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Autumnal changes in tissue nitrogen of autumn olive, black alder and eastern cottonwood

BENOIT CÔTÉ[1], CHRISTOPH S. VOGEL and JEFFREY O. DAWSON[2]
[1] *Faculty of Agriculture, Macdonald College of McGill University, Ste-Anne de Bellevue, Quebec, Canada H9X 1CO and* [2] *1301 West Gregory Drive, Department of Forestry, University of Illinois, Urbana, IL 61801, USA*

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Abstract

Two experiments were conducted to determine patterns of N change in tissues of autumn olive (*Elaeagnus umbellata* Thunb.) and black alder (*Alnus glutinosa* [L.] Gaertn.) during autumn in central Illinois, U.S.A. In the first study leaf nitrogen concentrations of autumn olive decreased 40% at an infertile minespoil site and 39% at a fertile prairie site throughout autumn whereas nitrogen concentrations in respective bark samples increased by 39% and 37%. Salt-extractable protein concentrations increased in bark and decreased in leaves over the sampling period. Free amino acid concentrations of autumn olive leaves decreased over the course of the experiment from peak concentrations in August. Asparagine, glutamic acid and proline were major constituents of the free amino acid pools in leaves. Total phosphorus concentrations of autumn olive leaves declined by 40–46% during autumn while bark concentrations of P did not significantly change.

In the second experiment non-nodulated seedlings of alder receiving a low level of N-fertilization did not exhibit net resorption of leaf N during autumn whereas foliar N concentration of contrasting nonactinorhizal cottonwood plants (*Populus deltoides* Bartr. ex. Marsh) under the same fertilization regime decreased by 27% after the first frost. A gradual but significant decrease of 38% in foliar N concentration of nodulated alder seedlings grown under a low N-fertilization regime was associated with the cessation of nitrogenase activity during autumn in nodules. Compared with the low N fertilization regime, the higher level of N-fertilization resulted in smaller autumnal decreases of foliar N concentration in nodulated alder (17%) and in cottonwood (20%); but there was no decrease in foliar N concentration in non-nodulated alder. The higher level of N-fertilization promoted a greater accumulation of N in the roots than in the bark of both tree species after the first frost.

Our results suggest that black alder lacking *Frankia* symbionts does not exhibit net leaf N resorption and that autumnal decreases in leaf N of *Frankia*-nodulated black alder result primarily from declining foliar N import relative to export due to low temperature inhibition of N_2 fixation. In contrast, autumn olive exhibited greater and more precipitous autumnal declines in foliar N concentration than those of alder, and the pattern of N decline was unaffected by site fertility.

Introduction

Resorption of foliar nitrogen (N) to perennial organs during autumnal senescence and before leaf abscission is an important strategy utilized by many species of temperate deciduous woody plants to conserve nutrients. The majority of N lost from leaves during autumnal senescence is due to the hydrolysis of proteins to amino acids which are subsequently retranslocated to reserve tissues. This ensures N availability for rapid growth of foliage and flowering structures of deciduous trees after

spring bud break (O'Kennedy and Titus, 1979; Tromp and Ovaa, 1971).

The quantity of foliar N resorbed in the fall can depend on site fertility and plant species. Sites low in available N tend to have proportionally more N resorption from tree foliage in the fall than sites high in available N (Stachurski and Zimka, 1975; Tilton, 1977) although exceptions are numerous (Chapin and Kedrowski, 1983; Côté and Dawson, 1986; Ostman and Weaver, 1982). Autumnal decreases in foliar N concentration for Populus, Tilia, Malus, Quercus, Betula, and Salix range from 40 to 80% (Côté and Dawson, 1986; Insley *et al.*, 1981; Murneek and Logan, 1932; Oland, 1963; Ostman and Weaver, 1982; Viro, 1956). Actinorhizal (*Frankia*-nodulated) black alder (*Alnus glutinosa* [L.] Gaertn.) resorbs less foliar N, as estimated from changes in foliar N concentration, than other deciduous broadleaved tree species (Côté and Dawson, 1986; Dawson and Funk, 1981; Rodriguez-Barrueco *et al.*, 1984). Tissues of black alder and other actinorhizal plants are generally high in N (Dawson and Funk, 1981; Rodriguez-Barrueco *et al.*, 1984), and because they readily fix N_2 in the spring (Johnsrud, 1978; Moiroud and Capellano, 1979; Trip *et al.*, 1979; Zitzer and Dawson, 1989), there seems to be little selective advantage for black alder and other actinorhizal species to conserve foliar N during autumn. Our first hypothesis was that actinorhizal autumn olive (*Elaeagnus umbellata* Thunb.) would show an autumnal pattern of change in leaf N components similar to that of black alder, with no rapid and appreciable (40–80%) decline in foliar N concentration in late autumn, particularly on a fertile site, and that no concurrent rapid increase in bark N would occur. We also hypothesized that the small proportion of net leaf N that is apparently translocated in black alder in autumn is a characteristic of the species and that sub-optimal levels of N-fertilization and the absence of nodulation would not increase the net resorption of alder leaf N in autumn.

