Effects of elevated CO₂ concentration on photosynthetic acclimation and productivity of two potato cultivars grown in open-top chambers

Ad H.C.M. SchapendonkAC, Marcel van OijenB, Paul DijkstraA, C. Sander PotA, Wilco J.R.M. JordiA and Geert M. StoopenA

APlant Research International, PO Box 16, 6700 AA Wageningen, The Netherlands.
BCentre for Ecology and Hydrology (CEH), Edinburgh Research Station, Bush Estate, Penicuik, EH26 0QB, Scotland.
CCorresponding author; email: mail@plant-dynamics.nl

Abstract. In two subsequent years, an early maturing potato cultivar with low leaf area index (LAI) and a late cultivar with high LAI were grown at concentrations of 350 and 700 µL CO₂ L⁻¹ in open-top chambers. The average increase of tuber dry matter yield by elevated CO₂ was 27% in 1995 and 49% in 1996. During the first weeks after planting, elevated CO₂ stimulated the light-saturated rate of photosynthesis (Amax) of both cultivars by 80%. However, Amax under elevated CO₂ declined to the level of the low-CO₂ treatment in the course of the growing season. In 1995 this convergence due to acclimation of photosynthesis was completed within 6 weeks, but in 1996, acclimation proceeded until the end of the growing season. Photosynthetic acclimation was accompanied by a reduced Rubisco content, and was correlated more closely with accumulation of sucrose than of starch. From fluorescence measurements it was concluded that, in contrast to the carboxylation efficiency, the efficiency of photosynthetic reactions centers was not affected by acclimation to elevated CO₂. The faster photosynthetic acclimation in 1995 coincided with overall lower values of Amax, crop growth rate and growth response to elevated CO₂. It is shown that the indeterminate growth pattern of potato with its large sink capacity does not preclude acclimation. The effect of acclimation on yield was quantified by computer simulations. The simulated results indicated that photosynthetic acclimation reduced the positive effect of elevated CO₂ on tuber yield by 50%.

Keywords: acclimation, climate change, elevated CO₂, photosynthesis, open-top chambers, potato, simulation model, Solanum tuberosum, source–sink.

Introduction

An elevated atmospheric CO₂ concentration stimulates the productivity of a broad range of agricultural crops (Kimball 1983; Lawlor and Mitchell 1991). However, the range of values obtained for the stimulation of Net Primary Production (NPP) by elevated CO₂ is often very large, even within species (e.g. 7–97% for wheat; Poorter 1993). In the comparison between species and cultivars it is essential to pay attention to the growing conditions. Interaction between temperature, light and phenology is quite decisive for the overall effect (Grashoff et al. 1995; Morison and Lawlor 1999). In a closed canopy of winter wheat, doubled CO₂ stimulated daily net photosynthesis by 5% in early spring, but 40% in late spring (Dijkstra et al. 1999).

Other important crop-specific properties such as the length of the vegetative stage (Acock et al. 1985), the time until canopy closure and start of senescence (Curtis et al. 1989; Grulke et al. 1990), the ability to store carbon in storage pools (Ammerlaan and de Visser 1993) and the use of carbohydrates as an energy source for nitrogen fixation (Ryle et al. 1992a, b) all have a significant influence on yield response to climate change. The initial stimulation of photosynthesis can be reduced by direct feedback inhibition due to accumulation of chemical intermediates and, in the longer term, by a decrease of the transcripts that encode for the nuclear-encoded small subunit of Rubisco (Rbcs) and other proteins involved in photosynthesis (Van Oosten and Besford 1994). It has been proposed that an increase in hexose sugars leads, via hexokinase-related signaling, to a repression of Rbcs and other genes leading to a decrease in Rubisco. An extensive review on this subject has recently been published by Moore et al. (1999).

Abbreviations used: Amax, photosynthetic rate of a leaf at light saturation; BSA, bovine serum albumin; Fm, maximum fluorescence during a saturating light pulse; Fo, minimum fluorescence in the dark; fw, fresh weight; LAI, leaf area index; OTC, open-top chamber; PAR, photosynthetically active radiation; Rbcs, small subunit of Rubisco; Rubisco LSU, large subunit of Rubisco; SDS, sodium dodecyl sulfate; SLA, specific leaf area; ΦPC, efficiency of energy transfer to open photosystem II reaction centres.

