SELECTION RESPONSE TO GLOBAL CHANGE OF BRASSICA JUNCEA (L.) CZERN.

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ABSTRACT

The potential for an adaptive response to global climatic change was evaluated for an annual C3 weed, Brassica juncea, by performing a selection on fecundity for eight generations. During the selection, atmospheric carbon dioxide and temperature were gradually increased from current levels (370 μ L·L⁻¹ CO₂, 20°C) to conditions predicted during the next century by climate models (650 μ L·L⁻¹ CO₂, 23.6°C, including heat stress events at 32°C/26°C day/night). At the end of the selection, a reciprocal transplant experiment was conducted to identify genetic differences between control selection lines of plants and those selected under increasing CO₂ and temperature. I observed a genetic adaptation of early vegetative growth to elevated CO_2 and temperature, which resulted in to 63%more biomass and 11% higher photosynthetic rates. Reproductive biomass, however, was decreased during the mainly due to temperature selection. stress, which disrupted flower development and induced strong maternal effects, counteracting the selection on fecundity.



RÉSUMÉ

Une plante annuelle à métabolisme en C₃, Brassica juncea, a été étudiée pour vérifier l'existence d'un potentiel aux changements climatiques d'adaptation génétique Pendant globaux prévus pour les prochaines décennies. huit générations, des plantes ont subi une sélection agissant sur la fécondité. Les conditions environnementales ont été modifiées graduellement dans le temps, passant des niveaux actuels (370 μ L·L⁻¹ CO₂, 20°C) aux conditions prédites par des modèles climatiques au cours du prochain siècle (650 μ L·L⁻¹ CO₂, 23.6°C, comprenant des vagues de chaleur à 32°C/26°C jour/nuit). Après la sélection, nous avons mené une expérience de transplants réciproques, pour vérifer la présence de différences génétiques entre les lignes de contrôle et celles exposées aux conditions Nous avons identifié une réponse adaptative changeantes. de la croissance pré-reproductive, traduite par une biomasse et des taux de photosynthèse majorés de 63% et Par contre, la reproduction des 11%, respectivement. plantes a été grandement inhibée durant la sélection, suite aux stress de chaleur, qui ont induit des effets maternels importants, amenant une réponse inverse à notre sélection sur la fécondité.

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GENERAL INTRODUCTION

1

The concentration of CO_2 in the atmosphere has been steadily rising for the last 100 years, and is likely to reach concentrations twice as high as pre-industrial levels during the second half of the next century (Firor 1990, Houghton and Woodwell 1989). In recent years, many studies have attempted to measure the potential impact of such a doubling in CO₂ concentration on plants, and have provided valuable insights on the physiological mechanisms of plant response to CO₂ (for reviews see Bazzaz 1990, Woodward et al. 1991, Poorter 1993, Hunt et al. 1991). The anticipated increase in atmospheric CO₂ concentrations is also predicted to affect temperature patterns around the globe. Overall, mean temperatures might increase by 2.5-4.5°C (Firor 1990). In turn, a rise in mean temperature increases the probabilities of extreme-temperature events, such as heat waves (Howarth 1991, Mearns et al. 1984). The study of the impact of global climatic change on plants should consider the joint increase in CO₂ and temperature, as they are both important factors in determining plant growth Temperature extremes also can affect or disrupt patterns. plant growth in a dramatic manner, especially during more vulnerable developmental stages, such as seedling establishment or the onset of reproduction (Howarth 1991).

The study of plant responses to multiple environmental factors is complicated by the fact that they

often interact (Chapin et al. 1987), and that the response to combined effect of multiple factors cannot the be extrapolated from studies of each factor separately. Long (1991) pointed out that CO_2 and temperature both had strong and contrasting effects on photosynthesis, and that the study of their combined effects was essential to the understanding of the potential impacts of global change on plants. These two factors can also have contrasting effects on biomass production and many other metabolic processes (Farrar and Williams 1991). In the same manner, the presence of stress. whether caused by extreme temperatures, drought, or any other factor, is equally likely to affect overall plant performance in future environments (Sionit et al. 1980, Howarth 1991).

Another important dimension of global climatic change is that it will not happen overnight, but rather progressively, over many years. Organisms with short lifespans, such as annual plant species, will be exposed during several generations to gradually changing environmental Conversely, long-lived individuals, such as conditions. trees and other perennials, will be exposed to a gradient of CO₂ and temperature within their lifetime. Studies of global change on annuals have mostly observed the plastic responses of plants to contrasting conditions. in experiments where two or more growth chambers reproduce present and future atmospheres, and the performance of the plants is evaluated in different

There is no assurance, though, that the gradual situations. shift in CO₂ concentration and temperature predicted by climate models will produce the same effect on plant growth as a direct exposure to enhanced conditions. Environmental changes spanning several generations could allow genetic adaptation to occur in response to natural selective pressures (Bradshaw and McNeilly 1991, Woodward et al. 1991). Mutations could modify individual genomes. The genetic structure of populations could be altered as a consequence of differential survival of individuals. In any case, natural selection would favor the fittest individuals which respond best to the new environmental conditions. No study has yet explored the question of possible adaptation of annual plants in the context of global climatic change.

This research addresses both the questions of the interactive effects of CO_2 and temperature on plant performance, and the consequences of a gradual change in atmospheric conditions on plastic and genetic responses of an annual plant species. Relatively few studies have attempted to look at the interacting effects of CO_2 and temperature, including temperature stress, on plant growth (e.g.: Hogan *et al.* 1991, Coleman *et al.* 1991, Acock *et al.* 1990, Sionit *et al.* 1987, Idso *et al.* 1987, Potvin 1985). A selection experiment, under either ambient conditions or gradually increasing CO_2 and temperature, was conducted over 8 generations on *Brassica juncea* (L.) Czern.

(Brassicaceae), a widespread annual weed present in Québec. Atmospheric CO_2 and temperature were modified simultaneously, and we observed the responses of plants to their combined effects, without attempting to partition them. The first portion of this thesis describes changes in biomass allocation, phenology, and correlations among fruit and seed characters during the selection process. The second chapter reports a reciprocal transplant experiment done at the end of the selection, to evaluate its effect on such traits as biomass allocation, growth patterns, phenology, and gas exchange, and the adaptive potential of the plants. I have also studied gas exchange relations during the selection process and reproductive characters with more detail. These results will be presented and discussed in later papers, and are not part of this thesis.

CHAPTER 1: CHANGES DURING SELECTION

INTRODUCTION

A great number of experiments designed to study global climatic change and its potential impact on plants are based on a doubling of CO₂ concentration (reviewed by Poorter 1993, Hunt et al. 1991, Woodward et al. 1991, Sometimes a rise in mean temperature Bazzaz 1990). accompanies this CO₂ enhancement, in order to study the interactive effect of the two factors (Baker and Allen 1993, Farrar and Williams 1991, Hogan et al. 1991). Such experiments focus on the acclimation response of plants to a very novel environment, to which they respond through physiological plasticity. A gradually changing climate, however, could allow plants to acclimate to the environment gradually, as it changes (Bradshaw and McNeilly 1991). Studying the effects of a direct doubling in CO₂ does not account for the possibility that a gradual change over time might affect plant responses differently. Changes in the environment over several generations, especially in the case of annual species, could allow evolutionary change to occur, modifying individual genomes and population structures (Woodward et al. 1991, Bradshaw and McNeilly 1991), and eventually leading to organisms which are better adapted. To my knowledge, no study on plants has reported the effects of a gradual

increase in CO_2 and temperature environment. Also, plants have never been selected to test for an evolutionary response to global change.

Increasing frequencies of high-temperature events a consequence of higher are expected as mean temperatures due to higher atmospheric CO₂ concentration (Mearns et al. 1984, Howarth 1991, Filor 1990). Heat stress is an important factor affecting plant growth, and can have considerable impact on plants, especially in the context of global change (Baker et al. 1992, Coleman et al. 1991, Howarth 1991, Hogan et al. 1991). Some stages of growth, such as seedling establishment or the onset of flowering, are particularly vulnerable to stress (Chiariello and Gulmon 1991, Howarth 1991). Plant reproduction has more temperature-sensitive than been noted to be vegetative growth (Potvin 1991, Polowick and Sawhney In this sense, heat stress could serve as an 1988). additional selective pressure confronting plants under global climatic change. Most early studies of global change wild plants ended before plants began their on reproductive phase (reviewed by Bazzaz 1990), and thus very little information is available on the impact of global change on the reproduction of non-crop species (Bazzaz 1990, Garbutt and Bazzaz 1984).

In this study, I examined a herbaceous C_3 weed, Brassica juncea (L.) Czern., throughout its growth cycle, including an analysis of its reproductive characteristics.

The experiment was designed to examine the combined effects of gradually increasing CO₂, mean temperature, and heat stress, over several generations. I did not attempt to partition these factors, but rather, concentrated on their joint effect, since it is the most crucial in understanding future impacts on plant growth. Environmental conditions were modified gradually, over eight generations of selection, to better reflect changes through time as they are predicted to occur. The final conditions reached at the end of the selection were close to a doubled CO_2 concentration from current levels, accompanied by an increase in mean temperature of almost 4°C, in accord with general climate model predictions (e.g. Firor 1990, Houghton and Woodwell In this chapter, I present results concerning 1989). changes observed during the selection process, particularly on biomass allocation patterns, phenology, and the relationship among reproductive characters.

