BIOLOGIA PLANTARUM 38 (1): 85-93, 1996

Gas exchange in leaves of the root hemiparasite *Melampyrum arvense* L. before and after attachment to the host plant

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Abstract

Gas exchange characteristics of a hemiparasite *Melampyrum arvense* L. before and after attachment to the host *Capsella bursa pastoris* (L.) Med. were compared. The net photosynthetic rates (P_N) on a leaf area basis were extremely low and in comparison to the value obtained for the host were about 15 % and 23 % for the unattached and attached hemiparasite, respectively. Also the concentration of photosynthetic pigments was low (as compared with the host the content of chlorophylls was about 33 % and 49 % and of carotenoids about 38 % and 36 % in the unattached and attached hemiparasite, respectively). On the other hand the rates of respiration were high (about 1.8 and 2.6 times higher in the unattached and attached hemiparasite, respectively, than in the host). In darkness stomatal conductance (g_s) of the host and the unattached hemiparasite was rapidly reduced to 10 % of the value obtained in light. g_s of the attached hemiparasite was decreased only by about 30 %. A total reduction of g_s occurred at relative water content (RWC) of 85 %, 75 % and 45 % for the unattached hemiparasite, the host, and the attached hemiparasite, respectively. The transpiration (E) rate in the preparasitic stage was very low, being 2.6 and 4.5 times smaller than in the host and the attached hemiparasite, respectively. In the attached hemiparasite WUE was 7.5 and 3 times poorer than in the host and in the preparasitic stage, respectively.

Additional key words: net photosynthetic rate, stomatal conductance, transpiration rate, WUE.

Introduction

High transpiration rate in both root and shoot angiosperm parasites (Atsatt 1983, Glatzel 1983, Ehleringer *et al.* 1985, Ullmann *et al.* 1985, Press *et al.* 1987) and

Received 17 July 1995, *accepted* 5 September 1995.

Abbreviations: Chl - chlorophyll; d.m. - dry mass; E - transpiration rate; f.m. - fresh mass; g_s -stomatal conductance to water vapour; I - irradiance; P_N - net photosynthetic rate; PS 2 - photosystem 2; R - respiration; Rubisco - ribulose-1,5-bisphosphate carboxylase-oxygenase; RWC - relative water content; WUE - water use efficiency

consequent lower water potentials of parasites (Glatzel 1983, Whittinghton and Sinclair 1988, Goldstein *et al.* 1989, Davidson *et al.* 1990) facilitates the transfer of solutes from the host to the parasite (Musselman 1980, Ehleringer *et al.* 1985).

Stomatal control of gas exchange is pure. Stomata of hemiparasites are fairly insensitive to darkness, moderate water stress, CO_2 concentrations, and application of abscisic acid (Press *et al.* 1987, Shah *et al.* 1987, Smith and Stewart 1990) and thus remain open almost continuously (Davidson *et al.* 1990, Smith and Stewart 1990). The modification of stomata response to environmental factors is connected with a pronounced accumulation of inorganic ions, particularly of potassium (Glazel 1983, Lamont 1983, Popp 1987, Smith and Stewart 1990). The association of hemiparasite and host changed many physiological features of the both plants (Press *et al.* 1987, Fer *et al.* 1994). Little is known about the tissue water relations and CO_2 exchange in the hemiparasite before attachment to the host (the preparasitic stage).

In the present work of photosynthesis and water relations in root hemiparasite *Melampyrum arvense* before and after attachment to the host *Capsella bursa pastoris* were investigated.

Materials and methods

Plant material: Seeds of the hemiparasite *Melampyrum arvense* L. and its host *Capsella bursa pastoris* (L.) Med. collected from nature and stored for 3 months at 2 °C were sown separately in pots (5 cm in diameter), containing a mixture of compost and sand. After 2 weeks the small pots were placed in 25-cm pots filled with a similar soil mixture (3 seedlings of the host and 1 seedling of the parasite). The cultivation of plants was carried out in a glasshouse in summer (May - September). Relative humidity was about 65 ± 5 %, day/night temperature was of 24/20 ± 2 °C, irradiance at noon was about 1 600 μmol m^{-2} s^{-1}. The third leaves of seedlings sampled at noon or at night were used for analyses.

