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Response of different white fir geographic provenances to *Trichosporium symbioticum* inoculation in California

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Abstract: We inoculated the fir engraver (*Scolytus ventralis* LeConte) associated fungus *Trichosporium symbioticum* Wright onto 56 white fir (*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.) trees planted in a common garden study near Camino, California, that represented five geographic provenances of this species. The objective was to determine if there is a differential lesion length response of white fir provenances with respect to provenance. We found a significant ($P < 0.019$) difference between the provenances from Arizona and those of eastern Nevada origins 28 days after inoculation. There was a significant interaction between the two *T. symbioticum* isolates and season of inoculation. Fall inoculations tended to have smaller lesions than those in the spring but this varied by isolate in that the one from eastern Nevada tended to produce longer lesions in the spring ($P = 0.0001$) whereas the isolate from the Camino plantation did not differ between spring and fall ($P = 1.000$). There is evidence for genetic variability relative to white fir provenance lesion length in response to *T. symbioticum* inoculation, and in future studies, isolate variability should also be taken into account.

Résumé : Nous avons inoculé le champignon *Trichosporium symbioticum* Wright associé au scolyte sculpteur du sapin (*Scolytus ventralis* LeConte) sur 56 sapins concolores (*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.) plantés dans le cadre d'une étude en jardin commun près de Camino, en Californie, qui compte cinq provenances géographiques de cette espèce. L'objectif consistait à déterminer si la longueur des lésions varie selon la provenance du sapin concolore. Nous avons observé une différence significative ($P < 0{,}019$) entre les provenances de l'Arizona et celles de l'est du Nevada 28 jours après l'inoculation. Il y avait une interaction significative entre les deux isolats de *T. symbioticum* et la saison d'inoculation. Les inoculations faites à l'automne avaient tendance à produire de plus petites lésions que celles du printemps mais cela variait selon l'isolat : l'isolat de l'est du Nevada avait tendance à produire des lésions plus longues au printemps ($P = 0{,}0001$) tandis que les lésions produites par l'isolat provenant de la plantation de Camino avaient les mêmes dimensions ($P = 1{,}000$) que l'inoculation ait eu lieu au printemps ou à l'automne. Il y a des indices que la longueur des lésions en réaction à l'inoculation de *T. symbioticum* reflète la variabilité génétique des provenances de sapin concolore et les études ultérieures devraient également tenir compte de la variabilité des isolats.

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Introduction

The fir engraver bark beetle *Scolytus ventralis* LeConte (Coleoptera:Scolytinae) is a serious pest in the western United States. The outbreak in California that arose during a protracted drought during 1988–1993 has resulted in extensive mortality in white fir (*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebr.), red fir (*Abies magnifica* A. Murray), and grand fir (*Abies grandis* (Douglas ex D. Don) Lindl.) stands. Sporadic outbreaks are usually associated with drought and have occurred nearly every decade of the 20th century (Berryman and Ferrell 1988).

As with most other bark beetles, the fir engraver vectors a symbiotic fungus, *Trichosporium symbioticum* Wright, which elicits a response when introduced into the phloem and cambium by the attacking beetle. This host response was first described by Wright (1935) and later employed by others to study bark beetle and fungal interactions (Berryman 1969; Wong and Berryman 1977; Raffa and Berryman 1982; Filip et al. 1989 among others). These studies generally demonstrated a rapid host response to *T. symbioticum* inoculation characterized by brown necrotic lesions and formation of induced traumatic resin canals. Some of these studies showed grand fir that was resistant to fir engraver attack tended to have more resin content (Raffa and Berryman 1982; Filip et al. 1989). They found little or no relationship to length of lesions and grand fir resistance to attack.

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Since the detailed investigation reported by Wright (1935), there have been few studies involving the fir engraver – *T. symbioticum* association in white fir. Ferrell et al. (1993) employed *T. symbioticum* inoculation in a field study determining susceptibility of white fir to fir engraver attack and mortality. In all of the studies cited above, effects of host provenance and fungal isolate origin were not addressed. Also, most of these studies were conducted on grand fir. The purpose of our study is to report on results from an experiment conducted in an established white fir common garden study involving five distinct geographic provenances of white fir as established by Hamrick and Libby (1972) and Libby et al. (1980) near Camino, California, and several other locations throughout the northern and central Sierra Nevada. Unfortunately, the Camino location is the only surviving plantation. The present study was designed to take advantage of this established common garden white fir plantation in the context of investigating response of five white fir provenances to inoculation with two isolates of *T. symbioticum*. Specifically, we explore if evidence of geographic provenance differences in lesion length response to artificial inoculation with this fungal symbiont exists. It is hoped we provide the basis for future, more detailed investigations concerning this bark beetle – fungal association.