MATERIALS AND METHODS

Plant material and general growth conditions

Seeds from a Rapid-cycling base population (RCBP) of Brassica juncea (L.) Czern. (Brassicaceae) were obtained from the Crucifer Genetics Cooperative (Madison, Wisconsin) (Williams and Hill 1986) and served as the base population for this selection experiment. This annual species is insect-pollinated, outcrossing and self-

incompatible, and has a C₃ metabolism. It has an indeterminate growth pattern, and flowers in racemes. Although the species is one of those cultivated for mustard seed and oil on the globe, it occurs as a widespread weed in eastern Canada (Sabourin et al. 1991). The rapid-cycling genotypes could complete their life-cycle within 49 days under our experimental conditions. Seeds were sown in 13-cm (1.13 L) plastic pots filled with Pro-Mix[™], a commercial mixture of peat, perlite, and vermiculite. The plants were placed in two growth chambers in the McGill University Phytotron. Growth conditions at the beginning of the experiment were chosen following recommendations for optimal growth of B. juncea, according to the seed supplier (Anonymous 1985). Lighting was provided both by incandescent bulbs and fluorescent tubes, with a 16h/8h day/night photoperiod. Photosynthetically active radiation (PAR) readings at the plant level varied between 350 and 450 $\mu E \cdot m^{-2} \cdot s^{-1}$. Relative humidity was maintained at 70%. Chamber air temperature and CO₂ concentration were initially set at 22°C/16°C and 370 μ L·L⁻¹, respectively, were modified thereafter for each generation, and depending on treatment, as will be described later. All plants were watered four times a week, including two weekly applications of 20:20:20 fertilizer, during the whole experiment.

Ten days after sowing, seedlings were thinned to one plant per pot. Flowering began during the fourth week of

Individual plants were surveyed daily and the growth. date of the opening of the first flower was recorded, except at generation 2 of one of the treatments. This measurement of the onset of flowering will be referred to from now on as flowering date. The great majority of plants in the same growth chamber began flowering within 5 or 6 days of each other, and each plant continually developed new flowers during approximately 10 days. When two-thirds of the plants within a given group had begun flowering, they were mass-pollinated by hand using a small paintbrush. I took particular care to ensure that flowers were pollinated randomly within a group, by brushing flowers repeatedly with the pollen-loaded instrument and by varying the order from time to time. Pollination was repeated at least every two days, until all the plants had completely stopped flowering. I harvested the plants after 49 days, when the siliques (seed pods) were formed and the seeds fully developed, but before the plants completed their senescence. The siliques were separated from the vegetative organs, and dried at 30°-35°C for 3 days to complete their senescence. The seeds were immediately ready to be sown again.

Experimental design and fecundity selection

At the beginning of the experiment, I placed two groups of 70 pots in each growth chamber, and grew them

under the initial conditions described above. Each group was pollinated separately, and led to a distinct line of plants. Four selection lines were established in this For the plants harvested from generations 2 manner through 7, fecundity selection was carried out to maximize chances of observing an adaptive response to the changing environmental conditions. After the plants were harvested and the siliques dried, I selected the 25% most productive plants within each selection line, based on total silique I then randomly chose 40 seeds from these 18 mass. mother plants, to sow four pots from each for the next generation, until generation 8. One of the mother plants, randomly chosen, provided 20 seeds for two pots, since space constraints did not allow more than 70 pots per The 70 pots of each line obtained in this manner group. were randomly placed in the growth chamber. Occasionally, when seed germination rates were low, I transplanted half-sibs between pots, prior to thinning the seedlings.

Selection treatments

Two replicate lines in each chamber were selected in conditions simulating either the present atmosphere ("Current" treatment), or gradually increasing CO₂ and temperature, designed to reflect future global climatic change ("Predicted" treatment). The "Current" selection treatment served as a control. Conditions were maintained at the initial temperature $(22^{\circ}C/16^{\circ}C)$ and CO_2 conditions $(370 \ \mu L \cdot L^{-1})$ for the 8 generations of the experiment. ln contrast, conditions in the "Predicted" selection treatment were changed gradually from one generation to the next (Table 1.1). Starting at generation 2, the chamber CO₂ level was raised by 40 μ L·L⁻¹ per generation, to reach a final concentration of 650 μ L·L⁻¹, close to double the initial concentration of 370 μ L·L⁻¹. Overall air temperature was gradually raised by a total of approximately 3.6°C over the eight generations. As predicted by climate modelers (e.g. Mearns et al. 1984), a doubling in atmospheric CO₂ concentration will cause a rise in mean air temperature, which in turn will increase the length and frequency of extreme temperature events, such as heat waves. Degreedays were calculated to model the desired changes in temperature over time, using a combination of increasing mean temperatures and more and more frequent heat Normal day/night temperatures were raised stress events. slightly (0.4°C) at each generation. In addition, at generation 2, a first heat wave (two days at 32°C/26°C) was programmed during the fourth week of plant growth. In subsequent generations, one day of heat stress was added per generation. Beginning with generation 3, the heat waves were split into two periods, during the fourth and sixth weeks of growth. By generation 8, the plants were exposed to normal day/night temperatures of

<u>Table 1,1</u> :	Summary	of the	growth	condition	s during
	selection of	Br assi	ca junce	ea under	the
	"Predicted"	treatn	nent.		

Genera- tion	CO ₂ level $(\mu L \cdot L^{-1})$	Normal day/night	No. of days of heat	Mean tempera-
number		temperature (°C)	stress (32°C/26°C)	ture (°C)
				(0)
1	370	22.0/16.0	0	20.00
2	410	22.0/16.0	2	20.41
3	450	22.4/16.4	3	20.99
4	490	22.8/16.8	4	21.55
5	530	23.2/17.2	5	22.10
6	570	23.6/17.6	6	22.63
7	610	24.0/18.0	7	23.14
8	650	24.4/18.4	8	23.64

24.4°C/18.4°C, and to a total of eight days of heat, grouped into two 4-day heat waves.

To account for possible chamber effects. plants from each treatment were sown in alternation in each of the two growth chambers, so that plants from both treatments, of the same generation, were successively grown in the same chamber. For example, seeds of generation 2 of the "Current" treatment were sown in the same chamber as those of generation 2 of the "Predicted" treatment, immediately after the latter were harvested. At the same time, the seeds for the third generation of the "Predicted" treatment were sown in the other growth chamber, and so on.

Biomass allocation and reproductive characters

The total mass of siliques produced by each plant was then determined using an electronic balance (Sartorius Handy H51). Vegetative organs were divided into aboveand below-ground components, except for generation 2 of the "Predicted" treatment. where vegetative biomass measurements were omitted. Roots were washed by hand to remove soil before drying. Above-ground organs (stems together with leaves), and roots were dried at 65°C for at least 2 days, and their dry mass was measured.

At generations 1, 3, 5, and 7 for both selection treatments, reproductive characters of individual plants were analyzed with greater detail. Siliques of each plant

were counted, then opened one by one, to determine total silique number and the mean number of seeds per silique for each plant. When all seeds were extracted, I determined total seed mass and number for each plant.. Finally, eight random samples of 25 seeds from each plant were weighed, to estimate mean individual seed mass.

Statistical analysis

All analyses were done on untransformed data, after verifying that they were normally distributed (Proc Univariate, SAS v. 6.0.4, SAS Institute Inc., 1988). Simple regression analysis was used (Proc REG, SAS v. 6.0.4) to examine the changes in vegetative dry mass (combined above- and below-ground biomass), reproductive mass, and flowering date, as selection progressed. Generation number was used as the independent variable. Slopes of the regression models were then tested for heterogeneity to show experimental treatment effects (Weisberg 1985). I tested the slopes for "Current" vs "Predicted" selection treatments, as well as the slopes for the replicate lines within treatments, to verify the constancy of the observed response. Relationships within individual plants, between flowering date (onset of flowering), total silique and seed mass, total silique and seed number, mean individual seed mass, and mean number of seeds per silique were

examined using Pearson's linear correlation coefficients (Proc CORR, SAS v. 6.0.4).

<u>Results</u>

There was a significant linear increase (Table 1.2, Fig. 1.1a) of vegetative dry biomass (stems, leaves and roots) for plants of the "Predicted" treatment, as selection progressed. Line 1 showed a significantly steeper increase (slope of 0.122 g-generation⁻¹) than line 2 (0.052 g-generation⁻¹) (Tables 1.2 and 1.3). This was the only significant difference observed between lines of а treatment, for all the recorded observations. "Current" selection lines, on the other hand, showed a slight but highly significant reduction in their vegetative dry mass over time (Table 1.2, Fig 1.1b), with no significant differences between the slopes of the two lines (line 1: -0.072 g·generation⁻¹; line 2: -0.095 g·generation⁻¹, Tables 1.1 and 1.2). The slopes were significantly different among selection treatments (F = 77.57, P < 0.01, Table 1.3). In comparing generations 8 and 1, plants of the "Predicted" treatment had 38% more vegetative biomass, whereas those of the "Current" treatment had 21% less vegetative biomass.

Table 1.2:Slopes of the linear relationships of vegetative
biomass, reproductive biomass, and flowering
date, as a function of generation number, during
selection of Brassica juncea under either
"Current" or "Predicted" CO2 and temperature
conditions.

Dependent variable	Selection line	Slope of the regression					
		"Cur	rent"	"Prec	"Predicted"		
		treat	ment	trea	treatment		
		Esti- mate	P (Ho: slope= 0)	Esti- mate	P (Ho: slope= 0)		
Vegetative biomass ^a	1 2	-0.072 -0.095	0.0001 0.0001	0.122 0.052	0.0001 0.0001		
Reproductive biomass ^a	1 2	-0.311 -0.334	$0.0001 \\ 0.0001$	-0.439 -0.447	$0.0001 \\ 0.0001$		
Flowering date ^b	1 2	0.317 0.353	0.0001 0.0001	-0.437 -0.344	$0.0001 \\ 0.0001$		

a: slopes expressed in g-generation-1

b: slopes expressed in days-generation-1

<u>Table 1.3</u>: Test of heterogeneity of slopes, within and between selection treatments on *B. juncea*, for the linear relationships of vegetative biomass, reproductive biomass and flowering date, as a function of generation number.

