SEASONAL CHANGES IN LEAF NITROGEN CONCENTRATION OF *ALNUS GLUTINOSA*, *A. RUGOSA*, AND *A. SERRULATA*

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Abstract.--Foliage nitrogen concentration of three *Alnus* species [*A. glutinosa* (L.) Gaertn., *A. rugosa* (Du Roi) Spreng., and *A. serrulata* (Ait.) Willd.] was measured at intervals of 4 weeks from late May until October or November when the last leaves remained on the trees. *A. rugosa* foliage was from a native stand in Freeport Marsh near Decorah, Iowa. *A. glutinosa* samples were from a naturalized stand in Urbana, Illinois and a plantation near Ambeer Creek in southern Illinois. *A. serrulata* leaves were obtained from the Ambeer Creek plantation. Results indicate that the seasonal pattern of leaf nitrogen concentration was different for the alders sampled than that reported for other temperate deciduous trees. An autumnal decrease in leaf N concentration measured prior to leaf fall in these stands ranged from 0% to 33% of the summer N concentration compared with average reported values of from 33% to 66% for other temperate deciduous tree species. Alder trees, therefore, seem to conserve nitrogen less effectively and pass nitrogen from foliage to soil more directly than other deciduous trees, which translocate considerable portions of their leaf nitrogen back to twigs and branches prior to leaf fall each autumn. This is a reasonable result since alders fix atmospheric nitrogen symbiotically with nodule-forming actinomycetes of the genus *Frankia* and are independent of substrate nitrogen for growth. The native stand of *A. rugosa* exhibited an anomalous midseason decline in leaf N content that reversed itself later in the season. This decline was associated with an unusually wet period and a raised water table in Freeport Marsh suggesting that environmental factors also play a role in determining leaf nitrogen concentration.

Additional keywords: Alder, nitrogen conservation, leaf senescence.

INTRODUCTION

Alder trees of various species tend to lack synchronous leaf fall and retain green foliage until early winter (Arno 1973, Hightshoe 1978). The fact that the leaves remain green suggests that they may retain organic nutrients, especially nitrogenous compounds, up to the time of leaf fall.

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Most temperate deciduous trees translocate a large percentage of their total leaf nitrogen back into nearby twigs and branches before autumn leaf fall. Apple trees, for instance, lose 40% to 80% of their leaf nitrogen (based on percentage of dry weight) before the leaves fall or are killed by freezing temperatures (Murneek and Logan 1932, Oland 1963). In Finland, Viro (1965) noted an average leaf nitrogen loss (on a percentage of dry weight basis) of 62% between early August and late September in species of Betula, Populus, Salix, and Sorbus. Other reports of autumnal nitrogen translocation out of leaves range from one-third to two-thirds of their nitrogen content (Kramer and Kozlowski, 1979).

Retranslocated nitrogen in twigs and branches is readily available for foliage growth the following spring and is usually found to be present in higher concentrations in spring foliage of temperate deciduous trees than at other seasons (Thomas 1927, Tromp 1970).

By mobilizing leaf nitrogen in the fall and storing it in nearby twigs and branches for use by foliage the following spring, temperate deciduous trees conserve nitrogen which is more often than not deficient in forest and other soils. Leaf nitrogen conservation is important as well because, in some instances, leaves may contain over 40% of the total nitrogen content in broadleaved trees (Kramer and Kozlowski 1979).

However, alders are able to fix atmospheric nitrogen via root-nodule symbiosis with actinomycetes of the genus Frankia (Bond 1967). Thus, alders benefit less from mechanisms of nitrogen conservation and less selection pressure for efficient means to conserve nitrogen probably exists.

The objective of this study was to determine the seasonal patterns of variation in leaf nitrogen concentration of two native and one exotic species of Alnus common in the central hardwood region. Changes in leaf nitrogen concentration of alder prior to leaf fall were compared with reports of seasonal nitrogen flux in other temperate, deciduous trees.

