



## Growth suppression of canola through wheat stubble II. Investigating impacts of hypocotyl elongation using simulated stubble

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### Abstract

In south-eastern Australia, surface-retained wheat stubble can reduce the growth and yield of canola as a result of reductions to the quality and quantity of light under the stubble and the associated elongation of the hypocotyl. This paper reports a series of pot experiments that examined the effect of hypocotyl elongation on the leaf area development of canola, the allocation of dry mass, and the absolute and relative growth rates compared to non-etiolated seedlings. The primary aim was to determine the magnitude of the growth reductions caused by hypocotyl elongation in canola seedlings under controlled conditions. Seedling hypocotyl elongation was induced by growing canola seedlings in narrow poly-pipe tubing of different lengths placed over the seedlings as they began to emerge through the soil, and removing the shade cloth covering the top of the tube when the cotyledons had reached it, to mimic the plant reaching and overtopping a stubble layer. Plants with longer hypocotyls had smaller root systems, less leaf area and less leaf and root biomass. These plants had lower relative growth rates than plants that allocated fewer resources to hypocotyls and more to roots and leaves. The magnitude of the growth responses observed in these experiments was similar to those of plants with long hypocotyls growing through stubble layers in previously reported field studies. This suggests that a significant portion of the effect of stubble observed in the field under stubble retention is due to the re-allocation of resources to the production and growth of the hypocotyls, rather than other biochemical effects of the stubble.

### Introduction

Retention of crop stubbles is a key component of conservation farming systems, which are promoted worldwide (Chan and Pratley, 1998). Retained crop stubbles reduce soil evaporation, erosion and degradation, increase water availability to crops and maintain soil organic matter (Chan and Pratley, 1998; Charman 1985). Despite these benefits to production and sustainability, adoption has been slow in many areas of the

world (Bruce, 2003; Lyon et al., 2004). Slow adoption of stubble retention by farmers can occur because it is either impractical or unprofitable; or because other significant constraints to adoption exist (Lyon et al., 2004).

A recent example of such constraints leading to low adoption of stubble retention is in canola production systems of south-eastern Australia, where sequences of canola (*Brassica napus*) and wheat (*Triticum aestivum*) are grown in the medium to high rainfall zones (>500 mm annual rainfall). Despite a general move toward minimum tillage and the routine retention of canola stub-

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bles when sowing wheat in these systems, the wheat stubble is invariably burnt prior to sowing canola. Bruce et al. (2005) showed that under most existing management systems, surface-retained wheat stubble loads typical at canola sowing (around 4–5 t/ha) reduced canola yields by 23%, although the role of various biochemical and physical factors involved in the growth suppression was unclear. A subsequent study concluded that the growth reductions primarily resulted from physical impacts of the stubble on the emerging seedlings, including reductions to the quality and quantity of light under the stubble and the associated elongation of the hypocotyl (Bruce et al., 2006). Seedlings growing through retained stubble had elongated hypocotyls, slower emergence rates, smaller leaf area, and slower root length development than those emerging from bare soil (Bruce et al., 2006). Despite strong evidence for alterations in light conditions constituting the key mechanisms of growth reduction, it was difficult to separate the physiological effects of hypocotyl elongation from the confounding effects of lower temperature and increased seedling disease in the field experiments. A study to quantify the impacts of elongated hypocotyls on canola seedling growth in the absence of these confounding factors was suggested (Bruce et al., 2006).

The elongation of the hypocotyl is a response to shade and increases a seedling's chance of ultimately emerging through surface stubble and/or soil (Holmes, 1983). Plants growing in shade experience altered spectral quality and quantity; specifically a reduction in blue (photosynthetically active radiation – PAR) and red wavelengths (Ballare et al., 1991; Ganade and Westoby, 1999; Schmitt and Wulff, 1993). A reduction in PAR can affect dry-mass accumulation (Holmes, 1983); while a reduction in the red to far red (R:FR) ratio can result in an increase in apical dominance, hypocotyl and stem extension and internode elongation (Ballare et al., 1991). Hypocotyl elongation is controlled more by spectral quantity than etiolation in the organs of adult plants (Ballare et al., 1991). Several studies have demonstrated the effect of spectral quality on plant growth and biomass partitioning (Ganade and Westoby, 1999; Kasperbauer and Hunt, 1992; Schmitt and Wulff, 1993), but few have examined the costs of these morphological

responses to relative growth rate and productivity, especially under agricultural conditions. In addition, most studies have examined the impact of shade on growth where the shade treatments are maintained for the entire experiment. Little is known about how the alteration of spectral quality and quantity, from low light conditions to high light conditions, during the early growth of a plant affects subsequent plant growth, which is of particular significance for plants emerging through a stubble layer.

This paper reports a series of pot experiments, which examine the effect of hypocotyl elongation on the leaf area development of canola, the allocation of dry mass, and the absolute and relative growth rates compared to non-etiolated seedlings. The primary aim of these experiments was to quantify the magnitude of the growth reductions caused by hypocotyl elongation in canola seedlings in the absence of other interacting factors and consider its role in the reduced growth of canola observed under retained wheat stubble in the field (Bruce et al., 2005, 2006). Such clarification would assist in the development of simple, effective management strategies, which avoid stubble burning, such as sowing techniques that push stubble off the seedling row and onto the inter-row.