Materials and methods

Experiment 1

Two study sites supporting stands of autumn olive shrubs were selected. The first site had a prairie-derived Catlin silt loam soil (fine silty, mixed mesic Typic Arguidoll) (Alexander *et al.*, 1974) at the University of Illinois experimental farms in Urbana, Illinois (40°07′ N, 88°12′ W). The shrubs averaged 3 m in height and were growing among other volunteer shrubs, trees and grasses in an abandoned field.

The second site was located 40 km east of Urbana (40°08′ N, 87°37′ W) on minespoil banks consisting of limestone and shale low in organic matter and nitrogen. The autumn olive plants averaged 3 m in height on this site. Plants on both sites were healthy and were effectively nodulated, although nitrogen fixation capabilities were not assessed for every plant.

Leaf samples were collected repeatedly from 5 randomly-selected autumn olive shrubs on each site at 2-wk intervals commencing 17 August, 1987 through leaf drop in late October. Current-year twigs were collected every 4-wk from 17 August through 19 November. Fully expanded leaves and associated branches were randomly selected with respect to orientation and height in crown. Samples were placed on ice immediately upon collection and were stored at minus 20°C prior to analyses. Specific leaf weights were determined for each sample.

Portions of the leaf and bark samples were dried at 65°C for 48 hr and ground to pass through a sieve with 0.2 mm diameter openings and digested using the method of Parkinson and Allen (1975), except that lithium sulfate was excluded from the digestion mixture. The digested plant material was analyzed for total N using a Wescan ammonia analyzer.

The first and last leaf and bark samples collected from each site were assessed for salt-extractable protein by extracting the tissues as described by Côté and Dawson (1986) except that a 3:1 ratio of PVPP to tissue was used in the extraction buffer. Two ml of the crude extract supernatant containing salt-soluble proteins were made to a volume of 5 ml with 50% trichloroacetic acid at 4°C and the mixture incubated on ice for 30 min, at which time they were centrifuged at 10,000 g for 15 min to pellet the precipitated protein. The supernatant was discarded and the protein pellets were dissolved in 1 ml of 0.5 *M* NaOH and measured for protein concentration using the protein dye-binding method of Bradford (1976) using bovine serum albumin as the standard.

Free amino acids were extracted from frozen samples (1.5 g) of leaf tissue using the methods of Côté and Dawson (1986). Prior to analysis the ethanolic amino acid extracts were filtered through 0.8 μm filters to eliminate fine plant residue not removed by centrifugation. The extracts were assessed for total free amino acid concentrations following the procedure of Yemm and Cocking (1955).

Aliquots of the leaf ethanolic extracts were pooled according to site and sample time for assessment of free amino acid compositions. Three sample times for each site were chosen; the first sample time, when free amino acid concentrations were greatest; at a point midway through the sampling period; and at a point just prior to the final and rapid decline in foliar amino acid concentrations near the end of the sampling period. The composite samples were concentrated using a rotary evaporator at 60°C and brought to an appropriate volume with 0.3 *M* Li citrate, pH 2.2, to give a final concentration of 2.5 micromoles free amino acids/ml. Amino acid compositions were determined using a Beckman amino acid analyzer model 6300.

Plant material digested as previously described was analyzed for total P using vanadomolybdophosphoric yellow color method in nitric acid (Jackson, 1958) in combination with a Perkin Elmer Lambda 3B UV/VIS spectrophotometer.

Data were analyzed statistically using a one-way analysis of variance procedure and means were compared using the least significant difference multiple range test at a probability level of 5%.