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Initial effects of CO₂ on photosynthesis and growth are thus often not sustained as a consequence of decreased leaf Rubisco content (Webber et al. 1994; Van Oosten and Besford 1995). Regulation of photosynthesis by this feedback mechanism is relatively well understood at the physiological level (Stitt 1991), but the consequences for productivity at the whole-plant and canopy levels are still speculative (Farrar and Williams 1991; Stitt and Knapp 1999).

The concept that photosynthetic acclimation is mainly expressed in determinate ‘sink-limited’ plants and absent in indeterminate or ‘source-limited’ plants is probably an oversimplification (Morison and Lawlor 1999). Potato is an excellent species to test the hypothesis related to this issue, that developmentally restricted capacity for growth or storage would lead to a feedback control on photosynthesis. Potato is not only an indeterminate plant; it also develops such large sinks that yield is expected to be limited by assimilate supply only, unless export is hampered by a blockage of phloem loading, or in the case that tuber filling is not a source-dependent as usually assumed. This was discovered in another major storage crop, sugar beet, that showed an unexpected sink control of CO₂ stimulation despite the large sink capacity of its tap root (Demmers-Derks et al. 1998).

Potato contains substantial genetic variation for tuber filling, reflecting phenological differences in sink activity. This makes comparison of potato cultivars an appropriate model system for study of sink–source interactions and acclimation under elevated CO₂. However, data from experiments with potato are scarce. The reported effects of CO₂ doubling on potato yield vary between slightly negative (Goudriaan and de Ruiter 1983) to a positive effect of 39% (Wheeler et al. 1991). In a Free Air CO₂ Enrichment (FACE) experiment, a potato crop grown in the field under otherwise natural conditions was exposed to elevated CO₂ by means of a sophisticated fumigation device (Miglietta et al. 1998), and plants were exposed to a gradient of CO₂ concentrations ranging from ambient to 460, 560 and 660 µmol mol⁻¹. An average increase in yield of 10% for each step of 100 µmol mol⁻¹ increase in CO₂ was observed. Similar stimulation numbers have been reported in a recent paper on potatoes in open-top chambers (OTCs) (Sicher and Bunce 1999).

Strong accumulation of starch in leaves of potato plants that were grown under elevated CO₂ has been observed (Goudriaan and de Ruiter 1983; Sicher and Bunce 1999). Attempts were made to relate this phenomenon to photosynthetic acclimation by Ludewig et al. (1998), but their results showed an inverse correlation between starch content of leaves and photosynthetic acclimation. It thus seems that not starch but an intermediate sugar is responsible for acclimation (Stitt and Knapp 1999). The occurrence of acclimation itself is often difficult to distinguish from senescence (Miglietta et al. 1998). Overall, the results obtained with potato suggest that photosynthetic acclimation is a complex process involving potentially interactive effects of elevated CO₂ with source–sink relationships and with the canopy energy balance.

We hypothesize that the effect of elevated CO₂ on potato yield is determined by the summed effect of two mechanisms, both of which affect early and late cultivars to different extent. First, CO₂ affects source strength by increasing photosynthesis and growth of biomass and leaf area index (LAI). The increase in LAI itself will enhance light interception and thereby cause additional growth stimulation, but mainly in early maturing cultivars because only they are characterized by low LAI at ambient CO₂. Elevated CO₂ will increase light interception in early cultivars, both by faster canopy closure and by less vulnerability to leaf losses during the stage of senescence. The second mechanism through which elevated CO₂ affects early and late cultivars differentially is by the interaction of CO₂ with sink strength. Late cultivars have higher tuber production rates, which provide them with a more durable sink for the enhanced carbohydrate production under elevated CO₂, so photosynthetic acclimation is expected to be less in late cultivars than in early ones. Because the ‘source mechanism’ described above favours early cultivars, and the ‘sink mechanism’ favours late ones, the overall effect of elevated CO₂ on yield of potato will be determined by the summed effect. Interactions with environmental conditions will determine whether acclimation effects have a negative effect on yield or not.

The work presented in this paper aims at quantifying both mechanisms and thereby identifying the one of major importance. The importance of inter-annual variation in temperature and irradiance with respect to the complex interaction between acclimation, senescence and yield is illustrated. We complete our analysis by using a simulation model to quantify the consequences of the photosynthetic acclimation for tuber yield and to assess any possible improvement of the CO₂ effect.

