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Temperature : effects on Fusarium subglutinans f. sp. pini infection on juvenile Pinus radiata (Monterey pine) and influence on growth of Fusarium subglutinans f. sp. pini isolates from California and Florida

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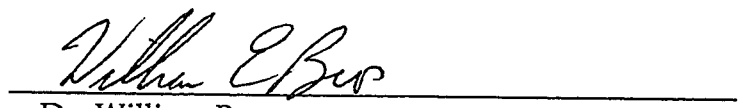

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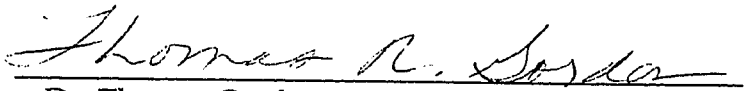

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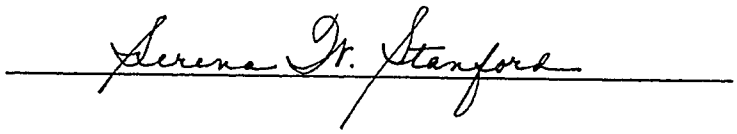

ABSTRACT

TEMPERATURE: EFFECTS ON *FUSARIUM SUBGLUTINANS* F. SP. *PINI* INFECTION ON JUVENILE *PINUS RADIATA* (MONTEREY PINE) AND INFLUENCE ON GROWTH OF *FUSARIUM SUBGLUTINANS* F. SP. *PINI* ISOLATES FROM CALIFORNIA AND FLORIDA

by Mark J. McDonald

This study examines the effect of temperature on growth of the pitch canker pathogen, *Fusarium subglutinans* f. sp. *pini*, on juvenile *Pinus radiata* (Monterey pine) branches. To examine the potential for spread in cool montane regions of California, growth rates of the fungus obtained from an *Ernobius* sp. (anobiid beetle) were examined from 14°C to 26°C. The potential effect of invasion of new fungal strains was examined by comparing temperature responses of Florida isolates to California isolates.

A nested one-way ANOVA indicated decreased temperatures inhibited F. *s. pini* growth in inoculated *P. radiata.* Correlation analyses showed no relationship between stain length and branch age, branch length, branch height, or branch circumference.

The relationship between fungal growth and temperature varied significantly among isolates, but was similar for both Florida and California. Orthogonal contrasts showed linear fungal growth in relation to temperature (9° to 18°C).

DEDICATION

This thesis is dedicated with love to my wonderful wife, Trish Rios-Gibson, whose wit, strength, support, and above all else, love, made this thesis possible.

I thank Bill Bros for his dedication, guidance, and hard work from the genesis of this thesis to its fruition. His enthusiasm carried me through the project. I am grateful to Tom Gordon for his direction and patience. And I thank Ron Stecker for support from my undergraduate years to today.

I thank all of my associates on the pitch canker project at UC Berkeley: Paul Dallara, Dr. Andrew Storer, Carolyn Warren, and Dr. Dave Wood. Thanks for all of your assistance and kindness.

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GENERAL INTRODUCTION

This thesis incorporates two closely related manuscripts that will be submitted to the Canadian Journal of Botany, concerning the effect of temperature on the potential spread of the newly introduced pitch canker disease throughout cooler regions of California. This is the first study concerning temperature preferences of the pathogen in California or potential effect of invasion of fungal strains from Florida (where the disease has been established for many years).

Temperature Effects on Infection of Juvenile *Pinus radiata* (Monterey Pines) by *Fusarium subglutinans* f. sp. *pini*

Introduction

Pine pitch canker, caused by *Fusarium subglutinans* f. sp. *pini*, was discovered in California in 1986 (McCain et al. 1987). The fungus infects branch tips, causing them to wilt and die. Characteristic resinous cankers are found on tree branches, boles and, less frequently, roots. Bole cankers produce excessive amounts of resin that can extend many feet from an infected site (Dwinell et al. 1985; Storer and Dallara, 1992). Tree mortality can result from an attack by bark beetles on a weakened tree (Storer and Dallara, 1992).

Twelve native pine species have been found to be susceptible to infection by the fungus (McCain et al. 1987; Storer and Dallara, 1992). Storer and Dallara (1992) have reported that the fungus attacks three commercially important species in Northern California: *Pinus radiata* D. Don (Monterey), *P. muricata* D. Don (Bishop), and *P. ponderosa* (Ponderosa). *Pinus radiata* has been identified as the most susceptible to infection by *F. s. pini* (Correll et al. 1991).

