REVIEW PAPER

Effects of elevated CO₂ concentration on seed production in C₃ annual plants

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Abstract

The response of seed production to CO₂ concentration ([CO₂]) is known to vary considerably among C₃ annual species. Here we analyse the interspecific variation in CO₂ responses of seed production per plant with particular attention to nitrogen use. Provided that seed production is limited by nitrogen availability, an increase in seed mass per plant results from increase in seed nitrogen per plant and/or from decrease in seed nitrogen concentration ([N]). Meta-analysis reveals that the increase in seed mass per plant under elevated [CO₂] is mainly due to increase in seed nitrogen per plant rather than seed [N] dilution. Nitrogen-fixing legumes enhanced nitrogen acquisition more than non-nitrogen-fixers, resulting in a large increase in seed mass per plant. In Poaceae, an increase in seed mass per plant was also caused by a decrease in seed [N]. Greater carbon allocation to albumen (endosperm and/or perisperm) than the embryo may account for [N] reduction in grass seeds. These differences in CO₂ response of seed production among functional groups may affect their fitness, leading to changes in species composition in the future high-[CO₂] ecosystem.

Key words: Carbon dioxide, functional groups, meta-analysis, nitrogen use, reproduction, seed nitrogen concentration, seed quality.

Introduction

Atmospheric CO₂ concentration ([CO₂]) has increased from 280 µmol mol⁻¹ before the industrial revolution to the present 390 µmol mol⁻¹. The rate of increase is currently 1.9 µmol mol⁻¹ per year and [CO₂] at the end of this century may reach 500–1000 µmol mol⁻¹ (IPCC, 2007). Because plant functions such as photosynthesis, transpiration, and biomass production are sensitive to [CO₂], elevated [CO₂] influences future ecosystem functions and agricultural yield.

Seed production is a vitally important event in plant life. For wild (undomesticated) plants, seed quantity and quality directly influence their fitness. Seeds are also important for humans as they provide foods, oil, and other useful materials. Several authors have reviewed CO₂ response of seed production (Kimball, 1983, 1986; Cure, 1985; Cure and Acock, 1986; Amthor, 2001; Jablonski et al., 2002; Ziska and Bunce, 2007). These reviews showed that elevated [CO₂] significantly enhanced seed mass per plant by 28–35%. However, considerable variations are observed in the extent of seed production enhancement. Miyagi et al. (2007) reported that the ratio of seed mass per plant in elevated [CO₂] to that in ambient [CO₂] ranged from 0.54 to 4.45 in the literature (Fig. 1).

Here, we analyse the interspecific variation in CO₂ response of seed production with respect to nitrogen use. Nitrogen is one of the most important resources that limit plant growth and seed production in natural and agricultural ecosystems (Aerts and Chapin, 2000). An increase in carbon availability due to elevated [CO₂] may enhance nitrogen limitation, leading to a reduction in plant nitrogen concentration ([N]). We hypothesize that nitrogen use in
reproductive organs accounts for the variation in CO₂ response of seed production.

**Variation in CO₂ response of seed production in annual plants**

Large variation in CO₂ response of seed production is found not only between species but also within a species. In our literature survey, the enhancement ratio of seed mass per plant by [CO₂] elevation ranged from 0.75 to 4.45 in rice (42 observations), from 0.93 to 1.87 in soybean (28 experiments), and from 0.88 to 2.07 in wheat (51 experiments). These variations were due to the different growth conditions in the studies. In addition, large variations were observed among species grown under the same conditions (Garbutt and Bazzaz, 1984; Garbutt et al., 1990). For example, 11 annual species showed that seed production enhancement due to elevated [CO₂] ranged from 0.84 to 2.12 (Miyagi et al., 2007; see Fig. 3a).

Why does the CO₂ response of seed production vary between species? The variation in seed production may reflect the variation in total plant mass. However, previous studies have demonstrated that seed production enhancement was not closely associated with vegetative growth enhancement. In the meta-analysis by Jablonski et al. (2002), the mean relative yield increase due to elevated [CO₂] was 12% and 25% in fruits and seeds respectively, which was lower than the response of total plant mass (31%). In some cases, elevated [CO₂] did not affect, or even reduced, reproductive yield, although vegetative mass was increased (Larigauderie et al., 1988; Fajer et al., 1991; Farnsworth and Bazzaz, 1995; Kinugasa et al., 2003; Lewis et al., 2003). Comparing six old-field annuals, Ackerly and Bazzaz (1995) demonstrated that the CO₂ effect on total mass was correlated with that on seed production; however, the correlation was rather weak. Therefore, CO₂ responses of reproductive yield should be considered differently from the CO₂ responses of vegetative growth.

