

## **I. GENERAL INTRODUCTION**

One of the pre-eminent and incontrovertible manifestations of global environmental change is the increase in atmospheric CO<sub>2</sub> concentration from approximately 280 up to 360 ppm over the last century or more (Schimel *et al.*, 1996). Global warming, which results from the increase in CO<sub>2</sub> and other greenhouse gases, has become a major scientific and political issue during the past decade. Despite the effort to reduce the emissions of greenhouse gases, the present rate of increase (an average of 1.5 ppm per year over the decade 1984-93; Schimel *et al.*, 1996; Gribbin and Gribbin, 1996) will continue until probably the next century, resulting in its concentrations doubling (560 ppm) as compared with those during the pre-industrial period (280 ppm) by the middle or second half of the twenty-first century (AD) (IPCC scenarios IS92e and IS92a, 1996, Schimel *et al.*, 1996). This increase has been primarily driven by the rapidly growing human population, and its high consumption of fossil fuel, cement manufacturing, and deforestation (Houghton *et al.*, 1990; Watson *et al.*, 1990; Vitousek, 1994). Recent climate model projections, taking into account greenhouse gases and aerosols, suggest a mean global surface air temperature increase of 1 to 4.5°C by 2100 AD (dependent on scenarios, Kattenberg *et al.*, 1996; IPCC, 1996). Indeed, the 0.3 to 0.6°C rise of mean annual surface air temperature over the last century may be the first discernible effect of these recent global atmospheric changes (Nicholls *et al.*, 1996).

Both temperature and CO<sub>2</sub> have been looked at to determine the response of plants to environmental change, because both temperature and CO<sub>2</sub> are very important environmental factors that directly affect plant growth, either as resources or as resource regulators (Long and Woodward, 1988; Bazzaz, 1990). Under a low rate change in these two factors, many plants have

adapted to the climate in their current locations, and their growth, development, and function are highly correlated with their current climate conditions (e.g. different growth season within a climate zone). However, current vegetations would be re-established by a rapid change in temperature and CO<sub>2</sub> preferences with regard to phenology, photosynthesis, respiration, germination and growth etc (Mayeux *et al.*, 1991; Johnson *et al.*, 1993; Ehleringer *et al.*, 1997; Street-Perrott *et al.*, 1997).

To understand how change in the global environment will affect current global vegetation, the relationship between the distribution of species and the environmental factors has been studied by many scientists. In particular, many studies have been carried out in order to deduce the geographic distribution of C3 and C4 plants (each with a different carbon fixation pathway) to predict how these plants' distribution will be changed under the increase in temperature with increasing CO<sub>2</sub>. C4 species evolve a photosynthetic pathway with the CO<sub>2</sub>-concentrating mechanism in the bundle sheath cells. This mechanism increases the effective concentration of CO<sub>2</sub> at the site of carboxylation, thereby masking photorespiration and apparently ensuing saturation of photosynthesis at current atmospheric CO<sub>2</sub> concentrations. The overall processes of "cooperative photosynthesis" (Karpilov, 1970) serve to elevate the CO<sub>2</sub> concentration in the bundle-sheath cells of C4 plants, thereby enabling ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in these cells to function nearer to CO<sub>2</sub> saturation. The concentrations achieved (>2000ppm; Furbank and Hatch, 1987) are about 10-100 times greater than those experienced by Rubisco in C3 plants. In effect, the C4 pathway has restored the photosynthetic carbon reduction cycle in the bundle-sheath cells that sustain an

internal CO<sub>2</sub> environment remarkably similar to the external atmosphere of the Cretaceous. Also, this can generally reduce water loss and increase photosynthetic carbon gain under warmed and arid conditions and low CO<sub>2</sub> concentration. For this reason, a concern has arisen about geographical partitioning related to differences in photosynthetic mechanism because of the advantages in productivity, water-use efficiency, and temperature tolerance that the CO<sub>2</sub> concentration mechanism confers on C4 plants over C3 plants. Black (1971), for example, suggested that the C4 plant is generally found in dry tropical, sandy and salty areas, depending on temperature. In Japan, the distribution of C3 and C4 grasses had a significantly higher correlation with the temperature, especially with yearly mean air temperature (Takeda, 1985). On the other hand, solar radiation and hours of sunlight had no significant correlation. Teeri and Stowe (1976) discovered a minimum July temperature to have a high correlation with the relative abundance of C4 grass species in a regional flora of North America; no strong relationship involving precipitation was established. Hasegawa (1979) suggested that C3 plant species are typically found in cool areas and seasons but C4 plant species are mainly found in hot areas and seasons with a low temperature at nighttime. Hattersley (1983) reported the distribution of C3 and C4 plants in relation to the climates in Australia: C4 species were most numerous in hot and wet summers and C3 species were numerous in cool and wet springs. The number of C4 species declines with decreasing temperature and/or decreasing summer rain fall; C3 species' numbers decline with increasing temperature and/or decreasing spring rain fall. Loomis and Gerakis (1975) concluded that C4 species were more productive at low latitudes and less productive at high latitudes. Generally

speaking, under the current environmental conditions, C4 plants have a higher temperature optimum for photosynthesis than co-occurring C3 species (de Jong *et al.*, 1982).