## Materials and methods

A series of three pot experiments was conducted using the canola cultivar Oscar. In all experiments, seedling hypocotyl elongation was induced using narrow poly-pipe tubing (20 mm diameter) of various lengths inserted vertically within a cardboard skirt and placed over the seedlings just as they emerged through the soil. The cardboard skirt ensured the tubes remained upright without the need to push them into the potting mix around the seedling. The top of the tube was covered with a piece of shade cloth (extra-heavy duty cream coloured; 84–90% UV and visible light reduction). This shading was less than the shade provided by a stubble layer in the field (PAR reduction 99%) as measured by LI-1800 portable spectrophotometer in Part I of this series (Bruce et al., 2006), but sufficient to elongate the hypocotyl. The shade cloth was removed from the top of each poly-pipe as soon as the

cotyledons had reached it, allowing the plant to continue growing above the tube opening. The aim was to mimic the plant growing through and overtopping a stubble layer. The extent of hypocotyl elongation was manipulated using different tube lengths (e.g. 20, 40 and 60 mm), designed to simulate the different thicknesses of stubble thatch, and compared with a control treatment (0 mm) where no pipe was inserted into the cardboard skirt.

Experiment 1 compared the leaf area development and dry mass allocation of etiolated and non-etiolated seedlings with sequential harvests based on days after emergence from the soil. Experiments 2 and 3 examined the effect of hypocotyl elongation on absolute and relative growth rates of seedlings. Sequential harvests of subsets of seedlings in all treatments were made according to phenological stage, to separate ontogenetic from treatment effects. Experiment 3 was conducted outside during winter in Canberra, Australia (149°06' E, 35°12' S), under environmental conditions more similar to those experienced by commercial canola crops. Seeds in all three experiments were within the weight range 2.5–3.0 mg to ensure that variation in the experiments was due to the treatment differences and not seed size. Table 1 summarises the experimental design and conditions in each experiment.

### Experiment 1

Pots (50 mm diameter, 70 mm high) were filled with potting mix (compost, gypsum 1 g/L). Two seeds of canola (cv. Oscar) were sown at 1 cm depth, germinated at 15 °C in the dark and thinned to one seedling per pot upon emergence.

Pots containing plants that had emerged the previous day were placed in flats (30 cm × 50 cm × 10 cm) filled with potting mix to ensure adequate drainage through the pots. Treatments were prepared using poly-pipe tubing of four lengths: 20 mm, 40 mm, 50 mm and 60 mm as previously described. A control treatment (0 mm) with no pipe was also established. The experiment was conducted in a growth cabinet at 15 °C with a 12 h day/night cycle (maximum PAR 360 mmol/m<sup>2</sup>/s). PAR was lower than under field conditions but comparable with other similar studies (Ganade and Westoby, 1999; Marañón and Grubb, 1993), and conditions were consistent and constant over time. Pots were watered with 25% Hoagland's solution daily. The pots were arranged in a randomised block design with six replicates. Due to destructive sampling each replicate contained six pots of each treatment.

Six seedlings were harvested from each treatment on six occasions, timed to coincide with the emergence of the cotyledons from the top of each poly-pipe tube treatment. For example, at harvest 1, the cotyledons of the control pots had opened and one seedling per replicate from each tube-length was harvested (5 tube lengths × 6 replicates = 30 seedlings). This was repeated as the plants in successive tube-lengths (20, 40, 50, 60 mm) had cotyledons opening at the top of the poly-pipe tube. Six replicates from each treatment were maintained and harvested 2 days after the 5th harvest, which coincided with the appearance of the cotyledons above the 60 mm tube. At each harvest, the seedlings were divided into root, hypocotyl, and leaf components, and root length, hypocotyl length, leaf area and dry mass of root, hypocotyl and leaf determined. Roots

Table 1. Summary of experimental design and conditions (location, light and temperature) for Experiments 1, 2 and 3

	Tube length (mm)	Harvests	Location	Light	Temperature
Experiment 1	0, 20, 40, 50, 60	Sequential, days after emergence	Growth cabinet	Maximum PAR 360 mmol/m <sup>2</sup> /s	Constant 15 °C
Experiment 2	0, 50	Phenological stage	Growth cabinet	Maximum PAR 360 mmol/m <sup>2</sup> /s	Constant 15 °C
Experiment 3	0, 50	Phenological stage	Bird-proof benches outside	Average 1260 mmol/m <sup>2</sup> /s on sunny day	0–15 °C

Hypocotyl length was induced using poly-pipe tubing of different lengths.

Table 2. List of abbreviations used in the growth analysis and derivation (revised from Atwell et al (1999))

Abbreviation	Units	Definition
RGR	g/g/day	Relative growth rate. Rate of mass increase per unit mass already present. Equivalent to $SLA \times LWR \times NAR$ . Calculated from $\frac{\ln W_2 - \ln W_1}{t_2 - t_1}$ , where $W$ is total plant mass and $t$ is time.
LAR	cm <sup>2</sup> /g	Leaf area ratio. Ratio of leaf + cotyledon area to total plant mass. Equivalent to $LWR \times SLA$ . Calculated from $\frac{1}{2} \left( \frac{A_1}{W_1} + \frac{A_2}{W_2} \right)$ , where $A$ is leaf area and $W$ is total plant mass.
NAR	g/cm <sup>2</sup> /day	Net assimilation rate. Rate of mass increase per unit leaf + cotyledon area per day. Calculated from $\left( \frac{W_2 - W_1}{t_2 - t_1} \right) \left( \frac{\ln A_2 - \ln A_1}{A_2 - A_1} \right)$ , where $A$ is leaf area, $W$ is total plant mass, and $t$ is time. Assuming biomass and leaf area are linearly related. It represents a plant's net photosynthetic effectiveness.
LWR		Leaf weight ratio. Ratio of leaf + cotyledon mass to total plant mass.
SLA	cm <sup>2</sup> /g	Specific leaf area. Ratio of leaf + cotyledon area to leaf + cotyledon mass. Gives an indication of leaf thickness.
SRL	cm/g	Specific root length. Ratio of root length to root mass. Gives an indication of root thickness.
RMR		Root mass ratio. Ratio of root mass to total plant mass.
HMR		Hypocotyl mass ratio. Ratio of hypocotyl mass to total plant mass.
SHL	cm/g	Specific hypocotyl length. Ratio of hypocotyl length to hypocotyl mass. Gives an indication of the thickness of the hypocotyl.
AGR	g/day	Absolute growth rate.