Materials and methods

During March 1966, nine seedlings from each of 39 white fir provenances, representing most of the species' natural range, were planted as a common garden study as 2-1 stock at Camino (1040 m elevation, 38°44′26.2″N, 20°39′48.0″W), Eldorado County, California (Libby et al.1980). Each provenance sample consisted of three replications, each of three seedlings, planted in an interlocked randomized noncontiguous plot layout designed to minimize microsite variation (Libby et al. 1980). One replication was removed by thinning in 1970.

For the purpose of this study, trees among remaining provenances were chosen to represent the five major morphological divisions of white fir as defined in previous studies (Hamrick and Libby 1972; Libby et al. 1980). The five divisions or provenance groups represent seed sources from (1) northern populations intermediate with *A. grandis* in central and southern Oregon (OR), (2) central var. *lowiana* populations in northern and central Sierra Nevada in California (CS), (3) southern populations intermediate between var. *lowiana* and var. *concolor* in southern California (SC), (4) interior north var. *concolor* populations in eastern Nevada and western Utah (EN), and (5) interior south var. *concolor* populations in Arizona and New Mexico (AZ). During the fall of 1990, trees of the various provenances representing the five groups were chosen a priori by randomly selecting trees from the Camino plantation inventory list on file at the USDA Forest Service, Institute of Forest Genetics, Placerville, California. Selected trees (Table 1) were located and flagged on site. Eight to 12 trees were selected from each of the five groups. Occasionally, alternates were chosen because the initial selection was deemed unsatisfactory due to mortality or heavy bark beetle attack. A total of 56 trees were selected for this experiment. Diameter measurements at 1.3 m height (DBH) were obtained on all selected trees.

Table 1. Number of trees and their source codes identifying trees at the Camino, California, white fir (*Abies concolor*) study used for inoculation with *Trichosporium symbioticum*.

Provenance group	Population code	No. of trees
Oregon (OR)	Q	4
	S	4
	U	4
	V	1
Central Sierra (CS)	AA	4
	AK	3
	AM	3
	BD	4
Southern California (SC)	AS	4
	AU	4
	AV	4
Eastern Nevada (EN)	AX	1
	BF	4
	BG	2
Arizona (AZ)	BB	2
	BC	4
	MSU	4

Note: Population codes are as designated by Hamrick and Libby (1972) and Libby et al. (1980).

Two isolates of *T. symbioticum*, one isolated from a lesion within a *S. ventralis* attacked white fir tree in the Camino plantation (TT-2) and one obtained from a lesion of an attacked white fir tree near Baker, Nevada (BG-3), were used in this study. Isolations from phloem tissue from these locations took place during late summer 1989. These isolates were maintained at the USDA Forest Service Pacific Southwest Research Station in Albany, California. Long-term storage of these cultures was in 3 mL vials containing 1% malt extract agar slants kept at 4 °C. The identity of the *T. symbioticum* isolates was determined morphologically by reference to a previously published description (Wright 1935) and from examination of *T. symbioticum* specimens obtained from the American Type Culture Collection, Rockville, Maryland. The two isolates of the fungus were grown for 10 days at 24 °C on malt extract agar (1.25% malt extract, 1.5% agar) in 100 mm × 15 mm Petri plates. After growing the fungus, inoculum was prepared by aseptically cutting agar plugs from the outer 3 cm margin of the colonies with a sterile 4 mm diameter cork borer. Subsequent cultures of these isolates were stored at 4 °C for future study. As of this time, only the isolate TT-2 survives in the culture collections of the USDA Forest Service, 320 Green St., Athens, Georgia.

Trees were prepared for inoculation by shaving sections of rough outer bark to a depth of 0.5 cm or less with a draw knife or sharp axe blade to remove excess, fissured bark. The shaved sections were arranged around the circumference of the bole at 1.2–1.5 m height. Occasional branch stubs and old *S. ventralis* attack scars prevented complete circumferential arrangement in a few trees. In those cases, shaved sections were located above or below the obstacle as close as possible to the others. Assignment of fungal isolate and control wound inoculations was randomized with respect to position on the bole. Shaved sections were marked with indelible pen as to the position of fungal isolates and control wound. Six sections were prepared for each tree, with the two *T. sym-*