Pitch canker is believed to be vectored in California by a variety of insects, including species of *Pityopthorus, Conopthorus, Ips* and *Ernobius*

species (Storer et al. in press). *Fusarium subglutinans pini* has been isolated from trapped insects, as well as individuals reared from infected pine branches (Fox et al. 1991; Dallara, unpublished).

Pitch canker has spread steadily since its discovery in 1986 in Santa Cruz County (McCain et al. 1987). The disease has also become established in Alameda and Monterey counties. Localized infections have also been found in Mendocino, San Luis Obispo, and Sonoma counties, and northern Santa Barbara county. Infected Christmas tree plantations have been reported in San Mateo, Santa Clara, Los Angeles and San Diego counties (Storer and Dallara, 1992).

With continued movement of pitch canker throughout California, there are economic implications for commercial and urban forestry. The susceptibility of timber trees such as *P. ponderosa* and *Pseudotsuga menziesii* (Douglas-fir) is of particular concern. The native ranges of these species extend into the Sierra Nevada, where there are large areas of susceptible commercial timber species (Storer et al. unpublished).

With reservoirs of infected beetles in *P. radiata* and other coastal pines, cross feeding by the beetles raises the possibility of continued cross inoculation into lumber species and Christmas tree varieties. Economic losses caused by pitch canker have included reduced growth and cone load, stained wood, and mortality (Dwinnell et al. 1985). Subsequent bark beetle

attacks on weakened trees could also create significant losses to the timber industry. A *P. radiata* Christmas tree plantation in Rosemead, California lost 5% of its crop in 1992, representing a potential market value of $75,000 (Storer pers. comm.). *Pinus radiata* in landscape settings has also suffered considerable damage throughout California. Dead and weakened trees also present a danger to life and property and are costly to remove.

This study examined a temperature range for pitch canker to determine its potential for becoming established in montane regions of California. *Fusarium subglutinans* f. sp. *pini* has not been recorded north of central Mendocino County and is restricted currently to coastal regions (Storer et al. in press). It is possible that growth of the fungus is inhibited by cooler temperature. The mean high temperature in the Sierra Nevada ranges from 16°C to 32°C during mid-Spring, Summer, and Fall (Owenby and Ezell, 1992). The purpose of this study was to determine if cooler temperatures (e.g. 14°C) would block or reduce growth of the fungus.

Materials and Methods

Pinus radiata (Monterey pine) saplings were obtained from two sources. Thirty potted pines were acquired from the California Department of Forestry and Fire Protection Nursery in Davis, CA and sixty potted *P. radiata* were obtained from the Lyon Tree Farm in Carmel Valley, CA. The pines

were at least 3 years old and at least 100 cm in height. All of the experimental trees were maintained outside in Berkeley and were watered daily, as needed to prevent stress.

The *Fusarium subglutinans* f. sp. *pini* isolate (JF119 described by Correll et al. 1991) was collected originally from a captured *Ernobius* sp. (anobiid beetle) in Santa Cruz County in 1988. The fungus was stored on filter paper at 4°C at University of California at Berkeley. Prior to experimentation the fungus was cultured on potato dextrose agar (PDA) for 7 days on petri plates at room temperature (23°±3°C). The plates were subsequently used to create suspensions.

Three healthy branches of at least 2 cm in circumference were inoculated on each tree with *F. s. pini.* Branches were wounded with a 1/8" drill bit (pretreated with a 0.5% sodium hypochlorite solution) until the phloem was reached at approximately 3 mm in depth. Five ul of a swirled suspension containing 2.5 x 10^6 microconidia/ml was pipetted onto the wound site. Inoculated trees were placed into one of three growth chambers set at 14, 18, or 26°C for 21 days. The growth chambers were set on a 16 hour photoperiod. The trees were watered daily.

Fusarium subglutinans growth was defined as the visible stain length on the pine phloem. At the end of the incubation period, branches were removed from the trees and dissected. Stain length was measured by peeling

the bark back from around the inoculation wound and measuring the stain along its longest axis to the nearest millimeter. A one-way nested analysis of variance (ANOVA) was used to analyze differences in average stain length among the three temperature groups (14, 18 and 26°C) (Zar, 1984). A nested design was used because three subsamples (branches) were collected from each individual tree.