Note leaf mass includes petiole.

were obtained by washing carefully over a 2 mm sieve to reduce the likelihood of loss of fine roots.

### Experiment 2

Seedlings were established as in Experiment 1 but only two tube lengths (0 and 50 mm) were included and larger pots were used (120 mm diameter, 140 mm high). Pots were watered daily with 25% Hoagland's solution. The pots were arranged in a randomised block design with six replicates. In contrast to Experiment 1, seedlings in each treatment were harvested according to phenological stage, when the 1st, 2nd, 3rd and 5th leaf appeared. Tubes were maintained on the seedlings until harvest. At each harvest, plants were treated as in Experiment 1.

### Experiment 3

The experimental design was similar to Experiment 2 except the plants were maintained outside on bird-proof benches (average PAR on sunny day in winter 1260 mmol/m<sup>2</sup>/s) in a randomised block design with six replicates. Blocks were bordered with extra unplanted-pots to minimise temperature extremes on the edge pots. Plants in

each treatment (0 and 50 mm) were harvested at the appearance of the 3rd, 5th and 11th leaf. Additional control plants were maintained until the harvest of the 50 mm tubes at the 11th leaf stage and these were harvested concurrently to allow a final growth comparison of the treatments. At each harvest, seedlings were treated as in Experiment 1.

### Growth analysis

In Experiments 2 and 3, seedlings were regularly checked for stage of development and harvested once the appropriate true leaf was 2 mm in length. It was not feasible to time the harvest according to the expansion of the true leaves, as the different treatments imposed on the plants may affect ontogenetic phase. That is, plants with elongated hypocotyls may have smaller leaf area at the same growth stage as plants with shorter hypocotyls. This policy of timing the harvests in relation to leaf appearance standardised the ontogenetic phase of the plants better than other methods used for estimating relative growth rate (RGR, see Table 2 for abbreviations), such as the leaf-area based method.

In all experiments, leaf width and length were measured at the widest point using 0.01 mm

precision electronic calipers (leaves were too small to measure at harvest 1). Due to the small size of the leaves, leaf area was determined using a calibration curve derived from relationships between the length and width of the photocopied cotyledons and leaves and their respective surface areas. Photocopied leaves were weighed and surface area determined based on the weight of a known area of paper. Cotyledon area (CA) and leaf area (LA) were determined using the following relationships:

$$CA = 0.9280(\text{length} \times \text{width}) - 2.142, r^2 = 0.96$$

$$LA = 0.7805(\text{length} \times \text{width}) - 5.493, r^2 = 0.99$$

Because cotyledons were green, persistent and increased in size in each experiment, results are presented as 'effective' leaf area (CA + LA).

Hypocotyl length was measured from the top of root system to the base of the cotyledon using 0.01 mm precision electronic calipers. Root length was measured using the computer-based digital scanning software winRHIZO<sup>®</sup> Version 3.10B (Régent Instruments Inc, Canada). Roots were washed in 50% ethanol, stained with 0.05% toluene blue for 10 min, rinsed to remove adhering stain not incorporated by the roots and spread in a transparent tray in a thin layer of water. The image was acquired using a flatbed scanner at 400 d.p.i and analysed using winRHIZO<sup>®</sup> software. Total dry weight was determined after drying the component parts for 48 h at 70 °C. Root mass was insufficient for accurate measurement in Experiment 1.

An analysis of the RGR, net assimilation rate (NAR) and leaf area ratio (LAR) was undertaken to investigate how hypocotyl elongation influenced plant growth (Table 2). Several methods exist for dividing RGR into its components, including methods based on the carbon economy of the plant (Poorter, 1989) and other methods based on plant nitrogen productivity (Lambers et al., 1989). In this study we focused on the carbon economy of the plant where:

$$RGR = NAR * LAR$$

and

$$LAR = LWR * SLA,$$

where LWR is leaf weight ratio and SLA is specific leaf area. Values estimating RGR, and other growth indices were calculated using paired plants, one from each harvest.

### *Statistical analyses*

Mean values for RGR and other growth indices were calculated for each pair of plants and analysed using ANOVA in Genstat version 5. Tube length  $\times$  days after emergence, and tube length  $\times$  growth stage interactions were used to identify significant differences in plant growth parameters. Plants were destructively harvested at each point in time (not repeated measures) allowing the use of ANOVA. In all cases the residuals were checked and, if necessary, the data were transformed to achieve normality or homogeneity of variance using the natural log transformation. In most instances, due to the large number of treatments and multiple comparisons in each experiment, the 'least significant difference' test (LSD) was not appropriate due to the likelihood of committing a type 1 error. For this reason, standard error of difference (SED) is reported. Significant treatment differences referred to in the results are statistically significant, at least to the 5% level.

## **Results**

### *Experiment 1*

Longer tubes resulted in longer hypocotyls (Figure 1a). Plants in the 60 mm tubes had hypocotyls three times longer than the controls. Hypocotyls were 20 mm long in the control treatment because hypocotyl length was measured from the top of the root system to the base of the cotyledon. Hypocotyls stopped elongating when the cotyledons reached the top of the tube. Plants growing in longer tubes had reduced leaf area from an early stage (Figure 1b). Although the leaves had opened and were green within the tube, they were slower to expand compared to the leaves of the control (Figure 1b). The specific leaf area (SLA) peaked and then declined for all plants regardless of tube length, and at the final harvest (day 10) there was no difference in SLA

