



Direct and Maternal Effects of Elevated CO₂ on Early Root Growth of Germinating *Arabidopsis thaliana* Seedlings

CHRISTOPHE ANDALO*, CHRISTIAN RAQUIN*, NATHALIE MACHON†, BERNARD GODELLE*‡ and MARIANNE MOUSSEAU*

* *Laboratoire d'Ecologie, Systématique et Evolution, URA CNRS 2154, Batiment 362, Université Paris Sud, 91405 Orsay Cedex, France*, † *Museum d'Histoire Naturelles, rue Buffon, Paris, France* and ‡ *Institut National Agronomique Paris-Grignon, rue Claude Bernard, Paris, France*

Received: 28 July 1997 Returned for revision: 26 August 1997 Accepted: 14 November 1997

Individuals of *Arabidopsis thaliana*, collected in different natural populations, were grown in controlled and elevated CO₂ in a glasshouse. Following germination, root growth of progeny of different lines of these populations was studied in control and elevated atmospheric CO₂. No significant direct effect of atmospheric CO₂ concentration could be demonstrated on root growth. An important parental effect was apparent, namely that root length and branching were decreased in seeds collected from a mother plant which had been grown in elevated CO₂. This was correlated with smaller seeds, containing less nitrogen. These parental effects were genetically variable. We conclude that CO₂ may affect plant fitness via parental effects on seed size and early root growth and that the genetic variability shown in our study demonstrates that *Arabidopsis* populations will evolve in the face of this new selective pressure.

© 1998 Annals of Botany Company

Key words: Root growth, root branching, seed, elevated CO₂, natural population, *Arabidopsis thaliana*, parental effect.

INTRODUCTION

Among the effects of elevated atmospheric CO₂ on plant growth, there is increasing recognition that a greater root biomass is produced (Rogers, Runion and Krupa, 1993; Day *et al.*, 1996). Changes in the distribution of roots may also be a critical component of a plant's response to elevated CO₂, changing the volume of soil explored by roots and the density of roots within a given volume of soil (Bernston, McConnaughay and Bazzaz, 1993). In natural situations, it may lead to greater competition between root systems by increasing the overlap of root foraging zones between individuals (Bazzaz, 1990).

Many studies have shown that elevated CO₂ results in the production of more, and longer, roots due to an increase in both root branching and root length of plants grown in elevated CO₂ (Del Castillo *et al.*, 1989; Rogers, Prior and O'Neill, 1992*a*; Rogers *et al.*, 1992*b*). Elevated CO₂ substantially affects elongation rates of root systems (Kaushal, Guehl and Aussenac, 1989; Jongen *et al.*, 1995) and root density (both biomass and length per unit volume of soil) especially in the upper layers of soil (Chaudhuri, Kirkham and Kanemasu, 1990; Curtis *et al.*, 1990; Day *et al.*, 1996). The overall morphology of root architecture is thus changed by the aerial environment (Bernston and Woodward, 1992).

At the seedling stage, it is difficult to make predictions of plant establishment and how seedlings will forage for minerals and water without prior knowledge of how root morphogenesis responds to elevated CO₂. There have been few systematic investigations into the effects of CO₂

concentration at early stages of root growth. Although it is generally recognized that increased temperature results in a greater rate of germination and a faster initial growth rate than cool temperatures, the influence of elevated CO₂ on this critical developmental stage is poorly understood. Conflicting results can be found in the literature regarding the role played by CO₂ concentration on germination: from an inhibition which was species-dependent (Bibey, 1948; Popay and Roberts, 1970), to no effect (Radoglou and Jarvis, 1993) but effective in combination with ethylene (Jones and Hall, 1979) or even enhancement (Ziska and Bunce, 1993; Yoshiokai *et al.*, 1995).

The physiological response of root growth is an important component of the response to rising CO₂. However, as the total number of seeds produced by a plant and their germination potential are life history traits closely related to plant fitness, they could also mediate an evolutionary response. Therefore, these characteristics have been studied to assess the potential selective effect of elevated CO₂. Some studies have reported variation in the response to elevated CO₂ among species, others among individuals of the same species. In a study on *Plantago lanceolata*, Wulff and Alexander (1985) found that high CO₂ increased germination rate and seed size of progeny. They suggested the existence of genetic variability in these characteristics. On the other hand, it has recently been found that the effect of elevated CO₂ during the maturation of seeds of *Arabidopsis thaliana* on the mother plant slowed germination and was genotypically variable (Andalo *et al.*, 1996).

