Chemical Constituents in Root Bark of Five Species of Western Conifer Saplings and Infection by *Armillaria ostoyae*

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ABSTRACT

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Ten years after being planted, five species of western conifer saplings were inoculated with two isolates of *Armillaria ostoyae*. Infection ratings assigned to *A. ostoyae* were highest in saplings of *Abies grandis* and *Pseudotsuga menziesii* and lowest in those of *Larix occidentalis*. Height and diameter growth were greater in saplings of *L. occidentalis*, *Pinus monticola*, and *Pinus ponderosa* than in those of *A. grandis* and *P. menziesii*. Concentrations of sugar and starch in root bark were higher in *A. grandis* and *P. menziesii* than in the other species. Concentrations of phenolics and protein-precipitable tannins were highest in root bark of *L. occidentalis*. Biochemical parameters of root bark were regressed with assigned ratings of infection by *A. ostoyae*; coefficients of determination (r^2) ranged from 0.06 (cellulose) to 0.56 (sugars). Ratios of the energetic costs of phenolic and lignin degradation relative to the energy available from sugars (E_{pd}:E_{as} and E_{ld}:E_{as}) were correlated with infection rate ($r^2 = 0.87$ and 0.76, respectively). Species that are more susceptible to infection by *A. ostoyae* may produce lower concentrations of phenolics and more sugar in root bark, thereby increasing the energy available to the fungus to degrade the phenolics and invade the host trees.

Additional keywords: energy from available sugars (E_{as}), energy of lignin degradation (E_{ld}), energy of phenolic degradation (E_{pd}).

Growth loss and mortality caused by the root pathogen *Armillaria* spp. are serious problems in forests of the United States (34). Although nine species of *Armillaria* have been identified in the northwestern United States and Canada (1,3), *Armillaria ostoyae* (Romagn.) Herink seems to be one of the few species of the genus that infects conifers (34). Forest pathologists recommend regeneration of forested areas affected by *Armillaria* spp. with conifer species resistant to the pathogen (15,17,24).

In the Pacific Northwest, *Larix occidentalis* Nutt. is considered on the basis of field observations to be resistant to root disease caused by *Armillaria* spp.; *Pinus ponderosa* Douglas ex. P. Laws. & C. Laws. and *Pinus monticola* Douglas ex D. Don are considered to have intermediate resistance; and *Pseudotsuga menziesii* (Mirb.) Franco and *Abies grandis* (Douglas ex D. Don) Lindl. are considered to be highly susceptible (15,24). Field observations are based on species performance under a wide variety of field conditions with tree vigor, and the amount of inoculum of *Armillaria* is often extremely variable on a particular site. Because field observations are taken over a large area, these variations seem to be negated by sample size. However, controlled studies involving known species of *Armillaria* are needed to support field observations.

The mechanisms of tree resistance to *Armillaria* spp. have recently been reviewed (25,26,32). Energy required to degrade compounds associated with tree defense, such as lignin and phenolic compounds, should constitute a major portion of the energy expended by the pathogen to colonize the tree root. Entry et al (11,12) have related the ratios of energy available to *A. ostoyae* from sugars (E_{as}) to energy required to degrade lignin (E_{ld}) or phenolic compounds (E_{pd}) to the frequency and severity of disease caused by this pathogen.

Our objectives in this study were to determine whether resistance of western conifer saplings to *A. ostoyae* could be detected when trees were subjected to controlled inoculation and to determine the tree defense compounds that might confer resistance to infection by *A. ostoyae* in saplings of five western conifers.