Dependent variable	Comparison of slopes within a treatment				Compa betw treatn	een nents
	(H _o : line	1 = line 2)	$(H_0: "C) = "Pred$	urrent" icted")
	"Cur F	rent" Pa	"Predi F	cted" P	F	Р
Vegetative biomass	0.96	n.s.	4.98	***	77.57	***
Reproduc- tive biomass	0.47	n.s.	0.06	n.s.	26.25	***
Flowering date	0.27	n.s.	1.12	n.s.	171.89	***

a: n.s.: non-significant (P > 0.05); ***: P < 0.01;



Biomass allocation between organs for Brassica Figure 1.1. juncea, during 8 generations of selection in the (a) "Predicted" or (b) "Current" treatment. For each treatment, only the results for one of the two selection lines are presented. Each column represents the mean values for each generation (N = 70).□: Siliques; □: Roots; Stems and leaves.



Reproductive biomass decreased significantly as selection progressed under both treatments, despite selection for high fecundity (Fig 1.1). The decrease of absolute yield was much more dramatic for the "Predicted" selection lines (average slope of -0.443 g-generation⁻¹, Fig. 1.1a, Table 1.2) than "Current" lines (average slope of -0.323 g generation⁻¹, Fig 1.1b, Table 1.2). This difference between treatments is highly significant (F = 26.25, P < 0.01, Table 1.3). The two lines within selection treatments did not differ significantly in the response observed (Table When the data are examined in terms of relative 1.3). allocation to reproduction (% of total biomass allocated to reproductive structures), it is apparent that plants of the "Predicted" treatment devoted an ever decreasing portion of their resources to fruit production as selection progressed, whereas those of the "Current" treatment quickly stabilized their reproductive effort at around 40% (Fig. 1.2).

Flowering date varied through time in very different ways, depending on selection treatment. By generation 8, there was a 6-day difference in the mean flowering date of plants in the two selection treatments (Fig. 1.3). In both "Predicted" selection lines there is a strong negative linear relationship between flowering date and generation number (slopes of -0.437 and -0.344 days generation⁻¹, Table 1.2), indicating that these plants flowered continually earlier as selection progressed. By contrast, flowering date



Figure 1.2. Reproductive allocation (% of total biomass allocated to reproductive organs) for *Brassica juncea*, during 8 generations of selection in the a) "Predicted" or b) "Current" treatment. For each treatment, only the results for one of the two selection lines are presented. Each column represents the mean value for each generation (N = 70).

a)



Generation

Figure 1.3. Mean flowering date over 8 generations of selection of *Brassica juncea*, in the "Current" or "Predicted treatments. Each symbol represents the mean of 70 plants. -=-: "Current" line 1;---: "Current" line 2;-+-: "Predicted" line 1;---: "Predicted" line 2: was delayed as selection progressed under the "Current" treatment, as shown by the positive slopes in Table 1.2 (line 1: 0.317 days·generation⁻¹; line 2: 0.353 days·generation⁻¹). In all cases, both lines within a selection treatment did not differ significantly (Table 1.3), and the response was significantly different among both selection treatments (F = 171.89, P < 0.01, Table 1.3).

For a given plant, all the measured silique and seed characters were significantly and positively correlated (P <0.0001, Table 1.4). The highest values of r were found between total silique mass, silique number, and seed mass, which all give an estimate of total plant yield (r = 0.837 or higher, Table 1.4). The lowest values of r were found between mean individual seed mass, mean number of seeds per silique, and total silique and seed number (r =0.354 to 0.583, Table 1.4). The strength and direction of linear correlations of seed and silique characters with flowering date varied with time and selection treatment (Table 1.5). At generation 1, a weak but significant negative correlation existed between flowering date and total silique number (r = -0.192, Table 1.5). By generation 7, selection under the "Current" treatment led to strong negative correlations between flowering date and total silique mass and number (r = -0.721 and -0.704), respectively), and also weaker but significant negative correlations between flowering date and mean number of seeds per silique and individual seed mass (r = -0.247 and

<u>Table 1.4</u>: Pearson's correlation coefficients, r, between reproductive characters of *Brassica juncea*, over the entire selection experiment (N = 1098).

ra	Total silique mass	Total seed mass	Total silique no.	Total seed no.	Mean indiv. seed mass	Mean no. of seeds / silique
Total silique mass	1.000	0.910	0.921	0.592	0.627	0.561
Total seed mass		1.000	0.837	0.605	0.743	0.657
Total silique no.			1.000	0.583	0.548	0.490
Total seed no.				1.000	0.354	0.481
Mean indiv. seed mass					1.000	0.446
Mean no. of seeds / silique						1.000

a: all values of r are significant at P = 0.0001.

Table 1.5:	Pearson's correlation coefficients, r , between
	flowering date and other reproductive
	characters of Brassica juncea, at different times
	during selection under a "Current" or "Predicted"
	environment (N = 140 at each generation).

Variable	r with flowering date (level of significance ^a)		
	Before	"Current"	"Predicted"
	selection	treatment	treatment
	Generation	Generation	Generation
	1	7	7
Total silique	-0.105	-0.721	0.055
mass	(n.s.)	(****)	(n.s.)
Total silique	-0.192	-0.704	0.102
number	(*)	(****)	(n.s.)
Mean number of seeds/silique	0.058 (n.s.)	-0.247 (**)	-0.096 (n.s.)
Mean indiv.	-0.066	-0.329	-0.120
seed mass	(n.s.)	(****)	(n.s.)

^a: n.s.: non-significant (P > 0.05); *: P < 0.05; **: P < 0.01; ****: P < 0.0001. -0.329). Earlier flowering was related to higher reproductive output under the "Current" treatment. By contrast, at the end of selection under the "Predicted" treatment, I did not observe any significant correlations between flowering date and seed or silique production (Table 1.5), suggesting that other important factors, such as the heat stress events, were linked to final reproductive biomass.

DISCUSSION

All the variables observed showed important changes as selection progressed, and were affected differently by the selection treatments. Independent replicate selection lines had been established in each treatment to verify the repeatability of the results. The two replicate lines within each selection treatment showed similar changes during the selection, for all the traits I observed, giving added confidence in the results.

During the eight generations of selection under increasing CO_2 and temperature in the "Predicted" treatment, plants consistently increased their vegetative biomass, both in absolute terms, as expressed by the positive slopes of vegetative biomass against generation number, and relative to the allocation to reproduction. Enhancement of vegetative biomass is regularly reported in studies of the effects of elevated CO_2 alone (Poorter 1993, Woodward *et al.* 1991, Bazzaz 1990), or when combined

with elevated temperatures (Kimball et al. 1993, Hunt et al. 1991, Acock et al. 1990, Patterson et al. 1988, Idso et al. However, many studies report a reduction in 1987). overall plant growth response during a long-term exposure to elevated CO_2 , with or without higher temperatures (reviewed by Farrar and Williams 1991). Reduction in plant response is thought to result from an imbalance between the various sinks in a plant, its capacity to effectively assimilate larger amounts of CO₂ via and the resources provided photosynthesis, bv the environment (Arp 1991, Farrar and Williams 1991). In our selection experiment, vegetative growth enhancement was The maintained through time, even in the long-term. growth enhancement of vegetative biomass in the "Predicted" treatment, expressed as the ratio of biomass in "Predicted" over "Current" conditions, was moderate during most of the selection, oscillating between 1.04 and 1.29, then rose sharply to 1.74 at the last generation of selection. Our selection scheme possibly allowed for genetic adaptation of plant assimilation and growth mechanisms to the changing atmospheric conditions. At the same time, the gradual nature of the changes in environment over several generations might have permitted plant growth and physiology to acclimate and remain in equilibrium with the environmental changes. Such a continuous adjustment of a plant might probably not be achieved when conditions are
modified abruptly, as is usually done in traditional experiments.

The few studies of wild plant reproduction under elevated CO₂ report varying results. Phenology is sensitive to CO₂ enrichment: earlier flowering is often reported for plants grown under elevated CO₂ (Sionit et al. 1987, Garbutt and Bazzaz 1984, Woodward et al. 1991). Garbutt and Bazzaz's (1984) study of wild plant reproduction under enhanced CO_2 reports that reproductive ratio was either increased (Phlox drummondii) or unaffected (Abutilon theophrasti) by elevated CO_2 . In the same study, Datura stramonium showed a decrease in relative reproductive effort, but no change in seed output. Studies of the interactive effect of increased CO_2 and temperature on crop plants, without the effect of stress, report variable but usually positive responses of reproductive yield (Baker and Allen 1993, Baker et al. 1992, 1989, Sionit et al. 1987, Havelka et al. 1984). The occurrence of temperature stress, however, can have important repercussions on plant reproduction (Chiariello and Gulmon 1991, Ceccarelli et al. The passage between vegetative and reproductive 1991). growth is particularly sensitive to stress in *B. juncea* (Singh et al. 1991).

My results show that the relationship between flowering date and other reproductive characters was modified by selection. Despite earlier flowering, reproductive biomass was dramatically decreased under

selection treatment. Plants of the "Predicted" the "Predicted" treatments experienced increasingly long heat waves during the fourth and sixth weeks of growth, which coincided approximately with the onset of flowering and with the maturation period of siliques and seeds. It is possible that, due to changes in flowering date, the stress events occurred at periods of greater sensitivity as selection progressed (Chiariello and Gulmon 1991). Plants probably experienced a disruption in floral development as a consequence of the first heat wave, which caused flower abortions or abnormal development of reproductive organs (Polowick and Sawhney 1988). This would lead to a corresponding reduction in number of siliques formed as well as in the number of seeds per silique.