The present work studied the effect of elevated CO₂ on early germination and root growth of *Arabidopsis thaliana*,

with special attention to the morphogenesis of root architecture, in order to see if the effect depends on the CO₂ treatment itself and/or on the CO₂ conditions during growth of the mother plant. Specifically, the questions we addressed were: (1) is the atmospheric CO₂ concentration acting directly on the early germination of *Arabidopsis*?; (2) is there a detectable effect of CO₂ during growth and seed production of the parent plant on subsequent germination of the seed and growth of the seedling?; and (3) is there genetic variation in these CO₂ responses?

MATERIALS AND METHODS

Plant material

Arabidopsis thaliana Heynh. (Brassicaceae) is a small, annual herb that lives in disturbed habitats (Meyerowitz, 1987). Three natural populations, one from England (Oxford Botanical Garden, B) and two from Loiret, France (C, shady area; D, sunny area) were chosen. In each population, five lines were used. Each line corresponds to the progeny (obtained in a glasshouse) of one plant sampled in the field and is considered to be genetically homogenous because of the large proportion of self-fertilization in this species (less than 1% outcrossing: M. Lefranc, Orsay, pers. comm.). Seeds were collected at maturity in the glasshouse in March 1993 for population B and in July 1993 for populations C and D, and stored in a seed cabinet at 5 °C and 35% humidity.

Three individual plants of the five maternal lines for each of the three populations were previously grown simultaneously in control (C) and in elevated (E) CO₂ conditions and the seeds of these individual plants were harvested at maturity in June 1994 and stored in the seed cabinet. This first treatment was termed the parental CO₂ treatment (Fig. 1A).

Seedling growth

Seeds were surface sterilized by soaking for 15 min in a mixture containing 90% (v/v) ethanol 95° and a 10% (w/v) aqueous solution of sodium dichloroisocyanurate and rinsed twice in 95° ethanol (C. Bellini, pers. comm.). After sterilization, seeds were sown in 12 × 12 cm square Petri dishes on a medium described by Estelle and Sommerville (1987) with three slight modifications: 2.5 mol m⁻³ KH₂PO₄ was replaced by 2 mol m⁻³ KH₂PO₄ and 0.5 mol m⁻³ M KH₂PO₄; sucrose was reduced to 29 mol m⁻³; and Phytigel 0.5% (w/v, Sigma) was used as gelling agent instead of agar so that the medium was directly buffered to pH 6.1 before autoclaving (20 min at 18 °C). Plates sealed with a surgical tape (Urgopore, Fournier, Chenove, France) permeable to gas could be cultivated vertically without sweating. They were then pre-incubated in a cold chamber in the dark at 4 °C for 7 d to break seed dormancy. This treatment is usually sufficient to induce germination of most of the seeds (M. Lefranc, unpubl. res.).

Experimental design

For each combination of genotype (five lines × three populations) and parental CO₂ treatment, 20 seeds (of one

individual) were sown into each of two Petri dishes in each growth chamber, as indicated in Fig. 1B, ten seeds of one individual being randomly associated with ten seeds of another one. In total, 1200 seeds were used for each experiment. Seeds were sown with their root pole orientated so that roots would grow down the surface of the nutrient medium (Fig. 1C). The plates were distributed between the chambers as shown in Fig. 1B. Plates were then placed in large CO₂ chambers (1 m × 1.20 m), made of an aluminium frame covered with transparent polypropylene film, located in a controlled environment room (phytotron chambers of Paris-Sud University). In order to avoid a progressive increase in CO₂ concentration in the phytotron, the chambers were continuously flushed with outside air ('Control') or with outside air + 350 μl l⁻¹ industrial CO₂ ('Elevated') at a constant rate. The light regime was 16 h d⁻¹ (PAR: 600 μmol m⁻² s⁻¹). Temperature was approximately regulated to 15 °C night/21 °C day, but the two chambers differed slightly. Therefore, the CO₂ conditions were permuted between two successive experiments to reduce any chamber effect and data from these two experiments were pooled (see 'Statistical analysis' below).

