

## Response of Western Coniferous Seedlings to Infection by *Armillaria ostoyae* Under Limited Light and Nitrogen

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Paper 2553 of the Forest Research Laboratory, Oregon State University. Submitted in partial fulfillment of requirements for a Ph.D. degree. This research was supported by National Science Foundation Ecology grant BSR-8717849 and United States Department of Agriculture grant FSTY 9-0224.

We thank Elaine Ingham and Neil Christensen for reviewing this manuscript, Jim Reaves and William Brandt for help and advice with fungal physiology, and Alison Moldenke for editing.

Accepted for publication 22 August 1990.

### ABSTRACT

Entry, J. A., Cromack, K., Jr., Hansen, E., and Waring, R. 1991. Response of western coniferous seedlings to infection by *Armillaria ostoyae* under limited light and nitrogen. *Phytopathology* 81:89-94.

During the first 16 mo of growth, seedlings of five western coniferous species were subjected to one of three physiological treatments: balanced light and nutrients, light limitation and adequate nutrients, or nitrogen limitation and adequate light. Four-month-old seedlings were inoculated with one of three isolates of *Armillaria ostoyae*. After 1 yr, disease severity was significantly greater when light or nitrogen were limited than when they were balanced. Seedlings responded to light or nitrogen limitation

by lowering the concentration of phenolic compounds and/or raising that of sugar in root tissue. Such changes lower the ratio of the energy required for phenol or lignin degradation ( $E_{pd}$  and  $E_{ld}$ , respectively) to the energy from available sugar ( $E_{as}$ ), which may indicate decreased disease resistance in seedlings. Decreasing  $E_{pd}:E_{as}$  ratios were correlated with increasing disease severity and may be important in explaining why pathogenic species of *Armillaria* preferentially invade stressed seedlings.

*Additional keywords:* phenolic:sugar ratio, thermochemical budgets.

Species of *Armillaria* are pathogens whose aggressiveness and role in disease of forest ecosystems may depend on the physiological condition of the trees (39,40). *Armillaria ostoyae* (Romagn.) Herink. has been identified as a primary pathogen that often has caused severe damage in forests of the western United States (41). Although numerous authors report that light and nitrogen stress predispose trees to *Armillaria mellea* (Vahl:Fr.) Kumm. sensu lato (29,33,34,39), information concerning the physiology of tree stress and attack by *A. ostoyae* is scarce.

Although the specific functions of light and nitrogen in plant cells are not yet completely elucidated, nitrogen limitation or reduced light may limit photosynthesis and, thus, reduce the amount of carbon available for producing defensive compounds (15,24). Such conditions can result in increased susceptibility to attack by *Armillaria* (13,29). Our objectives in this study were to determine the influence of light and nitrogen limitation on the physiological status of seedlings of five conifer species and the subsequent response of the seedlings to infection by *A. ostoyae*.

### MATERIALS AND METHODS

**Treatment conditions.** Before planting, seeds of *Pseudotsuga menziesii* (Mirb.) Franco, *Abies grandis* (Douglas ex D. Don) Lindl., *Pinus contorta* Douglas & Loud var. *latifolia* Engelm. ex Wats., *Pinus ponderosa* Douglas. ex P. Laws., and *Larix occidentalis* Nutt. were immersed in 1% NaOCl for 30 min, rinsed with cold water, soaked in 5%  $H_2O_2$  for 30 min, and rewashed with distilled water for 24 hr. Super cell potting containers (165 ml) were washed and sterilized with a 1% NaOCl solution for 20 min.

Five seeds were planted in each of 1,500 cells in a potting substrate containing equal volumes of vermiculite, perlite, and sphagnum peat moss. After 3 wk, seedlings were thinned to one

per cell and randomly assigned to treatment combinations in a factorial experiment arranged in a split split-plot design (22). Three physiological treatments (main plots) consisted of full light and complete Arnon's solution (LN) (5), limited light (shade cloth, Chicopee Mills, Gainesville, GA) and complete Arnon's solution (—L), and full light and limited nitrogen (0.05 mM  $NO_3^-$  L<sup>-1</sup> in tap water) (—N). Each treatment was applied to 60 seedlings of each species (subplot). After 4 mo of growth, 20 seedlings of each species in each physiological treatment were inoculated (as described below) with each of three isolates of *A. ostoyae*. Two hundred and twenty-five seedlings were inoculated (three physiological treatments  $\times$  five species  $\times$  three isolates  $\times$  five replicates). The entire experiment was repeated four times (a total of 900 seedlings). Two additional seedlings per physiological treatment  $\times$  species  $\times$  isolate (a total of 90 additional seedlings) were designated as controls and given the same treatments but were not inoculated with the fungus.

Seedlings were grown in a temperature-controlled greenhouse for 16 mo (June 1987–October 1988) and were watered three times weekly. Average photosynthetic photon flux density at summer maximum (measured in the greenhouse on a cloudless 23 June 1987 from 11 a.m. to 5 p.m.) was 700  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for fully illuminated seedlings and 100  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  for seedlings in the —L treatment. Day/night temperatures were maintained at 24/23 C.