Measurements of tree characteristics were made simultaneously to identify any correlation between tree attributes and the stain length. Branch age was measured to determine if branch maturity had any effect on the pathogen growth. The branch length and branch circumference at wound site were measured to determine if biomass was crucial to pathogen growth. The height of the branch above the soil was also recorded to see if there was any relationship between pathogen growth and spatial sites on the juvenile tree. A Pearson correlation (Zar, 1984) was used to examine for possible correlations between the phloem stain length and the tree variables.

Ten trees with a total of 30 inoculated branches were placed into each growth chamber, a total of 30 trees for all three temperature treatments. The experiment was conducted a total of three times. The replicate experiments were scored on 11/6/92, 12/18/92, and 1/21/93.

Results

The results of the one-way nested ANOVA showed that the temperature at which the juvenile trees were cultured did have an effect on *F. s. pini* growth (Table 1). A priori comparisons revealed that average stain length was lowest at 14°C, whereas the greatest average length was observed at 26°C (Figure 1). The decrease in growth with decreasing temperature appeared to be a linear function. A preliminary experiment revealed no visible lesion development at 10°C.

The measured tree variables appeared to have no effect on the extent of lesion development. The Pearson Product Moment correlations between branch age and stain length, branch length and stain length, circumference at the wound site and stain length, and branch height and the stain length were respectively, <0.01, -0.03, -0.05, and -0.02.

Discussion

Results from these laboratory experiments indicate that *Fusarium subglutinans* is able to grow at relatively cool temperatures in juvenile Monterey pine branches. A preliminary study indicated there was no fungal growth at 10°C. Although fungal growth was inhibited at 14°C, the fungus still grew.

The temperature range used in this study was within the typical

temperature range found in the Sierra Nevada from mid spring through early fall (Owenby and Ezell, 1992). The fungus may be able to establish itself in trees during these permissive seasons, if infected vectors are available. Subsequent growth of the fungus during the winter in the Sierra Nevada would depend on the amount of insulation provided by tree branches.

The fungus grew well at moderately warmer temperatures (26°C), indicating that pitch canker is unlikely to be limited by higher temperature. Research has yet to determine the upper temperature limit of the fungus, but it appears feasible that the disease will move from the coastal region into the warmer San Joaquin Valley and then to the cooler Sierra Nevada.

In addition to *P. radiata*, many other species of pine have shown susceptibility to the pathogen under greenhouse conditions (Storer and Dallara, 1992). McCain et al. (1987) also reported the commercially valuable *Pseudotsuga menziesii* (Douglas fir) was susceptible to the fungus; recently a naturally infected tree in California has been reported in California (Storer et al. in press).

A similar inoculation study using 1-2 year old *Pinus taeda* L. (loblolly) and *P. elliottii* Englem. var. *elliottii* (slash pine) seedlings determined the most efficient transfer of *F. subglutinans* inoculum was through a pin point wound (Kuhlman, 1987). This is congruous with the use of the drill bit in this study. Spore suspensions comparable to this study of 10^6 conidia/ml

were found to cause complete dieback of seedling branches (Kuhlman, 1987). Similar fungal inoculations on *P. radiata* saplings have eventually lead to death of the branch distal to the point of inoculation (Schultz and Gordon, unpublished).

An experiment examining the effects of temperature on soil inhabiting *Fusarium solani* f. sp. *pisi* and *F. oxysporum* f. sp. *ciceris* on chickpea found similar results to this study. Both fungi induced no disease symptoms at 10°C but showed severe symptoms (wilting) at higher temperatures (25° and 30°C) (Bhatti and Kraft, 1992). A linear regression relationship was discovered between disease severity of disease caused by *F. solani pisi* on chickpea and temperature (Bhatti and Kraft, 1992). This also appears true in this study of *F. s. pini* on pine.

The results of the Pearson correlation indicated that no measured physical attributes of the juvenile *P. radiata* influenced the growth of the pitch canker fungus. *Fusarium subglutinans* f. sp. *pini* grew equally well in all branch ages, branch circumferences, and branch height and lengths. Temperature was the most important factor influencing growth of the fungus.

These findings may indicate that the movement of pitch canker will not be limited by physical tree attributes or cool temperatures. Although the fungus didn't produce visible lesions at 10°C, the fungus was subsequently

isolated from these branches indicating it remained viable. The lower temperature limit for survival was not determined.

Further research is needed to establish the efficiency of insect vectors in transmitting *F. s. pini* to susceptible pines at cooler temperatures. Different species of bark and twig beetles could be treated with spore suspensions, then caged over a pine branch at various temperatures. Cooler temperature may decrease the efficiency of infection by the vectoring beetles.

