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Nitrate Reductase of Primary Roots of Red Spruce Seedlings[1]

EFFECTS OF ACIDITY AND METAL IONS

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ABSTRACT

Nitrate reductase activity (NRA) was found in primary roots, but not in foliage of red spruce (*Picea rubens* Sarg.) seedlings. Nitrate induced NRA: NH_4^+ did not induce and slightly depressed NRA in older seedlings. Induction required 8 hours and, once induced, NRA decreased slowly in the absence of exogenous NO_3^-. Seedlings were grown in perlite with a complete nutrient solution containing NH_4^+ to limit NR induction. Established seedlings were stressed with nutrient solutions at pH 3, 4, or 5 supplemented with Cl^- salts of Al, Cd, Pb, or Zn each at two concentrations. NRA in primary root tips was measured at 2, 14, 28, and 42 days. NRA induction was greatest at pH 3, and remained high during the period of study. NRA induction at pH 4 was lower. Metal ions suppressed NRA at pH 3 and 5, but enhanced NRA at pH 4. It is concluded that acidity and soluble metals in the root environment of red spruce are unlikely to be important factors in nitrogen transformations in red spruce roots.

Nitrogen availability is a limiting factor in natural forests (3), and red spruce (*Picea rubens* Sarg.) requires high N for best growth (14). In mountainous ecosystems, N in the acidic soil solutions is primarily as NO_3^- (12), deposition of nitrate from acidic precipitations is shifting the inorganic N ratio further towards NO_3^- (T Sherbatskoy, F Reed, RM Klein, unpublished data) and the leaching of NH_4-N is greater than for NO_3-N in acidic mountain soils (19).

There are few reports of the presence of NRA[2] in conifers roots. Li *et al.* (10) failed to find NRA in Douglas fir roots, although Bigg and Daniel (1) demonstrated it in seedling roots of Englemann spruce, lodgepole pine, and Douglas fir. For many conifers, NH_4^+ is a better N fertilizer than NO_3^- (18), but mixed sources may be as effective as ammonium alone (4).

Inasmuch as plants utilize reduced N (5), NRA is necessary for tree growth under natural conditions. Impairment of NRA by anthropogenic acidity and solubilized metals in soil solutions might be a factor in the decline of red spruce (20). This study was designed to determine whether endogenous NRA of primary roots of red spruce seedlings is affected by acidic, metal-containing nutrient solutions in which the plants were grown.

MATERIALS AND METHODS

Induction and Stability of NRA. Red spruce seed (Herbst Seed Co., Brewster, NY) were stratified 4 months at 4°C and germinated at 26°C in washed perlite in 1 L plastic refrigerator containers with bottom drainage. Different seed lots were used for the experiments reported here. Watered seedlings received 300 $\mu E/m^2 \cdot s$ from equal numbers of cool-white and red fluorescent lamps on a 15:9 photoperiod (15). To determine the effectiveness of NH_4–N and NO_3-N as inducers of NRA, seedlings grew for 4 weeks before receiving 0.1 × Ingestad's (7, 9) nutrient solution with N provided as KNO_3, NH_4NO_3, or NH_4Cl (3.57 mM N) or a N-free nutrient. Nutrient solutions (50 ml per container) were supplied to the plants every 2 d for 1, 2, or 4 weeks before root tips were analyzed for NRA.

To evaluate NR induction rates, roots of 2-week-old seedlings grown in perlite in 1 L plastic refrigerator containers with water only were assayed for *in vivo* NRA for 4, 8, and 12 h incubation periods. Two-week-old seedlings were supplied with 0.1 × Ingestad nutrient solution containing 3.57 mM N as NH_4NO_3 for 1 week and root tips assayed.

To determine decay of induced NRA, 2-week-old seedlings in perlite in 1 L plastic refrigerator containers were supplied with 0.1 × Ingestad nutrient containing 3.57 nM NO_3-N for 2 weeks, root tips assayed, and the remaining plants then supplied with 0.1 × Ingestad nutrient solution lacking N. Root tips were assayed 2 and 4 weeks after termination of N nutrition to determine if induced NRA would persist when the inducing ion was no longer supplied.

