SEASONAL LEAF NITRATE REDUCTASE ACTIVITY, NITROGEN AND NITRATE CONTENT, AND WATER RELATIONS OF *ATRIPLEX CANESCENS* IN THE NORTHERN CHIHUAHUAN DESERT

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ABSTRACT: Seasonal nitrate reductase (NR) activity of young, uppermost leaves of *Atriplex canescens* (Pursh) Nutt. (diploid genotype) growing *in situ* was maximal during reproductive growth. Because precipitation on the study site coincided with reproductive growth, higher NR activity during this period may have been due to new leaf growth and increased soil NO_3^- availability rather than the nitrogen sink in developing seeds. Seasonal leaf water content (percent) was significantly ($P<0.10$) correlated with NR activity. Stem xylem water potentials were not correlated with NR activity. Although thermal adaptation of NR would, in general, be advantageous for plants inhabiting a desert environment with widely fluctuating seasonal temperatures, there was no evidence to suggest that acclimation of NR occurs in *A. canescens* growing *in situ*.

INTRODUCTION

Perennial plants inhabiting the northern Chihuahuan desert are exposed to extended periods of drought, wide fluctuations in seasonal temperatures, and high solar insolation. These factors are intimately involved in regulating nitrate reductase (NR) activity. High temperatures rapidly inactivate NR (Onweunne and others 1971; Mattas and Pauli 1965), and water stress depresses NR activity through reduced synthesis of the enzyme (Morilla and others 1973). Irradiation is involved in both the synthesis (Hagemen and Flesher 1960) and maintenance (Klepper and others 1971) of NR. Of particular importance in a desert environment is the availability of soil NO_3^-, and its uptake and translocation to NR sites in canopy leaves. Since NR induction occurs in the presence of NO_3^-, the seasonal balance between leaf NR activity and NO_3^- levels may be critical in establishing reduced nitrogen concentrations of *Atriplex canescens* (Pursh) Nutt. plants during prolonged dry periods *in situ*. Optimal NR activity may occur only during a rather brief period following rainfall when new leaf growth is initiated and when more optimal soil- and plant-water relations permit increased leaf NO_3^- levels for NR induction.

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Harmer and Lee (1981) suggested that NR acclimates to low temperatures. It was not, however, evident whether their data support acclimation or, instead, an inhibition of NR under the colder growing temperature, thus leading to NO_3^- accumulation. The initial objective of the present study was to determine if NR is capable of thermal adaptation in *A. canescens* growing *in situ*. The second objective was to determine the seasonal NR activity, NO_3^- content, and reduced nitrogen levels of *A. canescens* growing *in situ* relative to leaf-water relations. A substantial increase in NR activity has been observed during the post-flowering reproductive growth period in *Phaseolus vulgaris* L. (Franco and others 1979) and a decline in NR activity occurred throughout a comparable period in *Glycine max* L. (Franco 1977; Harper and Hageman 1972) where there was no water stress. The final objective was to examine NR activity during reproductive growth *in situ* where soil water is often limited.

METHODS

Leaf samples (ca. 5 grams) from mature fourwing saltbush (*Atriplex canescens* [Pursh] Nutt.) plants growing *in situ* near Las Cruces, N. Mex. (diploid [2n=18] ca. 32° 37' N 106° 45' W were collected at approximately 25 day intervals from August 1981 through November 1982 for NR activity assays and nitrogen and NO_3^- content determinations. All samples were collected within 1.5 hours prior to solar noon except for September 25 and 26, 1981, when samples were collected prior to sunrise, at approximately solar noon, and 4.5 hours after solar noon. Sample collection days were cloudless except for the partly cloudy day of September 11, 1981. Young, fully expanded leaves located on the south side and top of the plant canopy were collected for subsequent analyses. The same four to six plants were used throughout the study period. The samples were transported to the laboratory in the dark at 32° F (0° C) for immediate NR activity analysis. Leaf water content was determined on three subsamples by drying the tissue for approximately 24 hours at 176° F (80° C). The dried samples were stored for subsequent determinations of nitrogen and NO_3^- content. Stem xylem pressure potentials were measured with a PMS pressure bomb. Precipitation received during the study period and ambient air temperatures were compiled from a U.S. Weather Bureau station located within 33 feet (10 meters) of the study site. NR activity and NO_3^- content were determined on three subsamples of leaf material from the field samples. Total

