PHENOTYPIC PLASTICITY OF CARBON ACQUISITION AND ALLOCATION

Phenotypic Plasticity of Carbon Acquisition and Allocation in Rapid Cycling *Brassica rapa* L. and the Androdioecious Species *Mercurialis annua* L. *s.l.* in Response to Light Quality

By

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Masters of Science

McMaster University

MASTERS OF SCIENCE
(Biology)

McMaster University
Hamilton, Ontario

TITLE: Phenotypic Plasticity of Carbon Acquisition and Allocation in Rapid Cycling *Brassica rapa* L. and the Androdioecious Species *Mercurialis annua* L. *s.l.* in Response to Light Quality

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NUMBER OF PAGES: x, 157pp.

ABSTRACT

Relatively few studies have examined whether traits associated with carbon acquisition in plants exhibit plastic responses as part of the phytochrome-mediated stem elongation response. Moreover, it has not been determined which environmental cues induce responses in carbon acquisition. This study examines the role of the ratio of red to far-red light (R:FR), stand density, water availability, and gender in the responses of carbon acquisition and allocation in two annual plant species.

In rapid cycling *Brassica rapa* L. (Brassicaceae), stem elongation and the responses in carbon acquisition traits were examined in plants grown in high and low water availabilities under low R:FR and neutral shade at low density, and under high R:FR and neutral shade at high density. Although the responses to density and water availability were inconclusive, this study clearly shows that at low density plants respond to low R:FR with increased stem elongation, reduced biomass accumulation, lower photosynthetic rates, and lower water-use efficiency relative to plants under the comparatively higher R:FR.

In the androdioecious species *Mercurialis annua* L. *s.l.* (Euphorbiaceae), sexual dimorphism in the responses of carbon acquisition to R:FR were examined in plants grown at high density under high R:FR, neutral shade, and normal sunlight. Although stem elongation only responded to light intensity, gas exchange responded to low R:FR as seen in *B. rapa*. Whereas sexual dimorphism was not found in trait means, the correlations between photosynthetic rate and allocation to vegetative and reproductive biomass showed significant gender differences. Phenotypic selection analysis revealed that significant differences between the sexes in natural selection on acquisition traits resulted from differences in allocation.

Similar patterns in carbon acquisition and allocation were associated with stem elongation in both species. Furthermore, both studies indicate that low R:FR results in reduced gas exchange, as was seen in *Impatiens capensis*. Taken together these findings suggest that responses in carbon acquisition may be a general phenomena associated with density-dependent stem elongation. It is hypothesized that the responses to R:FR in carbon acquisition and associated trade-offs would be costly where other aspects of the microenvironment within a plant stand do not serve to mitigate the imposed limitations on plant water-relations.

ACKNOWLEDGEMENTS

I would to start by thanking my supervisor Susan Dudley for her encouragement and patience. I would like to particularly thank her for her editorial comments during preparation of this thesis.

Cheers to John Pannell and Spencer Barrett for the opportunity to work on such an interesting plant and mating system.

I would like to thank Lara Armstrong, Mary DeGuzman, Alexis Fast, Catherine Jankowski, Chris Herlihy, Shawn McIntosh, and Daniella Pacifico for volunteering copious amounts of their valuable time to seeing me through all of this.

I would also like to thank Art Yeas and the staff at the Royal Botanical Gardens Arboretum for offering a little space in their facilities.

Finally, I would like to thank my parents and AHM for their enduring patience, support, and vigilance in keeping me focused. Thanks especially to my mother for enduring the difficult times.

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General Introduction

It is hypothesized that plants can adjust their morphological and physiological development across environments to better cope with the stresses experienced in each environment. The change in a plant's phenotype might represent the unavoidable consequence of a stressful environment, such as wilted leaves in a low water environment. However, the change may often be a response aimed at reducing the impact of the stress, such as increased solute concentrations in leaves to prevent wilting (Jones 1992). The potential for flexibility of design, phenotypic plasticity, is hypothesized to confer a selective advantage in stressful environments. For phenotypic plasticity to be considered adaptive, the environmentally induced responses must enhance fitness in the environment in which they are expressed (Sultan 1987, Thompson 1991). The adaptive value of environmentally induced plastic responses is most often based on functional arguments that the observed response increases performance and therefore fitness. However, interpretations of the adaptive significance of a given plastic response are often complicated by the fact that fitness results from numerous aspects of the phenotype (Schlichting 1989). Given that phenotypic plasticity may also occur in correlated yet functionally independent traits, the determination of whether or not a phenotypic response in a single trait is adaptive often remains tentative (Sultan 1995, Via et al. 1995). Interactions between traits that contribute to plant performance have been shown to be quite complex (Lechowicz 1984). Thus, it often becomes necessary to evaluate the degree of variability and the interactions among a suite of functionally related traits and the effects on fitness to be able to attribute an adaptive significance (Sultan 1995, Via and Lande 1985). An understanding of how phenotypes change across environments and characterization of the observed variability provides the first crucial steps towards addressing the fitness consequences of plastic responses.

Gas exchange and light capture in plants is a particularly responsive and variable system that serves as the interface between a plant and its environment (Gross 1986, Jones 1992). It is frequently hypothesized that the variability seen in these traits, which affect how plants acquire energy, represents plastic responses to increase energy acquisition. It is also suggested that the optimal attributes of traits associated with carbon uptake should vary among environments (Givnish 1986). Investigations of how carbon acquisition traits respond in different competitive environments can help us to understand how plants might make use of plasticity in their physiology to offset the stresses associated with competition. In this paper, I describe two studies on the plastic responses to the presence of competitors in traits associated with how plants acquire carbon.

The presence of competitors, which can be an important environmental factor affecting resource availability, has been shown to elicit plastic responses in the morphology and development of various plant characteristics (e.g. see Schmitt and Wulff 1993, Weiner et al. 1990, Weiner and Thomas 1992). For individual plants, competitors are other plants in close proximity that potentially reduce the availability of resources, such as light, nutrients, and water. Plants are particularly responsive to their light environment. Reductions in the intensity of light typically have a negative effect on growth and reproduction (Zelitch 1982). Plants growing in dense stands are subjected to a light environment that differs from normal sun light in at least two important aspects- a lower photosynthetic photon flux density; and an altered spectral distribution (Smith 1982). The changes in the spectral distribution of light that passes through a vegetation canopy are the consequence of the preferential absorption of blue and red wavelengths by photosynthetic pigments. The resultant spectral distribution of vegetation shade is a shift in the quantum ratios of 660 to 730 nm, or the red to far-red ratio (R:FR) (Morgan and Smith 1979, Smith 1982). The R:FR under normal sunlight is typically 1.10-1.25 and is as low as 0.10 under forest canopies (Lee 1988, Morgan and Smith 1979). It has been well documented that plants are capable of detecting the R:FR of direct incident light, as well as light reflected by other plants (Ballaré et al. 1987, Ballaré et al. 1988, Smith et al. 1990), via the light sensing phytochrome family of photoreceptors (for a recent review see Smith 1995). This ratio serves as a reliable cue to the presence of other plants (Smith 1982). Density dependent plastic responses have been observed to be elicited by changes in R:FR, as well as by blue light (Casal 1989, Morgan and Smith 1979, Schmitt and Wulff 1993).

Phytochrome-mediated plastic responses to R:FR are commonly considered to be part of the 'shade avoidance syndrome' (Smith 1982). The shade avoidance syndrome is characterized by a number of developmental and morphological changes in plant structure, which have been demonstrated to assist plants to compete more effectively for limited resources (Dudley and Schmitt 1996, Schmitt et al. 1995). Shade avoidance responses typically include marked stem elongation, reduced lateral branching, and redistribution of leaves to the top of the canopy (Ballaré et al. 1991, Geber 1989, Schmitt and Wulff 1993, Smith 1982, Weiner et al. 1990). The increase in stem elongation may allow plants to place their leaves higher in the canopy to receive more light. However, plants are not limited to morphological responses to increase light interception, they can also respond through changes in their physiology to increase carbon uptake. Carbon acquisition traits, such as leaf area (Mitchell and Woodward 1988, Thompson and Harper 1988), photosynthetic rate, and stomatal conductance have also been shown to respond to changes in the light environment (Maliakal et al. in press.). However, it is not known whether the responses in carbon acquisition traits represent an adaptation as part of the phytochrome-mediated shade avoidance syndrome.

It is hypothesized that plants which increase light interception and carbon uptake under competitive conditions will have a selective advantage (Ballaré et al. 1990, Casal and Smith 1989, Schmitt and Wulff 1993). However, according to resource partitioning models, in an environment where the acquisition of resources is limited, the allocation among traits is hypothesized to be constrained by trade-offs (Bloom et al. 1985, Gleeson and Tilman 1992). For example, in nutrient poor environments, some plants have been shown to redistribute a relatively high proportion of biomass to roots at the expense of above ground biomass (Crick and Grime 1987, Gedroc et al. 1996). Many allocation models assume that, in an environment where light is limited, stem elongation will compete for resources with other vegetative activities such as root growth. Therefore, responses to increase light interception may reduce the acquisition of below ground resources. Resource allocation to stem elongation at the expense of leaf and root biomass is expected to reduce growth and reproduction (Geber 1990, Givnish 1982, Schmitt 1995). In the absence of competition for light, the costs associated with the shade avoidance phenotype should result in a selective disadvantage when compared with non-elongated plants (Schmitt and Wulff 1993).

A number of studies have demonstrated that there are intrinsic costs of the stem elongation response on carbon uptake and water relations. However, the sources of these costs still need to be identified. While Ballaré *et al.* (1991) found no evidence for a cost of stem elongation on leaf and root biomass in *Amaranthus quitensis,* studies performed with other species, such as the work on *Impatiens capensis* by Maliakal *et al.* (in press), have demonstrated that a potential cost exists for plants in drier environments. Because carbon fixation is often dependent on stomatal conductance, an increased photosynthetic rate might increase water loss through increased transpiration (Farquhar and Sharkey

1982, Givnish 1986). In two other studies designed to test fitness consequences of stem elongation, phenotypic manipulations were done to obtain elongated and non-elongated plants in high and low density (Dudley and Schmitt 1996, Schmitt et al. 1995). These studies have demonstrated that elongated plants have higher fitness in high density conditions and lower fitness under low density conditions. Dudley and Schmitt (1996) found that the fitness advantages of elongation in high density could be explained through selection for increased height. However, the fitness disadvantages of elongated plants at low density could not be explained through direct selection on height. It was suggested that the apparent fitness cost of elongation is the consequence of unmeasured components of the shade avoidance response. Ballaré *et al.* (1991), having found that the opportunity costs of allocating resources to stem elongation were minimal, suggested that plasticity in traits associated with photosynthetic performance could also play a role in the shade avoidance response.

Maliakal *et al.* (in press), in an attempt to test this hypothesis, found that elongated plants in high density had lower root biomass, photosynthetic rates, and stomatal conductance compared to non-elongated plants growing at high density in high R:FR light. Compared to low density plants, elongated plants at high density still had lower root biomass. However, the photosynthetic rates and stomatal conductance were slightly higher when compared to the non-elongated plants. These results suggest that other aspects of the microenvironment within a plant stand may mitigate the effects of lowered root biomass on water relations and carbon uptake. Maliakal *et al.* (in press) have indicated that the R:FR cue may have an effect on the responses in gas exchange traits. However, these results do not rule out other possible causative factors, such as stand structure. Many studies have shown that stand density affects light intensity and quality, water availability, humidity, temperature, and wind (Jones 1992, Smith 1982). Physiological studies of photosynthesis and stomatal conductance have shown them to be dynamic and responsive to environmental variability associated with differences in stand structure (Farquhar and Sharkey 1982, Gross 1986, Jones 1992). From such studies it is arguable that the differences in stand structure, between the light quality experiment and density manipulations performed by Maliakal *et al.* (in press), may have had a significant role in determining the gas exchange of individual plants. Due to limitations of their design, Maliakal *et al.* (in press) were not able to provide conclusive evidence that R:FR has an effect on carbon acquisition traits, as part of a phytochrome-mediated response. The question still remains whether the responses in gas exchange traits and morphology are elicited by the R:FR cue or whether are elicited by some other condition associated with stand density.

In this paper, I present two studies on the effects of the shade avoidance response on the plasticity in biomass allocation and gas exchange traits. I compared the allocation patterns and gas exchange traits of plants grown in environments in which I manipulated the R:FR, stand density, and water availability. In the first experiment, I examined the responses in a rapid cycling cultivar of *Brassica rapa* L. (Brassicaceae) under controlled conditions. Here I grew elongated plants in high density conditions and at low density by lowering the R:FR to a degree that induced stem elongation (Dudley and Schmitt 1996, Smith 1982). For comparison, I grew non-elongated plants in low density conditions and at high density by raising the R:FR sufficient to suppress the elongation response (Ballaré et al. 1991, Rajapakse et al. 1992, Smith 1982). High and low water availability treatments were also applied to each light quality-density manipulation. I measured maximal photosynthetic rates, stomatal conductance, leaf size, and allocation to roots, stem, and leaves. In this experiment, I asked how plasticity in gas exchange traits differed in response to water availability and the R:FR independent of the effects of density. In the second experiment, I investigated the responses of *Mercurialis annua* L *s.l.* (Euphorbiaceae), an androdioecious species with both male and hermaphroditic individuals. In this experiment, all plants were grown at high density under controlled conditions. In the suppressed treatment, plants received high R:FR, which, though reduced by the canopy, would be sufficient to suppress an elongation response (Ballaré et al. 1991, Rajapakse et al. 1992, Smith 1982). For the elongated treatments, plants in full sun and under neutral shade received normal R:FR, which was reduced by the plant canopy. For this experiment, I measured reproductive allocation as a measure of fitness, as well as maximal photosynthetic rates, stomatal conductance, leaf size, and allocation to stem and leaves. In this experiment, I asked how gas exchange traits differed between males and hermaphrodites and how vegetative and reproductive allocation was affected by the R:FR independent of density. Through these studies of stem elongation and the plasticity in biomass allocation and gas exchange traits, I addressed the following questions: (i) how does the presence of competitors affect responses in carbon acquisition traits? (ii) which environmental signals are these responses linked to- i.e. the R:FR cue and/or density? and (iii) how does plasticity in gas exchange traits differ in response to water availability and R:FR?

Chapter II

Phenotypic plasticity in carbon acquisition of rapid cycling *Brassica rapa* L. in response to light quality and water availability.

Introduction

Phenotypic responses to changes in light quality associated with high density have been demonstrated to be adaptive (Dudley and Schmitt 1996, Schmitt et al. 1996). Typical responses to density and vegetation shade include marked stem elongation, reduced branching, and redistribution of leaves to the top of the canopy (Ballaré et al. 1991, Geber 1989, Schmitt and Wulff 1993, Smith 1982, Weiner et al. 1990). It has been demonstrated that many of these responses are mediated through phytochrome photoreceptors, which are sensitive to the lowered R:FR characteristic of vegetation shade. Greater vertical growth through stem elongation and increased apical dominance is hypothesized to be a shade avoidance response that enables plants to increase light interception and therefore performance in dense stands (Schmitt and Wulff 1993, Smith 1995). Though the morphological responses to density and R:FR are relatively well known, very little is know about the physiological responses associated with shade avoidance.

Though the placement of leaves have been the focus of related research, there is also evidence that the traits of leaves are affected. Changes in leaf area have frequently been observed in response to R:FR (e.g. see Berntson and Weiner 1991, Mitchell and Woodward 1988, Thompson and Harper 1988). One study suggests that photosynthetic rate and stomatal conductance also respond to changes in the light environment (Maliakal et al. in press.). In *Impatiens capensis*, it was observed that elongated plants, responding to the low R:FR cue associated with high density, had lower photosynthetic rates and stomatal conductance along with reduced ratios of root to shoot biomass when compared with plants also at high density, but under a manipulated high R:FR cue (Maliakal et al. in press.). These results suggest that gas exchange traits may be affected by the R:FR. However, the same study also found that elongated plants under high density conditions had higher photosynthetic rates and stomatal conductance, as well as a lowered root to shoot ratio when compared with non-elongated plants at low density. The results of Maliakal *et al.* (in press) indicate that the R:FR cue may have an effect on the responses in gas exchange traits. However, these results do not rule out other possible causative factors, such as stand structure. Moreover, it is arguable that the differences in stand structure, between the light quality experiment and density manipulations, may have had a significant role in determining the responses in gas exchange. The physiology of carbon uptake is particularly sensitive to many aspects of the microenvironment (Jones 1992), which are affected by stand density and structure.

Physiological studies of photosynthesis and stomatal conductance have shown them to be dynamic and responsive to environmental variability. Many studies have shown that stand density affects light intensity and quality, water availability, humidity, temperature, and wind (Jones 1992, Smith 1982). Rates of photosynthesis and stomatal conductance are affected by many environmental factors including light intensity, temperature, humidity, CO_2 concentrations, and wind (Farquhar and Sharkey 1982, Gross 1986, Jones 1992). Thus, any attempt to understand how competition affects gas exchange will require more explicit tests to assess the effects of R:FR independent of the other effects of density. An important context in which to examine plants for physiological responses to density is availability of water. The decreased root to shoot ratio of elongated plants (Maliakal et al. in press.) suggests that elongated plants may be less able to acquire water than suppressed plants. The higher water-use efficiency seen in the elongated plants is also typical of plants under drought stress (Cohen 1970, Toft et al. 1989). However, the lower water-use efficiency at high density is more typical of less water-stressed plants (Maliakal et al. in press.). The results of the study by Maliakal *et al.* (in press) suggest that density and water relations are strongly linked. However, the sources of the effects on the plasticity of carbon acquisition traits associated with the shade avoidance syndrome still need to be determined. The question still remains whether the responses in gas exchange traits and morphology are elicited by the R:FR cue or some other condition associated with stand density.

Though Maliakal *et al.* (in press) demonstrated that gas exchange traits differ significantly between elongated and suppressed plants in high density stands in *I. capensis*, more evidence from other species is needed to determine if this is a commonly occurring aspect of phytochrome-mediated responses. Plastic responses associated with the shade avoidance syndrome have been shown to vary between species in both magnitude and direction (Casal et al. 1987, Corré 1983, Morgan and Smith 1979). Here I present a study on the effects of the shade avoidance response on the plasticity in biomass allocation and gas exchange traits in a rapid cycling cultivar of *Brassica rapa* L. (Brassicaceae). These plants are well suited for laboratory investigations of many ecological questions due to their short generation time, small size, and lack of seed dormancy (Koning and Parker 1993, Miller and Schemske 1990, Williams and Hill 1986). Various studies of photomorphogenic mutant and wild-type variants have demonstrated that this species displays the characteristic responses associated with the shade avoidance syndrome (Devlin et al. 1992, Miller and Schemske 1990, Schmitt et al. 1995). Thus, this species should represent a useful model system for the investigations in this study.

In this study, plants were grown at low density under low R:FR, at high and low density under normal R:FR, and at high density under high R:FR to provide elongated and non-elongated plants at high and low densities. High and low water availability treatments were applied in a factorial design to the light and density manipulations. I measured maximal photosynthetic rates, stomatal conductance, leaf size, and allocation to roots, stem, and leaves. I asked the following questions: (i) how does the presence of competitors affects responses in carbon acquisition traits? (ii) which environmental signals are these responses are linked to- i.e. the R:FR cue and/or density? and (iii) how does plasticity in gas exchange traits differ in response to water availability and R:FR?

Materials and Methods

EXPERIMENTAL DESIGN

Wild type *Brassica rapa* L. seeds, rapid cycling cultivar (CGRC 1-1, Crucifer Genetics Cooperative, Madison, Wn, U.S.A.), were planted in 2.5x16 cm pine cells containing a mixture of 30% perlite and 70% turface MVP soil conditioner (Plant Products Co. Ltd. Brampton, Ontario Canada) on November 3rd, 1997. The seeds were placed on the surface of the media and allowed to germinate in full sun- at the McMaster University greenhouse- for a week with top watering performed twice daily. After this germination period the plants were taken to the greenhouse facilities at the Royal Botanical Gardens in Burlington, Ontario, where they were randomly assigned to the different treatments.

The purpose of the experimental treatments was to create a factorial design for density, elongation, and water availability. Each treatment consisted of one manipulation for each of: (i) three levels of light quality- low R:FR, normal R:FR, and high R:FR; (ii) two levels of water availability- high and low; and (iii) two stand densities- high and low. High density stands were placed under neutral shade to create high density elongated plants. High density stands were also placed in high R:FR to create non-elongated high density plants. Low density stands were placed under neutral shade to create non-elongated low density plants. Low density stands were also placed in low R:FR to create elongated low density plants. Within each block, a replicate of these light quality-density treatments was subjected to both high and low water availability treatments. The design included four blocks with one replicate per block of each light quality-density by water treatment combination for a total of 300 plants in 32 treatments. All replicates were randomized with respect to their position in each block every other week.

Unfortunately, the high density plants in neutral shade did not elongate relative to those in the low density-neutral shade treatment. The small stature and prostrate growth of the plants apparently did not reduce R:FR sufficiently to cue elongation. However, plants grown at low density under artificial vegetation shade did elongate. Therefore, instead of analyzing as a completely factorial design, I analyzed the experiment as four light quality treatments- (i) high R:FR, (ii) low R:FR, (iii) high density-normal R:FR, and (iv) low density-normal R:FR; each within the two water availability treatments.

At the time of harvest ~4.5% of plants had developed only a first internode, a further ~19.5% both a first and second internode, an additional ~36.2% to the third internode, as well as ~22.6% to the fourth internode, and finally ~3.5% to the fifth internode. Although many of the plants had developing flower buds, very few plants had begun to flower and none had developing fruits. Whereas surplus plants grown on a nearby bench without shading grew upright, all of the experimental plants grew prostrate and failed to grow sturdy and erect stems.

Stand Density:

High density stands, ~1076 plants m^{-2}, were achieved by placing nine experimental plants in a 3x3 array, in a pine cell tray, surrounded by two border rows of plants to mitigate edge effects. The low density stands, ~120 plants m^{-2}, consisted of ten plants equally spaced apart in alternating rows of two and three plants.

Water Availability Treatments:

For the high water treatment, trays were bottom-watered daily for approximately one hour and then drained. In the low water treatment, trays were bottom-watered on Monday, Wednesday, and Friday only. The plants were fertilized using a solution of 0.25 g/L 20:20:20 NPK for the Monday and Friday watering.

Light Quality Treatments:

All light treatments consisted of 43.8x74.3 cm filters placed at a height of 25 cm above the top of the trays and were matched for ~30% of the incident light intensity. The low R:FR treatment, planted at low stand density only, was achieved by using an artificial vegetation shade filter to selectively reduce the R:FR. This filter was created by applying a mixture of 11.2 g Purple (Hostaperm Violet RL02) and 2.8 g Yellow (Solvaperm Yellow G) Hoechst brand pigments in 180 ml clear varnish (Pratt & Lambert #38) and 20 ml mineral spirits paint thinner evenly with a paint roller to a clear 0.16 mm vinyl sheet (Dudley and Schmitt 1995, Lee 1985). In this treatment, removal of red wavelengths lowered the incident R:FR reaching the stand and induced the shade avoidance response (Dudley and Schmitt 1995, Smith 1982). The high R:FR treatment, planted at high density only, was created using a solution of 100 g/L of $CuSO_4$ in distilled water to selectively filter out far-red light (Ballaré et al. 1991, Rajapakse et al. 1992). The $CuSO_4$ solution was put to a depth of 3 cm in a 15.2x 45.7x76.2 cm box made of 0.95 cm clear acrylic plastic. The box had an open top covered with a fine screen that allowed the solution to be filled with distilled water as required. In this treatment, removing FR raises the incident R:FR reaching the stand and suppresses the shade avoidance response to neighbors (e. g. Ballaré *et al.* 1990, 1991, Schmitt *et al.* 1995, Dudley and Schmitt 1996).

The normal R:FR treatment, planted at both high (NH) and low (NL) stand densities, consisted of two layers of 50% black plastic shade cloth with a layer of clear thin vinyl to further reduce light intensity, as well as to match the microclimate of the previous two light treatments. In this treatment, the R:FR is not altered above the stand from that of the incident light. Here the plants are able to detect the presence or absence of close neighbors and to respond accordingly- i.e. increased shade avoidance in NH compared to NL (Schmitt and Wulff 1993, Smith 1982, Smith 1995).

