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Effects of Light Availability on Attached *Rhinanthus minor* (L.), an Angiospermatic Root Hemiparasite

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We studied the effect of light availability on the growth of an angiospermatic root hemiparasite, *Rhinanthus minor*. When attached to its host, height growth increased in response to shading, demonstrating that *R. minor* was able to detect alterations in light quality and/or quantity. However, this reduced illumination did not affect its biomass, number of haustoria, or the amount of ^{15}N transferred from the hosts, compared with its performance under non-shaded conditions. Therefore, *R. minor* is unlikely to have difficulty in extracting host resources under shading. This result may have been mediated by a lowered *R. minor* transpiration rate in response to fluctuations in external conditions, including shading and water stress, compared with non-parasitic plants. Therefore, we suggest that, as long as the extent of resources diverted from host to parasite is not significantly altered by shading, growth of the attached *R. minor* will be unaffected by reduced light availability *per se*.

Keywords: angiospermatic root hemiparasite, haustoria, light availability, *Rhinanthus minor*

Rhinanthus minor plants not only possess their own photosynthetic machinery (Seel et al., 1993; Press and Graves, 1995), but they also perform their own photosynthesis (Press et al., 1988; Seel and Press, 1994). However, the extent to which this contributes to their growth is not well understood. Because photosynthetic rates for *R. minor* clearly show a broad, positive relationship with growth (Seel et al., 1993), we would anticipate that both parameters are affected by light availability. Indeed, the lower survival rate of *R. minor* seedlings in dense host vegetation has been attributed to a decrease in light availability due to shading, subsequently leading to intense competition between *R. minor* seedlings (van Hulst et al., 1987). However, there have been no studies to determine the extent to which *R. minor* possesses traits enabling it to survive in the shade of its hosts and neighboring individuals.

Grime (1979) has described two basic responses of plant species to shade: 1) "shade avoiders" generally invest more biomass to stem elongation at the cost of leaf development, thereby providing the newer leaves with a greater chance to escape shade (Smith, 1978, 1981); or 2) "shade-tolerant" plants show characteristically slower growth rates, and utilize structural and biochemical adaptations that enhance the efficiency of photosynthetic energy transmission and reduce respiratory carbon loss (Boardman, 1977; Grime, 1979). Plants use chlorophyll and phytochrome pigments to detect shade by sensing changes in light quantity and quality (e.g., Red/Far-Red light ratio), respectively (Smith, 1982). Once a change has been sensed, the growing plants can then react to shade with morphological alterations, including stem elongation (Morgan and Smith, 1981; Maliakal et al., 1999). This phenomenon may also be true for *R. minor* plants in the field, e.g., increased height growth in response to the presence of tall host vegetation, compared with plants in plots from which the aboveground portions of the host vegetation have been removed (Hwangbo, 2000). Thus, *R. minor* might respond to alterations in light quality and/or quantity by growing taller and adopting a "shade-avoidance" strategy. It is also possible that, because *R. minor* extracts from its host some of the resources needed for growth (Seel and Jeschke, 1999), it might be able to tolerate a reduction in irradiance availability through increased dependence on host-derived materials, thereby compensating for the resources limited by shade. This latter scenario would demonstrate a "shade-tolerance" strategy.

The objectives in this study were to investigate how *R. minor* avoids and/or tolerates shade, and to determine whether its growth would be negatively affected when light availability was restricted to it, but not to

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its host. We used a ^{15}N tracer supplied to the host to determine whether *R. minor* growing under shade was more dependent on host-derived resources. Soil nitrogen was unavailable to *R. minor*, so the parasite plants had no alternative but to extract host-derived nitrogen. In general, the variation in the amount of ^{15}N present in the parasite should indicate the flux of solutes derived from the host. Much of the N transferred from host to parasite is in the form of amino acids (Seel and Jeschke, 1999). Nitrogen nutrition for xylem-tapping parasites (including *R. minor*) involves a carbon flux to the parasites equal to approximately three times the nitrogen flux (Raven, 1983). Therefore, ^{15}N tracers would also allow indirect measurement of the amount of carbon transferred from hosts by both shaded and unshaded *R. minor*.