Measurements

The length of each primary root was measured from hypocotyl to the root tip on day 6 and 9. Day 1 was the day on which the seeds were taken out of the cold chamber and placed in the growth chambers. On day 9, all the plants were killed with glutaraldehyde (250 mg per plate) and the number of roots greater than 1 mm in length was counted for each individual.

Before germination, length (L) and width (W) of a minimum of five seeds for three individuals of each line were measured under the microscope and the volume (V) was then calculated assuming that they were cylindrical using the formula

$$V = \pi(W/2)^2 L$$

Carbon and nitrogen contents of seeds from three individuals of the different lines of population B were determined from the combustion of seed samples (about 4 mg) in a Carlo Erba elemental analyser (type NA1500NCS, Thermoquest, Courtaboeuf, France).

Statistical analysis

Growth and branching of the early roots. Analysis of variance was performed using the SAS software (SAS Institute Inc. 1990). Data from the two successive experiments were pooled. To take account of the different environmental conditions (other than CO₂ concentration) created by these two successive experiments and by the two chambers, a variable called environment (Env) was created with environments 1 and 2 corresponding to expts I and II in elevated and controlled CO₂ chambers, respectively, and environments 3 and 4 corresponding to expts I and II in control and elevated CO₂, respectively. We tested the effect of environment (within the CO₂ treatment of the seeds), lines (within populations), CO₂ treatment of the seeds,