MATERIALS AND METHODS

Site descriptions and history. The three sites in this study are located in the Lonesome Creek drainage in the Panhandle National Forest, 10–16 km northeast of Coeur D'Alene, ID. The Huckleberry Mountain site (lat. 47°34′ N; long. 116°44′ W) is at a 340-m elevation on a 35% slope toward the south. The site is characterized as an *A. grandis-Clintonia uniflora* habitat type (8). The Treasure Mountain site (lat. 47°42′ N; long. 116°41′ W) is at a 300-m elevation on a 30% slope toward the southeast and has been characterized as a *Thuja plicata-Asarum caudatum* habitat type (8). The Kelley Mountain site (lat. 47°40′ N; long. 116°40′ W) is at a 280-m elevation on a 35% slope toward the east and has been characterized as a *Tsuga heterophylla-Clintonia uniflora* habitat type (8).

Mixed conifer stands on these sites were harvested in the summer and broadcast-burned in the fall of 1976. Tree stumps found to be infected with *Armillaria* spp. or *Phellinus weirii* (Murr.) Gilb. were physically removed from this site during logging. In the fall of 1977, 2-yr-old bareroot conifer seedlings were planted 2.5 m apart in a grid. *L. occidentalis*, *A. grandis*, *P. ponderosa*, *P. monticola*, and *P. menziesii* were planted in rows of 30 trees; each block contained six rows per species.

Sampling and analysis. *Experimental protocols.* The study was arranged in a randomized complete block design, replicated on three sites. Ten trees from each of five species were randomly chosen from each site for this study (a total of 150 trees, with 30 trees of each species; fifty trees total per site). The same 10 trees chosen at the beginning of the study were used for growth measurements, nutrient analysis, root bark analysis, and inoculations with *A. ostoyae*. Three additional trees from each species on each site (nine trees per species and 15 trees on each site)

were randomly chosen for controls. Control trees were used to test the effect of the inoculation method described below on tree growth and survival. Inoculated trees were then measured for nutrient and biochemical analysis as described below. Total height was measured on each tree, including uninoculated control trees, in September of 1987 and 1990. Each tree was then inoculated with isolates JR 1953 and DC 1 of *A. ostoyae*, as described below. Each tree was measured, sampled, and inoculated individually, thereby providing a measure of variation between tree species within each site and among the sites.

Tree growth measurements. Ten years after trees were planted, total height was measured on the 10 trees of each species on each site. In 1990, the height of the same trees was measured again.

Nutrient analysis. Foliar nutrient content of the 10 trees of each species in each plot was sampled in September 1987 with methods described by Comerford and Leaf (7). A composite sample of first-year needles was taken from the top, middle, and lower third of each tree. Samples were dried at 80 C and ground to <1 mm; a 1.0-g subsample of each was ashed at 525 C. The ash was dissolved in 6.0 ml of 1 N HCl, brought to 50 ml with deionized water, and analyzed for Al, B, Ca, Fe, Mg, Mn, K, P, S, and Zn (19) on a Jarrell-Ash 9000 inductively coupled, plasma spectrometer (Jarrell-Ash, Waltham, MA). Total N was analyzed by standard micro-Kjeldahl techniques modified to include nitrate (4).

Biochemical analysis of root bark. In September 1987, roots 7–10 cm long and 0.5–2.0 cm in diameter were selected from the north and south or the east and west quadrants of each tree on each site. The bark (living and nonliving periderm) was removed, dried at 80 C for 48 h, and ground to pass a mesh <1 mm. Samples (1.0 g) of ground bark (one from each side) were pooled for each tree and analyzed for total sugars and starch (18), cellulose and lignin content (28), phenolic compounds (Julkunen-Tiito [20], as modified in Kelsey and Harmon [21]), and protein-precipitable tannins (16). The tannin standard was isolated from Douglas-fir bark (21). Full details of the procedures are given in Entry et al (11,12).

***Armillaria ostoyae* isolates.** Isolates JR 1953 and TY 186 of *A. ostoyae*, (obtained from Jim Reaves of the Alabama Agricultural and Mechanical University, Normal, AL) were grown on 3% malt agar. Isolate DC 1 of *A. ostoyae* was collected from a dying *P. monticola* in the Deception Creek Experimental Forest in northern Idaho (14). All three *Armillaria* isolates were challenged against haploid isolates of known *Armillaria* spp. (3) and determined to be *A. ostoyae* (=North American Biological Species I).