CO₂ response differed between reproductive tissues in a species. Kimball et al. (2002) reported that free-air CO₂ enrichment enhanced the boll (seed+lint) yield of cotton by 40%, while it increased the lint fibre portion of the yield even more, by 54%. In soybean, elevated [CO₂] increased the pod-wall mass more than seed yield (Allen et al., 1988). Kinugasa et al. (2003) found that seed production in Xanthium canadense did not respond to [CO₂], but capsule mass increased by 86% with elevated [CO₂] (Fig. 2a). Elevated [CO₂] may increase the mass of a reproductive structure with a low [N] more than a structure with a high [N] (Hikosaka et al., 2005; Hirose et al., 2005). Indeed capsules had very low [N] in X. canadense (Fig. 2b).

**Nitrogen use in reproductive growth and CO₂ response**

Two hypotheses were postulated on the variation in CO₂ response of seed production in annuals (Miyagi et al., 2007):
(i) CO₂ response of seed production varies between species because the limiting factor of seed production differs from species to species. Elevated [CO₂] would enhance seed production only in the species whose seed production was limited by carbon as in capsules of *X. canadense*. If seed production is limited to a greater extent by nitrogen in species with high seed [N], as in seeds of *X. canadense*, a negative correlation is expected between seed [N] and CO₂ enhancement of seed production; (ii) alternatively, if seed production is always limited by nitrogen availability (Hirose et al., 2005), it would be enhanced by elevated [CO₂] only when nitrogen became more available for seed production. If there were no additional nitrogen available at elevated [CO₂], there would be no enhancement in seed mass per plant. The enhancement ratio of seed mass per plant would be correlated with the enhancement ratio of seed nitrogen per plant. These hypotheses were tested with 11 C₃ annuals grown at different [CO₂] (Miyagi et al., 2007). The enhancement of seed mass per plant by elevated CO₂ was not correlated with mean seed [N]; therefore, the first hypothesis was rejected. In contrast, a strong 1:1 relationship was observed between the enhancement ratio of seed mass per plant and that of seed nitrogen per plant (Fig. 3). This result supported the second hypothesis that seed production was enhanced only when nitrogen became more available at elevated [CO₂]. Seed production was limited by nitrogen rather than by carbon, and elevated [CO₂] increased seed production only in plants that exhibited increased nitrogen in seeds.

Why was seed nitrogen per plant enhanced to a greater extent in some species at elevated [CO₂] than in others? Seed nitrogen per plant may be enhanced if: (i) plants absorb more nitrogen during vegetative growth; or (ii) plants allocate acquired nitrogen more to reproductive growth, which involves nitrogen retranslocation from vegetative organs (Miyagi et al. 2007). Miyagi et al. (2007) found that the fraction of acquired nitrogen allocated to reproduction tended to decrease at elevated [CO₂], and that the increase in seed nitrogen per plant at elevated [CO₂] was due mainly to increased nitrogen acquisition. In addition, among the 11 species, legumes exhibited a more pronounced increase in nitrogen acquisition and enhanced seed production with elevated [CO₂] than non-leguminous species. Symbiotic bacteria perform nitrogen fixation, which entails carbon cost for nitrogen assimilation with nodule construction. As a greater amount of carbon is available at elevated [CO₂], legumes may allocate more carbon for nitrogen fixation, and hence increase nitrogen assimilation (Poorter, 1993; Soussana and Hartwig, 1996; Stewart and Potvin, 1996; Hebeisen et al., 1997; Lüscher et al., 2000; Jablonski et al., 2002; Lee et al., 2003). In fact, elevated [CO₂] enhanced nodulation and seed production increased to a greater extent in species that produced more nodules at elevated [CO₂] (Miyagi et al., 2007). Using an isogenic line of soybean that lacks the ability to form nodules, Oikawa et al. (2010) showed that elevated [CO₂] enhanced seed production in nodulated soybean but not in its non-nodulated mutant (Fig. 4).

Some non-nitrogen-fixing species exhibited increased nitrogen uptake at elevated [CO₂] but others did not. The different nitrogen uptake rate may be ascribed to differences in root size or in kinetics of nitrogen uptake. The former may be responsible if elevated [CO₂] enhanced root size, as has been shown in some studies (e.g. Rogers et al., 1992; Dippery et al., 1995). Some authors hypothesized that elevated [CO₂] increases nitrogen uptake per unit root mass (BassiriRad et al., 2001), because the nitrogen absorption rate in roots often increases when plant [N] is lowered (Lee and Drew, 1986; Lee and Rudge, 1986) and enhanced carbon availability is expected to provide additional energy for nitrogen absorption and assimilation. However, there are many reports that are not consistent with these.

