Decoupling of light intensity effects on the growth and development of C_3 and C_4 weed species through sucrose supplementation

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Abstract

Light availability has a profound effect on plant growth and development. One of the ways to study the effects of light intensity on plant growth and development without the confounding problem of photosynthate availability is sucrose injection/supplementation. A greenhouse experiment was conducted to evaluate the effects of light levels (0% and 75% shade) and sucrose injection (distilled water or 150 g sucrose l⁻¹) on three weed species: redroot pigweed (Amaranthus retroflexus L., C_4), lambsquarters (Chenopodium album L., C_3) and velvetleaf (Abutilon theophrasti Medic., C_3). The average total sucrose uptake was 7.6 and 5.9 g per plant for 0% and 75% shading, respectively, representing 47% of the average total weed dry weight. Plants injected with sucrose had greater dry weights and shoot-to-root ratios under both light levels. In spite of sucrose supplementation the reduction in dry matter due to shading was greater for roots and reproductive structures than vegetative shoot tissues, indicating light level regulation of morphological changes resulting in changed C allocation that are independent of photosynthate availability. Dry weights of plants injected with sucrose under 75% shading were not different from distilled water-injected unshaded plants. However, both sucrose-injected and control plants, regardless of their photosynthetic pathways, underwent similar changes in allocation of dry matter and morphology due to shading, suggesting that these effects are strictly due to light intensity and not related to photosynthate availability.

Key words: C_3 weeds, C_4 weeds, light levels, plant development, sucrose supplementation.

Introduction

Light plays a critical role in plant growth and development; both quantity and quality, as well as direction of light, are perceived by photosensory systems which, collectively, regulate plant development, presumably to maintain photosynthetic efficiency (Hangarter, 1997). Plant morphology and the pattern of C allocation are mediated by the quality of light through photoreceptors sensitive either to the red-to-far-red ratio (phytochromes) or to blue light (cryptochromes) (Pearcy and Sims, 1994; Aphalo and Ballaré, 1995; Board, 2001). Depending on plant species there are two types of morphological adaptation. In some plants changes in light quality lead to shade tolerance...
responses characterized by an increase in leaf area ratio and specific leaf area (Bourdot et al., 1984; Peace and Grubb, 1989; Heraut-Bron et al., 1999). Whereas in other plant species, the detection of low red-to-far-red ratios brings several morphological responses, such as increased stem internode elongation and reduced leaf-to-stem dry weight ratio (Ballaré et al., 1991; Aphalo and Letho, 1997); this is usually considered as a shade avoidance response. In the end, plants that are shade avoiders allocate a greater proportion of their C to shoot-support tissues.

Morphological changes can also be triggered by blue light. Gautier and Varlet-Grancher (1996) and Gautier et al. (1997) showed that the decrease in blue light associated with shading in white clover plants brought morphological responses that allowed the leaves to be positioned higher in the canopy. With a leaf area index of 4, the light spectral composition under a canopy is usually characterized by lower blue and red to far-red ratios (R:FR), which is about 1.15 in full sun while under a canopy it is as low as 0.2 (Holmes and Smith 1977a, b).

Photosynthesis, partitioning of photosynthates and growth need to be highly co-ordinated to allow efficient plant development and growth (Kehr et al., 1998). In healthy leaves, sucrose, glucose, fructose, soluble starch, and fructans account for approximately 80% of the total non-structural carbohydrate content (Kehr et al., 1998). The major carbohydrate is sucrose and its content is estimated to be 10–20 times higher than other non-structural carbohydrates. Thus, based on their proportion, sucrose concentrations in sources and sinks play a major role in substrate partitioning, as postulated by Farrar and Gunn (1996).