MATERIALS AND METHODS

Seeds of *Poa pratensis* were sown in trays of washed sand on 4 December 1998. After germination, the seedlings were transferred to individual split-pots (two 1-L pots taped together) on 25 January 1999. 50 mL of 3 mM nitrate-based Long Ashton solution (50% strength) was applied three times a week to the side of the pot that would not be receiving *R. minor*. The glasshouse temperature ranged from 18 to 25°C. Host plants were infected with *R. minor* on 30 April. Afterward, green-colored polyethylene mesh was shaped into boxes (10 × 10 × 25 cm), which were then used to shade selected *R. minor* plants beginning on 2 June. A 12-h photoperiod extended from 0800 to 2000 h. Between 11 June and 29 June, 50 mL of 3 mM N Long Ashton solution containing ^{15}N-enriched KNO_3 (10 atom %) was supplied a total of eight times to the halves of the split-pots without the parasite. Consequently, the *R. minor* plants were completely dependent on host-derived nitrogen, including ^{15}N, during this shading period.

We used a quantum meter (Skye Instruments, UK) to determine the Red/Far-Red (R/FR) ratio of daylight to shade between 1400 and 1500 h on 24 June. From 27 June (2200 h) to 28 June (2000 h), we measured, on an hourly basis, the leaf temperatures of the shaded and non-shaded *R. minor*, as well as the air temperature, relative humidity (RH), and light intensity in the shaded and non-shaded environments of the plants, using a Data-Hog (Skye Instruments, UK). VPD (vapor pressure deficit between the evaporating surface and the air above) of the two environments was calculated from the data for RH, and air and leaf temperatures (Pearcy et al., 1991):

$$\text{RH (\%)} = (e_a \times 100)/e_s$$

$$\text{VPD (kPa)} = e_l - e_a$$

where,

e_a = the actual water vapor pressure present in the atmosphere at a given air temperature;

e_s = the water vapor pressure in a saturated atmosphere at a given air temperature; and e_l = the saturated water vapor pressure of a leaf at a given leaf temperature.

Final heights of the *R. minor* with and without shading were measured on 30 June. All plant materials (host and parasite) were harvested on 1 July, and were separated into shoot and root portions. The number of haustoria was recorded prior to weighing, and the plant materials were then oven-dried at 80°C to a constant weight. Each tissue type was ground separately in a ball-mill grinder (Retsch, Germany), and carbon and ^{15}N contents were analyzed with a mass-spectrometer (Europa Scientific, UK). All data were statistically analyzed by Minitab software (one-way ANOVA).

RESULTS

Growth Conditions

During the day, the level of irradiance inside the mesh boxes was less than half of ambient (Fig. 1a). RH was maintained between 40 and 60% during the daytime, and from 80 to 100% at night, irrespective of the shading treatment (Fig. 1b). The air temperatures ranged from 20 to 25°C (day) and 15 to 20°C (night) for both treatments (Fig. 2a). Leaf temperatures of the shaded plants were slightly lower than those of the non-shaded plants during the daytime, but that difference disappeared at night (Fig. 2b). The ambient, daytime vapor pressure deficit (VPD) was higher than within the shade boxes (Fig. 3a). Shading induced negative VPD values at night (Fig. 3a). Compared with the ambient readings, the shaded *R. minor* plants showed significantly reduced Red/Far-Red light ratios (R/FR) (Fig. 3b).

Growth and Nutrient Status of Attached *R. minor*

Parasite biomasses and the number of haustoria did not differ between *R. minor* grown with and without shading (Table 1). However, height was significantly increased in response to shading (Fig. 4). Likewise,

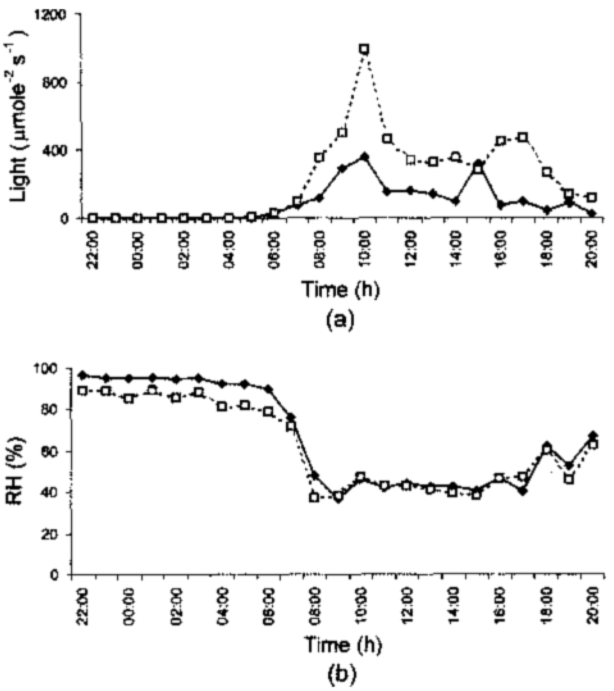

Figure 1. Variations in (a) light, (b) RH of shaded (solid-line) and non-shaded (dotted-line) environments. Measurements were made at one minute interval and averaged over an hour between 27th (22:00) and 28th (20:00) June 1999.

