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SEASONAL NITRATE REDUCTASE ACTIVITY OF THREE GENOTYPES OF *ATRIPLEX CANESCENS* IN THE NORTHERN CHIHUAHUAN DESERT

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SUMMARY

(1) Seasonal nitrate reductase (NR) activity of the young, uppermost leaves of three genotypes (diploid, tetraploid and hexaploid) of *Atriplex canescens* growing *in situ* showed maximal activity during reproductive growth. Because rainfall 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 an increased need for nitrogen due to developing seeds. The wing-like bracts of the immature fruits possessed NR and may have been a source of reduced nitrogen for seeds.

(2) Seasonal leaf water content was significantly correlated with NR activity in all genotypes, and water potentials in the stem xylem were correlated with NR activity only in the tetraploid plants.

(3) In a glasshouse the hexaploid plants had significantly higher NR activity and reduced-nitrogen concentrations than did either the diploid or tetraploid plants.

(4) Although thermal adaptation of NR would be advantageous for plants inhabiting a desert environment where seasonal temperatures fluctuate widely, 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 long droughts, wide fluctuations in seasonal temperature and high solar insolation. These factors are intimately involved in regulating nitrate reductase (NR) activity. High temperatures rapidly inactivate NR (Mattas & Pauli 1965; Onwueme, Laude & Huffaker 1971) and water deficiency depresses NR activity through reduced synthesis of the enzyme (Morilla, Boyer & Hageman 1973). Irradiation is involved in both the synthesis (Hageman & Flesher 1960) and maintenance (Klepper, Flesher & Hageman 1971) of NR. Of particular importance is the availability of soil NO_3^- and its uptake and translocation to the NR sites in the canopy leaves. Because NO_3^- induces NR synthesis, the seasonal balance between NR activity and NO_3^- concentrations in leaves may be critical in establishing reduced-nitrogen concentrations of *Atriplex canescens* plants in a desert. Optimal leaf NR activity may occur only during a brief period following rainfall when new leaf growth begins and when soil- and plant-water relations permit increased NO_3^- concentrations in leaves for NR induction.

Genotypic differences in NR activity and in the capacity to accumulate nitrogen have been demonstrated in several agronomic plants (Eilrich & Hageman 1973; Brunetti & Hageman 1976; Naik *et al.* 1982). In deserts, where soil nitrogen concentrations are generally low (Charley & West 1978) and nitrogen availability varies seasonally (Rychert

& Skujins 1979), different capacities to compete for and accumulate reduced-nitrogen would tend to segregate genotype populations of single plant species. Although *A. canescens* is uniformly distributed within the northern Chihuahuan Desert, there is a separation of its three genotypes (diploid, tetraploid and hexaploid) (Stutz & Sanderson 1979). In addition, diploid and tetraploid plants inhabit sandy soils while the hexaploid occurs mainly on the heavier, clay soils.

The initial objective of the present study was to determine if differences exist between the three genotypes with respect to leaf NR activity and total reduced-nitrogen concentrations in the leaves. The second objective was to quantify the seasonal changes in NR activity, NO_3^- content and reduced-nitrogen concentration of the three genotypes relative to phenological stage and leaf-water relations. The question of whether reproductive growth increases (Franco, Pereira & Neyra 1979), decreases (Harper & Hageman 1972; Franco 1977) or has no effect on NR activity in *A. canescens* was addressed. The final objective was to determine if NR of *A. canescens* growing *in situ* acclimates to seasonal fluctuation of temperature in the northern Chihuahuan Desert.

MATERIALS AND METHODS

Plant material

Mature plants of each genotype ($n = 9$) of *Atriplex canescens* (Pursh) Nalt. growing near Las Cruces, New Mexico, U.SA. were sampled at approximately 25-day intervals from August 1981 to the end of November 1982 from the following localities: diploid from 32°27′ N, 106°45′W; tetraploid from 32°22′N, 106°40′W; and hexaploid from 32°27′N, 106°50′W. Each sampling day was cloudless, except for 11 September 1981 which was partly clouded, and all samples were collected between 10.30 h and 12.00 h (local time). About 5 g of young, fully expanded leaves were collected from the south side of the top of the canopy of four to six plants of each genotype. The samples were transported to the laboratory in the dark at 0 °C for immediate NR-activity analysis. Leaf-water content was determined for each genotype by drying three samples of tissue for approximately 24 h at 80 °C. The sap-pressure-potentials of stem xylem were measured with a pressure chamber.