Materials and methods

Experimental set-up

Two OTC experiments were carried out in 1995 and 1996, on light clay soil (pH 7.5, organic matter content 6%), in Wageningen, the Netherlands (51°58’ N, 5°40’ E). The experiments were performed in a factorial set-up, with two levels of CO₂ and two cultivars, in three replicates, giving 12 OTCs in all, and a fifth treatment consisting of six ambient plots. The two cultivars of potato (Solanum tuberosum L.) were the early maturing cultivar Gloria and the late maturing cultivar Elles. Single eye pieces were cut from sprouted tubers in April, and placed in containers filled with white sand and supplied daily with a nutrient solution prepared according to Steiner (1961). After emergence, the young single stem sprouts were uprooted and transplanted in OTCs in the field on 16–17 May 1995 and 13 May 1996 (days of the year 136–137 and 133, respectively). Additional potato sprouts were planted in a 0.75-m-wide border outside the OTCs to prevent irradiation from the sides. CO₂ was supplied via tubes on the soil surface and via an airbag at 1.3-m height. Additional tubes on the soil surface were used for irrigation whenever the topsoil dried out visibly. Dry soil had a
mineral nitrogen content of 4.68 g kg⁻¹. In addition, 15 g m⁻² K₂O and 51.7 g m⁻² Ca(H₂PO₄)₂ were added to the soil in late October. Nitrogen was supplied at the beginning of April (17.5 g m⁻²), the beginning of June (15.0 g m⁻²) and the beginning of July (4.0 g m⁻²). The plantlets were planted 0.20 m apart with a spacing of 0.25 m between rows, giving a total density of 20 plantlets m⁻². Each plot consisted of 40 or 41 plants. Plots were watered through a drip-irrigation system. The experiments were set up as randomized block designs with three replicates. The CO₂ concentration was maintained at 350 µL L⁻¹ in the low-CO₂ treatment and 700 µL L⁻¹ in the high-CO₂ treatment.

**Statistical analyses**

Statistical analysis was carried out separately for the 1995 and 1996 experiments. All measurements were analysed using analysis of variance (ANOVA) corresponding to the two-factorial randomized block designs used; the ambient plots were not included in the ANOVAs.

**Open-top chambers**

The OTCs were constructed as equilateral hexagons with side width and length of 0.87 m, height of 1.95 m, and a volume of 3.8 m³. Ground area inside the chamber was 1.95 m². Chambers were made from 3-mm polycarbonate (Lexan®; GE Plastics Europe BV, Bergen op Zoom, The Netherlands), without chamber supports. A frustum of 0.25 m, an angle of 45° was added on top. The material was 88% transparent for PAR, but absorbed all UV-B below 385 nm. Air was blown into the chamber by a blower, through a series of manifolds and pipes placed in the soil before planting. Air entered the chamber through small straight upward pipes. A windbreaker, mounted over the pipes, reduced the wind speed from 30 m s⁻¹ in the pipes to less than 2 m s⁻¹ at soil level. A second air-inlet system was a circular flexible transparent PVC tube, at about 2/3 of the OTC height, well over the canopy. Chamber air was replaced 3.6 times min⁻¹. Temperature and CO₂ concentration were measured in all chambers every 6 min. Pure CO₂ was added at the ventilator inlet and thoroughly mixed inside the OTC. CO₂ concentration was measured and adjusted when needed. Average low-CO₂ levels were 335 to 380 µL L⁻¹ in both years, and high-CO₂ levels were 712 to 720 µL L⁻¹ in 1995, and 720 to 746 µL L⁻¹ in 1996. There was a higher average radiation level in 1995 than in 1996: 19.4 compared to 16.8 MJ m⁻² d⁻¹. Full light interception was reached around day 175. In 1996, full light interception was delayed for cultivar Gloria because of infestation by Verticillium dahliae, which occurred especially in OTCs where Gloria was grown at elevated CO₂. The data were not considered in the analyses.

