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TERMINAL REPORT

"CO₂ Exchange, Environmental Productivity Indices, and Productivity of Agaves and Cacti under Current and Elevated Atmospheric CO₂ Concentrations"

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SUMMARY

The research described in the proposal investigated net CO₂ uptake and biomass accumulation for an extremely productive CAM plant, the prickly pear cactus *Opuntia ficus-indica*, under conditions of elevated CO₂ concentrations for relatively long periods. The influences of soil water status, air temperature, and the photosynthetic photon flux (PPF) on net CO₂ uptake over 24-h periods were evaluated to enable predictions to be made based on an Environmental Productivity Index (EPI). Specifically, EPI predicts the fraction of maximal daily net CO₂ uptake based on prevailing environmental conditions. It is the product of indices for temperature, soil water, and intercepted PPF, each of which range from 0.00 when that index factor completely inhibits net CO₂ uptake to 1.00 when no limitation occurs. For instance, the Water Index is 1.00 under wet conditions and decreases to 0.00 during prolonged drought. Although the major emphasis of the research was on net CO₂ uptake and the resulting biomass production for *O. ficus-indica*, effects of elevated CO₂ concentrations on root: shoot ratios and on the activities of the two carboxylating enzymes were also investigated. Moreover, experiments were also done on other CAM plants, including *Agave deserti*, *Agave salmiana*, and *Hylocereus undatus*, and *Stenocereus queretaroensis*.

QUESTIONS ADDRESSED IN PROPOSAL

Five major questions were asked in the proposal. These questions were modified by the review process, which also recommended focusing the research on *Opuntia ficus-indica*. The five questions will be listed in the order originally presented and the corresponding findings briefly summarized. The numbers in

parentheses refer to the manuscripts based on the funded research that are listed at the end of this section and that contain the detailed information.

1. How do elevated CO₂ concentrations alter the diel gas exchange pattern of CAM plants? With regard to this question, reviewers recommended using field experiments in open-top chambers in addition to growth chambers, considering effects of plant age, and determining water-use efficiency.

The ambient CO₂ concentration was increased from the current mean value in the Los Angeles vicinity of about 360 to 370 $\mu\text{mol mol}^{-1}$ to 520 and 720 $\mu\text{mol mol}^{-1}$ for *Opuntia ficus-indica* planted in open-top chambers at the University of California, Riverside. Eight weeks after daughter cladodes (cladodes are flattened stem segments for prickly pear cacti) emerged on the basal (planted) cladodes, daily net CO₂ uptake was 35% higher at 520 $\mu\text{mol mol}^{-1}$ and 49% higher at 720 $\mu\text{mol mol}^{-1}$ than at 370 $\mu\text{mol mol}^{-1}$ CO₂(2). Water-vapor conductance was 9 to 15% lower at the doubled CO₂ concentration (2, 5). Daily water-use efficiency was 88% higher under the doubled CO₂ concentration for basal cladodes and 57% higher for daughter cladodes. The daily net CO₂ uptake capacity for basal cladodes increased for 4 weeks after planting and then remained fairly constant (depending on light level), whereas for daughter cladodes it increased with cladode age, became maximal at 8 to 14 weeks, and then declined gradually. But even at 13 to 15 months after emergence, daily net CO₂ uptake for daughter cladodes was 30 to 50% higher at 720 than at 370 $\mu\text{mol mol}^{-1}$ CO₂ (4, 6, 7), similar to enhancements observed for many C₃ plants but much greater than for C₄ plants. At the current CO₂ concentration, about 5% of the total daily net CO₂ uptake occurred during the daytime; the daytime contribution increased to 14 to 17% when the CO₂ concentration was doubled. Changes in gas exchange were accompanied by major changes in chloroplast ultrastructure (15).

2. How do elevated CO₂ concentrations affect the root-shoot ratio of CAM plants?

To examine effects on root-shoot ratios at current and doubled CO₂ concentrations, *O. ficus-indica* was grown in environmentally controlled rooms for 18 weeks in pots of three soil volumes (2,600, 6,500, and 26,000 cm³), the smallest of which was intended to restrict root growth (3). For plants in the medium-sized soil volume, basal cladodes tended to be thicker and the surface areas of both main and lateral roots tended to be greater at 380 μmol mol⁻¹ CO₂ than at 740 μmol mol⁻¹ CO₂. Increasing the soil volume 10-fold led to a greater stimulation of daily net CO₂ uptake and biomass production than did doubling the CO₂ level. At 18 weeks, root biomass doubled and shoot biomass nearly doubled as soil volume was increased 10-fold; the effects of soil volume tended to be greater for elevated CO₂. The cladode nitrogen per unit dry weight decreased as the CO₂ level was raised, as also occurs for other plants, and increased as soil volume increased, the latter suggesting that the effects of soil volume could be due to nitrogen limitations. For *O. ficus-indica* growing for 23 weeks in open-top chambers, the root: shoot ratio doubled when the atmospheric CO₂ level was doubled (2), which in turn affected the aboveground productivity (4).