leaf nitrogen was determined on three subsamples of a composite sample. To assay *in vitro* NR activity, leaf material (ca. 0.5 grams fresh weight per sample) was ground cold in a mortar with 5 ml extraction medium and 3 grams washed quartz sand. The grinding medium consisted of 25 mM K_2HPO_4 at pH 7.5, 5 mM EDTA·Na_2, 10 mM 2-mercaptoethanol and 3 percent (W/V) BSA, the last added to stabilize enzyme activity (Schrader and others 1974). After centrifugation at 30 000 g for 15 minutes, the supernatant crude extract was assayed for NR activity within 1 hour. The assay procedure was essentially that of Hageman and Hucklesby (1971), with the zinc acetate/phenazine methosulfate modification of Scholl and others (1974).

Oven dried leaf material was ground to 40-mesh for determination of nitrogen and NO_3^- content. Two tissue samples from each of the subsamples were digested (block digestor) for nitrogen determinations with 20:1 H_2SO_4:H_3PO_4 in the presence of 200:1 K_2SO_4:Se catalyst mixture. Nitrogen content was determined from an aliquot of the diluted digest, using the colorimetric method involving the reaction of ammonium with sodium salicylate, sodium nitroprusside, and sodium hypochlorite with absorption readings at 660 nm (Technicon Industrial Systems, Industrial Method No. 334-74W/B$^+$, 1977). Nitrogen content was calculated using standard NH_4Cl concentrations carried through the digestion and colorimetric procedure. Nitrate content was determined by the method of Cataldo and others (1975).

RESULTS

Diurnal NO_3^- Levels and NR Activity

Maximum NR activity occurred at solar noon and minimal activity occurred 4.5 h after solar noon (table 1). Leaf NO_3^- levels were highest prior to sunrise. These data suggest a diurnal NR activity rhythm, a pattern consistent with other findings (Bowerman and Goodman 1971; Lewis and others 1982). Diurnal NR activity and residual NO_3^- levels indicate considerable NO_3^- was translocated into the leaf tissue during the photoperiod.

Table 1.--Diurnal *in vitro* leaf nitrate reductase (NR) activity and NO_3^--N levels within the uppermost, young leaves of *Atriplex canescens* (Pursh) Nutt. growing *in situ* on September 25 (approximately solar noon) and September 26 (prior to sunrise and approximately 4.5 hours after solar noon), 1981. Values in parentheses represent ± 1 standard error of the means.

	Before sunrise	Solar noon	4.5 hrs past noon
NR activity (μmol $NO_2^-\cdot gDW^{-1}\cdot h^{-1}$)	4.71 (1.68)	8.61 (2.29)	2.95 (1.40)
NO_3^--N (mg $NO_3^-N\cdot gDW^{-1}$)	0.94 (0.01)	0.76 (0.15)	0.77 (0.05)

Seasonal NR Activity

Maximum leaf NR activity occurred during the initial sampling date of August 1981 (fig. 1A). At this time, mature fruits were present on the plants sampled, although a large proportion of the fruits had already abscised. NR activity decreased after this initial sampling date to a reasonably stable rate (approximately 6 μmol $NO_2^-\cdot gDW^{-1}\cdot h^{-1}$) that persisted through the winter to early June. Increased NR activity occurred in mid-June and coincided with the onset of reproductive growth and summer rainfall (fig. 1D). This increase in NR activity continued throughout the reproductive growth period (June through November). Mature fruits were abscising during the last sampling date of November 3, 1982. The high NR activity found during the initial sampling date in August 1981, was not evident during reproductive growth in 1982. NR activity has been shown to fluctuate in response to leaf water status in several plants (Beevers and Hageman 1980), and in *A. confertifolia* irrigated with various levels of NaCl (Kleinkopf 1975). In the present study, activity of NR and leaf water content (fig. 1C) followed similar seasonal trends and were significantly correlated ($P<0.05$; $r=0.47$). Seasonal xylem water potentials of the terminal portion (4 to 6 inches/10 to 15 cm) of stems bearing leaves representative of those assayed for NR activity were not, however, significantly correlated ($P<0.10$) with leaf NR activity.