**Seedling inoculation.** Three isolates of *A. ostoyae* (= *A. obscura* (Pers.) Herink, North American Biological Species [NABS] I) (26) were grown on 3% malt agar. Isolates JR1953 and TY186 were obtained from Jim Reaves, Alabama Agricultural and Mechanical University, Normal, AL (28), and isolate DC1 was collected from a dying *Pinus monticola* Douglas ex. D. Don in the Deception Creek Experimental Forest in northern Idaho (13). These isolates have been challenged with known haploid isolates of the eight North American species of *Armillaria* (2,4) and determined to be *A. ostoyae*.

Blocks of *Alnus rubra* Bong., 3 cm in diameter  $\times$  10 cm long, were washed and placed in a 0.89-L container with 50 ml of malt extract broth medium. The blocks and medium were auto-

claved for 60 min at 140 kPa and left to cool. Three 10-mm-diameter fungal plugs from petri dishes containing one of the isolates of *A. ostoyae* were placed on each block of *A. rubra*; blocks were then incubated for 9 mo. All blocks were well-colonized by the fungus before seedlings were inoculated.

Inoculation was performed by the method described by Shaw et al (31). A block of *A. rubra* colonized by *A. ostoyae* was fastened with two 0.5-cm-wide rubber bands to the primary root of each seedling; uncolonized blocks of *A. rubra* were attached to the roots of the control seedlings. Seedling roots were flooded at all times and were not wounded.

One year after inoculation, seedlings were removed from their containers, and their roots were carefully washed, first with tap water and then with distilled water. Three root sections with lesions, each approximately 2.5 mm long, were excised from each seedling and surface-disinfested by immersion in 1% NaOCl for 7 min and then in 3% H<sub>2</sub>O<sub>2</sub> for 5 min. Each root section was placed on 3% malt agar in a separate 60-ml test tube. Each seedling was rated as uninfected and living, infected and living, or infected and dead (13). Seedlings were also scored as infected (+) or not infected (−), and infection percentages were calculated. A seedling was considered infected if *A. ostoyae* was successfully recovered from root tissue. Inoculum blocks were then split in half; *A. ostoyae* was recovered from each inoculum block after immersion in 1% NaOCl for 7 min and in 3% H<sub>2</sub>O<sub>2</sub> for 5 min.

**Analysis.** All seedlings were dried at 80 C for 48 hr. Oven-dry weights of each root and shoot were recorded separately and then added to determine total dry weight of each tree. Shoot tissue from three seedlings of each species in each physiological treatment was ground to pass through a No. 40 (<1 mm<sup>2</sup>) mesh, and a 0.5-g subsample was ashed at 525 C in a muffle furnace. The ash was dissolved in 10% HNO<sub>3</sub>, brought to a 50-ml volume (18), and analyzed for Al, B, Ca, Cu, Fe, Mg, K, Na, P, and Zn with 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 (6).

**Physiological measurements.** Fresh secondary root tissue of three seedlings of each species in each physiological treatment was dried at 70 C and ground to pass through a No. 40 mesh; a 0.2-g subsample was analyzed for total sugars and starch by Hansen and Moller's (17) method. Cellulose and lignin were analyzed by procedures described by Van Soest (38). Phenolic compounds were extracted from a 10-mg subsample with 10 ml of 80% aqueous acetone (v/v) for 24 hr (19), and 5 ml of extract was diluted in 20 ml of 80% aqueous acetone (v/v). One milliliter of the diluted extract mixture was placed in a 30-ml test tube, followed by 1 ml of distilled water and 1 ml of Folin-Ciocalteu phenol reagent. The solution was mixed, 5 ml of 20% NaCO<sub>3</sub> (v/v) solution was added immediately, and the solution was thoroughly mixed again. After 20 min, the absorptivity of the mixture was read at 700 nm on a Bausch & Lomb spectrophotometer (Bausch & Lomb, Rochester, NY) (19). Phenol standards were dissolved in 80% aqueous acetone (v/v). A 50-μl subsample of the diluted extract mixture was analyzed for protein-precipitable tannins by radial diffusion assay (16). Standards were known concentrations of tannins extracted from Douglas-fir tissue (20).

**Growth efficiency of *A. ostoyae*.** Growth coefficients were calculated for the three isolates of *A. ostoyae* by growing each in Melin-Norkrans' medium (25) with concentrations of 10, 20, and 40 g L<sup>−1</sup> of glucose, fructose, and sucrose (8). Carbon sources were sterilized separately by dry autoclaving (14) and were added to the medium after it had cooled to 45 C. Disks 1 mm in diameter and containing no visible rhizomorphs were cut from stock cultures on 3% malt agar, and each was added to 30 ml of the medium in a 165-ml medicine bottle capped with a 20-mm serum cap. Cultures were incubated in the dark for 9 wk at 22 C. The medium and the fungus were then separated by filtration through a 4-mm glass-fiber filter, and the hyphae were oven-dried at 80 C for 48 hr and weighed.