ALDER SPECIES AND SITES

The species of alder chosen for this study are the two most common native alders and the single most commonly planted exotic alder of the United States east of the Great Plains. The native alders are Alnus rugosa (Du Roi) Spreng. (=A. incana ssp. rugosa, Furlow 1979) of the northeastern United States and A. serrulata (Ait.) Willd. of the southeastern United States. The exotic alder in this study is A. glutinosa (L.) Gaertn. These species are found in nature primarily along stream banks and in wetlands, though they also occur on drier sites. A. rugosa and A. serrulata are smaller than European alder and are of no commercial importance; however, they are of some value to wildlife as their shrubby habit, occurrence near water, and tendency to seed prolifically afford food and cover for many wildlife species. They are also probably important in fresh water ecosystems as a source of nitrogen. Unlike the common native alders of the eastern United States, A. glutinosa or European alder may achieve tree stature with stem diameters of 1m and heights of 30m attainable on the best sites. European alder is widely planted for mine spoil reclamation because it is tolerant of acid soils and fixes nitrogen symbiotically. Since European alder is nitrogen self-sufficient and is capable of

rapid growth rates, it is also of potential importance as a component of intensive sivicultural systems.

Trees included in this study were from a native stand of A. rugosa in northeastern Iowa, a naturalized stand of A. glutinosa in central Illinois, and plantings of A. glutinosa and A. serrulata in southern Illinois (Figure 1). The A. rugosa stand contained scattered, multiple-stemmed individuals

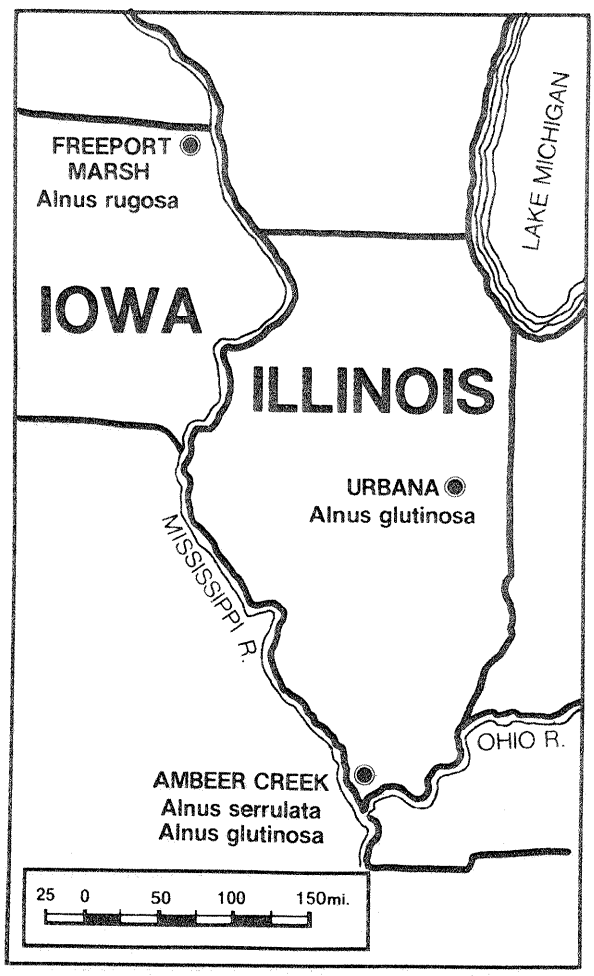

Figure 1.--Locations of alder study sites.

