]. Lower temperatures in the CR and LH after inoculation may have resulted in greater pathogen activity than the warmer conditions in the GH.

It is also possible the warmer temperatures in the GH after trees were inoculated were not favorable for the pathogen [10]. Isolation of the pathogen during late spring and summer from stems of almond trees was not successful [10]. Higher temperatures in the GH in our experiment may also have promoted stem wounds 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 wound gums in the layers of cells immediately subtending the wound surface. Resistance of wounds to fungal infection increases as wounds age [10, 11, 21, 22] and the rate of deposition of lignin and suberin in the wound depends on the time of the year [23], and temperature [11] when wounds are inflicted. Actively growing trees generally have a more rapid response to wounding than trees undergoing dormancy. Wound cork layers on apple bark form more quickly in summer compared to winter [24] and low temperature slows down lignin and suberin formation in almond stem wounds, resulting in low disease resistance [14].

**Table 2. Influence of Different Environmental Conditions Prior to Inoculation and After Inoculation on The Size of Lesions Caused by Inoculating Wounded Pear (*Pyrus communis*) OHF97 Rootstock Stems with *Phytophthora syringae* and Placing Trees in Locations with Different Minimum, Maximum, and Mean Temperatures**

Location After Inoculation <sup>z</sup>	Lesion Size (cm) <sup>y</sup>				Temperature (°C) <sup>w</sup>		
	LH1		GH1		Minimum	Maximum	Mean
GH2	1.730	aB <sup>x</sup>	0.000	aA	15.0	21.0	18.0
LH2	3.800	bB	1.020	aA	1.0	13.0	7.0
CR2	4.990	cB	2.600	bA	4.0	4.0	4.0

<sup>z</sup>Trees maintained in lath house (LH2), greenhouse (GH2), or cold room (CR2) after inoculation on 22 January 2005. Stems collected for analyses 8 weeks after inoculation.

<sup>y</sup>Trees maintained in lath house (LH1) or greenhouse (GH1) from 30 October 2004 to 22 January 2005.

<sup>x</sup>Means (n=10) followed by the same lower case letter within a column are not significantly different (THSD<sub>0.05</sub>). Means followed by the same upper case letter within a row are not significantly different (THSD<sub>0.05</sub>).

<sup>w</sup>Average daily maximum, minimum, and mean temperature between 22 January and 19 March 2005 in the GH, LH, and CR after inoculation with pathogen.

In our experiment, leaves on trees in the LH had abscised before trees were inoculated and leaves were still present on trees in the GH when they were inoculated. Smaller lesions on trees grown in the GH than trees grown in the LH may be a result of differences in stem N content (as described above); however, the ability of the trees in the GH to respond to stem wounding may also be higher than trees grown in the LH. The differences in tree susceptibility to *P. syringae* may therefore be a direct result of temperature on the pathogen or an indirect effect on the pathogen through the effects of temperature on plant metabolic activity.

During production, it is probably impossible to separate the effects of temperature on tree growth status and metabolic activity and tree susceptibility to *P. syringae*. Trees may be exposed to the pathogen when they are in different stages or dormancy (as in Experiment 1 and 2). In both cases, however, the temperatures after exposure to the pathogen appear to be extremely important for disease development.

### Experiment 3: Susceptibility of Actively Growing Trees

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. Tree stems were inoculated in September to determine whether environmental conditions after inoculation influence tree response to the pathogen before terminal bud set. When actively growing trees were inoculated with *P. syringae* before bud set, trees maintained in the GH and the LH after inoculation developed smaller lesions than trees moved to the CR (Table 3). The size of lesions on trees in the LH and in the GH was similar. After trees were inoculated differences between maximum and minimum daily temperatures in the GH and CR were less than 5 °C and differences between maximum and minimum daily temperatures in the LH was ~14 °C (Table 3). The temperature after inoculation remained relatively constant in the LH with mean, maximum, and minimum temperatures varying by less than 5 °C (Fig. 1C).

These results support the results from Experiment 1 and 2, indicating temperature after inoculation with *P. syringae* has a strong influence on disease development, regardless of whether the trees are in different stages of dormancy (Experiment 1 and 2) or whether trees are actively growing (Experiment 3). Temperatures after inoculation were lower in

the CR than in the GH and LH and this difference in temperature after inoculation may have resulted in the differences in lesion size between the treatments. Additionally, these results, combined with our results from Experiment 1 and Experiment 2 indicate the magnitude of the response of trees to the pathogen and temperature varies depending upon the growth status of the trees.

