

## The effect of canopy filtered light on the growth of white clover *Trifolium repens*

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**Summary.** Plants of white clover (*Trifolium repens*) were grown under canopies of clover leaves floating on shallow glass tanks of water and their growth was compared with that of plants under canopies of black polythene 'leaves'. The experimental design allowed the growth of the clover plants in canopy filtered light to be compared with that in unfiltered light at different intensities of Photosynthetically Active Radiation (P.A.R.). The effect of canopy filtered light was to exaggerate the effects of reduced P.A.R. especially in promoting petiole extension and inhibiting stolon branching. Two clones of white clover differed in their responses to P.A.R. and to light quality and there were significant interactions between the effects of the intensity of P.A.R. and type of shade. It is argued that it may be important for both ecologists and plant breeders to recognise the role of radiation quality in regulating the dynamics of pasture.

**Key words:** High quality – shade – Photomorphogenesis – Canopy filtered light – *Trifolium repens*

Most early studies of the effect of natural shading on plant growth concentrated on the role of different intensities of visible radiation (e.g. Blackman and Wilson 1951; Black 1957; Blackman and Black 1959). It was repeatedly demonstrated that the intensity of visible radiation diminished down through a canopy with often characteristic attenuation curves (Black 1960).

The attenuation curves have been studied in swards of *Trifolium* species by Brougham (1962), Black (1960), Stern (1960) and Harper and Clatworthy (1963) and in mixed grass clover swards by Stern and Donald (1962). In pure clover swards the leaf laminae are concentrated in a narrow zone and the intensity of visible radiation attenuates rapidly in the surface layers of the canopy. In a grass dominated sward, in contrast, attenuation is more gradual from the top to the bottom of the canopy.

Most studies on the activity of leaf canopies have focussed on the role of light as a resource factor in which mutual shading accounts for different photosynthetic rates of leaves held at different levels in the canopy (e.g. Donald 1963). However red and far-red wave bands have now been shown to affect germination (e.g. Borthwick et al. 1954) and other aspects of plant growth. A widely used measure

of the relevant spectral composition of radiation is the ratio ( $\zeta$ ) of the quantum flux in 10 nm wide wave bands in the red (660 nm) and far red (730 nm) (Monteith 1976; Holmes and McCartney 1976).

The average value of  $\zeta$  obtained by different investigators in natural daylight is 1.1 (Holmes and Smith 1977a; Holmes and McCartney 1976). More recently lower values have been reported e.g. 0.86 (Mitchell 1984). Values of  $\zeta$  remain remarkably constant over a wide range of conditions except at sunrise and sunset (Holmes and McCartney 1976; Monteith 1976; Holmes and Smith 1977a). When natural radiation passes through a vegetation canopy the red and blue wavelengths are preferentially absorbed, but far-red light is wholly transmitted so that  $\zeta$  values are reduced. Under canopies values of  $\zeta$  range from 0.09 to 0.97 depending on the type of vegetation and the Leaf Area Index (Smith 1982; Holmes and McCartney 1976; Holmes and Smith 1977b).

Most studies of the morphogenetic effects of  $\zeta$  have been made under quite unnatural conditions, e.g. brief light pulses, dark grown plants, monochromatic radiation, extended end-of-day treatments and produce effects that may be quite different from those observed in the field (McCree 1976; Young 1974).

In this paper we describe experiments made to study the effects of canopy filtered light on the branching pattern of *Trifolium repens*. It is known (e.g. Solangaarachchi 1986) that, when clover plants grow under grass (or clover) canopies, stolon branching is inhibited although the extension rate of the main stolons is barely affected. We were concerned to ask whether the suppression of branching was caused by reduced intensity of radiation, changed light quality or by more complex competitive interactions-e.g. between root systems.

The main problem with using real canopies to produce canopy filtered radiation is that real canopies are attached to roots and it is not easy to distinguish canopy effects from the effects of root competition. We therefore designed an experiment in which we used detached fresh leaves of clover, floating in shallow tanks of water to simulate a canopy. We then grew plants of *T. repens* beneath these floating canopies and determined the effects on clover growth form. To distinguish between the effects of light quality and quantity we also grew clover plants under floating canopies of black polythene 'leaves'. The densities of real and artificial leaves in the tanks could be adjusted so that P.A.R. was the same but  $\zeta$  differed. Black polythene

