Vegetative and reproductive plasticity of broccoli at three levels of incident photosynthetically active radiation

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Abstract

To study the effects of shading on the growth, development, dry matter partitioning, and plant architecture of broccoli, 'Legacy' hybrid plants were grown in pots in a greenhouse under black shading meshes to generate different levels of photosynthetically active radiation (PAR). The average incident PAR was 23 mol PPF m^{-2} d^{-1} under control conditions, 15.2 under a 35% mesh, and 6.7 under a 70% mesh. The air temperature was within the range 15-22°C. As shading increased so did the duration of the growth cycle and the leaf area (LA). Shading did not affect the number of leaves, although the upper ones were more erect. The stem length and the accumulated intercepted PAR (IPAR) were negatively related. Inflorescence diameter and commercial fresh weight decreased only with the 70% mesh. Shading did not affect stem dry weight (DW) but altered dry matter allocation in the root and spear. The DW of the leaves maintained an average 45.7% of the total DW despite the greater LA developed under shade. The net assimilation rate diminished with the reduction of IPAR, and the LA increased; the plant relative growth rate was therefore practically constant. With increased shading, the leaves and the stem became the dominant photosynthate sinks. The commercial fresh weight achieved with 15.2 mol photosynthetic photon flux (PPF) m^{-2} d^{-1} was equivalent to that obtained with 23 mol PPF m^{-2} d^{-1}, but the cycle was extended for 4.5 days. With 6.7 mol PPF m^{-2} d^{-1}, yield diminished because of the lower DW produced in a cycle extended by 15 days, and because more dry matter was allocated to the stem than to the spear. Based on these results, broccoli could be considered a shade-tolerant plant.

Additional key words: *Brassica oleracea* var. *italica*, dry matter allocation, leaf area, shading, source-sink, yield.

Resumen

Plasticidad vegetativa y reproductiva del brócoli ante tres niveles de radiación fotosintéticamente activa incidente

Para determinar los efectos del sombreado sobre el crecimiento, el desarrollo, la partición de materia seca y la arquitectura de la planta de brócoli se cultivaron plantas del híbrido 'Legacy' en macetas, en un invernadero con mallas de sombreado para generar diferentes niveles de radiación fotosintéticamente activa (PAR). El promedio de PAR incidente fue 23 mol PPF m^{-2} día^{-1} (testigo), 15,2 (malla del 35%) y 6,7 (malla del 70%). Las temperaturas estuvieron dentro del rango 15-22°C. A mayor sombreado, aumentó la duración del ciclo y el área foliar (LA). El sombreado no afectó al número de hojas, pero las superiores adoptaron una posición más erecta. La longitud del tallo y la PAR interceptada acumulada (IPAR) se relacionaron negativamente. El diámetro de la inflorescencia y el peso fresco comercial sólo disminuyeron con la malla del 70%. El sombreado no afectó al peso seco (DW) del tallo, pero alteró la distribución de materia seca entre raíces y pella. Las hojas mantuvieron una proporción promedio del 45,7% del DW total, a pesar de la mayor LA desarrollada con el sombreado. Con la disminución de la IPAR, la tasa de asimilación neta disminuyó y la tasa de área foliar aumentó: este comportamiento compensatorio mantuvo la tasa de crecimiento relativo de toda la planta prácticamente constante. Con el incremento del sombreado, las hojas y el tallo fueron los destinos dominantes. Con 15,2 mol PPF m^{-2} día^{-1}, el peso comercial fue equivalente al obtenido con 23 mol PPF m^{-2} día^{-1}, pero el ciclo se extendió en 4,5 días. Con 6,7 mol PPF m^{-2} día^{-1}, el rendimiento disminuyó debido al menor DW producido en un ciclo 15 días más largo, y a que la planta alojó más materia seca en el tallo que en la pella. Estos resultados indican que puede considerarse al brócoli como una planta tolerante al sombreado.

Palabras clave adicionales: área foliar, *Brassica oleracea* var. *italica*, fuente-destino, rendimiento, reparto de materia seca, sombreado.

Introduction[1]

Solar radiation is a major environmental factor affecting the growth and development of broccoli *(Brassica oleracea* L. var. *italica* Plenck), which is generally considered to be a sunny-environment vegetable (Drost and Johnson, 2005; Andersen, 2006). In humid broccoli-producing regions where many cloudy days are expected, such as on the damp plains of Argentina, the radiation received by plants may be significantly lower than in dry and sunny regions. Broccoli is being increasingly used in intercropping systems, and shading may occur when it is planted with, for example, corn (Sanchez and Wade, 2006). Also, when broccoli is planted at high densities, shading by neighbour plants may affect growth and development (Francescangeli *et al.*, 2006). Recently, Schonhof *et al.* (2007) demonstrated that it was possible to increase the concentration of health-promoting compounds in broccoli by growing plants under conditions of low temperature and moderate mean daily radiation. It is therefore important to know the response of broccoli to low radiation levels in terms of growth, development and yield.