Precipitation and air temperatures were compiled from a U.S. Weather Bureau station within 10 m of the site of the diploid plants.

NR activity

To assay *in vitro* NR activity, leaf material (0·5 g fresh weight per sample) was ground cold in a mortar with 5 cm^3 of extraction medium and 3 g of washed quartz sand. The medium was added in 2-cm^3 portions for the initial grinding stages and 3-cm^3 portions for the final ones. 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% (weight per volume) BSA; the last was added to stabilize the enzyme activity (Schrader, Cataldo & Peterson 1974). After centrifuging at 30 000 ***g*** for 15 min, the supernatant crude extract was assayed for NR activity within 1 h.

The assay procedure was essentially that of Hageman & Hucklesby (1971), with the zinc acetate-phenazine methosulphate modification of Scholl, Harper & Hageman (1974). Reaction mixtures, 4 cm^3 final volume, contained 25 mM K_2HPO_4 at pH 7·5, 10 mM KNO_3, and 0·2 mM NADH (omitted for blanks). Added enzyme aliquots equivalent to 0·2 cm^3 crude extract contributed 1·25 mM K_2HPO_4, 0·25 mM EDTA, 0·5 mM 2-

mercaptoethanol and 0·15% BSA to the reaction mixture. The assays were run routinely at 25 °C for 15 min, stopped by the addition of 0·4 cm^3 0·6 M zinc acetate, and treated with 0·4 cm^3 of 1·5 mM phenazine methosulphate to oxidize the excess NADH. After 20 min, including centrifuging at 3000 ***g*** for 10 min, 4·8 cm^3 colour developer were added. This consisted of combined equal volumes of 1% (weight per volume) sulphanilamide in 1·6 N HCl and 0·02% (weight per volume) N-1-naphthylethylene-diamine diHCl. After at least 20 min for colour development, the absorbance was read at 540 nm and the amount of nitrite produced calculated using standard nitrite concentrations. Duplicate assays were made of each of the three replicate sample extractions for each genotype.

Nitrogen and nitrate analysis

Oven-dried leaf material was ground to 40-mesh for the determination of nitrogen and NO_3^-. Duplicates from each of three samples were digested (block digester) for nitrogen determinations with 20:1 H_2SO_4:H_3PO_4 in the presence of 200:1 K_2SO_4:Se catalyst mixture. The nitrogen content was determined from an aliquot of the diluted digest; a colorimetric method involving the reaction of ammonium with sodium salicylate, sodium nitroprusside and sodium hypochlorite was used with absorption measured at 660 nm (Technicon Industrial Systems, Industrial Method 334–74W/B^+, 1977). The nitrogen content was calculated using standard NH_4Cl concentrations carried through the digest and colorimetric procedure. The NO_3^- content was determined by the method of Cataldo *et al.* (1975).

RESULTS

Seasonal NR activity

Diploid plants

The maximum NR activity in the leaves of the diploid genotype of *A. canescens* occurred on the initial sampling date in August 1981 (Fig. 1a). Mature fruit was then present on the plants sampled although a large proportion of the fruits had already abscised. The NR activity decreased after this to a reasonably stable rate (6 μmol NO_2^- g dry $weight^{-1}$ 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 period (June to November). Mature fruit were abscising on the last sampling date (3 November 1982). The high NR activity on the initial sampling date (August 1981) was not evident during reproductive growth in 1982.

Nitrate reductase activity has been shown to fluctuate in response to leaf water status in several plants (Beevers & Hageman 1980) and in *A. confertifolia* irrigated with various concentrations of NaCl (Kleinkopf, Wallace & Cha 1975). In the present study, activity of NR and leaf water content (Fig. 1c) followed similar seasonal trends and were significantly, but not highly correlated ($r = 0{\cdot}47$, $P < 0{\cdot}05$). Seasonal water potentials in the xylem of the terminal 10–15 cm of stems bearing leaves representative of those assayed for NR activity were not, however, significantly correlated ($P < 0{\cdot}10$) with leaf NR activity (data not presented).

