Effect of nitrogen application on growth And photosynthetic nitrogen use efficiency in two ecotypes Of wild strawberry, Fragaria chiloensis (L.) Duchn

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The relationships between increasing nitrogen fertilization and growth, maximum CO_2 assimilation and the initial slope of the CO_2 response curve were studied in two ecotypes of wild strawberry, Fragaria chiloensis (L.) Duchn. Nitrogen accumulation of CA11, an ecotype from a low-nutrient dune site, was greater at all nitrogen concentrations than that of RCP37, an ecotype from a higher-nutrient strand site. Maximum CO_2 assimilation, total Rubisco activity, dry weight, and initiation of leaves and crowns were higher in CA11 than RCP37 as nitrogen treatment was increased from 0 to 200 mg l^{-1}, whereas these parameters were lower in CA11 when fertilized at 300 mg l^{-1}, but not in RCP37. The mean leaf area of CA11 was greater than RCP37 when grown with no supplemental nitrogen, but mean leaf area of the two lines was similar under nitrogen fertilization. Maximum CO_2 assimilation and carboxylation efficiency increased with increasing leaf nitrogen in both clones. At equivalent concentrations of leaf nitrogen, RCP37 had higher CO_2 assimilation and carboxylation efficiency than CA11 and the difference between the 2 clones increased as leaf nitrogen increased. Thus, RCP37 had a higher photosynthetic nitrogen use efficiency than CA11. However, at a given applied nitrogen level, CA11 allocated more nitrogen to a unit of leaf area so that photosynthetic rates were higher than RCP37, except at the highest application of 300 mg l^{-1}. The high nitrogen accumulation capacity and resource allocation to fruiting structures (crowns) in CA11 lead us to suggest that this clone may possess genes that could increase fruit yield in cultivated strawberry.

Key words - Carboxylation efficiency, CO_2 assimilation, mineral nutrition, nitrogen use efficiency.

Introduction

Fragaria chiloensis (L.) Duchn. is one of the progenitor species of cultivated strawberry, Fragaria X ananassa Duchn., and has been an important source of insect and disease resistance in strawberry breeding programs (Sjulin and Dale 1987). Recent studies have shown that many clones of F. chiloensis have CO_2 assimilation (A) rates much higher than that of cultivated strawberry (Hancock et al. 1989; Cameron and Hartley 1990) and that high A is quantitatively inherited (Hancock et al. 1989). Native clones of F. chiloensis with high numbers of large fruit have been described that may provide the necessary genes to increase both A and resource allocation to fruit (Hancock and Bringhurst 1989).

Native North American F. chiloensis ranges from the coastal fog belt of Santa Maria, California, northward through western Alaska (Hancock and Bringhurst 1979). Colonies of F. chiloensis are found in diverse habitats including dunes (98% sand, low soil moisture availability and low nutrient availability) and woodland-meadows (higher silt, greater soil moisture and high nutrient availability) (Hancock and Bringhurst 1979). Photosynthetic capacity or efficiency may be related to the habitat in which these colonies of wild strawberry developed.

Species adapted to infertile soils have increased potential to absorb mobile ions such as nitrate and ammonium (Chapin et al. 1986). Since leaf nitrogen per area (N_a) is highly correlated with photosynthesis (DeJong 1982;

DeJong and Doyle 1985; Hunt et al. 1985; Sage et al. 1987; Sinclair and Horie 1989) these species might provide genes for increased A and yield, but only if useful traits are not linked to poor characters such as slow growth rate. Plants adapted to low-nutrient habitats are often co-limited by low soil moisture availability (Chapin 1987). Survival in these soils may be linked to low stomatal conductance (g'_s), and transpiration, which conserve limited soil moisture. Since low g'_s also limits A through low C_i (Farquhar and Sharkey 1982), these species tend to be slow growing. Thus, ecotypes from low-fertility sites require little fertilization, but likewise have poor inherent physiological capacity for yield response (Vose 1987). However, Antonovics et al. (1966) found wild populations of Lolium perenne adapted to either high or low N habitats and Goodman (1977) subsequently demonstrated selection for increased nitrogen uptake and yield in ryegrass. Hancock and Bringhurst (1979) have shown considerable ecological differentiation in wild populations of F. chiloensis, and in a greenhouse environment, they observed that plants from the more saline, nutrient poor sites performed better under salt and nutrient stress than those from a woodland meadows environment.

In preliminary studies we found distinct differences in leaf color and growth rate of clones of F. chiloensis, grown under low nitrogen availability. One clone from a low-nutrient dune habitat (CA11) and one from a higher nutrient strand habitat (RCP37) were chosen for further study. The purpose of this study was to characterize the growth and photosynthetic response of these clones from divergent habitats to increasing nitrogen availability. This will allow us to test the hypothesis that the clone from the low nitrogen habitat will have a greater capacity to assimilate nitrogen at low availability, whereas the clone from the high nitrogen habitat will have a greater PNUE and a greater capacity to increase growth with increasing nitrogen availability.