![Fig. 3.](image-url)
hypotheses (BassiriRad et al., 1996, 1997; Zerihun and BassiriRad, 2001). Different responses of nitrogen uptake to elevated [CO₂] among species (Roumet et al., 1996) would lead to different reproductive outputs and, subsequently, the number of offspring that potentially influence species composition in a future high-[CO₂] world.

It should also be noted that legumes are not always more responsive to elevated [CO₂] compared with non-legumes; CO₂ responses in whole-plant mass, leaf area index, and photosynthetic rates were not greater in legumes than in other functional types (Long et al., 2004; Ainsworth and Long, 2005; Ziska and Bunce, 2007). Some legumes even responded negatively to elevated [CO₂] (West et al., 2005).

Oikawa et al. (2010) found that biomass production was greater in the non-nodulated mutant than in the wild type in the vegetative growth phase, while the inverse was true in the reproductive growth phase. Because large quantities of carbohydrates are consumed by nodules, nitrogen fixation may be less advantageous when soil nitrogen is available. This may partly explain the variation across studies with regard to CO₂ responses in legumes.

**CO₂ response of seed [N] and its effect on seed production**

There was a strong 1:1 relationship between the enhancement of seed mass and seed nitrogen per plant (Fig. 3a). This was caused by seed [N], which did not change much with elevated [CO₂]. In contrast to that in seeds, [N] in vegetative tissues of many species decreased with elevated [CO₂] (Lutze and Gifford, 1998; Ishizaki et al., 2003; Miyagi et al., 2007). These results suggest that [N] in seeds is more conservative than [N] in vegetative tissues.

Other analyses, however, revealed that CO₂ response of seed [N] differed significantly between species. Jablonski et al. (2002) demonstrated a significant decrease in seed [N] in wheat and barley but not in soybean. Taub et al. (2008) also demonstrated a significant decrease in seed [N] in wheat, barley, and rice but not in soybean. They argued that seed [N] decreased at elevated CO₂ in non-legumes but not in legume species because of nitrogen fixation. However, these studies compared only a few species of legumes and grass species but no species from other groups, and thus the hypothesis has not been thoroughly tested.

If seed [N] decreased with elevated [CO₂], how does it impact seed production? A decrease in seed [N] may imply increment in seed production under limited nitrogen availability.

Here we present a simple model to evaluate CO₂ response in seed production. Seed mass per plant (M) is expressed as seed nitrogen per plant (N) divided by seed [N] ([N]):

\[ M = \frac{N}{[N]} \]

Then seed-mass response to elevated CO₂ is given as follows:

\[ R_M = R_N \times \frac{1}{R_{[N]}} \]

where \( R_M \), \( R_N \), and \( R_{[N]} \) are enhancement ratios, respectively, of M, N, and [N] due to elevated [CO₂]. This equation implies that, if seed production is limited by nitrogen availability, plants may enhance seed mass per plant at elevated [CO₂] by increasing seed nitrogen per plant or by reducing seed [N].

Figure 5 illustrates the relationship between the enhancement ratio of seed nitrogen per plant (R_N) and that of seed [N] (R_{[N]}). Isoclines represent the enhancement ratio of seed production (R_M). This figure indicates whether enhancement of seed production is due to enhancement of seed nitrogen per plant or reduction of seed [N] (Equation 2). Data are compiled from the literature (see Supplementary Data. 4. (a) Dry mass, (b) nitrogen concentration, and (c) nitrogen of seeds of nodulated soybean (Glycine max cv. Enrei) and the non-nodulated isogenic line (G. max cv. En1282) grown at ambient (white bars, 385 μmol mol⁻¹) and elevated (black bars, 593 μmol mol⁻¹) [CO₂]. Error bars represent ±SE (n=5). *** P<0.001, NS not significant (t-test). Data from Oikawa et al. (2010).
data), including 61 observations of 23 species from 22 studies, where plants were grown at ambient (350±50 μmol mol⁻¹) and elevated (700±50 μmol mol⁻¹) [CO₂]. We did not include data obtained under stressful conditions such as water deficit, ozone exposure, and UV enrichment. Because large variations were observed within species among different studies, data were presented for each observation rather than using a species average. We separated the species into three functional groups: grass (Poaceae), legume (Fabaceae), and non-legume dicot species. Most observations were distributed on isoclines Rₘ>1 (Fig. 5), indicating that seed mass per plant was enhanced by elevated [CO₂]. Total seed nitrogen per plant increased in 43 out of 61 observations (70%) and seed [N] decreased in 52 observations (85%); however, variation in seed [N] was 3.5 times lower than that of seed nitrogen per plant (standard deviation was 0.083 and 0.293, respectively; Fig. 5): seed [N] was less variable compared with seed nitrogen per plant. There were significant differences in CO₂ responses in seed nitrogen per plant and seed [N] between functional groups (Figs 5, 6).