Maximum NO_3^- concentrations coincided with minimal NR activity (Fig. 1a) and low leaf-water content (Fig. 1c) during winter. Similar increases in leaf-NO_3^- concentrations have been attributed to leaf-water shortage inhibiting NR activity (Plaut 1973; Srivastava

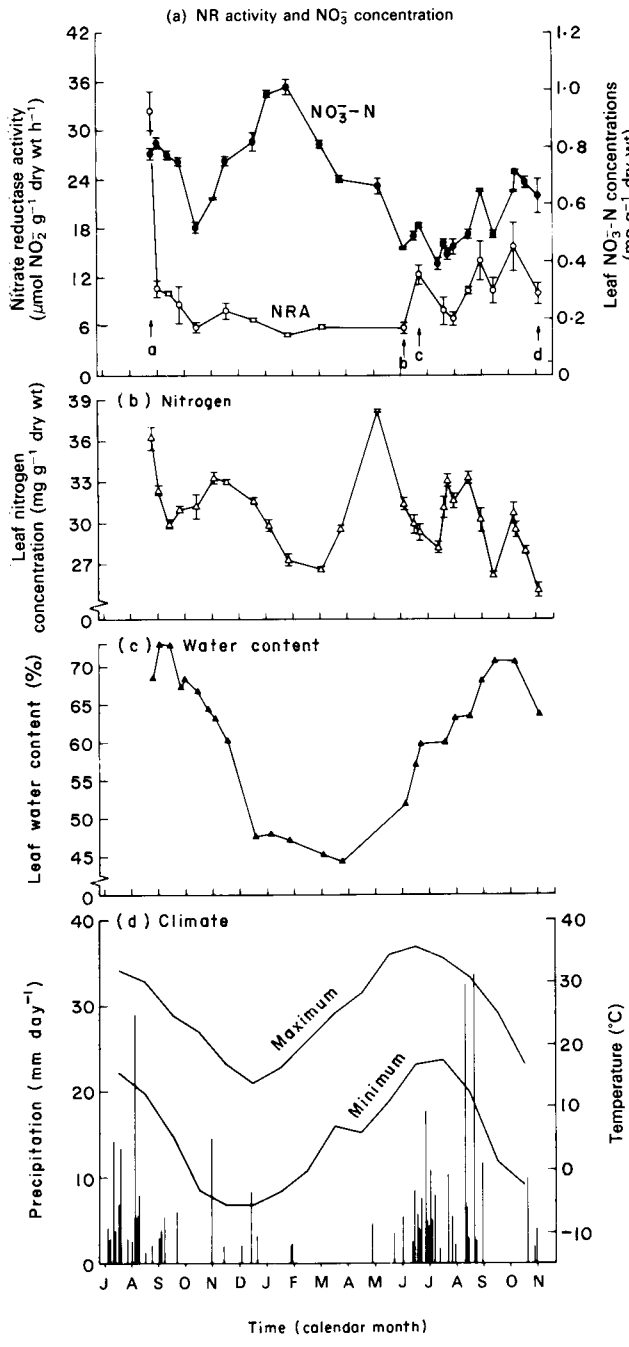

FIG. 1. Seasonal changes in (a), (○) leaf nitrate reductase activity (NRA) and (●) leaf NO_3-N; (b), leaf nitrogen concentration; and (c), leaf water content in the diploid genotype of *Atriplex canescens* in the northern Chihuahuan Desert, New Mexico, U.S.A., together with (d), mean monthly maximum and minimum temperatures and (vertical lines) daily precipitation approximately 10 m from the site, from August 1981 to October 1982. Bars are ±1 S.E. The arrows on Fig. 1a indicate reproductive stages as follows: a, mature fruit present and some fruit abscission; b, first observation of flowering; c, fruit growth initiated; d, seed set.

1980). Leaf-NO_3^- concentrations and NR activity followed similar trends from June to November. The precipitation received during this period (Fig. 1d) would tend to enhance root growth, and thus NO_3^- uptake and its translocation to NR sites within canopy leaves.

Total reduced-nitrogen concentration of the leaves varied between 38 mg N g dry weight^{-1} (May 1982) and 25 mg N g dry weight^{-1} (November 1982) (Fig. 1b). Leaf-N concentration declined by 35% during the initial 7-month period when leaf-water content and NR activity substantially declined. Thereafter, there was no discernible seasonal trend in nitrogen concentration.