Alternately, the strong reduction in yield of plants during selection under the "Predicted" treatment may partially be explained by strong environmental maternal effects acting on the developing gametes and seeds. Maternal effects are defined as the nongenetic contribution of the mother to the offspring phenotype (Roach and Wulff The temperature environment of a plant during its 1987). phase strongly affect reproductive can progeny performance (Chiariello et al. 1991). Potvin and Charest (1991) have reported reduced seed weight at high temperatures for barnyard grass (Echinochloa crus-galli), and attributed it to the higher respiration rates of inflorescences in warm conditions. Yield components of

Brassica spp. are known to be highly sensitive to the environment (Olsson 1960, Chay and Thurling 1989), and thus are likely prone to maternal effects. Maternal inheritance has been mathematically shown to cause timelags in the evolutionary response of a trait under selection, eventually even leading to a response opposite to the direction of selection (Kirkpatrick and Lande 1989). Our results would confirm this finding. To my knowledge, this is the first experiment to report a reversed response to fecundity selection due to environmental maternal effects in plants.

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Despite my selection for fecundity, I also observed a reduction in fecundity in the "Current" treatment, much less, however, than for the "Predicted" treatment. This suggests that our experimental design per se might have caused some reduction in plant fitness. Throughout the experiment, seeds were harvested and processed in the laboratory before sowing, according to the recommendations of the Crucifer Genetics Cooperative, which provided the seed for our base population (Anonymous 1985). Seed handling might have affected the plants' performance to some degree. It is also possible that basing the selection on total silique mass could have brought about unwanted effects of negatively correlated characters on yield, which could have greatly reduced the effectiveness of our fecundity selection (Antonovics 1976). This would be the case if high total silique mass was correlated with small individual seed mass. My results, however, show that all reproductive characters were significantly positively correlated. No trade-off exists between number and size of seeds and siliques, and number of seeds per silique. Among the variables I measured, total silique mass per plant had the highest correlation with total seed mass, which is the most direct measure of plant yield. Likewise, Olsson (1960), while not measuring silique mass, determined that among other estimates of yield in various species of *Brassica*, such as 1000-seed weight and number of seeds per silique, silique number was most closely related to total seed mass.

Another possible explanation for the observed decrease in fecundity in "Current" plants is that some reduction in overall fitness was due to inbreeding depression, as a consequence of our selection design. At each generation, four half-sibs from each selected mother plant were planted in the 70-plant group within which random mass-pollination was carried out. The number of related individuals within each selection line necessarily increased as selection progressed. Finite population size and inbreeding can both cause a reduction of the proportion of heterozygotes in a population (Charlesworth and Charlesworth 1987, Futuyma 1986) Population size was relatively small and probably led to some inbreeding during the selection. However, precautions were taken to maximize initial population size and to ensure random

outcrossing among individuals throughout the experiment. The seeds used at the beginning of the selection came from base population that had been mass propagated a specifically for use in genetic studies (Williams and Hill 1986, Anonymous 1985), and therefore one can assume that plants of generation 1 were unrelated individuals. Brassica juncea is a self-incompatible species, so not only is it safe to assume that self-pollination did not occur, but the self-incompatibility mechanisms of the species would have inhibited the growth of pollen tubes from close relatives with matching S-alleles (Nasrallah et al. 1991), thus preventing excessive inbreeding. In addition, by selecting the most fecund plants to provide seed for the following generation, we also selected those with the largest, bestquality seeds, since higher silique mass was positively correlated with individual seed weight. This should have contributed to reduce any negative effect of inbreeding An inbreeding coefficient could not be depression. determined for plants in this experiment, since the paternal parent was unknown, due to mass-pollination. We observed, however, that allocation to reproduction of "Current" plant lines decreased only marginally after generation 2, and was maintained at around 40% of total plant biomass. This value is not too far from Chiariello and Gulmon's (1991) estimate that, for an annual plant, seed yield is optimal at a reproductive effort near 50%. Given these elements, I believe that inbreeding depression, if

present, would only have caused a slight decline in plant fitness.

The outcome of this experiment and the interpretation of the results of selection under increasing CO_2 and temperature are not affected by the observation of reduced fecundity in the "Current" treatment. Seed handling or inbreeding depression likely had some impact on plant fitness, but this was only minor, compared to the magnitude of the strong treatment effects on every trait measured on plants in the "Predicted" treatment.

CHAPTER 2: TESTING THE OUTCOME OF SELECTION

INTRODUCTION

During the next century, we could encounter a global change in climate accompanying the rapid rise in atmospheric CO_2 and other greenhouses gases. lf predictions are met, the anticipated climate warming will be of a magnitude comparable to the last deglaciation, but at a rate of change much greater than any other climate event since man has inhabited the planet (Firor 1990, Huntley 1991). Historically, plant species have mostly responded to climate change by migration, tracking favorable conditions to avoid change (Huntley 1991, Bradshaw and McNeilly 1991, Firor 1990). However, accelerated changes in climate could exceed the capacity of species to migrate, which is estimated for trees at approximately 150-500 :n-year⁻¹ (Huntley 1991). In addition, human activities have created physical barriers restricting the movements of natural populations and In the context of rapid global change, communities. migration could therefore not be sufficient to assure plant success and survival (Huntley 1991, Bradshaw and McNeilly 1991). In order to cope with rapidly changing environments, plants will probably have to rely more and more on other mechanisms, such as acclimation (plastic changes in phenotype) or adaptation (genetic changes in genome and/or in population structure through time, in response to selection) (Bradshaw and McNeilly 1991). While physiological plasticity is likely to play some role, quickly rising CO_2 and temperature, as well as the increased frequency of heat stress, could exert strong selective pressures, and call for rapid population adaptations. Annual species in particular, with their short generation time, could benefit from rapid evolutionary change under these conditions.

The possibility for plants to respond to global climatic change by genetic adaptation has been suggested (Woodward 1993, Woodward et al. 1991, Bradshaw and McNeilly 1991), but not yet verified experimentally. Maxon Smith (1977) attempted without success to select for more efficient CO₂-utilization in greenhouse-grown lettuce, in the hope of increasing commercial yield. The possibility of a selection response to elevated CO_2 and temperature could deeply modify our vision and understanding of plant, population and community responses to global change. To my knowledge, this study is the first to specifically test for adaptation to increased CO_2 and temperature in a wild species. I selected plants for fecundity under either increasing CO_2 and temperature or ambient conditions (see Chapter 1 for details). The objective of the experiment reported here is to test the outcome of eight generations of selection under simulated global change on Brassica juncea (L.) Czern (Brassicaceae). At the end of the selection, I conducted a reciprocal transplant experiment to verify the existence of any adaptive and acclimation responses of the plants. This approach, inspired by the classic studies of Clausen, Keck and Hiesey (1948), has been used in ecological field experiments to test for population adaptations to their habitat (e.g. Jain and Bradshaw 1966, Potvin 1986, Woodward et al. 1991). It has less frequently been associated with artificial selection experiments, although some reports exist (Ågren and Schemske 1993, Maxon Smith 1977). By using this method, I was able to study both the intensity of selection on each line of plants, and their plastic response to contrasting conditions. Because the measure of a trait at the end of a plant's life-cycle is not always a true indicator of the effects of selection on that trait (Kelly 1992), I harvested plants at three different stages of their life-cycle. This allowed me to observe whether selection effects changed with time. In this chapter, I report results concerning biomass allocation and growth patterns, phenology, and gas exchange, resulting from our selection.

MATERIALS AND METHODS

Plant material and growth conditions

To determine whether the changes observed during the selection experiment (Chapter 1) were genetic or plastic, I grew plants from all four selection lines in a completely crossed design, under both environmental regimes (Fig. 2.1). The two growth chambers were set to the conditions at the end of the two selection treatments described in Chapter 1. The "Current" chamber was kept at day/night temperatures of 22°C/16°C, with a CO₂ concentration maintained at 370 μ L·L⁻¹. The "Predicted" chamber had a CO₂ level of 650 μ L·L⁻¹, and an overall mean temperature approximately 3.6°C higher than the "Current" chamber. Daily temperatures were set at 24.4°C/18.4°C, and two four-day heat waves (32°C/26°C) were programmed during the fourth and sixth weeks of growth.

Seeds were taken from 35 randomly chosen plants of each selection line. Four groups of 35 pots, each corresponding to a selection line, were sown in each chamber (Fig 2.1). The experimental design therefore produced four different treatment combinations which will be referred to by the following abbreviations: CC: "Current" selection lines grown in "Current" conditions; CP: "Current" selection lines grown in "Predicted" conditions; PC: "Predicted" selection lines grown in "Current" conditions;



Figure 2.1: Experimental design for testing the outcome of selection on *Brassica juncea*. Each of the patterns of shading represent a distinct selection line of plants.

PP: "Predicted" selection lines grown in "Predicted" conditions. Plants began to flower during their fourth week of growth. The date of opening of the first flower for each plant was recorded. Plants within each selection line were repeatedly mass-pollinated by hand, at random, using a small paintbrush.

Three partial harvests were made, at times where the plants were in the vegetative, early-reproductive (plants in full bloom, siliques beginning to develop) and latereproductive (siliques fully developed) stages of their lifecycle. The dates of harvest corresponded to the end of the third, fifth, and seventh week of the experiment (days 21, 35, and 49, respectively). For plants in the "Predicted" conditions, the vegetative harvest occurred before the onset of the first heat wave, and the early-reproductive harvest took place between the two heat wave events. Plants in the "Current" conditions did not receive any heat stress.

Biomass allocation and growth analysis

Above-ground vegetative organs were divided into leaves, stems and siliques where appropriate. On each plant, I counted the number of leaves and of stem ramifications, and measured the total leaf area using a Li-3100 (Li-Cor, Lincoln, Nebraska) leaf area meter. Roots were washed before drying. Stems, leaves, roots and siliques were then dried at 65°C and measured for dry mass, to determine the allocation of biomass between these different organs.

