Effects of Nitrate Application on *Amaranthus powellii* Wats. 1

I. CHANGES IN PHOTOSYNTHESIS, GROWTH RATES, AND LEAF AREA

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ABSTRACT

Physiological effects of different nitrate applications were studied using the *C₃* plant, *Amaranthus powellii* Wats. Plants were grown in a controlled environment chamber and watered daily with nutrient solutions containing 45, 10, 5, or 1 millimolar nitrate. Chloride and sulfate were used to keep the cation and phosphate concentrations constant. Total leaf nitrogen concentration, chlorophyll concentration, specific leaf mass, leaf area, relative growth rate, relative leaf growth rate, unit leaf rate (increase of dry mass per unit leaf area per day), net photosynthetic rate, and incident quantum yield decreased with decreasing nitrate concentration. The per cent decrease of unit leaf rate was similar to the decrease of light-saturated net photosynthetic rate; however, the decrease in relative growth rate was less than that of unit leaf rate because leaf area ratio (leaf area per unit dry mass) increased with decreasing nitrate concentration. Essential mineral concentrations per unit leaf area were about equal among all treatments. Leaf expansion, determined by stomatal density, decreased except for the 1 millimolar treatment which showed relatively more cell expansion but less cell division. Decreased nitrate application was correlated with higher osmotic potentials and lower pressure potentials (determined by pressure-volume curves), whereas leaf water potentials were equal among treatments. Even though total leaf area and shoot mass decreased with decreasing applied nitrate, the increase of the leaf area ratio may be related to selection for the highest possible growth rate.

Nitrogen is an essential element for plant growth and reproduction (16); however, the availability of nitrogen limits growth in most ecosystems (5). Several studies have related plant growth, either as biomass or leaf area, to different levels of applied nitrogen (18, 28). Since most nitrogen in leaves is used for synthesis of components of the photosynthetic apparatus (9, 18, 19), numerous investigations have related photosynthetic rate to various nitrogen treatments or to leaf nitrogen concentration, N₃ (4, 18, 19). The goals of this and two companion studies (13, 14) are to determine quantitatively the relationship of photosynthetic rate and stomatal conductance to N and to test hypotheses on the optimization of gas exchange rates. If daily transpiration is minimized and daily assimilation is maximized for a given nitrogen treatment, then it may be possible to show that plants have adapted to nitrogen deficiency. *Amaranthus powellii*, a *C₃* dicot, was chosen as a model plant for these studies because it is a native plant found in fertile disturbed habitats, and not artifi-

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MATERIALS AND METHODS

Growth Conditions and Nitrate Treatments. Seeds of *Amaranthus powellii* Wats., green pigweed (27), were obtained from one plant in the field located at the Matthaei Botanical Gardens, University of Michigan, Ann Arbor. Several plants were then grown in a greenhouse from the collected seed and the seeds from these plants were used for all experiments. Voucher specimens were prepared and deposited at the University of Michigan Herbarium, Ann Arbor.

There were four nitrogen fertilization treatments, 45, 10, 5, and 1 mm nitrate in a base nutrient solution (24, 26). Chloride and sulfate were used to keep the cation concentrations constant. Chloride concentrations were 16 μM, 32 mm 36 mm, and 40 mm, respectively. The sulfate concentrations were 2, 3, 4, and 4 mm, respectively. Concentrations of other essential nutrients (for all treatments) were 16 mm K, 15 mm Ca, 2 mm Mg, 1 mm P, 0.2 mm Fe, 0.2 mm Na, 9 μM Mn, 0.8 μM Zn, 0.3 μM Cu, and 0.1 μM Mo. Iron was supplied as ferric sodium (ethylene-dinitrilol)tetraacetate (Eastman Kodak, Rochester, NY).

