CO₂ Effects on Phasic Development, Leaf Number and Rate of Leaf Appearance in Wheat

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It has been predicted that the concentration of CO₂ in the air could double during the 21st century. Though it is recognized that CO₂-doubling could increase yield through its effects on plant photosynthesis and stomatal behaviour, it is unclear whether CO₂-doubling will change phasic development in wheat. A phytotron study was conducted with two contrasting cultivars of wheat, Condor (spring) and Cappelle Desprez (winter), to determine whether development is affected by a season-long exposure to 360 and 720 ppm CO₂. Plants were vernalized for 50 d (8/4 °C, 8 h photoperiod) before their exposure to the CO₂ treatments.

There were significant differences between cultivars in the duration of different phenophases as well as in the final number of leaves. However, CO₂ concentration had no effect in either cultivar on the duration of the early developmental phase to terminal spikelet initiation, or on the final number of leaves, though CO₂-doubling did slightly increase the later phase from terminal spikelet initiation to heading in Cappelle Desprez. Condor and Cappelle Desprez also differed markedly in the dynamics of leaf appearance. While the former had a constant rate of leaf appearance throughout development, the latter had a fast rate initially (between leaves 1 and 7), similar to that of Condor, which was followed by a slower rate after the appearance of leaf 7. Overall, CO₂-doubling did not significantly affect the rates of leaf appearance nor the shape of the relationship. Phyllochron for the first seven leaves was the same for both CO₂ concentrations. However, the change in phyllochron associated with CO₂-doubling for leaves 7–12 in Cappelle Desprez, although quite small (4%), accounts for part of the slightly increased duration of the phase from terminal spikelet initiation to heading under high CO₂ concentration in that cultivar. We conclude that CO₂ concentration does not influence development in wheat to a degree relevant to agronomy.

Key words: Carbon dioxide, climatic change, development, leaf number, phyllochron.

INTRODUCTION

It is well established that CO₂ levels have been rising from approx. 270 μmol CO₂ mol⁻¹ air in the pre-industrial era to the present 350 μmol CO₂ mol⁻¹ air (Neftel et al., 1985). Further increases depend on the trends of emissions into the atmosphere which in turn are dependent on economic factors determining the growth of energy usage (Pearman, 1988). Some estimates indicate that present concentrations of CO₂ in the air could double before the year 2050 (Pearman, 1988). Bowes (1993) in his review ‘Facing the inevitable’, based on the work of King, Emanuel and Post (1992), states that ‘without Divine intervention, the concentration will likely double in the next century’.

The increases in the atmospheric concentration of CO₂ and other ‘greenhouse gases’ may produce dramatic changes in the weather, in particular an associated increase in temperature, that will certainly affect agricultural productivity (e.g. Adams et al., 1990). The main effects of CO₂ are related to the increase of yield through direct effects on plant photosynthesis and stomatal behaviour (Gifford, 1988, Samarakoon et al., 1995). However, there is a parallel negative effect on yield of increased temperatures due to accelerated rates of development (Slafer and Rawson, 1994a, 1995a). Field studies in wheat considering increases in both CO₂ and temperature suggest that the interaction could result in unchanged or decreased yields, depending on the temperature (Rawson, 1995).

If we could predict the behaviour of wheat under future climatic scenarios, we should be able to design new cultivars and management practices to optimize the advantages and reduce the disadvantages of such climates. Some plant growth models have been modified to simulate the effect of global climate change on wheat growth and development (e.g. Nonhebel, 1990). In all cases, it is assumed that the negative aspect of future climates on wheat yield is due to faster development (shortened periods for radiation interception and organ formation) as a direct consequence of warmer environments. However, little is known about the effect of CO₂ on development itself, in particular from whole-season experiments.