Experiment 2

Seeds of black alder from a local source in central Illinois were germinated and potted in March 1987 in a 1:1 sterile mixture of sphagnum peat moss and calcined montmorillonite. Rooted cuttings of a clone of eastern cottonwood (*Populus deltoides* Bartr. ex. Marsh.) from Illinois, USA, were planted in mid April in the same medium for use as a nonactinorhizal comparison species. Both black alder seedlings and cottonwood cuttings were fertilized every week with 1/3-strength, N-free Hoagland's solution (Hoagland and Arnon, 1950) until the inoculation of half of the alders with *Frankia* ArI3 (Berry and Torrey, 1979) on April 27, 1987. One ml of 1% v/v (packed cells at 1000 *g* for 5 min) of *Frankia* was used to inoculate the roots of each alder seedling. In May, half of both the inoculated and non-inoculated alders, and half of the cottonwoods were randomly chosen to receive a low level of N and were fertilized weekly with N-free 1/3-strength Hoagland's nutrient solution. The other half of the plants were fertilized weekly with 20 ml of 1/3-strength Hoagland's nutrient solution containing N. Plants under the higher N-fertilization regime received 20 ml of 200 mg N l^{-1} as NH_4NO_3 three times a week until leaf senescence. Plants under the low N-fertilization regime received 20 ml of 100 mg N l^{-1} three times a week until mid-July and then received 20 ml of 50 mg N l^{-1} three times a week until leaf senescence. Other nutrients were supplied at levels equivalent to 20 ml of 1/3-strength Hoagland's nutrient solution three times per week.

Every two weeks beginning August 3, 1987, and continuing until leaf drop, five alders and four cottonwoods in each treatment were randomly harvested. Seedling heights were measured and alders checked for nodulation. All *Frankia*-inoculated alders were nodulated, and the few uninoculated alders that became nodulated were rejected. Leaves, branches with stems, and roots were separated. Secondary and tertiary roots of black alder, and the bark on the buried portion of cottonwood cuttings that generated roots were kept for analysis as root tissue. Leaves, bark and roots were dried at 65°C until constant weight was attained. Each sample was weighed and ground to pass through a sieve with 0.2 mm diameter openings and analyzed for total nitrogen concentration using the wet oxidation procedure of Parkinson and Allen (1975).

Nitrogenase activity of nodulated plants was assessed according to the method of Turner and Gibson (1980) by measuring the rate of acetylene (C_2H_2) reduction of washed roots of detopped plants every two weeks from September 29 until leaf drop or cessation of nitrogenase activity. C_2H_2 reduction during a 1-hour incubation between 1100 and 1300 h under ambient conditions was determined.

The effect of time was statistically tested using a one-way analysis of variance. Means within each combination of type of plant, level of fertilization

and tree component were compared using least significant difference (LSD) at a probability level of 5%.

Results and discussion

Experiment 1

Total N concentrations of autumn olive leaves decreased over the sampling period by 40% at the minespoil site and 39% at the prairie site (Fig. 1). The decreases in foliar N concentration determined in this study are at the low end of the range (40–80%) found for most temperate broadleaved deciduous trees (Chapin and Kedrowski, 1983; Grigal *et al.*, 1976), but are scarcely above maximum values established for actinorhizal *Alnus* species (0–38%) in this study and by others (Côté and Dawson, 1986; Dawson and Funk, 1981; Dawson *et al.*, 1980; Rodriguez-Barrueco *et al.*, 1984). N concentrations in respective bark samples increased by 39% and 37% (Fig. 2) suggesting a significant degree of translocation of N from leaves.

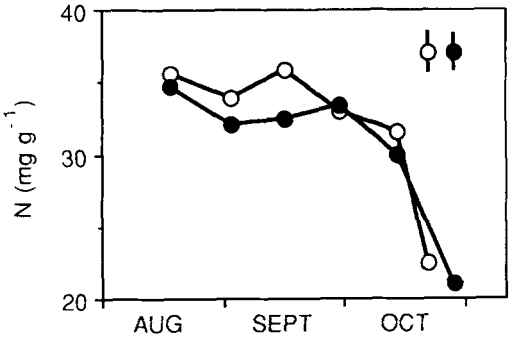

Fig. 1. Changes in foliar nitrogen concentrations on a dry weight basis of autumn olive on a prairie (○) and minespoil site (●). Vertical bars represent least significant difference at $P = 0.05$.