from 3m to 6m in height. All individual alders occurred within the several acres of Freeport Marsh near Decorah, Iowa. The soil of this marsh is a Caneek silt loam formed in light-colored silty alluvium. This soil is poorly drained, exhibiting grayish hues and mottles in the stratified material. The organic matter content of the soil averages 1.9% and the total nitrogen content is about .008g per 5g sample of dry soil. This soil contains large amounts of lime with soil pH values ranging from 7.1 to 7.4. The individual alder clumps consisted of many stems and sprouts of varying age. The central Illinois site is located in Crystal Lake Park, Urbana. The banks of Crystal Lake Park are populated by many European alders derived from a few parent trees planted near the lagoon more than 20 years ago. European alder produces large quantities of seed and does so as early as the fourth growing season (Funk 1965). For this reason and because European alder seeds contain air bladders that enable them to float, facilitating their dispersal by water, many alder trees of varying age and size have grown up along the banks of this park lagoon. The alluvial soil along the lagoon is a slowly permeable Colo silty clay loam with a very dark gray mottled subsoil indicating poor aeration. The pH of this soil ranges from 5.6 to 6.5. The organic matter content is 5% and the soil contains moderate amounts of nitrogen (.021g per 5g sample of dry soil). The trees from which foliage samples were taken varied from 6m to 15m in height and from 5 to 17 years in age. All trees at this location tended to produce single, straight main stems. The Ambeer Creek study site in southern Illinois is on U.S. Forest Service land near Diswood in Alexander County. Seedlings derived from local sources of native A. serrulata and A. glutinosa nursery stock of unknown seed origin were interplanted by rows with black walnut (Juglans nigra L.) in 1969. The overall spacing in these plantings is 8' x 12', with walnut spaced 8' x 12', 16' x 12' and 32' x 12', and alder planted in 0, 50, and 75 percent mixtures respectively. Most European alders in this plantation were fastigiate in form and had attained heights of approximately 12m in 9 years. The A. serrulata had developed a shrubby, multi-stemmed form over the same time period with heights of between 3 and 4 meters. The soil at the Ambeer Creek site is a Haymond silty loam underlain with coarse-textured material occurring at depths of 85 to 90cm. The soil thus has good internal drainage and is well aerated. The organic matter content of this soil averages 1.7% and the mean nitrogen content is .007g per 5g of dry soil. Soil pH values range from 5.2 to 5.5.

PROCEDURE

In order to determine seasonal changes in leaf nitrogen content, foliage sampling was begun at all sites during the last week in May or the first week in June of 1978. Sampling was repeated at 4-week intervals thereafter until leaf fall in the autumn. At each sample time and at all study sites branches were selected randomly with respect to the following: 1) azimuth of the branch with respect to the main stem or central vertical axis of the tree, 2) branch height, and 3) in larger trees with secondary branches greater than 1m in length, the distance of the secondary branch to be sampled from the main stem of the tree. All the foliage on each selected branch was separated by size class. A small size class included only young, expanding leaves with midveins less than 3cm in length. A medium size class consisted of leaves 3cm to 6cm in length along their midveins, and a large size class was limited to leaves with midveins greater than 6cm in length.

After collection, all leaf samples were promptly dried at 70°C for 24 hours. Then nitrogen contents, expressed as percentages of dry weight, were determined following microKjehldahl procedure B of Nelson and Sommers (1973).

In the planted alder stands at Ambeer Creek, which were uniform in height and age, 7 different trees of both A. glutinosa and A. serrulata were randomly selected every 4 weeks for foliage sampling. The trees were selected randomly without replacement so that no tree could be selected more than once. Nitrogen determinations for foliage samples from Ambeer Creek A. glutinosa trees were limited to 1 branch per tree for the 7 trees sampled at each sample time. This yielded 21 determinations at each of 7 sample times totalling 147 nitrogen determinations. The A. serrulata foliage samples from all branches and trees were pooled for each of 7 sample times so that only 21 sample determinations were made. Because of greater height and age variation of trees within stands of A. rugosa at Decorah and A. glutinosa at Urbana, 5 trees in each stand were randomly selected at the beginning of the sampling in 1978 and their foliage was repeatedly sampled throughout the growing season. The size of the trees was great enough and the size of the foliage sample was small enough that no noticeable effect on subsequent growth or vigor would be likely. For the A. rugosa foliage, leaf samples for each tree were combined. With 5 trees repeatedly sampled at each of 5 sample periods in the northern most study site, there were 15 nitrogen determinations per sample time and a total of 75 determinations. Foliage samples from the Urbana A. glutinosa site were taken at 7 sample times during 1978, with 5 trees repeatedly sampled at each sample time, 5 branches sampled per tree, and 3 leaf size-classes per branch for a total of 525 nitrogen determinations.