Sugar concentrations in the medium were determined at the

end of the experiment by diluting 1 ml of medium with 99 ml of deionized distilled water and analyzing at 625 nm on a Beckman DU-40 spectrophotometer (Beckman Instruments, Irvine, CA) by the methods of Hansen and Moller (17). Sugar concentrations are expressed in grams per liter, and sugars extracted from seedling roots are expressed in milligrams of sugar per gram of tissue. Because 1 ml of water weighs 1 g, the range of sugar concentrations was from 10 to 40 mg g<sup>−1</sup> of water, which is representative of the range of sugar concentrations extracted from seedling roots.

Growth efficiency was determined according to Cochrane (8) as follows: growth efficiency = mycelial dry weight/carbohydrate consumed × 100, where carbohydrate consumed is the amount of sugar in the medium at the start of the experiment minus the amount at the end. The results were used to determine thermochemical budgets, as described below.

**Thermochemical budgets.** *A. ostoyae* has been shown to grow substantially better with sugars as the carbon substrate than with any other substrate found in woody tissue (14). The energy available to *A. ostoyae* from sugars in root bark tissue is the largest portion of energy available for growth. Lignin and phenolic compounds have been shown to effectively inhibit growth of *A. ostoyae* (12,40). The energy required to overcome tree defense mechanisms, such as phenolic compounds and lignin, should contribute to the major portion of energy expended by the fungus to colonize the tree root. Therefore, the ratio of the energy available to *A. ostoyae* from sugars (E<sub>as</sub>) to the energy required to degrade phenolic compounds (E<sub>pd</sub>) or lignin (E<sub>ld</sub>), (E<sub>as</sub>:E<sub>pd</sub> or E<sub>as</sub>:E<sub>ld</sub>), may be related to the frequency and severity of disease caused by *A. ostoyae*.

Energy required to break a chemical bond between two compounds can be calculated by subtracting the enthalpy of formation (ΔH<sub>f</sub>) of the first compound from that of the second. (All ΔH<sub>f</sub> measurements are expressed in kilojoules per mole in water at 298.15 K.) The energy gained from available sugars (E<sub>as</sub>) in the host seedling was calculated as:

$$\begin{aligned}
 E_{as} &= [\Delta H_{f \text{ glucose (mol)}} + 6\Delta H_{f \text{ O}_2 \text{ (mol)}} \\
 &\quad - (6\Delta H_{f \text{ H}_2\text{O (mol)}} + 6\Delta H_{f \text{ CO}_2 \text{ (mol)}})] \\
 &\times \text{average growth efficiency of } A. \text{ ostoyae isolates} \\
 &\times \text{mol sugar g}^{-1} \text{ root tissue} \\
 &= -1,273.30 + 6(-498.4) \\
 &\quad - [6(-241.8) + 6(-391.51)] \\
 &\times 0.137 \times \text{mol sugar g}^{-1} \text{ root tissue} \\
 &= -63.54 \text{ kJ mol}^{-1} \times \text{mol sugar g}^{-1} \text{ root tissue.}
 \end{aligned}$$

Energy of dissociation of the phenolic bonds (E<sub>pd</sub>) was calculated from the energy required for a molecule of catechol to be degraded through the ortho pathway by fungi (7,9). For stable organic compounds of tyrosine, phenol, oxoadipeate, succinate, and CO<sub>2</sub>, ΔH<sub>f</sub> values are found in Pedley et al (27). The value for catechol is found in Khadikar et al (21) and that for muconate in Dolbneva et al (11):

$$\begin{aligned}
 E_{pd} &= \Delta H_{f \text{ catechol}} - \Delta H_{f \text{ muconate}} - \Delta H_{f \text{ oxoadipeate}} \\
 &\quad - \Delta H_{f \text{ succinate}} - \Delta H_{f \text{ CO}_2} \\
 &\times (\text{mol phenol g}^{-1} \text{ root tissue}) \\
 &= -374.11 - (-917.20) - (-1,026.20) - (-469.80) \\
 &\quad - (-391.51) \times (\text{mol phenol g}^{-1} \text{ root tissue}) \\
 &= 2,430.60 \text{ kJ mol}^{-1} \times (\text{mol phenol g}^{-1} \text{ root tissue}).
 \end{aligned}$$