Abbreviations - A, CO_2 assimilation; A_{max}, maximum CO_2 assimilation; C_i, intercellular CO_2 concentration; g'_m, carboxylation efficiency; g'_s, stomatal conductance to CO_2; N_a, leaf nitrogen per area; NUE, nitrogen use efficiency; PNUE, photosynthetic nitrogen use efficiency; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase; V_{max}, maximum enzyme velocity.

Materials and Methods

Plant Material and Experimental Layout

Plants of two clones of Fragaria chiloensis (CA 11 and RCP37) were potted into 4 l pots in soil:sphagnum peat:perlite (1:2:2, v/v/v), pH 6.2. The medium was amended with 744 g of treble superphosphate, 496 g potassium nitrate, 496 g magnesium sulfate, 4 kg ground calcitic limestone, and 62 g Peters Frit Industries Trace Elements No. 555 (Peters Fertilizer Products, Fogelsville, PA) per cubic meter. The plants were placed in a 22/17 °C greenhouse. Integrated natural irradiance averaged 20.2 ± 3.7 mol m^{-2} PPF per day. Plants were fertilized at each watering with N-P-K at 1) 0-43-200, 2) 100-43-200, 3) 200-43-200, or 4) 300-43-200. Fertilizer treatments 1-4 were created with (l^{-1}) 0.1 ml 75% (w:w) H_3PO_4 plus 1) 381 mg KCl, 2) 229 mg NH_4NO_3 + 144 mg KNO_3 + 275 mg KCl, 3) 457 mg NH_4NO_3 + 289 mg KNO_3 + 168 mg KCl, or 4) 686 mg NH_4NO_3 + 433 mg KNO_3 + 62 mg KCl. The total N supplied by these treatments was 40% NH_4-N and 60% NO_3-N. The experiment design was a randomized complete block with clone and nitrogen level as factors. Each plot was replicated 3 times in blocks. Four plants received each treatment combination, and the average of the 4 plants was used as the experimental unit for data analysis. Runners were pruned weekly as they formed.

Plants were harvested May 23, 1989, after 28 weeks of treatment. Growth data collected on each plant included number of crowns, crown dry weight, and total leaf area. A 10 g leaf blade sub-sample was used for nitrogen content analysis. Fully expanded young leaves were selected (2 leaves per pot) beginning May 14, 1989 through May 22, 1989 and were used for single time measurements of gas exchange or Rubisco activity.

Gas Exchange

Gas exchange measurements were made using an open system and a plexiglass chamber coated on the inside

with clear Teflon PTFE tape to reduce water vapor adsorption and absorption. Temperature within the chamber was controlled with a Peltier plate (Thermoelectric Cooperation of America, Chicago, IL, Model 150HC) and air circulation over a nickel plated aluminum heat exchanger was supplied with an small DC fan (Pammotor Inc., Burlingame, CA, Model L402). O_2, N_2, and CO_2 were mixed manually using multiple valves and flow rates into the mixing chamber were measured with mass flow meters (Hastings Models ST 2663 and ST 2664). Response of CO_2 assimilation (A) to increasing CO_2 was monitored by measuring CO_2 gas exchange in step increments from low (<100 μmol mol^{-1}) to high CO_2 (1000 μmol mol^{-1}). The reference, sample and the CO_2 differential concentrations between the inlet and outlet gas from the chamber were measured with an infrared gas analyzer (Analytical Development Co., Hoddesdon, Herts. ENG., Model LCA-2). Flow rate into the chamber was controlled at 4 l min^{-1} and the air temperature inside the chamber was controlled throughout at 20 ± 0.5 °C. Air and leaf temperatures were monitored with thin wire thermocouples. Vapor pressure deficit (VPD) was maintained at 0.5 to 1.0 KPa by saturating the gas stream in a temperature controlled water bath, and then drying part of the air stream with silica gel. Vapor pressure of the inlet gas was measured with a dew point hygrometer (EG & G Environmental Equipment Co., Burlington, MA, Model 911). Outlet vapor pressure was measured directly within the chamber by mounting a dew point hygrometer (EG & G Environmental Equipment Co., Burlington, MA, Model 200) sensor inside the chamber. Light supplied by a 1,000 W metal halide lamp was filtered through 8 cm of water and neutral density screens. The irradiance level was measured with a quantum sensor (LI-COR Inc., Lincoln, NB, Model LI-180) at the top of the leaves and was maintained between 1,400 and 1,600 (PPF μmol m^{-2} s^{-1}). Gas exchange was calculated using standard equations (Moon and Flore 1986). Total CO_2 and Mg^{2+} saturated Rubisco activity was measured as previously described (Perchorowicz et al. 1982). Total leaf nitrogen was measured by automated Micro Kjeldahl (Schuman et al. 1973).

Data Analysis

A logarithmic curve was fitted to the data for CO_2 assimilation (A) versus intercellular CO_2 (C_i). CO_2 assimilation at saturating CO_2 levels (A_{max}; C_i > 500 μmol mol^{-1}) was calculated from the plateau phase of the A/C_i curves. Carboxylation efficiency (g'_m) was determined from linear regression using the linear portion of the A/C_i curve (0 to 250 μmol mol^{-1} CO_2).