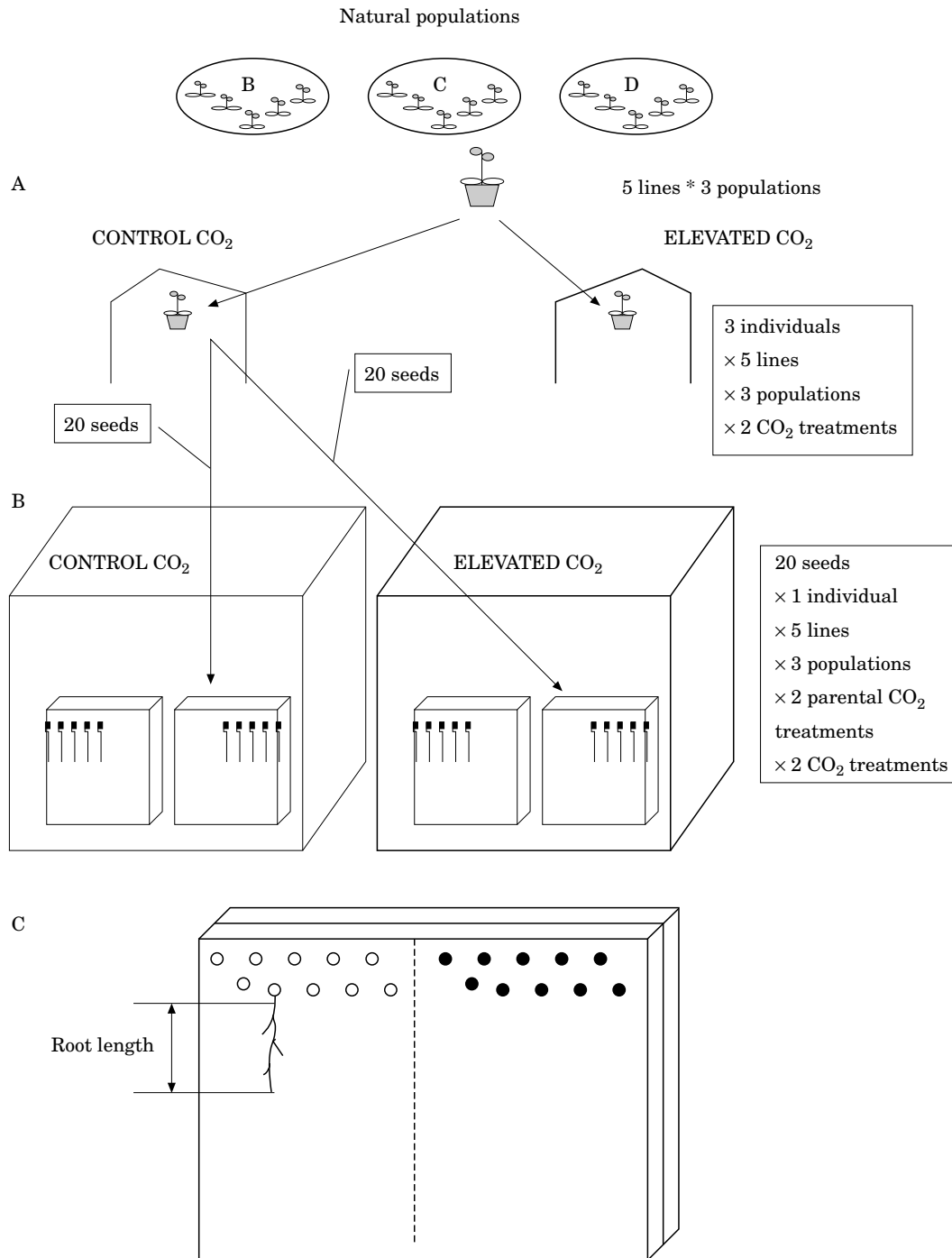


FIG. 1. Schematic design of the experiment (see text for details) showing the parental treatments (A), the CO₂ treatments during seed germination (B) and the seeds displayed in vertical Petri dishes (C). Black and white dots represent seeds from different origins (genotypes × parental CO₂ treatment).

parental CO₂ treatment, populations, and their interactions. The first two main effects were considered as random effects, the three last were considered as fixed. For all the measured traits, normality was approximate and the usual transformations did not improve normality and did not change the results of the analysis of variance.

Seed volume. For this trait, the analysis tested the effects

of individual (within lines and populations), parental CO₂ treatment, population, lines (within populations) and their interactions. Lines were considered a random effect.

Carbon and nitrogen content of seeds from population B. The model considered three effects: parental CO₂ treatment; lines; and their interaction. Lines were considered a random effect.

RESULTS

Direct effect of elevated CO₂ on radicle growth

Atmospheric CO₂ concentration had no significant effect on the length of the root axis on day 6 or 9. Elevated CO₂ increased the number of lateral roots in the first experiment, but slightly decreased it in the second. The global statistical analysis of the pooled data using the ANOVA model described above did not show a significant effect of CO₂. On the other hand, this analysis shows that environmental factors, other than CO₂ have a highly significant influence on root growth.

Effect of CO₂ concentration during growth on seed characteristics of progenies

Measurement of length and width of seeds from all populations and lines allowed us to compare the average seed volume of progenies. Table 1 shows a comparison for parents grown in control or elevated CO₂. In all populations, parents grown in elevated CO₂ produced smaller seeds than those grown in control CO₂, but this CO₂ effect depended on the line, as shown by the significant interaction between lines and CO₂ treatment ($P = 0.0471$).

The N and C content of seeds was measured in population B. Elevated CO₂ during growth of the parents decreased the nitrogen content of the seeds (Table 1). As a consequence, the C/N ratio increased. There was no significant interaction between lines and CO₂ treatment.

Effect of CO₂ treatment during parental growth on early root growth of progeny

There was a very significant parental effect both on main root growth of the seedling and on branching of these roots (Table 2). On the whole, progenies of parents grown in elevated CO₂ had smaller roots (both on day 6 and 9) and fewer laterals than the progenies of parents grown in control conditions. However, this effect of parental CO₂ treatment also depended on lines (Fig. 2), as the interaction lines × parental CO₂ treatment was significant for root growth and branching ($P < 0.01$).

A Pearson correlation coefficient (between lines) was calculated between the response of root growth to the CO₂

TABLE 2. Mean root length (in mm) at day 6 and 9 and average number of lateral roots (> 1 mm) for seeds from two parental treatments (control vs. elevated)

Parental treatment	Root length day 6	Root length day 9	Number of lateral roots
Control	28.2 (6.2)	71.5 (11.5)	10.4 (3.6)
Elevated	24.2 (6.9)	64.4 (14.0)	8.6 (4.0)
† Significance	**	**	**

Standard deviation is given in parentheses.