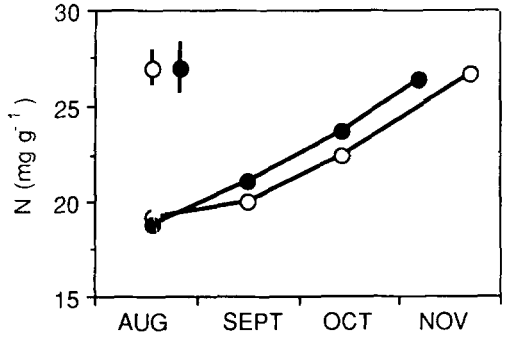

Fig. 2. Changes in bark nitrogen concentrations on a dry weight basis of autumn olive on a prairie (○) and minespoil site (●). Vertical bars represent least significant difference at $P = 0.05$.

Autumn olive bark samples harvested after leaf drop contained 178% and 91% more salt-extractable protein than August samples at the prairie and minespoil sites, respectively (Table 1). Corresponding leaf samples had 65% and 78% decreases in salt-extractable protein concentrations. Similar increases in bark concentrations of salt-extractable proteins during autumnal senescence were observed for apple, basswood, and eastern cottonwood (Côté and Dawson, 1986; Kang and Titus, 1980). Kang and Titus (1980) found the accumulation of a group of proteins in the bark of apple after leaf abscission and suggested they may play a role in the storage of N. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of our autumn olive salt-extractable proteins (data not presented) also suggest the preferential accumulation of certain proteins during autumnal senescence. These results are consistent with the leaf and bark N concentration changes established in this study and further suggest that leaf proteins were degraded and that the resulting amino acids were translocated from leaves to bark and used to synthesize bark proteins.

Leaf free amino acid concentrations were greatest at both sites at the start of the experiment, decreasing after 2 wk and remaining relatively stable for 3–4 wk (Fig. 3). Plants growing at the prairie site showed a steady decrease in foliar concentrations of free amino acids after Sept. 28 while a decrease in autumn olive foliage at the minespoil site was not apparent until after Oct. 12. These patterns contrast with those found for senescing

Table 1. Mean (n = 5) salt extractable protein concentrations (mg[g DW tissue]$^{-1}$) of autumn olive leaves and bark in August and after autumnal senescence collected from minespoil and prairie sites (± standard deviation)

	Minespoil	Prairie
Leaves		
August	26.6 ± 3.1	22.9 ± 1.7
Post senescence	5.9 ± 0.6	8.1 ± 3.0
Bark		
August	14.8 ± 4.0	12.9 ± 4.7
Post senescence	28.3 ± 5.2	35.8 ± 5.3

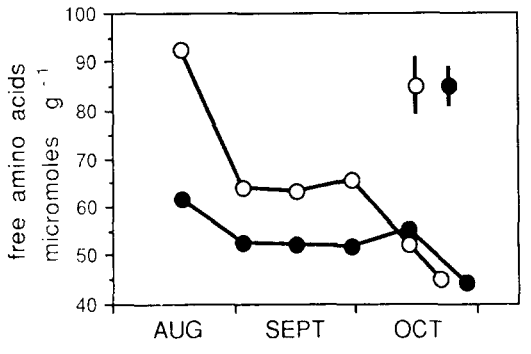

Fig. 3. Changes in foliar free amino acid concentrations on a dry weight basis of autumn olive on a prairie (○) and minespoil site (●). Vertical bars represent least significant difference at $P = 0.05$.

black alder in which foliar amino acid concentrations rapidly increased just prior to leaf abscission (Côté and Dawson, 1986). This suggests that autumn olive is capable of transferring amino acids from leaves at a rate that is at least comparable to their production during the breakdown of leaf N constituents.

The major free amino acids found in leaves of autumn olive in August were asparagine and glutamic acid, together accounting for 68% and 53% of the totals of the prairie and minespoil sites respectively. In October prior to leaf abscission, asparagine and glutamic acid concentrations had decreased by at least half from August concentrations. Asparagine has been shown to be an important transport amino acid in apple (Titus and Kang, 1982). In autumn olive asparagine and glutamic acid may be transport and/or products of transport amino acids exported from roots to shoots, and their decline in concentrations during autumn may reflect decreases in N-uptake and N_2-fixation by the roots of autumn olive. Proline concentrations increased in the period from August to October comprising at its maximal level 31% of all free amino acids at the prairie site and 56% at the minespoil site. Proline accumulation in plant tissues is associated with winter hardening as well as water stress (Levitt, 1980a; 1980b). The minespoil site tended to be droughty compared to the prairie site and may explain the proportionally greater increase in foliar proline concentration at this site compared with the prairie site.