Inoculum preparation. Blocks of red alder (*Alnus rubra* Bong.), 3 cm diameter × 10 cm long, were washed and placed in 1-L containers with 50 ml of malt extract broth. The blocks and medium were autoclaved for 60 min at 140 kPa, allowed to cool, and inoculated with isolate JR 1953 or DC 1. The blocks were incubated in the dark at 23 C for 9 mo and were well-colonized by the fungus before inoculation of the trees.

Inoculation techniques. In the fall of 1987, 10 randomly selected trees from each species on all three sites were inoculated with isolates JR 1953 and DC 1. Roots of each tree were inspected from the trunk to 0.75 m outward; signs or symptoms of infection by *Armillaria* spp. were not found on any tree. Two separate roots (0.5–2.0 cm diameter) were inoculated on each tree, one with a 3-cm-diameter × 10-cm-long block of *A. rubra* colonized by JR 1953 and one with a similar block colonized by DC 1. Tree roots were not wounded. The longitudinal axes of the blocks were pressed against the longitudinal axes on the underside of each root. Blocks were fastened to roots with 5.1-cm-wide plastic tape. Blocks and roots were then wrapped in plastic to prevent desiccation of the inoculum block. Three additional trees in each species per site were inoculated with similar, uninfected blocks of *A. rubra* to assess the effect of the inoculation method on tree growth and survival. Inoculated roots were covered with soil and left for 3 yr.

Verification of infection. After 3 yr, all inoculated sections of roots were removed and washed with tap water, followed by a distilled water wash. Three separate sections of roots at points showing signs or symptoms of disease, approximately 2.5 mm long, were surface-disinfected with 1% NaOCl solution for 7 min and then flamed for 5 s. Root sections were placed on 3% malt agar in 60-ml test tubes. The tubes were then capped and incubated in the dark for 9 wk at 20 C. To determine the viability of inoculum, we split in half the inoculum blocks that had been attached to the roots. Sections approximately 0.5 × 2.0 cm were immersed in 1% NaOCl for 7 min and then in 3% H_2O_2 for 5 min. *A. ostoyae* recovered from roots and blocks was isolated, and cultures from roots and inoculum blocks were identified as the *A. ostoyae* that was inoculated by the diploid-diploid culture challenge with original stock cultures of DC 1 and JR 1953 (2). Culture challenge was carried out on 3% malt agar in 100-mm-diameter petri dishes.

Incidence of infection was rated as follows: 0 = either isolate of *A. ostoyae* (JR 1953 or DC 1) recovered from any inoculation (tree alive); 1 = one isolate of *A. ostoyae* recovered from one of the inoculated roots (tree alive); 2 = both isolates recovered from their respective inoculated roots (tree alive); 3 = either isolate recovered from inoculated root (tree dead); 4 = both isolates recovered from their respective inoculated roots (tree dead). Thus, the total incidence of disease was the combination of the results from both isolates. Percentage of each tree species infected was scored as follows: 0 = neither isolate of *A. ostoyae* recovered from the inoculated roots; 1 = either or both isolates of *A. ostoyae* recovered. Tree could be alive or dead. The percentage of species infected = total number of trees per species infected/total number of trees for each species (30) × 100. Trees could be alive or dead.