Energy of lignin degradation (E<sub>ld</sub>) was calculated from the energy required for tyrosine to be degraded via the ortho pathway by fungi (23). Tyrosine is a central component of the lignin molecule (15); we calculate that tyrosine units comprise 70% of the model conifer lignin presented by Alder (1):

$$\begin{aligned}
E_{ld} &= \Delta H_{f_{\text{tyrosine}}} - \Delta H_{f_{\text{phenol}}} - \Delta H_{f_{\text{catechol}}} - \Delta H_{f_{\text{muconate}}} \\
&\quad - \Delta H_{f_{\text{oxoadipate}}} - \Delta H_{f_{\text{succinate}}} - \Delta H_{f_{\text{CO}_2}} \\
&\quad \times (\% \text{ lignin g}^{-1} \text{ root tissue} \\
&\quad \times \% \text{ tyrosine (w/w) in lignin}) \\
&= (-685.10) - (-165.1) - (-374.11) - (-917.20) \\
&\quad - (-1,026.20) - (-469.80) - (-391.51) \\
&\quad \times (\% \text{ lignin g}^{-1} \text{ root tissue} \times 0.70) \\
&= 2,658.82 \text{ kJ mol}^{-1} \times (\% \text{ lignin g}^{-1} \text{ root tissue} \times 0.70).
\end{aligned}$$

**Statistical analysis.** Root biomass, shoot biomass, total biomass, and root/shoot ratio data were not normally distributed. Log transformations were performed to normalize the data, which were then subjected to analysis of variance for a split split-plot factorial experiment (30). Analysis of variance of seedling biomass measurements indicated that physiological treatment  $\times$  seedling species  $\times$  isolate interactions were significant at  $P \leq 0.05$ . Contrasts on preplanned comparisons among individual treatment means were computed with Fisher's protected least significant difference (LSD) test at  $P \leq 0.05$ . Residuals were equally distributed with constant variance. Biochemical data were determined to be normally distributed via univariate procedures (30), and data were subjected to analysis of variance for a split split-plot factorial experiment (30). The residuals were normally distributed with constant variance. Because ANOVAs for biochemical characteristics of seedling roots did not indicate significance in physiological treatment  $\times$  species  $\times$  isolate, treatment  $\times$  isolate, or species  $\times$  isolate interactions, only physiological treatment  $\times$  species interactions may be discussed (35,36). Live uninfected seedlings, live infected seedlings, and dead infected seedling percentages and disease ratings were determined not to be normally distributed, and arc sine-square root transformations were performed to normalize the data. Transformed data were subjected to analysis of variance. Residuals were equally distributed with normal variance. Means are presented as untransformed numbers. Analysis of variance for disease ratings and percentage of seedlings infected in the treatment  $\times$  species  $\times$  isolate, treatment  $\times$  isolate, and species  $\times$  isolate interactions indicated no significance ( $P \leq 0.05$ ); only physiological treatments will be discussed (35,36). Correlations were analyzed, with disease severity as the  $x$  (dependent) variable and physiological or thermochemical parameters as the  $y$  (independent) variables.

## RESULTS

For all species, the root, shoot, and total biomass of seedlings in the LN treatment were greater than in the other two treatments (Table 1). Fully illuminated seedlings produced greater root biomass and higher root:shoot ratios than shaded seedlings. Nitrogen limitation produced seedlings with lower root, shoot, and total biomass but higher root:shoot ratios than seedlings receiving the LN treatment. No single isolate of *A. ostoyae* consistently affected root or total seedling biomass (Table 1). Nutrient concentrations in shoot tissues were more balanced in seedlings receiving the LN treatment than in the other two treatments (*data not shown*).

Sugar concentrations in root tissue were highest in secondary root tissue of seedlings receiving the -N treatment (Table 2). Sugar concentrations in root tissue did not differ among seedling species within physiological treatments. When seedlings received balanced light and nutrients or light limitation alone, starch concentrations in secondary root tissue did not differ among tree species. When seedlings were nitrogen-limited, starch concentrations were lower in secondary root tissue of *L. occidentalis* than in *P. menziesii*, *P. contorta*, or *P. ponderosa* (Table 2). Within each species except *A. grandis*, cellulose concentrations were highest in the -L and lowest in the LN treatment. Concentrations of lignin in secondary root tissue did not differ consistently among tree species within physiological treatments. Lignin concentrations were higher in seedlings receiving the LN

TABLE 1. Comparison of root, shoot, and total biomass of western conifer seedlings infected with isolates of *Armillaria ostoyae* under three physiological treatments: adequate light and nitrogen (LN), light limitation (-L), and nitrogen limitation (-N)<sup>a</sup>