In other studies it was also suggested that the geographical distributions of both C3 plants and C4 plants were related to CO<sub>2</sub> concentration. Using data based on the study of palaeovegetation from the palaeosols and palaeodiet from fossil tooth enamel, Cerling *et al.* (1993) suggested that the global expansion of C4 biomass may be related to lower atmospheric carbon dioxide levels because C4 photosynthesis is favored over C3 photosynthesis at low concentrations of carbon dioxide. Similarly, Polley *et al.* (1993) suggested that the increase in atmospheric CO<sub>2</sub> concentration from 160 ppm in the Last Glacial Maximum (LMG; about 18,000 years ago) to 280 ppm in 1800 altered species abundances by increasing the water-use efficiency of C3 plants' biomass production. Coie and Monger (1994) also found that changes in the atmospheric CO<sub>2</sub> concentration in the past might have caused changes in vegetation type, leading to an increase of global CO<sub>2</sub> concentration, as recorded in various ice cores, and causing a direct shift from C4 to C3 vegetation.

In addition, many experimental results from varying CO<sub>2</sub> concentrations also indicate that C3 plant species are more responsive to increasing atmospheric CO<sub>2</sub> enrichment than C4 plants. In general, in C3 plants representing more than 95% of all plant species in the world (Houghton *et al.*, 1990), atmospheric CO<sub>2</sub> enrichment will lead to a significantly increased net photosynthesis, caused by an increased ratio of CO<sub>2</sub> to O<sub>2</sub> at the catalytic site on Rubisco (ribulose biphosphate carboxylase oxygenase) and decreased diffusion limitation (Kimball, 1985). In C4 plants, this effect will be less marked

because phosphoenol pyruvate carboxylase catalyses the photosynthetic fixation of CO<sub>2</sub> in mesophyll cells and this eventually causes a high ratio of CO<sub>2</sub> and O<sub>2</sub> in the bundle sheath cells, where carboxylation occurs once more (Allen, 1990). For that reason, plants with the C3 photosynthetic pathway grown in elevated CO<sub>2</sub> often show an enhancement of photosynthesis, especially when other environmental resources such as light, water and nutrients are non-limiting (Bazzaz, 1990; Curtis *et al.*, 1989), whereas C4 species generally respond to a lesser degree (Potvin and Strain, 1985; Smith *et al.*, 1987). C3 plants grown in low CO<sub>2</sub> generally exhibited lower rates of photosynthesis when compared with plants grown at normal CO<sub>2</sub> (Overdieck 1989; Rowland-Bamford *et al.*, 1991; Thomas and Strain, 1991; Sage and Reid, 1992; Rozema, 1993; Tissue *et al.*, 1995). However, C4 species have similar rates of photosynthesis at low and normal CO<sub>2</sub> concentration (Byrd and Brown, 1989). Tissue *et al.* (1995) found that in C3 species, CO<sub>2</sub> partial pressure increased from 150 ppmv to 700 ppmv with increasing CO<sub>2</sub>, but this result did not occur in C4 species. Elevated temperature also markedly decreased the effect of elevated CO<sub>2</sub> on the biomass accumulation of C3 grass (Read and Morgan, 1996).

In summary, as the many studies cited above indicate, species with the C4 pathway are usually more abundant in tropical and warm temperature regions and low CO<sub>2</sub>-concentration eras, while species with the C3 pathway are more abundant in cool and high CO<sub>2</sub>-concentration conditions. Therefore, if global warming would progress with no change in the current CO<sub>2</sub> concentration, C4 species would have a competitive advantage compared with C3 species. By contrast, if atmospheric conditions would result in changing only the CO<sub>2</sub> concentration, C3 species would enjoy a competitive advantage over C4

species. However, atmospheric temperature and CO<sub>2</sub> have been observed to increase simultaneously and will certainly change. Therefore, it is very difficult to predict how the current distribution of C3 and C4 species would change as the CO<sub>2</sub> concentration and temperature increases.