Because of differences in sampling procedure and the pooling of foliage samples the nitrogen content data from the 4 combinations of species and sites were treated statistically as separate experiments. The data on nitrogen contents from the A. glutinosa stand in Urbana and the A. rugosa stand were analyzed separately using a blocked, two-way analysis of variance with trees as blocks. The Ambeer Creek data for the A. glutinosa stand was analyzed using a two-way ANOVA with a time x leaf interaction term. In the case of the Ambeer Creek A. serrulata data, in which the greatest number of leaf samples were pooled, the assumption that there was no time x leaf interaction was made and the data analyzed with a two-way ANOVA. Statistical procedures used are detailed in Cochran and Cox (1967).

All trees included in the study were checked for the presence of root nodules by partially excavating the root system near the root collar. A piece of root containing nodules was detached from each tree and immediately assayed for nitrogenase activity using root incubation techniques described in Dawson and Gordon (1979) and a portable gas chromatograph for the acetylene reduction assay (Mallard et al. 1977).

RESULTS

Results of the Urbana A. glutinosa foliage nitrogen data analysis indicate that seasonal effects (sample times), leaf-class effects, and their interactions all contributed significantly to the total variation (α=.01). The leaf nitrogen concentrations were high in the spring and remained fairly con-

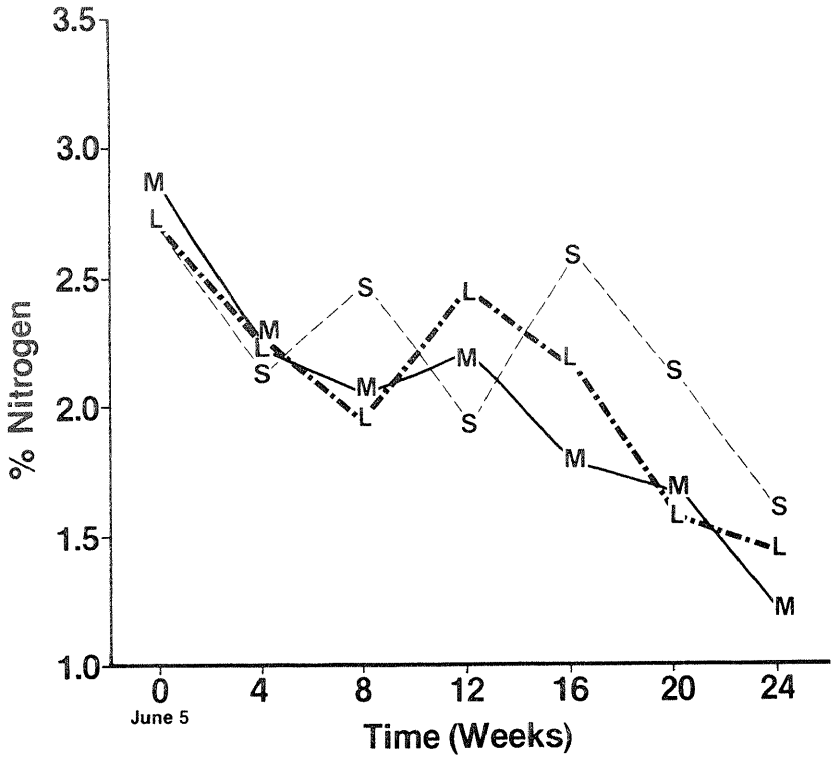

Figure 2.--Seasonal changes in mean nitrogen concentration of Alnus glutinosa foliage from a naturalized stand in Urbana, Illinois. S = leaves with midveins less than 3cm in length. M = leaves with midveins from 3-6 cm in length. L = leaves with midveins greater than 6cm in length. Nitrogen concentration is expressed as the percentage of dry weight.

stant during the summer months (Figure 2). During autumn up to the time of leaf fall, the leaf nitrogen concentrations decreased to about 2/3 of their midsummer average. The significant interaction between time of season and leaf size is attributable mainly to the fact that the small, slowly-expanding alder leaves had consistently higher leaf nitrogen concentrations than the larger leaves during autumn. The early August sample time at week 8 also showed higher leaf nitrogen concentrations in the small leaves compared to the larger leaves.