An effect of CO₂ on the rate of development in wheat could stem from two causes: a growth-related response or a direct effect on developmental processes (Marc and Gifford, 1984). The former would arise if there were a minimum organ size or amount of plant growth required before development could progress, a situation in which the rate of development would be limited by the source. Rawson (1993) showed that this can occur in wheat grown under high temperatures and winter radiation and Gifford (1977) had
were then transferred for 7 weeks to a growth-room with a
vermiculite between 20 at 53 d after the beginning of the experiment was an
developmental processes (Slafer and Rawson, 1994) anically identical. Temperature, a critical factor in de-
velopmental processes are affected by a season-long exposure to 'normal' and twice normal concentrations of CO₂ in the air in two
contrasting cultivars of wheat.

MATERIALS AND METHODS

A spring and a winter wheat cultivar, chosen to represent different developmental sensitivities to environmental factors, were grown between early March and late May 1993 in glasshouses in the Canberra phytotron (Morse and Evans, 1962). The cultivars were Condor (Australian spring wheat) and Cappelle Desprez (French winter wheat). Condor is a semi-dwarf cultivar, moderately sensitive to photoperiod and slightly sensitive to vernalization, while Cappelle Desprez is a standard-height cultivar which is strongly sensitive to both photoperiod and vernalization (Slafer and Rawson, 1995). Two glasshouse modules in the Canberra CSIRO Phytotron were set to maintain different carbon dioxide concentrations (360 and 720 μmol CO₂ mol⁻¹ air) throughout the study (see Samarakoon et al., 1995 for methods of control) by using automatic CO₂ injection and CO₂ scrubbing routines. Although plants subjected to different CO₂ concentrations were in different glasshouse modules, they were allocated equivalent positions within the modules and the modules were otherwise structurally and mechanically identical. Temperature, a critical factor in developmental processes (Slafer and Rawson, 1994), was the same in both modules during the experimental period. The temperature regime followed a diurnal sine wave between 25.5 and 15.5 °C for the first 7.5 weeks of the study and between 20.0 and 13.0 °C thereafter. The temperature step at 53 d after the beginning of the experiment was an unavoidable administrative decision.

Seeds were sown in a pre-soaked 50/50 mixture of vermiculite/perlite at a rate of three seeds per 100 × 150 mm pot, and retained at room temperature for 1 d. The pots were then transferred for 7 weeks to a growth-room with a light period of 8 h and set at 8/4 °C (day/night) to satisfy, at least partly, vernalization requirements.

On 9 Mar. defined as the beginning of the experiment when CO₂ treatments began, the pots were moved from the low temperature room to the glasshouse modules where natural radiation was artificially extended to 16 h with low-intensity incandescent lamps.

The study was a split plot design with CO₂ concentration as main plots and cultivars as subplots. There were five replications arranged in blocks. Within a block each treatment consisted of four pots. All pots were supplied with complete nutrient solution each morning and with water each afternoon.

Soon after the appearance of the third leaf on the main culm, the first harvest was made and plants were dissected to determine the stage of development of the apex (Gardner, Hess and Trione, 1985). One plant was selected randomly within each block at each harvest (five plants per treatment) and different pots were sampled at consecutive harvests, so that the sampling procedure was also a plant thinning mechanism throughout plant development. The frequency of harvests was twice weekly.

At terminal spikelet initiation, all pots were thinned to one plant. In each of the four plants left per replicate (i.e. 20 plants per treatment) the date of appearance of the tip of the glume of the uppermost spikelet above the flag-leaf sheath (heading) was recorded.

Two plants in each sub-plot (i.e. ten plants per treatment) were tagged with coloured rings at the beginning of the experiment. When necessary, some other leaves were also tagged individually on each plant. The number of leaves per main culm was recorded every 2–4 d following the method of Haun (1973). After anthesis the height of the main-culm was measured from the soil surface to the base of the spike and the number of elongated internodes (those longer than 1 cm) was counted in each of the four plants per subplot.