(2) On 24 July 1995 and 22 July 1996 (days of the year 205 and 203), i.e. before crop senescence, half of the area of each plot was harvested (first harvest). Border effects for the remaining area were avoided by placing shade cloth in the OTC at the borders of the harvested areas. The final harvests for both cultivars were on 14 August 1995 and 12 August 1996 (days 226 and 224). At each harvest, dry and fresh weight (fw) of leaves, stems and tubers were determined.
Simulation model

The potato crop growth model applied in the present study has been described by Spitters and Schapendonk (1990). Light interception by the canopy is calculated as a function of the LAI following Beer’s law (describing the exponential extinction of light in a certain medium). Thermal time determines how photosynthates are allocated to leaves, stems, roots and tubers. Death rate of leaves due to senescence depends on the maturity class of the cultivar. For the present analysis, the constant light use efficiency of the original model was replaced by a calculation of photosynthesis based on the biochemical model of Farquhar et al. (1980). The effects of acclimation on yield were estimated by comparing simulation runs with input data of non-acclimated $A_{\text{max}}$ values and input data of acclimated $A_{\text{max}}$ values.

Results

Ground cover and leaf area dynamics

Ground cover in spring was similar for all treatments (Fig. 1). The plots without OTCs (ambient) produced more leaves than the treatments in the OTCs, and maintained a higher LAI until the end of the season. In 1996, senescence started late, which coincided with lower temperatures and lower irradiances in that year compared with 1995 (Fig. 2). At the first harvest at the end of July (d 203–205), the weight of leaves (green and dead) and LAI were not significantly affected by elevated CO2 concentration (Tables 1 and 2) for both cultivars. The late cultivar, however, produced twice the

Fig. 1. Seasonal course of ground cover by green foliage in 1995 and 1996. Vertical bars indicate standard errors ($n = 3$).

Fig. 2. Seasonal course of daily global radiation and daily average temperature. The curves show 7-d moving averages.
leaf weight of the early cultivar, making it less vulnerable to leaf senescence. At final harvest, green leaf weight was higher under elevated CO2 in the early cultivar and lower in the late cultivar (significant interaction at \( P < 0.05 \)). During the 3-week period between the first and final harvest in 1995, the late cultivar lost 24\% of its green leaves at low CO2, but 57\% at elevated CO2. These results are in agreement with the decrease in ground cover of the late cultivar, especially in 1995 (Fig. 1). Senescence of the early cultivar did not respond to CO2 concentration (Table 1).

Specific leaf area (SLA) was significantly decreased by elevated CO2 in both years and for both cultivars (Table 2). This then compensated for the small CO2 stimulation in leaf weight, so LAI was not increased.

In summary, the results show that light interception is not affected much by elevated CO2, but that the underlying dynamics of leaf growth and senescence vary strongly with environmental conditions and cultivar. There are no effects of CO2 on LAI until canopy closure but a faster decline of LAI was observed at elevated CO2 under warm conditions.

**Other components of crop biomass**

Elevated CO2 had similar effects on stem biomass as on leaves. Biomass allocation between above-ground plant parts

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**Table 1. Data from destructive harvests in OTCs**