Gauss and Taylor (1969) and Marshall and Thompson (1987a,b) showed a positive correlation between daily mean radiation and spear growth. In five broccoli cultivars, Wurr *et al.* (1991) found that spear growth was best described when solar radiation values were included in degree-day models. Grevsen (1998) improved the adjustment of a model based on temperature to estimate spear diameter by including accumulated radiation during inflorescence growth. However, Pearson and Hadley (1988) found no effect of radiation on spear growth, although the effects of shading on plant morphogenesis has been demonstrated in several species (Blackman and Rutter, 1948; Grime and Jeffrey, 1965; Pearcy and Sims, 1994; Seigner *et al.*, 1994; Mourao and Hadley, 1998; Peil *et al.*, 2002). Broccoli plant responses to shading are reported to include a reduction in dry matter production, a retention of the photosynthate in the stem (at the expense of root growth), longer spear development, and bigger, thinner leaves (Wurr *et al.*, 1991; Mourao and Hadley, 1998). Maximum tolerance to shading is expressed as slow growth and a prolonged vegetative period (Grime, 1979). Few studies have related shading to broccoli growth and

development. Reduced irradiance due to shading is reported to result in reduced dry matter production and an increase in specific leaf area (Kläring, 1998; Mourao and Hadley, 1998). Increasing the leaf area ratio and specific leaf area are mechanisms for increasing the amount of intercepted radiation under conditions of shading. Other mechanisms, such as changes in plant architecture and dry matter partitioning might exist, but so far have not been reported in broccoli. Francescangeli *et al.* (2006) reported that the upper leaves of broccoli plants increase their erectness as a response to higher plant densities, suggesting this change in architecture may be due to shading by neighbour plants.

The aims of the present experiment were to study the effects of shading on growth, development, dry matter partitioning, and plant architecture in broccoli. These will increase the knowledge on the adaptability of broccoli to changes in radiation levels.

Material and Methods

Cropping system

Two experiments were performed during 2002 and 2003 at the INTA-San Pedro Agricultural Experiment Station (33°41'S, 59°41'W) in Buenos Aires, Argentina. Three-leaf plantlets of the broccoli hybrid Legacy, which has been evaluated for the region (Francescangeli *et al.*, 2003), were planted in 20-L black polyethylene pots filled with a 2:1 soil:sand (v:v) mixture (0.11% N, 0.68% C, 1.17% organic matter, pH = 6.3). The planting dates were August 23rd, 2002 and August 25th, 2003. The pots were placed in a greenhouse with 25% ventilation. Fertilizers were added as suggested by Dufault (1988).

To generate different levels of incident radiation (environments = treatments), two black polypropylene shading meshes, providing 70% (S70) and 35% (S35) shading, were randomly placed on 1/3 each of the greenhouse roof, leaving a control with no covering over the remaining third. Under field conditions, it has been shown that air temperature is not affected significantly by shading meshes, allowing for the independent quantification of radiation effects (Mourao and Hadley, 1998). Black meshes hardly modify the spectral balance

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[1] Abbreviations used: *DW (dry weight), IPAR (cumulative intercepted PAR), K (light extinction coefficient), LA (leaf area), LAI (leaf area index), LAR (leaf area rate), NAR (net assimilation rate), PAR (photosynthetically active radiation), PPF (photosynthetic photon flux), RGR (relative growth rate), RLER (relative leaf expansion rate), RUE (radiation use efficiency), SLA (specific leaf area).*

of transmitted radiation (Benavides Mendoza and Ramírez Rodríguez, 2002).

Plants were randomly allocated to the different PAR levels and placed at distances of 0.5×0.5 m (4 plants m^{-2}). One hundred plants (5 rows of 20 plants each) per PAR level were used. Eight plants for destructive measurements and 24 for commercial maturity measurements were randomly chosen from the two central rows on two occasions in 2002; in 2003, four plants were chosen for six destructive measurements every two weeks, and 16 for commercial maturity measurements. Additional plants were grown at the same level of incident PAR to replace those used in destructive measurements.

Cropping measurements

Growth and development records

Plant growth and development were recorded as indicated in Table 1.

The spear was considered to have initiated when the main apex of 10 randomly chosen plants was 1 mm in diameter (as determined through the opening of the upper leaves and using a hand-held 20-X magnifying glass).

The spear plus a 10 cm portion of the main stem was taken to represent the «commercial spear» (Baron *et al.*, 1996), while the main spear without the main stem portion was taken to represent the «botanical spear».

Commercial maturity was recorded in 10 plants chosen at random from each treatment. Leaf, stem, root, and spear dry weights (DW) were determined by drying at 75-80°C for 72 h. Leaf erectness was determined by measuring the angle between the main stem and the leaf petiole; this was measured in eight randomly chosen plants by recording the distance between the stem and the petiole 3 cm from the point of petiole insertion, and applying the sine theorem. These records were taken at 08:00 a.m. when the leaves were fully turgid.

Light and temperature records

Canopy air temperature was recorded with PT100 thermocouples. A dome-type sensor and a 1 m-long bar-type sensor were used to record global radiation (GR) above and below the canopy respectively. The average canopy air temperature beneath the shades (within the upper layers of the canopy for each shade condition), and the GR above the canopy for each shade condition, were determined hourly and the data stored using an ETG data logger (sensors and data logger manufactured by ETG, Florence, Italy). The below-canopy GR and the canopy-intercepted PAR, and its proportion with respect to the incoming PAR, were determined at 14-day intervals on sunny days between 11:00 and 13:00 a.m. (solar time) (Gallo and Daughtry, 1984). The bar GR sensor was placed transversally to the plant rows on top of two 1 m-long wooden supports placed along the rows at the height of the stem base. Four

Table 1. Variables recorded and frequency of their recording over the two years of the experimental period