Growth efficiency. The growth efficiency of the original cultures of isolates JR 1953, TY 186, and DC 1 was calculated by growing each isolate in sugar concentrations of 10, 20, or 40 g L^{-1} in Melin-Norkrans' medium (23). Isolate TY 186 was used only in determination of growth efficiency used in the calculation of thermochemical budget (11,12). The carbon source (glucose, fructose, or sucrose) was sterilized separately by dry autoclaving and was added to the basal medium after the latter had cooled to 45 C. Plugs (3.0 mm diameter) cut from stock cultures on 3% malt agar were transferred to 30 ml of medium in 180-ml medicine bottles capped with 20-mm serum bottle stoppers. Disks contained no visible rhizomorphs. Cultures were incubated for 9 wk at 23 C in the dark. Mycelia were separated from the medium by filtering the medium through a preweighed 0.2-mm glass fiber filter. Mycelia and filter were dried at 80 C for 48 h. Mycelial weight was determined by subtracting the filter weight from the weight of the fungus plus the filter. The following equation was used to determine growth efficiency (6): growth efficiency = mycelial dry weight/carbohydrate consumed × 100.

The experiment was arranged in a completely randomized factorial design; carbon source, carbon concentration, and isolates of *A. ostoyae* were treatments. The experiment contained five replications and was conducted four times. Sugar concentration in the medium at the end of the experiment was determined by diluting 1 ml of medium with 100 ml of deionized distilled water and by analyzing at 625 nm on a Beckman DU-40 spectrophotometer (Beckman Instruments Inc., Irvine, CA) with the methods of Hansen and Moller (18).

Calculation of thermochemical budget. The enthalpy of formation (ΔH_f, kJ mol^{-1}) of any compound is the energy of reaction by which it is formed. The energy required to break a chemical bond between two compounds can be calculated by subtracting the ΔH_f of the first compound from the ΔH_f of the second compound.

To obtain an accurate balance between energy obtained from sugars and the total work required for the fungus to degrade phenolics and lignin and colonize the tree, we calculated the ratio of energy required to degrade phenols (E_{pd}) or lignin (E_{ld}) to the energy gained from available sugars (E_{as}) in the root bark of host trees to give the ratios E_{pd}:E_{as} and E_{ld}:E_{as} (11,12). Concentration of sugars in root bark tissue was measured as described under biochemical measurements. The E_{as} for total sugars is -63.54 kJ mol^{-1} × mol sugar g^{-1} root bark (11,12). The growth efficiencies of isolates JR 1953, TY 186, and DC 1 were averaged

for the types and concentrations of media described in the preceding section. The overall mean growth efficiency used in our calculations was 0.137.

Energy of dissociation of phenolic compounds (E_{pd}) was calculated from the energy required for a molecule of an aromatic compound to be degraded through the ortho pathway for fungi via catechol (5,22). The $E_{pd} = 2{,}430.60$ kJ $mol^{-1} \times$ mol phenol g^{-1} root bark (11,12).

Energy required to degrade lignin (E_{ld}) was calculated from the energy required to degrade a molecule of tyrosine through the ortho pathway for fungi via catechol (5,9). The $E_{ld} = 2{,}658.82$ kJ $mol^{-1} \times$ (% lignin g^{-1} root bark $\times$ % tyrosine (w/w) in lignin) (11,12).

The concentrations of sugar, phenolic units, and lignin in root bark tissue were measured as described under biochemical analysis. The ratios E_{pd}:E_{as} and E_{ld}:E_{as} were then computed.

Statistical analysis. Normal distribution of the data was confirmed by univariate procedures (27). Disease percentages required a natural log transformation to normalize the data; data are expressed as original values. The data were analyzed by analysis of variance for a randomized complete block design for the field experiment and a completely randomized factorial design for the laboratory experiment (27). Residuals were normally distributed with constant variance. Individual treatment means were compared with Tukey's honest significant difference (HSD) test at $P \leq 0.05$.

RESULTS

Tree growth. Fungal infection caused minimal changes in the relative sizes of inoculated and uninoculated (control) saplings and among species. Both height and stem diameter of *L. occidentalis*, *P. monticola*, and *P. ponderosa* were significantly larger than those of *A. grandis* or *P. menziesii* saplings at the beginning and the end of the experiment. Heights of *P. monticola* and *P. ponderosa* increased the most relative to starting size, 79% and 63%, respectively.