<table>
<thead>
<tr>
<th>Year</th>
<th>Variable</th>
<th>Harvest yield (g dry mass m(^{-2}))</th>
<th>Statistical significance</th>
<th>CO2 effect (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elles, 350</td>
<td>Elles, 700</td>
<td>Gloria, 350</td>
<td>Gloria, 700</td>
</tr>
<tr>
<td>1995</td>
<td>First harvest (24 July)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves + stems + tubers</td>
<td>1054</td>
<td>1235</td>
<td>848</td>
</tr>
<tr>
<td></td>
<td>Green leaves</td>
<td>155</td>
<td>141</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Dead leaves</td>
<td>19</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>82</td>
<td>70</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Tubers</td>
<td>797</td>
<td>1008</td>
<td>739</td>
</tr>
<tr>
<td></td>
<td>Final harvest (14 August)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves + stems + tubers</td>
<td>1279</td>
<td>1539</td>
<td>1118</td>
</tr>
<tr>
<td></td>
<td>Green leaves</td>
<td>158</td>
<td>81</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Dead leaves</td>
<td>37</td>
<td>76</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>78</td>
<td>67</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Tubers</td>
<td>1007</td>
<td>1316</td>
<td>992</td>
</tr>
<tr>
<td></td>
<td>Roots</td>
<td>55</td>
<td>73</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Change from first to final harvest (i.e. in 3 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves + stems + tubers</td>
<td>276</td>
<td>304</td>
<td>271</td>
</tr>
<tr>
<td></td>
<td>Green leaves</td>
<td>2</td>
<td>–61</td>
<td>–42</td>
</tr>
<tr>
<td></td>
<td>Dead leaves</td>
<td>17</td>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>–4</td>
<td>–3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Tubers</td>
<td>210</td>
<td>308</td>
<td>252</td>
</tr>
<tr>
<td>1996</td>
<td>First harvest (22 July)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves + stems + tubers</td>
<td>850</td>
<td>1098</td>
<td>803</td>
</tr>
<tr>
<td></td>
<td>Green leaves</td>
<td>208</td>
<td>242</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>Dead leaves</td>
<td>6</td>
<td>18</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>140</td>
<td>170</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Tubers</td>
<td>497</td>
<td>669</td>
<td>671</td>
</tr>
<tr>
<td></td>
<td>Final harvest (12 August)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves + stems + tubers</td>
<td>1373</td>
<td>1899</td>
<td>1153</td>
</tr>
<tr>
<td></td>
<td>Green leaves</td>
<td>170</td>
<td>195</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>Dead leaves</td>
<td>57</td>
<td>76</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>148</td>
<td>141</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Tubers</td>
<td>999</td>
<td>1487</td>
<td>1025</td>
</tr>
<tr>
<td></td>
<td>Change from first to final harvest (i.e. in 3 weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leaves + stems + tubers</td>
<td>523</td>
<td>801</td>
<td>350</td>
</tr>
</tbody>
</table>
was not affected. Tuber weight, however, was increased by elevated CO₂ in both cultivars (Table 1). No significant CO₂–cultivar interaction was observed. In the temperate year 1996, the percentage increase by CO₂ was higher than in the warm year 1995. For the late cultivar, the CO₂ effect on tuber yield increased during the 3 weeks between the two harvests from 26 to 32% in 1995, and from 35 to 49% in 1996. These strong increases in tuber yield by elevated CO₂ contrast with the small effect of CO₂ on above-ground biomass. We observed not only a stimulation of total CO₂ assimilation under elevated CO₂, but also noticed that assimilates were preferentially exported to the tubers.

Roots were only assessed at the final harvest in 1995, and showed a similar stimulation by elevated CO₂ as the tubers (Table 1). The late cultivar had significantly more roots than the early cultivar.

Leaf photosynthesis and fluorescence

In both years, elevated CO₂ initially increased \( A_{\text{max}} \) of both cultivars by more than 80% (Figs 3a, 4a, 5a). In contrast, we did not find an effect of elevated CO₂ on the efficiency of electron transport (\( \Phi_{\text{PC}} \)) until senescence (Fig. 6). After the initial stimulation of \( A_{\text{max}} \) for both cultivars, we observed a gradual decrease of the CO₂ effect until the values of \( A_{\text{max}} \) under elevated CO₂ were equal to those at low CO₂. Marked differences in the rates of decline were observed between years and cultivars (Figs 3a, 4a, 5a). The dynamics of the weather conditions in the experimental years were completely different. In 1995, temperature and irradiance increased sharply to high values from day 165 onwards and remained high until the end of the experiment (Fig. 2). The period of increased irradiance and temperature coincided with strong photosynthetic acclimation. In 1996, radiation and temperature declined between days 160 and 175, and photosynthetic acclimation was delayed and less pronounced than in 1995. These results show that photosynthetic acclimation is correlated with high irradiance and high temperature. In 1995 with the strongest acclimation, the positive effect of CO₂ on accumulated biomass between the first harvest and the final harvest was 28 compared to 278 g m⁻² in 1996.