Gas exchange: CO_2 content of air entering and leaving the leaf chamber was measured using an *ADC series 225* differential infrared gas analyzer (*Analytical Development Co.*, Hoddesdon, England) connected with an electronic injection valve (*Leeds and Northrup*, North Wales) that controlled the release of compressed CO_2 from cylinder. Ambient concentration of CO_2 was held at 14.3 mmol m^{-3}. Air humidity in the chamber was measured with a dewpoint hygrometer (*Dew-10, General Estern*, Watertown, USA). Flow rate of air entering the leaf chamber was monitored with a mass-flow meter (*Model MFM-14 U, Matheson, Union Carbide*, Oevel, Belgium). Leaf temperature was measured by a copper-constantan thermocouple. Irradiance was provided by six 400 W mercury multivapour metal handle lamps and six 400 W high pressure sodium lamps (*General Electric Co.*, Cleveland, USA) filtered through a water filter (in thickness 10 cm) and a heat and UV filter (*KG 5, Schott*, Mainz, Germany). Irradiance inside and outside the chamber was monitored with quantum sensors (*LI-190 SA, Li-Cor Inc.*, Lincoln, U.S.A.). Conductance of adaxial and abaxial leaf surface was measured using a *LI-1600*

(*Li-Cor.*, Lincoln, USA.) steady-state porometer. The leaf area was measured using a *Li-Cor* portable area meter (*LI-3000*). The effect of water stress on leaf conductance was carried out by excising leaves and allowing them to dry in air (temperature of 25 °C, air humidity of 40 % and irradiance of 900 μmol m^{-2} s^{-1}).

Boundary layer resistances were calculated according to Parkinson (1985). These data were later used to calculate net photosynthetic rate (P_N), transpiration rate (E) and stomatal conductance (g_s) using the equations described by Von Caemmerer and Farquhar (1981). Irradiance response curves were fitted to function described by Reed *et al.* (1976). Parameters were fitted by nonlinear least squares regression.

Relative water content (RWC) was calculated from leaf fresh mass, dry mass and mass of full saturation obtained by floating the leaf on water according to the formula given by Beadle *et al.* (1985).

Chlorophyll (Chl) *a* and *b* contents were determined according to Arnon (1949) and content of carotenoids using the method described by Wellburn and Lichtenthaler (1984).

Results

CO_2 exchange and pigment content: P_N was saturated at I of about 300 and 900 μmol m^{-2} s^{-1} in the hemiparasite (both stages) and the host, respectively (Fig. 1). The relatively high I_{sat} and high P_N observed in *C. bursa pastoris* are typical for leaves adapted to sunny conditions. P_N of *M. arvense* on a leaf area basis was extremely low and under I_{sat} amounted to 15 % only in the unattached hemiparasite and to 23 % in that attached to the host, in relation to values obtained for *C. bursa pastoris*. With respect to Chl unit in both stages of hemiparasite the P_N constituted about 46 % of that found for the host. The fairly high R of *M. arvense* calculated per leaf area unit (about 176 % in the unattached and 255 % in the attached hemiparasite in comparison with the host) were observed. In consequence net carbon gain in *M. arvense* was small. In the preparasitic stage Chl (*a*+*b*) was only 33 % of the value