| Seedling species<br>Isolate of <i>A. ostoyae</i> | Biomass (mg dry weight) |          |          | Root:shoot<br>ratio |
|--|-------------------------|----------|----------|---------------------|
|  | Root                    | Shoot    | Total    |                     |
| LN treatment                                     |                         |          |          |                     |
| <i>Abies grandis</i>                             |                         |          |          |                     |
| JR   | 999 a                   | 984 a    | 1,983 a  | 1.02 a              |
| TY   | 1,443 b                 | 1,018 b  | 2,961 b  | 1.42 a              |
| DC   | 1,141 ab                | 961 ab   | 2,101 ab | 1.19 a              |
| <i>Larix occidentalis</i>                        |                         |          |          |                     |
| JR   | 1,927 a                 | 1,779 a  | 3,707 a  | 1.08 a              |
| TY   | 2,441 b                 | 2,455 b  | 4,897 b  | 1.00 a              |
| DC   | 2,263 ab                | 2,165 ab | 4,428 ab | 1.05 a              |
| <i>Pseudotsuga menziesii</i>                     |                         |          |          |                     |
| JR   | 2,452 b                 | 2,018 a  | 4,470 a  | 1.21 a              |
| TY   | 2,207 a                 | 1,884 a  | 4,090 a  | 1.17,a              |
| DC   | 2,254 a                 | 1,946 a  | 4,200 ab | 1.16 a              |
| <i>Pinus contorta</i>                            |                         |          |          |                     |
| JR   | 1,445 a                 | 1,378 a  | 2,823 a  | 1.08 a              |
| TY   | 1,956 b                 | 2,051 b  | 4,007 b  | 0.95 a              |
| DC   | 1,714 ab                | 1,674 a  | 3,388 ab | 1.02 a              |
| <i>Pinus ponderosa</i>                           |                         |          |          |                     |
| JR   | 2,258 a                 | 2,455 ab | 4,713 ab | 0.92 a              |
| TY   | 2,455 ab                | 2,330 a  | 4,586 a  | 1.05 a              |
| DC   | 2,683 b                 | 2,762 b  | 5,446 b  | 0.97 a              |
| -L treatment                                     |                         |          |          |                     |
| <i>Abies grandis</i>                             |                         |          |          |                     |
| JR   | 118 a                   | 285 b    | 404 b    | 0.41 a              |
| TY   | 158 b                   | 202 a    | 361 b    | 0.78 b              |
| DC   | 99 a                    | 199 a    | 298 a    | 0.50 a              |
| <i>Larix occidentalis</i>                        |                         |          |          |                     |
| JR   | 135 a                   | 352 b    | 487 b    | 0.38 a              |
| TY   | 155 b                   | 328 b    | 483 b    | 0.47 a              |
| DC   | 132 a                   | 246 a    | 378 a    | 0.53 a              |
| <i>Pseudotsuga menziesii</i>                     |                         |          |          |                     |
| JR   | 227 ab                  | 365 a    | 592 a    | 0.62 a              |
| TY   | 177 a                   | 380 a    | 558 a    | 0.46 a              |
| DC   | 262 b                   | 441 b    | 703 b    | 0.59 a              |
| <i>Pinus contorta</i>                            |                         |          |          |                     |
| JR   | 272 a                   | 596 b    | 868 b    | 0.46 a              |
| TY   | 242 a                   | 413 a    | 655 a    | 0.58 b              |
| DC   | 333 b                   | 590 b    | 923 b    | 0.56 b              |
| <i>Pinus ponderosa</i>                           |                         |          |          |                     |
| JR   | 248 a                   | 449 a    | 698 a    | 0.55 a              |
| TY   | 268 a                   | 544 a    | 812 ab   | 0.49 a              |
| DC   | 317 b                   | 622 b    | 940 b    | 0.50 a              |
| -N treatment                                     |                         |          |          |                     |
| <i>Abies grandis</i>                             |                         |          |          |                     |
| JR   | 177 a                   | 154 a    | 331 a    | 1.15 a              |
| TY   | 186 a                   | 153 a    | 340 a    | 1.22 a              |
| DC   | 431 b                   | 247 b    | 678 b    | 1.74 b              |
| <i>Larix occidentalis</i>                        |                         |          |          |                     |
| JR   | 275 b                   | 198 b    | 473 b    | 1.38 a              |
| TY   | 260 b                   | 194 b    | 454 b    | 1.34 a              |
| DC   | 184 a                   | 110 a    | 294 a    | 1.67 b              |
| <i>Pseudotsuga menziesii</i>                     |                         |          |          |                     |
| JR   | 174 a                   | 129 a    | 303 a    | 1.35 a              |
| TY   | 528 b                   | 288 b    | 817 b    | 1.83 b              |
| DC   | 262 a                   | 183 a    | 445 a    | 1.43 a              |
| <i>Pinus contorta</i>                            |                         |          |          |                     |
| JR   | 346 b                   | 272 b    | 618 b    | 1.27 a              |
| TY   | 372 b                   | 316 b    | 688 b    | 1.17 a              |
| DC   | 262 a                   | 184 a    | 447 a    | 1.42 b              |
| <i>Pinus ponderosa</i>                           |                         |          |          |                     |
| JR   | 498 c                   | 421 c    | 919 c    | 1.18 a              |
| TY   | 228 a                   | 188 a    | 416 a    | 1.21 a              |
| DC   | 361 b                   | 343 b    | 704 b    | 1.05 a              |

<sup>a</sup> Within physiological treatments and tree species in each column, values followed by the same letter are not significantly different ( $P \leq 0.05$ ) as determined by Fisher's protected least significant difference test;  $n = 15$ .