Results

CA11, the low-nutrient ecotype, initiated a significantly ($P < 0.001$) greater number of growing points (leaf and crown number) than did RCP37 (Fig. 1, Tab. 1). As N concentration increased both crown and leaf number increased except at the highest rate where reductions were observed in CA11 (Fig. 1), whereas RCP37 plateaued off at 100 mg l^{-1}. The reduction in crown number in CA11 with the highest nitrogen application rate was large enough to produce a significant ($P < 0.05$) interaction between nitrogen and clone (Tab. 1).

Leaf area per pot of CA11 was twice that of RCP37 when no supplemental nitrogen was applied (Fig. 1). However, leaf area of both clones was similar at concentrations of supplemental nitrogen (Tab. 1).

Leaf and total plant dry weight increased with increasing nitrogen application from 0 to 200 mg N l^{-1}. Little to no effect was observed on either parameter at 300 mg N l^{-1}. Although not statistically significant, leaf and plant dry weights were lower in RCP37 compared to CA11 at zero N, but were higher at all levels of supplemental nitrogen. CA11 had significantly ($P < 0.001$) higher crown dry weight than RCP37 (Fig. 2, Tab. 1), except at the high nitrogen treatment. Crown dry weight was 35 to 40% less in CA11 when nitrogen application was 200 compared to 300 mg l^{-1}.

Photosynthetic capacity of both RCP37 and CA11 increased in response to N application as indicated by the large differences in total Rubisco activity, A_{max}, and g'_m between 0 and 300 mg l^{-1} treatments (Figs 3 and 4). Carboxylation efficiency (g'_m) increased linearly with increasing nitrogen in both clones (Fig. 4). There was no significant difference in A_{max}, Rubisco activity or g'_m between CA11 and RCP37 (Tab. 1), whereas there was

a significant interaction between nitrogen and clone for A_{max} as a result of the low response of RCP37 at the 200 mg l^{-1} nitrogen treatment.

Leaf nitrogen was higher in both clones with increased nitrogen application between 0 and 200 mg l^{-1} (Fig. 4). CA11 accumulated more N_a than RCP37 ($P < 0.05$) at every level of nitrogen application (Fig. 4). As N_a increased, A_{max} and g'_m increased linearly in both clones (Fig. 5), but the rate of increase (slope) with increasing N_a was much higher in RCP37 than in CA11 for both A_{max} (0.32 to 0.26) and g'_m (1.38 to 0.96). The slope of the linear relationship between A_{max} and N_a has been defined as an estimate of PNUE (Sage et al. 1987). The nitrogen compensation points (x-intercepts) for the linear regressions of Amax and g'_m on N_a were lower for CA11 (47 and 43, respectively) than for RCP37 (52 and 63, respectively; Fig. 5).

Discussion

The relationship between photosynthesis and nitrogen has been studied many times because of the importance of photosynthesis to plant productivity and the status of nitrogen as a limiting essential element (Chapin 1980; Field 1983; Hunt et al. 1985; Chapin et al. 1987). In recent years widespread sources of germplasm have been sought, which might improve plant efficiency for N assimilation and utilization (Vose 1987). Use of wild germplasm to develop cultivars giving high yields with lower nitrogen fertilization would not only lower the energy and cost component involved in agricultural production, but also contribute to less groundwater contamination from the use of high nutrient application to maximize yields.

Here it was observed that a low-nutrient ecotype of F. chiloensis (CA11) had a greater nitrogen assimilation under low nitrogen availability. CA11 initiated more leaves and crowns, and had a lower nitrogen compensation point for photosynthesis than did the high-nutrient ecotype, RCP37. Similar responses have been reported for other species and ecotypes from low-nutrient sites (Chapin 1987). However, increased nutrient assimilation does not always contribute to plant productivity and yield when nutrient availability is increased (Chapin 1987). The high correlations reported between A and increasing leaf nitrogen for a number of plant species (DeJong 1982; DeJong and Doyle 1985; Hunt 1985; Chapin et al. 1987; Sage and Pearcy 1987; Sinclair and Horie 1989) are not surprising since the primary carboxylating enzyme in leaves, Rubisco, comprises a substantial fraction of the total soluble protein in the leaf (Chapin et al. 1987). In a survey of a number of species, Evans (1989) reported that with increasing N per unit leaf area, the proportion of total leaf N in structural components of photosynthesis (thylakoids) remained unchanged, whereas the proportion of leaf N in Rubisco and other Calvin cyclic enzymes increases. Thus, since A increases linearly with increasing Rubisco amount, N_a represents a primary "cost" component in the construction and maintenance of photosynthetic capacity. Nitrogen assimilation requires substantial amounts of energy, and plant maintenance respiration has been directly correlated with tissue nitrogen levels (DeJong 1982). The high "costs" of nitrogen uptake, assimilation, and transport would not contribute substantially to photosynthetic productivity unless A was limited by nitrogen (i.e., limited by the amount of Rubisco). Sage et al. (1987) reported that Chenopodium album (L.), a C_3 species, increased A by increasing the amount of Rubisco with increasing N_a, whereas Rubisco amount was increased very little in Amaranthus retroflexus (L.) a C_4 species, where Rubisco operates close to its V_{max} due to the CO_2 concentrating mechanism of this species.