Analyses of variance considering CO₂, cultivar and their interactions were performed on all the variables. Since cultivars were randomized within CO₂ concentrations, two different sources of error were estimated, and therefore two different least significant difference (L.s.d.) values were calculated for each variable (to make comparisons between CO₂ concentrations and between cultivars, respectively). Regression analyses were used to estimate the effect of CO₂ on rates of leaf appearance for each cultivar. For this purpose, the number of leaves on the main stem was regressed against time for each treatment, and the rate of leaf appearance was estimated as the coefficient of the regression. Phyllochron was then calculated for each treatment as the coefficient of regression of the relationship between days after beginning of the experiment and leaf number. This was preferred to simply calculating it as the reciprocal of the rate of leaf appearance, so that estimates of both phyllochron and its standard error could be obtained.

RESULTS

CO₂ concentration and the duration of phenophases

Cultivars differed significantly in their patterns of phasic development. For the two phenophases analysed, Cappelle
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Cultivars differed significantly in the final numbers of both leaf and spikelet primordia (Fig. 2). However, in neither cultivar were there any trends for change in final leaf number with CO$_2$-doubling (Fig. 2). This lack of sensitivity, together with the lack of effect of CO$_2$-doubling on the duration of the leaf initiation phase, indicates that the rate of leaf initiation (or its reciprocal, the plastochron) was also insensitive to CO$_2$. By contrast, the number of spikelets per spike was higher in the plants under high CO$_2$ concentration, though the difference was significant only for Cappelle Desprez (Fig. 2). Because duration between sowing and

**CO$_2$ concentration, final numbers of leaves and spikelets**

Desprez was later than Condor under both CO$_2$ concentrations, and relatively more so in the period to terminal spikelet initiation than in the following phase to heading (Fig. 1).

In both cultivars, the duration of the early developmental period to terminal spikelet initiation was insensitive to CO$_2$ (Fig. 1), and in Condor there was no significant response from terminal spikelet initiation to heading. Cappelle Desprez, however, showed a slight but significant response to increased CO$_2$ during the post-terminal spikelet initiation phase. In this cultivar the rate of development between these phenological stages was retarded by approx. 15% (from 0.040 to 0.034 d$^{-1}$) by CO$_2$-doubling. Consequently, the full period to heading was marginally longer (8%) under high CO$_2$ in Cappelle Desprez.

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**Fig. 1.** Effects of carbon dioxide concentration (360 ppmv, □; 720 ppmv, ■) on the duration of the phases from the beginning of the experiment (Beg) to terminal spikelet initiation (TSI), from TSI to heading (Hd) and from Beg to Hd in two contrasting wheat cultivars: Condor (Co) and Cappelle Desprez (CD). Lines represent the l.s.d. ($P = 0.05$) for comparisons between CO$_2$ concentrations. l.s.d. ($P = 0.05$) for comparisons between cultivars were 1.64, 3.58 and 3.32 d for the phases Beg-TSI, TSI-Hd and Beg-Hd, respectively.

**Fig. 2.** Effects of carbon dioxide concentration (360 ppmv, □; 720 ppmv, ■) on the final numbers of leaves and spikelets in two contrasting wheat cultivars: Condor (Co) and Cappelle Desprez (CD). Lines represent the l.s.d. ($P = 0.05$) for comparisons between CO$_2$ concentrations. LSD ($P = 0.05$) for comparisons between cultivars were 0.93 leaves, and 1.52 spikelets.

**Fig. 3.** Relationship between the number of leaves visible on the main culm and time after beginning of the experiment (seedlings were vernalised [8/4°C, 8 h photoperiod] for 50 d immediately preceding the beginning of the experiment) for two cultivars (A) Condor, and (B) Cappelle Desprez at two carbon dioxide concentrations (360, □, and 720, ■ ppmv). Lines were fitted by regression (see Table 1).
terminal spikelet did not respond to CO$_2$, the difference must be attributed to an increased rate of spikelet initiation under high CO$_2$.