One way to study the effects of low light intensities on plant development without the confounding effects of reduced photosynthetic rates would be to supply sucrose directly to the plant. During the last 15 years, several researchers (Grabau et al., 1986; Boyle et al., 1991; Ma and Smith, 1992; Ma et al., 1994a, b; Foroutan-pour et al., 1995; Zhou and Smith, 1996; Abdin et al., 1998) have succeeded in supplying exogenous solutions using injection techniques, whereby solutions are injected into the peduncle or stems of crop plants.

An injection system that can supply large amounts of sucrose to shaded plants could replace the reduced carbon that would have been supplied by photosynthesis and allow the examination of other low light-induced effects (changes in plant morphology and dry matter allocation), in the absence of the reduced photosynthate supply effect. In the absence of such effects, an injected plant might be expected to achieve the same size and shape as an uninjected plant under greater light intensities. Thus, the objectives of this work were to determine (a) whether injecting shaded plants with large amounts of sucrose will overcome the effects of shading on plant biomass accumulation and development and (b) whether C₃ and C₄ species respond to the sucrose injection and shading in a similar way.

**Materials and methods**

**Plant materials and seedling growth**

Seeds of lambsquarters (*Chenopodium album L., C₃*) and redroot pigweed (*Amaranthus retroflexus L., C₄*) were collected from a field under corn production at the Emil A Lods Agronomy Research Center of the Macdonald Campus of McGill University in the autumn of 1996. Seeds of velvetleaf (*Abutilon theophrasti Medic. L., C₃*) were also collected from the same research centre, but in the autumn of 1997. These weed species were chosen for this study because they are among the broad-leaf weeds that are most effective in competing for light with crops like soybean and corn, two of the main crops grown in the region. Prior to the start of the experiments, seeds of all weed species were placed in small bottles covered with water and stored at 4 °C for 48 h (stratification) in order to break dormancy (Totterell and Roberts, 1979). Seeds were planted in 11×21 cm trays divided into 32 small sections (5.5 cm deep cells) filled with a mixture of sand and Promix (1:3) (Premier Horticlure L’tee, Rivier-du-Loup, Quebec, Canada). Seedlings were left to grow until the 4-leaf stage and were watered as necessary. At this stage, vigorous seedlings were selected from the trays of each species and transplanted into plastic pots (15.5 cm diameter and 15 cm deep, four seedlings per pot) containing the same rooting medium as the trays. Approximately 2 weeks later the plants were thinned to one per pot and fertilized with 1.5 g l⁻¹ pot⁻¹ of NPK (20-20-20).

**The injection system**

Three days after thinning an injection system was established following the method of Abdin et al. (1998). Briefly, the system was composed of a supporting stand and syringe-tubing system. The needles attached to each end of the Tygon tubing were sealed in place with epoxy resin glue. The vacutainer needle was positioned at about 45° and inserted half way into the plant stem. The needles were sealed to the stems with latex (Vultex, General Latex Canada, QC, Canada). The latex was placed around the injection site in a cup formed by masking tape, and was allowed to set for 4–5 d. The injection systems were carefully tested for leakage and plants without leaks were then put under the various light regimes for injection. The plants were injected with either distilled water or a 150 g sucrose l⁻¹ solution. Uninjected plants were also included as controls on injection effects. The uptake of injected solutions was checked regularly and the syringe barrels were refilled as necessary. The injected plants were also checked regularly to make sure there were no leaks. The injected solutions were forced into the plants using construction type ceramic bricks (approximately 2.7 kg each), placed on top of the syringe plungers. One brick was added each day until reasonable flow rates were reached and, in this experiment, did not require more than 2.5 bricks. The plants were injected continuously for a period of 9 weeks.

**Shading and general growing conditions**

The first experiment tested lambsquarters and redroot pigweed using cages of 70×75×20 cm covered on all sides, except the bottom, with shading cloth (Tek Knit, Montreal, QC, Canada). The light intensity at the canopy level was approximately 700–800 μmol m⁻² s⁻¹, in the absence of shading. The shading level of 75% was calibrated using a Li-Cor a portable light meter (Li 6400). The second experiment included velvetleaf as an additional species. To accommodate the addition of the velvetleaf bigger cages were used; the size was increased to 100×100×200 cm. One side of the cage had a small
Medic grown for 9 weeks.