Most plants adapted to low-nutrient habitats are often co-limited by low moisture availability, and as such have been selected for features like low g'_s, transpiration and slow growth rate, which conserves limited soil moisture (Chapin 1987). Studies with many wild ecotypes have verified that plants adapted to sites with low resource availability respond poorly to increases in moisture and nutrients (Chapin 1987). In response to increased nutrient availability, such plants exhibit luxury consumption of nutrients (i.e. accumulate and store nutrient in leaves without increasing growth) and these nutrient stores support a slow growth over a long period of time. Luxury consumption of nitrogen does not result in large increases in dry matter yields because A is limited by the low g'_s and the ability to increase leaf area. However, luxury consumption of nitrogen may be of adaptive significance in ecotypes from low-nutrient habitats, since nitrogenous metabolic products associated with high

N_a may make these plants unattractive to herbivory (Chapin et al. 1987).

Hancock and Bringhurst (1989) have identified ecotypes of F. chiloensis which produce large fruit and high fruit numbers when grown under conditions of high moisture and nutrient availability. Here we report that CA11, which is a native dune clone, displays a greater number of growing points, total plant dry matter and A_{max} upon nitrogen application. CA11, unlike most low-nutrient ecotypes previously described, responds productively to increased fertilization. The presence of persistent fog during much of the day at the sites where dune species of F. chiloensis are found (Hancock and Bringhurst 1979), reduces high leaf-to-air vapor pressure deficits, and high transpirational demand that might select for low g'_s and slow growth rate found in other dune species. Thus, CA11 may possess the characteristics of high nutrient uptake capacity without the deleterious linkage of poor capacity for growth under high nutrient availability. The best measure of PNUE to compare species or ecotypes with different nitrogen uptake capacity is the slope of A versus N_a, which gives the increase in assimilation capacity per unit increase in nitrogen investment. The value of dA/dN_a tends to be larger in plants adapted to high nutrient availability than in plants adapted to low nutrient availability (Sage and Pearcy 1987). A_{max} increased linearly with increasing N_a in both CA11 and RCP37. A_{max} was greater in RCP37 than CA11 at identical N_a levels and this difference increased as N_a increased. The linear increase of A_{max} with increasing N_a was larger for RCP37 (slope = 0.32) than for CA11 (slope = 0.26). The PNUE of RCP37 was close to that reported for the C_4 species Amaranthus retroflexus (L.) where dA/dN_a ranges between 0.35 and 0.42 depending upon temperature (Sage and Pearcy 1987), whereas the slope of CA11 was similar to the C_3 species Chenopodium album (L.). However, for a given applied nitrogen level, CA11 had a greater N_a than RCP37 so that A_{max} was greater in CA11 except at the highest nitrogen application level. Thus, under low or moderate nitrogen application the high PNUE in RCP37 is offset by the greater uptake capacity of CA11 which allocates more nitrogen to leaves. However, the greater cost of nitrogen for photosynthetic capacity in CA11 may limit the allocation of nitrogen to leaf production. RCP37 had equal or greater leaf area per pot at all levels of supplemental nitrogen than did CA11.

The contribution of high PNUE to fruit yield is unknown. CA11 produces many more branch crowns than RCP37 at all levels of nitrogen application. Since flowers are initiated on branch crowns in strawberry, CA11 may possess the yield components to maximize fruit numbers and produce higher fruit yields. The greater capacity for nitrogen assimilation and the greater allocation to reproductive structures may more than offset the high PNUE of RCP37 unless fruit load in CA11 becomes so large as to become source-limited. Hancock and Bringhurst (1989) have reported that dune ecotypes of F. chiloensis have the largest fruit, and some of these clones also have high fruit numbers, which indicates that these clones are not source-limited. Thus, we suggest that CA11 may be a good germplasm source for high nitrogen uptake and high resource allocation to fruiting structures.

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Table 1. Significance levels of F-tests of the effect of nitrogen application on the vegetative growth, and photosynthetic potential of 2 clones of *Fragaria* chiloensis. NS = not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

	Significance		
	Nutrient	Clone	Interaction
Leaf number	***	***	NS
Crown number	***	***	*
Leaf area (cm^2)	***	NS	NS
Leaf DW (g)	***	NS	NS
Leaf N area $^{-1}$ (mmol m^{-2})	***	*	NS
Crown DW (g)	***	**	NS
Plant DW (g)	***	NS	NS
Rubisco activity (μmol CO_2 mg.chl^{-1} min^{-1})	***	NS	NS
A_{max} (μmol CO_2 $m^{-2}s^{-1}$)	***	NS	*
g'_m (mmol CO_2 m^{-2} s^{-1})	***	NS	NS