Although all groups (grass, legume, and non-legume dicot species) exhibited increased seed mass per plant with elevated [CO₂], legumes exhibited a greater extent of increased seed nitrogen per plant than other groups (Kruskal–Wallis test, \( P<0.05 \)). In contrast, seed [N] decreased in grass species by 12% and in non-legume dicots by 4% with elevated [CO₂] but not in legumes. A non-parametric multiple comparison test (Kruskal–Wallis test) indicated that grass species had a larger reduction in seed [N] with elevated [CO₂] than did legume species (\( P<0.001 \)) and also marginally larger than did non-legume species (\( P=0.057 \)), while there was no significant difference between legume and non-legume dicot species in this respect (\( P=0.37 \)).

These results suggest that contributions to the enhancement of total seed mass of the two underlying factors (seed nitrogen per plant and seed [N]) were different for different functional groups. Contributions may be calculated from Equation 2 after log₁₀ transformation.

\[
\log_{10} Rₘ = \log_{10} Rₙ + \log_{10}(1/Rₙ)
\]

Log transformation was performed because the ratios showed skewed distribution (0<R<∞). This, in turn, makes the equation additive; therefore, the contributions of the two underlying factors can be calculated as \( (\log_{10}Rₙ/\log_{10}Rₘ) \) and \( [\log_{10}(1/Rₙ)]/\log_{10}Rₘ \), respectively (sum to 1) (Poorter and van der Werf, 1998).

This calculation was applied for all observations that had >10% seed-mass enhancement (46 observations). Across all species, three-quarters of the variation in seed-mass enhancement with elevated [CO₂] was explained by an increase.
in seed nitrogen per plant and a quarter by reduction of seed [N] (median 75% compared with 25%). Within grass species, reduction of seed [N] was a relatively important contributor (median 37%) for seed-mass enhancement with elevated [CO₂] while the rest (63%) was due to the enhancement of seed nitrogen per plant (Fig. 7). In contrast, in legumes, reduction in seed [N] was approximately zero and seed-mass enhancement was nearly entirely due to increased seed nitrogen per plant. Non-legume dicot species showed an intermediate response; 85% (median) of seed-mass enhancement was due to increased seed nitrogen and the rest due to reduction of seed [N]. These results suggest that the reduction of seed [N] under elevated [CO₂] was an important mechanism in increasing seed production in grass species but not as much in other groups.

The presence of albumen (endosperm and perisperm) in seed may account for different CO₂ responses of seed production in different functional groups. Albumen stores large quantities of carbohydrates and its [N] is relatively low (e.g. Cakmak et al., 2010). Grass species produce albuminous seeds, while legumes produce exalbuminous seeds. In the present study, other non-legume dicot species consisted mainly of Asteraceae species, which also produce exalbuminous seeds. The embryo has higher [N] (Cakmak et al., 2010) and may not change its composition with elevated [CO₂]. If elevated [CO₂] increased the amount of albumen relative to the embryo, seed [N] would decrease. This is analogous to the amount of accessory tissues, such as lint in cotton and capsules in X. canadense that are more responsive to elevated [CO₂], as discussed above.

Then why did seed [N] not change in exalbuminous species with [CO₂] elevation? Seed [N] may be important for germination and subsequent growth. It has been shown that high seed [N] is advantageous for quick germination (Andalo et al., 1996, 1998; Hara and Toriyama, 1998) and in competition with other plants after germination (Parrish and Bazzaz, 1985; Huxman et al., 1998), especially under low nitrogen availability (Naegle et al., 2005). Seed [N] may be regulated to a certain level to maintain ‘quality’ of growth after germination. If so, we may hypothesize that reduction of seed [N] in grass species is due to an increased albumen content, not at the expense of seed quality. However, some species have exhibited decrease in both seed [N] and seed nitrogen per plant (Fig. 5). In such species, growth and survivorship of seedlings may be negatively affected by elevated [CO₂].

**Conclusion**

Nitrogen limitation is a key factor in understanding CO₂ responses of seed production in annual plants. Plants can increase seed production with elevated [CO₂] when they increase nitrogen acquisition or decrease seed [N]. Legumes are advantageous in this respect because they may increase nitrogen fixation with an increased supply of assimilates. Grass species increase seed production with elevated [CO₂] by decreasing seed [N] as well as increasing nitrogen acquisition. Decrease in seed [N] in grass may not imply reduction in seed quality if it occurs due to increased albumen content without reduced [N] in embryos. However, if reduction in seed [N] is associated with reduced seed quality, it will affect survivorship and growth of their progeny. Increasing atmospheric [CO₂] may affect species composition in future ecosystems through differential effects on seed quality and quantity in different plant functional groups.

**Supplementary data**

Supplementary data are available at JXB online.

Supplementary data are the references used in the meta-analysis shown in Fig. 5.
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References