L. retro¯exus leaf area ratio (leaf area per total plant biomass, cm² g⁻¹), and shoot (shoot plus root), leaf weight ratio (leaf weight per total biomass), fruits), shoot (leaf, stem and seeds plus fruits), and total dry weight assessed by calculating the dry weight of leaf, stem, seeds (including dried for weight determination. Dry matter allocation patterns were separated into leaf, stem, seed plus fruit, and root parts and oven-dried to complete senescence and at final harvest plant height was measured from the soil level to the tip of the stem and the harvested plants were separated into leaf, stem, seed plus fruit, and root parts and oven-dried for weight determination. Dry matter allocation patterns were assessed by calculating the dry weight of leaf, stem, seeds (including fruits), shoot (leaf, stem and seeds plus fruits), and total dry weight (shoot plus root), leaf weight ratio (leaf weight per total biomass), leaf area ratio (leaf area per total plant biomass, cm² g⁻¹), and shoot-to-root ratio (shoot biomass per root biomass). Total uptake (ml) of the injected solutions for the whole injection period was also calculated. All of the above mentioned variables are given on a per plant basis.

Table 1. Multiple pairwise means comparisons for interaction effects between shading levels and injection treatment on uptake of injected sucrose solutions and the size and interrelationships of plant part

The data represent the average of three species, Amaranthus retroflexus L., Chenopodium album L. and Abutilon theophrasti Medic. grown for 9 weeks.

<table>
<thead>
<tr>
<th>Shading level (%)</th>
<th>Injection treatment</th>
<th>Plant height (cm)</th>
<th>Root dry weight (g plant⁻¹)</th>
<th>Shoot dry weight (g plant⁻¹)</th>
<th>Total dry weight (g plant⁻¹)</th>
<th>Leaf area (cm² plant⁻¹)</th>
<th>Uptake (ml)</th>
<th>Shoot to root ratio (g g⁻¹)</th>
<th>Leaf area ratio (cm² g⁻¹)</th>
<th>Leaf weight ratio (g g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>122.6 bᵃ</td>
<td>2.09 b</td>
<td>15.4 b</td>
<td>17.5 b</td>
<td>757.0 b</td>
<td>0.0 e</td>
<td>7.9 c</td>
<td>48.8 b</td>
<td>0.25 b</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>120.1 b</td>
<td>2.51 a</td>
<td>22.6 a</td>
<td>25.1 a</td>
<td>1003.1 a</td>
<td>50.7 c</td>
<td>9.6 b</td>
<td>44.7 c</td>
<td>0.23 bc</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>112.8 c</td>
<td>2.04 b</td>
<td>15.1 b</td>
<td>17.2 b</td>
<td>736.0 b</td>
<td>87.4 a</td>
<td>8.2 c</td>
<td>48.1 b</td>
<td>0.25 b</td>
</tr>
<tr>
<td>75</td>
<td>None</td>
<td>137.0 a</td>
<td>1.41 c</td>
<td>10.1 c</td>
<td>11.6 c</td>
<td>594.3 c</td>
<td>0.0 e</td>
<td>9.0 bc</td>
<td>59.5 a</td>
<td>0.30 a</td>
</tr>
<tr>
<td></td>
<td>Sucrose</td>
<td>135.4 a</td>
<td>1.42 c</td>
<td>16.1 b</td>
<td>17.5 b</td>
<td>669.2 bc</td>
<td>39.6 d</td>
<td>13.5 a</td>
<td>41.5 c</td>
<td>0.20 c</td>
</tr>
<tr>
<td></td>
<td>Distilled water</td>
<td>135.6 a</td>
<td>1.42 c</td>
<td>10.3 c</td>
<td>11.7c</td>
<td>606.5 c</td>
<td>83.1 b</td>
<td>9.0 bc</td>
<td>59.7 a</td>
<td>0.30 a</td>
</tr>
</tbody>
</table>

ᵃ Values, in the same column, followed by the same letter are not different (P <0.05) based on a GLM protected LSD test.