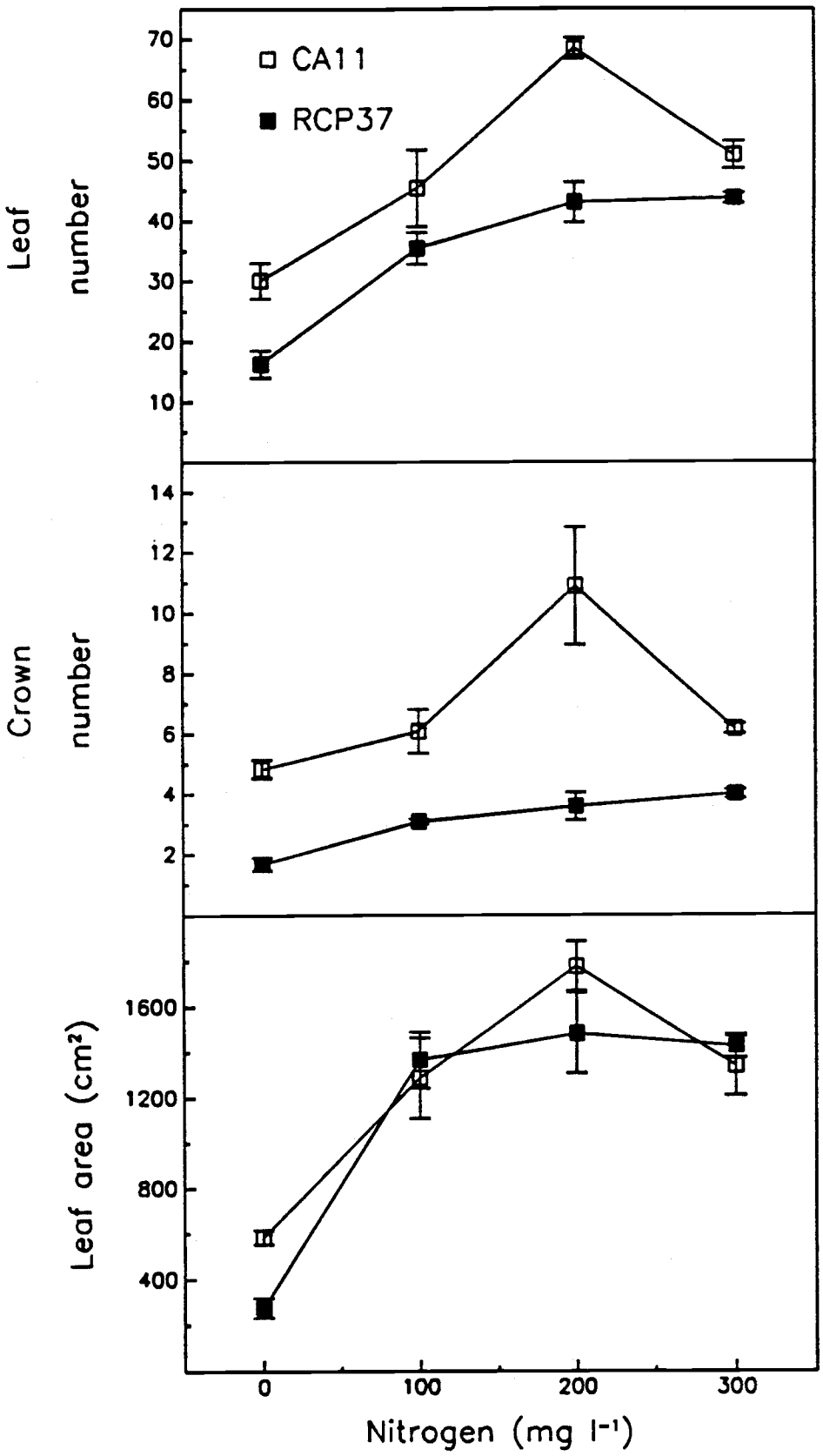

Fig. 1. Effect of nitrogen application on leaf number, crown number, and leaf area of F. chiloensis clones. Values are means ± SE.