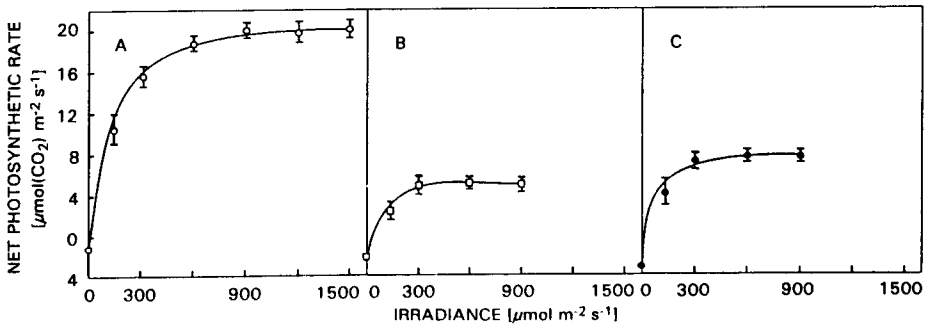

Fig. 1. Irradiance response curves for net photosynthesis in *C. bursa pastoris* (*A*, the host) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host (mean ± S.E, n = 9).

determined in the host (Table 1). After the hemiparasite attachment its level increased to about 46 %. The Chl *a/b* ratio in hemiparasite and host was similar. The concentration of carotenoids (carotenes + xanthophylls) was similar in the preparasitic and parasitic stage amounting to about 40 % of the value determined in the host.

Table 1. Photosynthetic characteristics of *C. bursa pastoris* (*A*, the host) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host (mean ± S.E., $n = 9$). All the gas exchange measurements were made at saturated I and 25 °C leaf temperature.

Parameter	A	B	C
Net photosynthesis [μmol (CO_2) m^{-2} s^{-1}]	18.82 ± 1.12	2.82 ± 0.17	4.27 ± 0.29
Net photosynthesis [μmol (CO_2) g^{-1}(Chl) s^{-1}]	2.80 ± 0.19	1.28 ± 0.09	1.29 ± 0.08
Dark respiration [μmol (CO_2) m^{-2} s^{-1}]	1.22 ± 0.07	2.15 ± 0.15	3.11 ± 0.19
Chlorophyll (*a+b*) [g m^{-3}]	6.73 ± 0.41	2.22 ± 0.15	3.31 ± 0.23
Chlorophyll *a/b* ratio	1.81	1.95	1.87
Carotenoids [g m^{-3}]	0.57 ± 0.04	0.22 ± 0.01	0.21 ± 0.01

Stomatal conductance: At I of 900 μmol $m^{-2}s^{-1}$ g_s of the attached *M. arvense* was 2 and 3 times higher than this of *C. bursa pastoris* and the unattached hemiparasite, respectively. When the plants were transferred from full I (1000 μmol m^{-2} s^{-1}) to darkness, g_s of *M. arvense* unattached to the host decreased very rapidly (Fig. 2). After 5 min darkness g_s was reduced by 60 %, after 20 min it decreased to about 7 % of the initial value and maintained on this level for 3 h. The residual conductance of 9.8 mmol m^{-2} s^{-1} probably represent cuticular conductance (Araus *et al.* 1991). Similar results were obtained with *C. bursa pastoris*, however the reduction of g_s

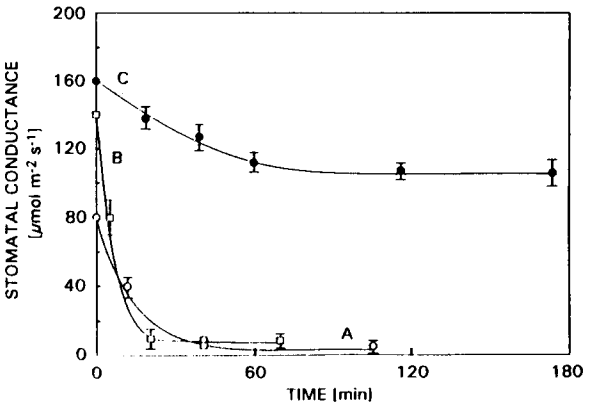

Fig. 2. Stomatal conductance after transfer of the plants from irradiance (1000 μmol m^{-2} s^{-1}) to darkness; *A* - *C. bursa pastoris* (the host), *B* - unattached and *C* - attached the hemiparasite *M. arvense* (mean ± S.E, $n = 9$).