Seeds were planted every 2 or 3 d such that at any time, one plant from each treatment would be available for use. Seeds were

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1 Abbreviations: PPFD, photosynthetic photon flux density; N, total leaf nitrogen concentration (per unit area); SLM, specific leaf mass (per unit area); RGR, relative growth rate; RLGR, relative leaf area growth rate; ULR, unit leaf rate; LAR, leaf area ratio; ψ, leaf water potential; ψₛ, leaf osmotic potential; ψₚ, leaf pressure potential.
germinated in a small pot filled with medium Vermiculite kept at pot capacity with distilled H₂O. When the first leaf was equal in size to the cotyledons (about 7 d after sowing), seedlings were transplanted into 10-cm diameter, 10-cm-deep plastic pots (potting mixture 1:1 medium Vermiculite:medium Perlite), one seedling per pot, and watered with distilled H₂O. The seedlings selected for transplanting were the easiest to remove with the roots intact. The next day, nutrient treatments were started. Seedlings were randomly assigned to each treatment. Each plant was watered in the morning with distilled H₂O to pot capacity and in the afternoon with 80 ml of nutrient solution. Plants were grouped by treatment and spread out evenly throughout the controlled environment chamber. Plants were moved within the treatment group every 2 or 3 d and each group was moved every 7 to 10 d to a different location in the chamber.

Plants that were watered twice daily with nutrient solution diluted 1:1 with distilled H₂O were not visibly different from plants that were treated as described above. Plants watered twice daily with undiluted nutrient solution were larger and greener. The differences between the two methods of application show that the total amount of applied nutrients was more important than the timing of application.

Plants were kept in a controlled environment chamber with a photoperiod of 16 h of incandescent light with 12 h of maximum light intensity provided by fluorescent lights. The PPFD ranged from 0.6 to 0.8 mmol photons m⁻² s⁻¹ depending on plant height. The thermoperiod was 14 h at 32°C (starting 1 h after the start of the incandescent lights) and 10 h at 24°C. Humidity was not controlled but extra water vapor was added during the winter months by placing two shallow pans filled with distilled H₂O inside the controlled environment chamber.

Leaf Measurements. The most recently mature leaf (90–100% full expansion) on plants 28 to 30 d old were used in experiments with single leaves. One-sided leaf area was measured with a LI-3000 leaf area meter (LI-COR, Lincoln, NE). Dry mass was measured after drying for 48 h at 70 to 75°C. The mass of the leaf divided by leaf area was SLM. Total leaf nitrogen was determined with a micro-Kjeldahl digest (heated for about 48 h in a sand bath and without added K₂SO₄) and Nessler's reagent (6, 17). Absorbance at 410 nm was measured with a Spectronic 21 spectrophotometer (Bausch and Lomb, Rochester, NY). Total Chl and Chl a and b concentrations were measured on leaf discs (3.6 cm²) following the method of Arnon (1) using the Spectronic 21 for total Chl and a Beckman 35 spectrophotometer (Beckman Instruments, Irvine, CA) for Chl a and b. Stomatal densities were measured using epidermal strips taken from the tip, sides, and center of the leaf excluding veins. The number of stomata was counted in randomly chosen fields of view of known area at ×400. The sample sizes for most of the leaf measurements from each treatment ranged from 15 to 35 with a mean of 25 measurements.

Xylem pressure potential was determined for four leaves from each treatment using a pressure chamber (PMS Instruments, Corvallis, OR) and was equated to Ψ. The volume of water lost for a specific chamber pressure was measured for two leaves from each treatment, by weighing the leaf and rubber stopper after the expressed water was removed. The data were plotted as 'pressure-volume' curves to obtain the Ψ, of each leaf (25). The Ψ, was the difference between Ψ and Ψ.

Essential Mineral Concentrations of Leaves. The essential mineral concentrations of leaves from each treatment were measured by neutron activation analysis. For each treatment, one leaf was chosen as described above from each of 10 plants. The leaves were dried and mixed together to obtain one sample per treatment for analysis. Phosphorus and sulfur were not measured.