Maximum NO_3^- levels (fig. 1A) coincided with minimal NR activity (fig. 1A) and low leaf water content (fig. 1C) during the winter. Similar increases in leaf NO_3^- concentrations have been associated with the inhibition of NR activity by leaf water stress resulting in an accumulation of the NR substrate, NO_3^- (Plaut 1973; Srivastava 1980). Leaf NO_3^- levels and NR activity followed similar trends for the remainder of the study. Precipitation during the latter period (fig. 1D) would tend to enhance root growth, and thus NO_3^- uptake and its translocation to leaves. Similarly, more favorable soil water relations following precipitation would enhance leaf water relations (fig. 1C) for efficient NO_3^- reduction by NR (Kleinkopf and others 1975). Total reduced nitrogen varied between 38 (May 1982) and 25 mg $N\cdot gDW^{-1}$ (November 1982) (fig. 1B).

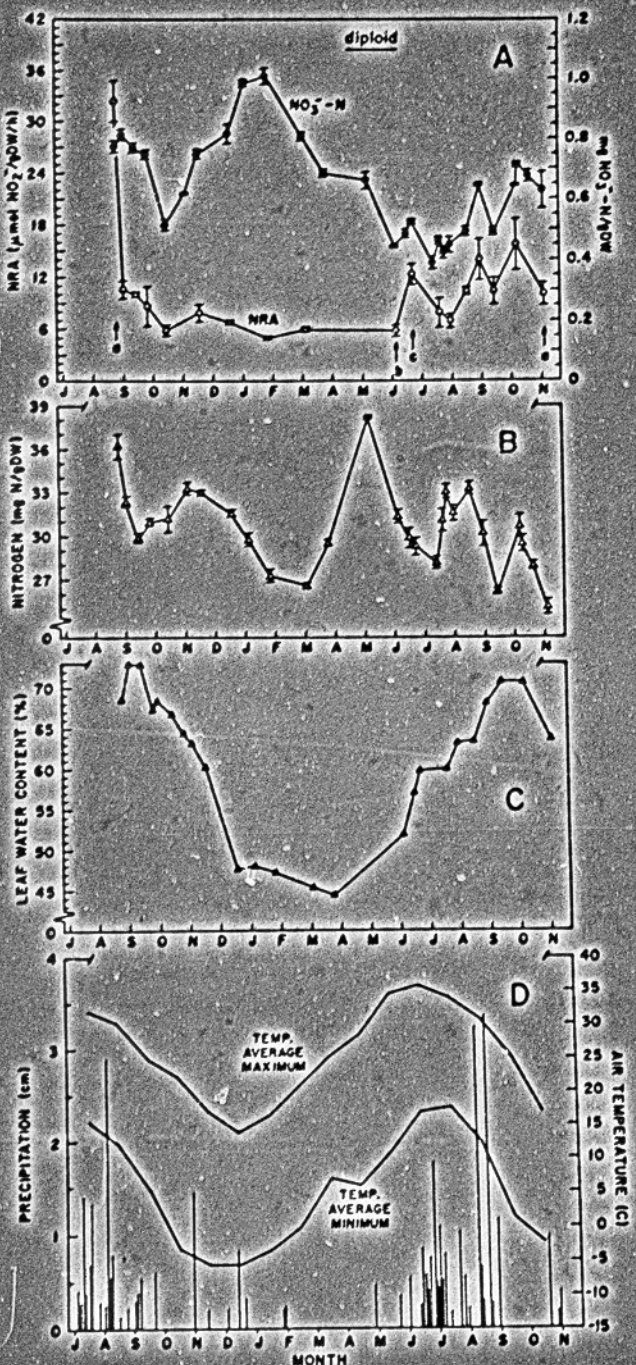

Figure 1.--Seasonal leaf nitrate reductase (NR) activity and leaf NO_3^--N (A), nitrogen (B), and leaf water content (percent) (C) of the diploid genotype of Atriplex canescens (Pursh) Nutt. growing in situ. Vertical bars represent ± 1 standard error of the means. Reproductive growth stages are indicated in A by arrows (a - mature fruit present and some fruit abscission; b - first observation of flowering; c - fruit growth initiated). Monthly ambient average high and low temperatures and precipitation (D) approximately 33 feet (10 meters) from the study site from August 1981 through October 1982.