Carbohydrate response to elevated CO₂

Potato leaves can store high amounts of starch. In microscopically examined leaf cross sections, we observed large starch grains in the mesophyll cells in elevated-CO₂ plants but not in the low-CO₂ treatment. We measured the starch content in the same leaves as those used for the photosynthesis measurements. The amount of accumulated starch was always lower in the low-CO₂ treatment (Figs 3b, 4b, 5b). Starch concentration increased 5-fold in young leaves between planting and the time that full light interception was reached. In the elevated-CO₂ treatments, the starch was clearly visible as large granules in the chloroplasts in microscopic cross sections of the leaves (not shown). Starch accumulation coincided with a strong increase in irradiance. In the late cultivar, a peak accumulation of starch only occurred at elevated CO₂. In agreement with the idea that the accumulation of starch was related to irradiance, we observed that the peak accumulation of starch was almost absent in 1996 because, contrary to the conditions in 1995, irradiance decreased in the corresponding period of the year (Fig. 2). In the early cultivar, carbohydrate concentrations, especially starch, peaked not only at elevated CO₂, but also at low CO₂. Similarly to the starch content of the leaves, sucrose levels were higher at elevated CO₂. The time patterns, however, were less variable, and the differences between the treatments were smaller than for starch (Figs 3c, 4c, 5c). Sucrose is present only in small amounts compared to the vast amounts of starch. The start of photosynthetic acclimation and the increase of carbohydrate accumulation coincide, but in contrast to carbohydrate accumulation, the acclimation effect was not reversible. The obvious decline of the carbohydrate content following a decline in irradiance levels did not reverse acclimation of \( A_{\text{max}} \).

Rubisco response to elevated CO₂

The Rubisco content of leaves under elevated CO₂ is consistently lower than in leaves grown under low-CO₂ conditions (Figs 3d, 4d, 5d). It was clear from these independently obtained samples in time that the Rubisco content of leaves over the season is declining over the season (1995), or stable.
Fig. 3. Seasonal course of physiological parameters, measured in 1995 on young fully expanded leaves of the late maturing cultivar Elles, grown and measured at either 350 or 700 µL CO₂ L⁻¹. (A) Photosynthetic rate at 1200 µmol PAR m⁻² s⁻¹. (B) Starch concentration. (C) Sucrose concentration. (D) Rubisco concentration. ● = high CO₂; ○ = low CO₂. Vertical bars indicate standard errors (n = 3).

Fig. 4. Seasonal course of physiological parameters, measured in 1996 on young fully expanded leaves of the late maturing cultivar Elles, grown and measured at either 350 or 700 µL CO₂ L⁻¹. (A) Photosynthetic rate at 1200 µmol PAR m⁻² s⁻¹. (B) Starch concentration. (C) Sucrose concentration. (D) Rubisco concentration. ● = high CO₂; ○ = low CO₂. Vertical bars indicate standard errors (n = 3).
until day 200 and declining afterwards in the senescence stage (1996). The differences between the CO₂ treatments persisted until the end of the season.

**Evaluation of photosynthetic acclimation by means of modelling**

What was the effect of photosynthetic acclimation on tuber yield? To answer that question, we ran a simulation model with measured $A_{\text{max}}$ values as input to drive the photosynthetic subroutine (Fig. 7). The results show that the simulated yield responses to elevated CO₂ were in good agreement with the measured yield at the final harvest, with a slight underestimation for the late cultivar and overestimation for the first harvests of the early cultivar (Fig. 7). The next step was to remove the acclimated $A_{\text{max}}$ values, for the elevated CO₂ treatment measured at elevated CO₂ and substitute them with the non-acclimated $A_{\text{max}}$ values of the low-CO₂ treatment, measured at elevated CO₂. The results indicate that CO₂ would have increased total dry matter production by about 36% in the absence of acclimation instead of the simulated 19% and observed 21% with acclimated photosynthetic rates. This shows that acclimation of leaf photosynthesis under elevated CO₂ is responsible for a major reduction of the CO₂ effect on potato productivity.

**Comparison with ambient plots**

There was a strong negative effect of the OTCs themselves on all yield components (Table 3). Total biomass declined in the range 21–42%. This is the result of a decrease in irradiance (−20%), an increased temperature (±2.1°C) due to heat dissipation in the OTCs by the ventilators, a reduced relative air humidity (−10%) and an overall change in the microclimatological conditions of the leaves due to artificial air movement patterns.

**Discussion**

The response of potato to elevated CO₂ concentrations was within the range reported for other crops (Kimball 1983; Lawlor and Mitchell 1991). The CO₂ effect on potato production in the present study ranged from 23% for the early variety to 49% for the late variety (Table 1), in agreement with the findings of Miglietta et al. (1998). Leaf area was hardly affected by CO₂ concentration. From previously made simulation studies it was predicted that doubling CO₂ concentration would stimulate potato yield, ranging from 20% for the late varieties to 30% for the early varieties (Schapendonk and Goudriaan 1995). These predictions were based on the expectation that a higher CO₂ concentration would increase light interception by the formation of more leaf area. This would be more beneficial to the early cultivar with its lower LAI than to the late cultivar with a predominantly high LAI.