3. How do elevated CO₂ concentrations affect the productivity of CAM plants? In response to this question, the reviewers recommended focusing on *O. ficus-indica*, which had already been demonstrated to have an extremely high productivity, as well as examining carboxylating enzymes to help gain a mechanistic interpretation at the cellular level.

Initiation of new daughter cladodes was monitored until canopy closure occurred (about 3 months after planting the detached cladodes in open-top chambers and adjacent field plots), after which bimonthly harvests maintained the plants for one year at a cladode area per unit ground area that is optimal for biomass

production (4). Doubling the CO₂ concentration slightly increased the number of first-order daughter cladodes growing on the basal cladodes after 3 months and nearly doubled the number and surface area of second-order daughter cladodes. When the CO₂ level was doubled, cladodes were 5% thicker after a few months and 11 to 16% thicker after one year (1). Although the productivity enhancement by elevated CO₂ tended to decrease during the year, the annual aboveground dry-mass gain was 37 to 40% higher when the CO₂ level was doubled, reaching the equivalent of 65 tons hectare⁻¹ year⁻¹ in a field plot.

The activities of RuBisCO and PEPCase measured *in vitro* for samples isolated from the chlorenchyma of mature cladodes were both lower when the CO₂ concentration was doubled (6, 7, 13). Enzyme activity increased with the age of second-order daughter cladodes, becoming maximal at 6 to 10 days. The effect of a doubled CO₂ concentration on RuBisCO and PEPCase activity declined with decreasing irradiance, especially for RuBisCO. Thus the observed increased net CO₂ uptake by *O. ficus-indica* occurred despite lower activities of both carboxylating enzymes. Such effects of the doubled CO₂ concentration were the result of a higher fraction of RuBisCO in the activated form *in vivo* and changes in K_m's (13).

4. How do elevated CO₂ concentrations affect the component indices of EPI?

For *O. ficus-indica* growing in controlled environment chambers, raising the total daily photosynthetic photon flux (PPF) from 5 to 13 to 20 mol m⁻² day⁻¹ increased daily net CO₂ uptake as well as the biomass of cladodes and roots (5). Averaged for the two CO₂ concentrations (370 and 720 μmol mol⁻¹), total plant dry weight increased 66% from the low to the medium PPF and 37% from the medium to the high PPF. First-order daughter cladodes from well-watered plants at day/night air temperatures of 25°C/15°C and a total daily PPF of 15 mol m⁻² day⁻¹ had 74% more total daily net CO₂ uptake at 730 than at 370 μmol mol⁻¹ CO₂ (4).

Under a doubled CO₂ concentration, less carbohydrate was translocated from basal to daughter cladodes than under current CO₂ concentrations (1). With the doubled CO₂ concentration, the percentage enhancement of daily net CO₂ uptake increased as the PPF was lowered, as the day/night temperature was raised or lowered, and as the soil water became more limiting during drought. Thus doubling the atmospheric CO₂ had subtle but measurable effects on the component indices of EPI.

5. How do the suite of environmental changes accompanying elevated CO₂ concentrations affect the productivity of CAM plants?

Based on the measured effects of elevated CO₂ concentrations on the component indices of EPI, predictions can now be made for net CO₂ uptake and hence productivity of *O. ficus-indica*. However, the generality of the effects of elevated CO₂ concentrations on CAM plants should first be checked. Preliminary experiments with two other cacti (*Hylocereus undatus* and *Stenocereus queretaroensis*) and two agaves (*Agave deserti* and *A. salmiana*) have shown that 20 to 60% enhancements in daily net CO₂ uptake accompany a doubling of the atmospheric CO₂ concentration for a period of 2 to 12 months for these species as well (9, 11, 14).