**CO$_2$ concentration and rate of leaf appearance**

For each combination of cultivar and CO$_2$ concentration, the accumulated number of leaves was plotted against time from the beginning of the experiment. The temperature step occurred after the flag leaf of Cappelle Desprez had appeared and therefore had no effect on phyllochron. The intercepts of the relationships were always positive because the experiment began after a vernalization pretreatment of 50 d during which some development occurred. At the beginning of the experiment the Haun stage of development was between 0.9 and 1.0 (Fig. 3).

A major difference in rate of leaf appearance was observed between Condor and Cappelle Desprez. The spring cultivar, Condor, exhibited a constant rate of leaf appearance throughout development (Fig. 3A). By contrast, the residuals of a linear regression for the winter cultivar showed a curvilinear pattern, implying the inadequacy of such a simple relationship, despite the fact that the coefficient of determination was high. The peak value of these residuals occurred at approximately the time of appearance of the seventh leaf. Consequently, a bi-linear model was fitted using the seventh leaf as the break. This model showed a faster rate of leaf appearance from the beginning of the experiment to the appearance of leaf 7 than between leaf 7 and leaf 12 (the flag leaf) (Fig. 3B). Each of the six linear regressions for cultivar × CO$_2$ explained more than 99% of the variation (Table 1).

The two cultivars had similar rates of leaf appearance for the first seven leaves (0.248 and 0.243 leaves d$^{-1}$, average of CO$_2$ levels for Condor and Cappelle Desprez, respectively). After the appearance of the seventh leaf, the rate of leaf appearance in Cappelle Desprez slowed substantially to 0.176 leaves d$^{-1}$, a reduction of more than 25% from the initial rate (Fig. 3B, Table 1).

**Table 1. Rates of leaf appearance (RLA) and standard error (s.e.), determined as the coefficient of regression (leaves d$^{-1}$) of the relationship between leaf number on the main culm and time after seedling emergence for the wheat cultivars Condor (spring) and Cappelle Desprez (winter) under normal (360 ppmv) and enriched (720 ppmv) CO$_2$ atmosphere.**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>CO$_2$ level (ppmv)</th>
<th>Leaves included in the analysis</th>
<th>RLA (leaves d$^{-1}$)</th>
<th>s.e.</th>
<th>$r^2$†</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Condor</td>
<td>360</td>
<td>1 to 7 (flag leaf)</td>
<td>0.2501 ± 0.0084</td>
<td>0.994***</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Condor</td>
<td>720</td>
<td>1 to 7 (flag leaf)</td>
<td>0.2457 ± 0.0086</td>
<td>0.997***</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>C. Desprez</td>
<td>360</td>
<td>1 to 7</td>
<td>0.2438 ± 0.0059</td>
<td>0.997***</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>C. Desprez</td>
<td>720</td>
<td>1 to 7</td>
<td>0.2410 ± 0.0079</td>
<td>0.995***</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>C. Desprez</td>
<td>360</td>
<td>7 to 12 (flag leaf)</td>
<td>0.1790 ± 0.0062</td>
<td>0.994***</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>C. Desprez</td>
<td>720</td>
<td>7 to 12 (flag leaf)</td>
<td>0.1727 ± 0.0063</td>
<td>0.993***</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

† The reported values of $r^2$ in this table were always highly significant ($P < 0.001$).