Variable	Frequency in 2002	Frequency in 2003
Number of leaves	Weekly up to 56 dat[1], at spear initiation, and at harvest	At 14, 28, 42, 56, 70 and 84 dat, at spear initiation, and at harvest
Leaf area	At 14 and 56 dat, and at harvest	At 14, 28, 42, 56, 70 and 84 dat
Leaf, root, spear, and stem dry weight	At 14 and 56 dat, and at harvest	At 14, 28, 42, 56, 70 and 84 dat, at spear initiation, and at harvest
Stem length and plant height	At 14 and 56 dat, and at harvest	At 14, 28, 42, 56, 70 and 84 dat, and at harvest
Time of spear initiation	Daily, starting at 35 dat	Daily, starting at 35 dat
Angle between the stem and the 10th, 15th and 20th leaf	—	At 70 dat
Spear fresh weight, diameter, and spear qualitative characteristics.	At harvest	At harvest

[1] dat: days after transplant.

instantaneous GR values were recorded, placing the meter at intervals of 0.25 m along the wooden supports. These four data were paired to those collected for the above-canopy GR by the dome-type sensor. The four pairs were termed a «station»; four of these stations were randomly chosen for each of the three central rows at each PAR level. Radiation data were analysed with rows nested within PAR levels, and stations nested within rows. The accumulated intercepted PAR (IPAR) was calculated according to Westgate *et al.* (1997) and Maddonni and Otegui (1996), the radiation use efficiency (RUE) according to Westgate *et al.* (1997), and the light extinction coefficient (K) according to Olesen and Grevsen (1997).

GR data were converted to PAR data by multiplying by 0.50 (Szeicz, 1974; Krug, 1997).

PAR data, in $J\ m^{-2}\ s^{-1}$, were converted to moles of photosynthetic photon flux (PPF) by multiplying by $4.54\ E^{-6}$ (since the amount of energy carried by photons corresponding to PAR wavelengths of 400 to 700 nm averages $220,000\ J\ mol^{-1}$) (Nobel, 1983). Even though this might underestimate the interception of PAR, since the interception of light by a crop will reduce the fraction of PAR in the light, this effect was assumed to be small; the same coefficient was therefore used for above and below the canopy. The same criterion was used by Andrade *et al.* (1992).

Growth variables

The relative growth rate (RGR), the net assimilation rate (NAR), the leaf area ratio (LAR), the leaf area index (LAI), the specific leaf area (SLA), and the relative leaf expansion rate (RLER) were calculated according to Nobel (1983) and Turner (2003). LA was calculated using the formula developed by Stoppani *et al.* (2003): LA = 0.63 (leaf length × leaf width at the widest point). The logarithmic model proposed by Grevsen (1998) was used to relate spear growth with intercepted PAR.

Statistical analysis

Data were subjected to ANOVA ($\alpha = 0.05$). The following procedures of the SAS package (SAS Inst., 1989) were used: radiation data were analysed in a nested design using the proc Nest routine, with rows nested within PAR levels, and stations nested within rows; proc Corr was used to determine the correlation between the IPAR and the time required to complete growth; proc Reg was used to evaluate models that related IPAR to days until spear differentiation or days until commercial maturity, and that related IPAR to LER; and the NLIN procedure was used to determine the relationship between the relative leaf expansion rate (RLER) ($m^2\ m^{-2}\ d^{-1}$) and air temperature $> 20^{\circ}C$. The Tukey test for non-additivity was used to check normality. Non-normally distributed variables (leaf length and width; commercial spear and botanical spear DW) were transformed into their square roots, although back-transformed data are presented. The significance of the trend (linear or quadratic) of the different variables on PAR levels was estimated by orthogonal comparisons. Correlation coefficients between variables and PAR were calculated.

Temperature and radiation data

Temperature and radiation data were analysed in terms of 14-day periods. Mean temperatures ranged from 14.5° to $22.9^{\circ}C$. The different shade conditions caused no differences in air temperature in the upper strata of the canopy. For the same 14-day period each year, average temperature differences were very low; the greatest difference ($2.0^{\circ}C$) was seen in the 0-14 day after transplanting (dat) period. The daily average quantity of accumulated photons at the top of the canopy under the different shade conditions was 23 (control), 15.2 (S35) and 6.7 (S70) $mol\ PPF\ m^{-2}$.

Results

The PAR level affected IPAR, spear initiation, cycle duration, leaf erectness and area, stem length and height, spear diameter, the weight of florets, the DW of different organs, and growth variables. The treatments had no effects on leaf and floret number. The physiological disorder known as «hollow stem» was not detected.

Intercepted accumulated PAR

The IPAR for every fortnight (data not shown), and consequently throughout the cycle, decreased as shading increased (Table 2). At the end of the cycle, with a different duration for each treatment (Table 3), S35 accumulated 76.2% and S70 42.4% (averages 2002-2003) of the control IPAR.

Table 2. Accumulated intercepted PAR (IPAR, mol PPF m^{-2} or mol PPF plant^{-1}) under the three shading intensities over the growth cycles of 2002 and 2003

	2002			2003		
	0	**35**	**70**	**0**	**35**	**70**
At spear initiation	207.80	180.82	134.77	227.91	207.57	153.23
At harvest	886.08	671.54	371.85	954.00	732.10	409.69
Cycle average (area basis)	11.22	7.99	4.13	11.36	8.31	4.18
Daily average (plant basis)	2.80	2.00	1.03	2.84	2.08	1.05

Spear initiation and cycle duration

The time until spear initiation and maturity was longer with shading (Table 3). Using data from both seasons, a significant ($P < 0.0001$) correlation was found between the IPAR (mol PPF plant^{-1} d^{-1}) and the time required to complete the vegetative period ($r = -0.94$) and the cycle length ($r = -0.89$). The best models that related these variables were ($Pr < 0.0001$):

— Days until spear differentiation = $63.0080 - 7.36$ IPAR; $R^2 = 0.90$.