Despite the predictions that both CO<sub>2</sub> and air temperature will rise together, very few field data are available to assess the potential interactive effects of elevated temperature and CO<sub>2</sub> on plant species. Limited data from studies with herbaceous species suggest that the combined effects of temperature and CO<sub>2</sub> are not necessarily additive and are therefore difficult to predict from a knowledge of their individual effects (Idso *et al.*, 1987; Idso and Kimball, 1989; Long, 1991; Farrar and Williams, 1991; Hogan *et al.*, 1991). Furthermore, many independent and interactive effects of temperature and CO<sub>2</sub> appear to be species-specific (Bazzaz, 1990; Bazzaz and Miao, 1993; Bazzaz *et al.*, 1990; Coleman and Bazzaz, 1992; Ackerly *et al.*, 1992). Consequently, to predict changes in the geographical distribution of plant species as a consequence of the increase in temperature occurring with increasing CO<sub>2</sub>, it is necessary to characterise the effect of elevated temperature and CO<sub>2</sub> on the key components of plant life history stages, since these stages determine the ability of a species to increase in population size and range. Such key components are likely to be the rate of the establishment and maturation of populations and their reproductive output (Beerling and Woodward, 1994).

Numerous studies have examined the effects of elevated CO<sub>2</sub> on plant productivity (usually measured in terms of biomass) for both crop and wild species. As a result of these studies, C3 species showed more positive responses to elevated CO<sub>2</sub> on growth than C4 species. These studies have

generally found this effect (e.g. Sionit *et al.*, 1982; Bazzaz, 1990; Bazzaz and Miao, 1993; Bazzaz *et al.*, 1993; Poorter, 1993; Reekie *et al.*, 1994; Reeves *et al.*, 1994), but not always (Bazzaz and Garbutt, 1988; Ferris and Taylor, 1993; Morse and Bazzaz, 1994). Although production acts as a strong carbon 'sink', and sink strength is thought to be an important determinant of the physiological response to elevated CO<sub>2</sub> (Bazzaz, 1990), it remains unclear whether physiological and vegetative responses to elevated CO<sub>2</sub> will have reproductive ability.

Moreover, at the population level, where the resources and microclimate are continuously modified by neighbors, the effects of temperature and CO<sub>2</sub> on growth are virtually unknown (Bazzaz and McConnaughay, 1992). There are very few studies on population responses to elevated temperature and CO<sub>2</sub> under field-like conditions. It is difficult to understand how the data from the individual response to elevated temperature and CO<sub>2</sub> should be interpreted at the population level. For example, it is very difficult to calculate how the productivity per unit ground area at current atmospheric conditions will change under the climate change. Also, the fluctuating diurnal, seasonal temperature and radiation can have more impact on the floral development of the plants through the responses of vernalization and seed dormancy. The environmental conditions in the field have substantial differences in the variability, coupling, and absolute values of key factors (controlled environments are often hot, wet, humid and poorly illuminated, compared with the field). A field-grown crop may experience rapid changes in temperature, water and radiation. These differences underline the need to confirm that the responses to CO<sub>2</sub> observed in the large number of controlled-environment experiments apply in agriculturally



or ecologically realistic situations (Lawlor and Mitchell, 1991). Thus to make any anticipatory statements regarding the plant population response to climate warming, one must consider the species-specific effects of temperature in combination with elevated CO<sub>2</sub> (Bazzaz *et al.*, 1996) under field-like conditions.

Consequently, if we are to predict the potential implications of changes in global atmospheric environments and plant communities, we need to quantify these effects throughout a growth period on the population levels, the subsequent performance of individual growth in relation with neighbors, and the effect of plant-plant interactions on the population size and structure (Morse and Bazzaz, 1994). Such all-season field studies on natural plants at elevated temperature and CO<sub>2</sub> can be utilized to obtain calibration and validation data for plant growth models. In order to predict change in the geographical distribution of C3 and C4 species as a consequence of elevated temperature and CO<sub>2</sub>, it is essential to characterize the effect of elevated temperature and CO<sub>2</sub> on key components of the plant life cycle, such as rate of growth, phenology, and reproductive output.

Currently, however, very few data have been available to assess the potential interactive effects of elevated temperature and CO<sub>2</sub> on most plant species (Bazzaz *et al.*, 1996; Morison and Lawlor, 1999). Therefore, a global assessment of the effects of CO<sub>2</sub> increase on (agro)ecosystem and vegetation requires careful analysis of the interaction between CO<sub>2</sub> enrichment and temperature (cf. Goudriaan *et al.*, 1990; Goudriaan and Unsworth, 1990). In this context, it is essential to conduct experiments on the responses of wild species under more realistically simulated global warming conditions. Such experiments, however, pose many difficulties because of the requirements for high-level

regulation techniques and the great expense to maintain proper experimental conditions and construct facilities for both CO<sub>2</sub> enrichment and warming. Although some modeling reports on the joint effects of CO<sub>2</sub> enrichment and global warming are available (Goudriaan *et al.*, 1990), experimental data are remarkably scarce. Not only photosynthesis and respiration under enriched CO<sub>2</sub> will be affected by change of temperature, but many other metabolic processes in plants are temperature-dependent as well (Warrick *et al.*, 1986; Campbell *et al.*, 1990; Baker *et al.*, 1989).