Relative growth rate (RGR, in mg.mg⁻¹.day⁻¹) and Net assimilation rate (NAR, in mg·cm⁻²·day⁻¹) were calculated for each treatment combination for the time intervals 0-21 days, 21-35 days, and 35-49 days, using the following equations (Beadle 1985):

RGR =
$$(\ln W_2 - \ln W_1)/(t_2 - t_1)$$

NAR = $[(W_2 - W_1)/(LA_2 - LA_1)]^*[(\ln LA_2 - \ln LA_1)/(t_2 - t_1)]$

where W_1 and W_2 represent total dry mass in grams, LA_1 and LA_2 denote total leaf area in cm², and t₁ and t₂, time in days. Indices 1 and 2 refer to the beginning and the end of a given time interval. The calculation of NAR assumed a linear relationship between W and LA. I used the average values of W and LA for each experimental group of plants for the calculations.

Gas exchange measurements

Leaf gas exchange was measured during the third week of growth, using a portable open-system infra-red gas analyzer (model LCA-2 by ADC, the Analytical Development Company Ltd., Hoddesdon, England). To assess the effects of selection on gas exchange parameters, I compared plants from both selection treatments in their "native" growth conditions by measuring CC and PP plants. In addition, I measured plants of the PC group to study the effects of transferring plants selected under "Predicted" conditions back to the "Current" environment, and to test hypotheses of reduced carboxylation efficiency and other mechanisms of photosynthetic acclimation following prolonged exposure to elevated CO₂. In all, three experimental groups were examined.

On the 70 plants of each group, two leaves were measured at each of three CO₂ concentrations. Readings were taken at the ambient chamber CO₂ level (either 370 or 650 μ L·L⁻¹, depending on the treatment), as well as at 157 and 225 μ L·L⁻¹, to document CO₂ assimilation rate (A) at limiting intercellular CO₂ concentrations (Ci). The slope of the linear portion of these A vs Ci curves, at low CO₂ concentrations, gives an estimate of the carboxylation efficiency of Rubisco (von Caemmerer and Farguhar 1981). Pots were moved to the same location in the growth chamber for all measurements, so that all readings were taken under uniform light conditions (PAR: 400-450 μ E·m⁻ $2 \cdot s^{-1}$). Readings taken at ambient chamber conditions were done at a relative humidity of approximately 70%. Compressed gas tanks were used to supply below-ambient CO₂ concentrations. In this case, air was passed through a humidifying column of ferrous sulphate (FeSO_{4.7H₂O) to} raise relative humidity to a minimum of 40%. Air flow was regulated at 250 mL·min⁻¹ using an ADC ASU-MF air pump.

Air temperature was 22°C in the "Current" chamber and 24.4°C in the "Predicted" chamber. The LCA-2 system is not equipped with dual humidity sensors necessary to adequately determine transpiration rates and stomatal conductance. A single sensor is located inside the leaf chamber. To obtain values of instantaneous air humidity in the growth chamber, I took readings with the leaf chamber open and empty every 10 minutes. Ambient relative humidity did not vary by more than $\pm 1\%$ between these measurements. These data were incorporated with the rest of the raw gas exchange data. CO₂ assimilation rate (A), transpiration rate (E), stomatal conductance (gs) and intercellular CO₂ concentration (Ci) were then recomputed according to von Caemmerer and Farquhar (1981). Wateruse efficiency (WUE) was calculated as the ratio of A over E.

Statistical analysis

Analyses were done on untransformed data, after testing for normal distributions of the measured variables (Proc Univariate, SAS v. 6.0.4). I performed three separate ANOVAs, one for each harvest, to account for the very different physiological status of plants through time. Type III sums of squares were used (Proc GLM, SAS v. 6.0.4), and the following variables were analyzed: vegetative dry mass, number of leaves, number of stem ramifications, and

total leaf area. The effects of selection regime, growth conditions, and selection line nested under selection regime, as well as their interactions, were tested in the model. The magnitudes of these experimental effects were estimated according to Winer (1971). Similar ANOVAs were performed on final reproductive mass and on flowering date. Tukey HSD post hoc tests were performed on significant interactions. A, gs and WUE measured at growth chamber CO₂ levels were analyzed using a two-way ANOVA model which included experimental Group ("Predicted" lines in "Predicted" conditions, "Predicted" in "Current" and "Current" in "Current") and Line(Group) as Tukey HSD tests were used to identify fixed factors. significantly different means among groups. No statistical analysis could be done on either RGR or NAR, since only a single value was obtained from means for each group of The slopes of the A vs Ci curves generated from plants. measurements at below-ambient CO₂ were calculated from mean values.

RESULTS

There is generally no significant effect of selection line within a treatment. Tables 2.1 to 2.4 indicate that the two replicate lines of each selection regime responded to the treatments in an identical manner. The only exceptions are leaf and stem number at the early-reproductive harvest (Table 2.1), and total leaf area at the late-

Table 2.1. Summary of ANOVA results for evaluating the outcome of selection on biomass allocation and phenology of *Brassica juncea*. The sources of variation refer to selection regime, growth conditions, and selection line nested under selection regime, as well as their interactions. The magnitude of each effect is expressed as the percentage of total variation explained by the effect. When an experimental effect was non-significant with F < 1, the calculated estimate of its magnitude was a nonsense negative value, which was approximated to 0.

Variable and	Source of	SS	df	F	р	Magni-
harvest	variation	(Type				tude
				· · · · · · · · · · · · · · · · · · ·		
Vegetative biomass						
Pre-	Selection	0.726	1	9.26	0.0032	7.96
reproductive	Condition	0.241	1	3.07	0.0836	1.99
harvest	Line(Sel)	0.161	2	1.03	0.3626	0.06
	Sel * Cond	0.884	1	11.28	0.0012	9.90
	Cond * Line (Sel)	0.169	2	1.08	0.3456	0.15
Early-	Selection	0.923	1	1.89	0.1741	1.12
reproductive	Condition	4.237	1	8.67	0.0045	9.62
harvest	Line(Sel)	0.732	2	0.75	0.4774	0
	Sel * Cond	0.957	1	1.96	0.1665	1.20
	Cond * Line (Sel)	0.348	2	0.36	0.7022	1.61
Late-	Selection	8.360	1	13.44	0.0005	14.78
reproductive	Condition	1.226	1	1.97	0.1651	1.15
harvest	Line(Sel)	0.176	2	0.14	0.8684	0
	Sel * Cond	0.673	1	1.08	0.3023	0.10
	Cond * Line (Sel)	0.240	2	0.19	0.8250	0
Reproductive	biomass					
Late-	Selection	0.389	1	0.83	0.3657	0
reproductive	Condition	114.06	1	243.25	5 0.0001	76.07
harvest	Line(Sel)	0.498	2	0.53	0.5904	0
	Sel * Cond	0.647	1	1.38	0.2445	0.12
	Cond * Line (Sel)	2.316	2	2.47	0.0924	0.92
Flowering date						
	Selection	247.52	1	94.51	0.0001	23.74
	Condition	280.33	1	107.03	0.0001	26.92
	Line(Sel)	9.938	2	1.90	0.1529	0.46
	Sel * Cond	9.188	1	3.51	0.0627	0.64
	Cond * Line (Sel)	0.104	2	0.02	0.9803	0

Table 2.2. Summary of ANOVA results for evaluating the outcome of selection on total leaf area, leaf number and stem number of *Brassica juncea* at the pre-reproductive harvest. Sources of variation and magnitude of experimental effects are as in Table 2.1.

Variable	Source of	df	F	pa	Magni-
	variation				<u>tude</u>
Total leaf	Selection	1	24.21	****	22.09
area	Condition	1	2.21	n.s.	1.15
	Line (Sel)	2	0.45	n.s.	0
	Sel * Cond	1	0.50	n.s.	0
	Cond * Line (Sel)	2	0.63	n.s.	0
Number	Selection	1	18 26	****	16.46
of leaves	Condition	1	6 4 4	*	5.19
or leaves	Line (Sel)	2	0.20	n.s.	2.78
	Sel * Cond	-	1.35	n.s.	0.33
	Cond * Line (Sel)	2	1.21	n.s.	0.40
Number	Selection	1	3,99	*	2.91
of stems	Condition	i	10.53	**	9.28
	Line (Sel)	2	1.89	n.s.	1.73
	Sel * Cond	1	2.45	n.s.	1.41
	Cond * Line (Sel)	2	2.99	n.s.	3.87

^a: n.s.: non-significant, P > 0.05; *: P < 0.05; **: P < 0.01; ***: P < 0.001; ****: P < 0.0001. Table 2.3. Summary of ANOVA results for evaluating the outcome of selection on total leaf area, leaf number and stem number of *Brassica juncea* at the early-reproductive harvest. Sources of variation and magnitude of experimental effects are as in Table 2.1.

Variable	Source of variation	df	F	pa	Magni- tude
Total loof	Coloction				
i otai leai	Selection	1	11.03	**	9.10
area	Condition	1	28.38	****	24.85
	Line (Sel)	2	1.35	n.s.	0.64
	Sel * Cond	l	1.13	n.s.	0.12
	Cond * Line (Sel)	2	0.97	n.s.	0
Number	Selection	1	0.60	n.s.	0
of leaves	Condition	1	25.18	****	23.20
	Line (Sel)	2	5.45	**	8.54
	Sel * Cond	1	0.18	n.s.	0
	Cond * Line (Sel)	2	1.19	n.s.	0.36
Number	Selection	1	6.88	*	4 47
of stems	Condition	1	46.25	****	34.42
	Line (Sel)	2	5.17	**	6.34
	Sel * Cond	1	0.09	n.s.	0
	Cond * Line (Sel)	2	1.46	n.s.	0.70

^a: n.s.: non-significant, P > 0.05; *: P < 0.05; **: P < 0.01; ****: P < 0.001; ****: P < 0.0001.

<u>Table 2.4</u>. Summary of ANOVA results for evaluating the outcome of selection on total leaf area, leaf number and stem number of *Brassica juncea* at the late-reproductive harvest. Sources of variation and magnitude of experimental effects are as in Table 2.1.