† Significance of difference in means: ** $P < 0.01$.

concentration for growth of parents (either the length of the primary root or the number of lateral roots) and the seed volume (Fig. 3). The correlation was always significant ($P < 0.05$).

DISCUSSION

Elevated CO₂ during germination did not influence early root length either directly or in interaction with the genotypes. This result contradicts the overall finding that CO₂ enrichment generally increases root growth (see Rogers *et al.*, 1992a, b), which was always related to increased photosynthesis of the aerial green parts. It must be remembered that the process we have studied here is less dependent on photosynthesis than classical growth experiments: the emergence of the first leaves occurred between day 6–9 in our experiment.

One may argue that looking for an effect of a doubled ambient CO₂ concentration on germination is unrealistic, since seeds are very often buried in the soil where the CO₂ concentration is about ten times greater than in the air above. This may not be the case for *Arabidopsis* seeds which, once dispersed, remain on the soil surface, and more generally for all seeds which need light to germinate. When germination has been observed specifically, either from radicle growth or from seed emergence, some authors have observed similar results, i.e. no detectable effect of elevated CO₂ (Garbutt, Williams & Bazzaz, 1990; Radoglou and Jarvis, 1993). In *Rumex palustris*, for example, CO₂ concentrations as high as 10% did not greatly influence root

TABLE 1. Average volume (mm³) of a seed produced by *Arabidopsis* cultivated under contrasted CO₂ treatments (control and elevated) and carbon and nitrogen content (% dry matter) of seeds produced by plants from one population (B)

Parental treatment	Volume of seed	Carbon content (Population B)	Nitrogen content (Population B)	C/N ratio (Population B)
Control	0.033 (0.008)	53.4 (2.85)	3.48 (0.27)	15.45 (1.74)
Elevated	0.030 (0.008)	54.68 (3.57)	2.97 (0.32)	18.6 (2.51)
† Significance	*	ns	*	*

Standard deviation is given in parentheses.

† Significance of difference in means: * $P < 0.05$; ns, non significant.

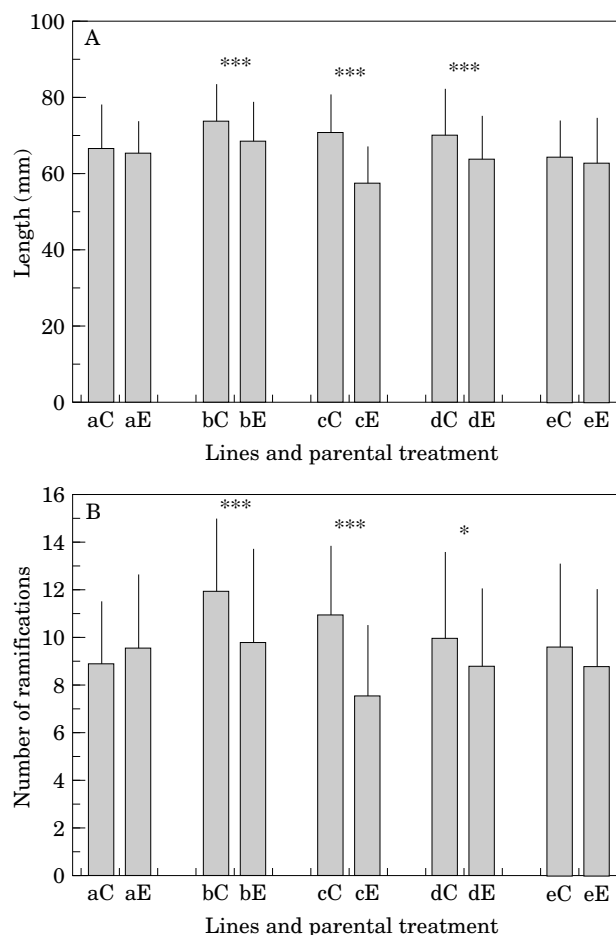


Fig. 2. Intrapopulation variation in the CO₂ response of radicle growth of different lines (a, b, c, d) of *Arabidopsis thaliana* issued from the same natural population B. A, Root length 9 d after sowing; B, branching 9 d after sowing. Parental growth conditions: C, control CO₂; E, elevated CO₂.

elongation and a complete absence of CO₂ hardly affected it (Visser *et al.*, 1997). In contrast, Ferris and Taylor (1994) found that fine root extension rate and cell elongation were sensitive to elevated CO₂. In all the species they tested, the plasticity of the cell wall increased.

