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# NITRATE-REDUCING CAPACITY OF ROOTS AND NODULES OF *ALNUS RUBRA* AND ROOTS OF *PSEUDOTSUGA MENZIESII*

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SUMMARY

Nitrate-reducing capability was demonstrated for root segments of red alder and, at more than twice that rate, for alder nodules. Root segments of Douglas-fir failed to reduce nitrate despite various treatments designed to induce such activity. The reported response of Douglas-fir to nitrate fertilizer may be ascribed either to microbial assimilation of nitrate ions with subsequent liberation of ammonium in the soil or to nitrate assimilation by fungi that form mycorrhizae with Douglas-fir roots.

Forest soils supporting the nitrogen-fixing red alder (*Alnus rubra* Bong.) have relatively high levels of nitrate nitrogen as compared to soils under conifers only [3]. Red alder leaves have nitrate-reducing capability, but no such capability has been detectable in needles of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco), a conifer commonly associated with red alder in western North America [9]. However, the nitrate-reductase system required by plants for assimilation of nitrate nitrogen is inducible [8], and presence or absence of the system in leaves may not necessarily coincide with that in roots. Determination of nitrate-reducing capability of roots is accordingly needed to interpret the implications of soil nitrate nitrogen for the mixed alder–conifer ecosystem. The reduction of nitrate to nitrite by plant tissue is assumed to demonstrate nitrate-reductase activity.

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## MATERIALS AND METHODS

Mulder *et al.*[14] demonstrated that grinding of cells disorganizes nitrate-reductase and electron donor systems, as evidenced by the ensuing lack of nitrate-reduction to nitrite. Therefore, we determined nitrate reductase with pieces of root [1]. Freshly collected rootlets, 4 to 5 mm in diameter, of red alder and Douglas-fir were collected from a mixed stand at the Cascade Head Experimental Forest, Oregon and cut into segments weighing 1 gram (fresh weight). Each segment was cross sectioned into pieces 1.5 to 2 mm long. For alder, similar samples were prepared from the nitrogen-fixing nodules.

The pieces from each sample were immediately immersed in 5.0 ml 0.06 *M* $Na_2HPO_4$–citric acid buffer at pH 7.0 [13] mixed with 1 ml 0.1 *M* succinic acid neutralized with NaOH plus 1 ml 0.1 *M* $KNO_3$ in Thunberg tubes. Air was evacuated from the tubes, readmitted, and again evacuated over a 30-min period. The evacuated tubes were sealed and placed in a water bath at 37°C for 2 hours. The amount of nitrite formed by the rootlet fragments and released into solution was determined by adding 1 ml of 1% sulfanilamide and 1 ml 0.02% N-(1-naphthyl)-ethylene-diamine dihydrochloride to 2 ml of the incubated solution. Enzyme activity was expressed as μ moles nitrite produced per gram fresh weight of rootlet in the 2-hour period.

Because the Douglas-fir samples showed no nitrate reduction, we attempted to induce nitrate reduction by using the same methodology except for modifying the solution. Respective attempts included addition to the solution described above of (1) potassium gibberellate at 200 ppm, (2) kinetin at 10 ppm, or (1) and (2) combined [10, 11]. These having failed, we then substituted 1 ml 0.1 *M* malic acid for the succinic acid in the original solution (H. J. Evans, Oregon State University, personal communication). Subsequent modifications of this malic acid solution included addition of (1) 0.75 mg triphosphopyridine nucleotide (TPN) plus 0.005 *M* $MnSO_4 . H_2O$ in 1.5 ml $H_2O$ (H. J. Evans, personal communication), (2) TPN + Mn + 1 mg $Mo^{+6}$ (as $Na_2MoO_4 . 2H_2O$ [16, 17]), (3) TPN + Mn + 200 ppm Gibrel, (4) TPN + Mn + 10 ppm kinetin, and (5) TPN + Mn + Gibrel + kinetin. Moreover, $KNO_2$ was substituted for $KNO_3$ in the starting succinic acid solution to determine if nitrite was disappearing due to a nitrite reduction by the Douglas-fir root tissue. All treatments were duplicated.

## RESULTS AND DISCUSSION

The results with roots of red alder and Douglas-fir parallel earlier results with leaves [9]: nitrate was reduced by red alder tissues, but not by those of Douglas-fir. The μmoles of nitrite formed per gram fresh weight of alder tissue in 2 hours incubation were, by replicate:

| Roots | Nodules |
| --- | --- |
| $1.83 \times 10^{-2}$ | $4.97 \times 10^{-2}$ |
| $1.83 \times 10^{-2}$ | $4.83 \times 10^{-2}$ |

Nitrate reduction by alder roots approximated that reduced by leaves in the earlier study [9] (recalculated to the same basis): $\pm 1.64 \times 10^{-2}$ µmoles. The leaf data is not strictly comparable, since that experiment entailed different methodology.