— Days until commercial maturity = $103.74 - 7.90$ IPAR; $R^2 = 0.80$.

Leaf number, leaf area and leaf expansion rate

Shading affected the dynamics of leaf initiation and loss. The number of leaves on plants in the shaded envi-ronments was smaller than in the control environment from 28 dat in S70 and 42 dat in S35 (data not shown). However, since leaf senescence was also delayed in the shady environments, the treatments did not affect the total number of leaves (34 leaves) or the number of leaves at harvest (27 leaves). At spear differentiation, plants in all environments showed abscission of the first leaf. The increase in LA at the end of the cycle was higher from 0 to 35% shading than from 35 to 70% shading (Table 4).

In 2003, a greater number of observations (Table 1) allowed the definition of the moment of maximum LA and a description of LA variation over a 14 day period (Fig. 1).

After a maximum LA at 56 dat, leaf loss in the control treatment was greater than leaf expansion, and a pro-gressive reduction in LA until harvest time was observed. In the S35 treatment, due to a rapid expansion between 42 and 56 dat, and slower expansion from 56 dat, plants reached the maximum LA on 70 dat. This LA was

Table 3. Spear initiation, duration of gowth cycle, steam and spear traits of plants grown at the three shading intensities

Year	Shading (%)	Days after transplant		Stem length (cm)	Plant height at the end of the cycle (cm)	Stem diameter at 10 cm from point of spear insertion (mm)	Diameter (cm)	Number of florets	Weight of commercial spear (g)	Weight of botanical spear (g)
		Up to spear initiation	Up to commercial maturity							
2002	0	40	79	28.1	34.8	34.0	12.4	42.5	291.4	125.0
	35	46	84	33.1	40.0	33.6	13.0	41.0	318.7	134.7
	70	55	94	37.6	43.8	32.2	10.1	41.2	207.8	103.5
	CV	2.2	1.5	7.3	9.3	2.8	10.4	5.7	9.9	11.1
	Pr > F[1]	Q < 0.0001	Q < 0.0001	L < 0.0001	L < 0.0001	L = 0.0023	Q < 0.0001	—	Q = 0.0206	Q = 0.0036
2003	0	43	84	26.5	32.7	34.1	11.8	43.0	263.7	119.9
	35	50	88	32.0	39.7	33.7	12.0	42.6	274.2	130.1
	70	56	99	36.5	42.5	32.4	9.1	41.5	198.1	97.6
	CV	1.9	2.3	6.9	7.7	3.0	13.0	8.3	9.5	7.3
	Pr > F[1]	Q < 0.0001	Q < 0.0001	L < 0.0001	L < 0.0001	L = 0.0074	Q = 0.0027	—	Q = 0.0071	Q = 0.0060

[1] Probability > F of the significant trends. L: linear. Q: quadratic.

Table 4. Leaf area at the end of the cycle (LAf), leaf area index at the end of the cycle (LAIf), maximum LA (LAmax), and maximum LAI (LAImax) of broccoli plants grown at the three shading intensities over the two experimental seasons, and the angle (radians) between the stem and petiole of selected leaves representing three canopy strata at 70 dat under the three shading conditions in 2003

Year	% shading	LA$_f$ (m^2 pl^{-1})	LAI$_f$	LAImax[a] (m^2 pl^{-1})	LAImax[a]	Angle stem-petiole of leaves		
						10°	15°	20°
2002	0	0.3195	1.27	—	—	—	—	—
	35	0.4271	1.70	—	—	—	—	—
	70	0.4625	1.85	—	—	—	—	—
	CV	7.0		—		—	—	—
	P > F[b]	Q = 0.0012		—		—	—	—
2003	0	0.3046	1.22	0.3725	1.49	90° 01′ 12′′	79° 04′ 55′′	64° 20′06′′
	35	0.4183	1.67	0.4196	1.68	65° 56′ 00′′	63° 46′12′′	58° 40′05′′
	70	0.4574	1.83	0.4574	1.83	60° 30′36′′	55° 17′12′′	50° 45′04′′
	CV	4.3		5.2		6.7	9.0	4.1
	P > F[b]	Q = 0.0115		Q < 0.0001		Q = 0.0003	L < 0.0001	L < 0.0001

[a] LA and LAI max were reached at different times depending on the percentage shading (see text). [b] Probability > F of the significant trends. L: linear. Q: quadratic.

maintained with little variation until the end of the cycle since leaf expansion compensated for leaf senescence. In the S70 treatment, leaf expansion was greater than leaf loss, and plants showed the maximum LA at commercial maturity.

Throughout the growth cycle, leaf size (leaves 17 to 32 in 2002 and leaves 18 to 31 in 2003) appeared as the main reason for differences in LA, with an increase of the leaf length and width as the quantity of intercepted PAR diminished (data not shown).

IPAR increased with LA development, reaching its highest point at the time of maximum LA (Fig. 2); the loss of the lower leaves had little impact on the IPAR (it was a little greater in the control than in the shaded environments). Differences between the highest interception percentages were not related to differences between maximum LAs. In both seasons, the maximum

percentage of canopy light interception reached by the control was 63%; this was 74% in the shaded environments. In 2003, even though the S70 treatment had an average maximum LA of 0.0378 m^2 plant^{-1} higher than that of S35, the maximum percentage of light interception of S70 was no higher than that of S35.