Foliar nutrients. Foliage of *L. occidentalis*, *A. grandis*, and *P. menziesii* contained greater concentrations of N than did *P. monticola* or *P. ponderosa* (data not shown). *L. occidentalis* also contained the highest concentrations of P, Ca, Mn, and B and the lowest concentration of Zn. Foliage of *P. monticola* and *P. ponderosa* was lowest in N and S but highest in Fe. The lowest level of K was in foliage of *P. menziesii*, although it was not significantly lower than in *A. grandis*. Magnesium was the only foliar element that did not differ among species.

Biochemical measurements in root bark. The concentration of sugar in the bark of 0.5–2.0-cm-diameter roots was higher in *A. grandis* and *P. menziesii* than in *L. occidentalis* or the species of *Pinus* (Table 1). The root bark of *A. grandis* also had the highest concentration of starch; root bark of *P. monticola* contained the least starch. Concentrations of phenolic compounds and protein-precipitable tannins and the phenolic/sugar ratio were greatest in *L. occidentalis*. The phenolic/sugar ratio for *L. occidentalis* was 35 times greater than the lowest ratio (that for *A. grandis*) and nine times greater than the phenolic/sugar ratio for *P. menziesii*. *A. grandis* contained the lowest concentration of phenolics and, with *P. menziesii*, the lowest concentrations of protein-precipitable tannins. Cellulose and lignin concentrations in the root bark of 0.5–2.0-cm-diameter roots did not differ among species.

Infection ratings. Attachment of blocks of *A. rubra* without *A. ostoyae* to roots did not affect the trees adversely. Survival, height, and diameter growth of such trees did not differ from those of untreated control trees of the same species on each site. In infected roots of inoculated trees, strands of white mycelium emerged from the point of contact with the inoculum block and advanced in the inner bark of the root 0–27 cm proximally and 0–10 cm distally. Areas of resinosis surrounded the point of contact, and proximal advance was as much as 12 cm in the inoculated root. Most, but not all, trees killed by the fungus had mycelial colonization of inner and sometimes outer bark, in addition to resin at the base of the tree on the outside of the bark. Twenty-three percent of the *A. grandis*, 10% of the *P. menziesii*, 6% of the *P. monticola*, 3% of the *P. ponderosa*, and 3% of the *L. occidentalis* died after infection by inoculum of *A. ostoyae*. Fungi isolated from infected trees were the same as those used for inoculations, as determined by culture challenge against the original stock cultures of *A. ostoyae* by diploid-diploid crossings (2).

Analysis of variance showed no significant difference between infection of tree roots by isolate JR 1953 and DC 1 (Table 2). *A. grandis* had the highest total disease rating and greatest percentage of infected roots, followed in order by *P. menziesii*, *P. ponderosa*, *P. monticola*, and *L. occidentalis*. *A. grandis* and *P. menziesii* had higher infection ratings and percentages of roots infected by both *A. ostoyae* isolates than the other species.

Total infection rate was not correlated with cellulose or lignin concentrations in root bark (Table 3). Concentrations of sugar, starch, phenolic compounds, and protein-precipitable tannins were correlated both linearly and curvilinearly with total infection ratings. The ratios of energy available to *A. ostoyae* relative to the energy required for phenolic degradation (E_{pd}:E_{as}) and lignin degradation (E_{ld}:E_{as}) correlated with total infection rates linearly ($r^2 = 0.57$ and 0.56, respectively) and curvilinearly ($r^2 = 0.87$ and 0.76, respectively) (Table 3; Figs. 1,2).