Effects of pH and Metals. Stratified red spruce seeds were germinated and grown at 22°C in washed perlite in 1 L plastic refrigerator containers with drainage holes under 400 $\mu E/m^2 \cdot s$ of cool-white fluorescent light on a 15:9 photoperiod. Seeds germinated in 7 to 9 d and seedlings received H_2O only until cotyledons were fully developed in 21 d. Seedlings were thinned to visual uniformity. Before seedlings were stressed by pH and metal ions, they received 0.1 × Ingestad nutrient solution with nitrogen supplied only as NH_4^+ (3.57 mM N) to avoid NR induction. When epicotyls were visible, 41 d after germination, 0.1 × Ingestad nutrient solutions were presented to paired containers for 42 d. Three pH levels (3, 4, or 5) and two concentrations of four metals plus metal-free controls at each pH were used, resulting in 27 treatment combinations. Nitrogen was supplied as NH_4NO_3 at 3.57 mM N; metals were provided as Cl^- salts. Seedling primary root tips were assayed for *in vivo* NRA 2, 28, and 42 d after the start of metal and acid nutrient treatments. Incubation time for this study on acid-metal ion effects was 4 h to avoid any additional RNA induction in the root tips.

***In Vivo* NRA Assay.** One cm long seedling root tips (0.015 mg dry weight) were excised, randomized in chilled 0.1 M K-phosphate (pH 7.5) containing 30 μg/ml chloramphenicol to prevent microbial activity and 1% *n*-propanol as a surfactant (8). Fifty root tips for studies of NRA induction or 30 tips for metal-pH studies were transferred to a vial containing buffer plus 2.76 mM N as KNO_3 (1.5 ml final volume). Anaerobiosis (8) was controlled by evacuating the vials with a water aspirator. Vacuum was released after 5 min to facilitate infiltration of buffer into roots (6, 11). Reevacuated dessicators were incubated at room temperature. The incubation period for NR induction and sta-

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[2] Abbreviation; NRA, nitrate reductase activity.

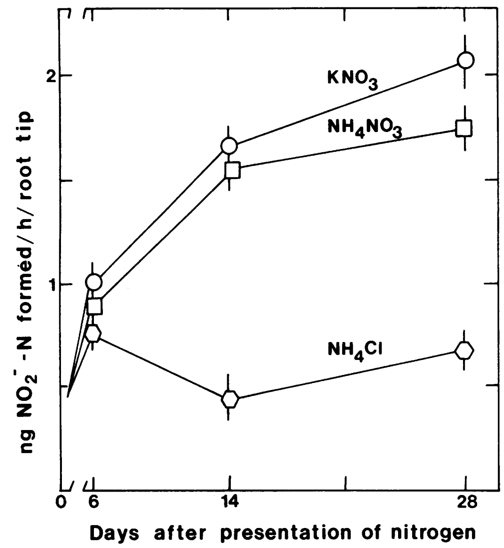

FIG. 1. Induction of NRA in primary root tips of red spruce seedlings. Seedlings were supplied with water for 14 d from sowing and then (0-time) with Ingestad's 0.1 × nutrient solution containing 3.57 mM N supplied as KNO_3, NH_4NO_3, or NH_4Cl. Vertical bars are SE of means of triplicate analyses.

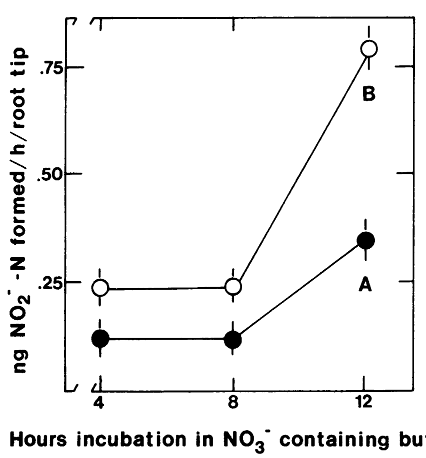

FIG. 2. Kinetics of NRA in primary root tips of red spruce seedlings grown without a N source for 14 d from sowing and primary root tips then analyzed for NRA (curve A). Seedlings were then supplied with KNO_3 for 7 d before assay of root tips (curve B). Vertical bars are SE of means of triplicate analyses.

bility studies was 6 h, and the incubation period for study of the interactions of acidity and metals on NRA was 4 h.