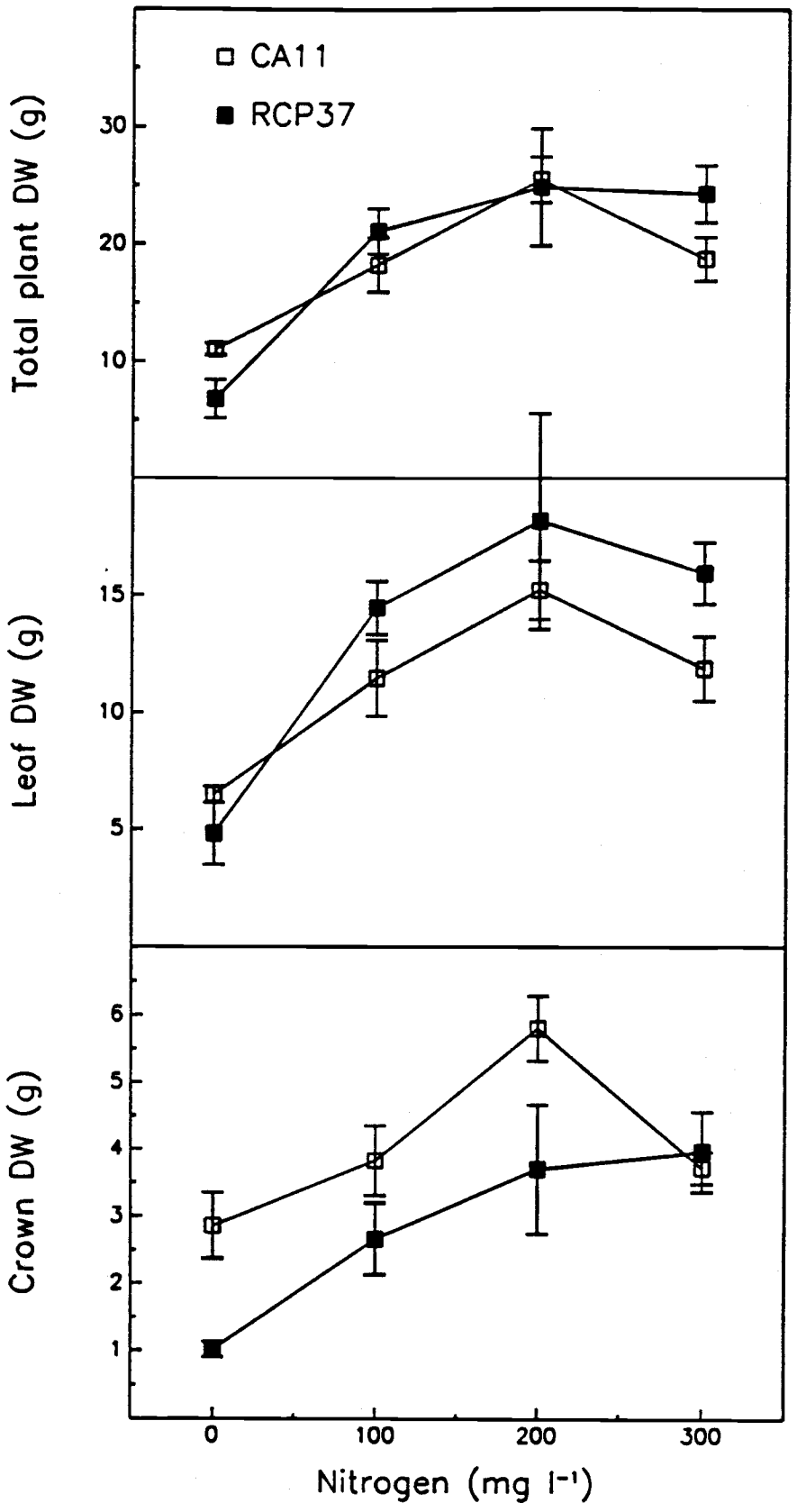

Fig. 2. Effect of nitrogen on dry matter accumulation of F. chiloensis clones. Values are means ± SE.