Leaf N content declined 35 percent during the initial 7-month period when leaf water content and NR activity substantially declined. Thereafter, a discernible seasonal trend in nitrogen levels was not evident.

The potential for thermal adaptation of NR to seasonal fluctuations in ambient temperature (fig. 1D) was determined on leaf samples collected from October 1981 through October 1982 (fig. 2). Maximum NR activity occurred at approximately 86° to 95° F (30° to 35° C) assay temperature, regardless of the sampling date. NR activity at 86° to 95° F (30° and 35° C) differed significantly ($P<0.05$; NR activity at 86° F > 95° F; 30° C > 35° C) only on July 22, 1982. Therefore, the ability of NR to acclimate to seasonal ambient temperature fluctuations was not apparent in the present study.

The thermal stability of NR was determined at 5-minute intervals for 20 minutes at 68°, 86°, and 104° F (20°, 30°, and 40° C) to determine if NR activity was stable throughout the incubation period (15 minutes) during routine assays (fig. 2, insert). NR activity remained fairly constant throughout this 20-minute period at 68° and 86° F (20° and 30° C), but decreased approximately 35 percent at 104° F (40° C). Thus, NR activity depicted in figure 2 at 104° and 113° (40° and 45° C) (and perhaps 95° F (35° C)) probably represents nonstable rates and is a function of the rate of thermal inactivation during the incubation period of routine NR activity assays.

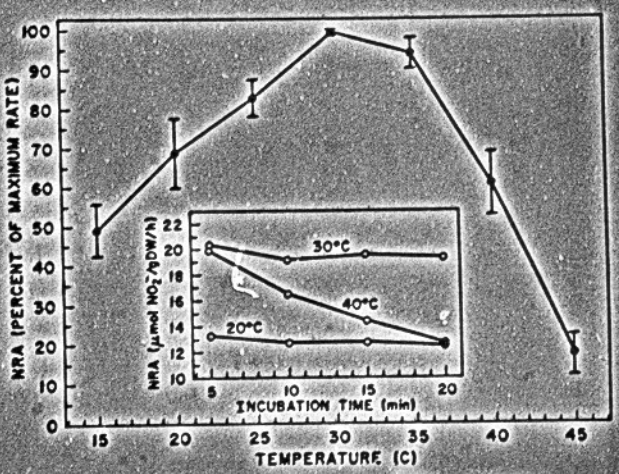

Figure 2.--Nitrate reductase (NR) activity response to temperature 59° to 113° F; 15° to 45° C) for Atriplex canescens (Pursh) Nutt. growing in situ from October 1981 through October 1982. Data from all analyses (10/20/81, 11/3/81, 1/14/82, 3/24/82, 5/6/82, 6/15/82, 7/22/82 and 10/8/82) were combined and are shown. Vertical bars represent 95 percent confidence intervals. Thermal stability of NR activity within the young, uppermost leaves during October 19, 1982, at 68°, 86°, and 104° F (20°, 30°, and 40° C) is shown in the insert.

DISCUSSION

Throughout this study, an attempt was made to collect random, but uniformly young, leaves from the south side and top of plant canopies. Therefore, it would be reasonable to assume that seasonal leaf samples were not representative of a single age group or include sequentially older leaves as the study progressed. The age composition of the leaves present, and hence leaf samples collected, varied throughout the year in response to leaf aging, abscission, and the initiation of new leaf growth. Thus, results of the present study are representative of the youngest, most physiologically active leaves present at any given time, where nitrogen assimilation primarily occurs (Srivastava 1980).

The effect of leaf age was readily demonstrated on August 25, 1981, when the older leaves of plants growing *in situ* possessed NR activity equivalent to 3 percent of that of younger leaves. Leaf water content of the older leaves was approximately 6 percent less than that of the younger leaves.