Other changes accompanying exposure to elevated CO₂ concentrations but at an anatomical level that affect gas exchange have also been investigated for *O. ficus-indica*. For instance, its chlorenchyma becomes thicker but contains less chlorophyll per unit area when the atmospheric CO₂ concentration is doubled (7, 8). Also, more epicuticular wax occurs and the cladode reflectance for PPF increases for elevated CO₂ concentrations. Experiments based on $\delta^{13}\text{C}$ to help determine substrate processing through RuBisCO versus PEPCase and hence daytime versus nighttime CO₂ fixation at elevated CO₂ concentrations proved inconclusive. In particular the introduced CO₂ is a byproduct from the petroleum industry and its low isotopic $\delta^{13}\text{C}$

value (-70 ‰) confused interpretations of the carboxylase discrimination because the carbohydrate partitioning pattern is not yet known for *O. ficus-indica*. In this regard, a technique has recently been developed using the stylets from female cochineal insects (*Dactylopius opuntiae*) that penetrate a few mm of tissue to reach the phloem (10). This has enabled an assessment of carbohydrate partitioning in this highly productive CAM species (12).

PUBLICATIONS RESULTING FROM THE ORIGINAL FUNDING

1. Luo, Y. and P.S. Nobel. 1993. Growth characteristics of newly initiated cladodes of *Opuntia ficus-indica* as affected by shading, drought and elevated CO₂. *Physiol. Plant.* 87:467-474.
2. Cui, M., P.M. Miller, and P.S. Nobel. 1993. CO₂ exchange and growth of the Crassulacean acid metabolism plant *Opuntia ficus-indica* under elevated CO₂ in open-top chambers. *Plant Physiol.* 103:519-524.
3. Nobel, P.S., M. Cui, P.M. Miller, and Y. Luo. 1994. Influences of soil volume and an elevated CO₂ level on growth and CO₂ exchange for the Crassulacean acid metabolism plant *Opuntia ficus-indica*. *Physiol. Plant.* 90:173-180.
4. Nobel, P.S. and A.A. Israel. 1994. Cladode development, environmental responses of CO₂ uptake, and productivity for *Opuntia ficus-indica* under elevated CO₂. *J. Exp. Bot.* 45:295-303.
5. Cui, M. and P.S. Nobel. 1994. Gas exchange and growth responses to elevated CO₂ and light levels for the CAM species *Opuntia ficus-indica*. *Plant Cell Environ.* 17:935-944.
6. Israel, A.A. and P.S. Nobel. 1994. Activities of carboxylating enzymes in the CAM species *Opuntia ficus-indica* grown under current and elevated CO₂ concentrations. *Photosyn. Res.* 40:223-229.

7. Nobel, P.S., M. Cui, and A.A. Israel. 1994. Light, chlorophyll, carboxylase activity, and CO₂ fixation at various depths in the chlorenchyma of *Opuntia ficus-indica* (L.) Miller under current and elevated CO₂. *New Phytol.* **128**:315-322.
8. North, G.B., T.L. Moore, and P.S. Nobel. 1994. Cladode development for *Opuntia ficus-indica* (Cactaceae) under current and doubled CO₂ concentrations. *Amer. J. Bot.*, in press.
9. Raveh, E., M. Gersani, and P.S. Nobel. 1994. CO₂ uptake and fluorescence responses for a shade-tolerant cactus *Hylocereus undatus* under current and doubled CO₂ concentrations. *Physiol. Plant.*, in press.
10. Wang, N. and P.S. Nobel. 1994. Phloem exudate collected via scale insect stylets for the CAM species *Opuntia ficus-indica* under current and doubled CO₂ concentrations. *Ann. Bot.*, in press.
11. Graham, E.A. and P.S. Nobel. 1994. Long-term and environmental effects of doubled atmospheric CO₂ concentration on the CAM plant *Agave deserti*. In preparation.
12. Wang, N. and P.S. Nobel. 1994. Influence of doubled CO₂ concentration on photoassimilate partitioning in source and sink tissues of *Opuntia ficus-indica*. In preparation.
13. Nobel, P.S. and A.A. Israel. 1994. Growth, CO₂ uptake, and responses of carboxylating enzymes to inorganic carbon for two highly productive CAM species at current and doubled CO₂. Submitted.
14. Nobel, P.S. 1994. Responses of cultivated CAM plants to doubled CO₂ concentrations and freezing temperatures -- Implications of global climate change. In preparation.
15. Balsamo, R.A. and P.S. Nobel. 1994. Chloroplast structure for the CAM species *Opuntia ficus-indica* at various times of day and irradiances for current and doubled CO₂ concentrations. Submitted.

PERSONNEL TRAINED DURING FUNDED PERIOD

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