Examination of lower inoculum levels in living trees should also be investigated. Only fragmentary information has been gathered regarding this topic. Determination of inoculum thresholds in combination with temperature would be useful in creating a predictive model comparable to a system for forecasting apple scab (Jones et al 1980). This information may be used to create models to predict the potential movement and efficacy of pitch canker infected vectors.

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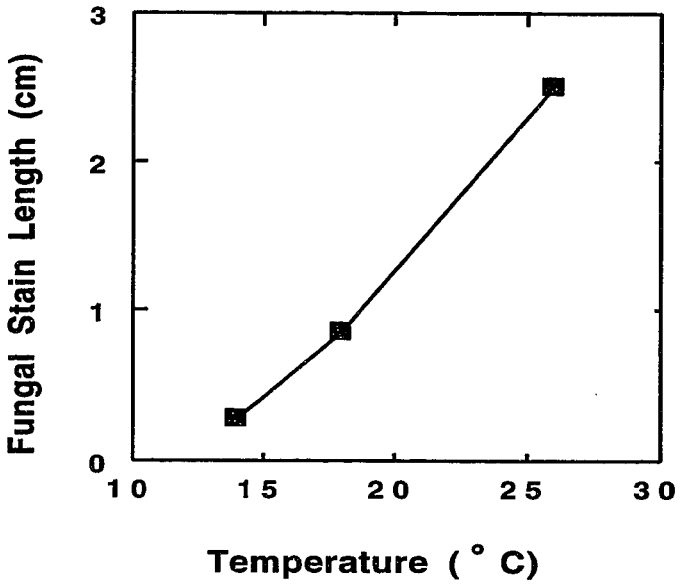

Figure 1: Mean stain length of *Fusarium subglutinans* f. sp. *pini* on branches of *Pinus radiata* saplings incubated at 14, 18 and 26 °C for 21 days.

Table 1: One-way nested analysis of variance of the effects of temperature on the stain length of *Fusarium subglutinans* on juvenile *Pinus radiata* branches.

Source	df	SS	F	P
Temperatures	2	221.469	42.1	<0.0001
A Priori Comparison				
14°vs. 18° & 26°C	1	94.018	35.785	<0.0001
18°vs. 26°C	1	126.542	48.164	<0.0001
Tree branch	77	202.302	2.204	<0.0001
Error	159	189.558		

The Influence of Temperature on Growth of *Fusarium subglutinans* f. sp. *pini* Isolates from California and Florida

Introduction

Pine pitch canker is a disease of pine trees caused by the fungus *Fusarium subglutinans* f. sp. *pini*. The disease was established in the southeastern United States and discovered in California in 1986 (McCain et al. 1987). Pitch canker is considered endemic on numerous southern pines including *Pinus elliottii* Englem. var. *elliottii* (slash), *P. taeda* L. (loblolly) and *P. virginiana* (Virginia) from Virginia to southern Florida and west to eastern Texas (Dwinnel et al. 1985). Since its discovery on *P. radiata* (Monterey pine) in Santa Cruz County, pitch canker has become well established in three coastal counties in California (Storer et al. in press).

Pitch canker causes many economic problems especially in pine plantations. An epidemic in 1974 infected over 51% of slash pines in east central Florida (Dwinell and Phleps, 1977). Normally, 2% of *Pinus elliottii* plantations in the southeastern United States are lost to the disease, but, during outbreaks, up to 25% mortality rates have been recorded (Oak et al. 1982). In 1992, Pitch canker was responsible for 5% mortality (3000 trees) of *P. radiata* in a Southern California Christmas tree plantation (Storer pers. comm.).

Introduction of exotic strains of *F. s. pini* to California from the southeastern Unites States is of economic concern for commercial forestry. If strains vary in their ability to reproduce in relation to environmental features, introductions of strains from other States or different lineages from the same State may allow the fungus to spread more rapidly. For example, the introduction of a strain capable of reproducing in cooler temperatures within the California Sierra Nevada range could cause an outbreak of pitch canker in large areas of susceptible timber species.

The purpose of this study was to determine if there is variation in the relationship of fungal growth to a range of cool temperatures among clonal descendants of various *Fusarium subglutinans* populations. Puhalla (1985) defined clonal lineages in *Fusarium oxysporum* as vegetative compatibility groups (VCGs). Variation among states was examined with isolates obtained from two States, California and Florida. Florida isolates were used as representatives of the *F. s. pini* population of the southeastern United States. The variation among California VCGs was examined with isolates from all known California strains. Variation among isolates was examined by including a number of isolates from two of the most common California VCGs (C1 and C2).