Gas Exchange Measurements. Net photosynthetic CO₂ uptake and transpirational H₂O loss were measured with the open flow differential gas exchange system described by Harley (12). A Series Five IR gas analyzer (Sensors, Inc., Ann Arbor, MI) was used to measure CO₂ concentration. Water vapor concentration was measured by a model 880 thermoelectric dew point hygrometer (EG & G International, Inc., Waltham, MA). Pure gases were mixed to provide any desired CO₂ and O₂ partial pressure by either Wösthoff gas mixing pumps (H. Wösthoff OHG, Bochum, Federal Republic of Germany) or model 8250 modular Dynamic Gas Mixers (Matheson Gas Products, East Rutherford, NJ) calibrated with the Wösthoff pumps. The airstream was humidified by bubbling it through distilled H₂O and passing it through an aluminum coil, both maintained at the desired dewpoint of the airstream. The leaf cuvette was made from nickel-plated copper and Teflon-coated plexiglass. Leaf temperature was maintained to ±0.1°C by water jackets above and below the leaf chamber. Light was provided by a metal halide lamp (OSRAM Powerstar, Berlin, Federal Republic of Germany). Different PPFDs were obtained by placing blackened screens between the lamp and cuvette.

Growth Analysis and Productivity. Shoot dry mass and total leaf area were determined for 15 to 23 plants from each treatment, harvested between the ages of 10 to 35 d from sowing. The RGR, RLGR, and LAR were calculated from the derivative at 28 d of a least-squares polynomial curve fitted through the data according to the method of Hunt (15). Unit leaf rate was calculated from RGR/LAR.

Statistics and Data Analysis. All statistical analyses were performed by computer using the Michigan Interactive Data Analysis System (MIDAS) written by the Statistical Research Laboratory, University of Michigan, Ann Arbor. One-way analysis of variance tests with unequal sample sizes were used to determine if any treatment means were significantly different. Tukey's confidence intervals of linear contrasts were used to determine which treatment mean was significantly different. Quantum yields were determined from the slope of a linear least-squares regression line fitted through the points at very low PPFD. The level of significance of P = 0.05 was used for all tests.

RESULTS AND DISCUSSION

Leaf Measurements. Leaf area, SLM, N, Chl concentration, and Chl a/b ratio decreased with decreasing applied nitrate (Table I). All of these differences are significant at P = 0.05. Differences in leaf nitrogen content per unit dry leaf mass were relatively smaller among the treatments compared to the differences per unit leaf area (Table I). The decrease in leaf area and N with decreasing applied nitrate have been well documented for other species (18, 19). However, SLM often increases with decreasing applied nitrate for many species (18, 20). The reciprocal of SLM is the specific leaf area which is important in the calculation of RGR (15).

The decrease in leaf area could be either from decreased cell expansion (21, 22) or from decreased cell division. Stomatal density on the abaxial surface increased with decreasing nitrate treatment from 45 to 5 mm, but the 1 mm nitrate treatment had the lowest stomatal density (Table II). The stomatal density on the adaxial surface was approximately 70% the abaxial density for all treatments. When the total number of stomata on the abaxial epidermis was calculated, the differences between the 45, 10, and 5 mm nitrate treatments were not significant at P = 0.05, however, the 5 mm treatment was lower. The number of stomata in the 1 mm nitrate treatment was much lower and significantly different than the other treatments (Table II). Epidermal cells were more fully expanded (not as convoluted) in the 45 and 1 mm treatments than in the 10 and 5 mm treatments. These data suggest the changes in leaf area between the 45, 10, and 5 mm treatments were due to decreased cellular expansion (22), and for the 1 mm treatment, decreased cellular division. However,
I. PHYSIOLOGICAL CHANGES FROM DIFFERENT NITRATE APPLICATIONS