The potential for thermal acclimation of NR to seasonal fluctuations in temperature (Fig. 1d) was determined on leaf samples collected between October 1981 and October 1982 (Fig. 2). Maximum NR activity occurred at approximately 30–35 °C assay temperatures, regardless of the sampling date. The NR activity at 30 °C was significantly higher than that at 35 °C ($P < 0{\cdot}05$) only on 22 July 1982. Therefore, there was no ability of NR to acclimate to seasonal fluctuations in temperature.

The thermal stability of NR was determined at 5-min intervals for 20 min at 20 °C, 30 °C and 40 °C to determine if NR activity was stable throughout the incubation period (15 min) during assays of NR activity for determining acclimation potential (Fig. 2, insert). The NR activity remained nearly constant throughout this 20-min period at 20 °C and 30 °C, but decreased by approximately 35% at 40 °C. Thus, the NR activities depicted in Fig. 2 at 40 °C and 45 °C (and perhaps at 35 °C) probably represent unstable rates which are functions of the rate of thermal inactivation during the incubation period.

Tetraploid plants

The seasonal trend in NR activity in the leaves of the tetraploid plants (Fig. 3a) was similar to that of the diploid plants (Fig. 1a). Maximum activity occurred when mature seed was present on the initial sampling date in August 1981. An increase in NR activity during reproductive growth in 1982 was preceded by low activity during the dry winter and

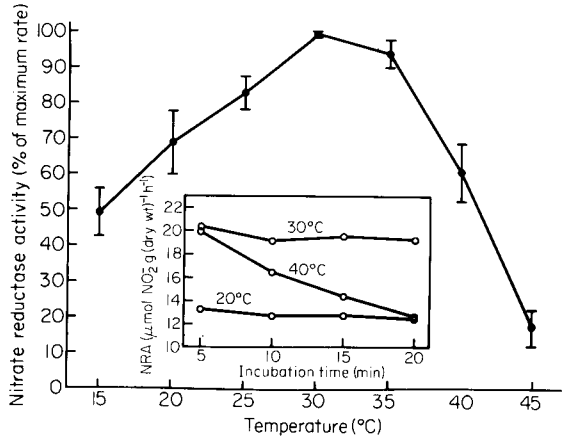

FIG. 2. Nitrate reductase activity (NRA) response to temperature in the diploid genotype of *Atriplex canescens* in the northern Chihuahuan Desert, New Mexico, U.S.A., based on analyses on eight dates from October 1981 to October 1982. Bars represent 95% confidence limits. Insert shows thermal stability of NRA on 19 October 1982 in the young uppermost leaves of the diploid genotype.