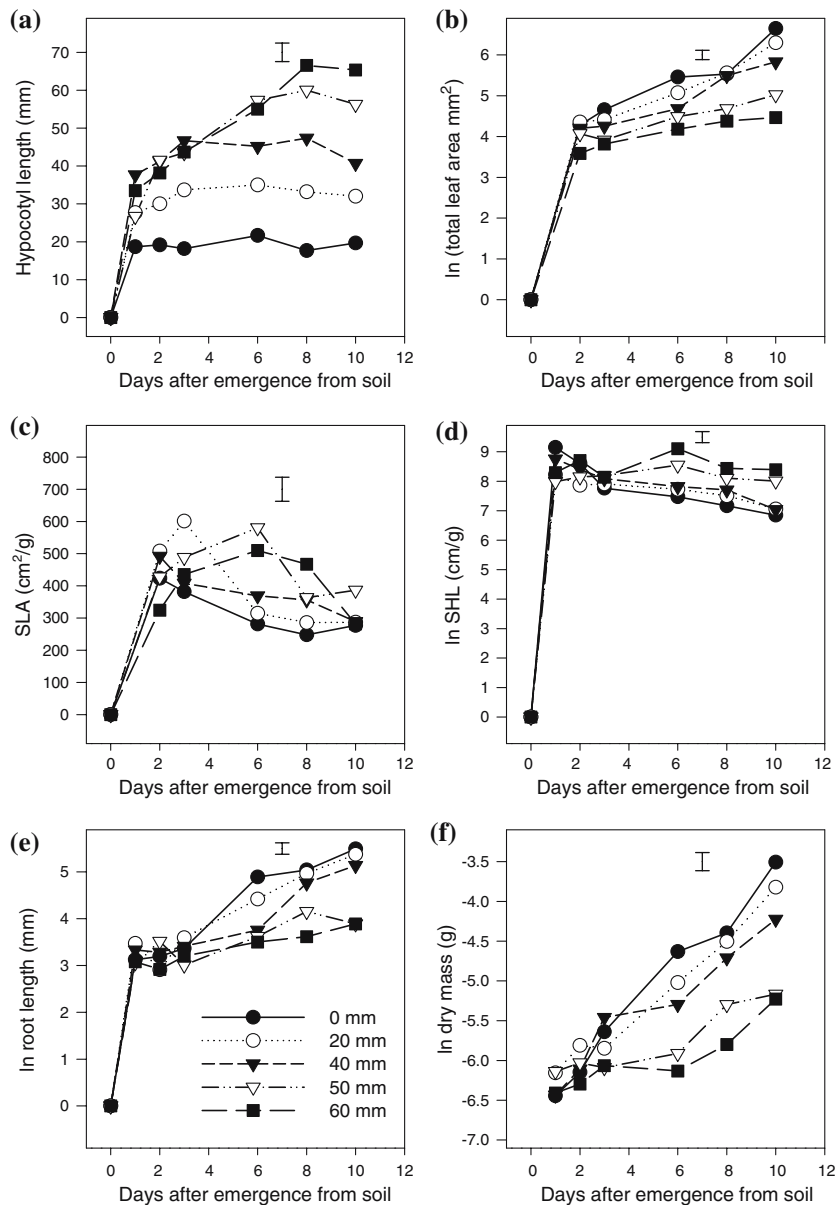


Figure 1. Effect of tube-length (0, 20, 40, 50, 60 mm) and days after emergence on (a) hypocotyl length; (b) ln (total leaf area); (c) specific leaf area (SLA); (d) ln specific hypocotyl length (ln SHL); (e) ln (root length); and (f) ln (dry mass) (Experiment 1). Harvest times were determined by the emergence of cotyledons from the top of each poly-pipe tube for each treatment (tube length). The final harvest occurred 2 days after the cotyledons emerged from the tallest tube. SEDs for the interaction between tube length and days after emergence are presented. Residual degrees of freedom are for (a) 138, (b) 110, (c) 109, (d) 137, (e) 135, (f) 136.

among the treatments (Figure 1c). In general, the decrease in SLA was delayed with increasing tube length and is possibly a result of delayed development (although the 40 mm tube length did not follow this pattern).

Hypocotyls increased in thickness over time for the plants in shorter tubes, while hypocotyls

in the 50 and 60 mm tubes became thinner until the 4th harvest (day 6) then increased in thickness until the final harvest (day 10) (Figure 1d). Plants growing in longer tubes had thinner hypocotyls at final harvest.

Root length and above-ground dry mass was lower with increasing tube length (Figure 1e,f),

and the differences between treatments increased over time. Figure 1f can also be used to estimate relative growth rate (RGR) between successive harvest times due to the relationship,  $R = \frac{d(\log e^W)}{dt}$ . RGR decreased as hypocotyl length increased.

There was no interaction between tube length and harvest time on leaf dry mass as a proportion of total above-ground biomass (data not shown), so only main effects of tube length and harvest time are considered. Leaf dry mass as a percentage of total seedling above-ground dry mass decreased with increasing tube-length, presumably as a response to the increased allocation of resources to hypocotyl dry mass (Figure 2a). There was also a trend for an increase in the proportion of leaf dry mass over time, regardless of tube length (Figure 2b).

### Experiment 2

The seedlings in the 50 mm tubes had hypocotyls 2.5 times longer on average than the plants with no tubes (0 :34 mm, 50 :75 mm; SED 1.6). Absolute growth rate (AGR) initially increased more rapidly for plants with shorter hypocotyls, but between the appearance of the 3rd and 5th leaf, AGR was similar for plants with long and short hypocotyls (Figure 3a). RGR was initially similar between the treatments, although between the appearance of the 2nd and 3rd leaf, RGR for the short hypocotyl plants was 2.6 times greater than for the long hypocotyl plants (Figure 3b). This difference disappeared between the appearance of the 3rd and 5th leaf. LAR was initially smaller in plants with long hypocotyls compared to control plants, however during the final growth period LAR was the same regardless of the length of the hypocotyl (Figure 3c). Both components of LAR (SLA and LWR) appeared to have an effect on RGR (Figure 3d, e). Indeed, SLA was initially reduced in plants with longer hypocotyls but this was reversed during the final growth period. LWR was lower for the plants with longer hypocotyls at all growth periods. There was no effect of growth stage or treatment on NAR (data not shown).