Table 1. Leaf and Pigment Characteristics of Recently Mature Leaves on 28-d-old Plants of Amaranthus powelli Grown at Four Nitrate Concentrations (Presented as Means ± sd)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>45 mM</th>
<th>10 mM</th>
<th>5 mM</th>
<th>1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm²)</td>
<td>29.7 ± 4.3</td>
<td>25.8 ± 3.9</td>
<td>20.8 ± 3.6</td>
<td>10.9 ± 3.0</td>
</tr>
<tr>
<td>SLM (g/m²)</td>
<td>60.8 ± 4.8</td>
<td>52.3 ± 4.9</td>
<td>49.7 ± 3.2</td>
<td>28.0 ± 3.8</td>
</tr>
<tr>
<td>N (mmol N/m²)</td>
<td>223 ± 27</td>
<td>148 ± 14</td>
<td>98 ± 12</td>
<td>48 ± 7</td>
</tr>
<tr>
<td>N (mg N/g)</td>
<td>51.4 ± 6.2</td>
<td>39.8 ± 3.9</td>
<td>27.7 ± 3.4</td>
<td>24.0 ± 3.5</td>
</tr>
<tr>
<td>Chl (µmol/m²)</td>
<td>954 ± 121</td>
<td>604 ± 118</td>
<td>457 ± 124</td>
<td>232 ± 52</td>
</tr>
<tr>
<td>Chl a/b ratio</td>
<td>3.91 ± 0.11</td>
<td>3.49 ± 0.05</td>
<td>3.25 ± 0.14</td>
<td>2.97 ± 0.13</td>
</tr>
</tbody>
</table>

Table II. Abaxial Stomatal Density and Total Number of Abaxial Stomata per Leaf for Amaranthus powelli Grown at Four Nitrate Concentrations (Presented as Means ± sd)

<table>
<thead>
<tr>
<th>Density (mm⁻²)</th>
<th>Leaf Area (cm²)</th>
<th>One-sided total</th>
</tr>
</thead>
<tbody>
<tr>
<td>222 ± 12</td>
<td>22.1 ± 1.4</td>
<td>4.90 × 10⁵</td>
</tr>
<tr>
<td>232 ± 13</td>
<td>20.9 ± 1.4</td>
<td>4.85 × 10⁵</td>
</tr>
<tr>
<td>256 ± 26</td>
<td>17.8 ± 2.4</td>
<td>4.54 × 10⁵</td>
</tr>
<tr>
<td>96 ± 10</td>
<td>10.4 ± 2.2</td>
<td>1.00 × 10⁵</td>
</tr>
</tbody>
</table>

Fig. 1. Net photosynthetic rate (Net PS) responses to PPFD for the leaves at or just before full expansion from 28-d-old plants grown at four nitrate concentrations. The ambient conditions were CO₂ partial pressure of 1945 µbar/bar and O₂ partial pressure of 200 mbar/bar. The leaf temperature was 35°C and the vapor pressure difference between the leaf and air ranged from 25 to 35 mbar/bar. Sample sizes for the 45 to 1 mM treatments were 7, 7, 6, and 4 leaves, respectively. Derived quantities from these data are presented in Table III.

changes in abaxial epidermal cell division may not be equal to changes in mesophyll cell division because the epidermis and mesophyll cells are formed by different initial cells (10).

Leaf xylem pressure potential for all treatments was -0.56 ± 0.03 MPa, which was equated to ψ. The mean ψ₉ for the 1 to 45 mM treatment was -1.06, -1.42, -1.56, and -1.70 MPAs, respectively. Therefore, ψ₉ increased from 0.50 to 1.14 MPa with increasing nitrate treatment, to keep ψ constant. The differences in ψ₉ may be the cause of the expansion differences because higher turgor pressure can exert more expansive force in cells with higher N (22). The means by which this was accomplished is different than in cotton or sunflower where low N plants had lower negative water potentials (21, 22). It is not known why the 1 mM treatment, which had the lowest ψ₉, had relatively fully expanded epidermal cells.

Essential Mineral Concentrations of Leaves. The amounts of K, Mg, Ca, Na, Mn, Fe, and Zn per dry leaf mass (in order of highest to lowest concentration) generally increased with decreasing applied nitrate concentration; whereas, the amounts per fresh mass or leaf area were approximately equal among treatments. The concentrations were greater than the amounts considered adequate for most plants except for Fe (9). The amount of Fe was one-third of the amount considered adequate; however, the experimental plants were not Fe-deficient according to published criteria (9). The amounts of Cu or Mo were too low to be detected. It is important to note that only one leaf age was used, since the mineral contents also vary with leaf age (5, 19, 23).