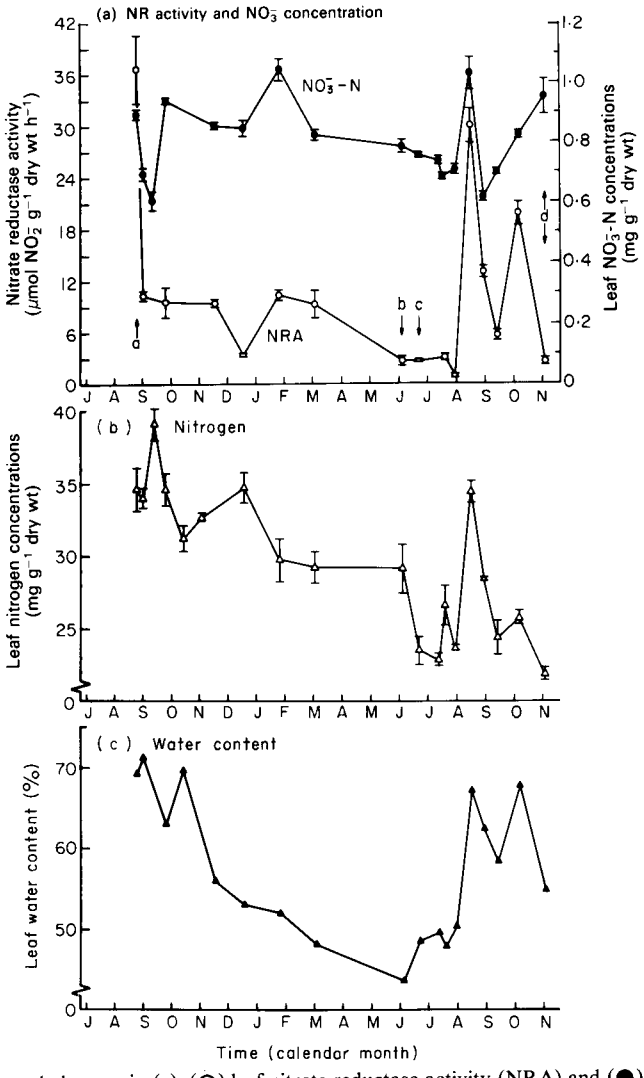

FIG. 3. Seasonal changes in (a), (○) leaf nitrate reductase activity (NRA) and (●) leaf NO_3-N; (b), leaf nitrogen concentration; and (c), leaf water content of the tetraploid genotype of *Atriplex canescens* in the northern Chihuahuan Desert, New Mexico, U.S.A. Bars represent ±1 S.E. The arrows on Fig. 3a indicate reproductive stages as follows: a, mature fruit present and some fruit abscission; b, first observation of flowering; c, fruit growth initiated; d, seed set.

summer months. However, this increase in NR activity did not coincide with the initial observation of flowering (4 June 1982) as occurred in the diploid plants. Substantial increases in NR activity, leaf-water content (Fig. 3c), NO_3^- concentration (Fig. 3a), and the production of new leaves occurred following rainfall during the first week of August 1982. There was no rain at this site between February and the first week in August. During June to August many leaves abscised. The low NR activity (0·88 μmol NO_2^- g dry weight^{-1} h^{-1}) determined just prior to the August rain was probably due to leaf water 'stress', as suggested by a mean stem water potential of −4·6 MPa, and the predominance of old leaves.

Leaf-water content (Fig. 3c) was correlated with NR activity ($r = 0{\cdot}70$, $P < 0{\cdot}05$) as

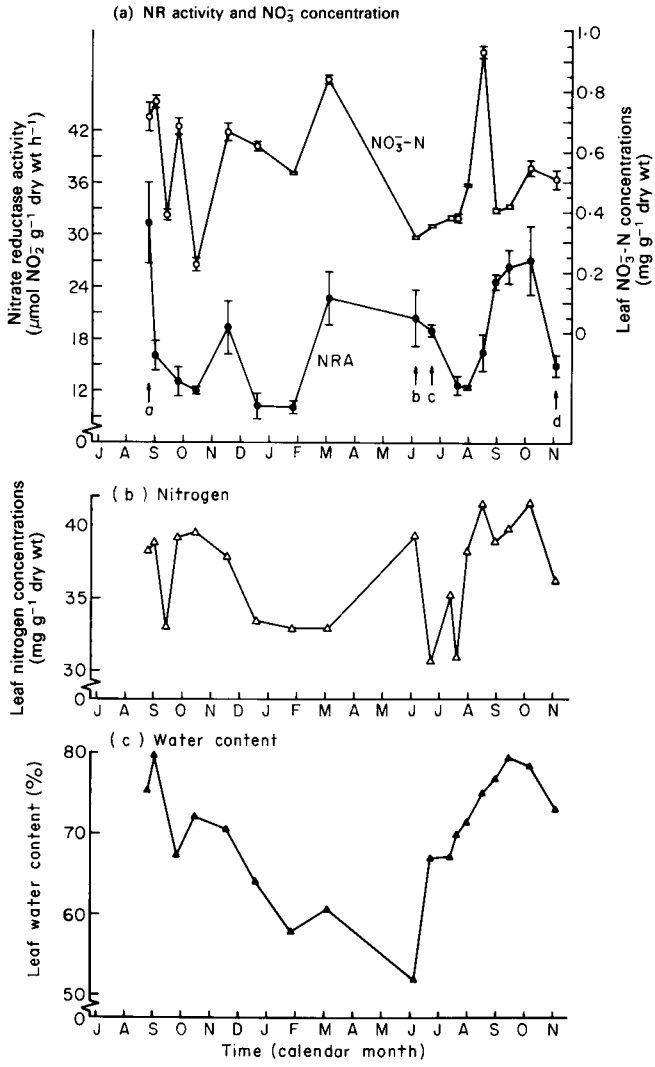

FIG. 4. Seasonal changes in (a), (○) leaf nitrate reductase activity (NRA) and (●) leaf NO_3-N; (b), leaf nitrogen concentration; and (c), leaf water content of the hexaploid genotype of *Atriplex canescens* in the northern Chihuahuan Desert, New Mexico, U.S.A. Bars represent ±1 S.E. The arrows on Fig. 4a indicate reproductive stages as follows: a, mature fruit present and some fruit abscission; b, first observation of flowering; c, fruit growth initiated; d, seed set.

was stem-water potential ($r = 0{\cdot}55$, $P < 0{\cdot}05$). Total leaf reduced-nitrogen tended to decrease throughout the study period (Fig. 3b). Leaf NO_3^- concentrations remained relatively constant except for the 32% decrease that occurred following the initial sampling date and the increases found in February and August; the latter was probably due to the rainfall.