Materials and Methods

A total 326 isolates of *F. s. pini* were previously collected from diseased pine tissue (Monterey, *Pinus radiata* D. Don; Bishop, *P. muricata*:; and slash, *P. elliottii* Englm. var. *elliottii*) insects (*Ernobius*, Cicadellidae), and air samples from Florida and California (Correll et al. 1991, 1992). The Florida isolates were collected from *P. elliottii* pine plantations at four sites in three counties: Gainesville, Alachua Co.; Maytown and Osteen, Volusia Co. and Carabelle, Franklin Co. during May 1988. The California isolates were collected from *P. radiata* and *P. muricata*, air samples and trapped insects in five counties between 1987 and 1989. The isolates were stored on dried filter paper until used in this study at 4°C.

One representative isolate was selected to represent each of the five California VCG groups. A total of thirty isolates, ten from Florida and ten each from C1 and C2, were selected randomly from the University of California, Berkeley collection. A California isolate was included with the Florida isolates as a standard.

The fungal isolates (Table 1) were cultured on potato dextrose agar (PDA) for 7 days on petri plates at 23±3°C, after which they were used to prepare spore suspensions. PDA plates were prepared for spore inoculation by piercing agar at the center of the plate once with an autoclaved toothpick. The puncture provided a reservoir for the suspension and a center point for

future measurement. Five ul of a swirled spore suspension (2.5 x 10^6 microconidia/ml) was pipetted into the reservoir. The plates were then placed into incubators set at 9, 12, 15, and 18°C where they were incubated in darkness for seven days. The incubator temperatures were rechecked at the end of the seven day incubation period.

Fusarium subglutinans f. sp.*pini* colony diameter was defined as the longest axis passing through the center point to edges of the visible mycelium. Fungal colony diameter was measured to the nearest millimeter. A two-way analysis of variance (ANOVA) was used to test the significance of differences in fungal growth among isolates at four temperatures (9, 12, 15 and 18°C). An orthogonal contrast test was used to determine if the data points were linear. Linear regression analyses (Zar, 1984) were used to determine the coefficients for each isolate.

A total of 16 repetitions for each isolate at each temperature (9, 12,15, and 18°C) was included. The four experiments were initiated on 4/30/93, 6/25/93, 7/9/93, and 8/13/93, respectively.

Results

The relationship between fungal growth and temperature varied among the individual VCGs for both California and Florida. The isolate*temperature interactions in the two-way ANOVA were highly

significant for isolates originating in both California (Table 2A) and Florida (Table 2B). The significant interactions indicated that the extent of radial growth varied among the isolates.

Fungal growth was found to increase linearly with temperature for both California (Figure 1) and Florida (Figure 2) isolates. Orthogonal contrasts for each isolate showed fungal colony diameter to be a linear function of temperature over the range of 9°C to 18°C. Rates of fungal growth as a function of temperature (i.e. slopes) varied from 0.389 cm/°C to 0.448 cm/°C for California (Table 3A) and from 0.391 cm/°C to 0.526 cm/°C (Table 3B). The California isolate included with the Florida VCGs exhibited a growth rate (0.484 cm/°C) within the Florida range.

A survey of all known California VCGs was reported in the preceding paragraphs. In summary, the relationship between fungal growth and temperature varied among individual isolates. The isolate*temperature interactions from the two-way ANOVA was highly significant (Table 2A). Orthogonal contrasts showed radial growth was a linear function of temperature for all of survey of California VCGs.

The relationship between the fungal growth and temperature varied among the individual isolates from both California VCGs (C1 and C2). The isolate*temperature interactions in the two-way ANOVA were highly significant for both C1 (Table 4A) and C2 isolates (Table 4B). The significant

interactions indicated that the extent of fungal growth varied among the isolates.

Fungal growth increased linearly with temperature for isolates associated with VCGs C1 and C2. Orthogonal contrasts for each isolate showed fungal colony diameter to be a linear function of temperature over the range of 9° C to 18° C. The slopes for isolates associated with both VCGs were similar and varied from 0.367 cm/°C to 0.492 cm/°C for C1 and from 0.399 cm/°C to 0.463 cm/°C for C2.

A linear regression analysis of all of the data combined yielded the relationship: Fungal diameter (cm) = 0.433-3.570*Temperature (°C). The data were combined for this analysis because all previous analyses indicated that the primary source of variation was between isolates rather than among States or among VCGs within States.