There was no difference in root length between the treatments until the appearance of the 5th leaf, when plants with shorter hypocotyls had greater root length (Figure 4a). RMR was highest

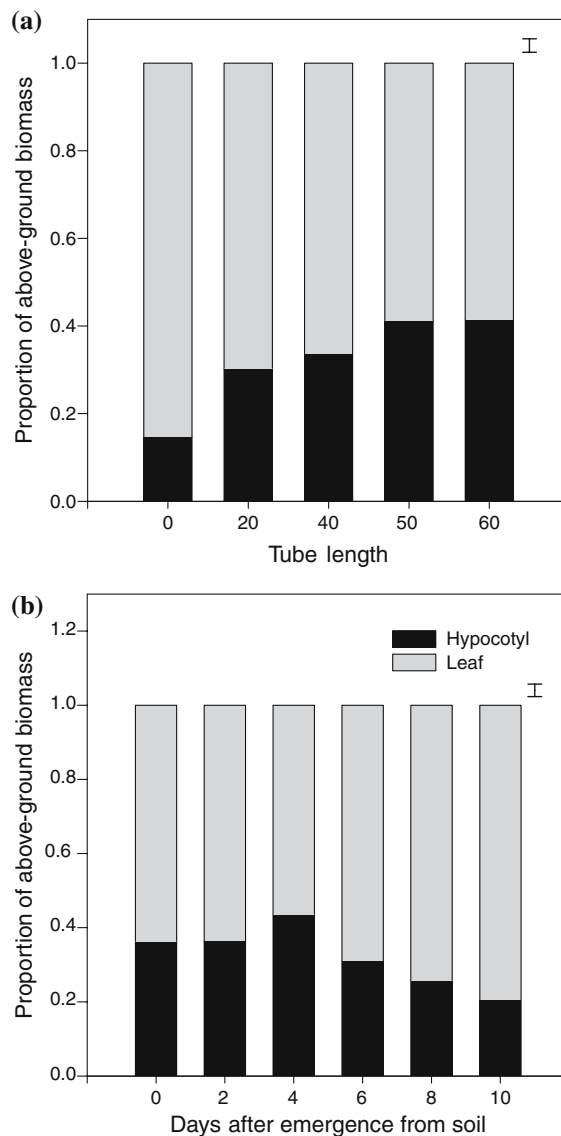


Figure 2. Effect of (a) tube length (0, 20, 40, 50, 60 mm) and (b) days after emergence on leaf and hypocotyl dry mass as a proportion of above-ground dry mass (Experiment 1). Harvest times were determined by the emergence of cotyledons from the top of each poly-pipe tube for each treatment (tube length). The final harvest occurred 2 days after the cotyledons emerged from the tallest tube. SEDs for main effects are presented because the interaction was not significant. Residual degrees of freedom are for both main effects are 142.

for both tube lengths at the appearance of the 2nd leaf but declined with the appearance of later leaves (Figure 4b). Plants with longer hypocotyls had the highest RMR at the appearance of the 1st and 2nd leaf although this difference disappeared at the appearance of the 3rd leaf. The rate