TABLE 2. Biochemical characteristics of secondary root tissue of western coniferous seedlings under three physiological treatments; adequate light and nitrogen (LN), light limitation (–L), and nitrogen limitation (–N)<sup>a</sup>

| Species                      | Sugars <sup>b</sup> | Starch <sup>b</sup> | Cellulose <sup>c</sup> | Lignin <sup>c</sup> | Phenolics <sup>d</sup> | Protein-precipitable tannins <sup>b</sup> |
|------------------------------|---------------------|---------------------|------------------------|---------------------|------------------------|---|
| LN treatment                 |                     |                     |                        |                     |                        |   |
| <i>Abies grandis</i>         | 33.45 a             | 50.53 a             | 68 b                   | 27 b                | 29.3 a                 | 11.66 a                                   |
| <i>Larix occidentalis</i>    | 36.80 a             | 52.37 a             | 68 b                   | 30 b                | 38.0 b                 | 12.53 b                                   |
| <i>Pseudotsuga menziesii</i> | 37.25 a             | 47.87 a             | 69 b                   | 27 b                | 38.7 b                 | 12.06 a                                   |
| <i>Pinus contorta</i>        | 37.69 a             | 48.16 a             | 69 b                   | 26 b                | 33.8 b                 | 11.80 a                                   |
| <i>Pinus ponderosa</i>       | 38.97 a             | 49.80 a             | 65 a                   | 30 b                | 36.8 b                 | 11.73 a                                   |
| –L treatment                 |                     |                     |                        |                     |                        |   |
| <i>Abies grandis</i>         | 30.18 a             | 35.91 b             | 79 a                   | 18 a                | 28.7 a                 | 11.10 a                                   |
| <i>Larix occidentalis</i>    | 35.77 a             | 33.18 b             | 80 a                   | 18 a                | 29.3 a                 | 11.26 b                                   |
| <i>Pseudotsuga menziesii</i> | 34.26 a             | 33.54 b             | 85 a                   | 16 a                | 28.7 a                 | 11.06 a                                   |
| <i>Pinus contorta</i>        | 31.26 a             | 34.03 b             | 81 a                   | 18 a                | 29.5 a                 | 11.04 a                                   |
| <i>Pinus ponderosa</i>       | 33.10 a             | 29.38 b             | 80 a                   | 19 a                | 29.0 a                 | 10.90 a                                   |
| –N treatment                 |                     |                     |                        |                     |                        |   |
| <i>Abies grandis</i>         | 65.05 b             | 40.03 ab            | 68 a                   | 27 b                | 28.7 a                 | 11.10 a                                   |
| <i>Larix occidentalis</i>    | 63.95 b             | 36.45 b             | 71 ab                  | 26 b                | 30.5 a                 | 11.70 b                                   |
| <i>Pseudotsuga menziesii</i> | 65.45 b             | 47.09 a             | 75 b                   | 26 b                | 28.7 a                 | 11.27 a                                   |
| <i>Pinus contorta</i>        | 48.61 a             | 45.01 a             | 71 ab                  | 27 b                | 29.5 a                 | 11.69 a                                   |
| <i>Pinus ponderosa</i>       | 45.61 a             | 45.08 a             | 71 ab                  | 27 b                | 29.5 a                 | 11.37 a                                   |

<sup>a</sup>In each column, values followed by the same letter are not significantly different ( $P \leq 0.05$ ) as determined by Fisher's protected least significant difference test.

<sup>b</sup>Milligrams per gram of dry root tissue.

<sup>c</sup>Percent dry weight of root tissue.

<sup>d</sup>Equivalent phenol units per gram of dry root tissue.

TABLE 3. Infection of seedlings of five western conifer species by *Armillaria ostoyae* (NABS I) under three physiological treatments: adequate light and nitrogen (LN), light limitation (–L), and nitrogen limitation (–N)<sup>a</sup>

| Physiological treatment | Uninfected living <sup>y</sup> | Infected living <sup>y</sup> | Infected dead <sup>y</sup> | Disease rating <sup>z</sup> |
|-------------------------|--------------------------------|------------------------------|----------------------------|-----------------------------|
| LN                      | 75 a                           | 23 a                         | 3 a                        | 1.3 a                       |
| –L                      | 50 b                           | 34 b                         | 16 b                       | 1.6 b                       |
| –N                      | 48 b                           | 23 a                         | 29 c                       | 1.7 c                       |

<sup>a</sup>All interactions and the variables seedling species and *A. ostoyae* isolate were not significant in the ANOVA ( $P \leq 0.05$ ); physiological treatment was the only significant variable. Values followed by the same letter are not significantly different ( $P \leq 0.05$ ) as determined by Fisher's protected least significant difference test.

<sup>y</sup>Percentage of 60 seedlings.