bioticum isolate inoculations and the control wound duplicated on each tree. The dimensions of each section to be wounded and inoculated were approximately 8–12 cm wide by 12–15 cm long, each section separated by a minimum of 5 cm. In the case of smaller diameter trees, sections to be inoculated were staggered at, above, or below the typical height. The section width ensured unlikelihood of lesion lateral spread and coalescence for the duration of the experiment. Inoculations were carried out using the cork borer method of Wright (1935) in late September 1990. The bole section to be inoculated was swabbed with 95% ethanol and a 4 mm diameter sterile cork borer was plunged into the bark section and appropriate force was applied to ensure penetration to the cambium. A 3–4 cm space was left between the three inoculation wound points in each section, ensuring discreetness of inoculation positions. The 4 mm diameter agar plugs from the fungal isolates on sterile malt agar control plates were placed into the hole. The bark plug was then replaced and each wound point was taped with a 3.5 cm square of duct tape. To eliminate the possibility of cross-contamination, the control wound inoculations were performed first. In a similar manner, the two fungal isolates were inoculated separately and with different sets of cork borers. The bark plug was replaced and duct tape was used as described in all control and fungal inoculations. Borers used during fungal inoculations and control wounds were sprayed and swabbed with 95% ethanol between trees.

After 28 days, the inoculated wounds were excised from the boles with a wood chisel, cutting approximately 7 cm long by 2.5 cm wide strips of phloem from each inoculation point to ensure complete lesion excision. Chisels were swabbed with 95% ethyl alcohol between inoculation treatments. Excised phloem sections were sealed in plastic bags immediately after excision and chilled in an ice chest in the field. Lesion lengths from all treatments were measured to the nearest millimetre. Slivers of phloem tissue samples were also plated onto cycloheximide amended malt extract agar (Harrington 1981) from 12 randomly selected trees with fungal treatments and control lesions equally represented to determine presence of *T. symbioticum*. The inoculated plates were incubated for 2 weeks prior to confirming presence or absence of the fungus via compound microscopy.

During early June 1991, the same trees employed in the fall inoculations were again prepared for inoculation. The wounds from the fall inoculation began to callus and did not appear to encroach upon the prepared sections for June inoculations. As in the fall, isolates BG-3 and TT-2 and control wounds were inoculated in the 1.2–1.5 m height zone on the tree boles, with duplicate sets of each treatment on each tree. Care was taken to ensure comparable placement and randomization of inoculum treatments within the height zone of each tree. After 4 weeks, lesions were excised, measured, and sampled for re-isolation of *T. symbioticum* as in the fall.

A three-factor mixed linear model analysis of variance was used to analyze the lesion length data. The experimental design was viewed as a split-split-plot design with the five provenance groups being the main-plot factor with replication completely at random by means of a sample of trees (7–14) within each provenance group. The split-plot factor was the lesion types (BG-3, TT-2, and control) applied to each tree. The split-split-plot factor was season (fall and spring), which was also considered as a repeated measures factor due to possible correlation between the fall and spring measurements on the same tree. In repeated measure designs, when observations have equal variance at all times, and pairs of observations on the same experimental unit are equally correlated regardless of the time between observations, the simpler univariate analysis of variance is valid and yields an optimal method of analysis (Littell et al. 1998). Data that meet these requirements are said to satisfy the Huynh–Feldt condition (Huynh and Feldt 1970). After performing graphical and statistical tests for normality and homogeneity of variance assumptions, we found that homogeneity was better achieved by adjusting the data for the control (which had a much smaller variance than the BG-3 and TT-2 lesions) at the split-plot level. This was done by subtracting the control reactions from those of the inoculated reactions associated with each tree and inoculation season to analyze the reaction due to fungal inoculation alone. This improved the homogeneity of variance assumption, and with only one remeasurement on a tree, the observations were equally correlated. Thus, the Huynh–Feldt condition was satisfied and univariate analysis of variance was used (SAS Proc Mixed), relaxing the requirement of using repeated measures and modeling the covariance matrix. Multiple comparisons based on the Bonferroni approach were performed using differences in least square means to test for fungal treatment and provenance group effects at the 0.05 level. We also performed a regression of lesion length on DBH to determine if DBH influenced lesion size.

Results

After 4 weeks, excised lesions exhibited resin soaking and brown staining defining the reaction zone and were consistent with those reported by Wright (1935) and Filip et al. (1989). No inoculated trees died during the course of the experiment. We recovered *T. symbioticum* from all 12 of the randomly selected excised lesions from each fungal treatment after plating tissue on1.25% cycloheximide amended malt agar. No *T. symbioticum* was isolated from excised control wounds. Mean lesion length and standard deviation of control wounds were 0.86 ± 0.23 cm in the fall inoculations and 0.72 ± 0.21 cm in the spring.