Variable	Source of variation	df	F	p ^a	Magni- tude
			-		
Total leaf	Selection	1	25.30	****	20.94
area	Condition	1	13.55	***	10.81
	Line (Sel)	2	4.82	*	6.58
	Sel * Cond	1	0.04	n.s.	0
	Cond * Line (Sel)	2	0.26	n.s.	0
Number	Selection	1	2.15	n.s.	1.37
of leaves	Condition	1	7.20	**	7.40
	Line (Sel)	2	2.34	n.s.	3.20
	Sel * Cond	1	2.48	n.s.	1.77
	Cond * Line (Sel)	2	0.15	n.s.	0
Number	Selection	1	0.12	n.s.	0
of stems	Condition	1	50.37	****	39.81
	Line (Sel)	2	1.27	n.s.	0.44
	Sel * Cond	1	3.39	n.s.	1.93
	Cond * Line (Sel)	2	0.29	n.s.	0

^a: n.s.: non-significant, P > 0.05; *: P < 0.05; **: P < 0.01; ****: P < 0.001; ****: P < 0.0001.

reproductive harvest (Table 2.4), where the main effect of line is significant, but accounts for only a relatively small portion of the total variation (8.54%, 6.34%, and 6.58%,respectively). Furthermore, the Condition x Line(Selection) interaction is never significant. Given this, most of the figures in this paper represent the pooled values of both replicate lines within each treatment combination.

Pre-reproductive harvest

As mentioned earlier, at the time of the first harvest, the two growth chambers differed only by the chamber CO_2 concentrations and mean day/night temperatures. At this time, there is a significant interaction between selection regime and growth conditions on vegetative biomass, accounting for 9.9% of the total variation (Table 2.1). The Tukey HSD post-hoc test on this interaction shows that plants of the PP group had a significantly higher biomass, 0.832 g, than the three other groups (CC: 0.512 g, CP: 0.423 g, PC: 0.548 g) (Fig. 2.2a). The only other significant effect on vegetative biomass at this harvest is the main effect of selection regime, which accounts for an additional 7.96% of the total variation (Table 2.1). Averaged over both growth conditions, biomass of plants from the "Predicted" selection lines was 47% higher than those of the "Current" selection lines. Growth condition did not affect vegetative biomass significantly at this harvest.

Fig. 2.2. Mean vegetative biomass of *Brassica juncea* plants at the a) pre-reproductive, b) earlyreproductive, c) late reproductive harvests. Plants of both selection lines within a treatment combination were pooled (Mean \pm standard deviation, n = 22). CC: "Current lines of plants in "Current" conditions; CP: "Current" lines in "Predicted conditions; PC: "Predicted" lines in "Current" conditions; PP: "Predicted" lines in "Predicted" conditions. Lower-case letters in a) designate significantly different means (P < 0.05), according to a Tukey HSD test on the significant Selection by Condition interaction.



Dry mass (g)

Although vegetative biomass responds to the Selection by Condition interaction, no significant interactions are found for leaf area, number of leaves, or number of stem ramifications (Table 2.2). Selection treatment has a significant effect on these three variables (Table 2.2). Averaged over both growth conditions, plants selected under the "Predicted" environment had mean values of 95.9 cm^2 for leaf area, 17.0 for leaf number, and 5.3 for number of stem ramifications, compared to mean values of 63.2 cm² for leaf area, 13.0 for leaf number, and 4.8 for number of stem ramifications in the "Current" lines (Fig. 2.3a). The effect of selection accounts for the largest portion of the total variation for total leaf area (22.09%) and for leaf number (18.26%), but only for 2.78% of the variation in stem number (Table 2.2). Growth condition also has a significant effect, regardless of past selection, on number of leaves and number of stems, but leaf area is not significantly affected (Table 2.2). Plants in the "Predicted" growth conditions, overall, had 16.2 leaves and 5.4 stems, significantly more than plants grown in "Current" conditions, which averaged 13.8 leaves and 4.7 stems (Fig. 2.4a and 2.5a). This effect of growth condition accounts for the greatest portion (9.28%) of the total treatment variation in stem number, but only for a modest portion (5.19%) of leaf number variation (Table 2.2).

Data on biomass allocation can further be used in a mathematical growth analysis, to understand the functional

Fig. 2.3. Mean leaf area of *Brassica juncea* plants at the a) pre-reproductive, b) early-reproductive, c) late reproductive harvests. Plants of both selection lines within a treatment combination were pooled (Mean \pm standard deviation, n = 22). Groups are identified as in Fig. 2.2.



Fig. 2.4. Mean number of leaves of *Brassica juncea* plants at the a) pre-reproductive, b) early-reproductive, c) late reproductive harvests. Plants of both selection lines within a treatment combination were pooled (Mean \pm standard deviation, n = 22). Groups are identified as in Fig. 2.2.



Fig. 2.5. Mean number of stem ramifications of *Brassica* juncea plants at the a) pre-reproductive, b) early-reproductive, c) late reproductive harvests. Plants of both selection lines within a treatment combination were pooled (Mean \pm standard deviation, n = 22). Groups are identified as in Fig. 2.2.



bases of biomass accumulation. Plants of the PP group had a 40% higher NAR, at 1.93 mg·cm⁻²·day⁻¹, than those of the three other experimental groups (CC: 1.52; CP: 1.39; PC: 1.22 mg·cm⁻²·day⁻¹, Fig. 2.6a). RGR, on the other hand, was very similar for all groups (Fig. 2.7a), with values ranging from 0.29 to 0.32 mg·mg⁻¹·day⁻¹.

Early-reproductive harvest

The second harvest took place after five weeks of growth (day 35), one week following the first heat stress for plants in the "Predicted" conditions. At this time, no significant interaction is found between selection treatment and growth conditions on any of the measured variables (Tables 2.1 and 2.3). Vegetative biomass responded significantly to growth condition, which accounts for 9.62% of the variation, but not to selection (Table 2.1). At this harvest, vegetative biomass of plants grown in the "Predicted" conditions was slightly (10%) lower than in the "Current" conditions (Fig. 2.2b).

Selection treatment has a significant effect on leaf area and number of stems (Table 2.3), but accounts for a relatively small portion of the total variation for these variables (11.03% and 6.88%, respectively). Plants from the "Predicted" selection lines had on average 16.7 stem ramifications, somewhat more than those from the "Current" lines, which averaged 13.9 stems (Fig. 2.5b).

Fig. 2.6. Net assimilation rate (NAR) of *Brassica juncea* plants between a) 0-21 days, b) 21-35 days, and c) 35-49 days after sowing. Values were computed using the mean of 11 plants from each selection line in each treatment combination. Groups are identified as in Fig. 2.2.



Legend: []: Line 1; []: Line 2.

Fig. 2.7: Relative growth rate (RGR) of *Brassica juncea* plants between a) 0-21 days, b) 21-35 days, and c) 35-49 days after sowing. Values were computed using the mean of 11 plants from each selection line in each treatment combination. Groups are identified as in Fig. 2.2.



Legend:
: Line 1;
: Line 2.
Unlike the pre-reproductive harvest, plants of the "Current" selection lines had a higher leaf area than those of the "Predicted" lines (mean of 310.2 cm², compared to 253.9 cm², Fig. 2.3b). No significant effect of selection was found on leaf number (Table 2.3). In addition, stem number, leaf number, and total leaf area were strongly affected by growth condition, which explains the largest portion of the total variation for this harvest (as much as 46.25% for stem number, Tables 2.1 and 2.3). Leaf and stem number were higher in the "Predicted" environment, with means of 50.4 leaves and 16.4 stems, compared to "Current" conditions (48.4 leaves and 13.9 stems, Fig 2.4b and 2.5b). Average leaf area was much lower in "Predicted" (234.1 cm²) than in "Current" conditions (330.0 cm^2 , Fig 2.3b).

There is a significant main effect of both selection treatment and growth conditions on the mean date of opening of the first flower (Table 2.1), which account for 23.74% and 26.92% of the total variation, respectively. Overall, there was a full 5-day difference between the mean number of days to first flower of the earliest blooming and latest-blooming group: PP plants flowered first on day 22, followed by those of the CP and PC groups, both around day 24, and finally by those of the CC group, at day 27 (Fig. 2.8). This implies that most plants of the PP group were already blooming before the onset of the first



Fig. 2.8. Mean number of days to first flower of Brassica juncea plants. Both selection lines within a treatment combination were pooled (Mean \pm standard deviation, n = 44). Groups are identified as in Fig. 2.2.

heat wave, whereas most of those of the CP group began to flower during the heat stress event.

Between days 21 and 35, NAR of the PP group was 0.85 mg·cm⁻²·day⁻¹, 22% less than the three others (PC: 1.17; CP: 1.29; CC: 1.10 mg·cm⁻²·day⁻¹) (Fig. 2.6b). Similarly, RGR of plants of the PP group, at 0.08 mg·mg⁻¹·day⁻¹, dropped to 41% of the RGR of plants from the three other experimental groups, which all had rates around 0.13 mg·mg⁻¹·day⁻¹ (Fig. 2.7b).

Late-reproductive harvest

The late-reproductive harvest was done at the end of the plant growth cycle (day 49), after the plants of the "Predicted" conditions had been exposed to two four-day heat waves. No significant interactions were observed between selection regime and growth condition on any of the measured variables. A significant main effect of Selection on final vegetative biomass, the only significant treatment effect for this variable, accounted for 14.78% of the total variation (Table 2.1). Figure 2.2c shows that plants of the "Current" selection lines overall had more vegetative dry mass with 4.15 g, compared to those of the "Predicted" lines (3.36 g).