The P concentration of autumn olive foliage declined 40% from an initial high value of 1.5 mg $(gDW)^{-1}$ during autumn on the prairie site and declined 46% from an initial high value of 1.3 mg $(gDW)^{-1}$ on the minespoil site. During the same period of time bark concentrations of P did not change significantly, averaging 1.2 mg $(gDW)^{-1}$ on the prairie site and 0.9 mg $(gDW)^{-1}$ on the minespoil site. The apparent lack of accumulation of P in the bark of autumn olive may be due to increases in dry weight of the bark causing a dilution effect or the translocation of P to other stem and root tissues, since these organs have been implicated in the storage of nutrients in other tree species (Nsimba-Lubaki and Puemans, 1986). Since P-containing compounds are not readily leached from leaves (Chapin and Kedrowski, 1983) and the specific leaf weights of autumn olive in this study declined (see below), it is likely that P was resorbed from the leaves to perennial organs for storage. Actinorhizal alder species have been shown to be efficient in their capabilities to take up soil P (Côté and Camiré, 1987; Prégent and Camiré, 1985; Binkley *et al.*, 1984), meeting requirements for high tissue P concentrations needed for N_2-fixation. The same may be true for autumn olive and the apparent resorption of P from leaves may reflect this plant's requirement for relatively high levels of P.

Autumn olive seemingly resorbed a greater proportion of foliar P than N at both sites during autumn. The decline in foliar P concentration in autumn olive was proportionally greater on the better prairie site compared with the minespoil site, but the proportional decline in foliar N concentration was similar for both sites. The same patterns have been observed for N_2-fixing *Alnus crispa* (Ait.) Pursh (Chapin and Kedrowski, 1983) and further supports the concept that actinorhizal species demand adequate P nutrition in order to maintain high rates of N_2-fixation and may have more efficient mechanisms for P resorption than for N resorption.

Specific leaf weights declined significantly only after October 12. Autumn olive leaves declined from an average of 8.9 to 6.3 mg cm^{-2} ($\pm$0.7SD) on the fertile prairie site and from 8.8 to 6.9 mg cm^{-2} ($\pm$0.7SD) on the minespoil site within 2 weeks after Oct. 12. Evidently a significant proportion of foliar nutrients were rapidly resorbed. This decline in specific leaf weight coincides with the precipitous drop in N that occurred after Oct. 12 and also with

the majority of the decline in P that occurred over the sampling period.

Actinorhizal autumn olive declined in foliar N concentration as well as in concentrations of free amino acids and salt-extractable proteins prior to autumnal leaf drop on two sites in Illinois. Concurrent increases in the concentration of bark N constituents suggest that much of the N translocated from leaves of autumn olive is stored in adjacent bark tissue. Furthermore, the rapid decline in foliar N concentration just prior to leaf abscission is more similar to the typical pattern for temperate deciduous trees than to that observed for alders. Zitzer and Dawson (1989) found a higher proportion of necrotic nodule tissue for *Elaeagnus angustifolia* than for *Alnus glutinosa* growing together on sites in central Illinois. We speculate that mature autumn olive shrubs, in conserving 39–40% of foliar N, may reduce their requirements for symbiotic dinitrogen fixation relative to alder.

Experiment 2

Foliar N concentration of nodule-free black alder did not decrease during autumn (Fig. 4B, E) but did decrease in eastern cottonwood (Fig. 4C, F) and nodulated black alder (Fig. 4A, D). Prior to the first frost on October 4, eastern cottonwood (Fig. 4C, F) and nodulated black alder under the high