All treatments were enclosed in frames that were 55.9 cm wide by 76.2 cm long by 43.2 cm high with their sides covered in aluminum foil. The foil was used to block diffuse light from the sides and reflect the imposed light quality back onto the stands. The high R:FR frames were constructed with pressure treated 2x4 studs while all others were made from PVC tubing.

Gas exchange measurements:

On January 20-27, 1998, the most recently fully expanded leaf on each plant was sampled for gas exchange measurements. The leaf area (cm^2), net photosynthetic rate (assimilation of CO_2 in μmoles m^{-2} s^{-1}), and stomatal conductance (mmoles H_2O $m^{-2}s^{-1}$) of this leaf were measured immediately prior to harvest. Gas exchange was measured with a

Ciras-1 portable photosynthesis system using a Parkinson Leaf Cuvette attached (PP Systems, Hitchin Herts, U.K.). To maintain a relatively consistent and uniform measurement environment, gas exchange was measured after the leaf had equilibrated for 45 s in the cuvette. The Ciras-1 unit was adjusted to the following settings- internal air flow rate ~200 ml s^{-1}, cuvette air temperature ~25 °C, and PAR 800 μmoles $m^{-2}s^{-1}$. In the late morning and early afternoon, the measurements were taken on attached leaves of plants selected in a random order. The pine cells in which the plants were growing were removed from the treatments and placed in a holder so that the attached leaf could be placed in the leaf cuvette. Boundary-layer resistance was determined using methods described in the Ciras-1 operator's manual (Version 2). Because boundary-layer resistance is correlated with leaf size, a calibration curve estimating resistance as a function of leaf size was obtained to use in calculation of photosynthetic rates and stomatal conductance. Photosynthetic rate and stomatal conductance were calculated according to equations described in the Ciras-1 manual. Calculated photosynthetic rates and stomatal conductance were covariate corrected for effects of date and time (Farris and Lechowicz 1990, Winn and Evans 1991).

Morphological measurements:

Immediately after the gas exchange measurements were performed, the measured leaf was sectioned into the portion within the cuvette and the remainder. The area of both portions was measured for the total leaf size using an AM-100 leaf area meter (Analytical Development Co. Ltd., Hoddeson, England). Total height as well as internode and hypocotyl lengths were measured for all plants. Plants were sectioned into roots, leaves, and stems. The roots were washed to remove the media and dried along with the leaves and stem portion at 65 °C for at least a week and then weighed.

STATISTICAL ANALYSIS

All tests were performed with SAS version 6.12 for Windows (SAS Institute Inc., 1996, Cary, NC, U.S.A.). An analysis of variance (ANOVA), with block, light quality treatment, and water availability as the main effects, was used to determine effects of the treatments on plant morphology and physiology. To determine significant treatment differences for internode lengths, height, weights, total biomass, and covariate-corrected values of photosynthetic rate (A), stomatal conductance (g), and water-use efficiency (WUE, the ratio of A/g), the ANOVA's were performed using the GLM procedure. An analysis of covariance (ANCOVA) was performed with PROC GLM to determine treatment differences in the relationship between total biomass and total height, root weight and shoot weight, root weight and leaf area. PROC CORR was used to determine correlation's between A, g, WUE, total height, and leaf size within and across treatments. Treatment means and standard errors were calculated for leaf size, A, g, WUE, internode lengths, total height, and dry weights of roots, stem, and leaves (PROC MEANS). Data that was found to have disproportionately large effects on regression analyses were omitted as outliers following the criterion of Sokal and Rohlf (1981).

Results

Light Quality Treatments

The effects of the light quality treatments on height and elongation were limited to differences between the low R:FR manipulation and the other treatments. Plants grown under low R:FR had greater values for height corrected for shoot weight, a measure of elongation, than plants in the other treatments (Fig. 1.1, Table 1.1). Although there were no significant treatment effects for total height, there was an effect of the low R:FR treatment on the height to the second internode (Table 1.2). Furthermore, significant effects of the low R:FR manipulation were found in the longer hypocotyls and first internodes (Fig. 1.2, Table 1.2). Because the plants did not elongate in response to density, there was no direct evidence for the suppression of elongation under high R:FR. There were no significant differences in elongation between the high R:FR and the neutral shade treatments. The small stature and prostrate growth of the plants did not reduce R:FR in high density to sufficiently induce elongation.

Significant treatment effects were found for biomass allocation patterns. The plants grown under the high R:FR manipulation and the low density-neutral shade treatment had significantly higher total biomass than plants in the high density-neutral shade treatment which, in turn, were significantly larger than plants under the low R:FR manipulation (Fig. 1.3a, Table 1.3). The same trend is also seen in total leaf weight and root weight (Fig. 1.3b). The shoot weight differed significantly between all treatments. The plants in the high R:FR manipulation showed the highest stem weight, followed by low density-neutral shade plants, then high density-neutral shade, and finally the plants under the low R:FR manipulation had the lowest shoot weight (Fig. 1.3b, Table 1.3). Although, analyses of specific leaf weight, a performance measure of leaf weight per unit area, revealed no significant differences between treatments (Table 1.4). The leaf used for gas exchange measurements showed significant treatment effects on its size and weight (Table 1.4). The effects on these two traits were seen in the smaller sized and lower weight leaves of the low R:FR manipulation to those of the other treatments (Fig. 1.4).

The light quality manipulations had highly significant effects on photosynthetic rates and marginally significant effects on stomatal conductance. Photosynthetic rates were highest under the high R:FR manipulation followed by the neutral shade treatments and finally the low R:FR manipulation (Fig. 1.5a, Table 1.5). It should be noted that many of the plants were respiring at the time of measurement, as indicated by negative photosynthetic rates, particularly in the low R:FR manipulation, although not significantly more plants than in the other treatments. However, effect of light quality on photosynthetic rate remains significant and similar trends are found when the respiring plants omitted from the analysis (ANOVA df=3, 203, F=9.14, p=0.0001). The effects of these treatments on stomatal conductance were less significant. Stomatal conductance was highest in the plants under the high R:FR manipulation, but no significant differences were found between the other treatments (Fig. 1.5b, Table 1.5). Stomatal conductance was more strongly correlated with photosynthetic rate in plants under the high R:FR manipulation than in the other treatments (Table 1.6). Furthermore, plants in the high

R:FR manipulation had significantly higher photosynthetic rates for a given stomatal conductance, a measure of water-use efficiency, than plants in the high density-neutral shade treatment which in turn was significantly higher than those in the low R:FR manipulation (Fig. 1.6a, Table 1.7). When instantaneous water-use efficiency, photosynthetic rate over stomatal conductance without correction, was analyzed it was found the differences were slightly more significant with similar trends (Fig. 1.6b, Table 1.5).

Water Availability Treatments

The water availability treatments had significant effects on patterns of biomass allocation. The plants in the high water availability treatments accumulated higher total biomass than the low water plants in the overall experiment (Fig. 1.7, Table 1.3). This effect was limited primarily to the level of interface between the plants and their environment, i.e. the roots and leaves, as shoot weight showed no effect of the water treatments (Table 1.3). The plants in the high water treatments had higher total leaf biomass and had more roots. However, this effect was found primarily in the plants in the low R:FR treatment. The mean number of internodes also showed significant effects of the water treatments. Plants in the high water availability treatments produced significantly higher mean number of internodes than the low water plants (Fig. 1.8) (ANOVA df=1, 219, F=3.72, p=0.055).

Significant treatment by water interactions were also found for total biomass. The plants grown in the high water availability treatments of the high R:FR manipulation accumulated higher total biomass than plants in all the other treatment combinations (Fig. 1.7, Table 1.3). Furthermore, the high water treatment plants were significantly different between the neutral shade treatments with the low density plants higher than the high density. The plants under the low R:FR manipulation in both water availability treatments were not significantly different from each other. However, both of the low R:FR water treatments accumulated significantly less biomass than all other treatment combinations. Significant treatment by water interactions were also found for allocation to leaves. The trends for the effects of the interactions on total leaf weight reflect those seen for total biomass (Table 1.3). It is likely that the differences in leaf biomass are resulting in the differences for total biomass. The growth patterns also show significant interactions. Significant treatment by water interactions were found for the mean number of internodes produced. The plants grown in both of the manipulated R:FR treatments showed a reduction in the mean number of internodes in the low water availability treatments (Fig. 1.8) (ANOVA df=3, 219, F=4.42, p=0.005). There were no significant differences between water availability treatments in either of the neutral shade treatments.

Although attempts were made to minimize block differences, the position of the blocks in the greenhouse suggests that differences in temperature may have had an influence. Tests for block effects revealed significant differences for total height, number of internodes, total leaf weight, and shoot weight, as well as total biomass. The plants from block 4 had significantly lower total heights than those in the other blocks (Table 1.2). Also, the plants from blocks 1 and 4 had significantly fewer mean number of internodes than plants from blocks 2 and 3 (ANOVA df=3, 219, F=8.95, p=0.0001). Plants in block 1 also had significantly lower total leaf weight than plants in the other blocks (Table 1.4). With regards to shoot weight, the plants in block 1 were significantly lower than plants from the other blocks, also plants in block 4 were significantly lower than plants from block 2 (Table 1.3). A similar trend is seen for total biomass where plants grown in block1 accumulated significantly less than those grown in blocks 2 and 3 (Table 1.3). From these results, it appears that blocks 1 and 4 had less favourable growing conditions. Significant block effects were controlled for by inclusion of block and block interactions in the general linear models.

Discussion

The wild type variety of rapid-cycling *Brassica rapa* L. did elongate in response to the low R:FR manipulation. The use of dyes to decrease the R:FR above low density stands effectively induced stem elongation, which is consistent with the results of other studies (Dudley and Schmitt 1996, Lee 1985). However, the plants grown at high density under neutral shade did not elongate, as was expected, relative to the plants grown at low density under neutral shade. This suggests that the stand density was ineffective at reducing R:FR sufficient to induce elongation. Presumably, this was due to the prostrate growth and small stature of the plants. The shade avoidance response typically requires a stand structure in which leaves are in a position to alter the light quality for neighbouring plants. Although I did not directly measure the change in light quality, observations of the stand structures in high density suggest the plants were ineffective at reducing the R:FR sufficient to induce elongation. Hence stem elongation was limited to the low R:FR manipulation. The use of copper sulfate has been demonstrated to be effective at increasing the R:FR sufficient to suppress elongation in other studies (e. g. Ballaré *et al.* 1990, 1991, Schmitt *et al.* 1995, Dudley and Schmitt 1996). Because the plants at high density under neutral shade did not elongate relative to those in the high R:FR manipulation, I was not able to explicitly test for the effects of density independent of light quality. However, the treatment differences in stem elongation and the responses in carbon acquisition traits did allow for examination of the role of the R:FR.

Although there were no differences between treatments for total height, the elongated plants under the low R:FR manipulation were taller to the height of their second internode. The differences observed in stem elongation were attributable mainly to increased lengths of the hypocotyl and first internode. This is consistent with previous studies that have shown that stem elongation typically occurs in the hypocotyl and early internodes (Dudley and Schmitt 1996). However, in many studies of stem elongation, elongated plants are usually significantly taller than non-elongated. The fact that the elongated plants in this study were not also taller, as would be expected, suggests that there are other differences that were imposing a cost to elongation.

Dudley and Schmitt (1996) and Maliakal *et al.* (in press) suggested that elongation may be costly in low light if limited resources were allocated to above ground biomass at the expense of below ground biomass. Maliakal *et al.* (in press) and Ballaré *et al.* (1991) found that plants that elongated in response to light quality showed significantly altered accumulation and allocation patterns compared to non-elongated plants. With respect to biomass accumulation, the results of this study are consistent with the results of the density experiment of Maliakal *et al.* (in press), where it was found that elongated plants at high density had lower biomass than non-elongated at low density. In this study biomass accumulation was lower in the elongated plants for all traits measured. However, no significant differences between elongated and non-elongated plants were found for allocation patterns. The trends in allocation between above and below ground biomass did not differ between the treatments. There appeared to be no trade-offs between leaf and stem biomass with root biomass. Given that there were no trade-offs between acquisition structures induced by differences in light quality, the fact that the elongated plants accumulated less biomass indicates that some other response to light quality was imposing a cost.

Maliakal *et al.* (in press) and Ballaré *et al.* (1991) proposed that elongated plants may be more efficient at light capture. Plants can be more efficient at light capture through a number of strategies including distribution of leaves and leaf area. Although I did not measure distribution of leaves or total leaf area, I did examine the size and mass of the most recently fully expanded leaf. The characteristics of the leaf used for measures of gas exchange are not consistent with the proposal that elongated plants were more efficient at light capture. The measured leaves of the elongated plants in this study were smaller and proportionately lighter in weight to those of the non-elongated plants. No differences were found for specific leaf weight. Furthermore, visual observations indicated that the elongated plants potentially had lower total leaf areas. Moreover, the reduced biomass accumulation is contrary to the proposal. Thus, the elongated plants were probably not more efficient at light capture. In contrast, the elongated plants in this study had reduced light harvesting capacities. The hypothesis that elongated plants could have greater light conversion into biomass, proposed by Ballaré *et al.* (1991), is not supported by my results either. If elongated plants were to have more efficient conversion of light energy to biomass, we would expect elongated plants to have significantly higher biomass for a given photosynthetic rate. This is not the case in this study. There were no significant differences between treatments for biomass corrected for photosynthetic rate. Furthermore, photosynthetic rates and water-use efficiency were significantly lower in the elongated plants.

I propose that at least part of the cost of elongation was the R:FR induced responses in gas exchange traits. Gas exchange responded to the light quality manipulations. Photosynthetic rates were lower in the elongated plants. Furthermore, the plants under the high R:FR manipulation had significantly higher rates than all other treatments. Stomatal conductance also shows a significant response to the high R:FR manipulation. Together these results support the proposal that gas exchange is capable of responding to light quality. And in responding to low R:FR, gas exchange appears to be imposing a cost to elongation. Within an environment smaller leaves have been observed to have higher photosynthetic rates per unit leaf area (Bhagsari and Brown 1986). Contrary to this the elongated plants, with smaller leaves, had significantly lower photosynthetic rates compared with the non-elongated plants. Although there were no treatment differences in stomatal conductance, which implies that the effects on photosynthetic rate do not result from stomatal control, the significant correlation of photosynthetic rate with stomatal conductance in the elongated plants is consistent with stomatal limitation on photosynthesis (Geber and Dawson 1990, Wong et al. 1985). Stomatal limitation results in lower photosynthetic rates when plants are drought stressed (Cohen 1970, Toft et al. 1989). Stomatal limitation coupled with the significantly lower water-use efficiency of the elongated plants, suggests that the R:FR induced responses in gas exchange plants are potentially costly in water-limited environments.

The low water availability treatments appear to have imposed a water-stress on the plants with respect to biomass accumulation. However, the lack of more significant effects of the water treatments, particularly with gas exchange traits, indicates that the impacts of the water treatments were not as pronounced as anticipated. A stronger water stress would be expected to result in lower stomatal conductance and higher water-use efficiencies for plants in the low water treatments. A smaller leaf size and higher specific leaf weight should have also resulted from the low water treatment. However, the reduced biomass accumulation in leaves and roots, as well as total biomass, indicate that the plants in the low water treatments were resource limited (Bloom et al. 1985, Gleeson and Tilman 1992). I propose that the lack of a significant effect of water availability treatment on gas exchange further supports the resource limitation hypothesis. Because the means for gas exchange were not affected by water availability, the observed differences in biomass suggest that water-availability was likely the limiting factor. Furthermore, the plants in the low water treatments developed significantly fewer mean number of internodes. These results are indicative of water-stress on the plants. However, the results for total biomass and total leaf weight reveal that the effect of water treatment is limited primarily to the high R:FR manipulation. The elongated plants under the low R:FR and the plants under high R:FR manipulations also show signs of drought stress in the mean number of internodes. As these plants were responding to comparatively strong R:FR cues, these results could indicate that plant water-relations and responses to light quality are strongly linked. Hence the responses in gas exchange in the elongated plants could exacerbate the effects of drought stress under more water-limited conditions.

A difference in leaf morphology noted in the plants under the low R:FR manipulation could represent a dimorphic response to light quality that could potentially further affect water relations. The leaves under low R:FR tended to be small, smooth, and oval-shaped on petioles, while the leaves in the other treatments were larger, wrinkled, longer, and predominantly sessile about the main stem. Typically, the larger and more rough textured a leaf, the higher its boundary layer resistance (Farquhar and Sharkey 1982, Jones 1992). The difference in morphology could potentially result in a significant reduction in stomatal conductance for a given leaf area. Although no significant difference was found between elongated and non-elongated plants for the correlation of stomatal conductance with leaf size, the difference in leaf morphology is a characteristic that should be examined in more detail. Changes in leaf shape could represent a potentially costly or adaptive plastic response associated with the shade avoidance syndrome.

Responses in gas exchange to the R:FR may also carry intrinsic costs through imposed limits to light-harvesting capabilities. It is reasonable to assume that the plants have adjusted their physiology to the conditions in which they were grown (Jones 1992). Plants that grow in low light environments, such as those found in this study, commonly have lower maximal photosynthetic rates (Boardman 1977). Also leaves which are shade adapted become saturated at lower light levels. When exposed to comparatively high light levels these leaves can be overwhelmed or damaged and may begin to respire. Higher temperatures could also exacerbate this effect. There was a slight tendency for a larger number of respiring plants in the low R:FR manipulation, though not significantly more than other treatments. It is important to note that the gas exchange measurements in this study were relative measures of maximal CO_2 exchange. The plants were removed from their treatments and measured under higher light intensity and higher temperatures than were experienced by the plants in the treatments. Thus the measurements are not indicative of the performance of individual plants in their respective treatments. However, the leaves of the elongated plants may have been more sensitive to the effects of higher light intensity and higher temperatures. As treatments were matched for light intensity, this potential difference appears to be influenced by R:FR. More explicit tests will be required to determine if the apparent inability to make use of higher light intensity and reduced ability to cope with higher temperatures could be a further potential cost associated with stem elongation in response to the R:FR.

Stem elongation is hypothesized to be an adaptive response to increase carbon acquisition in the presence of competitors. Various morphological traits have been shown to respond to the light environment associated with competitive conditions (e.g. see Schmitt and Wulff 1993, Weiner et al. 1990, Weiner and Thomas 1992). Maliakal *et al.* (in press) and Ballaré *et al.* (1991) suggested that gas exchange may also show plastic responses associated with stem elongation. However, they were unable to determine which environmental cues were inducing changes in gas exchange. In this study I attempted to test the effects of light quality independent of the effects of density and the effects of water availability. Because the plants did not elongate in response to density, I was not able to explicitly test for the effects of density. However, I was able to show that gas exchange does respond to changes in the R:FR as part of the stem elongation response. The response to low R:FR, which induced stem elongation, resulted in lower photosynthetic rates and lower water-use efficiency, as well as reduced biomass accumulation. I suggest that the responses in gas exchange to low R:FR indicate a potential cost to elongation. Although no significant effects of water availability were found for gas exchange and biomass allocation patterns, the cost of elongation is expected to be more significant in water limited environments. I further suggest that the responses observed in this study are indicative of potential trade-offs between elongation and photosynthetic capacity. Though the shade avoidance hypothesis predicts that elongation is adaptive, plants that are elongating could be doing so at the expense of allocation to leaves and the photosynthetic machinery in the leaves. The question of the fitness consequences of these responses and the potential trade-offs remain open.

Table 1.1. Analysis of covariance of treatment and water availability on height with shoot biomass. The regression of height with shoot biomass, a measure of elongation, revealed significant treatment effects. The effects of block were not significant and were omitted from the analysis.

		Height		
Source	$MS_{denominator}$	df	F-ratio	p-value
Shoot biomass (S)	MS_{error}	1	226.20	0.0001
Treatment (T)	MS_{error}	3	1.83	0.1425
S × T	MS_{error}	3	7.31	0.0001
Water (W)	MS_{error}	1	1.13	0.2895
S × W	MS_{error}	1	1.20	0.2745
T × W	MS_{error}	3	0.90	0.4410
S × T × W	MS_{error}	3	0.68	0.5675
Error		264		

Table 1.2. Analysis of variance of treatment and water on stem characteristics. Significant treatment effects were found for height to second internode, and the lengths of the hypocotyl and first internodes.

			Total Height		Length of hypocotyl		Length of first internode		Height to second internode	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	MS_{error}	3	1.25	0.2933	12.80	0.0001	11.28	0.0001	4.04	0.0080
Water (W)	MS_{error}	1	0.18	0.6727	1.56	0.2129	1.54	0.2152	0.75	0.3758
T × W	MS_{error}	3	0.45	0.7203	1.49	0.2187	0.91	0.4347	0.14	0.9350
Block (B)	MS_{error}	3	3.83	0.0105	0.66	0.5772	1.12	0.3412	0.87	0.4590
B × T	MS_{error}	9	1.33	0.2215	1.78	0.0727	1.37	0.2045	1.09	0.3696
B × W	MS_{error}	3	2.65	0.0494	2.47	0.0624	0.71	0.5469	0.99	0.3964
B × T × W	MS_{error}	9	1.16	0.3233	0.53	0.8536	1.92	0.0501	1.19	0.3021
Error		248								

Table 1.3. Analysis of variance of treatment and water on allocation. Significant treatment effects were found for all biomass characters. Significant effects of water availability were found for total, leaf, and root biomasses. Leaf and total biomass also showed significant treatment by water interactions.

			Total biomass		Leaf biomass		Shoot biomass		Root biomass	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	MS_{error}	3	17.43	0.0001	16.93	0.0001	22.37	0.0001	9.75	0.0001
Water (W)	MS_{error}	1	5.62	0.0185	3.90	0.0494	1.60	0.2072	6.25	0.0131
T × W	MS_{error}	3	2.73	0.0443	3.11	0.0272	0.70	0.5557	0.81	0.4917
Block (B)	MS_{error}	3	3.29	0.0214	3.57	0.0147	4.27	0.0058	0.68	0.5659
B × T	MS_{error}	9	1.77	0.0753	1.55	0.1319	2.24	0.0203	1.15	0.3292
B × W	MS_{error}	3	1.35	0.2573	0.73	0.5356	1.26	0.2882	0.81	0.4913
B × T × W	MS_{error}	9	1.18	0.3070	1.22	0.2846	1.31	0.2319	1.45	0.1684
Error		242								

Table 1.4. Analysis of variance of treatment and water on the characteristics of the leaf used for gas exchange measures. Significant treatment effects were found for leaf area and leaf dry weight.

			Leaf area		Leaf dry weight		Specific leaf weight	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	MS_{error}	3	18.80	0.0001	21.07	0.0001	1.81	0.1459
Water (W)	MS_{error}	1	0.99	0.3214	2.24	0.1358	0.46	0.4998
T × W	MS_{error}	3	0.15	0.9297	0.34	0.7965	1.02	0.3828
Block (B)	MS_{error}	3	1.02	0.3854	0.98	0.4006	0.56	0.6443
B × T	MS_{error}	9	0.89	0.5319	0.72	0.6874	0.75	0.6657
B × W	MS_{error}	3	0.55	0.6507	0.26	0.8522	0.90	0.4414
B × T × W	MS_{error}	9	0.54	0.8428	0.45	0.9089	0.79	0.6278
Error		252						

Table 1.5. Analysis of variance of treatment and water on gas exchange measures. Significant treatment effects were found for photosynthetic rate, stomatal conductance, and water-use efficiency.

			Photosynthetic rate		Stomatal conductance		Water-use efficiency	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	MS_{error}	3	16.58	0.0001	2.79	0.0411	12.53	0.0001
Water (W)	MS_{error}	1	0.50	0.4788	0.20	0.6535	0.72	0.3977
T × W	MS_{error}	3	2.02	0.1118	1.99	0.1159	0.56	0.6425
Block (B)	MS_{error}	3	0.79	0.5025	0.81	0.4894	0.99	0.3987
B × T	MS_{error}	9	1.30	0.2390	1.15	0.3248	1.10	0.3609
B × W	MS_{error}	3	1.07	0.3621	0.77	0.5096	1.01	0.3871
B × T × W	MS_{error}	9	2.15	0.0258	1.54	0.1336	1.70	0.0902
Error		252						

Table 1.6. Pearson correlation coefficients for photosynthetic rate and stomatal conductance within light quality treatments. Photosynthetic rate and stomatal conductance were significantly correlated in the high R:FR and the normal R:FR, low density treatments. (p<0.10 †, p<0.05 *, p<0.01 **, p<0.001***)

	Photosynthetic rate (A)			
Treatment	High R:FR	Low R:FR	Normal R:FR, high density	Normal R:FR, low density
Stomatal conductance (g)	0.31*	0.22†	-0.05$^{n.s.}$	0.27*

Table 1.7. Analysis of covariance of treatment and block on photosynthetic rate with stomatal conductance. The regression of photosynthetic rate with stomatal conductance revealed marginally significant differences between treatments.