Table 2. Multiple pairwise means comparisons for interaction effects between shading levels and weed species (Amaranthus retroflexus L., Chenopodium album L., and Abutilon theophrasti Medic.) on growth variables

<table>
<thead>
<tr>
<th>Shading levels (%)</th>
<th>Weed species</th>
<th>Plant height (cm)</th>
<th>Stem dry weight (g plant⁻¹)</th>
<th>Root dry weight (g plant⁻¹)</th>
<th>Shoot dry weight (g plant⁻¹)</th>
<th>Total dry weight (g plant⁻¹)</th>
<th>Shoot to root ratio (g g⁻¹)</th>
<th>Leaf weight ratio (g g⁻¹)</th>
<th>Leaf area ratio (cm² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Redroot pigweed</td>
<td>91.0 cᵃ</td>
<td>9.0 b</td>
<td>1.93 c</td>
<td>15.4 c</td>
<td>17.3 b</td>
<td>8.8 c</td>
<td>0.24 c</td>
<td>43.8 c</td>
</tr>
<tr>
<td></td>
<td>Lambquarters</td>
<td>125.9 b</td>
<td>11.6 a</td>
<td>2.21 b</td>
<td>19.5 a</td>
<td>21.7 a</td>
<td>9.7 bc</td>
<td>0.23 c</td>
<td>40.7 c</td>
</tr>
<tr>
<td></td>
<td>Velvetea</td>
<td>138.4 a</td>
<td>9.6 b</td>
<td>2.50 a</td>
<td>18.3 b</td>
<td>20.8 a</td>
<td>7.3 d</td>
<td>0.27 b</td>
<td>57.1 b</td>
</tr>
<tr>
<td>75</td>
<td>Redroot pigweed</td>
<td>129.0 b</td>
<td>7.1 c</td>
<td>1.28 d</td>
<td>10.7 f</td>
<td>12.0 e</td>
<td>10.2 b</td>
<td>0.22 c</td>
<td>44.5 c</td>
</tr>
<tr>
<td></td>
<td>Lambquarters</td>
<td>152.2 a</td>
<td>9.0 b</td>
<td>1.12 d</td>
<td>14.2 d</td>
<td>15.3 c</td>
<td>14.9 a</td>
<td>0.23 c</td>
<td>43.6 c</td>
</tr>
<tr>
<td></td>
<td>Velvetea</td>
<td>127.8 b</td>
<td>5.8 d</td>
<td>1.85 c</td>
<td>11.6 e</td>
<td>13.4 d</td>
<td>6.3 d</td>
<td>0.34 a</td>
<td>72.7 a</td>
</tr>
</tbody>
</table>

ᵃ Values, in the same column, followed by the same letter are not different (P <0.05) based on a GLM protected LSD test.

Door that could be opened and closed to aid in data collection. The research was conducted in the greenhouse of Plant Science Department of McGill University. A photoperiod of 16 h was maintained with high-pressure sodium lamps to extend the daylength when required. Day/night temperatures were 25/20±3 °C, respectively, at a relative humidity of 60±5%. Each treatment had four plants.

Experimental design, data collection and data analysis

The experiment was designed as a split-split-plot with three blocks. Shading level (0% and 75%) was the main plot and injected solutions (distilled water and sucrose) was the subplot. The three weed species were treated as sub-subplot factors. To determine the leaf areas of the weeds, a random collection of leaves was made for each treatment at plant maturity. The areas of these leaves were measured using leaf area meter (Delta-T Devices Ltd., Cambridge, UK). Total leaf area of each treatment was calculated based on the leaf area and the injected solutions for the whole injection period was also calculated. All of the above mentioned variables are given on a per plant basis.