Seasonal leaf NR activity of *A. canescens* growing *in situ* was significantly correlated ($P<0.10$) with leaf water content, consistent with results from other studies (Huffaker and others 1970; Morilla and others 1973; Tischler and others 1978). On a seasonal basis, stem xylem water potentials were not correlated ($P<0.10$) with NR activity. During periods of low NR activity and leaf water content, NO_3^- accumulated in the leaves. Nitrate accumulation and lower NR activity during water stress have been attributed to a decline in the synthesis of NR (Morilla and others 1973). Shaner and Boyer (1976a, b) demonstrated that NR activity recovery after cessation of water stress was dependent upon protein synthesis and correlated with NO_3^- flux into the leaves rather than the leaf NO_3^- content. Thus, the controlling influence of optimal soil water content on NO_3^- availability and uptake, and the need for favorable leaf water content for efficient NR activity within plants growing *in situ*, is evident from the present study and others.

Reproductive growth during the post-flowering period resulted in substantial NR activity increases in *Phaseolus vulgaris* L. (Franco and others 1979). In contrast, a continuous decline in both NR activity and NO_3^- uptake occurred during this same period in soybean (*Glycine max* L.) (Franco 1977; Harper and Hageman 1972). In the present study, reproductive growth by *A. canescens* appeared to have little or no influence on leaf NR activity since low NR activity coincided with flowering (June 4, 1982). Rather, the enhanced levels of NR activity that occurred during reproductive growth were probably due to rainfall on the study site and new leaf growth. Hence, leaf NR activity coincident with reproductive growth of *A. canescens* growing *in situ* appears dependent upon favorable plant and soil water relations. These conditions would enhance soil NO_3^- availability and uptake, its translocation to leaves, and the production of new leaves. The wing-like bracts of the fruits possess the enzyme (2.1 μmol $NO_2^- \cdot gDW^{-1} \cdot h^{-1}$, September 1982) and thus, the primary site of nitrogen reduction for developing seeds may be within these bracts, independent of leaves.

There was no evidence in the present study suggesting that NR of *A. canescens* growing *in situ* is capable of thermal adaptation to seasonal ambient temperature fluctuations (fig. 2). Maximum NR activity occurred at assay temperatures of 86° to 95° F (30° to 35° C) throughout the year, though monthly average temperatures varied from a maximum of 97° F (36° C) in July to a minimum of 21° F (-6° C) in January. Harmer and Lee (1981) suggested acclimation of NR appeared evident in two grass species because NR activity increased after transferring the plants from a 68° F (20° C) growth temperature to one of 41° F (5° C) for 1 week. However, lack of pertinent associated data such as assay temperature, tissue NO_3^- levels, and particularly NR activity over a range of temperatures tends to obscure an interpretation concerning the occurrence of acclimation. For example, in the present study, NR activity at an assay temperature of 86° F (30° C) was 4.3 μmol $NO_2^- \cdot gDW^{-1} \cdot h^{-1}$ in October and 9.0 μmol $NO_2^- \cdot gDW^{-1} \cdot h^{-1}$ during November when ambient average daily temperatures were 59° and 48° F (15° and 9° C), respectively. Although higher NR activity occurred in leaves during the colder month of November, acclimation was not evident because maximum NR activity during both months occurred at 86° F (30° C) when assayed at temperatures between 59° and 113° F (15° and 45° C). As a general phenomenon, thermal acclimation of NR would be adaptive for evergreen plants inhabiting environments with large annual fluctuations in ambient temperature. However, there appears little evidence at the present time that NR is capable of such acclimation.

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PUBLICATIONS CITED

Beevers, L.; Hageman, R. H. Nitrate and nitrite reduction. In: Miflin, B.J., ed. The biochemistry of plants, Vol. 5, Amino acids and derivatives. [New York: Academic Press]; 1980: 116-159.

Bowerman, A.; Goodman, P.J. Variation in nitrate reductase activity in *Lolium*. Ann. Bot. 35: 353-366; 1971.

Cataldo, D.A.; Haroon, M.; Schrader, L.E.; Youngs, V.L. Rapid colorometric determination of nitrate in plant tissue by nitration of salicylic acid. Comm. Soil Sci. Plant Anal. 6: 71-80; 1975.

Franco, A.A. Nutritional constraints for tropical grain legume symbiosis. In: Vincent, J.M.; Whitney, A.S.; Bose, J.; eds. Exploiting the legume-rhizobium symbiosis in tropical agriculture; Misc. Publ. 145; College of Tropical Agriculture, University of Hawaii, Honolulu, HI. 1977: 237-252.