Our results support previous reports from field studies of *P. syringae* actively causing disease in cold conditions [5, 10, 11, 20]. With pear trees, we also determined that the pathogen can cause disease in both actively growing and dormant trees if the temperatures after exposure to the pathogen are conducive to pathogen activity and when temperatures increase tree susceptibility. Similarly dormant lilac plants were also reported to be more susceptible to *P. syringae* than actively growing plants; however, the pathogen could still infect actively growing plants under certain conditions [25]. The seasonal activity of the host and the influence of environmental factors such as temperature determine the time course of epidemics caused by *Phytophthora* spp. [9]. In rhododendrons, 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, even under low temperatures [8]. *Phytophthora syringae* was not able to infect lilac [12], apple [25, 26] and almond [10] during the summer. Under natural conditions, the lack of disease caused by *P. syringae* in summer and susceptibility of dormant plant tissue may be coincidental to the effects of temperature on plant growth and pathogen activity. Our results indicate both actively growing and dormant pear trees are susceptible to *P. syringae* when they were placed into a cold room after inoculation. These results suggest temperature after exposure of the tree to the pathogen was more important than tree growth status.

### Experiment 4: Relationships Between Susceptibility, Tree N Status, and Wound Age

The influence of tree N status and wound age on susceptibility of pear trees to *P. syringae* was evaluated by spraying trees with Urea+CuEDTA in the autumn after terminal bud set and wounding the stems at different times before inoculation as trees developed dormancy in the autumn. When trees were maintained in the LH after inoculation, 0 d and 1 d old wounds had the highest disease incidence and largest lesions

**Table 3. Influence of Different Environmental Conditions After Inoculation on the Size of Lesions Caused by Inoculating Actively Growing Wounded Pear (*Pyrus communis*) OHF97 Rootstock Stems with *Phytophthora syringae* and Placing trees in Locations with Different Minimum, Maximum, and Mean Temperatures**

Location After Inoculation <sup>z</sup>	Lesion Size (cm)	Temperature (°C) <sup>x</sup>		
		Minimum	Maximum	Mean
GH	0.837 a <sup>y</sup>	15.0	21.0	18.0
LH	1.075 a	8.0	22.0	15.0
CR	5.850 b	4.0	4.0	4.0

<sup>z</sup>Trees moved to a greenhouse (GH), lath house (LH) or cold room after inoculation 13 September 2004. Stems collected for analyses on 11 October 2004.

<sup>y</sup>Means (n=8) followed by the same lower case letter within a column are not significantly different (K-W<sub>0.05</sub>).

<sup>x</sup>Average daily maximum, minimum and mean temperature between 13 September 2004 and 11 October 2004 in the GH, LH, and CR after inoculation with pathogen.

and wounds 7 d old and older had lower disease incidence and smaller lesions (Table 4). Similarly, when trees were moved to the CR after inoculation, 0 d and 1 d old wounds had the highest disease incidence and largest lesions and wounds 7 d old and older had the lowest disease incidence and smallest lesions. Moving trees to the CR increased disease incidence in 1 d and 7 d old wounds, increased the size of lesions in 1 d old wounds, and decreased the size of lesions in fresh (0 d) wounds. Spraying trees with Urea+CuEDTA had no influence on disease incidence of trees maintained in the LH or CR after inoculation and no influence on the size of lesions when trees were maintained in the LH after inoculation (Table 4). Spraying trees with Urea+CuEDTA decreased the size lesions on trees moved to the CR after inoculation.

The temperature in CR was 4°C during the experiment. In the LH the daily minimum, maximum, and mean temperature between when trees were inoculated and the end of experiment varied by ~6 °C after inoculation (Fig. 1). The average minimum, maximum, and average daily temperature during the 8 weeks after inoculation was 7.8 °C, 1.4 °C, and 4.6 °C, respectively.

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* [5, 12]; however our data indicate the length of time between wound

formation and exposure to the pathogen dictates whether infection will occur.