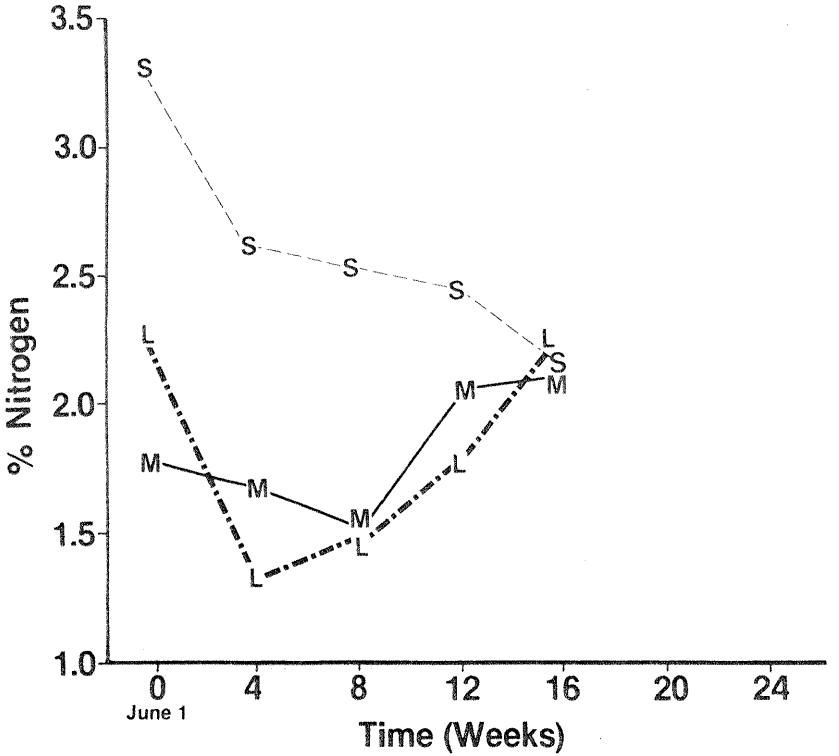

Figure 3.--Seasonal changes in the mean nitrogen concentration of Alnus rugosa foliage from a native stand at Freeport Marsh in Iowa.

The A. rugosa stand seemed to respond to a midsummer elevation in the soil water table with a concurrent decrease in leaf nitrogen concentration (Figure 3). A noticeable drying occurred after midsummer at Freeport Marsh so that July's flooded and unusually moist soil surface became gradually drier and firm. Results of statistical analysis indicate that seasonal effects, leaf-class effects, and their interaction contributed significantly to the total nitrogen concentration variation (α=.01). In this case the significant interaction is due to the fact that the nitrogen concentration in small, expanding leaves did not decrease to the extent that the nitrogen concentration decreased in larger leaves during the midsummer wet period when the water table at the marsh became elevated.

Trees in the Ambeer Creek plantation of A. glutinosa behaved unlike most deciduous trees in that the foliage leaf nitrogen concentration was low in the spring, rose, and remained stable throughout the summer with very little