We did not find any significant direct effect of atmospheric CO₂ on root length and branching. The mechanisms by which elevated CO₂ could affect germination and early growth are still poorly understood. Contrasting effects of CO₂ have been found in different species (Yoshioka *et al.*, 1995) and different mechanisms have been suggested: CO₂ enrichment may indirectly regulate primary carbon metabolism via ethylene production, which is enhanced under elevated CO₂ and is an internal growth regulator (Grodzinski, 1992). Sangwan, Singh and Plaxton (1992) suggested that during the early phase of seed germination, PEPcarboxylase has a critical function in building up the metabolic pools required to initiate significant growth. Thus it could be possible that elevated CO₂ could increase activity of this enzyme in the radicle.

One of the striking effects of elevated CO₂ found in this study is the parental effect on early seed germination. This

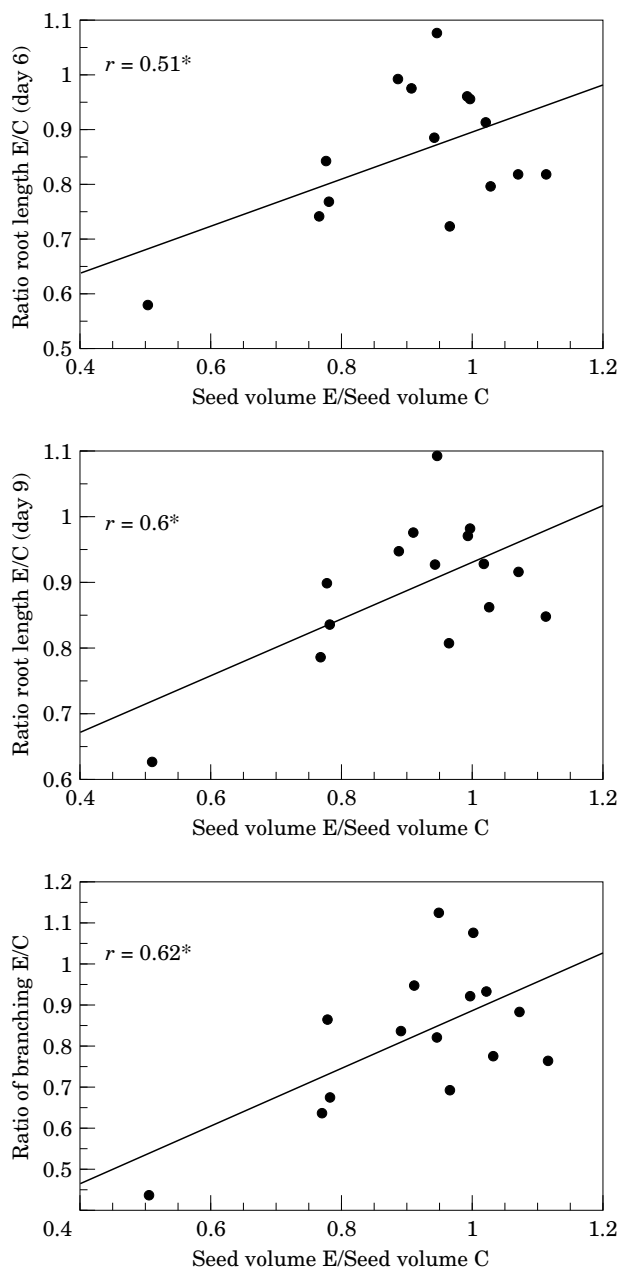


Fig. 3. Correlation between the effect of elevated CO₂ on seed volume and its relative effect between lines (3 × 5 lines) on root length at day 6 and 9 and on the number of lateral roots. Plant growth conditions: C, control CO₂; E, elevated CO₂.

effect had already been noted by Andalo *et al.* (1996), but it is documented here in more detail: growth of the root both in length and number of branches was decreased when seeds were collected from a mother plant which was grown in elevated CO₂.