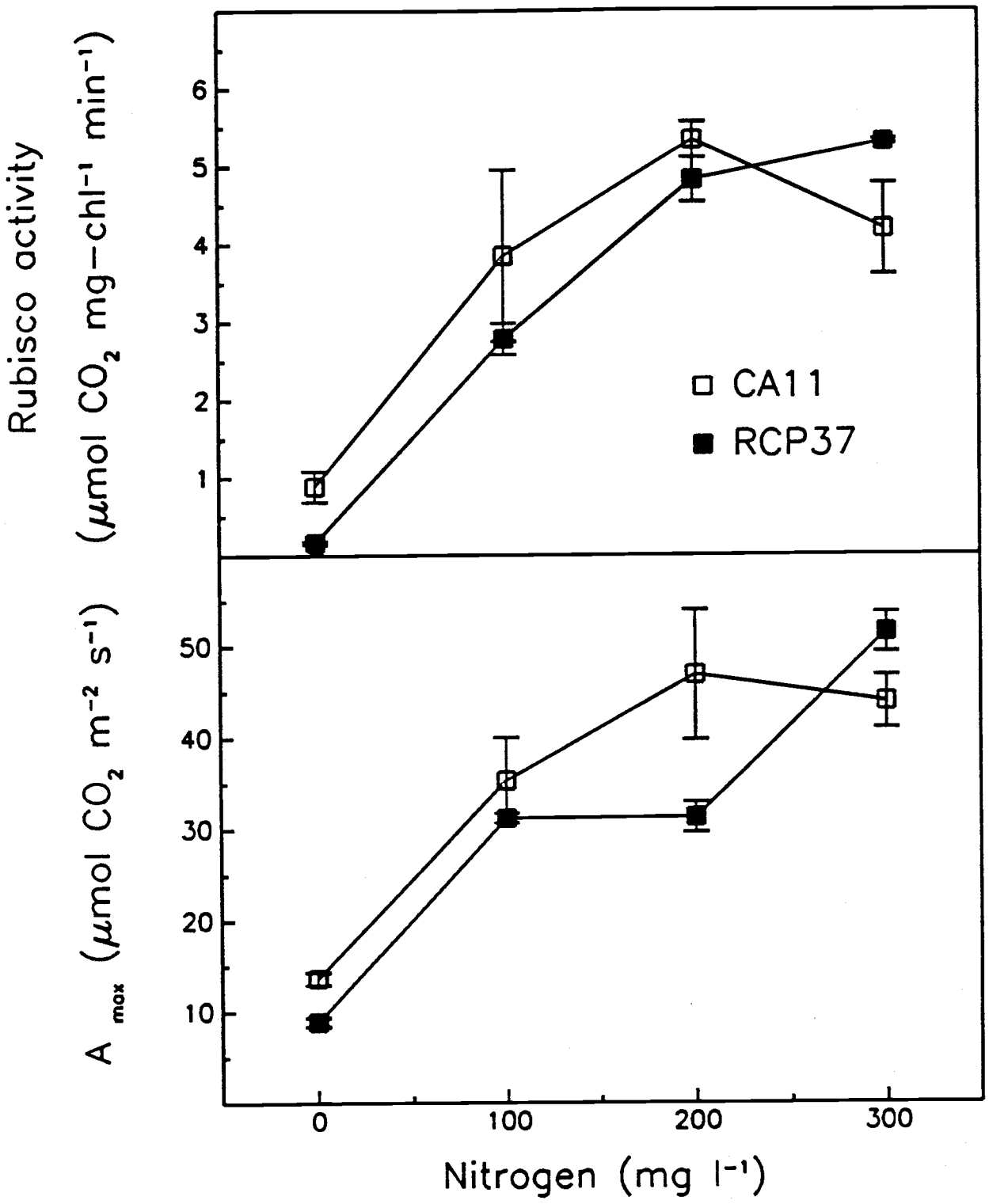

Fig. 3. Effect of nitrogen application on maximum CO_2 assimilation (A_{max}) and total Rubisco activity. A_{max} was measured at leaf temperatures of 20 °C, irradiance of 1500 μmol m^{-2} s^{-1} PPF and at saturating intercellular CO_2 (C_i > 500 μmol mol^{-1}). Total Rubisco activity is the CO_2 and Mg^{2+} saturated rate of fixation at 25 °C. Values are means ± SE.