Provenance group, inoculation season, and fungal isolates were significant as was the interaction between fungal isolate and season (Table 2). Trees from the AZ provenance group had significantly longer lesions than the EN groups at Bonferroni-adjusted $\alpha = 0.05$ (Table 3). Least square means and standard errors for lesion lengths from fall and spring inoculations with the two *T. symbioticum* isolates are given in Table 4. The *T. symbioticum* isolates differed in their lesion responses between fall and spring inoculations, with spring inoculations yielding greater lesion lengths. Isolate TT-2 lesion length differed from BG-3 lesion length in the fall ($P < 0.0001$) but not in the spring ($P = 0.88$). Also, we did not detect a difference between spring and fall lesion lengths for TT-2 ($P = 1.00$) but did so for isolate BG-3 ($P < 0.0001$). Lesion lengths from TT-2 spring inoculations differed from BG-3 fall inoculations ($P < 0.0001$). The differences in least square means and their associated probabilities for this interaction are given in Table 5.

Table 2. Analysis of variance for lesion length data from white fir (*Abies concolor*) inoculated with *Trichosporium symbioticum*.

Effect	df (numerator)	df (denominator)	*F*	Probability
Provenance	4	51	2.9	0.0309
Isolate	1	151	4.23	0.0413
Provenance × isolate	4	151	0.73	0.5722
Season	1	152	18.72	0.0001
Provenance × season	4	151	1.72	0.1489
Isolate × season	1	151	17.02	0.0001
Provenance × isolate × season	4	151	0.87	0.4824

Table 3. Least square means of lesion length response of white fir (*Abies concolor*) provenance groups at the Camino, California, common garden plantation 4 weeks after inoculation with *Trichosporium symbioticum*.

	Provenance groups				
	AZ	CS	EN	OR	SC
Lesion length (cm)	3.70 (0.29) a	3.00 (0.25) ab	2.22 (0.35) b	2.78 (0.26) ab	2.99 (0.27) ab

Note: Least square means not sharing letters indicate significant differences at Bonferroni-adjusted $\alpha = 0.05$. Standard errors of least square means are in parentheses. Provenance groups: AZ, Arizona; CS, central Sierra; EN, eastern Nevada; OR, Oregon; SC, southern California.

Table 4. Mean spring and fall lesion length response of two isolates of *Trichosporium symbioticum* on white fir (*Abies concolor*) provenances.

Isolate	Fall	Spring
Camino TT-2	3.07 (0.18)	3.09 (0.18)
E. Nevada BG-3	2.19 (0.18)	3.38 (0.18)

Note: Numbers in parentheses are standard errors of least square means.

There are statistical differences for DBH among the provenance groups (Table 6). The AZ, CS, and SC groups had larger DBH than the EN and OR groups ($P < 0.0001$). The regression of DBH with lesion length as the dependent variable was significant for isolates TT-2 in fall and spring ($P = 0.026$, $R^2 = 0.09$ and $P = 0.020$, $R^2 = 0.10$, respectively) and BG-3 in fall and spring ($P = 0.007$, $R^2 = 0.13$ and $P = 0.000$, $R^2 = 0.25$, respectively).

Discussion

There is evidence indicating a host genetics component in white fir responses to *T. symbioticum* inoculation. The statistical difference in lesion length between the AZ provenances and the EN one is interesting in that these populations are both *A. concolo*r var. *concolor* but are genetically separate based upon morphology, phenology, and cortical monoterpene composition (Hamrick and Libby 1972; Zavarin et al.1975; Libby et al. 1980). On the other hand, lesion length did not differ among the other provenances or between AZ provenances and the others. Thus it appears there are other associated factors governing response of white fir to *T. symbioticum* besides provenance. There may be some other dynamics within the AZ group and among the other groups affecting their interactions with this fungus. An important point is that this study obviously addresses only trees surviving a protracted drought episode that resulted in considerable mortality in the Camino common garden site, the subject site in this study (Ferrell and Otrosina 1996). Our study took place during that protracted drought (1987–1992) and, being a common garden study, the inoculation responses we found may be a differential reaction of the provenances to the drought. Initial assessments of stress based upon predawn water potential measurements in this plantation did not reveal differences among provenances (Ferrell and Otrosina 1996 and unpublished data). The AZ, EN, and SC provenances are regarded as more drought resistant and sustained significantly less mortality during this fir engraver outbreak than the CS and OR provenances (Ferrell and Otrosina 1996). The AZ, EN, and SC populations also have different cortical monoterpene composition than that of the other two groups (Zavarin et al.1975). Perhaps because more drought susceptible trees were eliminated, particularly those of CS and OR origins, the genetic basis for host–fungus interactions relative to surviving individuals within provenances could have changed or become skewed. The original experimental design of the Camino plantation study minimized microsite and positional variation (Hamrick and Libby 1972) and the trees in this study were randomly selected.