The slower root growth rate of seedling progenies of mother plants from populations grown in elevated CO₂ is, very probably, related to the seed size, as shown by comparison of Tables 1 and 2. Even in very small seeds, such as those of *Arabidopsis*, the quantity of metabolic reserves is very likely to be related to seed size. In the only population

tested (population B, Table 1), composition of the seed reserves was correlated with root growth: analysis shows an increase in the C/N ratio of the seeds produced by parents grown in elevated CO₂, which indicates that their protein content should be smaller than in seeds from control parents. The increased C/N ratio of plant tissue under CO₂ enrichment is a classical result, but it has not often been described in seeds. Wheat grown in elevated CO₂ showed differences in seed quality, namely alteration in lipid composition (Williams *et al.*, 1995) and decreased protein content (Blumenthal *et al.*, 1996; Sanhewe *et al.*, 1996) but these were not related to a subsequent change in germination capacity and potential survival.

The effect of parental CO₂ treatment on root growth depends on the line but, as shown by correlation coefficients (Fig. 3), the genetic variability in root growth response may be explained by the genetic variability in seed volume. In other words, elevated CO₂ affects the seed volume and, consequently, root growth of the seedling produced. However, all genotypes do not respond in the same way. Krannitz, Aarssen and Dow (1991) have shown that, independent of root growth, seedlings from genotypes of *Arabidopsis thaliana* with larger seeds survived longer than seedlings from genotypes with smaller seeds when nutrient supply was deficient. Indeed, the variation in seed size may be an important adaptive factor in the future changing environment (Westoby, Jurado and Leishman, 1992).

In conclusion, the increasing CO₂ concentration will probably have a large influence on fitness via its impact on the parent plant, and the genetic variability shown in our study demonstrates that populations will evolve in the face of this new selective pressure.

ACKNOWLEDGEMENTS

The authors thank Dr David Lawlor for thorough language revision of this paper. They also acknowledge the skilful technical assistance of Madeleine Lefranc. Thanks are due to C. Bellini for kindly providing us with an easy seed sterilization procedure.