Others have reported 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 [10]. Fresh wounds on almond have also been reported to be more susceptible to infection by *P. syringae* compared to aged wounds [11]. 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 [10, 14, 21, 23]. The rate deposition of lignin and suberin in the wound depended on the time of the year and temperature when wound are inflicted [11]. Wound cork layers on apple bark formed quickly in summer than winter [24]. Low temperature slowed down lignin and suberin formation in almond wounds, resulting in reduced disease resistance [14].

Our data indicate 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 the relatively low temperatures during incubation in both LH (max, min, and mean temperature of 7.8 °C, 1.4 °C, and 4.6 °C, respectively) and CR (4 °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

**Table 4. Influence of Spraying Foliage with a Combination of Urea and Copper Chelate (CuEDTA) in October on the Size of Lesions and Disease Incidence Caused by Inoculating Wounded Pear (*Pyrus communis*) OHF97 Rootstock Stems with *Phytophthora syringae* Either 0, 1, 7, 14, 21, or 28 d After Wounding and Storing Trees in a Lathhouse (LH) or Cold Room (CR)**

Factors <sup>z</sup>	Treatments <sup>y</sup>	Lesion Size (cm)		Disease Incidence (%)	
		LH	CR	LH	CR
<b>Urea x Incubation Location</b>					
	<b>Water</b>	1.669 aA <sup>x</sup>	2.252 bB	35.4 aA <sup>x</sup>	41.7 aA
	<b>Urea</b>	1.727 aA	1.812 aA	33.3 aA	45.8 aA
<b>Wound Age x Incubation Location</b>					
	<b>0</b>	7.300 cB	5.900 bA	100.0 bA	100.0 cA
	<b>1</b>	2.500 bA	5.056 bB	81.2 bA	100.0 cB
	<b>7</b>	0.218 aA	0.912 aA	12.5 aA	43.7 bB
	<b>14</b>	0.169 aA	0.325 aA	12.5 aA	18.7 aA
	<b>21</b>	0 aA	0 aA	0 aA	0 aA
	<b>28</b>	0 aA	0 aA	0 aA	0 aA

<sup>z</sup>Specific treatment comparisons evaluated using Kruskal-Wallis test for interactions between Urea x Incubation Location (n=48) and Wound Age x Incubation Location (n=16).

<sup>y</sup>Trees sprayed with water (Water), or a combination of 3% urea solution with 1% CuEDTA (Urea) on 22 October 2004 and wounded on 29 October (28 d), 5 November (21 d), 12 November (14 d), 19 November (7 d), and 25 November (0 d) before inoculation with pathogen and maintained for 8 weeks in a lath house (LH) or coldroom (CR). Stems collected for analyses on 21 January 2005.

<sup>x</sup>Means followed by the same lower case letter within a column and factor or followed by the same upper case letter within a variable, row, and factor are not significantly different (K-W<sub>0.05</sub>).

the northern hemisphere) [4, 5]. *Phytophthora syringae* can cause cankers on almond branch segments at temperatures between 2 °C to 20 °C but not at 27 °C [10]. The development of resistance to infection by *P. syringae* on bark wounds of almond trees is slowed when temperatures are low [11]. In our study the average daily temperature in the CR and the LH after inoculation were similar (<1 °C difference) and differences between the LH and the CR after inoculation were less than 4 °C. 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 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 Urea+CuEDTA did not physiologically alter pear tree susceptibility to *P. syringae* infection. Others have reported immature leaf scars, remaining after early defoliation, can serve as primary avenues for infection by *P. syringae* [5, 11, 12]. 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*.

## CONCLUSIONS

In summary, using a simple disease assay system we determined the susceptibility of pear trees to *P. syringae* varies between actively growing and dormant trees, and incubation conditions after exposure to the pathogen, particularly temperature, are important for disease development regardless of tree growth stage. Under conditions favorable to the pathogen, *P. syringae* can infect stems of both actively growing and dormant pear trees. The ability of *P. syringae* to infect wounds on stems of pear may be restricted by the age of the wound, even in dormant trees. In our test conditions, as trees became more dormant in the autumn their susceptibility to *P. syringae* was not increased by the influence of urea of tree N status. This information is useful to nursery management practices in areas infested with *P. syringae* or during transport or cold storage when the potential for exposure to the pathogen is high and highlights the important of inspection and treatment of both dormant and actively growing trees to prevent or reduce the build up of inoculum and avoid epidemics.

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