One ml aliquots were removed from each vial and 5 ml of 0.1% sulfanilamide (Sigma) in 2 N HCl was added. This coupling reaction required 5 min. Five ml of 0.02% *N*-(1-naphthyl) ethylenediamine diHCl (Sigma) was added and O.D. determined at 540 nm (17). Activity was calculated from a standard curve and is presented as ng NO_2^--N/h·root tip. All incubations and assays were conducted in triplicate using root tips pooled from plants grown in several plastic containers. Vials containing tissue were heated for 15 s in a microwave oven to provide tissue blanks. As determined in a preliminary trial, increase in NO_2^- was linear for 24 h, indicating that measured NRA was not due to bacterial contamination.

RESULTS AND DISCUSSION

NRA Induction and Stability. Red spruce root tips excised from plants not supplied with exogenous nitrate showed low NRA levels that averaged 0.4 ng NO_2-N formed/h·root tip (Fig. 1). When plants were supplied with KNO_3 or with NH_4NO_3 for 7 d, root tip NRA doubled and by 28 d of exposure to KNO_3, activity had increased 4-fold. Ammonium was not a NRA inducer, but did not repress NRA induction by NO_3^- measured at 6 and 14 d after presentation of N; by 28 d, root tips from plants receiving NH_4NO_3 showed less NRA than root tips from plants grown with KNO_3.

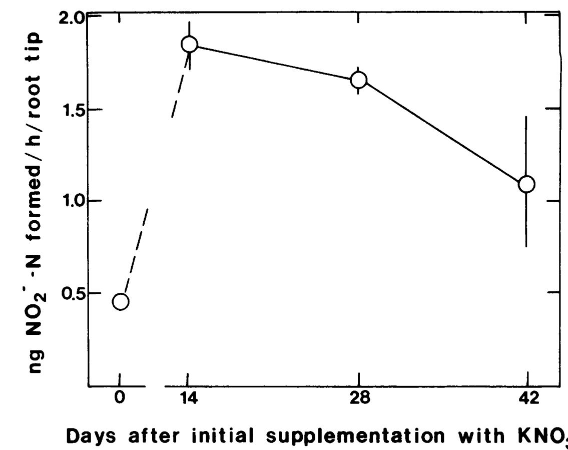

FIG. 3. Decay of NRA in primary root tips of red spruce seedlings. Seedlings were grown in nutrient solution with KNO_3 for 14 d from seed sowing and then in nutrient solutions without N. Root tips analyzed for NRA. Vertical bars are SE of means of triplicate analyses.

Root tips from plants grown 2 weeks without nitrogen and incubated with 2.76 mM N as KNO_3 showed low levels of NRA for the first 8 h after which NRA increased (Fig. 2, line A). Seedlings that received KNO_3 for 1 week after the first assay had a higher baseline NRA and, after an 8 h induction period, the rate of newly induced NRA was higher than in seedlings that had not had NRA induced (Fig. 2, line B). The concentration of NO_3-N in the nutrient solution, 1.78 mM, was that found in lysimeter-collected soil solutions from our field study area (T Sherbatskoy, F Reed, RM Klein, unpublished data). It was half that used in the root tip NRA assay (2.76 mM) and was apparently inadequate to cause full NRA induction. Compared to maize, where induction requires 30 min (13), NRA induction in red spruce roots is sluggish.

When NO_3^- nutrition of seedlings was stopped after 14 d, NRA of the primary root tips decayed slowly (*cf.* 16) over a 4 week period (Fig. 3). NRA was not detected under aerobic conditions. It was not measurable in whole or diced red spruce needles grown with or without NO_3^- in the nutrient medium. Clement and Le Tacon (2) found very low NRA in needles of *Picea abies*.

Effects of Acids and Metals. NRA induction in primary roots of red spruce was differentially affected by the nutrient solution pH (Fig. 4). NRA at 2 d was lower at pH 4 than at either pH 3 or 5. By 42 d of exposure to NO_3-containing media, the NRA of root tips from plants grown at all three pH levels was roughly similar. The differences are probably not of ecological significance.

NRA induction in red spruce root tips by 48 h after presentation of nutrient solutions containing NO_3^- and metal ions varied with pH, the metal ion and metal-ion concentration (Fig. 4). At pH 3, both concentrations of Zn and Al and the higher concentration of Cd and Pb repressed NR induction, while at pH 5, Pb, Cd, and the lower concentration of Al repressed NRA induction. At pH 4, Zn, Pb, Cd, and Al stimulated NRA induction.