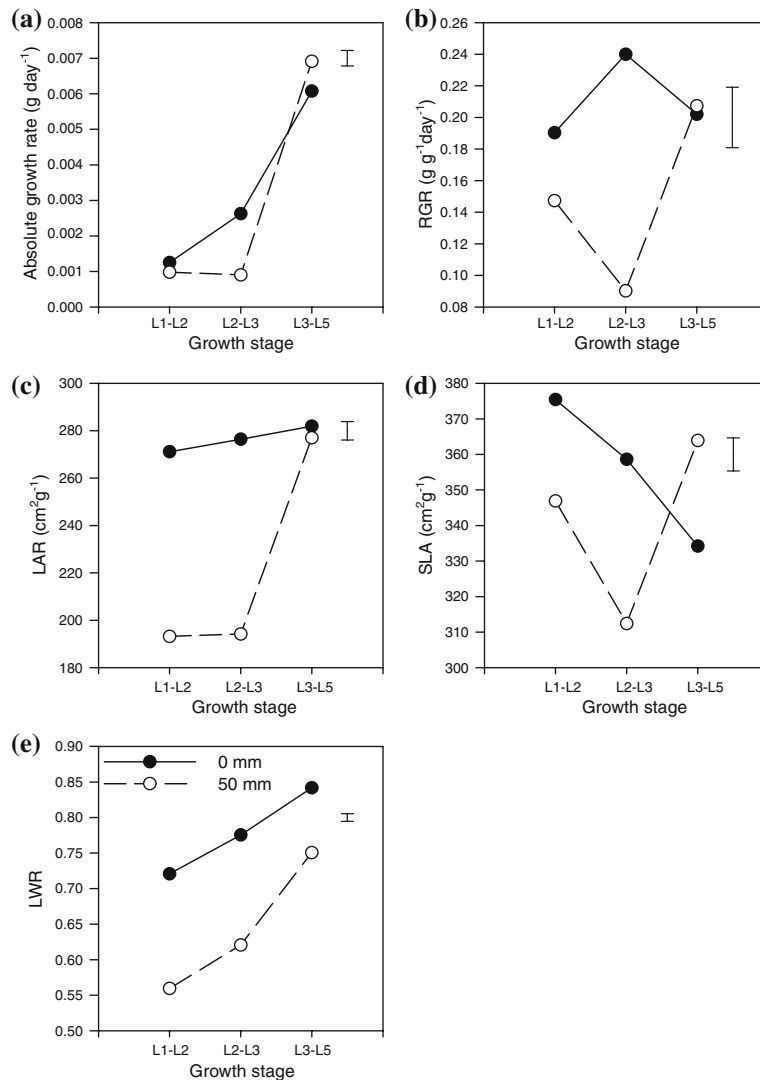


Figure 3. Effect of tube length (0 and 50 mm) and growth stage [Leaf 1–Leaf 2 (L1–L2), Leaf 2–Leaf 3 (L2–L3), Leaf 3–Leaf 5 (L3–L5)] on (a) absolute growth rate (AGR); (b) relative growth rate (RGR); (c) leaf area ratio (LAR); (d) specific leaf area (SLA); and (e) leaf weight ratio (LWR) (Experiment 2). Treatments were harvested according to phenological stage, when the 1st, 2nd, 3rd and 5th leaf appeared. SEDs are presented for the interaction between tube length and growth stage. Residual degrees of freedom are for (a) 23, (b) 24, (c) 23, (d) 23, (e) 24.

of decline in RMR after the appearance of the 2nd leaf was greater for the seedlings with the longer hypocotyls. Total biomass increased over time for both treatments, although the control treatment had slightly higher total biomass at the 3rd leaf stage (Figure 4c). Specific root length (SRL) was higher in plants with short hypocotyls (0 mm:  $39 \times 10^3$ , 50 mm:  $24 \times 10^3$ ; SED  $3 \times 10^3$ ) and did not change with leaf stage (data not shown). Hypocotyl mass ratio (HMR) was always higher in plants with long hypocotyls (Figure 4d),

although the absolute value declined over time with leaf stage.

### Experiment 3

Hypocotyls were longer in the seedlings growing in the 50 mm tubes (Table 3). In contrast to Experiment 2, there was no interaction between tube length and growth stage, so only main effects of tube length and growth stage are considered. AGR increased for the seedlings with

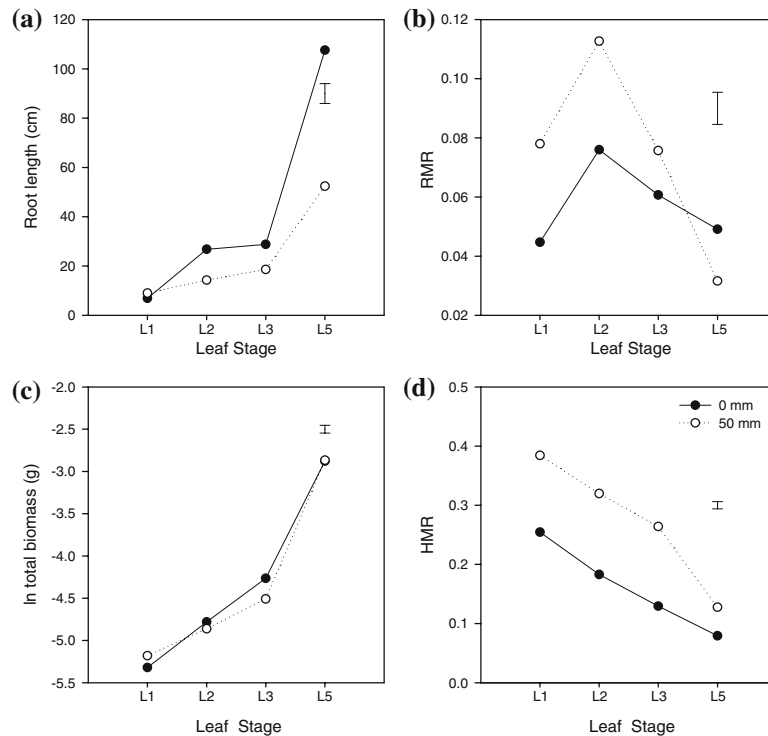


Figure 4. Effect of tube length (0 and 50 mm) and developmental stage (indicated by leaf number) on: (a) root length (cm); (b) root mass ratio (RMR); (c) ln total dry mass; and (d) hypocotyl mass ratio (HMR) (Experiment 2). Treatments were harvested according to phenological stage, when the 1st, 2nd, 3rd and 5th leaf appeared. SEDs are presented for the interaction of tube length and developmental stage. Residual degrees of freedom are for (a) 39, (b) 38, (c) 39, (d) 39.

Table 3. Effect of two tube lengths, 0 and 50 mm; and two growth periods, 3rd leaf–5th leaf (L3–L5) and 5th leaf–11th leaf (L5–L11) on growth characteristics [absolute growth rate (AGR), relative growth rate (RGR), leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA) and net assimilation rate (NAR)] (Experiment 3)

Variable	Tube length			Growth stage			df
	0 mm	50 mm	SED	L3–L5	L5–L11	SED	
Hypocotyl length (mm)	23.6	70.5	1.2	–	–	–	30
ln AGR (g/day)	–4.2	–4.5	0.11	–5.4	–3.4	0.1	20
ln RGR (g/g/day)	–2.0	–2.8	0.1	–2.0	–2.3	0.1	20
LAR (cm <sup>2</sup> /g)	171	164	ns	179	155	4	19
LWR	0.81	0.77	0.01	0.77	0.81	0.01	17
SLA (cm <sup>2</sup> /g)	213	214	ns	232	195	4	20
NAR (g/cm/day)	$8.0 \times 10^{-4}$	$6.9 \times 10^{-4}$	$5.1 \times 10^{-5}$	$7.6 \times 10^{-4}$	$7.3 \times 10^{-4}$	ns	20

SEDs and residual degrees of freedom (df) are presented. Only main effects are presented, as there was no interaction between tube length and growth stage.

short hypocotyls and increased with growth stage. RGR declined with growth stage and increased in plants with short hypocotyls. Changes in both LAR and NAR were associated with changes in RGR in response to the treatments. In particular,