No significant relationship was seen between the daily LER and IPAR for the whole cycle in 2003, suggesting that radiation was not the only factor affecting leaf expansion. However, when two-weekly periods were examined separately, a linear relationship was seen between the daily LER and IPAR. This was positive up to 56 dat, with a change of direction from that moment on (Fig. 3).

The relationship between the RLER and air temperatures from 56 dat for the three environments was negative and quadratic (Fig. 4).
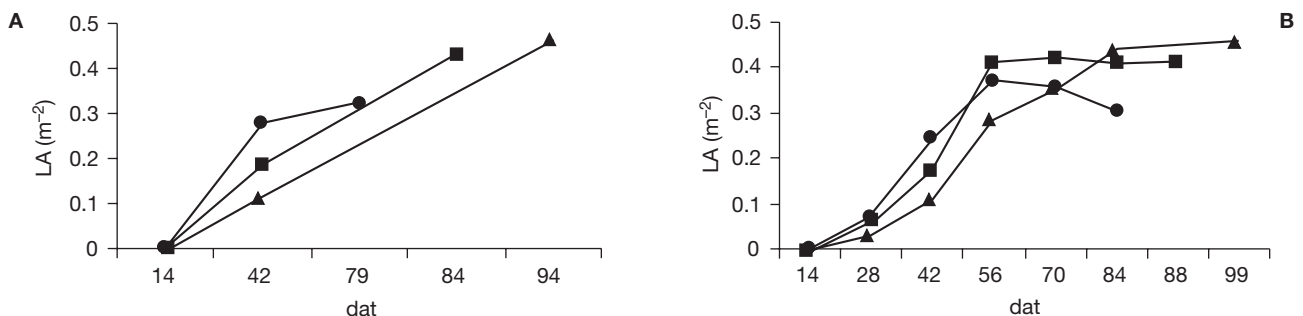

Figure 1. Change in LA (m^2 plant^{-1}) in broccoli plants over the growth cycle under the three shading intensities (● 0%; ■ 35%; ▲ 70%) in (A) 2002 and (B) 2003. dat: days after transplant.
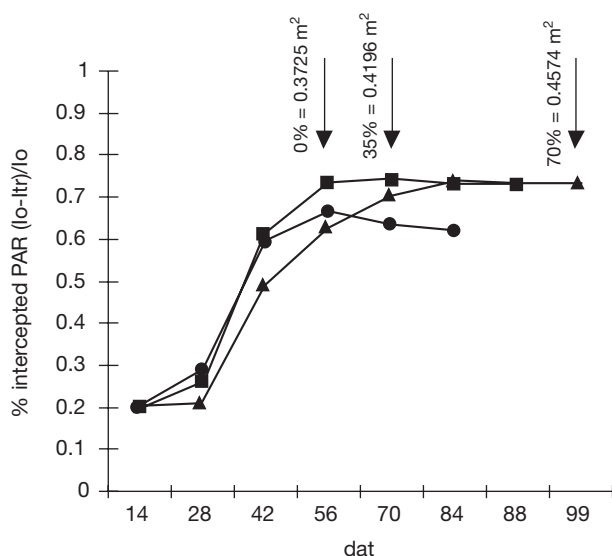
Figure 2. Change in the proportion of intercepted PAR (Io-Itr)/Io by the canopy of broccoli plants grown at the three shading intensities (● 0%; ■ 35%; ▲ 70%) in 2003. Arrows indicate the moment and values of maximum LA. Io: incoming PAR. Itr: PAR at the height of the base of the stems.

Extinction coefficient and leaf angle

In 2003, the light extinction coefficient (K) when LA was maximum (control: 56 dat, S35: 70 dat, S70: at harvest) decreased as shading increased ($K = 7E-05x^2 - 0.0082 x + 0.6275$, where $x = \%$ shading). This indicates that the lower level of intercepted radiation in the shaded environments stimulated a more
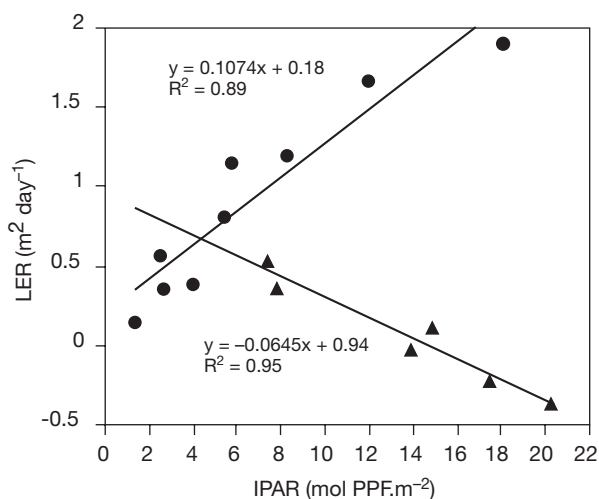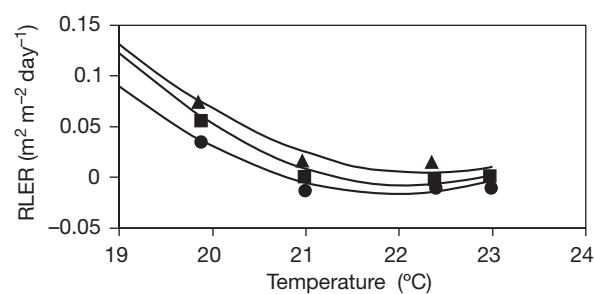

Figure 3. Relationship between LER (m^2 d^{-1}) and daily IPAR (mol PPF m^{-2}) in two periods of the cycle of broccoli plants grown at the three shading intensities in 2003 (● transplant up to 56 ddt; ▲ 56 ddt up to harvest).