With these positive results, no further experiments were conducted with alder. In contrast, Douglas-fir root tissue reduced no nitrate with the succinic acid system. When $KNO_2$ was substituted for $KNO_3$ in the succinic acid solution, equal amounts of nitrite ions were present at the end of the experiment as were added originally, indicating that no nitrite reduction was occurring. All other treatments failed to show measurable nitrate reduction. Similar results have indicated the absence of a nitrate-reducing system in leaves and roots of *Vaccinium* sp.[18].

Douglas-fir seedlings and saplings have responded particularly well to fertilization with the nitrate form of nitrogen [7,15]. The apparent anomaly of this response by a species lacking nitrate-reducing capability in either roots or leaves [9] can be speculatively explained by either or both of two alternatives. Nitrate ions in the soil could be microbially transformed to other nitrogen compounds more readily assimilated by Douglas-fir. Or, the mycorrhizal fungi of Douglas-fir could absorb and reduce the nitrate ions prior to translocating them to the root tissue. Lundeberg [12] demonstrated that several species of mycorrhizal fungi associated with Douglas-fir [19] can utilize nitrate ions in pure culture: *Amanita muscaria* (L. ex Fr.) Pers. ex Hooker, *Boletus edulis* Bull. ex Fr., *B. piperatus* Bull. ex Fr., *B subtomentosus* L. ex Fr., *Cenococcum graniforme* (Sow.) Ferd. & Winge, *Hebeloma crustuliniforme* (Bull. ex St. Am.) Quel., *Lactarius deliciosus* (L. ex Fr.) S. F. Gray and *Paxillus involutus* (Batsch ex Fr.) Fr. Another Douglas-fir mycorrhizal associate, *Suillus caerulescens* Smith & Thiers, has shown nitrate-reductase activity [20].

It might be suggested that Douglas-fir root hairs or roots smaller than those we studied are active in nitrate reduction. The experiments of Van den Driessche [22], however, reinforce the hypothesis that mycorrhizal fungi rather than root hairs or small rootlets are responsible for nitrate utilization by Douglas-fir. Greenhouse seed-

lings were grown from seed for periods ranging from 15 weeks to 6 months in subirrigated sand culture or sand-peat mixtures. Two forms of nitrogen were tested as components of the nutrient solution: $Ca(NO_3)_2$ and $(NH_4)_2SO_4$, both separately and in combination. The seedlings responded quite poorly to the $Ca(NO_3)_2$ as compared to the $(NH_4)_2SO_4$. In these experiments, mycorrhiza formation could be expected to be lacking, or at best, long-delayed for lack of abundant inoculum in the sand and peat. Comparison of the two forms of nitrogen as fertilizer in nursery experiments, however, produced quite different results. By the end of 1 year, the ammonium form showed significant advantages over the nitrate form in terms of nitrogen content of seedling shoot and, when pH effects were averaged out, in terms of seedling dry weight. By the end of the second year, however, these differences had disappeared. The nursery-grown seedlings were undoubtedly mycorrhizal, since Douglas-fir is incapable of normal growth in nurseries in the absence of mycorrhizal fungi [21]. In other words, nitrate nitrogen was a relatively poor nitrogen source where mycorrhiza formation was most likely lacking or delayed but was as good as the ammonium form in nurseries after mycorrhizae were doubtlessly well formed on the root system.

Nitrate reduction in alder nodules proceeded at more than twice the rate of that in alder roots. The difference can be attributed at least in part to the nodule endophyte, doubtlessly an Actinomycete; species of Actinomycetes characteristically contain nitrate-reductase [4]. Possibly the nitrate-reductase activity of the nodules is positively correlated with nitrogen-fixing capacity of the endophyte, as has been demonstrated for *Rhizobium* spp. in legume nodules (Cheniae and Evans [5 6]).

Nitrate-reductase in roots of alder is useful in nitrogen assimilation from soil, but its utility in leaves is less obvious. Both nitrate and ammonium forms of nitrogen leach from foliage and stems of red alder [2]. Presumably, these ions are translocated from roots to the leaves in surplus. The nitrate-reducing capacity of leaves might well be a mechanism for preventing toxic accumulations of nitrate ions during dry periods, when the removal of surplus nitrogen from the leaf would depend on assimilation or volatilization as opposed to washing by precipitation.

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