**Table 1.** Measures of the growth of clover and values of  $\zeta$  under canopy tanks with no leaves (control) or with fresh leaves of clover or black polythene 'leaves' adjusted to give 50% or 30% of the values of P.A.R. under control tanks. Values of  $\zeta$  were compared with Tukey's HSD value and values sharing the same letter do not differ significantly at  $P > 0.05$ . Results are for the experiments started on 30 August 1984 (underlined), 11 October 1984 (b) and 20 June 1985 (italics).

| Variable             | Control                                  | Polythene 'leaves' P.A.R.                 |  | Clover leaves P.A.R.                     |  |
|----------------------|--|---|--|--|--|
|                      |  | 50%                                       | 30%                                      | 50%                                      | 30%                                      |
| Red/far-red          | 0.83 a<br><u>0.84 a</u><br><i>0.87 a</i> | 0.84 ad<br><u>0.87 a</u><br><i>0.92 a</i> | 0.85 d<br><u>0.84 a</u><br><i>0.91 a</i> | 0.70 b<br><u>0.74 b</u><br><i>0.66 b</i> | 0.63 c<br><u>0.64 c</u><br><i>0.55 c</i> |
| Main stolon          |  |   |  |  |  |
| Node number          | 10.63<br><u>13.75</u><br><i>13.83</i>    | 8.75<br><u>11.33</u><br><i>12.44</i>      | 8.25<br><u>10.56</u><br><i>10.79</i>     | 8.50<br><u>11.38</u><br><i>11.80</i>     | 6.50<br><u>10.25</u><br><i>10.11</i>     |
| Length (cm)          | 15.46<br><u>21.43</u><br><i>23.41</i>    | 10.63<br><u>15.33</u><br><i>23.25</i>     | 8.97<br><u>10.53</u><br><i>19.15</i>     | 9.73<br><u>15.25</u><br><i>21.57</i>     | 4.19<br><u>10.59</u><br><i>18.17</i>     |
| Branched nodes       | 7.63<br><u>10.66</u><br><i>10.90</i>     | 3.63<br><u>5.41</u><br><i>8.42</i>        | 2.38<br><u>2.94</u><br><i>6.64</i>       | 2.25<br><u>4.19</u><br><i>8.02</i>       | 0.38<br><u>2.00</u><br><i>4.22</i>       |
| Rooted nodes         | 8.88<br><u>11.99</u><br><i>11.57</i>     | 6.88<br><u>7.04</u><br><i>9.80</i>        | 5.44<br><u>6.00</u><br><i>8.61</i>       | 5.75<br><u>7.55</u><br><i>8.95</i>       | 1.94<br><u>5.81</u><br><i>7.97</i>       |
| Internode length     | 2.12<br><u>2.20</u><br><i>2.15</i>       | 1.76<br><u>1.69</u><br><i>2.39</i>        | 1.61<br><u>1.26</u><br><i>2.43</i>       | 1.65<br><u>1.74</u><br><i>2.46</i>       | 0.91<br><u>1.49</u><br><i>2.41</i>       |
| Petiole length (cm)  | 9.94<br><u>10.81</u><br><i>10.57</i>     | 9.93<br><u>10.77</u><br><i>13.73</i>      | 10.27<br><u>10.92</u><br><i>14.22</i>    | 11.04<br><u>12.35</u><br><i>15.20</i>    | 10.30<br><u>13.16</u><br><i>17.03</i>    |
| Whole plant          |  |   |  |  |  |
| Total leaf area      | 73.6<br><u>113.6</u>                     | 28.8<br><u>41.6</u>                       | 20.4<br><u>27.4</u>                      | 24.7<br><u>44.4</u>                      | 10.7<br><u>24.7</u>                      |
| Mean area per leaf   | 3.70<br><u>2.90</u>                      | 2.69<br><u>1.87</u>                       | 2.38<br><u>1.89</u>                      | 3.01<br><u>2.18</u>                      | 1.99<br><u>2.03</u>                      |
| Total node number    | 75.28                                    | 52.71                                     | 28.06                                    | 33.86                                    | 18.90                                    |
| Dry weight of leaves | 0.81                                     | 0.39                                      | 0.20                                     | 0.32                                     | 0.18                                     |

leaves (cut to the shape and size of an average clover leaf) were used to absorb radiation over the whole relevant range without affecting its quality.