Control: $y = 0.0184x^2 - 0.7948x + 8.3248$ $R^2 = 1$
35% shading: $y = 0.0143x^2 - 0.6318x + 6.9635$ $R^2 = 0.97$
70% shading: $y = 0.0113x^2 - 0.5044x + 5.6379$ $R^2 = 0.92$

Figure 4. Relationship between the relative leaf expansion rate RLER (m^2 m^{-2} d^{-1}) and air temperature > 20ºC beneath the meshes and above the canopy in plants grown at the three shading intensities (● 0%; ■ 35%; ▲ 70%) in 2003.

vertical position of the leaves, which in turn let the light reach the lower leaves.

At 70 dat, when the final number of leaves was reached in all three treatments, the angle formed by the stem and the 10th leaf petiole decreased in a quadratic manner with shading; for the 15th and 20th leaves, however, the reduction was linear (Table 4). Leaves in S35 and S70 were more erect than those of the controls as a response to shading.

When the incident PAR decreased by 35% and 70%, the LA at the end of the cycle (with the same number of leaves) increased 35.4% and 47.9% respectively (means for 2002-2003).

Stem and plant height

At the end of the cycle, plant height and stem length up to the point of spear insertion increased as the radiation received decreased, whereas the stem diameter at 10 cm from the spear insertion point was reduced (Table 3).

Fresh spear quality

Commercial and botanical spear diameter and weight decreased with 70% shading in both seasons (Table 3). Neither the total IPAR nor the IPARs for different periods affected spear diameter or commercial or botanical weight (data not shown). The same results were obtained when the Grevsen logarithmic model was used (Grevsen, 1998). The level of shading did not affect floret number per inflorescence (Table 3). Re-

Table 5. Dry weight (g plant⁻¹) of broccoli plant organs at harvest, and total dry weight at 84 dat of broccoli plants grown at the three shading intensities in 2003

Year	% shading	Root	Stem	Leaves	Commercial spear[a]	Stem portion of commercial spear	Botanical spear	Plant	Plant at 84 ddt
2002	0	24.42	30.84	60.69	26.61	9.45	17.17	133.11	—
	35	22.89	32.46	58.19	25.48	6.68	18.80	132.34	—
	70	17.30	30.67	47.50	14.97	2.91	12.06	107.52	—
	CV	10.3	6.7	8.4	6.5	11.6	5.8	5.1	
	P > F[b]	Q < 0.0001	—	Q = 0.0321	Q = 0.0031	L < 0.0001	Q < 0.0001	Q = 0.0246	
2003	0	26.82	25.59	59.71	24.29	9.31	14.98	127.10	127.10
	35	24.24	26.99	57.29	23.94	7.27	16.67	125.19	121.86
	70	18.57	24.14	48.18	13.51	3.95	9.56	100.45	81.12
	CV	6.5	6.0	5.9	9.3	7.2	11.2	3.6	5.8
	P > F[b]	Q < 0.0001	—	Q = 0.0031	Q = 0.0004	L = 0.0373	Q = 0.0007	Q = 0.0030	Q = 0.0381

[a] Commercial spear: botanical spear + 10 cm stem. [b] Probability > F of the significant trends. L: linear. Q: quadratic.

gardless of the reduction in the IPAR, all spears were commercially acceptable.

Dry weight

In both seasons, root, leaf, botanical and commercial spear DW decreased in a quadratic manner with shading at the end of the cycle (Table 5). In all cases, the reduction in DW between S70 and S35 was higher than that between S35 and the control. Shading did not affect stem DW. In 2003, the total dry matter yield of the plants was not reached at the same time in the three environments due to already mentioned differences in cycle duration. Table 5 shows the total DW of plants in the shaded environments at control harvest (84 dat). The dry weight of the spear stem portion decreased linearly with shading in both seasons (Table 5) in agreement with the reduction in its diameter (Table 3).

At 70% shading the plants allocated proportionally more dry matter to their stems (28% *vs.* 23% in the control in 2002, and 24% *vs.* 20% in the control in 2003) at the expense of the roots and commercial spear, while that allocated to the leaves did not vary between treatments (44.6% in 2002, 46.9% in 2003) (Table 6).

Throughout the cycle, and on a per plant basis, the RUE decreased in a quadratic manner as the radiation level increased (Table 7).

Table 6. Percentage dry matter at harvest allocated to different organs in broccoli plants grown at the three shading intensities in 2002 and 2003

Year	% shading	Root	Stem	Leaves	Commercial spear[a]	Botanical spear
2002	0	18.34	23.17	45.59	19.99	12.89
	35	17.29	24.52	43.97	19.25	14.20
	70	16.08	28.52	44.18	13.92	11.21
	CV	9.7	10.3	7.9	10.6	9.7
	P > F[b]	Q = 0.0001	Q = 0.0273	—	Q = 0.00173	Q < 0.0001
2003	0	21.10	20.13	46.98	19.11	11.78
	35	19.36	21.56	45.76	19.12	13.31
	70	18.48	24.03	47.96	13.45	9.51
	CV	10.2	9.4	9.7	9.3	11.8
	P > F[b]	Q < 0.0001	Q < 0.0001	—	Q = 0.0099	Q = 0.0064

[a] Commercial spear: botanical spear + 10 cm stem. [b] Probability > F of the significant trends. L: linear. Q: quadratic.