		Photosynthetic rate		
Source	$MS_{denominator}$	df	F-ratio	p-value
Stomatal conductance (g)	MS_{error}	1	10.20	0.0016
Treatment (T)	MS_{error}	3	3.73	0.0119
g × T	MS_{error}	3	2.47	0.0628
Block (B)	MS_{error}	3	2.12	0.0979
g × B	MS_{error}	3	2.02	0.1113
T × B	MS_{error}	9	1.25	0.2679
g × T × B	MS_{error}	9	1.61	0.1123
Error		252		

Fig. 1.1. Height, cm, versus shoot biomass, g, in the light quality treatments. Height was significantly correlated with shoot biomass in all treatments. The plants under low R:FR were significantly more elongated than in the other treatments. Significance level given is taken from an analysis of covariance of height with shoot biomass. Lines shown represent the slope of the regression for each treatment.

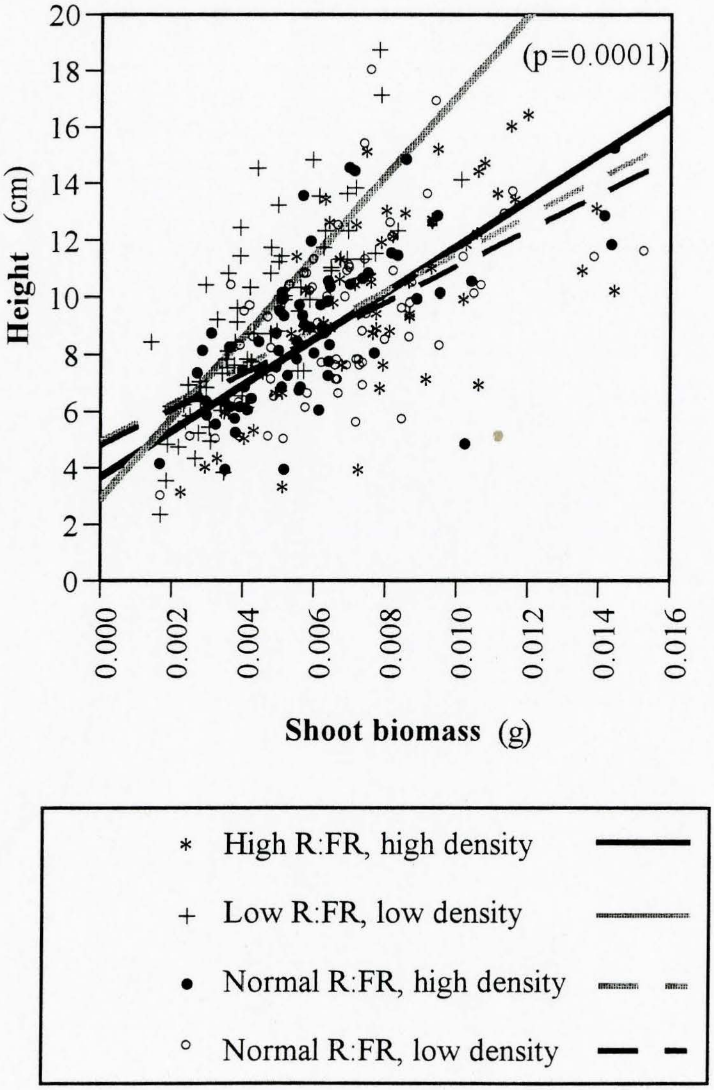

20
18
16
14
12
10
8
6
4
2
0
Height (cm)
(p=0.0001)
0.000
0.002
0.004
0.006
0.008
0.010
0.012
0.014
0.016
Shoot biomass (g)
* High R:FR, high density
+ Low R:FR, low density
● Normal R:FR, high density
○ Normal R:FR, low density

Fig. 1.2. Means for the length of the hypocotyl and first internode, cm, in the light quality treatments. The hypocotyl and first internode were significantly longer in plants under low R:FR. Significance level given is taken from an analysis of variance for hypocotyl and first internode. Bars represent ±1 S.E.

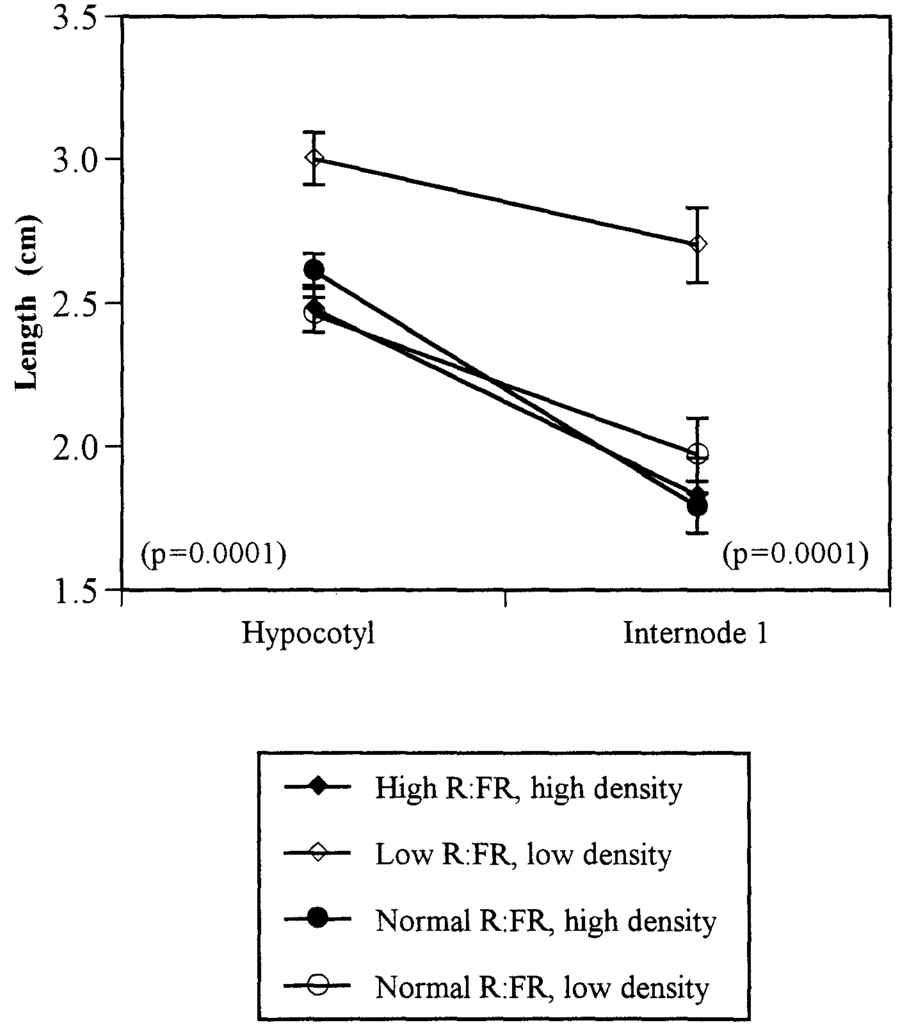

3.5
3.0
2.5
2.0
1.5
Length (cm)
(p=0.0001)
(p=0.0001)
Hypocotyl
Internode 1
High R:FR, high density
Low R:FR, low density
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.3a. Means for total dry biomass, g, in the light quality treatments. Total biomass was significantly lower in plants under low R:FR while higher under high R:FR and neutral shade at low density. Significance level given is taken from an analysis of variance for biomass. Bars represent ±1 S.E.

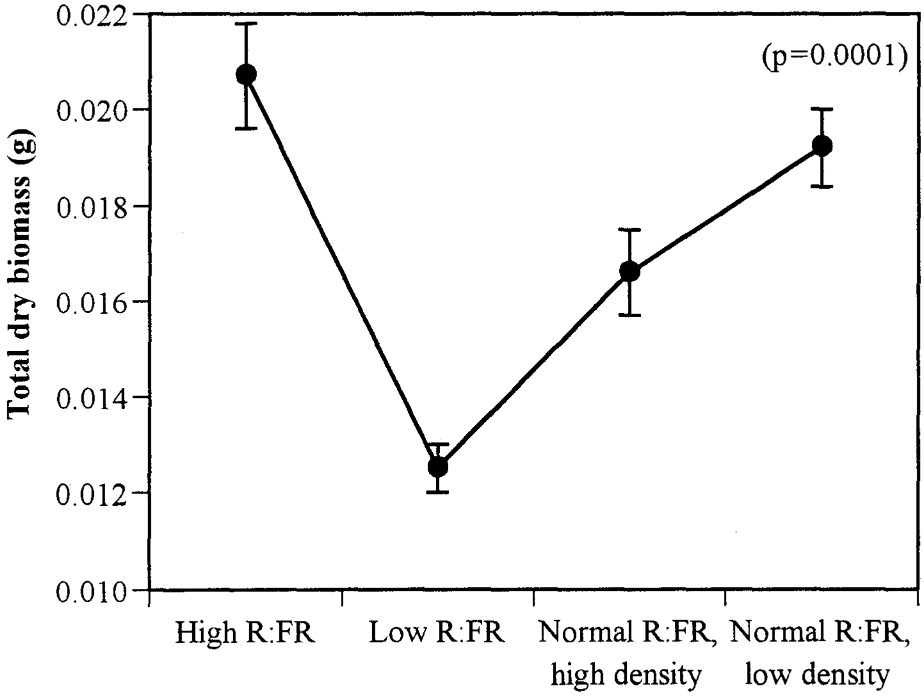

Total dry biomass (g)
0.022
0.020
0.018
0.016
0.014
0.012
0.010
(p=0.0001)
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.3b. Means for biomass allocation, g, to leaves, shoot, and roots in the light quality treatments. The biomass for all traits were significantly lower in plants under low R:FR and higher under high R:FR and neutral shade at low density. Whereas leaves and roots did not differ significantly between high R:FR and neutral shade at low density, shoot biomass differed significantly between all treatments. Significance level given is taken from an analysis of variance for each trait. Bars represent ±1 S.E.

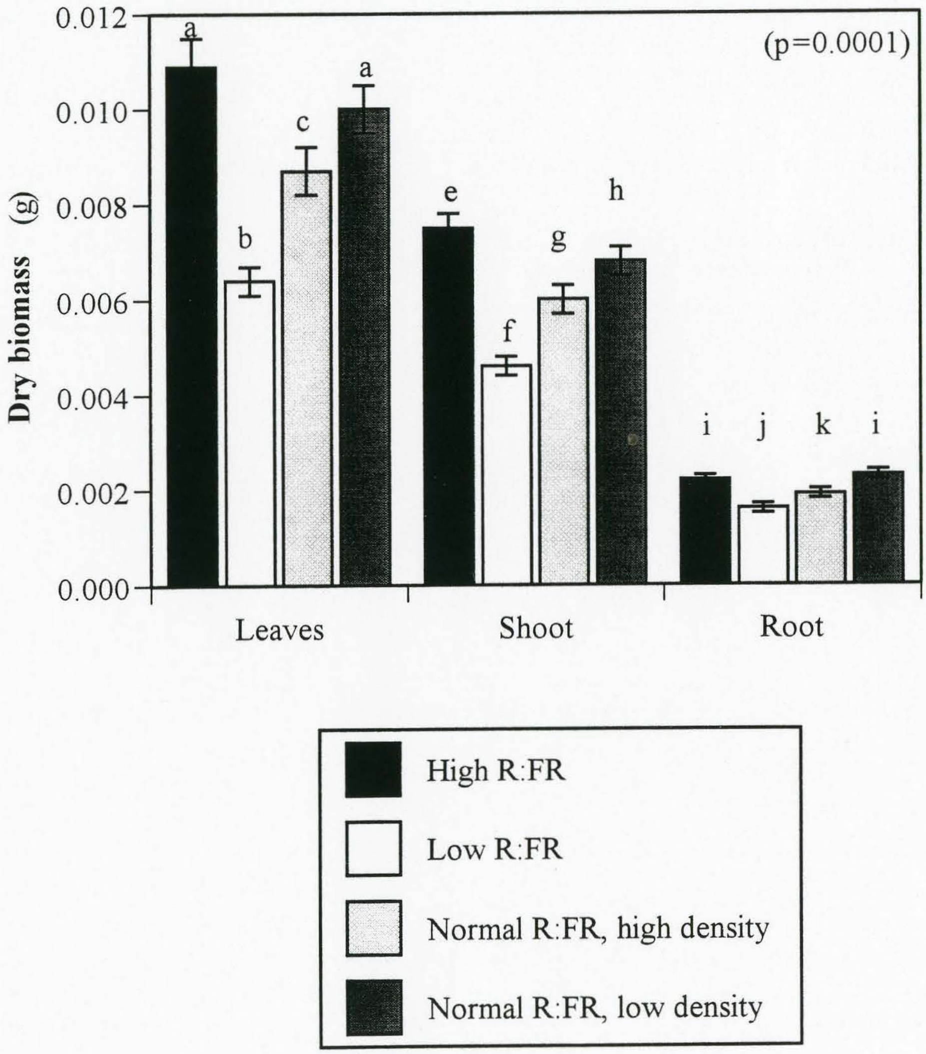

Dry biomass (g)
0.012
0.010
0.008
0.006
0.004
0.002
0.000
(p=0.0001)
a
b
c
a
e
f
g
h
i
j
k
i
Leaves
Shoot
Root
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.4. Means for the area of the leaf used for measures of gas exchange, cm^2, in the light quality treatments. Leaf size was significantly lower in plants under low R:FR. Significance level given is taken from an analysis of variance for leaf size.

Bars represent ±1 S.E.

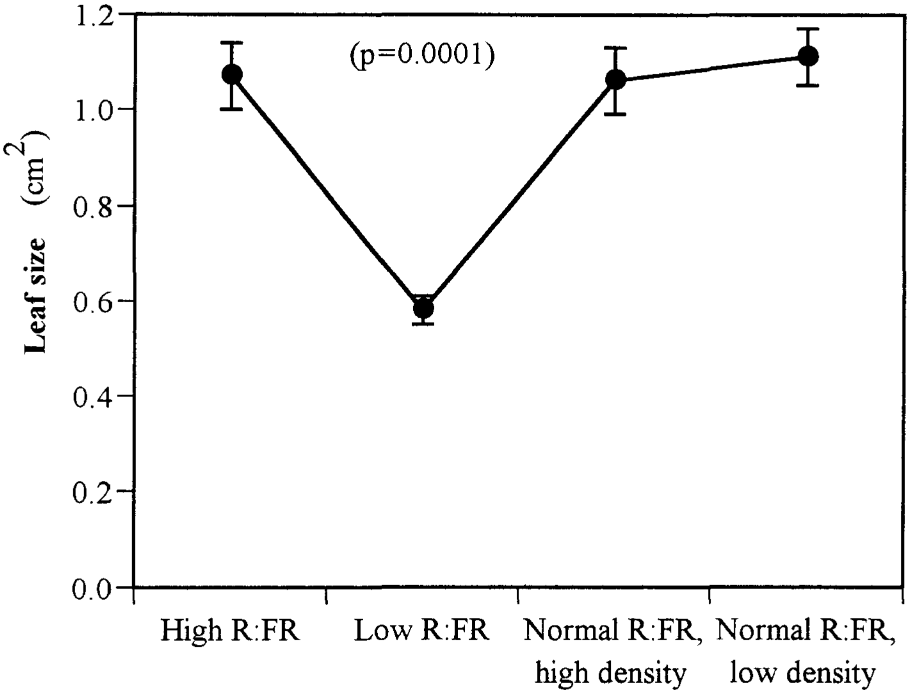

(p=0.0001)
Leaf size (cm^2)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.5a. Means for photosynthetic rate, μmole CO_2 m^{-2} s^{-1},in the light quality treatments. Photosynthetic rate was significantly lower in plants under low R:FR while higher under high R:FR. Significance level given is taken from an analysis of variance for photosynthetic rate. Bars represent ±1 S.E.

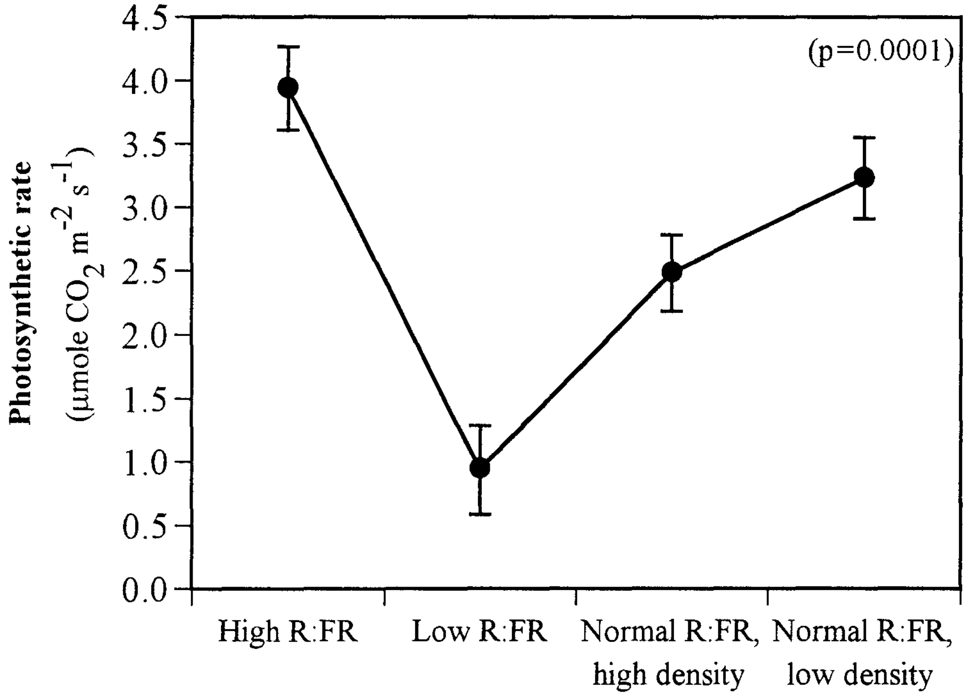

Photosynthetic rate
(µmole CO2 m-2 s-1)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
(p=0.0001)
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.5b. Means for stomatal conductance, mmole H_2O m^{-2} s^{-1},in the light quality treatments. Stomatal conductance was significantly higher in plants under high R:FR. Significance level given is taken from an analysis of variance for stomatal conductance. Bars represent ±1 S.E.

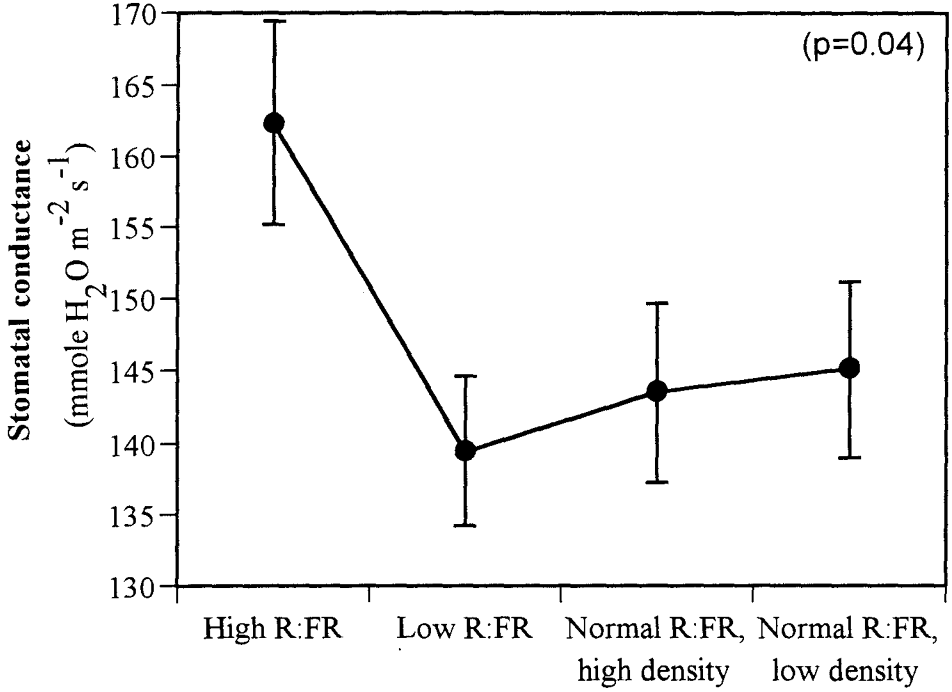

Stomatal conductance
(mmole H2O m-2 s-1)
170
165
160
155
150
145
140
135
130
(p=0.04)
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.6a. Photosynthetic rate versus stomatal conductance in the light quality treatments. Photosynthetic rate was significantly correlated with stomatal conductance under manipulated R:FR. The plants under high R:FR had marginally significant higher photosynthetic rates for a given stomatal conductance than under low R:FR. Significance level given is taken from an analysis of covariance of photosynthetic rate with stomatal conductance for treatment differences. Lines shown represents the slope of the regression for each treatment.

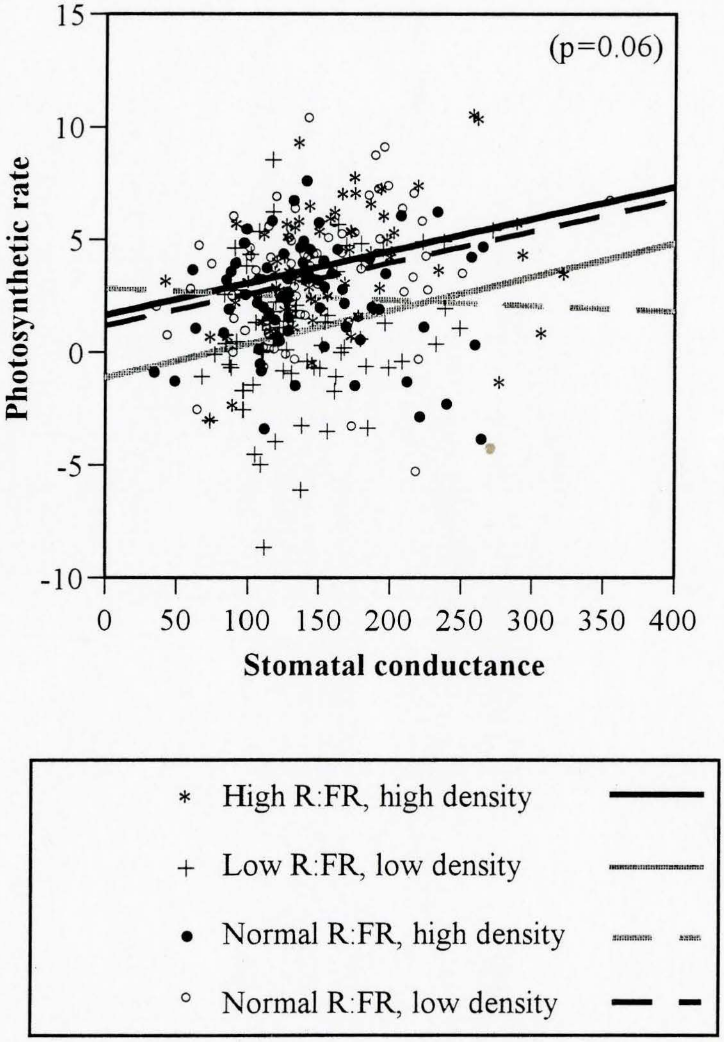

(p=0.06)
Photosynthetic rate
15
10
5
0
-5
-10
0
50
100
150
200
250
300
350
400
Stomatal conductance
* High R:FR, high density
+ Low R:FR, low density
● Normal R:FR, high density
○ Normal R:FR, low density

Fig. 1.6b. Means for water-use efficiency, μmole CO_2/mmole H_2O,in the light quality treatments. Water-use efficiency was significantly lower in plants under low R:FR while higher under high R:FR. Significance level given is taken from an analysis of variance for water-use efficiency. Bars represent ±1 S.E.