LAR decreased with growth stage but was not different between the different tube lengths. In contrast, NAR did not differ between the different growth stages but was higher in the control treatment (Table 3). Both the components of

Table 4. Effect of two tube lengths (0 and 50 mm); and developmental stage (appearance of leaf 3, leaf 5 and leaf 11) on root morphology [root length, specific root length (SRL)] and whole plant measures [root mass ratio (RMR), hypocotyl mass ratio (HMR)] (Experiment 3)

Variable	Tube length			Developmental stage				
	0 mm	50 mm	SED	L3	L5	L11	SED	df
ln root length (cm)	5.89	5.64	ns	3.67	5.33	8.28	0.17	30
ln RMR	-2.14	-2.39	0.11	-2.48	-2.39	-1.91	0.13	30
SRL (cm/g) $\times 10^3$	30.8	32.6	ns	32.0	34.4	28.6	2.38	30
ln total biomass (g)	-2.30	-2.34	ns	-4.19	-2.72	-0.06	0.08	30
HMR	0.08	0.15	0.01	0.19	0.09	0.07	0.01	29

SEDs and residual degrees of freedom (df) are presented. Only main effects are presented, as there was no interaction between tube length and developmental stage.

Table 5. Comparisons of growth and development of the control (0 mm) and the 50 mm tube at 67 days after sowing

Variable	Control (0 mm)	50 mm tube	SED	df
Leaf Number	17	10	0.93	10
Shoot biomass (g)	1.7	0.74	0.12	10
Total biomass (g)	2.0	0.87	0.16	10
Hypocotyl length (mm)	22	70	2.4	10
SLA (cm <sup>2</sup> /g)	163	164	ns	10
Root length (cm) $\times 10^3$	9.4	4.0	1.6	9
RMR	0.17	0.16	ns	10
SRL (cm/g) $\times 10^4$	2.4	3.1	ns	10

Additional control plants were maintained until the harvest of the 50 mm tubes at the 11-leaf stage, and these were harvested concurrently to allow a final growth comparison of the treatments. SED and residual degrees of freedom (df) are presented for the main effect of tube length.

LAR influenced RGR. Indeed, SLA and RGR decreased with increasing growth stage, while LWR and RGR decreased with increasing hypocotyl length.

Root length did not differ with hypocotyl length but increased with plant development (Table 4). Root mass ratio (RMR) declined in plants with long hypocotyls, and increased with plant age. SRL did not differ between the tube lengths, nor between the appearance of the 3rd and 5th leaf, however, by the appearance of the 11th leaf, SRL had decreased. Total biomass increased with plant development stage, although there was no difference in biomass between the treatments. HMR was higher in the 50 mm tubes and decreased with plant age.

Control plants that were maintained and harvested at the same time as the final harvest of the 50 mm tubes (67 days after sowing) had 17 leaves compared to 10 for the plants in the

50 mm tubes (Table 5). Hypocotyls were shorter in the controls than plants in the 50 mm tubes. Shoot and total biomass, as well as root length, were larger in the controls than in the 50 mm tubes. SLA, RMR and SRL were not different between the control and the 50 mm tube.

## Discussion

### *Impact of hypocotyl elongation on biomass allocation and growth*

This study has quantified the impact of early seedling etiolation caused by an initial period of altered light conditions, on dry mass allocation and growth in canola during early seedling growth. Plants grown in longer tubes had longer hypocotyls, shorter root systems, smaller leaf area and less leaf and root dry mass. Together,

these factors are likely to reduce the ability of these plants to capture CO<sub>2</sub> and nutrients compared with the control treatment. In Experiment 1 it was clear that the elongation of the hypocotyl occurred at the expense of root and leaf production due to diversion of resources; however, the effects of hypocotyl elongation on growth were confounded by the clear delay in plant development. The smaller roots and shoots measured at each harvest may have been a function of the different growth stages rather than by the longer hypocotyls *per se*. In Experiments 2 and 3, where valid comparisons of treatment effects on net partitioning could be made, an increase in hypocotyl length was also associated with biomass re-allocation from the roots and the leaves to the hypocotyls.

Plants can respond to low light by maximising light interception by increasing the specific leaf area (SLA) (Poorter and Van der Werf, 1998); however, this is not necessarily the case when light conditions suddenly change during growth. Plants grown in low light, with high SLAs, transferred to high light show a rapid reduction in SLA to values similar to those of plants grown in high light (Blackman and Wilson, 1954; Poorter and Van der Werf, 1998), partly to reduce water-loss and partly to prevent photo-inhibition (Poorter and Van der Werf, 1998). This pattern was observed in Experiment 1. In Experiment 2, plants with long hypocotyls initially had lower SLA and LAR than plants with shorter hypocotyls; between the appearance of Leaf 3 and Leaf 5, these differences disappeared. In Experiment 3, long hypocotyls had no effect SLA or LAR; the appearance of true leaves coincided with the increase in irradiance (the removal of the shade cloth from the tubes), and as the appearance of true leaves occurred under the same irradiance levels for both treatments, it is not surprising that the SLA was similar between the treatments. The slower response to the removal of the shade cloth in Experiment 2 may be due to the lower total irradiance in the growth cabinets; the difference in hypocotyl length between the two experiments lends support to this hypothesis. The overall impact for a seedling growing through the stubble thatch might be an initial increase in SLA to increase light interception; on emergence through the stubble layer a reduction in SLA is

likely to reduce the likelihood of water-loss and photo-inhibition.