Discussion

Results from these laboratory experiments indicate that *Fusarium subglutinans* f. sp. *pini* isolates from various vegetative compatibility groups collected from Florida and California have similar slope range variability. While there is a range of variability among isolates within each state, the slope range is consistent among states. *F. s. pini* in Florida was found to be much more diverse (45 VCGs) than in California's population (five VCGs).

Even within a single Florida tree plantation there was greater VCG diversity than found in the entire California population (Correll et al. 1992). The results of this study support a report that the pathotype of *Fusarium subglutinans* that infects the central coast of California is the same that is found in the Southern U.S. (Dwinell, 1980).

The variability among isolates all known California VCGs is similar to the interstate findings between Florida and California. There is variability among the five California VCGs, but the slope range is consistent with isolates found in Florida.

The variability among isolates from within California VCG (C1 and C2) was also consistent with the inter- and intrastate results. There was variability among individual isolates from both C1 and C2, but the slope range was consistent among isolates.

The fungal growth variation among States and among VCGs within States can be attributed to variability between isolates. The consistency of the fungal growth relationship to temperature and VCG indicates that fungal growth responds similarly to temperature, regardless of an isolates' vegetateve compatability grouping or geographic origin. The source of variability is unknown, but the random design precluded experimental bias.

Ploetz (1990) compared isolates of *F. oxysporum* f. sp. *cubense* associated with various vegetative compatibility groups over a range of

temperatures (8°-26°C) in a similar study. The mean growth rates differed frequently between VCGs of the pathogen by up to 5% between the slowest and fastest growing. The range difference between the slowest and fastest growing *F. s. pini* was much greater, from 14% to 25%. The linear growth rate of *F. o. cubense* peaked at approximately 26°C and declined to no growth at 36°C (Ploetz, 1990). While the temperature range of this study was more restricted, *F. s. pini* growth has showed no growth inhibition at the 26°C (McDonald, unpublished).

The similarity of slope variability of all isolates indicates that the temperature growth parameter is a locked in trait for all vegetative compatibility groups of *F. s. pini*. All of the isolates were able to grow at the lowest temperature, 9°C, quite well, thus there is no evidence of variation in growth, at the lowest temperature on which selection might act. Vegetative compatibility groupings are assumed to identify clonal lineages, with which asexual *F. s. pini* isolates, are associated. Isolates that are able to anastomose and form stable heterokaryons are associated with the same vegetative compatibility group (VCG) (Puhalla, 1985). VCGs often share traits such as colony size, isozyme patterns and virulence (Leslie, 1993). Because the formation of anastomoses is a prerequisite for exchange of genetic material through the parasexual cycle, isolates that aren't vegetatively compatible would constitute genetically isolated populations (Puhalla, 1985). Vegetative

compatibility of isolates used in this study was previously established by using nitrate nonutilizing (*nit*) mutants (Correll et al. 1987).

Because there were no significant differences between isolate growth rates (ie. slopes), management of pitch canker in California must be concerned with both internal and exotic sources of inoculum. If infected material from Florida was introduced into California, the new isolates would be able to compete against all known California VCGs with regard to temperature. Any of the tested isolates could infect susceptible trees in cooler regions of California, if vectors are available and other conditions are favorable.

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GENERAL DISCUSSION

Fusarium subglutinans f. sp. *pini* ability to grow at low temperatures typically found from mid-spring through early fall in California's Sierra Nevada creates the potential of movement of the fungus into these montane regions. The findings that there is no significant growth difference between *F. s. pini* isolates from Florida or California leads to the possibility that any infection, regardless of origin, has the potential to colonize any region of California, including the Sierra Nevada, if vectors are available and favorable conditions are found.

Management of pitch canker should be focused on four points: prediction of infected vector movement, removal of infected trees, prevention of importation and internal movement of inoculum sources throughout California, and, for a long term solution, development of resistant tree varieties.