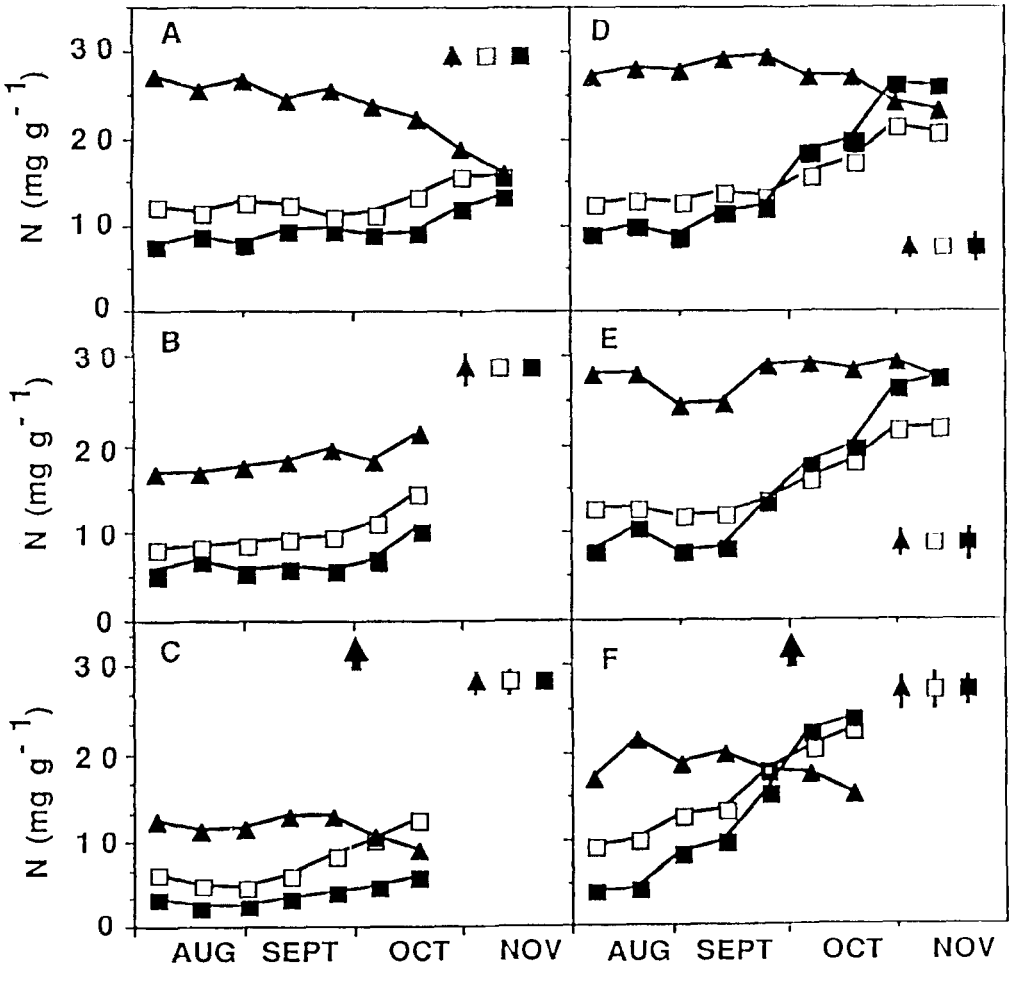

Fig. 4. Changes in nitrogen concentration on a dry weight basis in leaves (▲), bark (□), and roots (■) of *Frankia*-nodulated black alder, non-nodulated black alder, and eastern cottonwood under a low fertilization regime (**A, B, C**) and under a high fertilization regime (**D, E, F**). Samples were collected every two weeks from August 3, 1987 until leaf senescence. First frost occurred on Oct. 4, as indicated by the arrow. Vertical bars represent least significant differences at $P = 0.05$.