By 42 d root tips from plants grown with Pb and Al at all 3 pH values reached about the same level of NRA as root tips from plants not exposed to metal ions. There was less NRA in

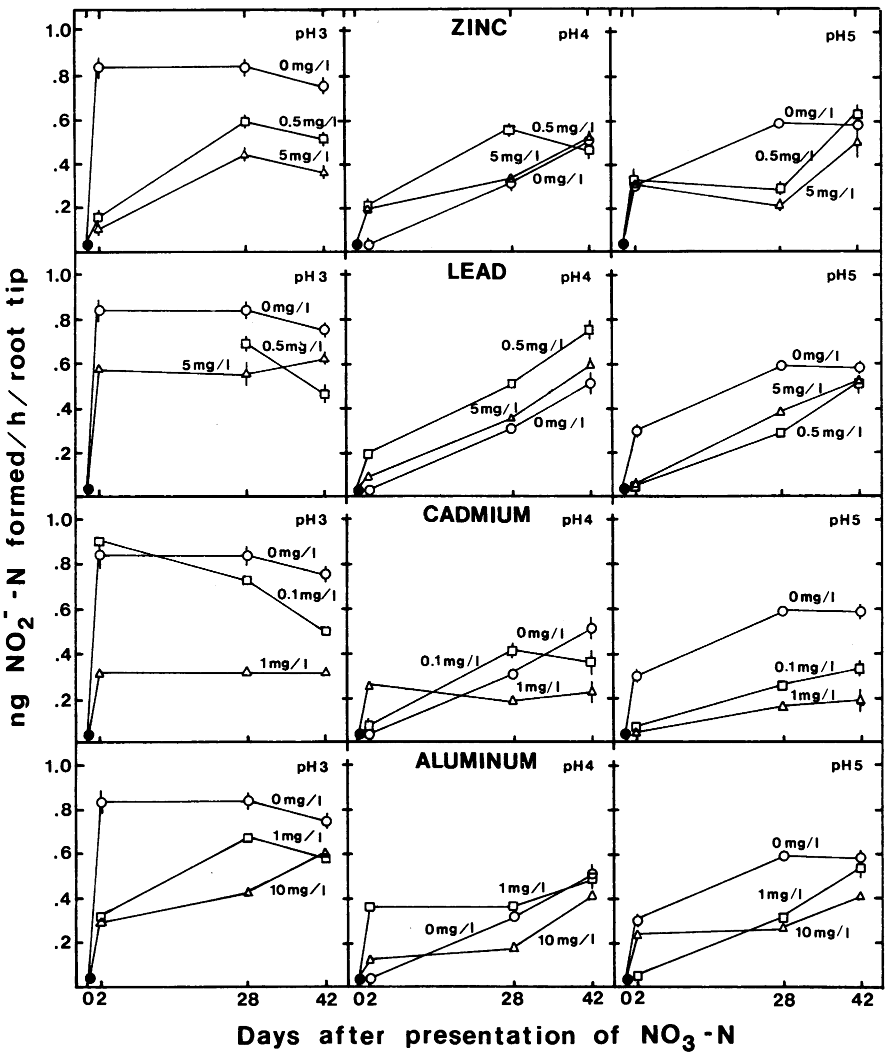

FIG. 4. Effect of pH and metals on NRA of primary root tips of red spruce seedlings. Seedlings grown in nutrient solutions containing 3.57 mM N as NH_4NO_3 at pH 3, 4, or 5 in the presence of two concentrations each of Al, Cd, Pb, or Zn as Cl^- salts. Root tips analyzed for NRA on indicated d after start of nutrient plus metal treatments. Vertical bars are SE of means of triplicate analyses. Value for Pb at 0.5 mg/L, pH 3 is missing.

root tips from plants stressed with Cd and those receiving Zn at pH 3.

With the exception of Cd, red spruce roots have the ability to adjust to toxic metals in their immediate environment (the rhizosphere) and can induce and maintain NRA needed to reduce oxidized nitrogen. This conclusion is reinforced by the fact that the Cd, Pb, and Zn concentrations used in this study exceed by a factor of over 10 those found in spring-collected lysimeter soil solutions when the soil solution pH was 3.3 (T Sherbatskoy, F Reed, RM Klein, unpublished data). Thus, acidity and metal-ion contaminations are unlikely to be significant perturbing factors in the ability of red spruce roots to induce and maintain NRA.

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