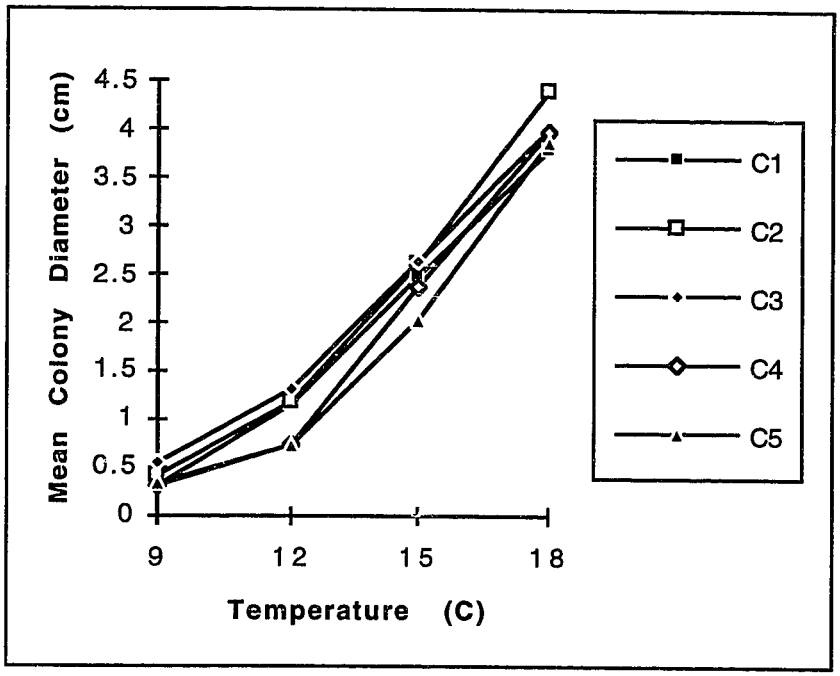

Figure 1: Variation in growth (colony diameter in cm) of *Fusarium subglutinans* f. sp. *pini* in relation to temperature (9 to 18°C) for various isolate representatives of California vegetative compatibility groups (VCGs). This illustrates the significant Isolate*Temperature interaction from the analysis in Table 2A.

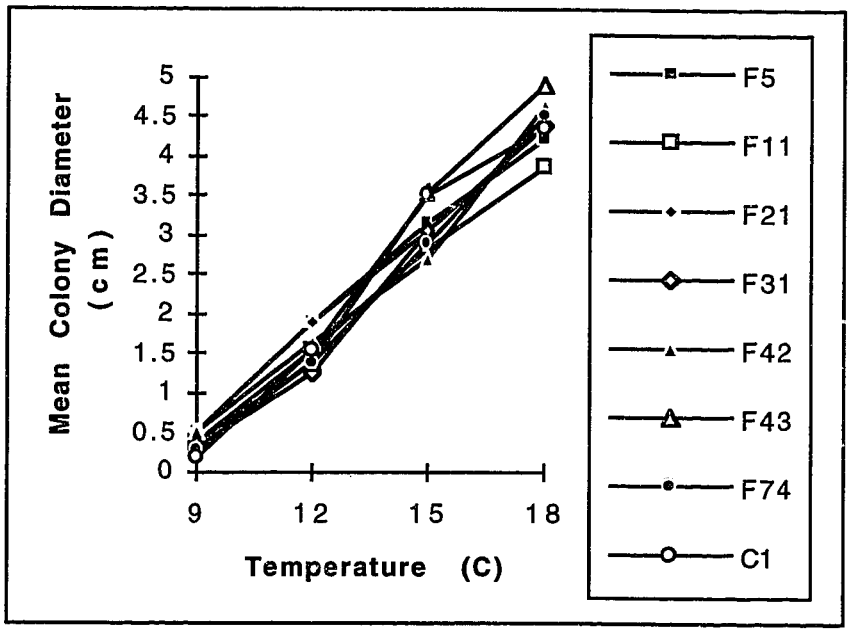

Figure 2: Variation in growth (colony diameter in cm) of *Fusarium subglutinans* f. sp. *pini* in relation to temperature (9°to 18°) from various isolate representatives of Florida vegetative compatibility groups (VCGs). This illustrates the significant Isolate*Temperature interaction from the analysis in Table 2B.

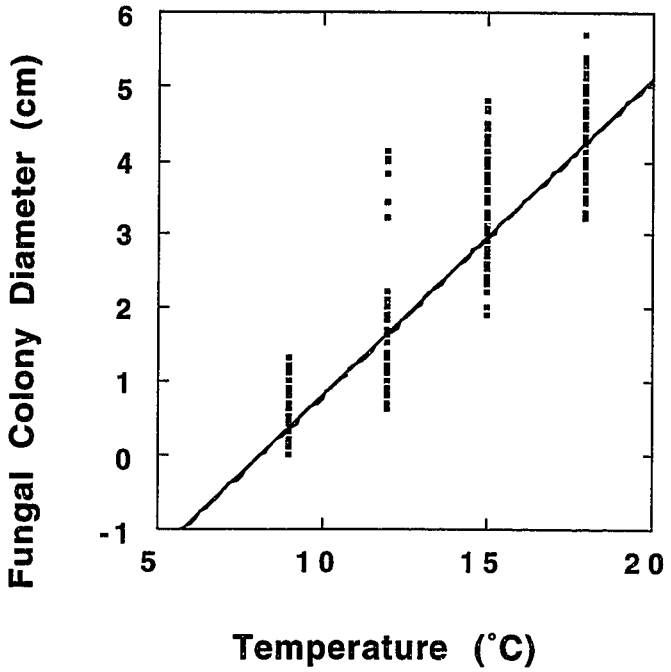

Figure 3: Regression plot of all *Fusarium subglutinans* f. sp. *pini* isolates from California and Florida.