### Materials and methods

Five canopy tanks 100 × 100 cm in area and 8 cm deep were constructed from glass 5.5 mm thick. The tanks contained 1 cm depth of water and were supported 60 cm above the ground in a glasshouse maintained at ca. 20° C. The effect of the water and glass of the tanks on the P.A.R. beneath them was measured using the Crump Cat. No 550 Quantum/Radiometer/Photometer. P.A.R. was reduced by ca. 15% beneath the tank. Values of  $\zeta$  above and below the tanks were determined using a Multisensor (the design and calibration of which are described by Woodward 1983 and Mitchell 1984). Values of  $\zeta$  were  $0.829 \pm 0.005$  above the tank surface and 0.803 below. The values of  $\zeta$  above the tank are close to those obtained by Mitchell for natural radiation using a Multisensor of the same design. The differences in  $\zeta$  above and below the tanks were very small

compared to those produced by the presence of floating leaves. Values of  $\zeta$  beneath the tanks were not altered by the canopies of floating polythene 'leaves'. Values of  $\zeta$  were reduced under canopies of fresh clover leaves and scarcely altered over a period of 4 days.  $\zeta$  increased if leaves were left for more than 4 days.

The tanks were allocated to the following 5 treatments: (1) Control; a canopy tank with water but without leaves, (2) A canopy of black polythene leaves to give 45–50% P.A.R., (3) A canopy of clover leaves to give 45–50% P.A.R., (4) A canopy of black polythene leaves to give 25–30% P.A.R., (5) A canopy of clover leaves to give 25–30% P.A.R. Each % P.A.R. value was obtained by adding 'leaves' to the tanks. Both natural and artificial leaves were renewed at 4-day intervals. Algal growth in the water was prevented by adding an algicide. Tanks were surrounded by black polythene to ground level so that experimental plants grown beneath them received light only through the tanks.

Plants of white clover were grown for the experiments

**Table 2.** Significance levels of the effects of treatments and interactions in the three experiments started on 30 August 1984 (a), 11 October 1984 (b) and 20 June 1985 (c).

## Treatment

1 = Control compared to shaded treatments.

2 = Genotypes × (control compared with shading and interaction between P.A.R. level and type of shade).

3 = 50% P.A.R. compared with 30% P.A.R.

4 = Leaf shade compared with polythene shade.

5 = Genotype × (50% P.A.R. compared with 30% P.A.R.)

6 = Genotype × (leaf shade compared with polythene shade).

7 = Interaction between P.A.R. level and type of shade

| Treatments           |   | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|----------------------|---|----|----|----|----|----|----|----|
| Degrees of freedom   |   | 1  | 2  | 1  | 1  | 1  | 1  | 1  |
| Main stolon          |   |    |    |    |    |    |    |    |
| Node number          | a | ** | NS | ** | ** | ** | NS | ** |
|                      | b | ** | NS | ** | NS | ** | NS | NS |
|                      | c | ** | ** | ** | ** | NS | NS | NS |
| Length               | a | ** | *  | ** | ** | NS | NS | ** |
|                      | b | ** | NS | ** | NS | NS | ** | NS |
|                      | c | ** | ** | ** | ** | ** | NS | NS |
| Branched nodes       | a | ** | ** | ** | ** | NS | NS | ** |
|                      | b | ** | NS | ** | ** | NS | NS | NS |
|                      | c | ** | ** | ** | *  | ** | NS | NS |
| Rooted nodes         | a | ** | ** | ** | ** | NS | NS | ** |
|                      | b | ** | NS | ** | NS | NS | NS | NS |
|                      | c | ** | ** | ** | ** | NS | NS | NS |
| Internode length     | a | ** | ** | ** | ** | NS | NS | ** |
|                      | b | ** | *  | ** | ** | NS | *  | NS |
|                      | b | ** | ** | NS | NS | ** | ** | NS |
| Petiole length       | a | *  | *  | NS | ** | ** | NS | ** |
|                      | b | ** | ** | *  | ** | ** | NS | NS |
|                      | c | ** | ** | ** | ** | ** | *  | ** |
| Total leaf area      | a | ** | NS | ** | ** | NS | NS | NS |
|                      | b | ** | NS | ** | NS | NS | NS | NS |
| Mean area per leaf   | a | ** | ** | ** | NS | ** | NS | ** |
|                      | b | *  | NS | NS | ** | NS | NS | NS |
| Total node number    | c | ** | ** | ** | ** | NS | ** | ** |
| Dry weight of leaves | c | ** | ** | ** | ** | ** | NS | *  |

\* =  $P < 0.05$ ; \*\* =  $P < 0.01$ ; NS = not significant. Analyses were carried out on square root transformed data. In experiments (a) and (b) total degrees of freedom were 70 and in experiment (c) were 329

as single node cuttings in compartmented trays filled with John Innes No 1 compost. Cuttings were taken from two clones which differed in the compactness of the swards that they formed when grown in field plots. Both clones had been obtained from an old permanent pasture at Henfaes, Abergwyngregyn, Near Bangor. After 14 days, when the transplants bore 3–4 leaves, uniform plants were selected and transplanted to trays  $22 \times 17.5 \times 5$  cm filled with the same compost. After a 3 day recovery period the trays were placed under the canopy tanks. Eight replicates of each of the two genotypes were used in each treatment. Plants were watered on alternate days and the position of trays under the canopy was rerandomised every four days.