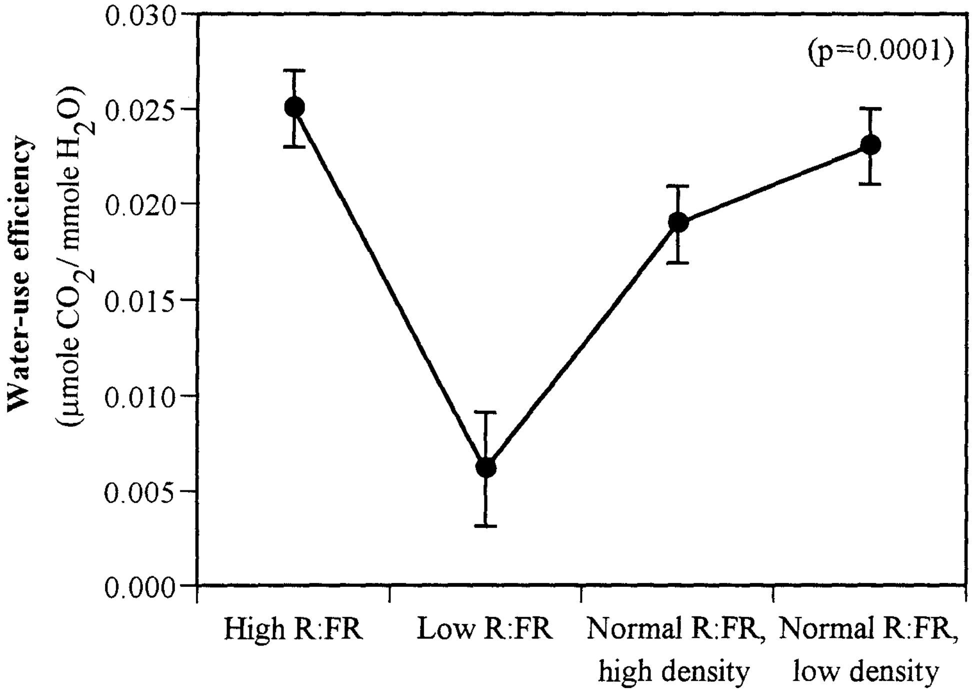

Water-use efficiency
(µmole CO2/ mmole H2O)
0.030
0.025
0.020
0.015
0.010
0.005
0.000
(p=0.0001)
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.7. Means for total dry biomass, g, in the light quality within the water availability treatments. Biomass was significantly lower in plants under low R:FR in both water availabilities. An effect of water availability on biomass was only found in plants under high R:FR. The effect of light quality is more pronounced in high water. Significance level given is for the effect of water availability and was taken from an analysis of variance for biomass. Bars represent ±1 S.E.

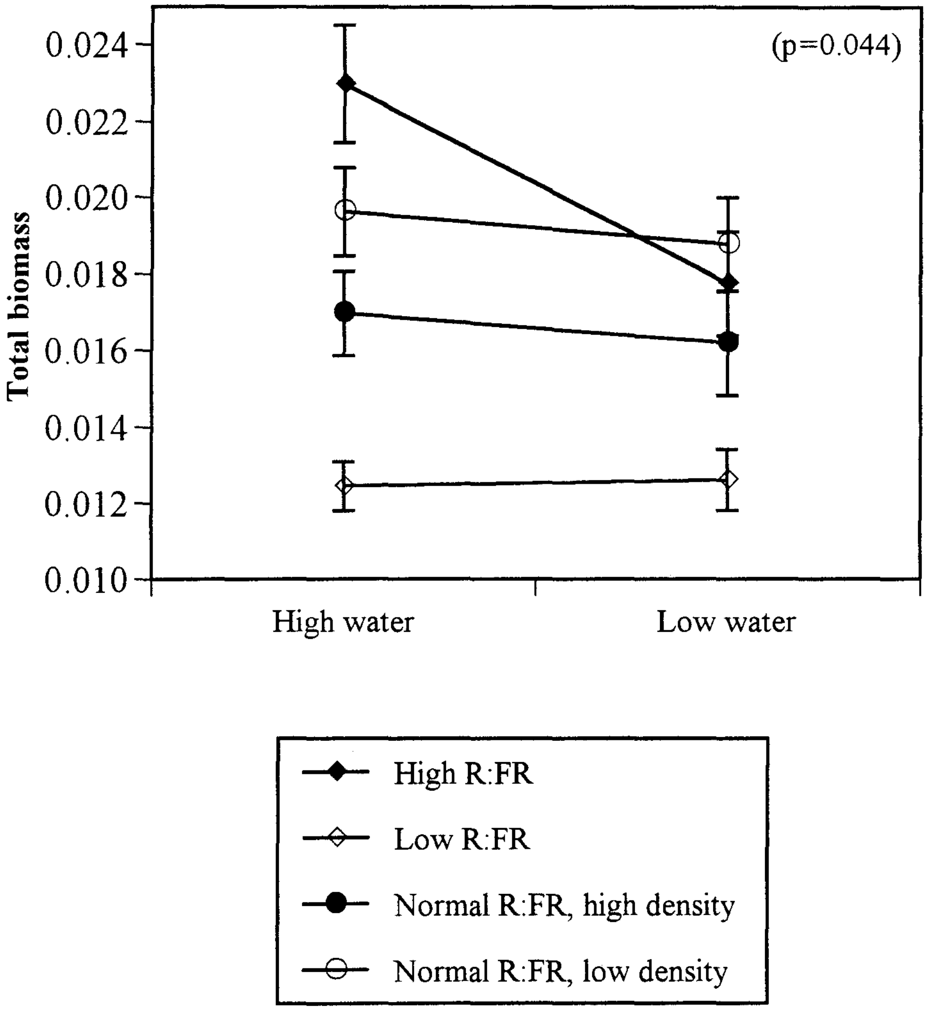
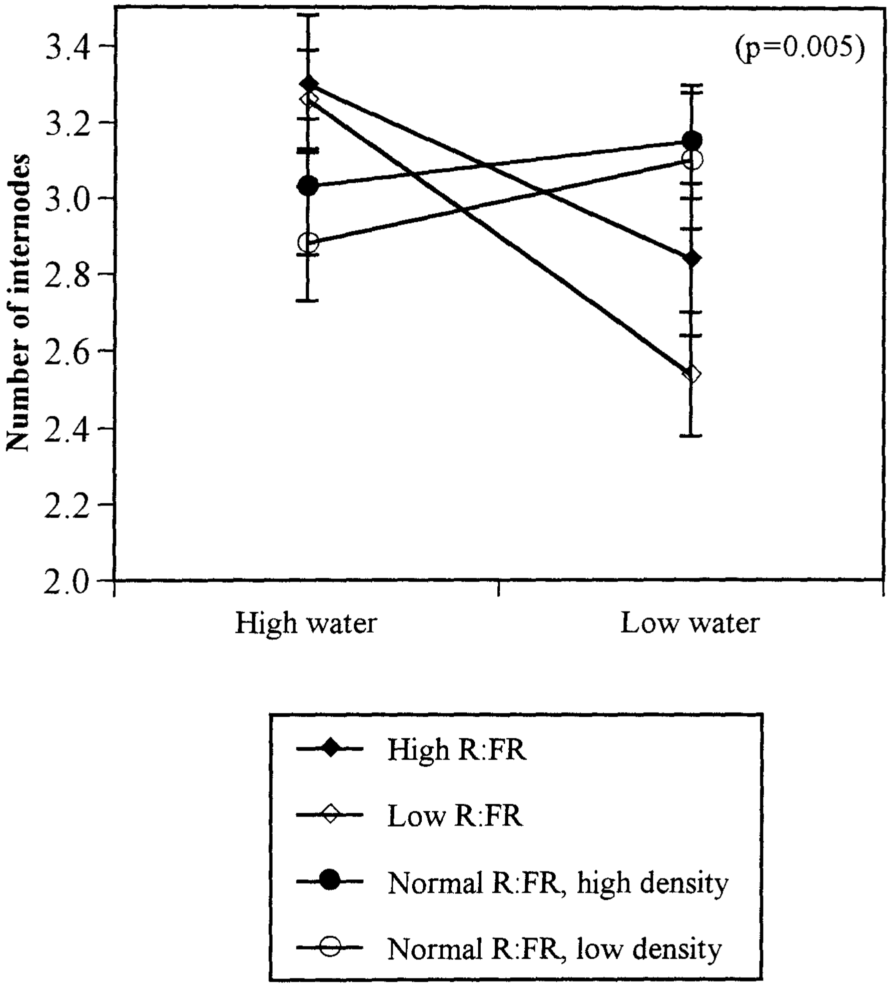

Total biomass
0.024
0.022
0.020
0.018
0.016
0.014
0.012
0.010
(p=0.044)
High water
Low water
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Fig. 1.8. Means for number of internodes in the light quality within the water availability treatments. Number of internodes was significantly lower in plants in the low water availability under low R:FR and high R:FR. Significance level given is for the effect of water availability and was taken from an analysis of variance for number of internodes. Bars represent ±1 S.E.


Number of internodes
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
(p=0.005)
High water
Low water
High R:FR
Low R:FR
Normal R:FR, high density
Normal R:FR, low density

Chapter III

Sexual dimorphism in carbon acquisition of the androdioecious species, *Mercurialis annua* L *s.l.*, in response to light quality.

Introduction

It is commonly suggested that the genders in plants will have different resource requirements and allocation strategies because they are performing different functions in reproduction- e.g. pollen versus seed production. Differences in resource allocation between genders have been well documented in various dioecious species (Conn 1981, Cox 1981, Korpelainen 1992, Lloyd and Bawa 1984, Zimmerman and Lechowicz 1982). It is further postulated that these differences can lead to sexual dimorphism in resource acquisition as well as allocation (Freeman et al. 1976Bawa, 1980 #2642, Thomson and Brunet 1990). Most studies on sexual dimorphism and correlated traits have focused on differences in morphology and resource allocation between the genders (Conn 1981, Geber and Charnov 1986, Korpelainen 1992, Lloyd and Bawa 1984), as well as the evolution of dioecy as a life history trait (Bawa 1980, Quinn 1991, Thomson and Brunet 1990). Comparatively few studies have examined sexual dimorphism in both morphology and physiology. The results vary among studies that have examined physiological differences in resource acquisition between sexually dimorphic species. Evidence for differences was found in a number of studies that found males tend to be more drought tolerant despite lower water-use efficiencies (Dawson and Bliss 1989, Gehring and Monson 1994, Marshall et al. 1993). Zimmerman and Lechowicz (1982) also found males to be more drought tolerant than females, but did not find differences in gas exchange between the genders. It was suggested that the differences they found between the genders were related to patterns of allocation and not due to differences in gas exchange and water-use efficiency. It is apparent from these studies that the genders may respond in their acquisition and allocation in different ways. However, it is not clear if these differences are affected by the environment or what environmental cues could elicit the observed responses. The variability in the results for different species indicate that further studies are needed which incorporate morphological and physiological traits to better understand how the genders differ in their resource acquisition and allocation strategies. In this paper, I describe a study on sexual dimorphism in traits associated with how plants acquire carbon and the responses induced by differences in light quality.

Various studies have examined how plants can respond in their patterns of biomass allocation to the presence of competitors and the associated changes in the light environment (for a recent review see Smith 1995). Plants growing in dense stands often show clear density-dependent responses in their growth and development. Typical phytochrome mediated plastic responses to the low R:FR cue associated with vegetation shade include marked stem elongation, reduced branching, and redistribution of leaves to the top of the canopy (Schmitt and Wulff 1993, Smith 1982, Smith 1995). These responses are generally considered to be shade avoidance responses which enable plants to avoid the negative effects of reduced light availability in dense stands (Schmitt and Wulff 1993, Smith 1995). The results of the previous chapter and those by Maliakal *et al.* (in press) indicate that the responses to density and water relations are strongly linked. The morphological and physiological traits associated with carbon acquisition have been shown to respond to the R:FR and water availability. However, it is not known if there is sexual dimorphism in carbon acquisition traits of dioecious plants. It is also important to ask how gender differences in resource acquisition strategies will affect the patterns of allocation.

To my knowledge, no study has attempted to examine both carbon acquisition and allocation strategies in an androdioecious mating system, which is characterized by both male and hermaphroditic individuals. Functionally androdioecious hexaploid populations of *M. annua* are found distributed around the Mediterranean (Pannell 1997a, Pannell 1997b). These populations consist of male individuals with staminate flowers on long peduncles held away from the plant and monoecious individuals with both male and female flowers clustered in the leaf axils. Pannell (1997a) found evidence for differences in allocation strategies between the genders in the androdioecious species *Mercurialis annua* L *s.l.* (Euphorbiaceae), as well as differential responses to the effects of shade and density. Although it has been shown that in this species the genders differ in their allocation strategies, it is not known how traits related to carbon acquisition, such as photosynthetic rate and stomatal conductance, will vary in conjunction with morphological responses associated with shade avoidance.

In the previous chapter I considered how environmental variables- light quality, stand density, and water availability; were affecting gas exchange traits as part of the shade avoidance response. In this chapter I present a study on how gas exchange traits are affected by gender and reproductive allocation in association with the shade avoidance response in *M. annua*. I compared the allocation patterns and gas exchange traits of plants grown in environments in which I manipulated the R:FR. In this experiment, all plants were grown at high density under controlled conditions. In the suppressed treatment, plants received high R:FR, which, though reduced by the canopy, would be sufficient to suppress an elongation response (Ballaré et al. 1991, Rajapakse et al. 1992, Smith 1982). For the elongated treatments, plants in full sun and under neutral shade received normal R:FR, which was reduced by the plant canopy. I measured reproductive allocation as a measure of fitness, as well as maximal photosynthetic rates, stomatal conductance, leaf size, and allocation to stem and leaves. I asked (i) is there evidence for sexual dimorphism in allocation and gas exchange, (ii) is there sexual dimorphism in the responses associated with stem elongation, and (iii) do the genders differ in their reproductive and vegetative allocation strategies?

Materials and Methods

EXPERIMENTAL DESIGN

Mercurialis annua L. *s.l.* seeds were collected by Pannell (1997b) from natural populations in the precincts of Pabellon de Cuba in southern Spain during March 1994. On September 3rd, 1997, seeds were planted in ten 8.0x29.5x37.0 cm plastic trays containing pure, washed river sand. The seeds were set 1.0 cm below the surface of the media in 7 rows with 350 seeds per row providing high density stands of ~22500 plants m^{-2}. The seeds were allowed to germinate in full sun at the greenhouse facilities at the Royal Botanical Gardens in Burlington, Ontario, with top watering performed daily for the duration of the experiment.

This experiment was conducted as part of a larger study on the effects of light quality and nutrients on sex ratio (Pannell, Sleeman, Dudley, and Barrett, unpublished). For the overall design, five of these trays had a 1 L solution of 2.0 g/L 20:20:20 NPK fertilizer added to the media while the remaining five received 1 L at 4.0 g/L. After the majority of plants had emerged, the trays were randomly assigned to the different light quality treatments with two trays, one of each nutrient level, placed into each treatment. The effects of nutrient availability were not considered in the study I describe in this chapter. Therefore only the plants from the trays that received the higher nutrient solution were measured. At the time of harvest ~25.6% of plants had developed both a first and second internode, an additional ~57.4% to the third internode, and finally ~16.9% to the fourth internode. Many of the plants had begun to flower and had developing fruits. Of the male plants, ~96% of plants were producing male flowers. With respect to the hermaphroditic plants, ~90% were producing both male and female flowers, of which ~75% had developing fruits. Seed maturation and dispersal was also noted in ~26% of hermaphrodites.

Light Quality Treatments:

The experiment incorporated three light treatments- full sun (FS), high R:FR, and neutral shade (NS). The design included two replicates of each the high R:FR and NS treatments and one replicate of the FS treatment. All replicates were randomized with respect to their position on the bench every other week. From each replicate 20 male and 20 hermaphrodites were randomly selected for measurement of gas exchange and biomass allocation.

For the full sun treatment, one of each of the two nutrient level trays were placed together on the bench. In this treatment, the plants received altered light only from their close neighbors. The two altered light treatments consisted of 43.8x74.3 cm filters placed at an initial height of 25 cm above the top of the trays and were matched for ~45% (45% ± 5%) of the full sun light intensity. The light filters were subsequently raised at 18 cm intervals when plants grew as tall as the level of the treatment. Both treatments were raised at the same time to maintain comparable conditions between them.

The high R:FR treatment was imposed using a solution of 45 g/L of $CuSO_4$ in distilled water to selectively filter out far-red light (FR: 710-730 nm) (Ballaré et al. 1991, Rajapakse et al. 1992). This solution was put to a depth of 3 cm in a 15.2x 45.7x76.2 cm box made of 0.95 cm clear acrylic plastic. The box had an open top and was filled with distilled water as required. In this treatment, removing FR raises the incident R:FR reaching the stand and suppresses the shade avoidance response to neighbors (e. g. Ballaré *et al.* 1990, 1991, Schmitt *et al.* 1995, Dudley and Schmitt 1996). The neutral shade treatment consisted of one layer of 50% black plastic shade cloth with a layer of 0.16 mm clear thin vinyl to further reduce light intensity and to help match the microclimate of the high R:FR treatment. In this treatment, the R:FR is not altered from that of the incident light. Here the plants are able to detect the presence of neighbors and to respond to the associated changes in the light quality (Schmitt and Wulff 1993, Smith 1982, Smith 1995).

The high R:FR and NS treatments were enclosed in frames that were 56 cm wide x 81 cm long x 101.5 cm tall with their sides covered in aluminum foil. The foil was used to block diffuse light from the sides and to reflect the imposed light quality back onto the stands. The high R:FR frames were constructed with spruce 2x3 studs while the NS frames were constructed with spruce 2x2 studs.

Gas Exchange Measurements:

Twenty plants of each sex, male and hermaphrodite, were randomly selected from each of the replicates. Plants near the edges of trays were excluded by selecting plants at least five positions in from the edge in rows two through six. On November 12th, 1997, the most recently fully expanded leaf on each plant was sampled for gas exchange measurements. The net photosynthetic rate (assimilation of CO_2 in μmoles m^{-2} s^{-1}), and rate of stomatal conductance (mmoles H_2O $m^{-2}s^{-1}$) of this leaf were measured within 60 s of having cut the plant's stem at its base to remove it from the tray. Gas exchange was measured with a Ciras-1 portable photosynthesis system using a Parkinson Leaf Cuvette attached (PP Systems, Hitchin Herts, U.K.). To maintain a relatively consistent and uniform measurement environment, measurements were taken after the leaf had equilibrated for 45 s in the cuvette. The Ciras-1 unit was adjusted to the following settings- internal air flow rate ~200 ml s^{-1}, cuvette air temperature ~25 °C, and PAR 800 µmoles $m^{-2}s^{-1}$. Boundary-layer resistance was determined using methods described in the Ciras-1 operator's manual (Version 2). Because boundary-layer resistance is correlated with leaf size, a calibration curve estimating resistance as a function of leaf size was obtained to use in calculation of photosynthetic rates and stomatal conductance. Photosynthetic rate and stomatal conductance were calculated according to equations described in the Ciras-1 manual. Calculated photosynthetic rates and stomatal conductance were covariate corrected for effects of date and time (Farris and Lechowicz 1990, Winn and Evans 1991).

Morphological Measurements:

Immediately after the gas exchange measurements were performed, the measured leaf was sectioned into the portion within the cuvette and the remainder. The area of both portions was measured for the total leaf size using an AM-100 leaf area meter (Analytical Development Co. Ltd., Hoddeson, England). Internode lengths and total height were measured for all plants. Plants were stripped of remaining leaves, male and female flowers, and fruits. The leaves, flowers, fruits, and stem portion were dried at 65 °C for at least a week and then weighed.

STATISTICAL ANALYSIS

All tests were performed with SAS version 6.12 for Windows (SAS Institute Inc., 1996, Cary, NC). An analysis of variance (ANOVA) with light treatment as the main effect was used to determine effects of the treatments on plant morphology and gas exchange traits. To determine significant treatment differences for internode lengths, height, dry weights for vegetative and reproductive biomass, and covariate-corrected values of photosynthetic rate (A), stomatal conductance (g), and water-use efficiency (WUE, the ratio of A/g), analyses were performed using the GLM procedure. An analysis of covariance (ANCOVA) was performed with PROC GLM to determine treatment differences in the relationship between height and shoot weight- a measure of elongation. PROC CORR was used to determine correlations between A, g, WUE, height, and leaf size within and across treatments. Means and standard errors were calculated for leaf size, A, g, WUE, internode lengths, total height, and dry weights of stem, leaves, and vegetative and reproductive biomass (PROC MEANS). Data that was found to have disproportionately large effects on regression analyses were omitted as outliers following the criterion of Sokal and Rohlf (1981).

To test hypotheses about direct selection through fitness on height, leaf size, photosynthetic rate, and stomatal conductance, I performed a phenotypic selection analysis on males and hermaphrodites following Lande and Arnold (1983). Directional standardized selection gradients, for both genders in each treatment, were obtained from a linear regression of relative fitness on standardized traits (Dudley 1996, Lande and Arnold 1983). The selection gradients were used in a path analysis to examine the functional dependency between height, leaf size, and gas exchange traits and fitness (Arnold 1983, Dudley 1996). I constructed a priori path models based on well-known and understood functional relationships. The correlations and covariance between traits were tested and the path model refined to reflect selection observed in this study. The path model was constructed to allow for height to affect fitness through photosynthetic rate and vegetative biomass, and leaf area and gas exchange traits to affect fitness through vegetative biomass (Fig. 2.1). Path coefficients were estimated using PROC GLM to obtain the appropriate regression coefficients on the standardized variables and relative fitness. Analysis of covariance (PROC GLM) was used to determine if path coefficients or selection gradients differed between treatments or genders.

Results

The plants in both of the reduced light intensity treatments were taller than plants in full sun (Fig. 2.2, Table 2.1). The hypocotyl, first internode, and second internode were significantly longer in both of the low light treatments (Fig. 2.3a-c, Table 2.1). The plants in both of the low light treatments were significantly taller for a given vegetative biomass, a measure of elongation, than plants in full sun (Fig. 2.4a, Table 2.2). Between the low light treatments, hypocotyls were significantly longer for plants under neutral shade, as expected (Fig. 2.3a). However, the plants under high R:FR had longer first internodes (Fig. 2.3b). Moreover, the low light treatments did not differ significantly in the length of the second internode (Fig 2.3c, Table 2.1). Furthermore, the plants grown under neutral shade were not more elongated, than plants grown under high R:FR (Fig. 2.4a). The elongation of the plants under high R:FR and neutral shade compared to those under full sun suggests that stem elongation in *M. annua* may be regulated by a blue-light receptor, which signals for light intensity (Casal 1989, Jenkins et al. 1995, Liscum and Hangarter 1994).

An analysis of variance revealed no significant differences between treatments or genders for vegetative biomass, a measure of plant size (Table 2.3). Contrary to findings by Pannell (1997a) there were no significant differences between genders, as well as between treatments, for male reproductive biomass. However, the plants grown in full sun produce significantly more fruits than those in the low light treatments (Fig. 2.5, Table 2.3). Reproductive allocation was observed to be relatively low compared to *M. annua* in other studies (Pannell per conversation, Pannell 1997a). This suggests that the plants may have been relatively early in their reproductive stage, which was further supported by the relatively few plants that had dispersed seeds. Relative male and female fitness was significantly correlated in both of the low light treatments, as well as the overall experiment, but not in the full sun plants (Table 2.4). The partial correlation between fitnesses including vegetative biomass, to test for trade-offs between male and female function in hermaphrodites, revealed no evidence for trade-offs between relative male and female fitness within treatments. For a given plant size, relative male fitness increased marginally with an increase in relative female fitness (Table 2.4). Males and hermaphrodites in all treatments were producing the same male reproductive biomass on average.