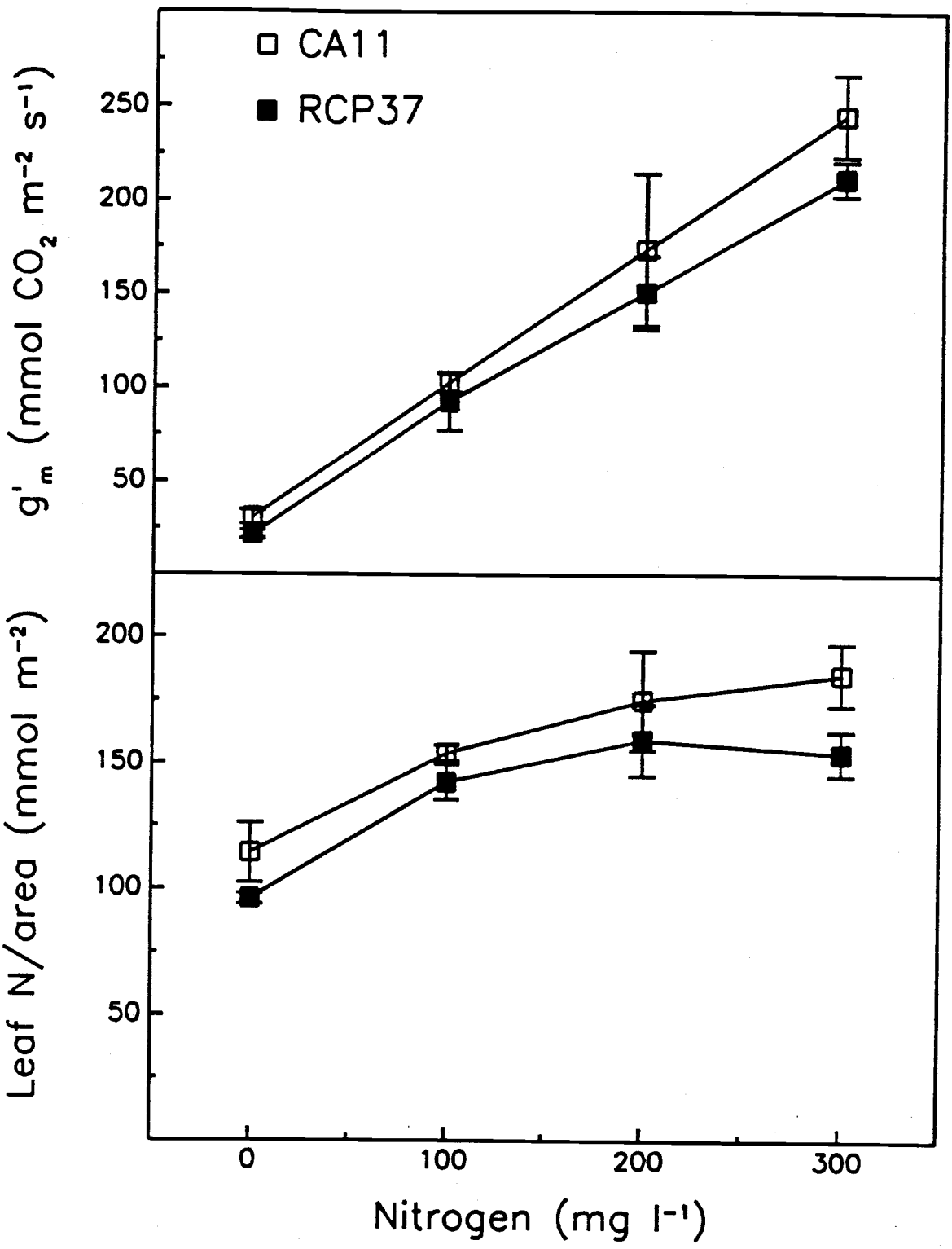

Fig. 4. Effect of nitrogen application on carboxylation efficiency (g'_m) and leaf nitrogen per area. Carboxylation efficiency was estimated from the linear portion of the A/C_i curves (0 to 250 μmol mol^{-1}). Values are means ± SE.

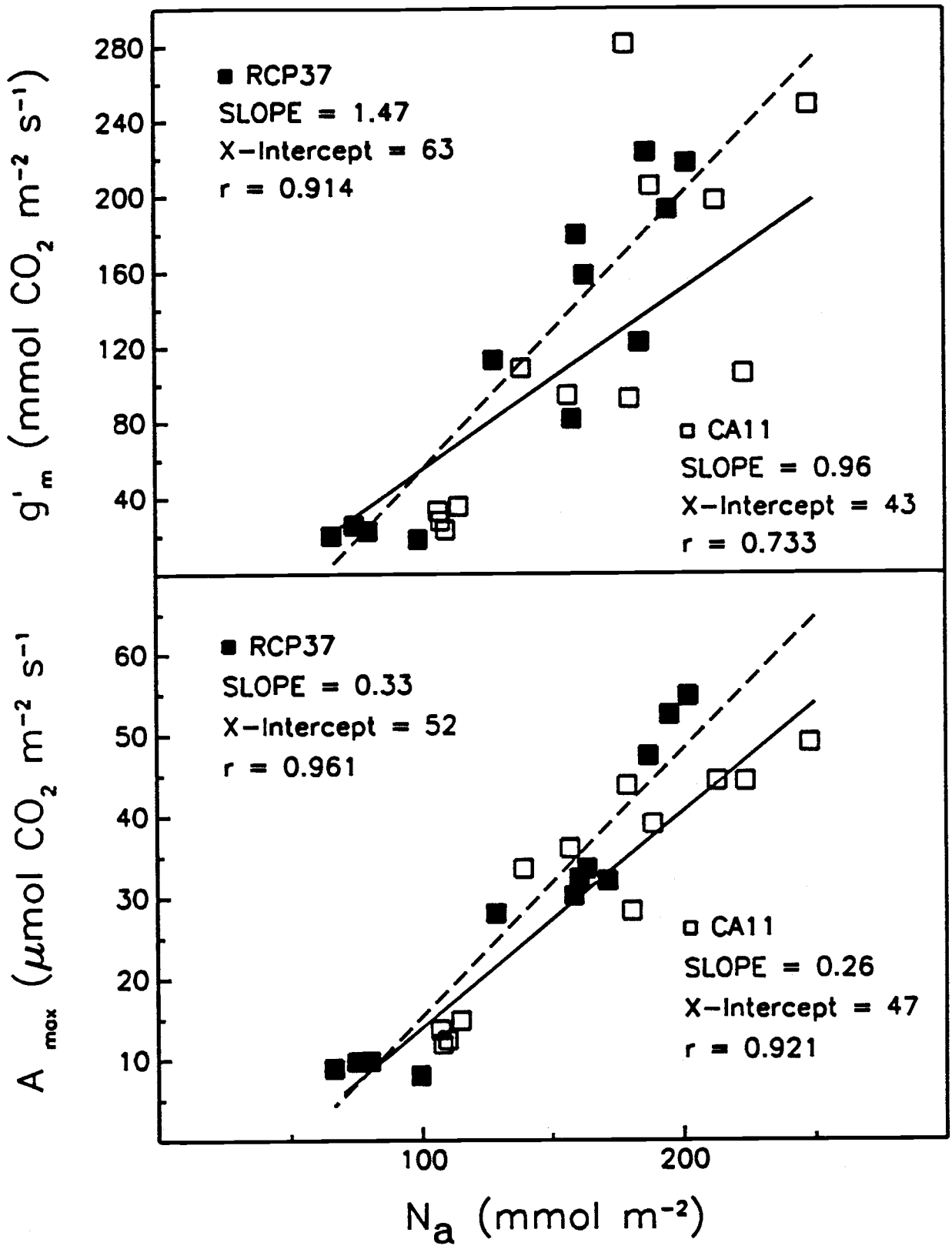

Fig. 5. Linear regression of A_{max} and g'_m with increasing leaf nitrogen. Data for A_{max} are those from Fig. 3, whereas data for g'_m are from Fig. 4.