Franco, A.A.; Pereira, J.C.; Neyra, C.A. Seasonal patterns of nitrate reductase and nitrogenase activities in Phaseolus vulgaris L. Plant Physiol. 63: 421-424; 1979.

Hageman, R.H.; Flesher, D. Nitrate reductase activity in corn seedlings as affected by light and nitrate content of the nutrient media. Plant Physiol. 35: 700-708; 1960.

Hageman, R.H.; Hucklesby, D.P. Nitrate reductase from higher plants. Meth. Enzymol. 23: 491-503; 1971.

Harmer, R.; Lee, J.A. Some effects of temperature on nitrate reductase in upland and lowland populations of pasture grasses. Plant Sci. Lett. 21: 295-303; 1981.

Harper, J.E.; Hageman, R.H. Canopy and seasonal profiles of nitrate reductase in soybeans (Gycine max L. Merr.). Plant Physiol. 49: 146-154; 1972.

Huffaker, R.C.; Radin, T.; Kleinkopf, C.E.; Cox, E.L. Effects of mild water stress on enzymes of nitrate assimilation and of the carboxylative phase of photosynthesis in barley. Crop Sci. 10: 471-474; 1970.

Kleinkopf, G.E.; Wallace, A.; Cha, J.W. Sodium relations in desert plants: 4. Some physiological responses of Atriplex confertifolia to different levels of sodium chloride. Soil Sci. 120: 45-48; 1975.

Klepper, B.; Flesher, D.; Hageman, R.H. Generation of reduced nicotinamide adenine dinucleotide for nitrate reduction in green leaves. Plant Physiol. 48: 580-590; 1971.

Lewis, O.A.M.; Watson, E.F.; Hewitt, E.J. Determination of nitrate reductase activity in barley leaves and roots. Ann. Bot. 49: 31-37; 1982.

Mattas, R.E.; Pauli, A.W. Trends in nitrate reduction and nitrogen fractions in young corn (Zea mays L.) plants during heat and moisture stress. Crop Sci. 5: 181-184; 1965.

Morilla, C.A.; Boyer, J.S.; Hageman, R.H. Nitrate reductase activity and polyribosomal content of corn (Zea mays L.) having low water potentials. Plant Physiol. 51: 817-824; 1973.

Onwueme, I.C.; Laude, H.M.; Huffaker, R.C. Nitrate reductase activity in relation to heat stress in barley seedlings. Crop Sci. 11: 195-200; 1971.

Plaut, Z. The effect of soil moisture tension and nitrogen supply on nitrate reduction and accumulation in wheat seedlings. Plant Soil 38: 81-94; 1973.

Scholl, R.L.; Harper, J.E.; Hageman, R.H. Improvements of the nitrate color development in assays of nitrate reductase by phenazine methosulfate and zinc acetate. Plant Physiol. 53: 825-828; 1974.

Schrader, L.E.; Cataldo, D.A.; Peterson, D.M. Use of protein in extraction and stabilization of nitrate reductase. Plant Physiol. 53: 688-690; 1974.

Shaner, D.L.; Boyer, J.S. Nitrate reductase activity in maize (Zea mays L.) leaves. I. Regulation by nitrate flux. Plant Physiol. 58: 499-504; 1976a.

Shaner, D.L.; Boyer, J.S. Nitrate reductase activity in maize (Zea mays L.) leaves. II. Regulation by nitrate flux at low leaf water potential. Plant Physiol. 58: 505-509; 1976b.

Srivastava, H.S. Reglation of nitrate reductase activity in higher plants. Phytochem. 19: 725-733; 1980.

Tischler, C.R.; Parvis, A.C.; Jordan, W.R. Factors involved in in vitro stabilization of nitrate reductase from cotton (Gossypium hirsutum L.) cotyledons. Plant Physiol. 61: 714-717; 1978.

In: Tiedemann, Arthur R.; McArthur, E. Durant; Stutz, Howard C.; Stevens, Richard; Johnson, Kendall L., compilers. Proceedings--symposium on the biology of Atriplex and related chenopods; 1983 May 2-6; Provo, UT. General Technical Report INT-172. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station; 1984. 309 p.