In addition, the average irradiance at the leaf surface will be higher in the early cultivar because there is less internal shading. Since the response of $A_{\text{max}}$ to elevated CO₂ will increase with increasing irradiance, this also would stimulate photosynthesis of the early variety more than the late variety. None of the above assumptions, however, appeared to be true and therefore the ‘source-part’ of our hypothesis for CO₂ effects on potato cultivars, which stated that the early cultivar in particular would profit from elevated CO₂ through faster leaf formation, less vulnerability to senescence and a higher light interception, must be rejected. Elevated CO₂ had no effect on light interception during early spring, and hardly any effect on total leaf formation in general. The only effect was on senescence of leaves, which was accelerated by a combination of elevated CO₂ and high irradiance/high temperature, especially in the late cultivar. Thus, the amount of intercepted light remained equal, and it is therefore concluded that photosynthesis on a leaf area basis is the most important attribute to explain the observed yield increases.

The second, sink-strength related part of our hypothesis, concerning CO₂ effects on potato cultivars, stated that stimulation of photosynthesis by elevated CO₂ would be maintained longer in late cultivars, which have greater sink activity than early cultivars because of their prolonged tuberizing capacity. This was investigated by comparing the responses in cultivars that were known to differ significantly with respect to tuber growth rates and by investigating the interaction with light intensity and temperature. The very high starch content under elevated CO₂ indicates that the export to the tubers is either limited by an export blockage or that tuber growth in potato is more sink-limited than we would expect from the large sink potential of the growing tubers. Wheeler et al. (1991) demonstrated that doubling the CO₂ concentration increased tuber dry matter by 32% under 12-h photoperiods but had no effect under a 24-h photoperiod regime. This difference was attributed to faster tuberization under the shorter photoperiod, providing a greater sink for assimilates.

Sink limitation or carbon export blockage may even cause irreversible damage of the chloroplasts due to starch accumulation (Goudriaan and de Ruiter 1983). The negative feedback mechanism that down-regulates the photosynthetic rate was apparently not sufficient to prevent starch accumulation. In agreement with these results, we found that the chloroplasts of leaves from the elevated CO₂ treatments contained much larger starch granules than leaves from the low-CO₂ treatments. Even though starch was reduced in the newly formed leaves later in the tuber-filling stage, this was not accompanied by a recovery of the photosynthetic rates (Figs 3a, b, 5a, b) or the Rubisco content. It is therefore doubtful that starch has a signaling function. By leaf-specific antisense repression of the key enzyme of starch synthesis (ADPglucose pyrophosphorylase), it was shown by Ludewig et al. (1998) that the accumulated starch itself does not cause down-regulation, but on the contrary they assumed that the starch pool was necessary as a kind of overflow to allow a constant rate of CO₂ assimilation.
It is more likely that carbohydrate regulation of photosynthetic gene expression is mediated by pathways intermediate to starch synthesis. Sucrose repression of several photosynthetic genes including the genes that code for Rubisco may be involved in hexose metabolism via hexokinase or directly as a signal metabolite (Moore et al. 1999). It has been argued that the Rubisco content, in general, remains in excess of that required to support the measured photosynthetic rates, also at elevated CO₂, and that acclimation would be caused by other limiting factors such as the regeneration of orthophosphate in the chloroplasts (Sage et al. 1989). However, it is difficult to debate on this topic in general terms, because acclimation can only be assessed indirectly by studying processes and enzymes that are strictly regulated by the internal energy balance of plants, which depends on temperature and absorbed irradiance. Our results show that transients of temperature–irradiance combinations have a big impact on acclimation of photosynthetic enhancement. In addition, it is clear that apparent acclimation and senescence are difficult to separate and that both are often linked to the same phenomenology (Miglietta et al. 1998).