**CO$_2$ concentration, number of internodes and culm height**

As expected, cultivars differed significantly in culm height, with Cappelle Desprez, the standard-height genotype, on average 32 cm (i.e. 62%) taller than Condor. The proportional difference was constant across CO$_2$ concentrations (Fig. 4). The cultivar difference was partly due to differential development which resulted in Cappelle Desprez averaging almost one elongated internode more than Condor (Fig. 4), and also the elongated internodes of the...
Carbon dioxide concentration affected culm height of both cultivars significantly and consistently. Plants of both cultivars grown under 720 ppmv CO$_2$ were 17% taller than those under 360 ppmv (Fig. 4). As with final number of leaves, CO$_2$ concentration did not affect the number of elongated internodes significantly in either of the two cultivars (Fig. 4). Consequently, differences in height arising from CO$_2$-doubling were due to differences in the average length of individual internodes. Internodes of Condor averaged 1.2 cm longer and those of Cappelle Desprez 2.5 cm longer. Assuming that culm elongation begins at terminal spikelet initiation (Kirby, Appleyard and Fellowes, 1985; Masle, 1985; Stapper and Fischer, 1990), the duration of culm elongation was not altered by CO$_2$ concentration (see above). Thus differences in culm height due to CO$_2$ concentrations reflected differences in culm elongation rate.

**DISCUSSION**

The cultivars chosen for the present study differed markedly in most of the variables measured, and if there were any common responses to CO$_2$ between cultivars, it is likely that those responses would be common to many wheat cultivars. CO$_2$ concentration affected few of the variables measured, and the interaction between the factors was mostly negligible. In this discussion we will consider each of the factors using the averages across the other factor. To allow for direct comparison with field studies and other papers in the literature, thermal units will be used occasionally, assuming a base temperature of 0°C. This base temperature was chosen because of its general usage rather than because of any physiological meaning (see Slafer and Rawson, 1994a, 1995a).

**Differences between the cultivars**

Cappelle Desprez had a longer period to heading and a greater number of leaves than Condor, possibly indicating that Condor is intrinsically earlier than Cappelle Desprez (Flood and Halloran, 1984), confirming results of earlier studies (Slafer and Rawson 1995b, c). Additional to any ‘intrinsic’ difference between the cultivars in their earliness, it is also possible that the daylength or vernalization period used in the present study (16 h and 50 d, respectively) or the temperature used in the vernalization room (8/4 °C), were inadequate to satisfy completely the requirements of Cappelle Desprez. This is evident from a comparison of this and a previous study (Slafer and Rawson, 1994b) conducted under 18 h photoperiod and after 50 d of vernalization at 4/4 °C. In the present study, final leaf number (approx. 12) was greater than in the previous study (approx. 8), as was the length of the period to terminal spikelet initiation (820 vs. 680 °C d). This comparison suggests that the difference observed between cultivars in the present study was greater than could be explained by difference in ‘intrinsic earliness’.

The differences between the two cultivars were apparent in both subphases of development considered here, although the difference was much stronger in the early phase to terminal spikelet initiation (approx. 820 vs. 270 °C d for Cappelle Desprez and Condor, respectively) than in the later phase to heading (545 and 395 °C d). This could reflect earlier observations (a) that the differences between these cultivars in intrinsic earliness were found to be smaller in the late than in the early developmental phases (Slafer and Rawson, 1995a), or (b) that the quantitative responses to photoperiod or vernalization are generally stronger before than after the initiation of the terminal spikelet (Slafer and Rawson, 1994a).

A single rate of leaf appearance (corresponding to a phyllochron of approx. 90 °C d) was appropriate for Condor, but Cappelle Desprez exhibited a reduced rate after the appearance of leaf 7 (its phyllochron increased from approx. 90 to approx. 125 °C d). Some authors have also reported bi-linear rather than a simple linear model to explain the relationship between leaf number and time or thermal time (Hay and Delécalle, 1989; Stapper and Fischer, 1990; Cao and Moss, 1991a), while many other studies support a constant rate of leaf appearance throughout plant development (e.g. Gallagher, 1979; Cao and Moss, 1991b; Kirby, 1993; Slafer et al., 1994b; Slafer and Rawson, 1995c). The result in the present paper indicates that the conflict in the literature may be due to genotypic variation in the dynamics of leaf appearance, such that while some cultivars show a single rate during their development, others exhibit a downward change in rate. The causes of these different patterns, however, appear to be complex: Cappelle Desprez, the cultivar showing a bi-linear response in the present study, had a simple linear relationship in a previous study (Slafer and Rawson, 1995c). In the present study, leaf number of Cappelle Desprez was approx. 50% higher than that reported by Slafer and Rawson (1995c), possibly indicating that the shift to a lower rate of leaf appearance after some leaves had already appeared could be a mechanism for delaying anthesis in a non-inductive environment (responsible for the higher number of leaves as well). In other words, when the lack of satisfaction of the requirements for vernalization and photoperiod produces a delay in phasic development, there could be a reduction in rate of leaf appearance, which in turn further increases the delay in phasic development caused by the increased final number of leaves (Slafer, Connor and Halloran, unpubl. res.).