Hexaploid plants

Two sustained periods of high NR activity occurred in the hexaploid plants (Fig. 4a); in early spring and summer (March to June 1982) and during autumn (September–October 1982). Reproductive growth occurred during both periods. Mean leaf-water content was

higher during the second (autumn) period of enhanced NR activity (Fig. 4c). Water potential in the stem xylem during both these periods was similar ($\bar{x} = -2{\cdot}2$ MPa and $-2{\cdot}5$ MPa). Seasonal changes in leaf-water content were significantly ($P < 0{\cdot}05$) correlated with NR activity while water potentials in the stem xylem were not ($P < 0{\cdot}10$).

No consistent trend in leaf-NO_3^- concentration (Fig. 4a) was apparent relative to season, leaf water content or NR activity. Similarly, considerable seasonal variability occurred in total reduced-nitrogen concentration (Fig. 4b).

The wing-like bracts of the immature fruits possessed NR activity of 2·1 μmol NO_2^- g dry weight^{-1} h^{-1} in September. Thus, these fleshy, chlorophyll-containing bracts may be a site of NO_3^- reduction and a source of reduced-nitrogen for developing seeds.

NR activity in glasshouse-grown plants

Heritable, genotypic differences in NR activity have been demonstrated in several agronomic plants and may be related to their differential abilities to accumulate total reduced-nitrogen (Naik *et al.* 1982). To determine if genotypic differences for NR activity and nitrogen accumulation occur in *A. canescens*, leaf-NR activity, NO_3^- and nitrogen were assayed in plants transplanted from the field and grown in sand in a uniform environment in a glasshouse for 4 months. All plants received 60 cm^3 of 10 mM NO_3^- in Long Ashton solution daily for two days prior to and during the day of analysis.

The hexaploid genotype possessed significantly ($P < 0{\cdot}10$) higher total reduced-nitrogen and NR activity in the young leaves than either the diploid or tetraploid plants (Table 1). The hexaploid leaves also possessed significantly ($P < 0{\cdot}10$) lower NO_3^- concentrations. These results are consistent with the hypothesis of Naik *et al.* (1982) that NR activity is related to total reduced-nitrogen in leaf tissue.

TABLE 1. Leaf nitrate reductase (NR) activity and nitrogen and NO_3^- concentrations of three genotypes of *Atriplex canescens* transplanted from their native field locations in the northern Chihuahuan Desert, New Mexico, U.S.A., and grown in sand for 4 months in a glasshouse. Values are means ± 1 S.E.

Genotype	NR activity (μmol NO_2^- g dry weight^{-1} h^{-1})	NO_3^--N (mg g^{-1} dry weight)	N (mg g dry weight^{-1})
Diploid	7·02 ± 0·39[a]	0·85 ± 0·09[a]	17·7 ± 0·67[a]
Tetraploid	6·01 ± 0·57[a]	0·76 ± 0·14[b]	15·1 ± 0·38[a]
Hexaploid	13·19 ± 2·57[b]	0·59 ± 0·25[c]	23·9 ± 0·93[b]

Means within columns followed by a similar superscript are not significantly different at $P < 0{\cdot}10$.

DISCUSSION

In *Atriplex canescens* the age composition of the uppermost canopy leaves present, and hence the samples collected, varied throughout the year in response to leaf aging, abscission and the initiation of new leaf growth. Thus the results of the present study are representative of the youngest, most physiologically active leaves present at any given time; these are where nitrogen assimilation primarily occurs (Srivastava 1980).

The effect of leaf age was readily demonstrated on 25 August 1981, when the older leaves of plants growing *in situ* possessed NR activity equivalent to 3%, 4% and 52% of the younger leaves of the diploid, tetraploid and hexaploid plants, respectively. Yet the

leaf-water content of the older leaves averaged only 6% less than that of the younger leaves.