Final leaf area was most strongly affected by selection, which accounts for 20.94% of its total variation (Table 2.4). Plants from "Current" selection lines had

considerably more leaf area, with an average of 357.7 cm², than those of "Predicted" selection lines, with 276.3 cm² (Fig. 2.3c, Table 2.4). The effect of growth condition on leaf area was also significant. Plants in "Current" conditions had, on average, slightly more leaf area with 347.7 cm^2 , compared to "Predicted" conditions (286.3 cm^2 , Fig. 2.3c). Leaf and stem number were not affected significantly by selection, but the effect of growth condition was very important, explaining as much as 39.81% of the total variation in the case of stem number. Leaf and stem number were higher in the "Predicted" environment (Average values for "Predicted" conditions: 96.5 leaves and 40.4 stem ramifications; "Current" conditions: 86.1 leaves and 24.8 stem ramifications, Fig. 2.4c and 2.5c). Plants exposed to the "Predicted" conditions therefore had many more leaves and stems, though leaves were much smaller, compared to those of the "Current" conditions.

Nowhere is the impact of growth condition more important than for final reproductive biomass (Table 2.1, Fig. 2.9), where it accounts for over 76% of the total observed variation. For plants from both selection regimes, reproductive output was reduced 3 to 4-fold in the "Predicted" environment (average of 0.949 g), compared to plants in "Current" growth conditions, which produced on average 3.459 g of siliques.

During this period, plants in the "Predicted" conditions also had a lower NAR (0.59 mg·cm⁻²·day⁻¹) and



Fig. 2.9. Reproductive biomass of *Brassica juncea* plants at the late-reproductive harvest. Plants of both selection lines within a treatment combination were pooled (Mean \pm standard deviation, n = 22). Groups are identified as in Fig. 2.2.

RGR (average of 0.03 mg·mg⁻¹·day⁻¹) than those in the "Current" environment (NAR: 0.87 mg·cm⁻²·day⁻¹; RGR: 0.06 mg·mg⁻¹·day⁻¹, Fig. 2.6c and 2.7c).

Gas exchange

I measured leaf gas exchange during the third week of growth, when plants were in their pre-reproductive lifestage and had not yet been exposed to any heat stress. Three groups of plants were studied: PP, CC, and PC. PC plants were measured upon transfer in a growth chamber programmed to the "Current" growth conditions. The three experimental groups had significantly different CO₂ assimilation rates (Tables 2.5 and 2.6). No significant differences among replicate lines are detectable (Table 2.6). PP plants had a significantly higher CO₂ assimilation rate, with 18.6 µmol·m⁻²·s⁻¹, than CC plants, at 16.66 µmol·m⁻²·s⁻ 1 (Table 2.5). This represents an 11% increase in photosynthetic rate. However, stomatal conductance and water-use efficiency did not differ significantly between PP and CC plants. Average values for these two groups were a gs of 0.635 mol·m-2·s-1 and WUE of 3.26 mmol CO₂·mol H₂O-¹ (Table 2.5). PC plants showed a significantly depressed A, gs, and WUE, compared to both PP and CC plants (Table 2.5). CO_2 assimilation rate of PC plants was only 15.42 µmol·m⁻²·s⁻¹, which is 20% less than for PP plants, and 7%

<u>Table 2.5.</u> CO₂ assimilation rate (A), stomatal conductance (gs), and water-use efficiency (WUE) of *Brassica juncea* after 8 generations of selection under a current or projected atmosphere (Mean of two pooled replicate lines \pm standard deviation, N = 70). Means in a same row designated by different letters are significantly different (P < 0.05), as determined by a Tukey HSD test.

Selection environment:	"Current"	"Predicted"		
Measurement [CO ₂] (μL·L ⁻¹)	370	370	650	
A (μ mol CO ₂ ·m ^{-2.s-1})	16.66 ± 2.65 (a)	15.42 ± 1.30 (b)	18.59 ± 2.03 (c)	
gs (mol H ₂ O·m ⁻² ·s ⁻¹)	0.62 ± 0.24 (a)	0.51 ±0.14 (b)	0.66 ± 0.37 (a)	
WUE (mmol CO_2 mol H_20^{-1})	3.32 ± 6.83 (a)	2.89 ± 4.74 (b)	3.21 ± 7.83 (a)	

Table 2.6. Summary of ANOVA results on CO₂ assimilation rate (A), stomatal conductance (gs), and water use efficiency (WUE) measurements taken during the third week of growth, at the chamber CO₂ concentration. The sources of variation refer to experimental group (PP, CC or PC), and to selection line nested under group.

Variable	Source of variation	SS (Type III)	df	F	Р
А	Group	326.4	2	38.71	0.0001
(µmol CO ₂ ·m ⁻² ·s ⁻¹)	Line(Group)	25.0	3	1.98	0.1185
gs	Group	7842.8	2	5.66	0.0041
(mol H ₂ O·m ^{-2.} s ⁻¹)	Line(Group)	4932.3	3	2.37	0.0715
WUE	Group	6676.1	2	7.71	0.0006
(mmol CO ₂ ·mol H ₂ 0 ⁻¹)	Line(Group)	2763.8	3	0.21	0.8875

less than CC plants. PC plants had values of gs and WUE averaging 0.507 mol·m⁻²·s⁻¹ and 2.89 mmol CO₂·mol H₂O⁻¹, respectively, equivalent to reductions of 20% in gs and of 11% in WUE, compared to the PP and CC groups.

Besides measuring photosynthesis under the two growth chamber conditions, I also examined A at limiting CO₂ concentrations, to approximate the linear portion of the A vs Ci curve for the three groups of plants. The slope of the linear portion of this curve, at low CO_2 , gives an estimate of the carboxylation efficiency of Rubisco (von Caemmerer and Farquhar 1981). This slope is much steeper for PP plants, at 69.1 mol·m-2·s-1, compared to CC and PC plants, with 44.2 mol·m⁻²·s⁻¹ and 41.4 mol·m⁻²·s⁻¹, respectively (Fig. 2.10). The carboxylation efficiency of PP plants was thus more than 50% higher than both groups of plants measured in "Current" conditions. However, the slope was almost identical for CC and PC plants, and seems therefore unaffected by selection regime.



Figure 2.10. Relationship between CO₂ assimilation rate (A) and intercellular CO₂ (Ci) at below-ambient CO₂ concentrations, for *Brassica juncea* plants during their third week of growth. Each point represents the mean of 70 plants. The three groups are identified as in Fig. 2.2. --- : CC; --- : PC; ->- PP plants.

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DISCUSSION

Most studies of the potential impacts of global change have not examined the possibility of an adaptive response of the plants. To my knowledge, only two studies have approached this question. Woodward (1993) located populations of Boehmeria cylindrica (an annual) occurring naturally near cold springs in Florida, where CO₂ emanations create stable sites of varying atmospheric CO₂ concentrations (350-550 μ L·L⁻¹). Seeds were harvested from these populations, and grown under laboratory various CO₂ regimes. conditions with **Population** differences in the plant growth responses to CO₂ were detected after 8 weeks, suggesting an adaptation to high CO_2 . Populations from the highest CO_2 environment (500) $\mu L \cdot L^{-1}$) had a greater growth rate in high CO₂ than populations from the lower CO_2 environment, but no differences between populations were observed at the lower CO₂ levels. The results, however, cannot demonstrate that the observed population differences were directly the result of selection at elevated CO_2 , since CO_2 was confounded with other site-specific characteristics (Woodward 1993, Woodward et al. 1991). It is also not known how long the populations had been isolated from one another and were evolving independently. In another study, Maxon Smith (1977) attempted to select for more

efficient CO₂-utilization in glasshouse lettuce (Lactuca sativa) by performing a selection experiment over 8 generations under enhanced CO₂ concentration and day temperatures. This study differs from mine in two major First, plants were selected on the basis of vegetative ways. traits of commercial interest, rather than fecundity. Second. the conditions were growth kept constant throughout the selection. The experiment was not aimed at studying ecological questions related to global change. Although the selection produced stable, true-breeding lines of plants, there was no evidence of any adaptive response of the plants. The plants responded positively to the CO2enriched environment, regardless of whether the plants had been selected under these conditions or not.

To my knowledge, the present study is the first to effectively test for and identify the adaptive response of a wild annual to gradual changes in CO₂ and temperature. Based upon population genetics, I hypothesized that if plants did adapt during the selection process under contrasting environments, then a significant Selection x Condition interaction would exist (Jain and Bradshaw 1966, Maxon Smith 1977). This was confirmed at the prereproductive harvest, and suggests that for vegetative biomass accumulation, genetic adaptation had occurred in response to selection of *B. juncea* in the "Predicted" environment. Both replicate lines within a selection treatment behaved in a similar way, and generally showed no significant differences, which further strengthens my results. I estimated the intensity of the selection effect by calculating selection coefficients (Antonovics 1976, Jain and Bradshaw 1966) for vegetative biomass of the plants at the pre-reproductive harvest. A selection coefficient, s_{1} quantifies the selective pressure against a population or genotype grown in a novel environment. Values of s c an range from 0, in the absence of any selective pressure, to 1, if there is complete selection against a population in the In this experiment, the selection new environment. pressure against PC plants, (PP-PC)/PP for vegetative biomass, was 0.34, twice the value of s against CP plants, 0.17, expressed by (CC-CP)/CC. Jain and Bradshaw (1966) reported values of s as low as 0.05 and as high as 0.99 as evidence of selective pressures existing against a population. Our value of s of 0.17 suggests a low selective pressure, if any, against "Current" lines of plants transferred to "Predicted" conditions. By contrast, an s of 0.34, observed for "Predicted" plant lines transferred back to the "Current" environment, is comparable to other reports of moderate selective pressures against a population in a novel environment (Jain and Bradshaw 1966, Potvin 1986). These results are in accord with published acclimation results.