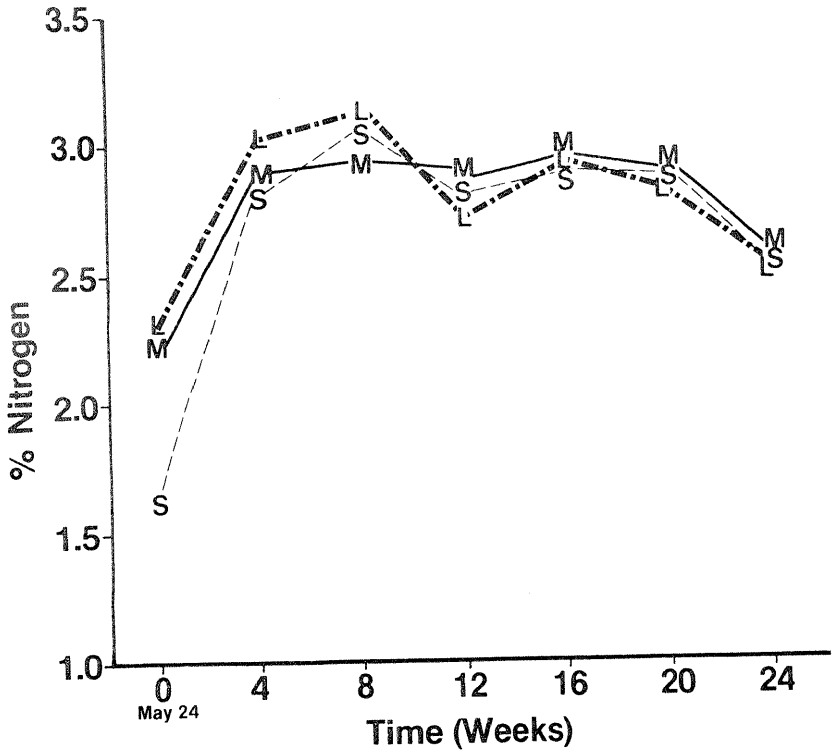

Figure 4.--Seasonal changes in the mean nitrogen concentration of Alnus glutinosa foliage from a plantation at Ambeer Creek in southern Illinois.

decrease during autumn (Figure 4). Results of statistical analysis indicate that there was a significant seasonal effect, but that there were no significant effects for either leaf size class or time x leaf interaction (α=.01). The Ambeer Creek site affords better soil aeration, a low soil nitrogen content, a longer, warmer growing season, and greater annual precipitation than the other sample sites. These conditions are ideal for symbiotic nitrogen fixation in alder root nodules. From late June until November, leaf nitrogen concentrations remained at higher levels when compared with other sites and species, and decreased very little during the autumn.

The A. serrulata stand at Ambeer Creek (Figure 5) exhibited no significant seasonal changes in leaf nitrogen concentration whatsoever.

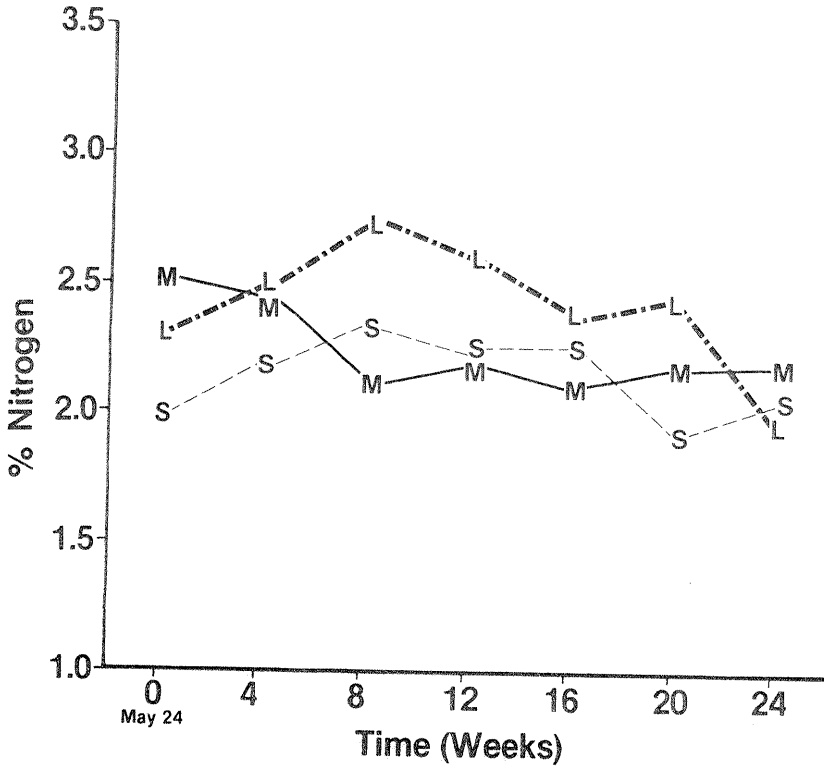

Figure 5.--Seasonal changes in the mean nitrogen concentration of Alnus serrulata foliage from a plantation at Ambeer Creek in southern Illinois.

All alders sampled had root nodules that were effective in reducing acetylene to ethylene, indicating nitrogenase (nitrogen-fixing) activity. Some individual nodules were greater than 5cm in diameter.