N-fertilization regime (Fig. 4D) had maintained stable leaf N-concentration values, while nodulated black alder under the low fertilization regime (Fig. 4A) exhibited a gradual decline in foliar N concentration. After the first frost, eastern cottonwood ramets receiving low and high levels of fertilization decreased their foliar N concentration by 27% and 20%, respectively (Fig. 4C, F). Nodulated black alder seedlings receiving low and high levels of fertilization decreased in foliar N concentration by 38% and 17%, respectively, over the last two months prior to leaf fall (Fig. 4A, D). The decrease in foliar N concentration in nodulated alders was gradual and did not decline more rapidly during the last stages of leaf senescence, as is characteristic of other deciduous broadleaved tree species (Côté and Dawson, 1986; Insley *et al.*, 1981; Oland, 1963; Ostman and Weaver, 1982; Sanchez-Alonso and Lachica, 1987), including autumn olive in experiment 1. This rapid decrease in foliar N concentration is usually associated with a rapid breakdown of RuBP carboxylase/oxygenase and other N-containing substances in the leaves (Titus and Kang, 1982). The decrease in foliar N concentration of nodulated alders was greatest in seedlings grown under the low N-fertilization regime. N_2 fixation probably accounted for more of the plant N-uptake in the low N treatment because initial rates of acetylene reduction in September were more than twice as high as rates of alders receiving high levels of N (Fig. 5). The rate of acetylene reduction decreased 82% for seedlings receiving low levels of N-fertilization and 89% for seedlings receiving higher levels of N fertilizer after the first frost. Foliar N in nodulated alders also declined more rapidly after the first frost. Foliar N concentration did not decrease in non-nodulated alders suggesting that the input of N from the roots to the leaves as well as the rate of breakdown and output of N-containing substances from the leaves remained balanced. Therefore, the decrease in foliar N concentration that occurred in nodulated alders could be associated with a decreasing N input from N_2 fixation to the leaves relative to the rate of breakdown and export of N-containing substances from the leaves.

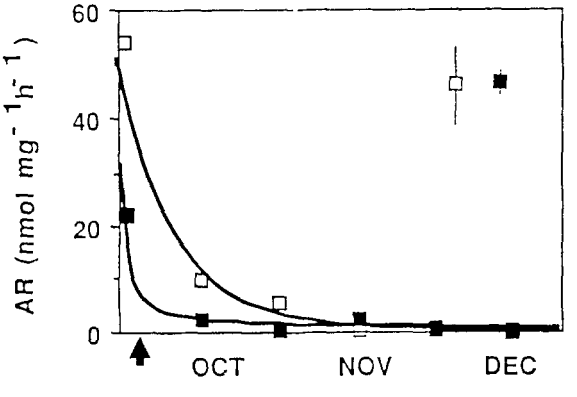

Fig. 5. Changes in nitrogenase activity (acetylene reduction [AR] in nmol ethylene per dry mg of nodule) of *Frankia*-nodulated black alder under low (□) and high (■) fertilization regime. Samples were assayed every two weeks from September 29, 1987 until leaf senescence. First frost occurred on Oct. 4, as indicated by the arrow. Vertical bars represent least significant differences at $P = 0.05$.

At the end of October, nodule-free black alder receiving a low level of fertilization averaged 46 ± 4 cm in height compared to 79 ± 11 cm for nodulated alder under the same regime of fertilization. The low N-fertilization regime probably limited eastern cottonwood growth as well, since their foliar N concentrations were below the limiting N levels determined by Bernier (1984) for *Populus* spp. N-deficient eastern cottonwood continuously dropped lower yellow leaves throughout the summer. In contrast, lower leaves of N-deficient black alder did not yellow during the summer nor did they drop prior to autumn. This suggests that black alder is less able than cottonwood to internally reallocate N from older leaves to young, expanding leaves. Non-nodulated alders under the low N-fertilization regime did, however, drop some leaves during the last month of senescence as shown by the decrease in total foliar N content per plant at the end of the sampling period (Fig. 6). The decline in foliar N content of N-deficient alders at the same time that total foliar N concentration increased indicates that the lower leaves that abscised were lower in N concentration than the remaining attached leaves. Some leaf N may have been translocated to other tissues in non-nodulated black alder during autumn, but the lack of net resorption of leaf N based on whole plant samples indicates that internal movement of N in alder is certainly less extensive than in eastern cottonwood. We conclude that the lack of N decrease in the leaves of black alder during the autumn is a characteristic of this species, and perhaps of the genus Alnus, since other species of alders maintain green

foliage longer and show less net translocation of foliar N than associated temperate-deciduous trees (Arno, 1973; Côté and Dawson, 1986; Dawson *et al.*, 1980; Hightshoe, 1978; Rodriguez-Barrueco *et al.*, 1984).