Leaf morphology also showed significant treatment differences. The size of the most recent fully expanded leaf, used for gas exchange measurements, differed significantly between all treatments- neutral shade plants had the largest and full sun the smallest on average (Fig. 2.6a). Specific leaf weight, a measure of weight relative to size, also showed significant treatment differences (Table 2.5). The mean specific leaf weight was highest in full sun and lowest under neutral shade (Fig. 2.6b). A higher specific leaf weight, commonly a thicker leaf for a given area, is typical of plants experiencing water stress, as well as phenotype for leaves in high light. The full sun treatment was observed to dry more quickly and require more frequent watering than the other treatments. This indicates that there may have been a potential for drought stress on the plants in the full sun treatment.

Gas exchange also responded to the light quality, as well as intensity. Significant treatment differences were found for photosynthetic rate and stomatal conductance (Table 2.5). Photosynthetic rates were highest in plants grown under high R:FR and lowest in full sun plants (Fig. 2.7a). There was also a marginally significant sex effect on photosynthetic rate, primarily in full sun, with hermaphrodites tending to have higher rates (Fig. 2.7a). Stomatal conductance differed significantly between all treatments- highest in plants under high R:FR and lowest in full sun (Fig. 2.7b, Table 2.5). Although photosynthetic rate and stomatal conductance were found to be significantly correlated in the treatments and genders, which was indicative of stomatal limitation on photosynthesis, there was only a marginal non-significant difference in water-use efficiency between plants under high R:FR and full sun (Table 2.5). However, hermaphrodites (0.045±0.002 μmole CO_2/mmole H_2O) had a marginally significant higher mean water-use efficiency than males (0.041±0.002 μmole CO_2/mmole H_2O).

PHENOTYPIC SELECTION ANALYSIS

Although the means for various morphological and physiological traits examined revealed only marginal differences between the genders, the correlations among traits showed significant sexual dimorphisms. The correlations between acquisition and allocation traits tended to be significant and positive in hermaphrodites while weak and non-significant in males. Males produced relatively constant reproductive biomass over a range of photosynthetic rates, while hermaphrodites show increased male biomass with higher rates (Fig. 2.8). This suggests there could be a potential difference in selection between the genders through male fitness on photosynthetic rates. The standardized selection differentials for photosynthetic rate, which are a sum of direct and indirect selection based on the correlation between the standardized trait and relative fitness, revealed that selection through male fitness tends to be weak and non-significant for males in all treatments (Table 2.6). However, photosynthetic rate is selected to be higher in hermaphrodites through both male and female fitness, except for plants in full sun (Table 2.6). Selection for increased height was significant only in hermaphrodites in the low light treatments, except for the significant selection through female fitness in full sun. With respect to stomatal conductance, selection was only significant in hermaphrodites under neutral shade. The trend for leaf size was similar, except for an additional significant positive selection through female fitness and significant negative selection in males in full sun.

In both the males and hermaphrodites, photosynthetic rate was positively correlated with height and vegetative biomass, but the relationship does not differ between the genders (Fig. 2.9a and 2.9b). However, males and hermaphrodites appear to differ in their reproductive strategies. In hermaphrodites, allocation to male reproductive biomass was significantly associated with vegetative biomass for the plants in the low light treatments; larger plants are producing more flowers (Fig. 2.10, Table 2.6). However, in males, allocation to reproduction and vegetative growth were decoupled, except for a marginally significant negative relationship in full sun (Fig. 2.10, Table 2.6). Males tended to produce the same reproductive biomass over a range of plant sizes. Further interpretations and inferences based on a path analysis of directional selection within the genders and treatments will be examined in the discussion section of this paper.

Discussion

This study examined how gas exchange traits differed between males and hermaphrodites and how vegetative and reproductive allocation was affected by the R:FR independent of density. Although there were significant treatment differences, the genders showed little difference in allocation patterns and physiology. Hermaphrodites and males showed similar elongation responses and leaf measurements. There was tendency for hermaphrodites to have a higher photosynthetic rate for a given stomatal conductance, primarily in the full sun treatment. This likely contributed to the marginally significant difference between the genders in water-use efficiency. Hermaphrodites were found to have slightly higher, but not significantly, water-use efficiency. Taking this finding into account in conjunction with the finding that the genders showed no difference in allocation to male reproductive biomass and vegetative biomass indicates that males appear to be more drought tolerant despite lower water-use efficiency. This result is consistent with studies of other dioecious species (Dawson and Bliss 1989, Gehring and Monson 1994, Marshall et al. 1993). Surprisingly, this was the extent to which the genders differed in the means for the traits examined. There was no evidence for sexual dimorphism in allocation and gas exchange and the responses associated with stem elongation.

Furthermore, there was no evidence for trade-offs between vegetative and reproductive allocation in the genders. Allocation to vegetative and male reproductive biomass did not differ between the genders contrary to expectations. With the additional female reproductive function, hermaphrodites were expected to have lower relative allocation to male biomass (Freeman et al. 1976, Pannell 1997a). Moreover, the significant positive correlation between male and female fitness in hermaphrodites shows a lack of trade-offs between reproductive functions. As previously stated, the plants appeared to be relatively early in their reproductive stage as suggested by the seemingly low reproductive allocation. This may have contributed to the apparent lack of trade-offs found in this study. However, potential trade-offs are suggested by other differences in allocation between treatments. Hermaphrodites under full sun received more light than plants in the low light treatments and produced more fruits, but did not increase allocation to male biomass. It should be noted that a more detailed examination of total lifetime fitness could potentially reveal significant trade-offs. Nevertheless, the lack of significant interactions of gender by treatment with many of the traits examined indicate that potential differences between the genders were not affected by the R:FR.

The significant treatment effects on the carbon acquisition traits did reveal that *M. annua* was sensitive to the R:FR. The responses in carbon acquisition traits were consistent with the findings of other studies, as well as for *B. rapa* in the previous chapter. Between the low light treatments, photosynthetic rates and stomatal conductance were lower in neutral shade, where the plants were aware of the presence of neighbours. The significant differences in leaf size and specific leaf weight suggest resources are diverted to larger, thinner leaves where neighbour proximity is signaled by reduced R:FR. It also appears that light quality was not the only factor to which the plants were responding.

Leaves of plants grown in relatively high light intensities are typically thicker for a given area and commonly have higher maximal photosynthetic rates than shade-adapted leaves (Boardman 1977). The lower photosynthetic rate and stomatal conductance coupled with the smaller leaf size and higher specific leaf weight, indicating smaller thicker leaves, suggests that the full sun plants may be responding to drought stress, as well as the higher light intensity (Farris 1984).

Light intensity appears to be the most important factor regulating elongation in these plants. Plants in the high R:FR and the neutral shade treatments were significantly elongated relative to the full sun plants. However, the plants in both of the low light treatments elongated to a similar extent. The lack of suppression of elongation and the patterns of elongation, in the high R:FR treatment compared to neutral shade, suggest that stem elongation in *M. annua* is regulated by a blue-light receptor rather than by strictly by phytochromes. Elongation in response to changes in light intensity are commonly cued by blue-light receptors (Casal 1989, Jenkins et al. 1995, Liscum and Hangarter 1994). Patterns of elongation in other species regulated by blue-light receptors are consistent with those seen in this study. However, more explicit tests of this hypothesis will be required to assess the role of blue-light receptors in *M. annua*.

Stem elongation may also be controlled to an extent by the R:FR in this species. Evidence for this can be seen in the differences in elongation patterns between the low light treatments. Plants under neutral shade have significantly longer hypocotyls and shorter first internodes than those under high R:FR. This result is similar to the findings of

Dudley and Schmitt (1996) of elongation in *Impatiens capensis* in response to R:FR manipulation. They found that the potential for continued elongation in latter internodes was reduced in continually elongating plants relative to plants that were induced to elongate after hypocotyl development. It was suggested that this may indicate an intrinsic cost of elongation in response to R:FR. The neutral shade plants in this study may have exhibited a similar response to the R:FR- a hypothesized adaptive early rapid elongation response to the presence of neighbours followed by reduced ability to elongate relative to the plants elongating in response to low light under high R:FR. However, the issue of whether or not *M. annua* is showing stem elongation in response to changes in the R:FR remains speculative. Other tests will need to be performed to assess the role of the R:FR on stem elongation in this species.

Although the plants in this study did not show significant differences in elongation and mean reproductive and vegetative allocation in response to the R:FR cue, the treatment differences for gas exchange, in conjunction with difference between the genders in the correlations between acquisition and allocation, raise interesting questions about selection in this species. The correlations between photosynthetic rate and allocation to vegetative and reproductive biomass showed significant sexual dimorphisms. For the traits examined, significant directional selection, as seen in the selection differentials, was found in hermaphrodites only. For the most part, selection was significant only in hermaphrodites for both male and female fitness. Selection in hermaphrodites also revealed a potential effect of the R:FR. Selection differentials indicated that selection was strongest under neutral shade. Furthermore, there were some differences between the low light treatments, particularly for male fitness in hermaphrodites. The lack of significant selection in males indicates that males are using a somewhat different reproductive strategy than hermaphrodites.

However, the selection differentials only provide limited insight into selective mechanisms. Selection differentials, the sum of all direct and indirect selection, indicate the amount that fitness is expected to change for a unit change in the given trait without controlling for other traits. Selection differentials represent the observed responses. To gain more insight into how selection was operating between traits, I performed a path analysis of selection. The method of selection analysis I used is based on making inferences from partial regressions and correlations between performance and fitness. Hence, there are a number of potential issues that must be addressed. First, I must acknowledge that unmeasured correlated characters may be the actual focus of selection (Arnold 1983, Lande and Arnold 1983). To reduce this problem the large number of traits examined incorporated the three categories of characters outlined by Farris and Lechowicz (1990) as required in such an analysis- i) reproductive output, ii) growth and architectural characters, iii) and physiological traits associated with resource acquisition. Furthermore, the analyses in this paper are based on well-known and understood functional relationships.

A further problem is that the total force of selection can only be assessed from lifetime fitness (Arnold 1983, Lande and Arnold 1983). In this study, I examined early fitness to imply how selection could operate. Early lifetime selection has also been assessed and has been found to be a significant determinant in total lifetime fitness in *Impatiens capensis* (Mitchell-Olds and Bergelson 1990b). Moreover, differences in early selection can be expected to result in dimorphic responses between genders. Geber (1991) found that early reproductive success in *Polygonum arenastrum* was largely determined by early reproductive allocation. Later success was strongly associated with plant size. Thus, early allocation in *M. annua* is of interest to examine, particularly for differences in selective forces between the genders.

PHENOTYPIC SELECTION ANALYSIS

To further explore direct selection on acquisition and allocation, I constructed a priori models based on understood functional relationships consistent with the result in this study. Then I analyzed the correlations and covariance between traits to refine the path model. The path analysis, a form of linear regression on standardized variables used to quantify causal links between correlated characters, revealed significant treatment and gender differences. Following the model through (Fig. 2.1), it can be seen that I have included the relationship between height and photosynthetic rate- taller plants will have higher photosynthetic rates. I hypothesize that plants with higher photosynthetic rates and bigger leaves will acquire more carbon (get bigger) and that the cost of water loss through stomatal conductance may reduce size. I further hypothesize that acquisition traits show indirect selection, as indicated by the double-headed arrows. And finally, the bigger plants become the more flowers they can produce. In effect, this path can be seen as the acquisition and allocation of carbon- with both acquisition and allocation important in determining selection and fitness.

An evaluation of the standardized directional selection gradients from the physiological and morphological traits on relative male and female fitness, as presented in the path analysis, revealed significant differences between the genders (Table 2.7a). Significant direct selection on height, photosynthetic rate, leaf size, and vegetative biomass was present in the hermaphrodites only. Selection on stomatal conductance was non-significant. Selection in the males for all traits was weak and non-significant. However, the selection gradients for relative male fitness differed significantly between the genders for all traits except stomatal conductance. This suggests that significant selection on acquisition was only occurring in hermaphrodites, as modeled in the path analysis. Males were following a different reproductive strategy that was not identified in this study.

Selection gradients for relative male fitness within the treatments, with pooling of the genders, revealed light quality and quantity also affected selection. Significant direct selection for increased height, photosynthetic rate, and vegetative biomass was present primarily in the neutral shade treatment (Table 2.7b). The gradients for height, photosynthetic rate, and vegetative biomass differed significantly between treatments. Although only significant for neutral shade, selection for increased height differed significantly between neutral shade and full sun. For photosynthetic rate, selection differed significantly between all treatments and was marginally significant for plants under high R:FR. Direct selection for increased vegetative biomass through fitness, significant for high R:FR, although stronger under neutral shade, also differed significantly between all treatments. The selection gradients for relative female fitness within treatments for hermaphrodites show a somewhat different trend than those of male fitness. Direct selection on height was significant in all treatments (Table 2.7c). For vegetative biomass, selection was significant in both of the low light treatments and marginally significant in full sun. Selection for a higher photosynthetic rate, significant under neutral shade and marginally significant under high R:FR, differed significantly only between neutral shade and full sun. To briefly summarize, the selection gradients indicate that selection, as modeled, was significant primarily for hermaphrodites under neutral shade, particularly through male fitness.

Analyses of covariance, to assess the significance of treatment and gender on selection, in the path model, revealed further differences between treatments and the genders (Table 2.8a-c). Path coefficients, which are based on regression coefficients, represent the indirect selection on a trait occurring through another trait as modeled. The regression of photosynthetic rate with height showed a significant effect of treatment (Table 2.8a). Thus, the path coefficients were distinct between treatments for indirect selection for increased height through its effects on photosynthetic rate (Fig. 2.11a-c). The magnitude and significance of the path coefficients indicate that selection for increased height through higher photosynthetic rates was relatively strong and significant in the low light treatments (Fig. 2.11a,b). Selection on height was weak and non-significant in full sun (Fig. 2.11c).

The regression of vegetative biomass with photosynthetic rate, stomatal conductance, and leaf size showed no significant treatment or gender differences (Table 2.8b). Thus selection on the carbon acquisition traits through their effects on allocation to plant size did not differ between treatments. Path coefficients for photosynthetic rate and leaf size were found to be significant and relatively strong overall (Fig. 2.11a-c). Selection on stomatal conductance was weak and non-significant. However, indirect selection between the acquisition traits differed between the genders within treatments. Photosynthetic rate was significantly correlated with stomatal conductance between males and hermaphrodites in both of the low light treatments, but did not differ significantly between the genders or these treatments (Fig. 2.11a,c). However, the correlation between photosynthetic rate and stomatal conductance in full sun was relatively strong and significant, but only in males (Fig. 2.11c). Also the indirect selection between photosynthetic rate with leaf size differed between treatments and genders. In plants grown under high R:FR, the correlation was significant and moderate in strength, again only in males (Fig. 2.11a). Under neutral shade, the correlation was significant in both genders, but stronger in males (Fig. 2.11b). However, in full sun the correlation tended to be negative in both genders, though non-significant (Fig. 2.11c). Indirect selection between stomatal conductance and leaf size was non-significant for all but hermaphrodites under high R:FR. For the hermaphrodites under high R:FR, there was a significant negative relationship between leaf size and stomatal conductance, indicating that selection favored reduced water loss with increased leaf size. This further supports the finding that hermaphrodites were more water-use efficient and suggests a potential selective mechanism for the difference.

The phenotypic selection analysis as modeled indicated that males and hermaphrodites are somewhat different in their acquisition strategies. Hermaphrodites showed relatively moderate to strong selection on allocation in the path analysis, whereas males showed little significant selection. Height was a strong predictor of photosynthetic rate in both of the low light treatments for both genders. For the acquisition traits, photosynthetic rate and leaf size contributed moderately to plant size in all treatments. Stomatal conductance did not affect fitness through plant size. Although the genders did not differ significantly in the relationship between photosynthetic rate and stomatal conductance, the indirect selection between these two traits and leaf size revealed significant differences between the genders and in response to the R:FR. Indirect selection between photosynthetic rate and leaf size was strongest for males, particularly in neutral shade. Although not significant, the moderately negative correlation selection in full sun is also stronger in males. This suggests that selection in males was acting predominantly at the level of acquisition. For both males and hermaphrodites, the relationship between photosynthetic rate and leaf size is strongest under neutral shade where they can detect the proximity of neighbours. This is consistent with functional adaptive hypotheses that predict leaf size and photosynthetic rate will be more responsive in competitive environments were light resources are limited.

Males and hermaphrodites also differ somewhat in allocation strategies. Regressions of relative male and female fitness with vegetative biomass revealed significant treatment and gender differences (Table 2.8c). The path coefficients for vegetative biomass to relative fitness indicate the magnitude and significance of direct selection on plant size. Selection for increased plant size through fitness was significant primarily in hermaphrodites under the low light treatments (Fig. 2.11a,b). For these plants selection was relatively moderate, except for the strong selection through male fitness in the neutral shade plants (Fig. 2.11b). Males in the low light treatments showed weak, non-significant selection. Selection in full sun was also weak, but marginally significant for hermaphrodites (Fig. 2.11c). However, for males in full sun selection on plant size was moderately negative, though only marginally significant. Plant size does not appear to affect fitness in males. However, increased plant size resulted in increased fitness in hermaphrodites under low light. This effect was more pronounced in plants under neutral shade, particularly for male fitness. This suggests that hermaphrodites were responding through plant size and male fitness to the presence of competitors, cued by the R:FR.

The responses in fitness of hermaphrodites to the low light treatments are somewhat consistent with the findings of Pannell (1997a). In response to reduced light intensity, hermaphrodites increased male reproductive output relative to female output. The relatively stronger selection of male fitness on plant size in the low light treatments reflects this. However, the increased effect under neutral shade is not consistent with the findings of Pannell (1997a) with respect to density. Increased density, which results in a lowered R:FR, was found to result in a shift to a more female bias in hermaphrodites. In this study, it appears that the response to lowered R:FR would result in a shift similar to the response to reduced light. Given that Pannell (1997a) looked at lifetime reproduction, the apparent discrepancies are not entirely unexpected. Moreover, the findings of this study are not inconsistent with functional adaptive hypotheses that predict hermaphrodites should increase male reproductive output in response to increased competition. Thus, the stronger selection to increase male fitness in hermaphrodites under neutral shade provides evidence for hypothesized adaptive responses. Furthermore, it appears that the R:FR ratio has a significant role regulating these responses in hermaphrodites.

Both light intensity and light quality appeared to play significant roles in the developmental and reproductive strategies in *M. annua*. Analyses of treatment means suggested that light intensity was the main factor affecting stem elongation and selection. The R:FR was found to affect carbon acquisition traits, however, there was no evidence for sexual dimorphism in these plastic responses. Furthermore, there was no conclusive evidence for trade-offs between reproductive function in hermaphrodites. However, the correlations between acquisition and allocation, raised interesting questions about selection. The correlations between photosynthetic rate and allocation to vegetative and reproductive biomass showed significant sexual dimorphisms. In these early reproducing *M. annua*, differences in selection are a consequence of males and hermaphrodites following different rules in their reproductive strategies. Selection in males appeared to be operating at the level of acquisition through indirect effects on fitness. Hermaphrodites were under strong direct selection through allocation. It is quite surprising that even with such a conservative path I was able to find such striking differences. Despite the apparent lack of sexual dimorphism, the differences I found in the functional relationships in allocation are suggestive of a mechanism by which sexual dimorphism in acquisition could evolve.

Table 2.1. Analysis of variance of treatment and sex on leaf size and stem characteristics. Significant treatment effects were found for leaf size, total height, and the lengths of the hypocotyl and first internodes. A significant effect of sex was found for the length of the first internode.

			Total Height		Length of hypocotyl		Length of first internode		Length of second internode	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	$MS_{Tray[T]}$	2	39.69	0.0001	126.14	0.0001	29.96	0.0001	22.58	0.0001
Sex (S)	MS_{error}	1	1.38	0.2417	1.35	0.2462	4.47	0.0358	0.66	0.4161
Tray[T]	MS_{error}	2	9.00	0.1000	269.52	0.0037	4.95	0.1680	3.02	0.2487
T × S	MS_{error}	2	1.48	0.2311	0.97	0.3800	0.93	0.3962	1.49	0.2272
Error		185								

Table 2.2. Analysis of covariance of treatment and sex on height with vegetative biomass as a covariate. The regression of height with biomass, a measure of elongation, revealed effects of both treatment and sex.

		Height		
Source	$MS_{denominator}$	df	F-ratio	p-value
Vegetative Biomass (B)	MS_{error}	1	265.6	0.0001
Treatment (T)	$MS_{Tray[T]}$	2	19.39	0.0001
B × T	MS_{error}	2	5.39	0.0054
Sex (S)	MS_{error}	1	0.04	0.8332
B × S	MS_{error}	1	3.88	0.0503
T × S	MS_{error}	2	2.08	0.1283
B × T × S	MS_{error}	2	1.43	0.2426
Tray[T]	MS_{error}	2	5.11	0.1637
Error		176		

Table 2.3. Analysis of variance of treatment and sex on vegetative and reproductive allocation. No significant effects of treatment or sex were found for vegetative biomass (plant size) and allocation to male reproductive biomass. A significant effect of treatment was found for number of fruits produced by hermaphrodites.

			Vegetative biomass		Male reproductive biomass		Number of fruits	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	$MS_{Tray[T]}$	2	0.70	0.4962	0.31	0.7340	7.83	0.0007
Sex (S)	MS_{error}	1	1.36	0.2442	1.22	0.2698		
Tray[T]	MS_{error}	2	1.13	0.4685	0.13	0.8866	102.34	0.0097
T × S	MS_{error}	2	0.88	0.4146	1.40	0.2494		
Error		187						
(fruits)		(94)						

Table 2.4. Correlation coefficients of relative male fitness with relative female fitness in hermaphrodites within the treatments. The partial correlation including vegetative biomass (plant size) is given to test for trade-offs between male and female function. Relative male and female fitness was significantly correlated in both of the low light treatments, as well as the overall experiment. The partial correlation revealed no evidence for trade-offs between male and female fitness within treatments. Significance levels are taken from Pearson correlation coefficients between male and female fitness.

(† p<0.10,* p<0.05, ** p<0.01, *** p<0.001)

Treatment	Correlation between fitness	Partial correlation including biomass
Copper Sulfate (high R:FR)	0.41**	0.28†
Neutral Shade (normal R:FR)	0.48**	0.16
Full sun (normal R:FR)	0.28	0.26
Pooled treatments	0.43***	0.25*

Table 2.5. Analysis of treatment and sex on gas exchange traits and specific leaf weight. Significant treatment effects were found for photosynthetic rate, stomatal conductance, and specific leaf weight. Marginally significant sex effects were seen in water-use efficiency.

			Photosynthetic rate		Stomatal conductance		Water-use efficiency		Specific leaf weight	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p	F-ratio	p	F-ratio	p
Treatment (T)	$MS_{Tray[T]}$	2	45.84	0.0001	28.76	0.0001	2.11	0.1245	113.83	0.0001
Sex (S)	MS_{error}	1	2.18	0.1415	0.41	0.5227	3.21	0.0747	0.00	0.9639
Tray[T]	MS_{error}	2	16.55	0.0570	3.58	0.2184	2.20	0.3130	334.50	0.0030
T × S	MS_{error}	2	2.36	0.0970	1.33	0.2681	1.48	0.2307	0.46	0.6347
Error		185								

Table 2.6. Selection differentials for relative male and female fitness through physiological and morphological variables for the genders within treatments. The selection differentials for the traits, as presented in the path analysis, revealed differences between treatments and genders within treatments. Significant selection of these traits on fitness was present primarily in hermaphrodites in the neutral shade. Significance levels are taken from the Pearson correlation coefficients between the trait and the fitness components.