DISCUSSION

In our study, only the A. glutinosa stand in Urbana (Figure 2) fit the general seasonal pattern of leaf nitrogen concentrations for temperate deciduous trees. However, the autumnal decrease in nitrogen concentration was about 33% and is low in comparison to similar reports on other temperate zone deciduous species (Murneek and Logan 1932, Oland 1963, Viro 1965, Kramer and Kozlowski 1979). The A. glutinosa stand at the Ambeer Creek site (Figure 4) had a dissimilar pattern with low nitrogen concentrations in the spring foliage and a decrease of about 16% in leaf nitrogen concentration from midsummer to November. Red alder (A. rubra Bong.) has, in one instance, exhibited a seasonal pattern of leaf nitrogen concentrations similar to that of A. glutinosa from the Ambeer Creek site (Tripp et al. 1979). No seasonal change whatsoever was observed in the foliage of A. serrulata at the Ambeer Creek site (Figure 5). A. rugosa foliage nitrogen concentration seemed to be in-

fluenced by a midsummer raising of the marsh water table, which coincided with the lowest average leaf nitrogen concentration (Figure 3). During July, the weather station at Decorah, Iowa recorded 190mm of precipitation, which was 72mm above normal levels. At this time the marsh water level was high and much of the alder rhizosphere soil was saturated. The oxygen concentration may be reduced in clay soils or water logged soils, resulting in substantial inhibition of nitrogen fixation in nodulated plants (Pate 1976, Sprent 1976). Reduced rates of nodular nitrogen fixation due to water logging may have resulted in the observed decrease in midsummer foliage nitrogen concentration at Freeport Marsh. Small, expanding leaves decreased less in nitrogen concentration than did the larger leaves, exhibiting a nitrogen sink effect at the time of root flooding.

We speculate that high levels of tissue nitrogen in alders, due to symbiotic nitrogen fixation, may in some way directly or indirectly interfere with the processes of autumnal leaf senescence usually observed in temperate deciduous trees. Cytokinin levels in plants that are well supplied with useable forms of nitrogen are higher than in plants supplied with low levels of nitrogen (Buban et al. 1978, Salama and Wareing 1979). Cytokinins, which originate in plant roots, have been demonstrated to delay senescence in the leaves of many plant species (Bonner and Varner 1976). High cytokinin levels have been described in A. glutinosa nodules, and these levels in mature trees remain fairly constant throughout most of the year, showing a large increase only near the time of bud burst (Henson and Wheeler, 1977). The gross effect of high nitrogen levels in plant tissues has long been noted by nursery managers who commonly refrain from nitrogen fertilization in late summer and early fall to avoid delays in the winter hardening of tree seedlings.

We cannot discount the possibility that genetic factors may, in a way unrelated to nitrogen concentration, determine how alders respond to decreasing temperature and day length in autumn. In other deciduous trees these environmental changes result in the mobilization and export of organic nutrients, including nitrogen, from leaves to branches prior to leaf abscission (Kramer and Kozlowski 1979). Since alders are symbiotic nitrogen fixers, there is little advantage to alder trees in conserving nitrogen in this manner, and there may have been little genetic selection pressure in alders for efficient means to trigger an autumnal nitrogen mobilization.

CONCLUSIONS

Three species of alder occurring in Iowa and Illinois did not lose foliage nitrogen in the autumn to the extent recorded for other deciduous broadleaved trees. Nitrogen retention in their autumnal foliage may help to explain why alder litter is found by researchers such as Mikola (1958) to be rich in nitrogenous compounds.

Soil moisture seemed to influence leaf nitrogen concentrations in a native stand of A. rugosa. When surface soil became waterlogged leaf nitrogen concentrations declined, though the small, expanding leaves had a significantly smaller decrease than larger leaves.

Expanded seasonal studies that include stem and branch nitrogen concentration determinations, an accounting for foliage nitrogen leaching, litter

nitrogen determinations, and determination of endogenous levels of cytokinin in root, leaf and nodular tissue would go much further toward obtaining an understanding of the aboveground dynamics of nitrogen in alder stands. Precise measurements of soil moisture availability and nodule nitrogenase activities during the season should also be included in future studies for comparison with aboveground nitrogen changes.