Gas Exchange Measurements. The maximum PPFD saturated net photosynthetic rate and the PPFD required for saturation decreased with decreasing applied nitrate (Fig. 1, Table III). The quantum yield on an incident quantum basis and leaf absorption of photons between the wavelengths of 400 to 700 nm decreased with decreasing applied nitrate (Table III). However, when the quantum yield was expressed on an absorbed quantum basis, the differences between the 45, 10, and 5 mM treatments were not significant (P = 0.05). The absorbed quantum yield for the 1 mM treatment was significantly lower than the other treatments (Table III). Residual or dark respiration rates were calculated as the extrapolated y-intercept of the quantum yield regression and show a nonsignificant decreasing trend with decreasing applied nitrate treatment (Table III).

The above effects of different applied nitrogen treatments on photosynthetic rate and leaf Chl concentration (Table I, Table III, Fig. 1) have been well documented (18, 19). El-Sharkawy et al. (8) found that a lower light intensity was required for the saturation of photosynthetic rate in older leaves of A. edulis. Under the assumption that the old leaves in that study have less leaf nitrogen than recently expanded leaves (22), the data are consistent with the data for A. powelli (Fig. 1, Table III). The relationships among net photosynthetic rates, stomatal conductance, and N for A. powelli will be analyzed in the companion studies (13, 14).

The quantum yield on an absorbed quantum basis for the 45, 10, and 5 mM treatments (Table III) are about equal to the quantum yields calculated for A. tricolor, A. palmeri, and A. retroflexus (7). The reduction in absorbed quantum yield for the 1 mM treatment suggests a relatively larger non-Chl absorption in leaves that have low Chl content (11). The trend of decreasing respiration rate and Chl a/b ratio (Tables I and III) with decreasing applied nitrate is consistent with the data of other workers (3, 4). The lower PPFD for saturation of photosynthesis rate (Table III), Chl a/b ratio, and SLM (Table I) at the lower applied nitrogen concentrations are qualitatively similar to shade plants.
or sun plants grown in the shade (2).

Growth Analysis and Productivity. The total shoot mass and total one-sided leaf area for 28-d-old plants decreased significantly with decreasing applied nitrate (Table IV). The decreases in total leaf area resulted from smaller area per leaf and fewer leaves. Decreases in shoot mass and leaf area were largely caused by decreases in lateral branch growth. Root dry mass could not be determined accurately because Vermiculite and Perlite adhered to the roots. Root mass was visually estimated to increase progressively from 1, 5, 10, and 45 mm nitrate.

The RGR, ULR, and RLGR decreased with decreasing applied nitrate (Table IV). The per cent decrease in ULR was much larger than the per cent decrease in RGR because LAR increased with decreasing applied nitrate (Table IV). The decrease of ULR between the 45 and 1 mm treatments was 90% (Table IV), and was similar to the 81% decrease of the PPFD-saturated net photosynthetic rates for the same treatments (Fig. 1).

The effects of nitrogen application on LAR and RLGR have usually been attributed to be the major cause of the decrease of RGR due to nitrogen deficiency (15, 28). The reciprocal of LAR was similar to SLM (Table I) for each treatment even though LAR includes the stem dry mass whereas SLM does not. As for the case with SLM, nitrogen deficiency often causes the LAR to decrease (18, 20). However, because the LAR increases (SLM decreases), and mineral concentrations and ψ remain equal among treatments with decreasing applied nitrate, the data may indicate that *A. powellii* maintains the largest possible leaf area during nitrogen deficiency, even with the result of having lower ULR and net photosynthetic rates than possible. This speculation is supported by the fact that the epidermis from leaves of the 1 mm treatment showed full cellular expansion, similar to the cellular expansion of the 45 mm treatment leaves. However, it may be possible that LAR will decline in the 1 mm nitrate treatment after 35 d, even though LAR was constant between 10 and 35 d (for all treatments). Presumably, the maintenance of the largest possible leaf area (but with fewer and smaller leaves) may be related to selection for an increased growth rate compared to neighboring plants which are under similar conditions. However, this idea cannot be tested with the techniques used in this study.

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**LITERATURE CITED**


18. NATR L 1975 Influence of mineral nutrition on photosynthesis and the use of assimilates. *In JP Cooper, ed. Photosynthesis and Productivity in Different