was much slower. Residual conductance (8 mmol $m^{-2}s^{-1}$) was attained after about 40 min. In *M. arvense* attached to the host g_s was reduced during 60 min by about 30 %, and maintained later at the same level. At night the normal closure of stomata was not observed in the attached hemiparasite, while in the host plant and in the preparasitic stage of *M. arvense* stomata were closed in darkness (Lechowski 1995). g_s decreased with the reduction of RWC (Fig. 3). In the unattached *M. arvense* RWC of about 85 % brought about a total reduction of g_s. The host plant manifested a greater water stress resistance and the completely reduced g_s occurred at RWC of about 75 %. In *M. arvense* attached to the host a decrease in RWC to 80 % did not significantly change g_s. A further decrease in RWC was connected with a reduction in g_s and at RWC of 45 % g_s approximated to 10 %.

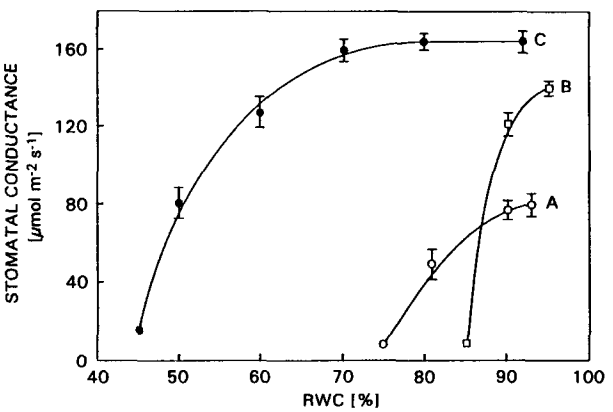

Fig. 3. Relationship between stomatal conductance and relative water contents of *C. bursa pastoris* (*A*, the host) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host (mean ± S.E., $n = 9$).

Transpiration rate and water use efficiency: E of *M. arvense* in the preparasitic stage was low and constituted about 37 % of that obtained for *C. bursa pastoris* (Table 2). Attachment of the hemiparasite to the host resulted in an increase in E which were about 4.5 and 1.7 times higher than in *M. arvense* in the preparasitic stage and in the host plant, respectively. Moreover, the host plant and the unattached *M. arvense* showed a circadian rhythm of E. In dark periods E was reduced by about 91 and 83 % in host and unattached hemiparasite plants, respectively. In hemiparasite attached to the host plant, a depression of E in dark did not exceed 20 %. High E values are generally associated with parasitism. Under the experimental conditions employed here, WUE was 2.5 times lower in unattached *M. arvense* than in *C. bursa pastoris*. The hemiparasite attachment to the host reduced WUE by about 7.5 times in relation to the host (Table 2). During the closure of stomata in the preparasitic stage of *M. arvense* the rate of water loss was reduced to a greater degree than that of carbon gain, leading to an increase in WUE.

Table 2. Leaf transpiration rates of *C. bursa pastoris* (*A*, the host) and the hemiparasite *M. arvense* unattached (*B*) and attached (*C*) to the host (mean ± S.E., $n = 9$).

Parameter	A	B	C
Transpiration - day [mmol (H_2O) m^{-2} s^{-1}]	4.80 ± 0.34	1.81 ± 0.12	8.12 ± 0.56
Transpiration - night [mmol (H_2O) m^{-2} s^{-1}]	0.41 ± 0.03	0.11 ± 0.01	6.52 ± 0.45
Night/day ratio	0.08	0.06	0.80
WUE [mmol(CO_2) mol^{-1}(H_2O)]	3.92 ± 0.31	1.56 ± 0.12	0.52 ± 0.03