Our study is the first of its kind involving white fir response to *T. symbioticum* inoculation relative to geographic provenance. Other studies involved inoculation of grand fir trees with *T. symbioticum* in assessments of tree vigor relationships relative to *S. ventralis* attack. They showed no relationship between lesion length and tree vigor (Filip et al. 1989). Wong and Berryman (1977), Raffa and Berryman (1982), and Filip et al. (1989) all found monoterpene or resin concentrations to be a better indicator of wound response relative to tree vigor than lesion length alone. Wound response in relation to *T. symbioticum* inoculation in fir engraver resistant grand fir trees is apparently influenced by tree age (Raffa and Berryman 1982). Trees older than age 90 and younger than age 50 had lower monoterpene concentrations than middle-aged trees. In that study, lesion length was not related to age, crown status, monoterpene content, or survivorship from *S. ventralis* attack. Tree age and crown position variation was not an issue in our study. Ferrell et al. (1993) found lesion lengths to be a poor predictor of white fir mortality or attack by the fir engraver based on white fir tree

Table 5. Least square means differences in lesion lengths and associated probabilities for the interaction between isolate and season 28 days after inoculation of white fir (*Abies concolor*) provenances with *Trichosporium symbioticum*.

	Isolate, season		
Isolate, season	TT-2, spring	TT-2, fall	BG-3, spring
BG-3, fall	0.900 (0.000)	0.870 (0.000)	1.191 (0.000)
BG-3, spring	–0.291(0.883)	–0.320 (0.659)	
TT-2, fall	–0.029 (1.000)		

Note: Least square means differences of comparisons are outside parentheses and associated Bonferroni-adjusted probabilities are inside parentheses.

Table 6. Mean diameter at breast height (DBH) of inoculated white fir (*Abies concolor*) trees in the Camino, California, geographic provenance study.

Provenance	DBH (cm)
Arizona (AZ)	24.10 (1.47) a
Central Sierra (CS)	23.21 (1.24) a
Eastern Nevada (EN)	10.00 (1.76) b
Oregon (OR)	16.25 (1.29) b
Southern California (SC)	24.38 (1.34) a

Note: Means sharing the same letters are not significant at Bonferroni-adjusted $P < 0.05$. Numbers in parentheses are standard errors.

vigor compared with stand basal area. We found DBH of selected trees in this study to differ among provenances (Table 6) and regression of lesion length and DBH for isolates BG-3 and TT-2 in fall and spring inoculations was significant but had low r^2 values. We conclude DBH does not appear to robustly influence lesion length although this possibility merits further investigation.

We found lesion length is also dependent upon season. Lesions from fall inoculations tended to be shorter than those of the spring. However, interpretation of the significant interaction between isolates and season is complex in that the two isolates of *T. symbioticum* had differential responses relative to season (Table 5). Because only two isolates were employed in this study, it is not possible to draw inferences about the basis of these differences vis-à-vis isolate geographic origin or host provenance. The results clearly demonstrate that isolate variability exists and similar studies addressing this insect fungal complex should take fungal isolate variability into account.

Incidentally, a recent taxonomic revision of ambrosia beetle fungal associates (Harrington et al. 2010) provides information implying the *T. symbioticum* taxonomic position should be revised and perhaps placed in the genus *Hyalorhinocladiella* Upadhyay and Kendrick. A taxonomic revision would be an important undertaking in that it will clarify the relationships between the *T. symbioticum* – *S. ventralis* association and other conifer bark beetle – fungal associations, aiding research on the role these fungi play in bark beetle attacks and conifer mortality.