The urgent need to predict the consequences of global change has led to steady innovation in facilities used to study CO<sub>2</sub> effects. Facilities that provide CO<sub>2</sub> enrichment to field plots for extended periods and that have minimal influence on light, temperature and other environmental variables have been developed by scientists who have made an effort to obtain more accurate data for plant response to global warming in the future; these included the Open Top Chamber (OTC, e.g. Rogers *et al.*, 1983; Wong *et al.*, 1992; Leadley and Drake, 1993; Field *et al.*, 1996; Leadley *et al.*, 1997; Norby *et al.*, 1997), the growth chamber with fixed temperature (e.g. Jones *et al.*, 1984; Imai *et al.*, 1985; Bazzaz, 1990; Wayne *et al.*, 1998), Free-Air CO<sub>2</sub> Enrichment (FACE, e.g. Hendrey *et al.*, 1993; Hendrey *et al.*, 1999), Free Air Temperature Increase (FATI, Nijs *et al.*, 1996), SACC (Screen-Aided CO<sub>2</sub> Control; A middle ground between FACE and OTC, e.g. Leadley *et al.*, 1997) and the Temperature Gradient Chamber (TGC, e.g. Idso *et al.*, 1987; Rawson, 1992; Horie *et al.*, 1995; Hadley *et al.*, 1995; Okada *et al.*, 1995; Sinclair *et al.*, 1995). Ultimately, we search for a more realistically designed facility because we are interested in determining the true effects of global warming on current natural ecosystems

(Morison and Lawlor, 1999). Therefore, the best facility to study the effects of global warming should consider the increase in both temperature and CO<sub>2</sub> because these are the essential environmental factors for all plants. With increasing sophistication, however, has come increasing expense (Kimball, 1992). Partly as a result, enrichment studies rarely consider more than two CO<sub>2</sub> concentrations. Non-linear or threshold responses of plants and ecosystems that may be critical to future dynamics go undetected (Ackerly and Bazzaz, 1995; Körner, 1995).

Two general types of experiments, chamber exposures (indoor or outdoor growth chambers, greenhouses, OTC) and open-field exposures (FACE systems), have been widely used. In the former, the various types of enclosures have been devised to provide close control over the concentrations of CO<sub>2</sub>, but their microenvironmental conditions were quite artificial for test plants. Microclimate conditions and resultant plant growth in such chambers have been known to differ markedly from those in open fields (Olszyk *et al.*, 1980, Clark *et al.*, 1983). FACE systems may be efficient for the study of CO<sub>2</sub> enrichment effects on natural plant populations. However, they have three main drawbacks. First, they cannot reveal the effects of warming, which inevitably occurs with CO<sub>2</sub> increase. Second, the operation and construction costs of CO<sub>2</sub> release are very high (e.g. \$668 m<sup>-2</sup>, Hendrey *et al.*, 1993), and special techniques are necessary in order to operate these systems. Third, CO<sub>2</sub>-enriched conditions in FACE fields depend closely on wind direction and wind velocities because injected CO<sub>2</sub> gases are transported by wind. Therefore, the poorest concentration control occurs especially at low wind velocities and the accuracy for the CO<sub>2</sub>-enriched condition is not always satisfactory (Hendrey *et al.*, 1993).

The purpose of this study is to determine the responses of C3 and C4 annuals to elevated temperature and CO<sub>2</sub> throughout the full life cycle from seed to senescence in population level. In this study I have attempted to improve a temperature gradient chamber (TGC) based on an excellent idea developed by Mihara (1971) and to make a new CO<sub>2</sub>-temperature gradient chamber (CTGC) compounded with a CO<sub>2</sub> concentration gradient. These chambers allow for a wider utilization, since they require only simple techniques and have low operating costs. Growth experiments for C3 and C4 populations were conducted using the TGC and CTGC. I measured phenology, vegetative response, and reproductive output of one annual C3 species (*C. album*) and two annual C4 species (*E. crus-galli* and *S. viridis*) under ambient, 2°C higher condition than ambient with ambient CO<sub>2</sub>, 4°C higher condition than ambient with ambient CO<sub>2</sub>, 2°C higher condition than ambient with 1.4 fold of ambient CO<sub>2</sub>, and 4°C higher condition than ambient 1.8 fold of ambient CO<sub>2</sub>.