<sup>z</sup>Average of 60 seedlings, each rated as 1 (healthy, not infected), 2 (live and infected), or 3 (dead and infected). (Infection refers to successful recovery of *A. ostoyae*.)

and –N treatments and lowest in seedlings receiving the –L treatment. With the exception of *A. grandis*, concentrations of phenolic compounds were higher in secondary root tissue of seedlings receiving balanced light and nutrients than of those that were light- or nitrogen-limited. In all physiological treatments, the concentrations of protein-precipitable tannins in secondary root tissue were higher in *L. occidentalis* than in the other seedling species. The ratio of phenolic compounds to sugars was lowest in secondary root tissue of seedlings receiving the –N treatment.

**Disease ratings.** When challenged against the original inoculum of *A. ostoyae* by diploid-diploid crossings on 3% malt agar (3), fungi isolated from infected seedlings were the same as those used for inoculations. Resinous lesions appeared on 63% of infected seedlings (both living and dead combined). All dead seedlings were infected.

Because disease rates did not differ significantly among tree species or the three isolates of *A. ostoyae*, we present results for disease ratings with regard only to physiological treatments (34,35) (Table 3). *A. ostoyae* infected an average of 26% of seedlings receiving the LN treatment, 50% of those receiving the –L treatment, and 52% of those receiving the –N treatment.

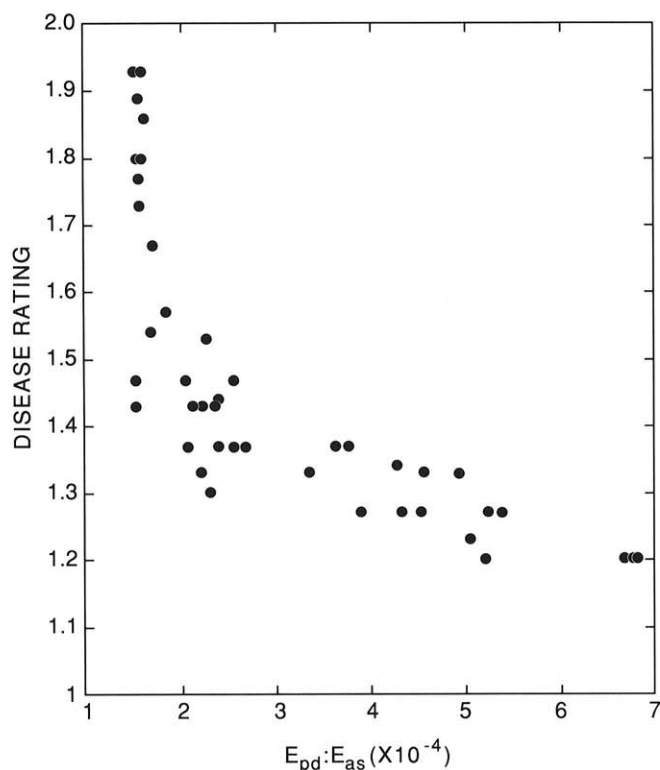
More trees were infected but living in the –L treatment than in the LN or –N treatment. More trees died as a result of infection by *A. ostoyae* in the –N treatment than in the –L or LN treatments. Disease ratings were higher in the –L and –N treatments than in the LN treatment and were higher in the –N treatment than the –L treatment (Table 3). Disease ratings did not differ significantly among isolates of *A. ostoyae* or seedling species.

Disease ratings did not correlate linearly with sugar, starch, cellulose, lignin, or protein-precipitable tannins nor curvilinearly with starch, cellulose, or lignin. Disease ratings correlated both linearly ( $r^2 = 0.39$ ) and curvilinearly ( $r^2 = 0.41$ ) with total phenolics. Disease ratings also correlated both linearly ( $r^2 = 0.43$ ) and curvilinearly ( $r^2 = 0.44$ ) with the phenolic:sugar ratio. Concentrations of phenolic compounds correlated with those of lignin ( $r^2 = 0.28$ ) and protein-precipitable tannins ( $r^2 = 0.51$ ). Concentrations of lignin correlated with those of protein-precipitable tannins ( $r^2 = 0.47$ ). The ratios  $E_{pd}:E_{as}$  (Fig. 1) and  $E_{ld}:E_{as}$  (Fig. 2) correlated curvilinearly with disease ratings ( $r^2 = 0.73$  and  $0.71$ , respectively).