Some insights on the physiological basis of the adaptive changes I observed are given by the study of gas exchange and growth patterns. Pre-reproductive growth of

our plants was related to differences in CO₂ assimilation, as expressed by NAR. Mechanisms of carbon uptake and assimilation appear to be central in understanding plant response to the "Predicted" selection treatment. Many examined long-term responses of authors have photosynthesis to elevated CO_2 in C_3 species, and reported down-regulation of photosynthetic activity, sometimes even below initial levels (Arp and Drake 1991, Arp 1991, Besford et al. 1990, Bowes 1991, Ziska et al. 1991, Sage 1990). It is also often alleged that, although photosynthetic rates might rise, the carboxylation efficiency of Rubisco decreases during a long-term exposure to elevated CO₂ (Long 1991, Ziska et al. 1991, Hogan et al. 1991, Bowes 1991, Sage 1990, Besford et al. 1990). This is reflected by a decreased slope in the linear portion of the A vs Ci curve, which measures Rubisco activity (von Caemmerer and Farquhar 1981). A common explanation for this phenomenon is a decrease in the amount of Rubisco protein and/or in the activation state of the enzyme, to compensate for a limitation in the rate of RuBP regeneration by the of electron transport and/or activities phosphate regeneration (Bowes 1991, Sage 1990, Long 1991, Besford et al. 1990). This line of thought implies that the different components of the carbon fixation process are in disequilibrium, and one of the physiological steps has become limiting. In this experiment, I found no evidence of any down-regulation of photosynthetic capacity, nor did I observe a reduction in carboxylation efficiency for "Predicted" lines of plants, either in "Current" or in "Predicted" conditions. Selection therefore seems to have altered the type of response to doubled CO_2 and increased temperature, and allowed for enhanced CO_2 assimilation in *B. juncea* to be maintained without later reduction. There are also countless reports of reduced gs and increased WUE for C₃ plants in elevated CO_2 conditions (see reviews by Bazzaz 1990, Hunt *et al.* 1991, and Hogan *et al.* 1991). My results indicate that PP and CC plants did not differ significantly in either stomatal conductance or water-use efficiency, which again would suggest that selection modified the long-term response of plants to enhanced CO_2 and temperature.

Conversely, the coefficients of selection reported above suggest that plants from "Predicted" lines of plants disadvantage when grown in "Current" were at a This result was predicted by several authors, conditions. who suggested that adaptation to high CO_2 could reduce performance under ambient CO₂ (Woodward 1993, Maxon The observed reduction in growth and Smith 1977). photosynthesis of PC plants is not due to a loss in carboxylation efficiency and must be linked to other aspects of carbon uptake or assimilation. PC plants had significantly reduced gs and WUE, compared to both PP and CC groups. This would suggest that the reduced A they exhibit is due to increased stomatal resistance to CO₂ diffusion into the leaf. Farquhar and Sharkey (1982) note that stomatal limitation of photosynthesis is rather rare, and is usually associated with water stress or abscisic acid accumulation in leaves. My study suggests that a back transfer to current CO_2 levels, after several generations of exposure to increasing CO_2 , might also induce stomatal limitation of photosynthesis. The mechanism involved is at present unknown.

The gas exchange results bring interesting nuances to our understanding of plant responses to global change, that could affect our predictions of their potential impacts of The result of the increasing CO_2 and temperature. differences in photosynthetic rates among groups are apparent through differences of pre-reproductive biomass. When comparing PP and CC plants, vegetative biomass is increased by a factor of 1.63. This growth modification factor is slightly higher than previous reports of plant responses to increased CO_2 and temperature. Kimball *et al.* (1993) report an average growth modification factor of 1.56 for five C₃ species, while Hunt et al. (1991) obtained an average biomass modification factor of 1.43 in 11 C₃ species, with only one non-crop species exceeding 1.6 (Chamerion angustifolium). Poorter (1993) also reports that the average increase in vegetative growth under doubled CO_2 , in terms of biomass accumulation, is 41% for C₃ plants, with crops usually exhibiting a larger response (58%) than wild species (23%). Our results would indicate

that B. juncea selected under "Predicted" conditions showed a strong vegetative growth enhancement to elevated CO_2 and temperature. However, we have also shown that there significant short-term was vegetative growth no enhancement for "Current" plants grown in "Predicted" conditions, since biomass accumulation of CP plants was little affected by the enhanced CO_2 and temperature conditions. This would suggest that *B. juncea* is normally rather insensitive to a short-term exposure to enhanced CO_2 and temperature. A direct CO_2 -doubling experiment would probably have shown a slight or no response in prereproductive *B. juncea* plants. Because of our selection treatment, however, we observed a tremendous effect on vegetative biomass accumulation, which increased by 63%. The selection effect we found thus completely altered the type and magnitude of response to global change shown by this species.

My results also show that the initial adaptive response is not present at all stages of the growth cycle. Reproductive growth responded to the treatments very differently from earlier vegetative growth. The heat stress events in the "Predicted" conditions are likely to be the main cause for the observed reduction in reproductive effort of *B. juncea*. Many authors recognize that reproductive growth, and particularly the onset of flowering, are highly vulnerable to heat and/or moisture stress (e.g. Chiariello and Gulmon 1991, Setter 1990, Singh et al. 1991, Coleman et al. 1991). Both the amount of biomass and leaf area of a plant about to flower (Chay and Thurling 1989), and the amount of floral meristems serving as sinks (Bazzaz et al. 1987), can have great repercussions on biomass allocation to reproduction. Brassica is no exception, as its reproductive development and yield are highly related to environmental conditions (Olsson 1960, Chay and Thurling 1989), and are particularly vulnerable to stress (Polowick and Sawhney 1988, Singh et al. 1991).

The heat stress treatment recreates temperatures which are not uncommon in regions where B. juncea grows wild, such as in Québec (Sabourin et al. 1991). These same temperatures, however, when supplied as a continuous regime, were shown to induce serious morphological deformations and abortion of floral development for closely related Brassica napus plants (Polowick et al. 1988). The first heat wave event, during the fourth week of growth, possibly disrupted flower meristem development in the already flowering plants. After the first heat stress, plants in the "Predicted" conditions showed a sharp increase in the number of developing new lateral stems and leaves. This would support the hypothesis of floral meristem damage. The stressed plants compensated for reduced sinks (damaged floral buds and meristems) by initiating new branches which eventually developed into new flower Evans (1991) also demonstrated that rapidracemes.

cycling plants of *Brassica campestris* responded to decreased sinks by altering allocation patterns.

 CO_2 and temperature can interact to affect other aspects of plant physiology as much as photosynthesis and allocation patterns (Potvin 1985). It has been suggested that CO_2 alleviates the effects of high temperature stress (Hogan et al. 1991), through an increase in radiative heat loss from the leaf, as a consequence of decreased stomatal conductance and higher leaf temperature. My results, however, do not support this hypothesis. Coleman et al. (1991) have similarly found that CO_2 did not improve a plant's resistance to four-hour heat shocks. Both vegetative and reproductive growth were negatively affected by the heat shock treatments. This study also showed that Sinapsis alba (a related mustard species) was the most sensitive to heat shock among the three species examined. In another study, Sionit et al. (1980) found that, although elevated CO_2 increased yield potential in wheat, water stress limited reproductive growth, such that plants exposed to both high CO_2 and water stress did not show higher grain yields than unstressed plants under ambient CO₂.

The experiment I report here confirms that temperature stress can deeply modify a plant's response to enhanced CO_2 and mean temperature. Heat stress became the main factor determining reproductive and late vegetative growth on *B. juncea*, above and beyond the

adaptive response to "Predicted" conditions expressed at the pre-reproductive stage of growth. This factor should not be neglected in future studies of plant responses to global change, as it could become a central factor affecting plant growth and reproduction, and consequently, have repercussions on all levels of an ecosystem.

CONCLUSION

My study was aimed at testing whether the potential for an adaptive response to global change exists in an annual plant, and at comparing plant response to gradual changes in environment, spanning several generations, to an abrupt increase in CO_2 and temperature. Brassica juncea was selected for fecundity during eight generations, under conditions of gradually changing CO_2 and temperature, matching predictions of future changing climate. After the selection, a reciprocal transplant experiment tested for genetic differences between lines of plants from the "Predicted" and "Current" (control) treatments.

Results show that there exists a potential for an adaptive response to simulated global change in *Brassica juncea*. Due to the selection under gradually rising CO₂ and temperature conditions, vegetative growth and assimilation were continually increased during the experiment. Changing the growth conditions gradually over several generations seems to prevent the acclimation response most often observed in long-term experiments under elevated CO₂ and temperature (Farrar and Williams 1991). The carboxylation efficiency of Rubisco also was not reduced. As was suggested by Bradshaw and McNeilly (1991), it is probable that some effects of global change on annual wild plants will be seen through genetic adaptation.

Changes in genome and population structure are likely to populations. have an impact on communities and ecosystems in future environments. The selection response of different plant species to global change needs to be examined, in order properly to include adaptation mechanisms among the factors determining plant responses to global change.

The present study examined the temporal response of plants to a dynamically changing environment. The strength and magnitude of the response to increased CO_2 and temperature were shown to be affected not only by the final conditions reached in the treatments, also by the rate of the changes in environment. When growth conditions were modified over several generations, a rather insensitive species to a direct increase in CO_2 and temperature was shown to have a very important Studies in which CO_2 and temperature conditions response. are modified abruptly might not truly reflect the potential impact of global change on some species.

This experiment was designed to examine the combined effect of enhanced CO_2 and temperature on plant performance. Heat stress played a major role in determining the reproductive success of *Brassica juncea*. I observed a reversed response to the selection on fecundity, due to environmental maternal effects linked to stress during flowering in "Predicted" conditions. It is likely that high temperature and other types of stress, such as chilling

or drought, will largely affect the response of plants to rising CO₂ and mean temperature. Predictions about plant performance in this context should include the effects of stress on vegetative growth and reproductive success. More information is needed on the reproduction and allocation patterns of wild species under global change. If weedy species respond differently than cultivated plants to the changing environment, competition between crop and non-crop species could be modified and affect cultural The composition of natural ecosystems could also practices. be altered if interspecific differences in plant responses to global change exist, leading to changes in competitive ability of co-occurring species. In order to predict impacts of global change on agriculture and natural environments, more information is necessary on interspecific relations and on reproduction in non-crop plant species in this context.

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