(† p<0.10,* p<0.05, ** p<0.01, *** p<0.001)

Treatment	Copper Sulfate (high R:FR)			Neutral Shade (normal R:FR)			Full sun (normal R:FR)		
Sex	Male	Hermaphrodite		Male	Hermaphrodite		Male	Hermaphrodite	
relative fitness	male	male	female	male	male	female	male	male	female
Height	0.13	0.29†	0.41**	0.08	0.53***	0.53***	-0.29	0.08	0.47*
Photosynthetic rate	-0.06	0.41**	0.30†	0.20	0.61***	0.52***	-0.06	0.22	-0.34
Stomatal conductance	-0.04	0.04	0.02	0.26	0.40**	0.31*	0.35	0.18	-0.05
Leaf size	0.14	0.20	0.14	0.08	0.55***	0.33*	-0.46*	0.16	0.50*
Vegetative biomass	0.09	0.47**	0.39*	0.10	0.79***	0.50***	-0.40†	0.12	0.43†

Table 2.7a. Selection gradients on physiological and morphological traits through relative male and female fitness within genders with pooling of treatments. The gradients are taken from regression coefficients of the traits on relative fitness preserving the path analysis structure. Height and vegetative biomass were regressed individually, whereas photosynthetic rate, stomatal conductance, and leaf size were regressed simultaneously. Significant direct selection on height, photosynthetic rate, leaf size, and vegetative biomass was present in the hermaphrodites only. The selection gradients for relative male fitness differed significantly between the genders for all traits except stomatal conductance. Standard errors are given in parentheses. Significant differences between selection gradients between the genders for relative male fitness are shown in bold and p-values are given. († p<0.10,* p<0.05, ** p<0.01, *** p<0.001)

Trait	Hermaphrodite				Male		
	female fitness		male fitness		male fitness		p-value
Height	0.40***	(0.08)	**0.43*** **	(0.12)	**0.03**	(0.09)	0.0096
Photosynthetic rate	0.20*	(0.09)	**0.41*** **	(0.10)	**-0.02**	(0.14)	0.0114
Stomatal conductance	0.04	(0.11)	0.14	(0.14)	0.14	(0.10)	0.3076
Leaf size	0.16*	(0.08)	**0.31** **	(0.10)	**-0.01**	(0.11)	0.0443
Vegetative biomass	0.33***	(0.07)	**0.60*** **	(0.09)	**0.002**	(0.11)	0.0002

Table 2.7b. Selection gradients for relative male fitness on physiological and morphological traits within treatments with pooling of the genders. The gradients are taken from regression coefficients of the traits on relative fitness preserving the path analysis structure. Height and vegetative biomass were regressed individually, whereas photosynthetic rate, stomatal conductance, and leaf size were regressed simultaneously. Significant direct selection on height, photosynthetic rate, and vegetative biomass was present primarily in the neutral shade treatment. The gradients for height, photosynthetic rate, and vegetative biomass differed significantly between treatments. Standard errors are given in parentheses. Significant differences between selection gradients between treatments are shown in bold and p-values are given.

(† p<0.10,* p<0.05, ** p<0.01, *** p<0.001)

Trait	Selection gradients on relative male fitness						p-value
	Copper Sulfate (high R:FR)		Neutral Shade (normal R:FR)		Full sun (normal R:FR)		
Height	0.18	(0.11)	**0.36****	(0.13)	**-0.13**	(0.17)	0.0352
Photosynthetic rate	**0.21†**	(0.11)	**0.36***	(0.12)	0.04	(0.20)	0.3151
Stomatal conductance	-0.06	(0.13)	0.14	(0.14)	0.27	(0.20)	0.2640
Leaf size	0.11	(0.11)	0.15	(0.16)	-0.04	(0.17)	0.6211
Vegetative biomass	**0.29****	(0.11)	**0.63*****	(0.12)	**-0.07**	(0.17)	0.0034

Table 2.7c. Selection gradients for relative female fitness on the physiological and morphological traits within treatments. The gradients are taken from regression coefficients of the traits on relative fitness preserving the path analysis structure. Height and vegetative biomass were regressed individually, whereas photosynthetic rate, stomatal conductance, and leaf size were regressed simultaneously. Significant direct selection on height, photosynthetic rate, and vegetative biomass was present in the low light treatments. Gradients for photosynthetic rate differed significantly between treatments. Standard errors are given in parentheses. Significant differences between selection gradients between treatments are shown in bold and p-values are given.

(† p<0.10,* p<0.05, ** p<0.01, *** p<0.001)

Trait	Selection gradients on relative female fitness						
	Copper Sulfate (high R:FR)		Neutral Shade (normal R:FR)		Full sun (normal R:FR)		p-value
Height	0.43**	(0.15)	0.47***	(0.12)	0.24*	(0.11)	0.4746
Photosynthetic rate	**0.29†**	(0.13)	**0.37***	(0.16)	**-0.18**	(0.12)	0.0658
Stomatal conductance	-0.19	(0.26)	0.08	(0.18)	0.07	(0.11)	0.7213
Leaf size	0.04	(0.13)	0.11	(0.15)	0.27*	(0.12)	0.6157
Vegetative biomass	0.30*	(0.11)	0.43***	(0.12)	0.20†	(0.10)	0.4418

Table 2.8a. Analysis of covariance of treatment and sex on photosynthetic rate with height. The regression of photosynthetic rate, as presented in the path analysis, revealed a significant effect of treatment.

		Photosynthetic rate		
Source	$MS_{denominator}$	df	F-ratio	p
Height (H)	MS_{error}	1	52.23	0.0001
Treatment (T)	MS_{error}	2	0.03	0.9708
Sex (S)	MS_{error}	1	1.69	0.1955
H × T	MS_{error}	2	10.05	0.0001
H × S	MS_{error}	1	1.14	0.2876
H × T × S	MS_{error}	2	0.87	0.4212
Error		181		

Table 2.8b. Analysis of covariance of treatment and sex on vegetative biomass with photosynthetic rate, stomatal conductance, and leaf size. The regression of vegetative biomass, as presented in the path analysis, revealed significant effects of photosynthetic rate and leaf size. All sex and treatment interactions with these traits were non-significant.

		Vegetative biomass		
Source	$MS_{denominator}$	df	F-ratio	p
Photosynthetic rate (A)	MS_{error}	1	39.61	0.0001
Stomatal conductance (g)	MS_{error}	1	0.00	0.9539
Leaf size (L)	MS_{error}	1	92.85	0.0001
Treatment (T)	MS_{error}	2	0.00	0.9991
Sex (S)	MS_{error}	1	0.97	0.3259
A × T	MS_{error}	2	1.86	0.1583
A × S	MS_{error}	1	0.02	0.8922
A × T × S	MS_{error}	2	0.03	0.9744
g × T	MS_{error}	2	0.09	0.9171
g × S	MS_{error}	1	0.00	0.9907
g × T × S	MS_{error}	2	0.07	0.9314
L × T	MS_{error}	2	0.01	0.9878
L × S	MS_{error}	1	0.66	0.4172
L × T × S	MS_{error}	2	0.09	0.9150
Error		171		

Table 2.8c. Analysis of covariance of treatment and sex on relative male and female fitness with vegetative biomass. The regression of relative fitness, as presented in the path analysis, revealed a significant effect of vegetative biomass (plant size). The interactions of biomass with treatment and sex were also significant.

			Relative male fitness		Relative female fitness	
Source	$MS_{denominator}$	df	F-ratio	p	F-ratio	p
Vegetative biomass (B)	MS_{error}	1	8.71	0.0036	17.19	0.0001
Treatment (T)	MS_{error}	2	0.04	0.9561	0.07	0.9305
Sex (S)	MS_{error}	1	0.47	0.4949		
B × T	MS_{error}	2	5.88	0.0033	0.79	0.4575
B × S	MS_{error}	1	14.65	0.0002		
B × T × S	MS_{error}	2	0.99	0.3746		
Error		185				
(female fitness)		(93)				

Fig. 2.1. Path model of the hypothesized relationships between morphological and physiological traits and fitness. The straight, single-headed arrows between two traits indicate a causal relationship, while a curved, double-headed arrow reflects correlation without causation. Selection on height from fitness is modeled as an indirect effect through photosynthetic rate and vegetative biomass. Physiological carbon acquisition traits are hypothesized to be under indirect selection from fitness through vegetative biomass, as well as indirect selection between the traits. Selection from vegetative biomass on male and female fitness is modeled as a direct effect.

Path Analysis

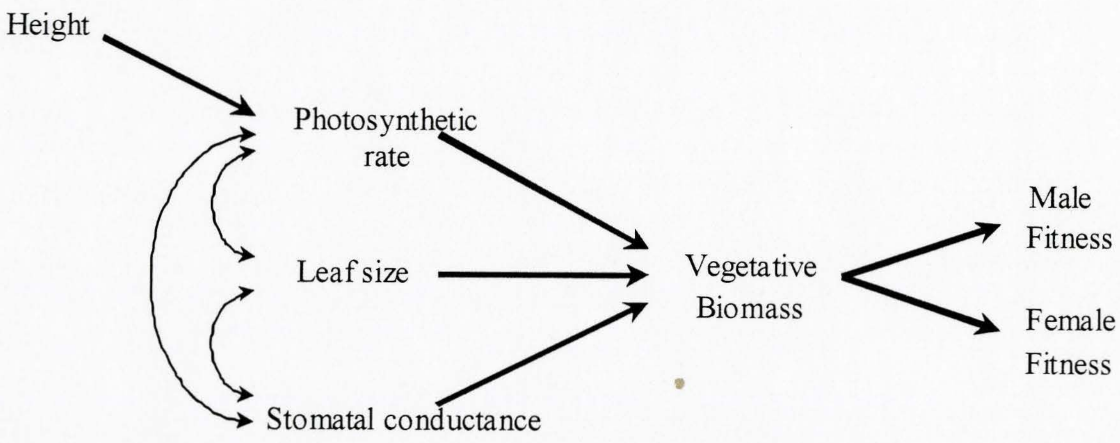


Fig. 2.2. Sex within treatment means for total height. The genders did not differ in total height within treatments. However, the plants in the low light treatments were significantly taller than the full sun plants for both genders. Bars represent ±1 S.E. The significance level shown is for the effect of treatment.

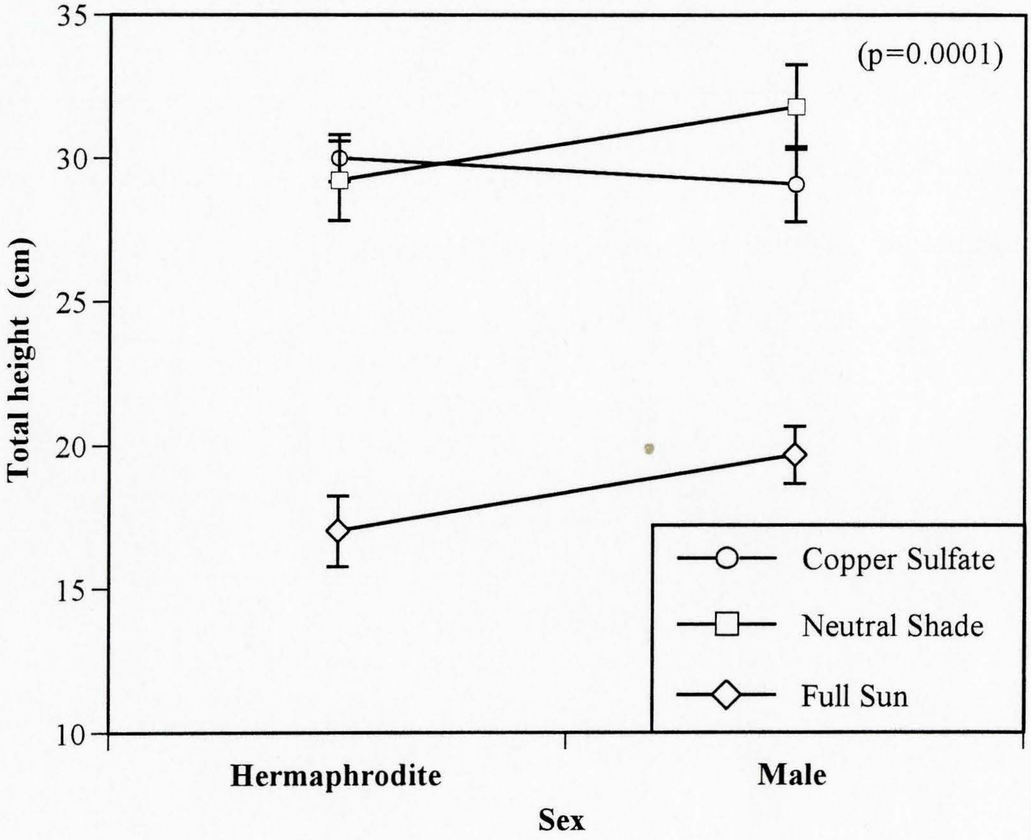

35
30
25
20
15
10
Total height (cm)
(p=0.0001)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

Fig. 2.3a. Sex within treatment means for length of hypocotyl. The genders did not differ in hypocotyl length within treatments. However, all treatments differed significantly for both genders. Bars represent ±1 S.E. The significance level shown is for the effect of treatment.

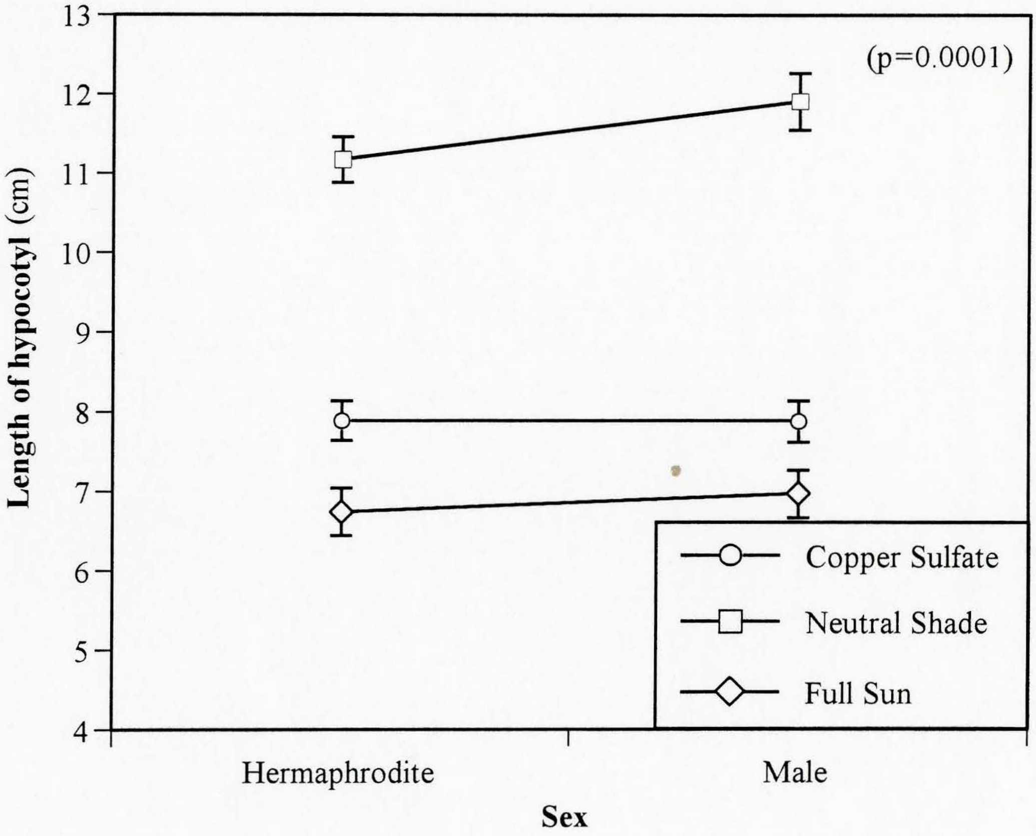

(p=0.0001)
Length of hypocotyl (cm)
13
12
11
10
9
8
7
6
5
4
Hermaphrodite
Male
Sex
Copper Sulfate
Neutral Shade
Full Sun

Fig. 2.3b. Sex within treatment means for length of the first internode. The genders differed significantly only within the full sun treatment. However, all treatments differed significantly for both genders. Bars represent ±1 S.E. The significance levels given are for the effect of treatment ($p=0.0001$) and the effect of sex ($p=0.04$).

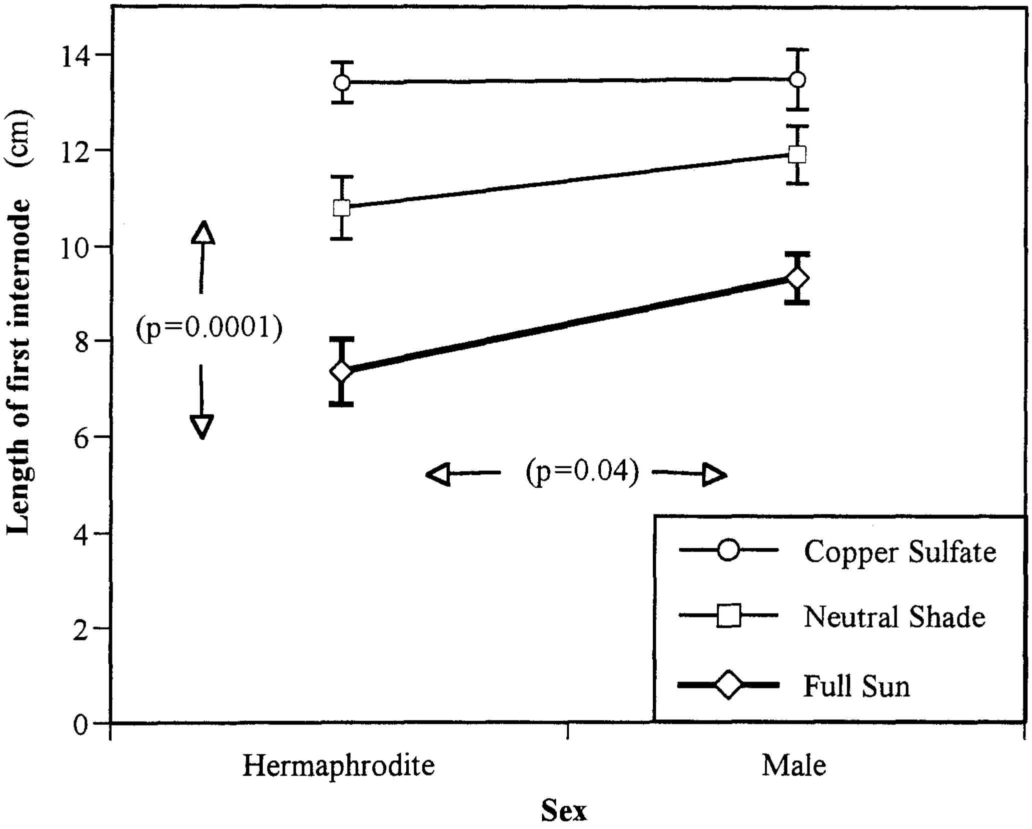

Length of first internode (cm)
0
2
4
6
8
10
12
14
(p=0.0001)
(p=0.04)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

Fig. 2.3c. Sex within treatment means for length of the second internode. The genders did not differ in second internode length within treatments. However, the plants in the low light treatments had significantly longer second internodes than the full sun plants for both genders. Bars represent ±1 S.E. The significance level shown is for the effect of treatment.

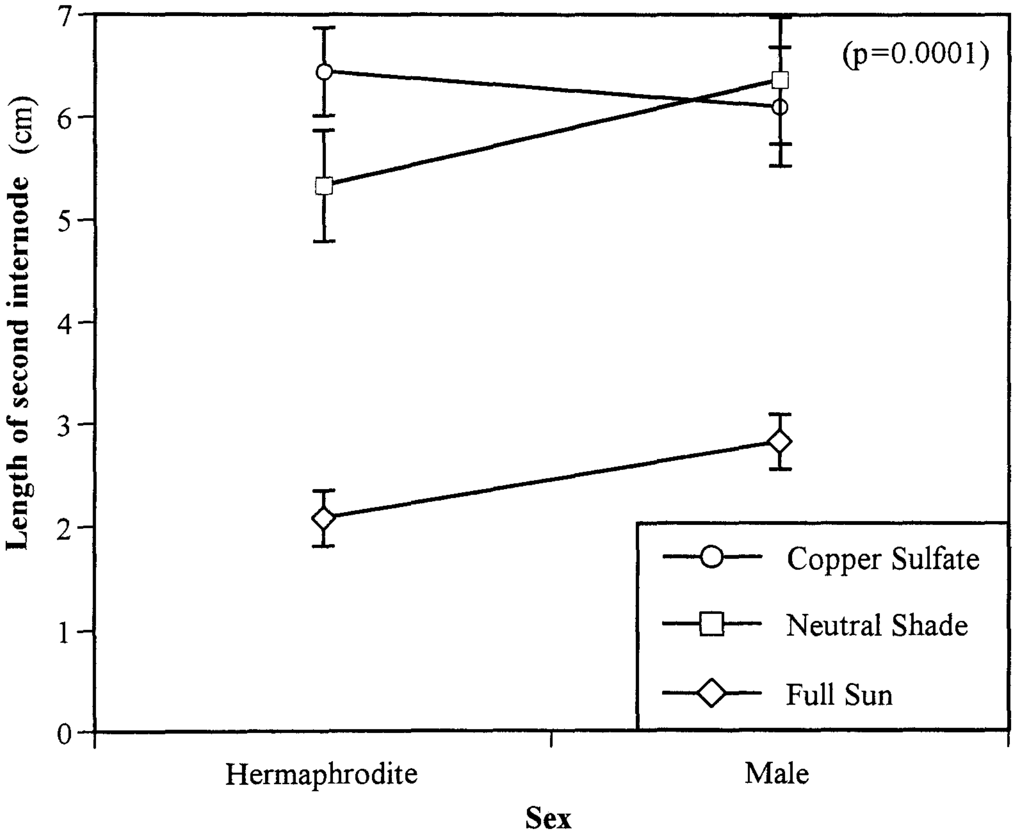

Length of second internode (cm)
0
1
2
3
4
5
6
7
(p=0.0001)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

Fig. 2.4a. Least squares means for height corrected for vegetative biomass, a measure of elongation, within treatment. The plants in the low light treatments were significantly more elongated from the full sun treatment. Bars represent ±1 S.E.

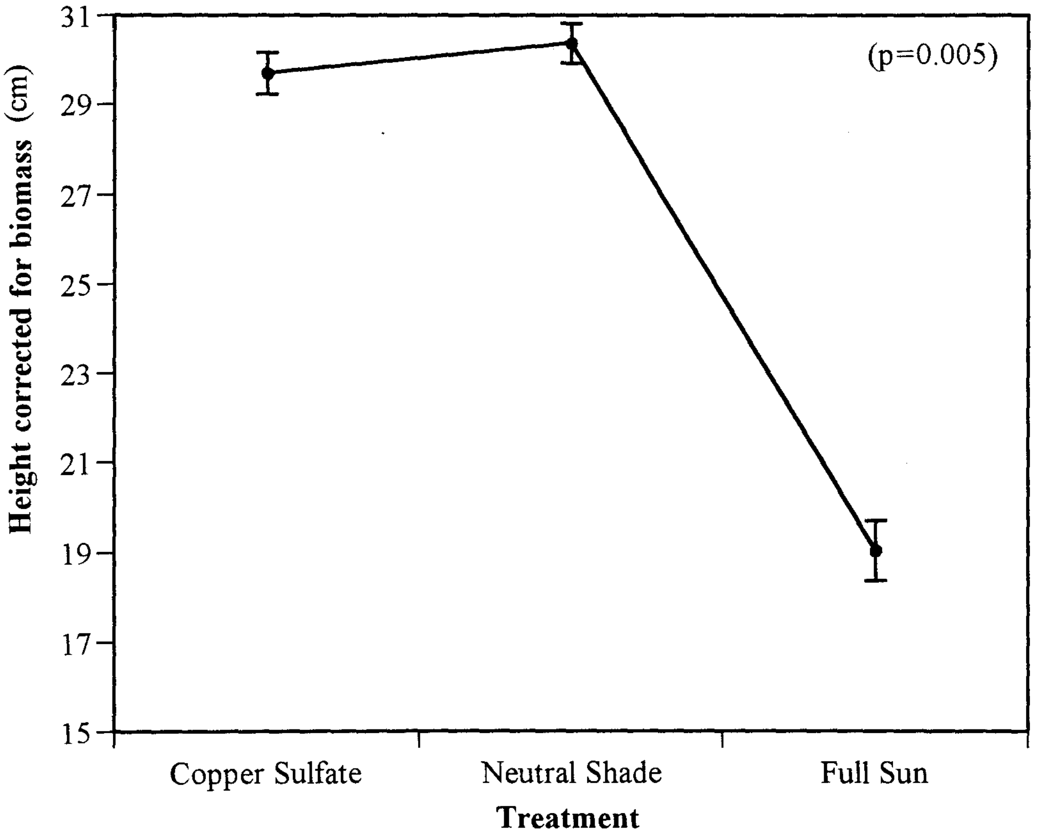

31
29
27
25
23
21
19
17
15
Height corrected for biomass (cm)
(p=0.005)
Copper Sulfate
Neutral Shade
Full Sun
Treatment

Fig. 2.4b. Least squares means for height corrected for vegetative biomass (elongation) between genders. Males were significantly more elongated than hermaphrodites in the overall experiment. Bars represent ±1 S.E.