Differences in root structure, assessed by the ratio of root length to dry mass (SRL) and ratio of root mass to total plant mass (RMR) closely paralleled those of shoots. SRL was lower in plants with long hypocotyls in Experiment 2, but was not different in Experiment 3. In Experiment 2, RMR was higher in plants with long hypocotyls until the appearance of the 3rd leaf, after which the differences disappeared. Interestingly, the rate of decline in RMR was faster in plants with long hypocotyls suggesting that if the experiment had continued then RMR would have been lower in plants with long hypocotyls at later growth stages. Indeed in Experiment 3, which was conducted up to the appearance of the 11th leaf, RMR was lower in plants with long hypocotyls. Hence, plants with a longer hypocotyl had less root mass and in some instances lower SRL than the control plants, suggesting that canola seedlings emerging through a stubble layer would have reduced opportunity to capture water and nutrients compared to seedlings in the absence of stubble. The higher RMR observed in plants with long hypocotyls in Experiment 2 before the appearance of the 3rd leaf is likely to be a result of development stage, low light conditions or a delayed response to the shifting of allocation from the roots to the shoots. Because RMR is an instantaneous measure, a change in partitioning will not be detectable until the increments in mass added to the shoots or hypocotyl have been accumulated sufficiently to shift the ratio. Therefore, it seems likely that the change in RMR at the 5th leaf in Experiment 2 (Figure 4b) was a delayed response to the shifting of allocation from the roots to shoots.

The experiments indicated that an initial period of reduced light increased hypocotyl elongation and consequently reduced the allocation of resources to leaves and roots. It is likely that seedlings emerging through a stubble layer with small leaf areas and leaf mass, and root systems with low total length to weight ratios and root mass would potentially reduce the water, nutrient and light acquisition that is required to support a high RGR (Reich et al., 1998), thereby potentially reducing the RGR compared to seedlings in the absence of a stubble layer.

*Is variation in RGR related to allocation, plant structure or other plant traits?*

The elongation of the hypocotyl led to a decrease in the absolute growth rate (AGR) and relative growth rate (RGR) of canola seedlings. Variation in RGR was associated with both root and leaf morphology (SLA and SRL) and the whole-plant measures LAR, LWR and RMR. Plants with larger root systems, higher leaf mass and higher surface areas for light, CO<sub>2</sub>, water and nutrient acquisition had higher RGRs, while plants that allocated more resources to hypocotyls at the expense of roots and leaves had lower RGRs. Because RGR is the instantaneous product of LAR × NAR, variation in RGR should be a function of these variables. Indeed in Experiment 2, increases in RGR were associated with increases in LAR in plants with short hypocotyls, although NAR did not have an effect on RGR. In Experiment 3, LAR did not have an effect on the RGR between the treatments; instead, increases in both LWR and NAR were observed. It is not surprising that the role of NAR was more important in Experiment 3, as NAR increases with increasing irradiance (Blackman and Wilson, 1951). The later increase in RGR of the plants with long hypocotyls in Experiment 2 to levels similar to that of the controls seems likely to be a result of the low irradiance in the growth cabinets, since the trend for the lower RGR of the longer hypocotyl plants was maintained in Experiment 3 under higher irradiance conditions outside.

*Can hypocotyl elongation explain poor canola growth through stubble? – comparison with field experiments*

A key aim of these experiments was to consider the role that elongated hypocotyls arising from the altered light conditions under retained stubble in the field might play in reducing seedling growth. Table 6 presents a summary of the comparisons using re-calculated field data presented from Part I of this study (Bruce et al., 2006). Hypocotyl length in the field study was recorded from ground level; however, for consistency in this comparison we have adjusted the hypocotyl lengths in Bruce et al., (2006 – Table 2) assuming a seed depth of 10 mm. With this assumption,

Table 6. Comparison of the impact of stubble (stubble vs. bare) in the field experiment (Bruce et al., 2006) and the impact of tubes [50 mm vs. control (0 mm)] in the laboratory experiment on growth parameters of canola cv. Oscar

Variable	Percentage change	
	Field	Laboratory
Leaf number	-12	-40
Hypocotyl length (mm)	+100	+216
Shoot biomass (g/plant)	-65	-56
Total biomass (g/plant)	-65	-57
Root length	-64	-57

Data presented as the percentage change from the bare or control treatments and recalculated from data from Part I, Bruce et al. (2006) and Table 4. Hypocotyl length for the field experiment has been adjusted to account for a sowing depth of 10 mm.

the change in length of the hypocotyl in response to the presence of stubble is similar to the change in length of the hypocotyl in response to the increased tube length (Table 6). Of the seedling growth parameters measured, most responded to a similar extent to the presence of stubble in the field or the presence of the 50 mm tube in the laboratory experiments reported here. This provides strong evidence that hypocotyl elongation alone, and the physiological impacts it has on seedling growth plays an important role in the growth reductions of canola observed in the field experiment (Table 6). Shoot biomass, total biomass and root length were reduced by an average of 65% in the stubble treatments compared to the bare treatments in the field experiment, and a similar effect of stubble on shoot biomass (56% reduction) was observed in other field studies (Bruce et al., 2005). In the current study, the growth reduction caused by the hypocotyls elongation in the 50 mm tubes was on average 57%.

Elongation of the hypocotyl in canola in response to reduced and/or altered quality and quantity of light during emergence, delayed development, caused a re-allocation of resources away from the roots and leaves, and reduced absolute and relative growth rates. The magnitude of the growth reduction in these experiments, in the absence of other potential growth limiting factors such as disease or low temperatures, was similar to that observed in etiolated canola seedlings growing through retained wheat stubble in the field. This suggests that a significant portion of the growth restriction caused by

retained stubble in the field may result from a similar re-allocation of resources to the production and growth of the hypocotyl associated with the altered light quality and quantity caused by stubble. This observation provides important information to assist in the development of strategies to reduce the impact of surface-retained wheat stubble in the field, such as: sowing techniques that push stubble off the seeding row and onto the inter-row; or the rapid screening of canola varieties that have the ability to compensate for early reductions in growth caused by elongation of the hypocotyl.

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