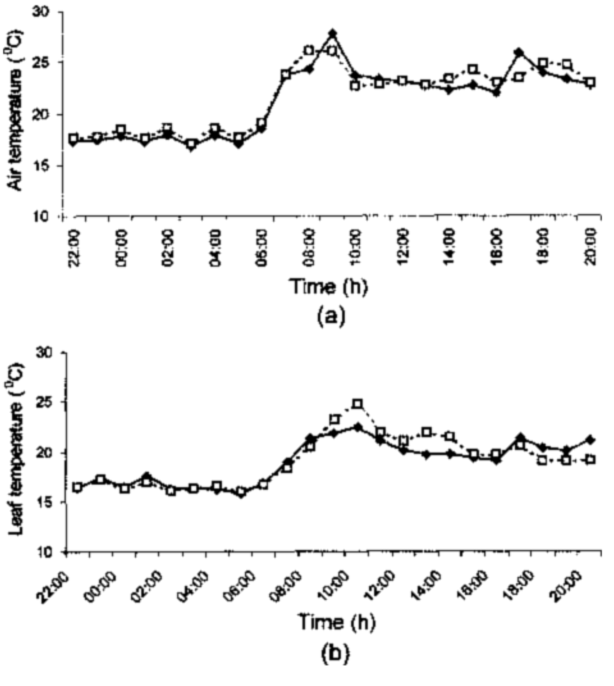

Figure 2. Variations in (a) air temperature and (b) leaf temperature of *R. minor* in shaded (solid-line) and non-shaded (dotted-line) environments. Measurements were made at one minute interval and averaged over an hour between 27th (22:00) and 28th (20:00) June 1999.

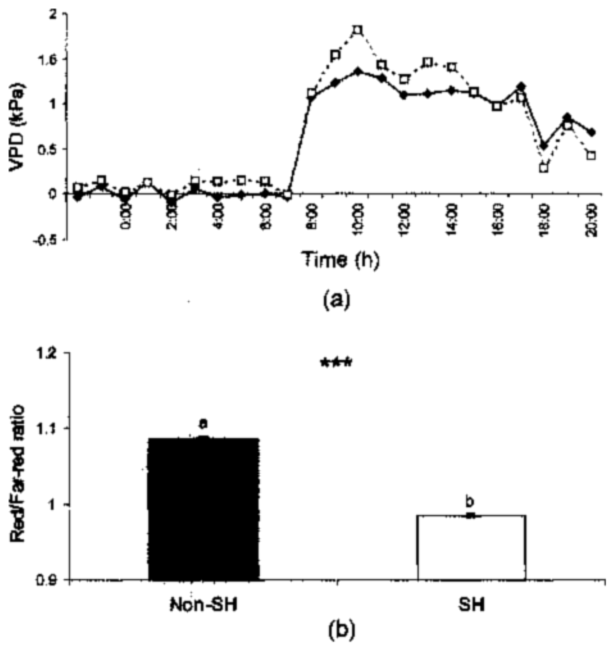

Figure 3. Variations in (a) VPD (Vapour Pressure Deficit) based on leaf, air temperature and RH (%) measurements and (b) Red/Far-Red light ratio on *R. minor* under non-shaded (Non-SH) and shaded (SH) environments, which were recorded 30 times between 14:00 and 15:00 on 24th June 1999. Bars represent means ± 1 S.E. Significant differences between bars are represented by asterisks (one-way ANOVA, ***: $P < 0.001$).

Table 1. Dry weight, number of haustoria, and nutrient status of *R. minor* without and with shading treatment. Values represent means ± 1 S.E. ($n = 6$).

	R. minor without shading	*R. minor* with shading
DW (g/plant)	0.15 ± 0.05	0.14 ± 0.04
No. of haustoria/plant	186.00 ± 39.80	184.00 ± 24.50
N (% DW)	1.79 ± 0.11	2.21 ± 0.16
C (% DW)	41.90 ± 1.13	39.00 ± 0.99
^{15}N (mg/mg DW)	0.03 ± 0.01	0.03 ± 0.01

the whole-plant carbon concentration in shade-grown *R. minor* plants did not differ significantly from those not shaded (Table 1). In addition, whole-plant nitrogen and ^{15}N concentrations in *R. minor*, which was completely dependent on its host for nitrogen nutrition, were not different between shading treatments (Table 1).