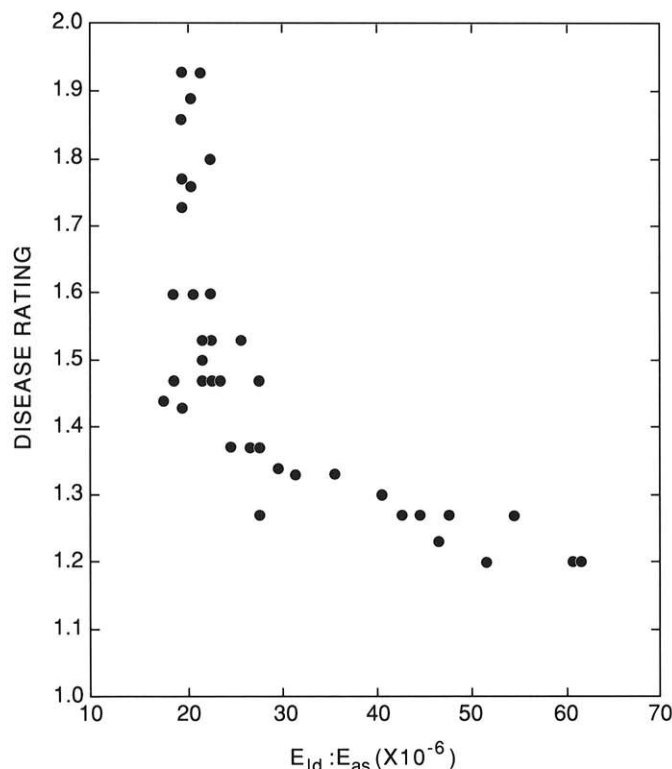
## DISCUSSION

Seedlings grown with full light and balanced nutrition produced far greater biomass than those for which light or nitrogen was limited. Secondary root tissue of the latter also exhibited lower concentrations of lignin, total phenolic compounds, and protein-precipitable tannins. These compounds may be an important part of a plant's primary defense against fungal attack (10,37). Severity of disease caused by *A. ostoyae* was greater in seedlings that had lower concentrations of defensive compounds resulting from limited light or nitrogen.

Results of this investigation confirm earlier findings. In greenhouse experiments, Redfern (29) reported a higher rate of infection by *A. mellea* (sensu lato) in *P. menziesii*, *A. grandis*, *Picea sitchensis* (Bong.) Carrière, *Larix kaempferi* (Lamb.) Carrière, and *Pinus sylvestris* L. when light intensity was reduced. Singh (33,34) noted that *Picea abies* (L.) H. Karst., *P. sitchensis*, *P. mariana*, and *P. sylvestris* were infected by *Armillaria* to a greater extent when N, K, and Ca were deficient. Shields and Hobbs (32) found that lower soil pH and N, P, and Ca concentrations were associated with greater infection of *P. menziesii* and *A. grandis* by *Armillaria*.



**Fig. 1.** Plot of the  $E_{pd}:E_{as}$  ratio (energy required for phenolic degradation:energy available from sugars) against disease ratings in five species of western conifer seedlings inoculated with *Armillaria ostoyae* ( $r^2 = 0.73$ ,  $n = 45$ ). Disease rating =  $1.88 + [E_{pd}:E_{as} \times (1.712 \times 10^{-9})] + [E_{pd}:E_{as} \times 2 \times (-4.097 \times 10^{-16})] + [E_{pd}:E_{as} \times 3 \times (3.678 \times 10^{-24})]$ .



**Fig. 2.** Plot of the  $E_{ld}:E_{as}$  ratio (energy required for lignin degradation:energy available from sugars) against disease ratings in five species of western conifer seedlings inoculated with *Armillaria ostoyae* ( $r^2 = 0.71$ ,  $n = 45$ ). Disease rating =  $2.004 + [E_{ld}:E_{as} \times (8.48 \times 10^{-7})] + [E_{ld}:E_{as} \times 2 \times (-2.912 \times 10^{-12})] + [E_{ld}:E_{as} \times 3 \times (6.382 \times 10^{-24})]$ .

in northern Idaho. Although the factors that determine infection of host species by *Armillaria* are not completely understood, nutrient-deficient seedlings are known to be more vulnerable to attack (34).

Growth efficiency coefficients for fungi that can grow well on media containing easily degradable carbohydrates range from 10 to 25% (8). In our study, growth coefficients for the three tested isolates of *A. ostoyae* indicated that the fungi can grow faster on media containing high amounts of soluble sugars, especially glucose (14). In the presence of phenolic compounds, *A. ostoyae* grows faster in media supplemented with higher sugar concentrations (12).

Thermochemical calculations rarely have been applied to forest host-pathogen relationships. In our study, however, the parameters that correlated best with disease severity were  $E_{pd}:E_{as}$  and  $E_{ld}:E_{as}$  ratios. Thus, energy relationships appear to predict accurately the relative success of attack by *A. ostoyae* on western coniferous seedlings and may provide greater understanding of pathogen invasion. The thermochemical relationship with disease ratings indicates that there are thresholds of  $E_{pd}:E_{as}$  and  $E_{ld}:E_{as}$  below which resistance to infection is drastically reduced, and colonization of the root by the pathogen occurs. This threshold ratio can be reached by an increase in the sugar concentrations of roots and/or a decrease in phenolic and lignin concentrations in root tissue. Further knowledge of the phenolic compounds in root bark tissue and their thermochemical equilibria would allow more precise estimation and more accurate interpretation of those relationships.

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