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LITERATURE CITED

Arno, S. F. 1973. Discovering Sierra trees. U. S. National Park Service, Yosemite National Park. p. 61.

Bond, G. 1967. Fixation of nitrogen by higher plants other than legumes. Annu. Rev. Plant Physiol. 18: 107-126.

Bonner, J. and J. E. Varner. 1976. Plant biochemistry. p. 727. Academic Press, New York.

Buban, T., A. Varga, J. Tromp, E. Knegt, and J. Bruinsma. 1978. Effects of ammonium and nitrate nutrition on the levels of zeatin and amino nitrogen in xylem sap of apple rootstalks. Z. Pflanzen Physiol. 89: 289-295.

Cochran, W. G. and G. M. Cox. 1967. Experimental designs. Second Edition. John Wiley and Sons, Inc. New York.

Dawson, J. O. and J. C. Gordon. 1979. Nitrogen fixation in relation to photosynthesis in Alnus glutinosa. Botanical Gazette 140(Suppl.): 70-75.

Funk, D. T. 1965. Silvics of European alder. Central States Forest Experiment Station, Columbus, Ohio. 20 p.

Furlow, J. J. 1979. The systematics of the American species of Alnus (Betulaceae). Rhodora 81: 1-121.

Henson, I. E. and C. T. Wheeler. 1977. Hormones in plants bearing nitrogen-fixing root nodules: distribution and seasonal changes in levels of cytokinins in Alnus glutinosa (L.) Gaertn. J. Exp. Bot. 28: 205-214.

Hightshoe, G. L. 1978. Native trees for urban and rural America. p. 30. Iowa State University Press, Ames, Iowa.

Kramer, P. J. and T. T. Kozlowski. 1979. Physiology of woody plants. p. 305 and p. 313. Academic Press, New York.

Mallard, T. M., C. S. Mallard, H. S. Holfeld and T. A. LaRue. 1977. Portable gas chromatograph for the acetylene reduction assay for nitrogenase. Analytical Chemistry 49: 1275-1277.

Mikola, P. 1958. Liberation of nitrogen from alder leaf litter. Acta Forest. Fenn. 67: 1-10.

Murneek, A. E. and J. C. Logan. 1932. Autumnal migration of nitrogen and carbohydrate in the apple tree. Mo. Univ. Agr. Exp. Sta. Res. Bull. 171.

Nelson, D. W. and L. E. Sommers. 1973. Determination of total nitrogen in plant material. Agron. J. 65: 109-112.

Oland, K. 1963. Changes in the content of dry matter and major nutrient elements of apple foliage during senescence and abscission. Physiol. Plant. 16: 682-697.

Pate, J. S. 1976. Physiology of the reaction of nodulated legumes to environment. In "Symbiotic Nitrogen Fixation in Plants" (P. S. Nutman, ed.), pp. 335-360. Cambridge Univ. Press, Cambridge.

Salama, A. M. S. El-D. A. and P. F. Wareing. 1979. Effects of mineral nutrition on endogenous cytokinins in plants of sunflower (Helianthus anuus L.). J. Exp. Bot. 30: 971-981.

Sprent, J. I. 1976. Nitrogen fixation by legumes subjected to water and light stresses. In "Symbiotic Nitrogen Fixation in Plants" (P. S. Nutman, ed.), pp. 405-420. Cambridge Univ. Press, Cambridge.

Thomas, W. 1927. Nitrogenous metabolism of Pyrus malus. III. The partition of nitrogen in the leaves, one- and two-year branch growth and non-bearing spurs throughout a year's cycle. Plant Physiol. 2: 109-137.

Tripp, L. N., D. F. Bezdicek and P. E. Heilman. 1979. Seasonal and diurnal patterns and rates of nitrogen fixation by young red alder. Forest Sci. 25: 371-380.

Tromp, J. 1970. Storage and mobilization of nitrogenous compounds in apple trees with special reference to arginine. In "Physiology of Tree Crops" (L. C. Luckwill and C. V. Cutting, eds.), pp. 143-159. Academic Press, London.

Viro, P. J. 1956. Investigations of forest litter. Communicationes Instituti Forestalis Fenniae 45.6, p. 15 and p. 17.