Data from the two weed species included in both experiments (i.e. redroot pigweed and lambsquarters) were pooled when the hypothesis of homogeneity of variances and means were tested and accepted by a Bartlett’s test (Steel and Torrie, 1980) and thereafter all data were subjected to analysis of variance with the PROC GLM procedure of SAS (SAS Institute, 1994). Multiple-pairwise means comparisons were made with a general linear models (GLM) analysis protected LSD test at the 0.05 probability level (Steel and Torrie, 1980).

Results

The average total uptake of injected sucrose was 50.7 and 39.6 ml for 0% and 75% shading levels, respectively; this represented 7.6 g and 5.9 g of sucrose per plant for the shaded and unshaded conditions, respectively (Tables 1, 2). In this study, the amount of injected sucrose, averaged over light levels and plant species, represented 47% of total plant dry weight. The uptake of injected solutions, averaged over shading levels, was lower for lambsquarters and redroot pigweed than for velvetea (data not shown). Under both the light levels, biomass, above-ground biomass in particular, was much greater for plants injected with sucrose than plants injected with distilled water or non-injected plants (Table 2). Shaded plants produced less biomass than unshaded plants as a result of reduced light availability for carbon assimilation; however, shaded
plants seemed to benefit to a greater degree from the availability of an additional reduced carbon source (injected sucrose) than unshaded plants. With the availability of a large alternative carbon source, sucrose-injected shaded plants were as large as unshaded, distilled water-injected or uninjected plants (Table 2). Thus, heavily shaded plants can achieve the same degree of biomass production as plants grown in full sun when supplemented with reduced carbon.

The morphological traits of all the three plant species were affected by shading. Lambsquarters, a C3 plant, and redroot pigweed, a C4 plant, were much taller under shade than full sunlight. Although redroot pigweed plants were shorter than lambsquarters, their response to reduced light intensity was greater (41% versus 20% taller) (Table 1). As expected, both above- and below-ground dry weight of weed species, regardless of their photosynthetic pathway, were much greater for plants under full sunlight than under shade (Table 1). Under shade, both C3 and C4 plants allocated a greater proportion of their assimilates to shoots.

There were no differences amongst injection treatments (distilled water, sucrose, or not injected) for plant height at both light levels (Table 1). This may be partially explained by a greater allocation of assimilates to leaves, seeds, and stems with no increase in overall stem elongation, when sucrose was injected (Tables 1, 3). Under both light levels shoot and total dry weight were greater for sucrose-injected treatments. However, the root dry weights of sucrose-injected plants were higher only for full sunlight plants, while root dry weights of plants under lower light levels were unchanged (Table 1). Overall, the values of these variables were greater for lambsquarters than for redroot pigweed and velvetleaf (Table 2). Seed dry weight, averaged over injection treatments and weed species, was reduced by as much as 53% by shading (Table 3).

Under both light regimes, leaf area generally increased due to sucrose injection. However, the increase due to sucrose supplementation was much greater for unshaded than shaded plants (25% versus 12%), (Table 1). A higher average leaf area was recorded for the two C3 species (velvetleaf, 909.9 cm²; lambsquarters, 707.9 cm²) than the C4 species (redroot pigweed, 565.3 cm²). Although the leaf area of lambsquarters was greater than that of the redroot pigweed, under both light levels, the leaf weight and leaf area ratios of redroot pigweed were generally similar to those of lambsquarters, whether the plants were sucrose supplemented or not (Table 1). However, the leaf weight and leaf area ratios of the C3 species velvetleaf were higher under shade than full sunlight conditions.