LITERATURE CITED

- Andalo C, Godelle B, Lefranc M, Mousseau M, Till-Bottraud I. 1996. Elevated CO₂ decreased seed germination in *Arabidopsis thaliana*. *Global Change Biology* **2**: 129–137.
- Bazzaz FA. 1990. Response of natural ecosystems to the rising CO₂ levels. *Annual Review of Ecology and Systematics* **21**: 167–196.
- Berntson GM, McConnaughay KDM, Bazzaz FA. 1993. Elevated CO₂ alters deployment of roots in 'small' growth containers. *Oecologia* **94**: 558–564.
- Berntson GM, Woodward I. 1992. The root system architecture and development of *Senecio vulgaris* in elevated CO₂ and drought. *Functional Ecology* **6**: 324–333.
- Bibey RO. 1948. Physiological study of weed seeds germination. *Plant Physiology* **23**: 467–484.
- Blumenthal R, Rawson HM, McKenzie E, Gras PW, Barlow EWR, Wrigley CW. 1996. Changes in wheat grain quality due to doubling the level of atmospheric CO₂. *Cereal Chemistry* **73**: 762–766.
- Chaudhuri UN, Kirkham MB, Kanemasu ET. 1990. Root growth of winter wheat under elevated carbon dioxide and drought. *Crop Science* **30**: 853–857.
- Curtis PS, Balduman LM, Drake BG, Whigham DF. 1990. Elevated atmospheric CO₂ effects on below ground processes in C₃ and C₄ estuarine marsh communities. *Ecology* **71**: 2001–2006.
- Day F, Weber WP, Hinkle CR, Drake BG. 1996. Effects of elevated atmospheric CO₂ on fine root length and distribution in an oak-palmetto scrub ecosystem in central Florida. *Global Change Biology* **2**: 143–148.
- Del Castillo D, Acock B, Reddy VR, Acock MC. 1989. Elongation and branching of roots on soybean plants in a carbon dioxide enriched aerial environment. *Agronomy* **81**: 692–695.
- Estelle MA, Somerville C. 1987. Auxin resistant mutants of *Arabidopsis thaliana* with an altered morphology. *Molecular and General Genetics* **207**: 200–206.
- Ferris R, Taylor G. 1994. Increased root growth in elevated CO₂: a biophysical analysis of root cell elongation. *Journal of Experimental Botany* **45**: 1603–1612.
- Garbutt K, Williams WE, Bazzaz FA. 1990. Analysis of the differential response of five annuals to elevated CO₂ during growth. *Ecology* **71**: 1185–1194.
- Grodzinski B. 1992. Plant nutrition and growth regulation by CO₂ enrichment. *Bioscience* **42**: 517–525.
- Jones JF, Hall MH. 1979. Studies on the requirement for carbon dioxide and ethylene for germination of *Spergula arvensis* seeds. *Plant Science Letters* **16**: 87–91.
- Jongen M, Jones MB, Hebeisen T, Blum H, Hendrey G. 1995. The effects of elevated CO₂ concentrations on the root growth of *Lolium perenne* and *Trifolium repens* grown in a FACE system. *Global Change Biology* **1**: 361–371.
- Kaushal P, Guehl JM, Aussenac G. 1989. Differential growth response to atmospheric carbon dioxide enrichment in seedlings of *Cedrus atlantica* and *Pinus nigra* ssp. *Laricio* var. *Corsicana*. *Canadian Journal of Forest Research* **19**: 1351–1358.
- Kranitz PG, Aarssen LW, Dow JM. 1991. The effect of genetically based differences in seed size on seedling survival in *Arabidopsis thaliana* (Brassicaceae). *American Journal of Botany* **78**: 446–450.
- Meyerowitz EM. 1987. *Arabidopsis thaliana*. *Annual Review of Genetics* **21**: 93–111.
- Popay AI, Roberts EH. 1970. Factors involved in the dormancy and germination of *Capsella bursa-pastoris* L. and *Senecio vulgaris* L. *Journal of Ecology* **58**: 103–122.
- Radoglou KM, Jarvis PG. 1993. Effects of atmospheric CO₂ enrichment on early growth of *Vicia faba*, a plant with large cotyledons. *Plant, Cell and Environment* **16**: 93–98.
- Rogers HH, Petterson CM, McCrimmon JN, Cure JD. 1992b. Response of plant roots to elevated atmospheric carbon dioxide. *Plant, Cell and Environment* **15**: 749–752.
- Rogers H, Prior AA, O'Neill E. 1992a. Cotton root and rhizosphere responses to free-air CO₂ enrichment. *Critical Review in Plant Sciences* **11**: 251–263.
- Rogers HH, Runion GB, Krupa SV. 1993. Plant responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**: 155–189.
- Sangwan RS, Singh N, Plaxton WC. 1992. Phosphoenolpyruvate carboxylase activity and concentration in the endosperm of developing and germinating castor oil seeds. *Plant Physiology* **99**: 445–449.
- Sanhewe AJ, Ellis RH, Hong TD, Wheeler TR, Batts GR, Hadley P, Morison JIL. 1996. The effects of temperature and CO₂ on seed quality development in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **47**: 631–637.
- Visser EJW, Nabben RHM, Blom CWPM, Voeselek LACJ. 1997. Elongation by primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. *Plant, Cell and Environment* **20**: 647–654.
- Westoby M, Jurado E, Leishman M. 1992. Comparative evolutionary ecology of seed size. *TREE* **7**: 368–372.
- Williams M, Shewry PR, Lawlor DW, Harwood JR. 1995. The effects of elevated temperature and atmospheric carbon dioxide concentration on the quality of grain lipids in wheat (*Triticum aestivum* L.) grown at two levels of nitrogen application. *Plant, Cell and Environment* **18**: 999–1009.

Wulff RD, Alexander HM. 1985. Intraspecific variation in the response to CO₂ enrichment in seeds and seedlings of *Plantago lanceolata*. *Oecologia* **66**: 458–460.

Yoshioka T, Ota H, Segawa K, Takeda Y, Esashi Y. 1995. Contrasted effects of CO₂ on the regulation of dormancy and germination in

Xanthium pennsylvanicum and *Setaria faberi* seeds. *Annals of Botany* **76**: 625–630.

Ziska LH, Bunce AJ. 1993. The influence of elevated CO₂ and temperature on seed germination and emergence from soil. *Field Crops Research* **34**: 147–157.