Table 1: Experimental vegetative compatibility groups (VCGs) of *Fusarium subglutinans* examined.

VCG	State	County	Source
C1	CA	Santa Cruz	*P. radiata*
C2	CA	Santa Cruz	*Ernobius* sp.
C3	CA	Los Angeles	*P. radiata*
C4	CA	Santa Cruz	*P. radiata*
C5	CA	Los Angeles	P. radiata
C1	CA	Santa Cruz	air sample
C1	CA	San Diego	*P. radiata*
C1	CA	San Diego	*P. radiata*
C1	CA	Santa Cruz	*Ips* sp.
C1	CA	San Luis Obispo	*P. radiata*
C1	CA	Alameda	*P. radiata*
C1	CA	Alameda	*P. radiata*
C1	CA	Alameda	*P. radiata*
C2	CA	Santa Cruz	air sample
C2	CA	Santa Cruz	*P. muricata*
C2	CA	Santa Cruz	*P. muricata*
C2	CA	Santa Cruz	Cicadellidae
C2	CA	Santa Cruz	*P. radiata*
C2	CA	Santa Cruz	*P. radiata*
F5	FLA	undetermined	*P. elliottii*
F11	FLA	undetermined	*P. elliottii*
F21	FLA	undetermined	*P. elliottii*
F31	FLA	undetermined	*P. elliottii*
F42	FLA	undetermined	*P. elliottii*
F43	FLA	undetermined	*P. elliottii*
F74	FLA	undetermined	*P. elliottii*

Table 2:Two-way analysis of variance on the effects of temperature and vegetative compatibility group in relation to *Fusarium subglutinans* f. sp. *pini* colony diameters for California and Florida.

A. Survey of all Californian VCGs

Source	df	SS	F	P
Temperature	3	620.972	6715.936	<0.0001
Isolate	4	8.198	66.494	<0.0001
Temp*Isolate	12	4.963	13.420	<0.0001
Error	300	9.246		

B. Survey of Florida VCGs

Source	df	SS	F	P
Temperature	3	1086.575	4651.989	<0.0001
Isolate	7	7.519	13.796	<0.0001
Temp*Isolate	21	20.552	12.570	<0.0001
Error	454	35.347		

Table 3: Slopes and y-intercepts from California and Floridia vegetative compatibility groups of *Fusarium subglutinans*.

A. Slopes and y-intercepts for the five California VCGs.

VCG	Slope	Intercept	df	P
C1	0.392	-3.349	62	<0.0001
C2	0.422	-3.838	62	<0.0001
C3	0.448	-3.885	62	<0.0001
C4	0.395	-3.593	62	<0.0001
C5	0.389	-3.118	62	<0.0001

B. Slopes and y-intercepts for isolates from Florida and the California standard (C1).

VCG	Slope	Intercept	df	P
F5	0.410	-3.093	61	<0.0001
F11	0.391	-3.119	62	<0.0001
F21	0.427	-3.299	62	<0.0001
F31	0.467	-4.057	62	<0.0001
F42	0.450	-3.708	61	<0.0001
F43	0.526	-4.550	62	<0.0001
F74	0.477	-4.163	62	<0.0001
C1	0.484	-4.133	38	<0.0001

Table 4: Two-way analysis of variance for the effects of temperature and isolates on the *Fusarium subglutinans* f. sp. *pini* colony diameters for California vegetative compatibility groups C1 and C2.

A. C1 isolates

Source	df	SS	F	P
Temperature	3	1067.963	4327.864	<0.0001
Isolate	7	3.920	6.809	<0.0001
Temp*Isolate	21	21.752	12.593	<0.0001
Error	472	38.824		

B. C2 isolates

Source	df	SS	F	P
Temperature	3	830.576	4.322	<0.0001
Isolate	6	2.599	6.762	<0.0001
Temp*Isolate	17	14.689	13.489	<0.0001
Error	387	24.791		