A recent review by Lieutier et al. (2009) about elicitation of conifer defenses toward Ophiostomatoid fungi carried by other bark beetles opens an intriguing line of questions relative to the *T. symbioticum* – *S. ventralis* association. They hypothesize that bark beetle aggressiveness is correlated with the ability of the associated sap stain fungus to stimulate host defenses. Could the *S. ventralis* – *T. symbioticum* association be an evolutionary mechanism whereby the insect fungal association serves as a probe of host defenses? For example, an *S. ventralis* attack introducing *T. symbioticum* to firs with an optimal physiological state would cause the tree to elicit a rapid and vigorous, induced resinous response to the fungus. Such a response could then render the tissue unsuitable for further insect colonization. Trees under stress, on the other hand, would not have such a strong response, thereby increasing the likelihood for insect and fungus colonization. Resin chemistry studies of the induced response of white fir provenances and fungal isolate interactions are merited and would also help interpret the intricacies of this bark beetle and fungus association.

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References

Berryman, A.A. 1969. Responses of *Abies grandis* to attack by *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. **101** (10): 1033–1041. doi:10.4039/Ent1011033-10.

Berryman, A.A., and Ferrell, G.T. 1988. The fir engraver in western states. *In* Dynamics of forest insect populations. *Edited by* A.A. Berryman. Plenum, New York. pp. 555–577.

Ferrell, G.T., and Otrosina, W.J. 1996. Differential susceptibility of white fir provenances to the fir engraver and its fungal symbiont in northern California. *In* Dynamics of forest herbivory: quest for pattern and principle. *Edited by* W.J. Mattson, P. Niemela, and M. Rousi. U.S. For. Serv. Gen. Tech. Rep. NC-183. pp. 187–191.

Ferrell, G.T., Otrosina, W.J., and DeMars, C.J., Jr. 1993. Assessing susceptibility of white fir to the fir engraver, *Scolytus ventralis* Lec. (Coleoptera: Scolytidae), using fungal inoculation. Can. Entomol. **125**(5): 825–901. doi:10.4039/Ent125895-5.

Filip, G.M., Christiansen, E., and Parks, C.A. 1989. Secondary resin production increases with vigor of *Abies grandis* inoculated with *Trichosporium symbioticum* in northeastern Oregon. U.S. For. Serv. Res. Note PNW-RN-489.

Hamrick, J.L., and Libby, W.J. 1972. Variation and selection in western U.S. montane species. 1. White fir. Silvae Genet. **21**: 29–35.

Harrington, T.C. 1981. Cycloheximide sensitivity as a taxonomic character in *Ceratocystis*. Mycologia, **73**(6): 1123–1129. doi:10.2307/3759682.

Harrington, T.C., Aghayeva, D.N., and Fraedrich, S.W. 2010. New combinations in *Raffaelea*, *Ambrosiella*, and *Hyalorhinocladiella*, and four new species from the red bay ambrosia beetle, *Xyleborus glabratus*. Mycotaxon, **111**(1): 337–361. doi:10.5248/111.337.

Huynh, H., and Feldt, L.S. 1970. Conditions under which mean square ratios in repeated measures designs have exact F-distributions. J. Am. Stat. Assoc. **65**: 1582–1589.

Libby, W.J., Isik, K., and King, J.P. 1980. Variation in flushing time among white fir population samples. Ann. For. **8**: 123–138.

Lieutier, F., Yart, A., and Salle, A. 2009. Stimulation of tree defenses by Ophiostomatoid fungi can explain attack success of bark beetles on conifers. Ann. For. Sci. **66**(8): 801. doi:10.1051/forest/2009066.

Littell, R.C., Henry, P.R., and Ammerman, C.B. 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. **76**(4): 1216–1231. PMID:9581947.

Raffa, K.F., and Berryman, A.A. 1982. Accumulation of monoterpenes and associated volatiles following inoculation of grand fir with a fungus transmitted by the fir engraver, *Scolytus ventralis* (Coleoptera: Scolytidae). Can. Entomol. **114**(9): 797–810. doi:10.4039/Ent114797-9.

Wong, B.L., and Berryman, A.A. 1977. Host resistance to the fir engraver beetle. 3. Lesion development and containment of infection by resistant *Abies grandis* inoculated with *Trichosporium symbioticum*. Can. J. Bot. **55**(17): 2358–2365. doi:10.1139/b77-269.

Wright, E. 1935. *Trichosporium symbioticum*, n. sp., a wood-staining fungus associated with *Scolytus ventralis*. J. Agric. Res. **50**(6): 525–539.

Zavarin, E., Snajberk, K., and Fisher, J. 1975. Geographic variability of monoterpenes from the cortex of *Abies concolor*. Biochem. Syst. Ecol. **3**(4): 191–203. doi:10.1016/0305-1978(75)90002-2.