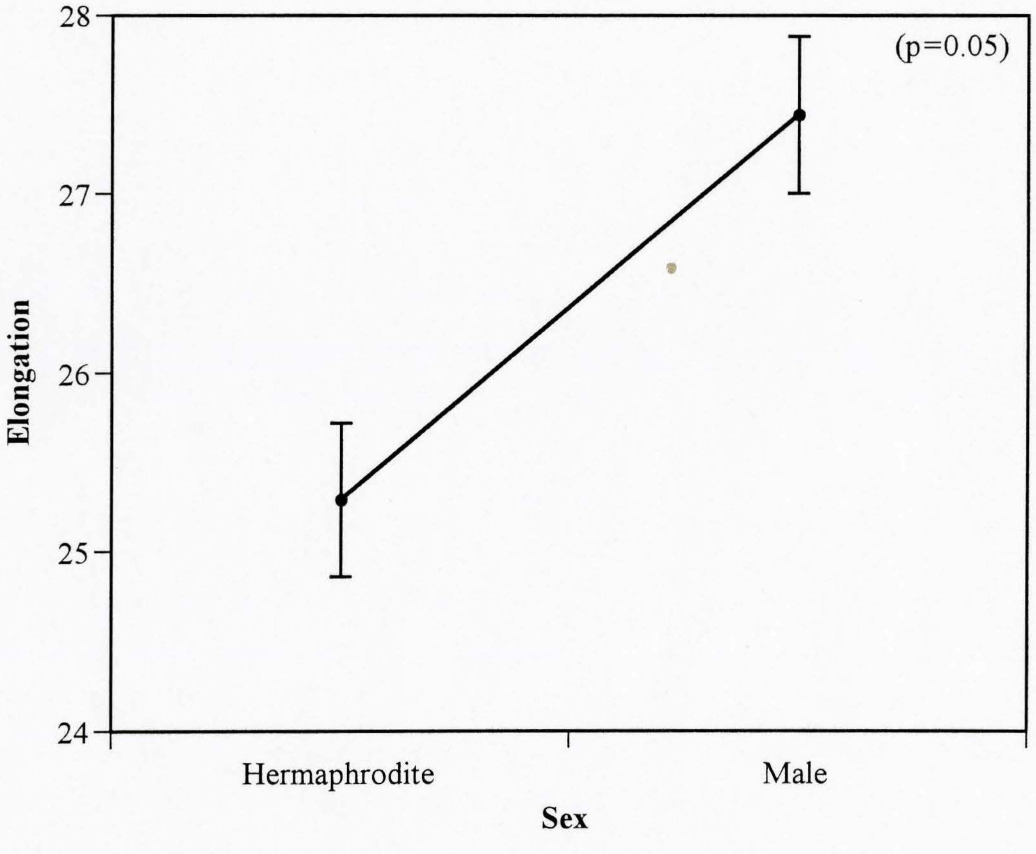
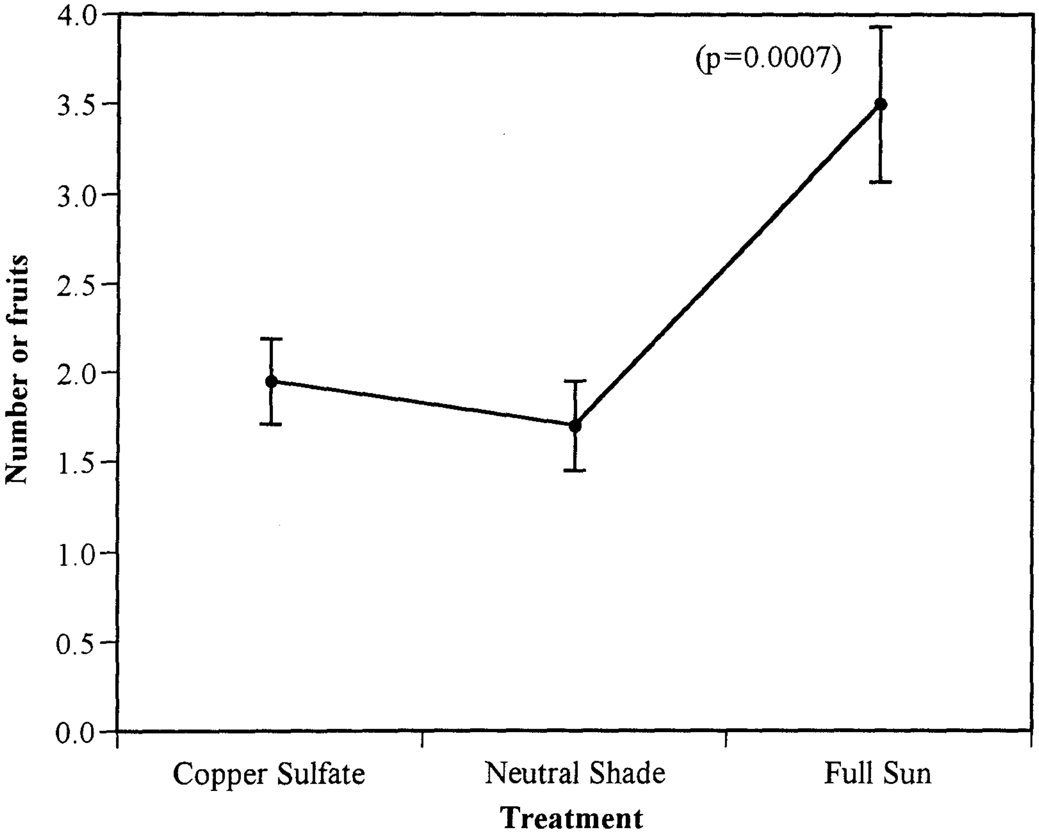
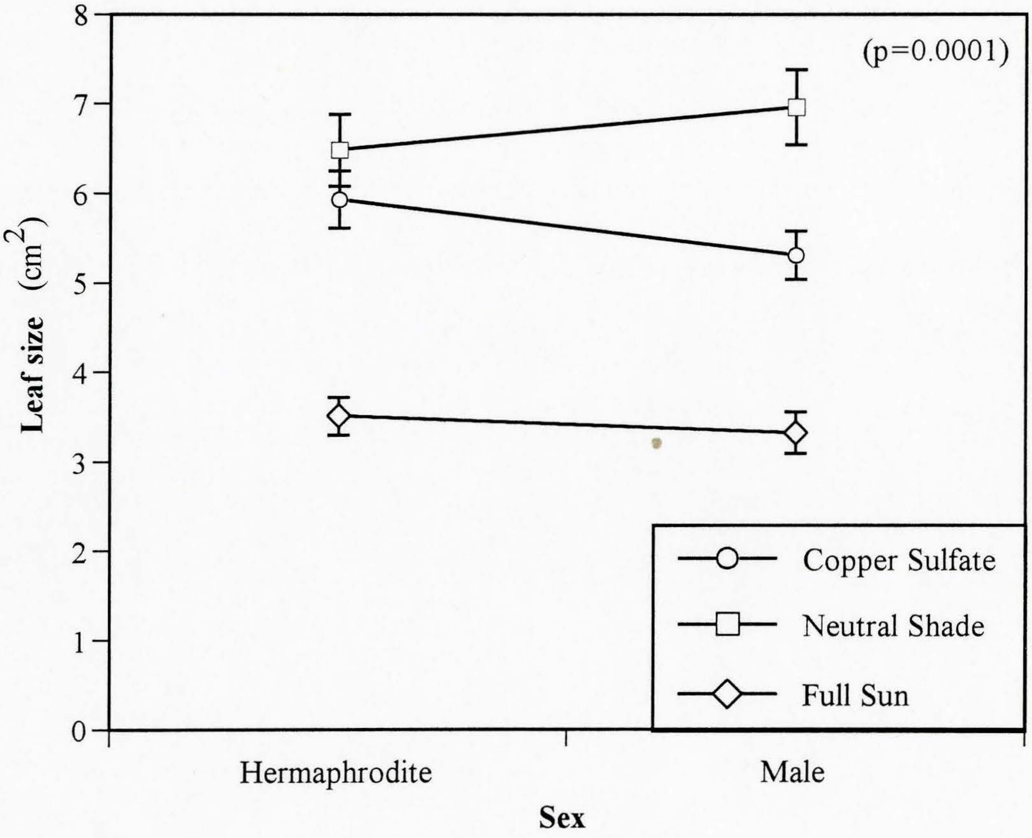

28
27
26
25
24
Elongation
(p=0.05)
Hermaphrodite
Male
Sex

Fig. 2.5. Treatment means for number of fruits produced by hermaphrodites. The plants in the full sun treatment produced significantly more fruits than plants in the low light treatments. Bars represent ±1 S.E.

Fig. 2.5. Treatment means for number of fruits produced by hermaphrodites. The plants in the full sun treatment produced significantly more fruits than plants in the low light treatments. Bars represent ±1 S.E.


4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Number or fruits
(p=0.0007)
Copper Sulfate
Neutral Shade
Full Sun
Treatment


8
7
6
5
4
3
2
1
0
Leaf size (cm^2)
(p=0.0001)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

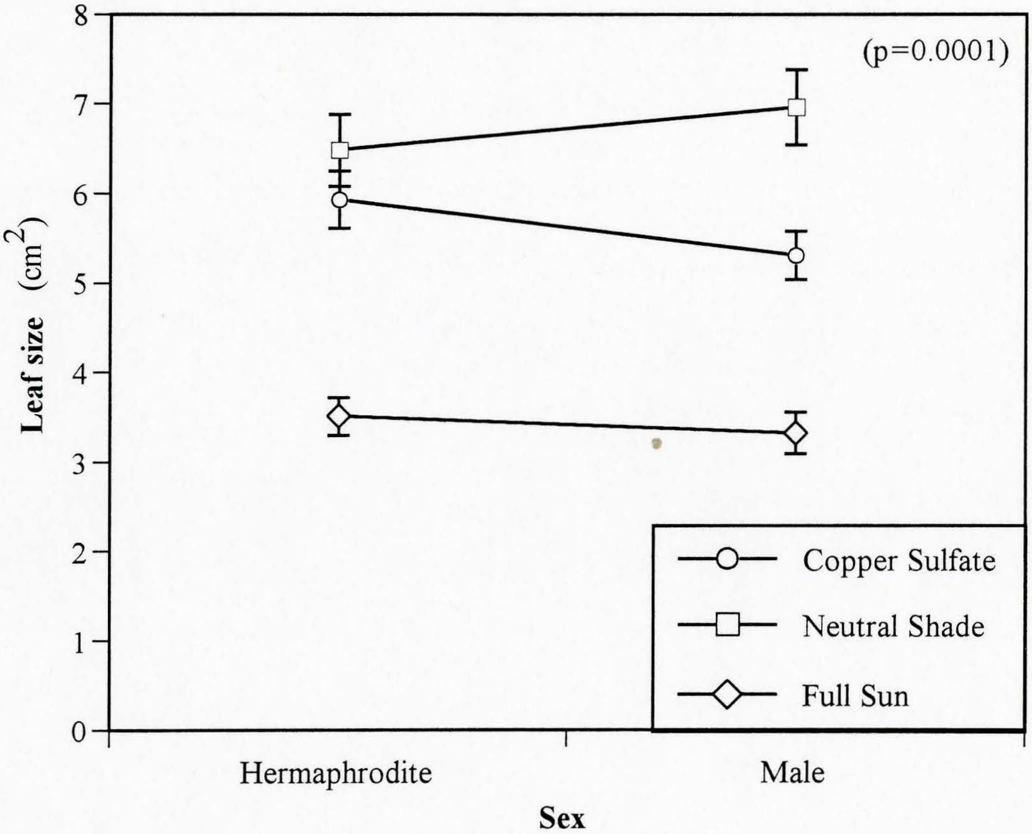

8
7
6
5
4
3
2
1
0
Leaf size (cm^2)
(p=0.0001)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

Fig. 2.6b. Sex within treatment means for specific leaf weight. The genders did not differ in specific leaf weight within treatments. However, the plants in the low light treatments had significantly higher specific leaf weights than the full sun plants for both genders. The significance level shown is for the effect of treatment. Bars represent ±1 S.E. and may be smaller than the data point.

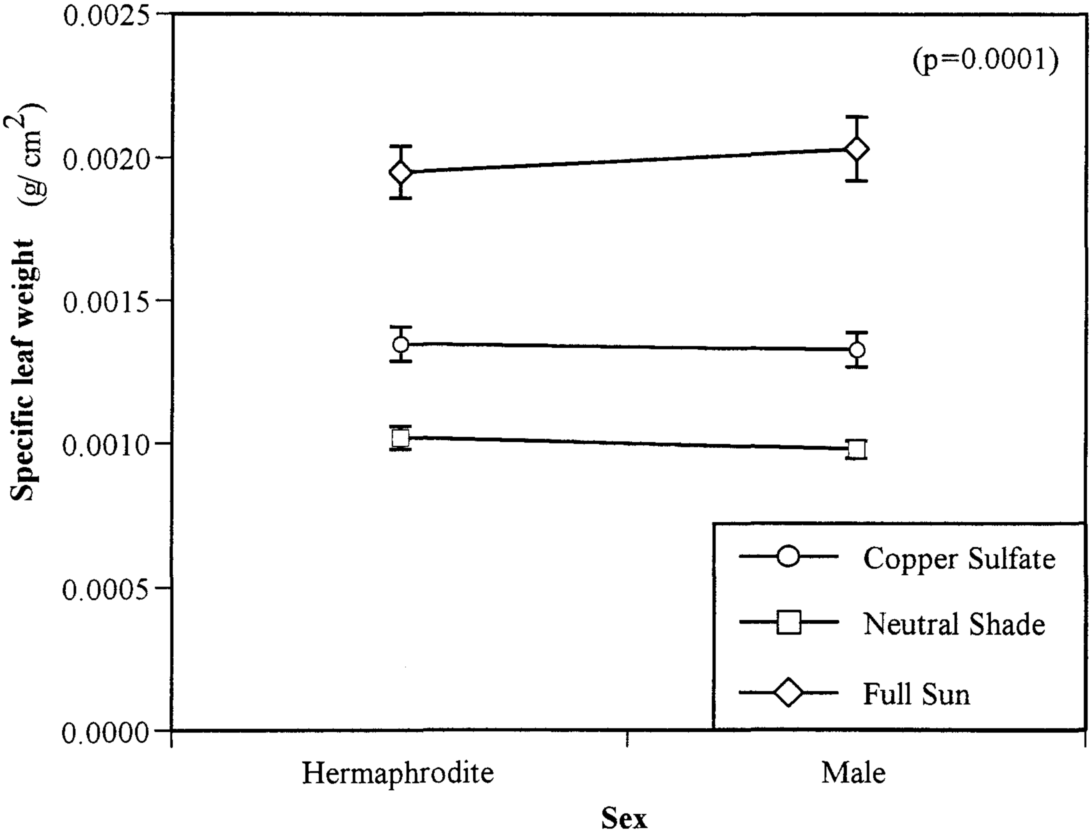

0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
Specific leaf weight (g/ cm^2)
(p=0.0001)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

Fig. 2.7a. Sex within treatment means for photosynthetic rate. The genders did differed significantly only within the full sun treatment. However, all treatments differed significantly for both genders. The significance levels given are for the effect of treatment (p=0.0001) and the effect of sex (p=0.01). Bars represent ±1 S.E.

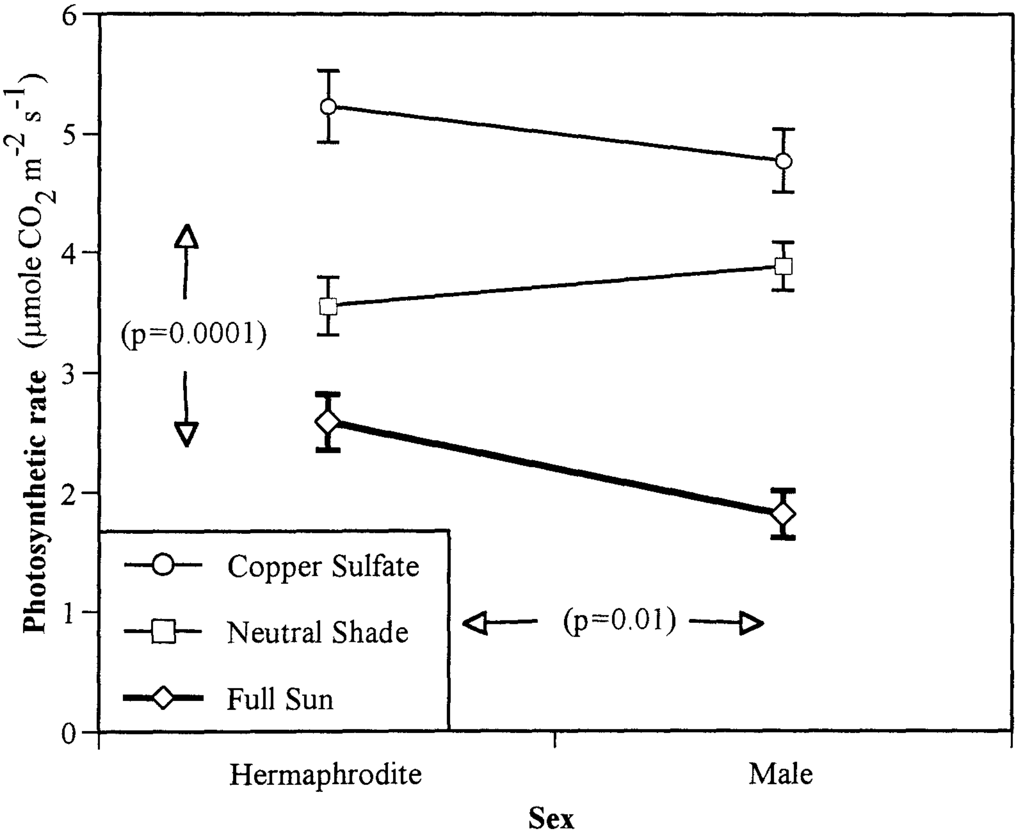

Photosynthetic rate (µmole CO2 m-2 s-1)
0
1
2
3
4
5
6
Hermaphrodite
Male
Sex
(p=0.0001)
(p=0.01)
Copper Sulfate
Neutral Shade
Full Sun

Fig. 2.7b. Sex within treatment means for stomatal conductance. The genders did not differ in stomatal conductance within treatments. However, all treatments differed significantly for both genders. The significance level shown is for the effect of treatment. Bars represent ±1 S.E.

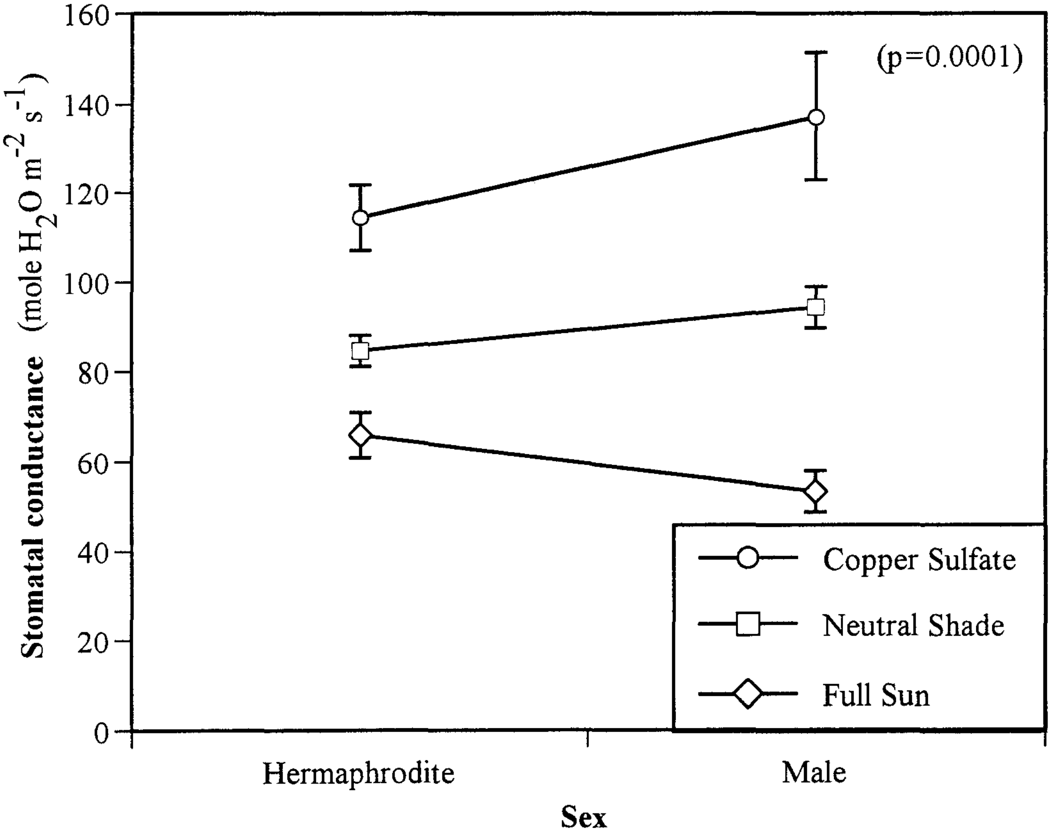

160
140
120
100
80
60
40
20
0
Stomatal conductance (mole H_2O m^{-2} s^{-1})
(p=0.0001)
Copper Sulfate
Neutral Shade
Full Sun
Hermaphrodite
Male
Sex

Fig. 2.8. Allocation to male reproductive biomass, g, versus photosynthetic rate, µmoles CO_2 m^{-2} s^{-1}, in the genders. Hermaphrodites showed a significantly stronger positive relationship than males. Significance level given is taken from an analysis of covariance for male reproductive biomass with photosynthetic rate. Line shown represents the slope of the regression.

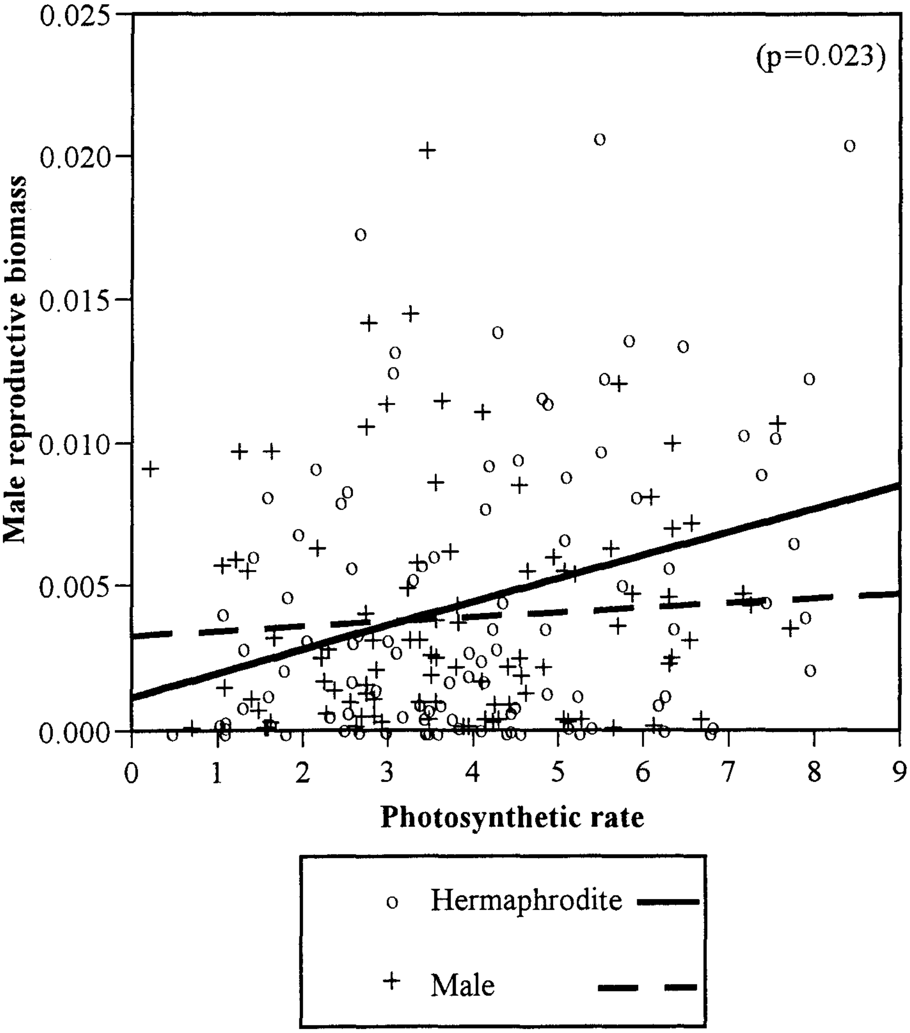

(p=0.023)
Male reproductive biomass
0.025
0.020
0.015
0.010
0.005
0.000
0
1
2
3
4
5
6
7
8
9
Photosynthetic rate
o Hermaphrodite
+ Male

Fig. 2.9a. Photosynthetic rate, μmoles CO_2 m^{-2} s^{-1}, versus total height, cm, in the genders. Photosynthetic rate and height were positively correlated in both genders. An analysis of covariance of photosynthetic rate with height revealed no significant difference between the genders. Line shown represents the slope of the regression.