In a recent study, it was concluded that the Rubisco content was unaffected by elevated CO₂, and that this would eliminate the possibility that major leaf proteins were responsible for photosynthetic acclimation in Solanum.
The observed progressive decline of the photosynthetic enhancement were attributed to inhibitory effects of as yet unknown compounds on Rubisco activity. Our results, however, show a clear coherence between the temporal variation of $A_{\text{max}}$ and the temporal variation of the Rubisco content, which declines over the season and more so at elevated CO$_2$. However, a direct proof that the correlation is a causal one can not be given yet, for the obvious reason that the observed effects are the result of complex mechanisms, with negative feedback of sink–source balance induced by high temperature and high irradiance levels.

Late varieties have greater sink capacities than early varieties. This is illustrated by the low amount of starch accumulation in the low-CO$_2$ treatment of the late variety and the strong starch accumulation in the early variety in 1995, during the period in which irradiance increased sharply (Figs 3b, 5b). When the environmental conditions are such that sink limitations are eliminated, the CO$_2$ effect on yield might increase, and this trend will be more positive for late than for early cultivars. Interestingly, we observed a preferential allocation of carbon to roots and tubers under elevated CO$_2$, especially in the late maturing cultivar Elles. Preferential allocation of carbon to below-ground parts has also been commonly observed in other crops. For potato, it means that the sink function of the tuber itself is stimulated by elevated CO$_2$. However, it is evident that this increase in sink strength was not enough to prevent acclimation.

**Interaction with environment**

The source–sink balance is strongly influenced by the environment (Long 1991; Grashoff et al. 1995). In that sense, it was a coincidence that the two experimental years were contrasting for irradiance and temperature during the critical periods that acclimation occurred. This created the opportunity to get a good impression of the environmentally induced variability on acclimation and the time course of $A_{\text{max}}$.

We observed a stimulation of $A_{\text{max}}$ in the beginning of the growth season. This might be due an acceleration of the physiological development of the leaves under elevated CO$_2$, leading to a faster gain of maximum photosynthetic capacity. A shift in the timing of the normal photosynthetic stages of leaf ontogeny would also result in an earlier onset of the photosynthetic decline (Miller et al. 1997). Our results are partly consistent with this suggestion. However, we did not observe any ontogenetic shift due to elevated CO$_2$ on reaction centre efficiency, because the time courses of $\Phi_{\text{pc}}$ were equal until senescence, and it is unlikely that this would have been the case if development had been affected. $A_{\text{max}}$ was higher at elevated CO$_2$, but the effect diminished in time. Limitations in the capacity to export the assimilated carbon could be the reason for that. Because the differences in photosynthetic capacity existed only during the 4–8 weeks after planting, this seems to be the critical period for the CO$_2$ effect.

**General implications for OTC experiments**

The observed interactions with the environment also have implications for OTC experiments in general. The average higher temperature in the OTCs and the lower irradiance will have had an influence on the observed CO$_2$ responses. The aspect of higher temperatures in OTCs compared with ambient temperatures was assessed by Van Oijen et al. (1999). They found that for wheat, cooling of the OTC mainly extended the period before leaf senescence, but that light use efficiency was not affected. The cooling decreased the magnitude of the CO$_2$ effect. It would be too speculative to try to estimate the separate effects of irradiance and temperature from our experimental results. However, we think that because the climatic variation between the two years is large, it covers a major part of the bandwidth of irradiance and temperatures, including the offset caused by the OTCs. Nevertheless, it cannot be excluded that OTC experiments will slightly overestimate the CO$_2$ effect (Van Oijen et al. 1999).
Conclusions

Maximum leaf area and total light interception are not affected much by elevated CO₂. Therefore, we reject the hypothesis that early cultivars with a low LAI will profit more than late cultivars.

Under elevated CO₂, the allocation of assimilates becomes more directed to the tubers, but not enough to prevent acclimation due to sink limitation.

Photosynthesis acclimated under elevated CO₂, especially under high radiation and temperature. Acclimation was accompanied by a decrease in the Rubisco content and an accumulation of starch and sucrose, more so in the early than in the late variety. This resulted in a lower stimulation of yield and total biomass.

Model calculations showed that the acclimation of photosynthesis under elevated CO₂ reduced the response to CO₂ concentration by 50% compared to a hypothetical situation where this acclimation did not occur. If photosynthetic acclimation can be decreased through breeding or management, potato, especially late varieties, could profit more from an increase in the atmospheric CO₂ concentration than indeterminate crops such as cereals.

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References


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