DISCUSSION

In this study, we found the following order of resistance to *Armillaria* infection: *L. occidentalis* > *P. monticola* = *P. ponderosa* > *P. menziesii* > *A. grandis*. The results of this study are consistent with and validate the resistance ranking that pathologists have given to western conifers from field observations (15,24). We found no statistically significant difference between the two isolates of *A. ostoyae* in infection rates or percentage

TABLE 1. Biochemical measurements of bark collected in 1987 from roots (0.5–2.0 cm in diameter) of five species of 10-yr-old conifers before inoculation with *Armillaria ostoyae*[u]

Species	Sugar[v]	Starch[v]	Cellulose[w]	Lignin[w]	Phenolics[x]	Protein-precipitable tannins[y]	Phenolic/sugar ratio[z]
Abies grandis	19.96 a	44.71 a	62 a	35 a	5.3 d	24.8 c	0.26 d
Larix occidentalis	3.16 c	22.41 b	65 a	32 a	27.8 a	34.8 a	9.09 a
Pinus monticola	4.81 c	10.31 c	62 a	34 a	19.7 b	27.6 b	2.12 b
Pinus ponderosa	4.87 c	22.68 b	61 a	32 a	21.7 b	28.7 b	3.03 b
Pseudotsuga menziesii	11.68 b	29.45 b	59 a	35 a	11.2 c	26.5 c	0.99 c

[u] Values are means of 30 samples. Within each column, values followed by the same letter are not significantly different at $P \leq 0.05$, as determined by Tukey's honest significant difference (HSD) test.
[v] Milligrams per gram of dry root tissue.
[w] Percentage dry weight of tissue.
[x] Calculated as phenol equivalents per gram of root bark.
[y] Expressed in milligrams of tannin per gram of root bark.
[z] Calculated as phenol equivalents per gram of root bark/milligrams of sugar per gram of root bark.

of roots infected on any of the conifer species. Long-term studies of tree species on sites infested with *Armillaria* spp. would further validate results of this and other experiments.

Mineral nutrition may be an important physiological component of host resistance, although its role in host physiology and infection by *Armillaria* spp. is poorly understood. The effect of infection by *A. ostoyae* on the mineral nutrition of host trees was not analyzed in this study. Entry et al (13) found that *P. menziesii* infected with *Armillaria* spp. had lower concentrations of foliar N and S than trees that were not infected. There was no difference in the concentrations of B, Ca, Cu, Fe, K, Mg, Mn, P, and Zn between infected and noninfected trees. In this study, species resistant to *A. ostoyae* had higher foliar concentrations of K, Ca, Mn, and B, whereas less resistant species were characterized by low foliar concentrations of Ca and B.

In this study, the soils on which the tree species were grown were common among species. Trees were not deficient in any elements analyzed; therefore, differences in foliar elements apparently were not due to environmental deficiencies, but rather represented genetic or physiological differences.

TABLE 2. Percentage of roots infected by *Armillaria ostoyae* and mean disease ratings of five conifer species 13 yr after planting and 3 yr after inoculation with isolates JR 1953 and DC 1

Species	Total infection[x]	
	Percentage[y]	Rating[z]
Abies grandis	57 a	1.47 a
Larix occidentalis	10 d	0.18 d
Pinus monticola	25 c	0.46 c
Pinus ponderosa	23 c	0.37 c
Pseudotsuga menziesii	40 b	0.73 b

[x] Infection percentages required natural log transformation to normalize data. Within each column, values followed by the same letter are not significantly different ($P \leq 0.05$), as determined by Tukey's honest significant difference (HSD) test.

[y] Percentage of each tree species infected ($n = 30$): Total number of trees of each species infected/total number of trees for each species (30) × 100. Trees could be alive or dead. Ratings: 0 = neither isolate of *A. ostoyae* recovered from the inoculated roots; 1 = either or both isolates of *A. ostoyae* recovered.

[z] Total infection was rated as follows: 0 = neither isolate recovered from any inoculation; 1 = either isolate DR 1953 or isolate DC 1 recovered from one of the inoculated roots (tree alive); 2 = both isolates recovered from their respective inoculated roots (tree alive); 3 = either isolate recovered from the inoculated root (tree dead); 4 = both isolates recovered from their respective inoculated roots (tree dead). Values are means of 60 samples.