Seasonal leaf NR activity of the three genotypes of *A. canescens* growing *in situ* was significantly correlated ($P < 0{\cdot}10$) with leaf-water content. Maximum and minimum water potentials of stem xylem were −0·88 MPa to −5·31 MPa and coincided with high and low NR activity, respectively. However, on a seasonal basis, water potentials in the stem xylem were correlated ($P < 0{\cdot}10$) with NR activity only in the tetraploid plants. During periods of low NR activity and leaf-water content, NO_3^- accumulated in the diploid plants and, to a lesser extent, in the hexaploid and tetraploid plants. Nitrate accumulation and lower NR activity during water 'stress' have been attributed to a decline in the synthesis of NR (Morilla, Boyer & Hageman 1973). However, Shaner & Boyer (1976a, b) demonstrated that the recovery of NR activity after the cessation of water stress was dependent upon protein synthesis and correlated with NO_3^- flux (concentration of nitrate in xylem × transpiration rate) into the leaves rather than the leaf NO_3^- content. The requirement for a NO_3^- flux into leaves for NR synthesis could be advantageous to *A. canescens* in a desert —energy needs to be expended to maintain high NR levels only when soil- and plant-water conditions favour NO_3^- uptake and its translocation to NR sites within the canopy leaves.

Reproductive growth after flowering resulted in substantial NR activity increases in *Phaseolus vulgaris* (Franco, Pereira & Neyra 1979). In contrast, a continuous decline in both NR activity and NO_3^- uptake occurred during this same period in *Glycine max* (soybean) (Harper & Hageman 1972; Franco 1977). In the present study, reproductive growth by *Atriplex canescens* appeared to have little or no influence on leaf NR activity. Rather, the enhanced NR activity that occurred during reproductive growth coincided with rainfall and with new leaf growth. For example, the initial observation of flowers (4 June 1982) on all genotypes coincided with low NR activity in the diploid (Fig. 2a) and tetraploid (Fig. 3a) plants, and relatively high activity in the hexaploid plants (Fig. 4a). Immature fruit was present on the following sampling date (21 June 1982) and NR activity increased only in the diploid plants. Nitrate reductase activity increased approximately 30 days after the initiation of fruit growth in the tetraploid plants and coincided with both precipitation and new leaf growth. Thus, leaf NR activity coincident with reproductive growth of *A. canescens* growing *in situ* appears dependent upon favourable plant- and soil-water relations enhancing soil NO_3^- availability and uptake, its translocation to the leaves and the production of new leaves. The wing-like bracts of the fruits possess the enzyme and thus reduced-nitrogen for developing seeds may partially originate within these bracts.

Genotypic differences in NR activity and the capacity to accumulate reduced nitrogen have been demonstrated in several agronomic plants (Naik *et al.* 1982). In the present study, the hexaploid genotype of *A. canescens* possessed higher NR activity and total reduced-nitrogen concentrations than did either the diploid or tetraploid plants when grown in a uniform environment and soil (Table 1). However, the NR activity of plants growing *in situ* was shown to be regulated by several endogenous and environmental factors that would limit the extrapolation of the results obtained under controlled environments. Therefore, nitrate uptake and nitrogen assimilation by the three genotypes growing in close proximity and under the same conditions in a uniform soil should be studied before assessing differences in the adaptiveness of NR between genotypes.

There was no evidence in the present study that NR of *A. canescens* growing *in situ* is capable of thermal adaptation to seasonal fluctuations in temperature (Figs 1d and 2). Maximum NR activity occurred at assay temperatures of 30–35 °C throughout the year,

though mean monthly temperatures varied from a maximum of 36 °C in July to a minimum of −6 °C in January. Harmer & Lee (1981) suggested that acclimation of NR was evident in two grass species because NR activity increased after transferring the plants from a 20 °C growth temperature to one of 5 °C for one week. However, the lack of pertinent associated data, such as tissue NO_3^- concentrations and particularly NR activity over a range of temperatures, tends to obscure an interpretation of acclimation. As a general phenomenon, thermal acclimation of NR would be adaptive for evergreen plants inhabiting environments with large annual fluctuations in temperature. However, there appears little evidence at present that NR is capable of such acclimation.

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