Nodulated black alder seedlings in this experiment showed a gradual decline in foliar N concentration (Fig. 4A, D) that was similar to the decline exhibited by black alders in the field (Côté and Dawson, 1986; Dawson and Funk, 1981). However, the decrease in foliar N concentration observed in nodulated alders receiving a low level of N fertilization was almost twice as large as that observed in the field. We believe that nitrogenase activity of nodulated black alder seedlings growing in small, non-insulated containers in our experiment was prematurely diminished by low temperatures compared to alders growing in the field. Temperatures below 2–8°C inhibit nitrogenase activity in alders (Huss-Danell *et al.*, 1987; Waughman, 1977; Winship and Tjepkema, 1985). We believe that nitrogenase activity of nodulated black alder seedlings in our experiment was prematurely and abruptly diminished by cold soil temperatures compared to alders growing in the field and resulted in the large decrease of foliar N concentration of nodulated alders under the low N-fertilization regime. Foliar N changes of alders grown in the field in Illinois' fertile soils (Dawson *et al.*, 1980) are similar to those observed in nodulated alders grown under the high N-fertilization regime. This suggests that their dependence on N_2 fixation is similarly proportional.

Nitrogen concentration changes in bark and roots of plants receiving low levels of fertilization followed similar patterns, with bark N concentration being consistently greater than root N concentration (Fig. 4A, B, C). In contrast, root N concentration of plants under the high fertilization regime became greater than bark N concentration after the first frost (Fig. 4D, E, F). Eastern cottonwood started to accumulate N in bark and roots before black alder and the high level of N fertilization initiated earlier accumulation of bark and root N. The accumulation of N in bark and roots was generally accelerated after the first frost. The more rapid increase of N concentration and content in roots than in bark of alder and cottonwood after the first frost (Figs. 4, 6) indicates that roots are an important N-storage tissue in seedlings of these two

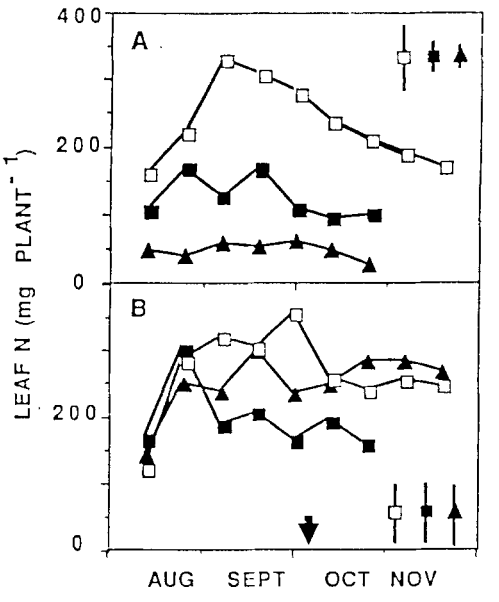

Fig. 6. Changes in leaf nitrogen content of *Frankia*-nodulated black alder (□), non-nodulated black alder (▲), and eastern cottonwood (■) under a low fertilization regime (**A**) and under a high fertilization regime (**B**). Samples were collected every two weeks from August 3, 1987 until leaf senescence. First frost occurred on Oct. 4, as indicated by the arrow. Vertical bars represent least significant differences at $P = 0.05$.

tree species. Black alder commonly exhibits little net N resorption during the fall. The increase in bark and root N was apparently due to a shift in importance of N sinks within the plant for root assimilated N. The overall demand for N for shoot growth after budset and for leaf growth and maintenance during leaf senescence certainly declined. Our experimental design did not allow us to evaluate specifically the contribution of foliar N resorption to the increase of N in bark and roots during autumn.

Foliar N content per seedling peaked in September before the first frost, except for cottonwoods receiving high levels of fertilization which peaked in foliar N content during August (Fig. 6). Foliar N content decreased rapidly in eastern cottonwood and non-nodulated black alder under the low fertilization regime, with decreases starting near the time of the first frost (Fig. 6A). Foliar N content of nodulated black alder receiving low levels of N started to decrease at the end of August, more than a month before the first frost; the decrease was gradual and coincided with the decrease in foliar N

concentration. Foliar N content of seedlings receiving a high level of N fertilization also declined gradually during the last six weeks of leaf senescence (Fig. 6B).

Results demonstrate that black alder seedlings lacking nodules do not exhibit net resorption of foliar N during autumn. Autumnal decreases in leaf N of *Frankia*-nodulated black alder seem to result primarily from declining N import from N_2 fixation to the leaves relative to export of foliar N-containing substances. In contrast, autumn olive has net resorption of 39–40% of foliar N during autumn in both fertile and infertile soils.

Acknowledgments

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