DISCUSSION

As with non-parasitic plants that usually exhibit morphological alterations (including stem elongation)

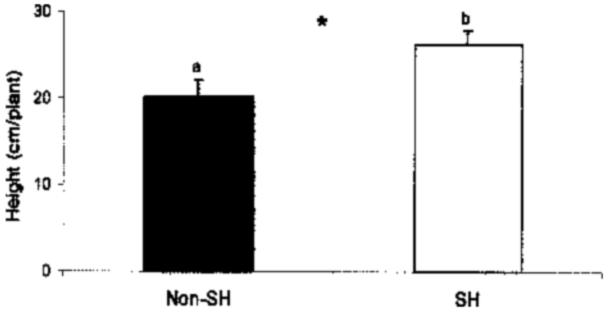

Figure 4. Final height of *R. minor* under non-shaded (Non-SH) and shaded (SH) environments (n = 6). Bars represent means ± 1 S.E. Significant differences between bars are represented by asterisks (one-way ANOVA, *: $P < 0.05$).

in response to shade (Grime and Jeffrey, 1965; Morgan and Smith, 1981; Smith, 1982; Maliakal et al., 1999), our attached *R. minor* plants showed increased heights under shading compared with those receiving ambient irradiance. This is consistent with the findings of Matthies (1991), who reported that *Melampyrum arvense*, an angiospermatic root hemiparasite, invested more biomass into height growth under a moderate light regime than under full light.

In natural environments, shade changes both the light quality (R/FR) and quantity that reaches the plants, but their response to these alterations varies by component (Smith, 1982). For instance, *Chenopodium album* maintains its leaf-development process at the expense of stem elongation in response to reduced irradiance without an alteration in spectrum, whereas stems elongate at a cost to leaf growth when the R/FR ratio is reduced at constant irradiance (Morgan and Smith, 1981). The typical R/FR ratio is approximately 1.2 at midday under natural sunlight, but has lower values in the shade of vegetation because of the preferential absorption of red light by chlorophyll (Grace, 1983). Therefore, the increased stem extension in our shaded *R. minor* plants may have resulted from the significantly reduced R/FR ratio, which is detected by the phytochrome pigment family (Smith, 1982; Grace, 1983). As a facultative root hemiparasite, *R. minor* uses increased extension growth in sensing and responding to shade-induced alterations in light quality, a tactic similar to that of non-parasitic, shade-avoiding plants (Smith, 1982).

R. minor is able to assimilate its own carbon (Seel and Press, 1994; Press and Graves, 1995), although the extent to which those photo-assimilates contribute to growth is unknown. It is thus possible that 1) shading by the neighboring individuals and/or hosts might negatively affect the growth of parasites by reducing the amount of carbon fixed, and/or 2) shading might force them to rely more on their host. However, in our study, dry weights and carbon concentrations of shaded *R. minor* plants were not significantly different from those without shading, implying that light availability may not be a critical growth determinant of chlorophyllous *R. minor* attached to a host. Furthermore, the absence of differences between shaded and unshaded *R. minor* for both tissue-nitrogen concentrations (including ^{15}N) and the number of haustoria demonstrates that shading did not cause this species to become more dependent on host-derived resources.

Given that the transpiration rate of parasites plays an essential role in determining the amount of resources transferred from the hosts (Press et al., 1988; Ackroyd and Graves, 1997), the lack of any difference in ^{15}N concentration for *R. minor* indirectly suggests that shading might not negatively affect the rate. This may be partly supported by 1) the absence of any large differences in % RH, air and leaf temperatures between the shaded- and non-shaded environments (all known factors affecting transpiration; see Cowan, 1977), and/or 2) the insensitive responses of parasite transpiration to fluctuations in external conditions involving darkness and water stress, compared with those of non-parasitic plants (Press et al., 1987; Press and Graves, 1995).

Therefore, it is likely that light availability is not as essential to growth of attached *R. minor* as it is to non-parasitic plants. In addition, a decrease in light availability does not negatively affect the parasite transpiration rate, so that shaded *R. minor* has no difficulty in acquiring host resources compared with its well-lit counterparts. The latter may be possible because annual root hemiparasites, including *R. minor*, have been shown to have similarly high transpiration rates per unit leaf area both night and day (Press et al., 1988). However, given that our *R. minor* plants were almost five weeks old and beginning to flower when shading commenced, we cannot exclude the possibility that its vegetative growth may have been out-competed by its maintenance and/or reproductive needs, in terms of resource utilization. This may partially account for the absence of any growth differences between shaded and non-shaded *R. minor*.

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