Table 7. Radiation use efficiency (RUE, g mol^{-1}) of he intercepted PAR in broccoli plants grown at the three shading intensities in 2002 and 2003

Year	% shading	RUE between (dat)					
		0-harvest	14-28	28-42	42-56	46-70	70-harvest
2002	0	0.51	—	—	—	—	—
	35	0.68	—	—	—	—	—
	70	0.98	—	—	—	—	—
	CV	4.1					
	P > F[1]	Q = 0.0105					
2003	0	0.53	0.44	0.96	0.75	0.53	0.10
	35	0.71	0.44	0.68	0.90	0.83	0.59
	70	1.05	0.31	0.66	0.81	1.04	1.83
	CV	9.4	7.2	12.3	3.0	10.1	12.7
	P > F[1]	Q = 0.0112	Q = 0.0395	Q = 0.0069	Q = 0.0002	L = 0.0097	Q = 0.0275

[1] Probability > F of the significant trends. L: linear. Q: quadratic.

Growth variables

The NAR between transplant and harvest decreased, and the LAR and SLA increased with shading in both seasons (Table 8). Even though the RGR decreased with shading in a quadratic fashion (Table 8), the reduction was negligible and the RGR can be understood as constant for practical purposes.

Shading affected the SLA from the beginning of the cycle in S70 conditions, and from 28 dat in S35 conditions (data not shown). Throughout the cycle, there was a reduction of 76% in SLA in the control treatment, and of 67% in the S35 and S70 treatments.

With the increase of shading, the leaves and stem, rather than the spear, were the dominant photosynthate sinks between spear differentiation and harvest in 2003 (Table 9). In S35 conditions, a cycle extension of 4.5 days occurred (Table 3, averages 2002-2003); this allowed the equivalent commercial weights to be reached, but this did not occur with the S70 treatment even with a 15 day extension of the cycle (Table 3).

Discussion

The vegetative period ends with the development of the last leaf (length > 10 mm), at which moment the spear is normally initiated (Gauss and Taylor, 1969). However, in this experiment no such coincidence of events was seen. Although a delayed initiation of visible leaves was observed in the shady environments, still no coincidence was seen between these events.

Table 8. Growth variables of broccoli plants grown at the three shading intensities in 2002 and 2003[1]

Year	% shading	RGR cycle (kg DW gained kg DW^{-1} d^{-1})	NAR cycle (kg DW gained m^2 LA^{-1} d^{-1})	LAR at the end of the cycle (m^2 kg DW^{-1})	SLA at the end of the cycle (m^2 kg leaf DW^{-1})
2002	0	0.012312	0.004746	2.565544	4.363965
	35	0.012278	0.003064	3.966233	6.998532
	70	0.012173	0.001883	6.458324	10.033674
	CV	0.14	4.4	5.1	12.0
	P > F[1]	Q = 0.0301	Q = 0.0026	Q = 0.0256	Q = 0.0322
2003	0	0.011884	0.004768	2.494228	4.994268
	35	0.011829	0.002953	3.971432	7.200352
	70	0.011314	0.001783	6.350839	10.383280
	CV	0.07	3.2	5.4	4.9
	P > F[1]	Q = 0.0072	Q = 0.0023	Q = 0.0198	Q = 0.0483

DW: dry weight. [1] Probability > F of the significant trends. L: linear. Q: quadratic.

Table 9. Relative growth rate (RGR = kg DW gained, kg DW day^{-1})[a] between spear differentiation and harvest of different organs of broccoli plants grown at the three shading intensities in 2003

% shading	Root	Stem	Leaves	Spear	Total
0	0.003693	0.004084	0.006672	0.004860	0.019317
35	0.004135	0.004752	0.008709	0.004579	0.022175
70	0.004174	0.005442	0.010061	0.003807	0.023486
CV	6.0	1.2	2.9	7.3	1.2
P > F[b]	Q = 0.0028	L < 0.0001	Q = 0.0085	Q = 0.0234	Q = 0.0030

[a] DW: dry weight. [b] Probability > F of the significant trends. L: linear. Q: quadratic.

Shading caused changes in the growth and development of the broccoli plants. In previous work (Francescangeli *et al.*, 2006), the erectness of the upper leaves increased as plant density increased, suggesting that shading by neighbour plants elicits this change in plant architecture. The results of the present study confirm that shading causes the upper leaves to be more upright.

Shading changed the partitioning of dry matter. The highest percentage reduction in dry matter allocation was seen in the spear. When light is limiting, more dry matter is partitioned in the stem and less in the roots (Pearcy and Sims, 1994). This change in the distribution of dry matter is considered an indicator of tolerance to shading (Grime, 1979). The plants did not vary the percentage of dry matter allocated to the leaves with shading as a mean of increasing the LA. The largest LA resulting from an increase in shading (Table 4) did not modify the percentage of photosynthates allocated to the leaves. Stems, rather than the spears or roots, were probably the dominant photosynthate sink. Differences recorded in root, leaf and spear dry weight between S35 and the control treatments are relatively small compared to those seen between the latter and S70 (Table 5). Therefore, there must have been a radiation threshold. Below this threshold, the carbohydrate level would be lower than that with higher radiation levels, despite the greater LA and the same proportion of photosynthate resources kept in the leaves. The results show that 'Legacy' broccoli can be cultivated without yield reduction in systems where IPAR at harvest is reduced by approximately 25%, but lower yields can be expected if radiation is reduced by about 60%. The effect on yield of reductions in IPAR at harvest of between 25 and 60% remains to be determined.