The experiment was repeated on three occasions, 30 August to 25 September, 1984, 11 October to 11 November, 1984 and 20 June to 18 July, 1985. For the last experiment further canopy tanks had been constructed and the experiment then involved using two replicate tanks per treatment and more replicate plants per treatment.

### Experimental results

The results of the three experiments are given in Table 1 and the relevant significance levels in Table 2. The black polythene 'leaves' had no detectable effect on  $\zeta$  but the clover leaf canopies produced the expected shifts in  $\zeta$  which were more pronounced under the thicker canopy. The response of the clover plants to the treatments was generally similar in the three experiments although they were carried out at different seasons of the year – the second experiment was made in late autumn when the level of incident radiation was low. The third experiment was made in summer when P.A.R. was high and involved the more sensitive experimental design.

For all the variables measured there were significant differences between clover growth in the controls and the shaded treatments. The clover made greater growth under the control treatment in every measured respect except that the lengths of internodes and petioles were shorter.

The responses of the two clones differed significantly averaged over all treatments. Clone A developed more nodes, more branched nodes, and longer stolons.

At the same levels of P.A.R. the type of shade (black polythene or real clover leaves) had significant effects on all the variables. Mean petiole length was longer under the shade of clover leaves than under polythene 'leaves'. Most other variables were depressed more under a clover canopy than under polythene 'leaves'. The overall impression is that the effect of shading with a real canopy was to exaggerate the effects of shading with polythene 'leaves' at the similar levels of P.A.R. used.

The major differences in the response of clover to the two types of canopy occurred at the lower values of P.A.R.. The most dramatic of these differences was in the extent of branching and in the total number of nodes developed. Under the denser clover canopy branching was almost wholly suppressed.

### Discussion

The abundance of clover in grassland declines when a sward is allowed to grow tall and can be increased by heavy grazing—especially in late spring and summer (see e.g. the classic experiments of Jones (1933)). Solangaraachchi (1986) has made detailed maps of the growth of clover clones as they meet and grow into each other or as they grow into grass swards. In both cases clover changes from a richly branched habit to a largely linear growth form as the stolons enter the canopies of neighbours. At the same time petiole length tends to increase and often also the length of stolon internodes. The results of the experiments reported here suggest that these effects are due *both* to changes in the level of photosynthetically active radiation *and* to changes in light quality.

The use of canopy tanks in this experiment allows the effects of real canopies to be separated from the effects of root competition which have made the interpretation of interactions in real swards so difficult. Clearly there are problems with canopy tanks. The levels of P.A.R. reaching the plants are reduced by the layer of glass and water by ca 15% and  $\zeta$  is also changed, though only slightly, from 0.83 to 0.80, compared with the reduction to 0.55–0.64 under the denser canopies. The more severe problem comes from the need to float the real and simulated leaves on a water surface which confines them to a monolayer. It may be possible to design more sophisticated canopy tanks in which petioled leaves are held upright in the tanks so that deeper canopies can be produced. This would allow more realistic Leaf Area Indexes to be used in such studies. The great advantage of canopy tanks is that they give changes in light quality that are real leaf filtration effects. They do not assume which wavelengths are relevant and – perhaps as important – they produce a dappled shade effect that is a more realistic reproduction of the field conditions.

It was a feature of the results of this experiment that despite its simplicity of design, highly significant interactions were detected. The effects of altering P.A.R. and the nature of the canopy were not simply additive. This might be expected since the change in spectral quality of incident radiation caused by the leaf shade increases as P.A.R. decreases. Moreover there were on occasions strongly significant interactions between genotypes and both the level and type of shading. The results of this study suggest that a

clover breeder anxious to improve the persistence of clover might well concentrate on the selection of genotypes that maintain active branching at low  $\zeta$  levels.

There is recent evidence that the dormancy of grass buds may also be controlled by light quality. Deregibus et al. (1985) showed that the tillering of grasses in a sward could be enhanced by changing the near red/far red ratio under the sward by supplementing the near red radiation in the base of the sward with light-emitting diodes. It may be that our understanding of the behaviour of grass swards has been misled by assuming that mutual shading effects are due to changes only in the intensity of radiation and neglecting its quality.

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