### Discussion

In this study, the amount of injected sucrose, on average, represented 47% of total plant dry weight. The injection of similarly large proportions of plant carbon, such as sucrose, has been previously reported (Abdin et al., 1998). In their study they reported that injected sucrose made up as much as 65% of the plant dry weight of soybean plants. Shaded plants injected with sucrose produced as much biomass as unshaded plants that were not injected, suggesting that the responses to light level were through direct light level detection, and not related to photosynthetic activity. Wilson et al. (1998) reported an increase in dry mass and leaf area due to sucrose addition to growth media, but a decrease in soluble sugars in stems of broccoli seedlings during storage regardless of illumination although levels remained higher in illuminated seedlings than seedlings in the dark. They postulated that red light, and not white or blue light, was the main reason for increasing or maintaining leaf and stem soluble sugars in illuminated plants. Several researchers have reported greater above- and below-ground biomass for unshaded than shaded plants (Edward and Myers, 1989; Kolb and Steiner, 1990; Messier, 1992).

Light plays a decisive role in morphogenesis and resource allocation pattern in plants. It is well-established that shaded plants allocate much of their photosynthates into the shoot structures to allow interception of more light. Robin et al. (1992) reported a morphogenetic response of clover to changes in light quality in the form of an increase in the proportion of C allocated to shoot tissues associated with "light foraging". This decreased the C available for root growth. Generally, the plant species evaluated here showed a decrease in assimilate allocation to root and reproductive parts of the plants in response to reduced light availability, which is also the typical strategy of plants adapting to shade (Tables 1, 2, 3). This was due to an allocation of more dry matter to vegetative than reproductive structures when light intensity was lower, even with the addition of sucrose as an external C source.

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**Table 3. Overall main effects of weed species, injection treatment, and shading levels on leaf area, stem and seed dry weights**

<table>
<thead>
<tr>
<th>Weed species</th>
<th>Leaf area (cm²)</th>
<th>Injection treatment</th>
<th>Stem dry weight (g)</th>
<th>Shading level (%)</th>
<th>Stem dry weight (g)</th>
<th>Seed dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Redroot pigweed</td>
<td>565.30 a</td>
<td>None</td>
<td>7.25 b</td>
<td>0</td>
<td>10.11 a</td>
<td>3.20 a</td>
</tr>
<tr>
<td>Lambsquarters</td>
<td>707.89 b</td>
<td>Sucrose</td>
<td>11.68 a</td>
<td>75</td>
<td>7.30 b</td>
<td>1.68 b</td>
</tr>
<tr>
<td>Velvetleaf</td>
<td>909.86 a</td>
<td>Distilled water</td>
<td>7.19 b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* a Values, in the same column, followed by the same letter are not different (P <0.05) based on a GLM protected LSD test.*
Bello et al. (1995) found up to 94% reduction in seed production by velvetleaf grown under 76% shade. Similar adjustments in response to reduced light has been reported in soybean, lambquarters, velvetleaf, eastern black nightshade, and tumble pigweed (Stoller and Myers, 1989). Allard et al. (1991) reported a reduction in dry matter for tall fescue under reduced irradiance relative to plants under high irradiance, while the shoot to root ratio and leaf area ratio were higher for plants grown at low irradiance than for those grown at high irradiance.

In conclusion, despite the presence of substantial levels of exogenously supplied photosynthate, and regardless of photosynthetic pathway differences, light intensity played a key role in the development and morphology of the plant species that was independent of growth (increase in biomass). When plants were provided with sucrose as an additional source of reduced carbon, they weighed more than uninjected plants. When shaded plants were injected with sucrose, shading effects on growth were overcome, while shading effects on development were not substantially affected. Reductions in dry matter due to shading were more pronounced for below-ground and reproductive parts than for leaves and stems. Interestingly, C3 and C4 species exhibited similar responses to shading and an alternative source of photosynthate. Thus, although sucrose injection provided an alternative source of carbohydrate large enough to overcome the effects of heavy shading on plant biomass accumulation, it did not alter the low light intensity-induced effects on plant development, morphology and dry matter allocation patterns. This study has provided the first report of a successful decoupling of low light effects on photosynthesis and other light processes that control plant responses. The findings reported herein suggest that photosynthate availability is less important to morphological plant responses than light intensity.

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