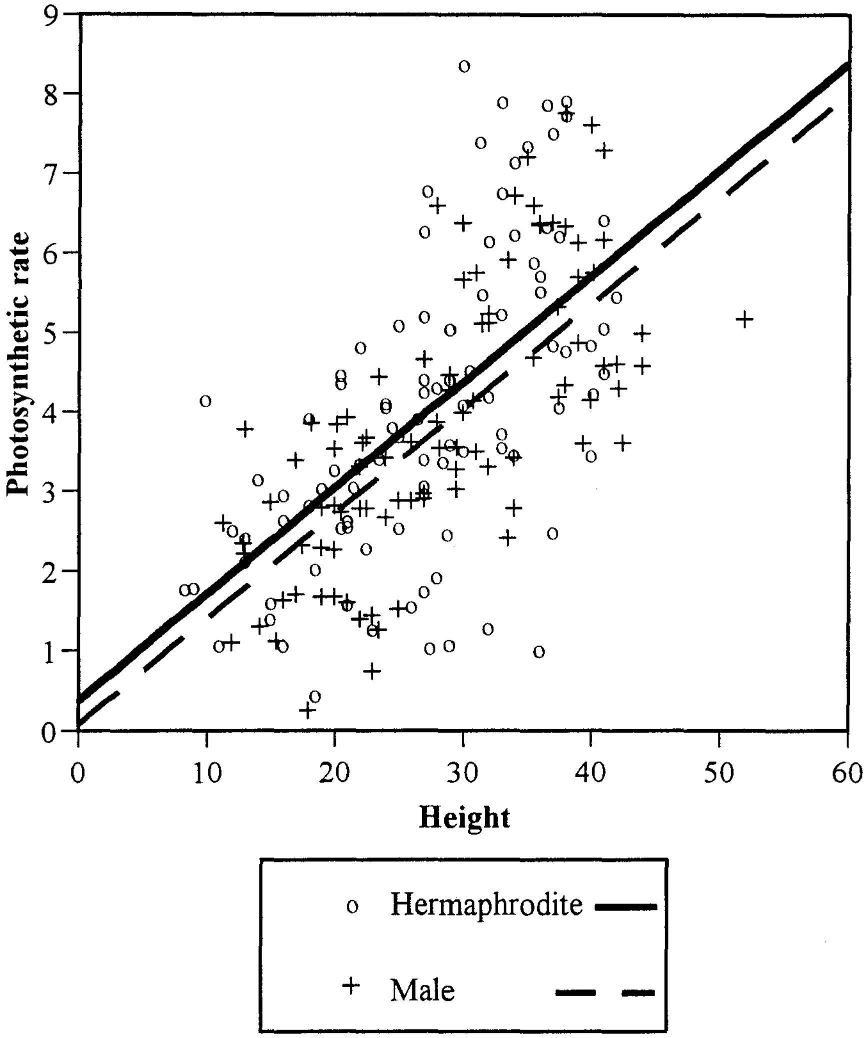

Photosynthetic rate
9
8
7
6
5
4
3
2
1
0
0
10
20
30
40
50
60
Height
o Hermaphrodite
+ Male

Fig. 2.9b. Photosynthetic rate, µmoles CO_2 m^{-2} s^{-1}, versus vegetative biomass, g, in the genders. Photosynthetic rate and vegetative biomass were positively correlated in both genders. An analysis of covariance of photosynthetic rate with vegetative biomass revealed no significant difference between the genders. Line shown represents the slope of the regression.

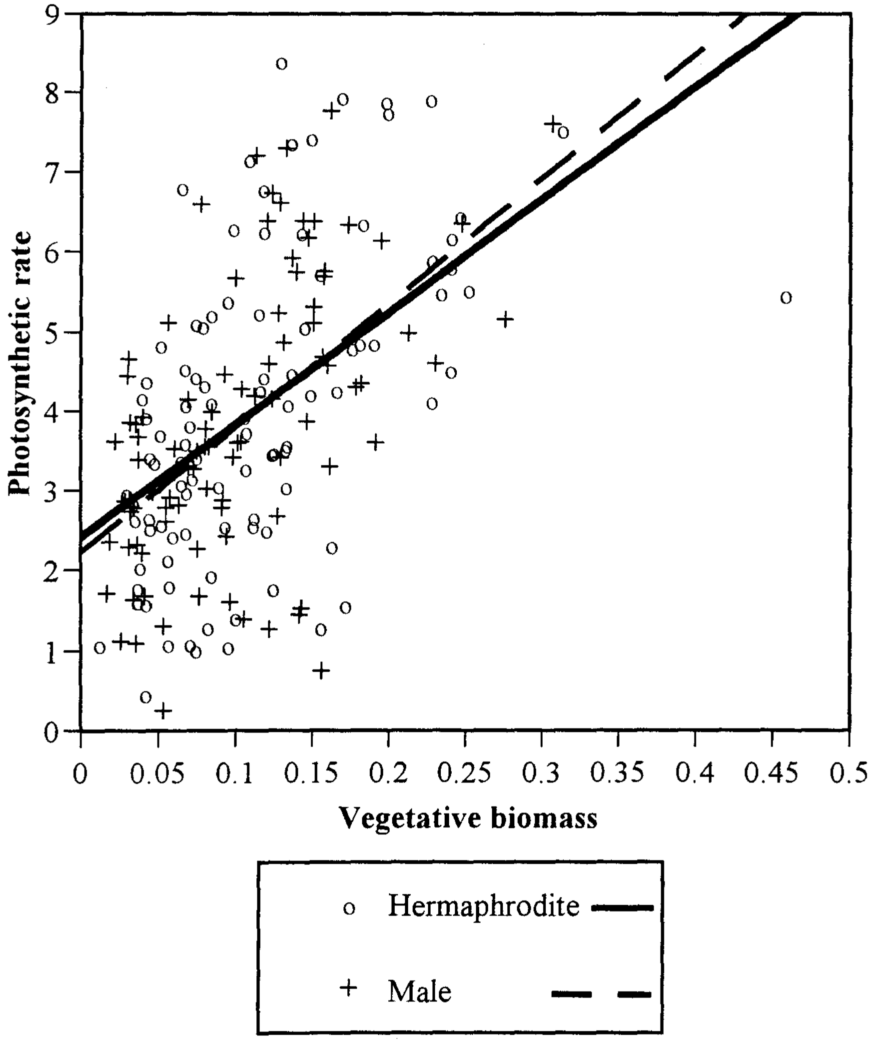

Photosynthetic rate
0
1
2
3
4
5
6
7
8
9
Vegetative biomass
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
o Hermaphrodite
+ Male

Fig. 2.10. Allocation to male reproductive biomass, g, versus vegetative biomass, g, in the genders. Hermaphrodites showed a significantly stronger positive relationship than males. Males tended to allocate relatively constant reproductive biomass over a range of plant sizes. An analysis of covariance for male reproductive biomass with vegetative biomass revealed a significant difference between the genders. Line shown represents the slope of the regression.

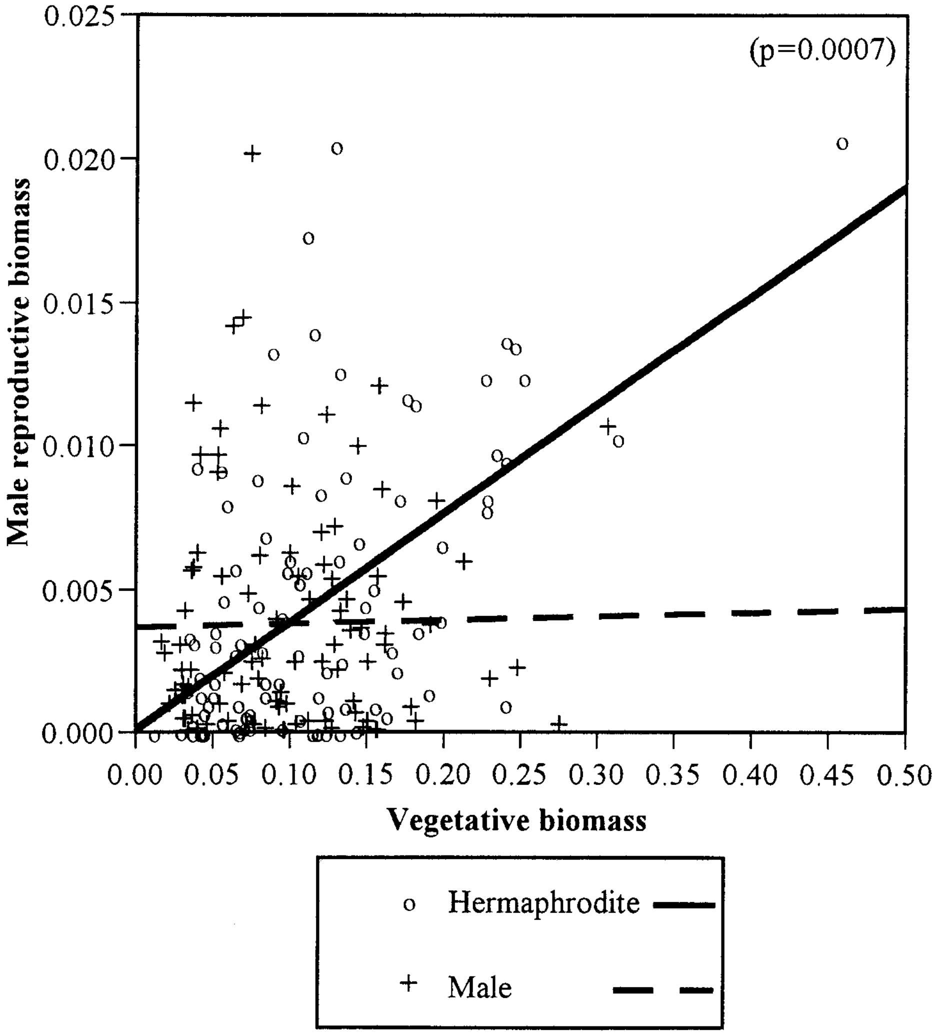
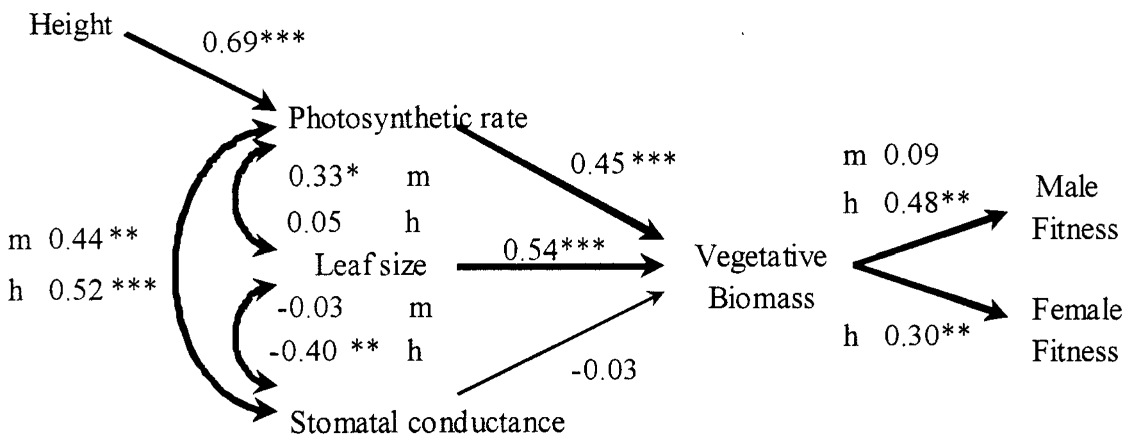

(p=0.0007)
Male reproductive biomass
0.025
0.020
0.015
0.010
0.005
0.000
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50
Vegetative biomass
o Hermaphrodite
+ Male

Fig. 2.11a. Path model of the hypothesized relationships between morphological and physiological traits and fitness in the high R:FR treatment (Copper Sulfate). Thick lines indicate a significant causal relationship between traits. Thick double-headed lines indicate significant correlation without causation between acquisition traits. Path coefficients are given for both males (m) and hermaphrodites (h) where the genders differ in the strength and/ or significance of selection, reflected in the value and significance level respectively. Significant paths are indicated by heavy lines.

(† $p<0.10$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$)

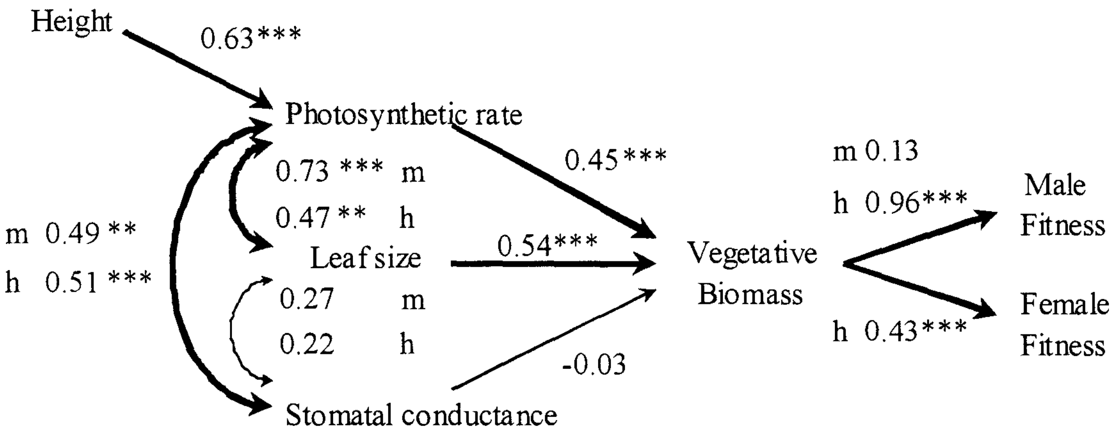


Copper Sulfate

Fig. 2.11b. Path model of the hypothesized relationships between morphological and physiological traits and fitness in the neutral shade treatment (Neutral Shade). Thick lines indicate a significant causal relationship between traits. Thick double-headed lines indicate significant correlation without causation between acquisition traits. Path coefficients are given for both males (m) and hermaphrodites (h) where the genders differ in the strength and/ or significance of selection, reflected in the value and significance level respectively. Significant paths are indicated by heavy lines.

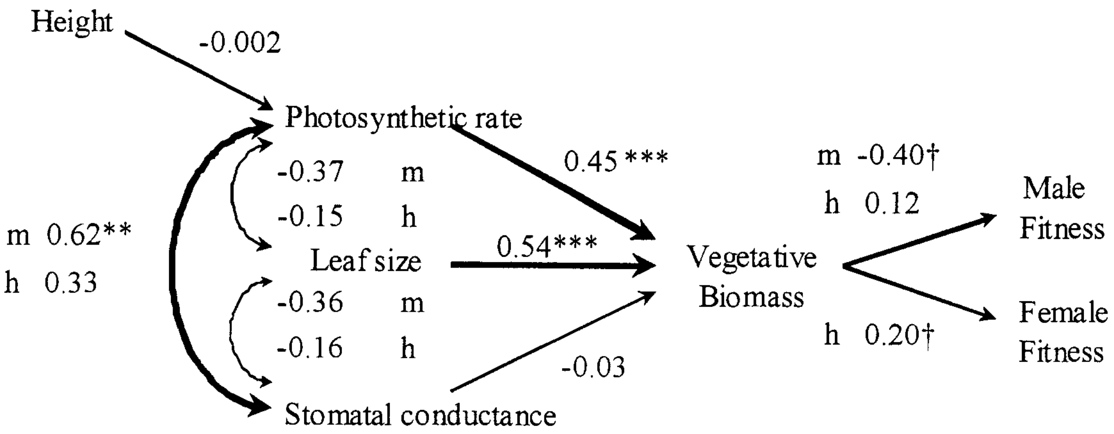
(† $p<0.10$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$)

Neutral Shade



2.11c. Path model of the hypothesized relationships between morphological and physiological traits and fitness in the full sun treatment (Full Sun). Thick lines indicate a significant causal relationship between traits. Thick double-headed lines indicate significant correlation without causation between acquisition traits. Path coefficients are given for both males (m) and hermaphrodites (h) where the genders differ in the strength and/ or significance of selection, reflected in the value and significance level respectively. Significant paths are indicated by heavy lines.

(† $p<0.10$, * $p<0.05$, ** $p<0.01$, *** $p<0.001$)



Full Sun

Height
-0.002
Photosynthetic rate
-0.37 m
-0.15 h
Leaf size
-0.36 m
-0.16 h
Stomatal conductance
m 0.62**
h 0.33
0.45 ***
0.54***
-0.03
Vegetative Biomass
m -0.40†
h 0.12
h 0.20†
Male Fitness
Female Fitness

Chapter IV

General Conclusion

Stem elongation is hypothesized to be an adaptive response to increase carbon acquisition under competitive conditions. Redistribution of an individual plant's biomass to place its leaves above those of neighbouring plants has been observed to confer a selective advantage in a number of species. Various other morphological traits have been shown to respond to the light environment associated with competitive conditions. However, the responses associated with stem elongation have also been found to carry intrinsic costs in less favourable environments. Maliakal *et al.* (in press) and Ballaré *et al.* (1991) suggested that gas exchange may also show plastic responses associated with stem elongation. In an attempt to test for intrinsic costs of elongation, Maliakal *et al.* (in press) found that elongated plants in high density had lower root biomass, photosynthetic rates, and stomatal conductance compared to non-elongated plants growing at high density in high R:FR light. These results suggest that the plastic responses in gas exchange traits in response to R:FR could be costly under water-limited conditions. However, Maliakal *et al.* (in press) were unable to show which environmental cues were inducing changes in gas exchange.

In rapid cycling *Brassica rapa* L., the treatment differences in stem elongation and the responses in carbon acquisition traits allowed for examination of the role of the R:FR. I was not able to explicitly test for the effects of density, because the plants did not elongate in response to density. The findings for the effects of water availability were also inconclusive. However, I was able to show that gas exchange does respond to changes in the R:FR. The response to low R:FR, which induced stem elongation, resulted in reduced biomass accumulation, lower photosynthetic rates, and lower water-use efficiency relative to plants under comparatively higher R:FR. I suggest that the responses in gas exchange to low R:FR indicate that there are potential costs associated with stem elongation. The costs associated with stem elongation appear to be strongly linked to plant water relations and are expected to be more significant in water limited environments.

The costs associated with stem elongation seen in *B. rapa* were not as evident in *M. annua*. Although stem elongation in *Mercurialis annua* L. *s.l.* appeared to be driven by light intensity rather than quality, the responses in carbon acquisition traits were more dependent on R:FR. The R:FR was found to affect gas exchange as seen in *B. rapa*. However, there was no conclusive evidence for trade-offs between reproductive and vegetative function or biomass allocation patterns as seen in *B. rapa*. Furthermore, indications of potential trade-offs did not appear to be influenced by R:FR. Also, there was no evidence for sexual dimorphism in these plastic responses. Gas exchange in the genders was responding in a likewise manner. The plastic responses, particularly in carbon acquisition, are somewhat suggestive of potential costs to R:FR induced responses as seen in *B. rapa*. However, the effect of R:FR on fitness and selection in *M. annua* is potentially of more consequence.

Both light intensity and light quality contributed significantly to the developmental and reproductive strategies of *M. annua*. The correlations between photosynthetic rate and allocation to vegetative and reproductive biomass showed significant sexual dimorphisms. Significant differences in selection through acquisition and allocation were found between the genders. The differences in selection were a consequence of males and hermaphrodites following different rules in their reproductive strategies. Hermaphrodites were under strong direct selection through allocation. However, selection in males appeared to be operating at the level of acquisition through correlational effects on fitness. The correlational relationship between acquisition traits appeared to be responsive to the R:FR. Indirect selection was strongest under neutral shade where plants could detect the proximity of neighbours. Direct selection through allocation in the hermaphrodites was also found to be responsive to R:FR. These findings suggest that R:FR could have a significant role in the development of sexual dimorphism through its effects on selection. However, the differences between the findings in this study and those of Pannell (1997a) suggest that total lifetime allocation and fitness may reveal different patterns in selection. The total force of selection can only be assessed from lifetime fitness, hence a more complete study will be required (Arnold 1983, Lande and Arnold 1983). Therefore, the role of R:FR in affecting selection through acquisition and allocation will need to be examined more thoroughly.

The variation in acquisition and allocation should also be explored in more detail. Positive correlations can result when the variation in acquisition is larger relative to the variation in allocation (van Noordwijk and de Jong 1986). It is possible that significant trade-offs in life history traits are masked by the relative variation in acquisition and allocation. For trade-offs between allocation to be clearly demonstrated variation in acquisition should be minimized. Van Noordwijk (1996) suggested that variation in acquisition could be reduced through increased average levels of acquisition to minimize differences in individual performance. The relatively low light intensity in both of these studies suggests that an increase in light availability could reveal the expected trade-offs. Closer examination of the relative variations could yield more insight and potential trade-offs in responses associated with R:FR may be revealed.

Although light quality has been demonstrated to elicit a number of morphological and physiological responses, further tests will be required to determine its role in relation to other environmental factors. In light of the blue light dependent findings for *M. annua*, as well as the density issues for *B. rapa*, more explicit tests of stand density should be performed. Stand density can affect light intensity and quality, water availability, humidity, and temperature (Jones 1992, Smith 1982). Photosynthesis and stomatal conductance have shown them to be responsive to environmental variability associated with differences in stand structure (Farquhar and Sharkey 1982, Gross 1986, Jones 1992). It is arguable that many of differences in stand structure may also have had a significant role in determining the gas exchange of individual plants. The issue of water availability will need stronger testing as well. Particularly in light of the costs hypothesized to be associated with stem elongation. However, the results of this study provides strong evidence for the role of R:FR in affecting carbon acquisition.

The extent to which other physiological traits and performance traits are affected by removal of far-red light is not known. The assumption that the effect of the copper sulfate filter is limited to suppressing stem elongation should also be tested. Because photosynthetic pigments effectively do not absorb far-red light, it is believe that removal of far-red light will have little effect other than on stem elongation. Comparison of plants grown at high and low density under copper sulfate filters should provide a reasonable test of this assumption.

In both *B. rapa* and *M. annua*, low R:FR resulted in reduced gas exchange, as was found in *Impatiens capensis*. Taken together these findings suggest that responses in carbon acquisition may be a general phenomena associated with density-dependent stem elongation. Studies on stem elongation and carbon acquisition in other species will be required to test this hypothesis. Although R:FR has been strongly implicated in this study, the question still remains whether the responses in gas exchange traits and morphology are elicited primarily by the R:FR cue or some other conditions associated with stand density. I suggest that the responses observed in this study are indicative of potential trade-offs between elongation, photosynthetic capacity, and plant water relations. The responses in leaf morphology and physiology to R:FR in this study are indicative of intrinsic costs of stem elongation. The shade avoidance hypothesis predicts that elongation is adaptive.

However, plants that are elongating could be doing so at the expense of allocation to leaves and the photosynthetic machinery in the leaves. Such trade-offs would be costly and potentially maladaptive in conditions where other aspects of the microenvironment within a plant stand do not serve to mitigate the limitations associated with the responses in carbon acquisition traits.

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