It was assumed that black meshes do not modify the spectral balance of transmitted radiation (Benavides Mendoza and Ramírez Rodríguez, 2002). However, as for all wavelength fractions they cause a reduction in the amount of blue light (B, 400-500 nm). Different plant responses considered to represent «shade avoidance», including stem lengthening, have been reported when B light is diminished (Casal and Alvarez, 1988; Erwin and Heins, 1995; Gautier *et al.,* 1997). In the present study, since the plant density was uniform, the differences in stem length in the shaded conditions were possibly defined by the reduction in the amount of this light. However, the effect of the long-wavelength light fractions should not be ruled out. As light filters through the canopy, the amount of reflected far-red (FR) light increases, and a quicker passage of the FR light-absorbing form of phytochrome, Pfr, into the red light-absorbing form, Pr, is promoted. As Pfr decreases so does auxin degradation, promoting an increase in stem elongation (Schmitt *et al.,* 1999). The shade increased as the plants grew bigger, and this could have contributed to an increase in Pr concentration and auxin accumulation, as seen in plants growing at high density (Quail, 1991; Xiong *et al.*, 2002).

It needs to be confirmed whether the different light signals work together in broccoli development, i.e., whether their effects are additive, as in other species (Gautier *et al.*, 1997). Under natural shading, the reduction in B light is combined with a reduction in PPF and in the red:far red light ratio. Therefore, it is important to understand how the plant integrates these different signals, and whether their effects are additive, as has been shown for the reduction in the red:far red ratio and irradiance.

Throughout the cycle, and on a per plant basis, the RUE decreased in a quadratic manner as the radiation level increased (Table 7). This agrees with the findings of Olesen and Grevsen (1997). However, the change in the RUE was different across environments in the 2003 season. The higher the percentage shading the later the plants reached their maximum RUE. In the

control and S35 treatments, the RUE declined towards the end of the cycle, while in S70 it increased until harvest. The effect of shading on the RUE varied with each period throughout the cycle; in the early stages the trend was a reduction with shading, while the opposite occurred in the final stages (Table 7).

The more frequent records taken in 2003 than in 2002 showed that the effects of shading on growth started in the first fortnight of the cycle (data not shown). Although during this time the increase in LA was larger in the control, the related rates (LAR, SLA) were never higher than those of the shaded plants (data not shown). The reduction in LAR starting on 56 dat in all three environments coincides with the beginning of leaf abscission, and according to the change in LA (Fig. 1) and RLER (Fig. 4), the reduction in the LAR was proportionally higher and faster in the control.

The higher values for LAR and SLA in the S35 and S70 treatments (Table 8) could be due to the increase in leaf area and a reduction in thickness caused by shading. Nobel (1983) indicated that leaves in the sun are usually thicker than those growing in the shade.

The increase in LAR with shading was compensated by an equivalent reduction in NAR, thus the RGR was almost constant. The reduction of 63-65% in the radiation received per plant when shading increased from 0 to 70% (Table 2) caused a reduction in the RGR of just 0.01% in 2002 and 0.04% in 2003. The compensatory change in NAR and LAR keeping RGR almost constant has also been reported for the shade-tolerant *Impatiens parviflora* (Nobel, 1983); therefore it seems to be a mechanism of some plant species to help them cope with shading.

The negative relationship between the RLER and air temperature from 56 dat for the three environments was of the same type (quadratic), although the significant equation for each shading level was different (Fig. 4). This probably means that leaf expansion was affected by the interaction between radiation and other factors.

The period until spear initiation and maturity was longer with shading. These findings confirm observations made by Wiebe (1972, cf: Hadley and Pearson, 1998) and Hand and Atherton (1987) on delayed flower induction at low light levels.

Most of the models suggested to predict flower differentiation and commercial maturity in broccoli are based on the accumulation of thermal units (Miller, 1985; Dufault, 1997; Tan *et al.,* 2000b), with little or no inclusion of solar radiation (Tan *et al.,* 2000a). Marshall and Thompson (1987a,b) showed the im-

portance of solar radiation for determining base temperature in degree-day models; Taborda (2000) found that sensitivity to PAR is cultivar-dependent when certain temperature thresholds are exceeded. Under the conditions of this experiment, with equal temperatures and consequently with a similar accumulation of thermal units, the hybrid 'Legacy' showed sensitivity to the quantity of intercepted and accumulated PAR in terms of floral differentiation and yield, whatever method might be used for heat unit calculation. Possibly, differences in the incident and intercepted PAR recorded between environments were higher than those observed between planting dates in other studies on the effects of temperatures and radiation on broccoli (Fontes *et al*., 1967; Pearson and Hadley, 1988; Wiebe, 1990; Mourao and Hadley, 1998; Tan *et al*., 2000a). Delayed flower differentiation is one of the most widely used traits to classify a cultivar as shade-tolerant (Grime, 1979).

In conclusion, shading caused morphological changes in broccoli plants (taller plants and greater LA) and changes in dry matter partitioning (percentage dry matter in stems increased with shading at the expense of the roots and spears), which can be interpreted as mechanisms for increasing IPAR. About half of the total dry matter produced was allocated to the leaves irrespective of the radiation level. Commercial yield seems to be reduced only at high shading levels. Thus, broccoli can be used in production systems with diminished radiation. As radiation levels decrease, the vegetative and reproductive stages become longer, the RUE increases, and a compensatory behaviour occurs between the LAR and the NAR to keep the total plant RGR almost independent of the radiation level.

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