TABLE 3. Coefficients of determination (r^2) of infection rating of conifer trees infected by *Armillaria ostoyae* with biochemical parameters measured in the bark of roots (0.5–2.0 cm diameter), 13 yr after planting and 3 yr after inoculation[x]

Parameter	Total incidence of infection	
	Linear	Curvilinear
Sugar	0.56*[y]	0.26*
Starch	0.51*	0.50*
Cellulose	0.06	0.06*
Lignin	0.08	0.08*
Total extractable phenolics	0.55*	0.52*
Protein-precipitable tannins	0.26*	0.30*
Sugar/phenolic	0.57*	0.21*
$E_{pd}:E_{as}$[z]	0.57*	0.87*
$E_{ld}:E_{as}$[z]	0.56*	0.76*

[x] $n = 45$.

[y] * Indicates a correlation that is significantly different from a straight line drawn throughout the data points ($P \leq 0.01$).

[z] E_{pd}, the energy required to degrade phenols; E_{ld}, energy required to degrade lignin; E_{as}, energy available from sugars.

The incidence of infection by *A. ostoyae* was lower in species with root bark containing relatively high concentrations of phenolic compounds and low concentrations of sugars. This result is consistent with previous greenhouse and field studies (11,12).

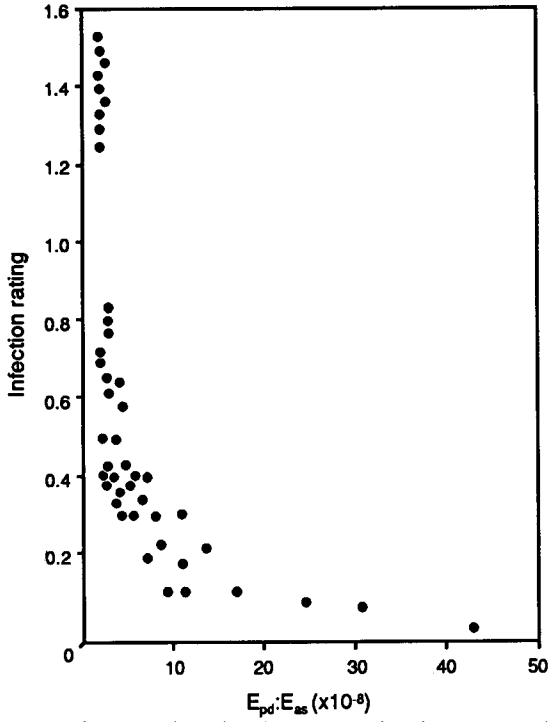

Fig. 1. Plot of the relationship of $E_{pd}:E_{as}$ (ratio of energy required for phenolic degradation/energy available from sugars) to total *Armillaria ostoyae* infection ($r^2 = 0.87$; $P \leq 0.001$) in five coniferous species. Infection rating = $0.148 + [(E_{pd}:E_{as}) \times 3.183 \times 10^7 + (E_{pd}:E_{as})^2 \times (-2.31 \times 10^{-14})] + [(E_{pd}:E_{as})^3 \times (5.144 \times 10^{-20})]$.