Discussion

Unattached hemiparasites can autotrophically live for few weeks but their growth rates are very low (Fer *et al.* 1994, Lechowski 1995). In the preparasitic stage of *M. arvense* *a*) P_N was extremely low, *b*) the relative low I was necessary to ensure the saturation of P_N, *c*) the concentration of photosynthetic pigments was low, and *d*) the rate of respiration was fairly high. The low P_N of the unattached hemiparasite is only partly due to the low concentration of Chl. In comparison with autotrophic species of *Scrophulariaceae* low P_N in hemiparasites resulted from *a*) the poor activity of Rubisco (Press *et al.* 1986), *b*) the low activity of PS 2 (Sale *et al.* 1987), and *c*) high rates of R (Press *et al.* 1987). After attachment to the host the P_N of *M. arvense* was higher (by about 50 %) than that reported for the unattached parasite, nevertheless its value was only 23 % of the rate obtained for the host while the high rate of R (about 2.6 times higher than in the host) results in a small daily net carbon gain. These data may suggest that the growth and development of the hemiparasite is highly dependent on its host for the supply of carbon. The transfer of labelled organic solutes from the host to the parasite or carbon isotope ratios, gave direct or indirect evidence for the transfer of reduced carbon from the host plant to some hemiparasites (Press *et al.* 1986, 1987, Graves *et al.* 1989, Marschall and Ehleringer 1990, Stewart and Press 1990, Simier *et al.* 1993). Similar conclusions may suggested by the observation reported by Shah *et al.* (1987) concerning attached albino plants of *Striga hermonthica* that appear to grow as rapidly as normally pigmented plants.

Stomatal conductance showed a good correlation with stomatal aperture observed in both the parasite and the host (Lechowski 1995). In darkness stomata were closed in the host and in the unattached *M. arvense*, while in the attached hemiparasite they were only partially closed. The behaviour of stomata in attached hemiparasites could be explained by a lack of response to one or more of the factors which control stomatal aperture (Fisher 1983, Glatzel 1983, Ullmann *et al.* 1985, Whittington and Sinclair 1988, Davidson *et al.* 1990, Smith and Press 1990). On the other hand no sufficient information is found in the literature concerning the reaction of stomata of the preparasitic stage to environmental factors. The unattached *M. arvense* was characterized by a great sensitivity to RWC and at RWC of about 85 % a total reduction of g_s occurred (Fig. 3). A similar result was obtained with about 75 % and

45 % RWC for the host and the attached hemiparasite, respectively. In the association *Sorghum-Striga,* the stomata of *Sorghum* were virtually closed within the first 20 min (RWC 94 %) but these of *Striga hermonthica* were still open after 150 min (RWC 35 %) (Smith and Stewart 1990). This specific sensitivity of g_s of the unattached *M. arvense* to decreases in RWC might be connected with *a*) a fairly large water content in leaf tissues of the parasite (about 91 %) (Lechowski 1995) and *b*) inadequate ability of the hemiparasite root (*Scrophulariaceae*) to supply the required amount of water (Kostytschev 1924). Among other factors the attachment of the hemiparasite to the host changed the physiology of the plant in response to the constant inflow of water and solutes through the connection of haustoria with xylem of the host (Klaren and Janssen 1978, Fer *et al.* 1994, Lechowski 1995).

Transpiration rate of the unattached *M. arvense* was very low, being 2.6 and 4.5 times less than in the host and the attached hemiparasite, respectively (Table 2). Similar the data were obtained for the preparasitic stage of *Thesium humile* where E was about one third of that assessed in the attached hemiparasite (Fer *et al.* 1994). Moreover, the different E under light and dark was observed in the unattached *M. arvense* and in the host. In the attached *M. arvense* high rates of E occurred both in light and in darkness. In general, high E values were connected with parasitism (Musselman 1980, Ehleringer *et al.* 1985, Marschall and Ehleringer 1986, Shah *et al.* 1987, Press *et al.* 1988). The capture of water and solutes from the host requires a gradient of decreasing water potential towards the parasite and high E rates are required to maintain this gradient (Stewart and Press 1990). Low P_N and high E caused low WUE for the attached hemiparasite. For the attached *M. arvense* the value of WUE was about 7.5 and 3 times lower than that determined for the host and the unattached *M. arvense*, respectively. After the attachment of *M. arvense* stomatal behaviour did not maximize WUE similarly like in *Striga hermonthica* (Press *et al.* 1987, Shah *et al.* 1987 Smith and Stewart 1990).

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