**Effects of CO$_2$ concentration**

In general, there was no effect of CO$_2$ on the timing of either the initiation of the terminal spikelet or the emergence of the head nor was there any effect on the final number of leaves. The exception was the length of the phase from terminal spikelet initiation to heading which was extended when plants were grown under elevated CO$_2$. Marc and Gifford (1984) observed a slight advance of floral initiation (of approx. 3 d) in wheat grown under elevated compared with ambient CO$_2$, but the plants under different CO$_2$ environments reached anthesis simultaneously, indicating that those plants under elevated CO$_2$ experienced a slight extension of the period from floral initiation to anthesis. However, if we put the data in an agronomic context,
although the difference may be statistically significant in some cultivars, the magnitude of the CO₂ effect in our studies was negligible. This supports the general statement made by Rawson (1992) that only ‘little (if any) effects of CO₂ on development would be expected’.

Neither the pattern nor the rate of leaf appearance were modified by CO₂ concentration. The bi-linear model found in the relationship between leaf number and time in Cappelle Desprez was not modified by CO₂. The change in rate of development occurred at the same Haun stage in both environments. Phyllochron was virtually the same value for both CO₂ concentrations. The difference in phyllochron between high and normal CO₂ for the first seven leaves was 1.5 and 1 °C d in Condor and Cappelle Desprez, respectively. This agrees with Schönfeld et al. (1989) who also found negligible (approx. 2%) differences in phyllochron for wheat plants grown at 350 and 700 ppmv CO₂.

The difference in the second phase of the bi-linear relationship in Cappelle Desprez, although still quite small, was higher (4.5 °C d) than that found for the appearance of the first seven leaves. This accounts, at least in part, for the longer duration of the phase from terminal spikelet initiation to heading when CO₂ concentration was doubled.

**CONCLUSION**

This study was conducted to examine whether development in wheat plants is changed by CO₂-doubling. Two cultivars of contrasting developmental characteristics were chosen to explore the possibility of a cultivar × CO₂ concentration interaction. The mean squares of this interaction were negligible for all the variables.

Cultivars differed markedly in all the variables considered in the present study. Condor produced fewer leaves and reached heading earlier than Cappelle Desprez. Although both cultivars had similar rates of leaf appearance initially, this rate slowed after the appearance of the seventh leaf. Plant height was markedly different since Cappelle Desprez is a standard-height cultivar whilst Condor is semi-dwarf.

Both cultivars responded to CO₂ concentration as expected in terms of growth parameters such as culm length. However, developmental processes were mostly unresponsive to CO₂ concentration: the rate of development early in the growing season (up to terminal spikelet initiation) was completely insensitive in both cultivars, and there was only a minor, negligible effect on the length of the phase from terminal spikelet initiation to heading in Cappelle Desprez, but not in Condor. Finally, neither the rate of leaf appearance nor the final number of leaves on the main culm were significantly affected by the CO₂ concentration. It is concluded that, in an agronomic context, CO₂ does not influence either the rate of phasic development or the rate of leaf appearance in wheat.

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**LITERATURE CITED**


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