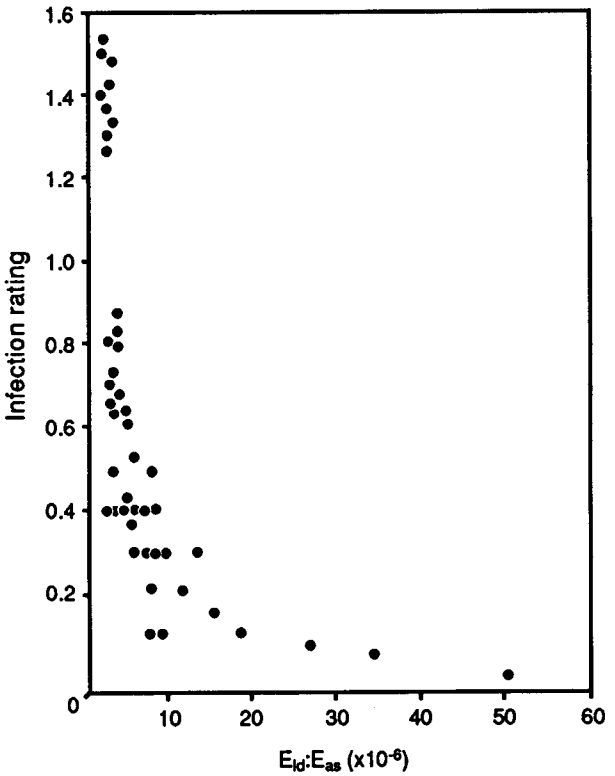

Fig. 2. Plot of the relationship of $E_{ld}:E_{as}$ (ratio of energy required for lignin degradation/energy available from sugars) to total *Armillaria ostoyae* infection in five coniferous species ($r^2 = 0.76$; $P \leq 0.01$). Infection rating = $0.1383 + [(E_{ld}:E_{as}) \times (7.262 \times 10^5)] + (E_{ld}:E_{as})^2 \times (6.716 \times 10^{11})] + [(E_{ld}:E_{as})^3 \times (-1.144 \times 10^{-23})]$.

Wargo (31) proposed that pathogenic species of *Armillaria* infect hosts, because the energy available to the fungus through sugars, nitrogen, and ethanol in root bark is great enough to overcome host defense compounds such as tannins and other phenolic compounds. This study and previous studies (11,12,29,33) support this hypothesis. High concentrations of phenolic compounds inhibit growth of *A. ostoyae* (10). Increased glucose concentration enables *Armillaria* spp. to grow in the presence of inhibitory concentrations of phenolic compounds (30).

Fungi can degrade phenolic compounds only when an additional carbon source is present; the rate of degradation is directly proportional to the amount of additional growth substrate (22). When infection occurs, the fungus must penetrate the root bark of the host tree. Because the energy base (inoculum block) was the same for all inoculations, the concentrations of sugars, phenolics, and lignin in the root bark of these trees were relative to the susceptibility of the tree to infection. The low sugar concentrations in root bark of tree species that are more resistant to *A. ostoyae* may have provided less energy for the fungus to degrade phenolic compounds and tannins. Susceptible tree species may allocate more carbon to sugar and cellulose and less to the shikimic acid pathway, which produces defense compounds such as lignin, phenolics, and tannins.

Although the infection process is poorly understood, thermochemical relationships may be a new and useful concept to explain the mechanism of infection of host tissues by root pathogens. The correlation of E_{pd}:E_{as} and E_{ld}:E_{as} with total infection rates in this study and our previous studies (11,12) indicates that these parameters may provide dependable assays of the physiological response of trees to attack by *Armillaria* spp. and may be important in explaining the mechanisms of infection by *A. ostoyae*. Thermochemical relationships with disease ratings indicate that there are thresholds of E_{pd}:E_{as} and E_{ld}:E_{as} below which resistance to infection is reduced and infection of the root by *A. ostoyae* occurs. Such thresholds have been found in our previous studies (11,12). These thresholds can be reached by an increase in the concentration of sugars or a decrease in the concentration of phenolic compounds or lignin in root bark.

The E_{pd} and E_{ld} that we used are based on the pathway of lignin degradation by fungi (9), particularly *Phanerochaete chrysosporium* (22). We could not include the energy requirements necessary for these conversions, because the specific identities of each phenolic compound are unknown, and the energy requirements of demethylation and decarboxylation vary greatly with each specific phenolic molecule but are relatively small when compared to the energy required for cleavage of a benzene ring. The equations would be more accurate if the identities of the phenolic compounds in root bark of trees and the biological pathways of phenolic degradation by *Armillaria* spp. were elucidated.

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