

Hydathode trichomes actively secreting water from leaves play a key role in the physiology and evolution of root-parasitic rhinanthoid Orobanchaceae

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- **Background and Aims** Root hemiparasites from the rhinanthoid clade of Orobanchaceae possess metabolically active glandular trichomes that have been suggested to function as hydathode trichomes actively secreting water, a process that may facilitate resource acquisition from the host plant's root xylem. However, no direct evidence relating the trichomes to water secretion exists, and carbon budgets associated with this energy-demanding process have not been determined.
- **Methods** Macro- and microscopic observations of the leaves of hemiparasitic *Rhinanthus alectorolophus* were conducted and night-time gas exchange was measured. Correlations were examined among the intensity of guttation, respiration and transpiration, and analysis of these correlations allowed the carbon budget of the trichome activity to be quantified. We examined the intensity of guttation, respiration and transpiration, correlations among which indicate active water secretion.
- **Key Results** Guttation was observed on the leaves of 50 % of the young, non-flowering plants that were examined, and microscopic observations revealed water secretion from the glandular trichomes present on the abaxial leaf side. Night-time rates of respiration and transpiration and the presence of guttation drops were positively correlated, which is a clear indicator of hydathode trichome activity. Subsequent physiological measurements on older, flowering plants indicated neither intense guttation nor the presence of correlations, which suggests that the peak activity of hydathodes is in the juvenile stage.
- **Conclusions** This study provides the first unequivocal evidence for the physiological role of the hydathode trichomes in active water secretion in the rhinanthoid Orobanchaceae. Depending on the concentration of organic elements calculated to be in the host xylem sap, the direct effect of water secretion on carbon balance ranges from close to neutral to positive. However, it is likely to be positive in the xylem-only feeding holoparasites of the genus *Lathraea*, which is closely related to *Rhinanthus*. Thus, water secretion by the hydathodes might be viewed as a physiological pre-adaptation in the evolution of holoparasitism in the rhinanthoid lineage of Orobanchaceae.

Key words: Ecophysiology, holoparasite, hydathode trichome, *Lathraea*, parasitic plant, respiration, *Rhinanthus alectorolophus*, rhinanthoid Orobanchaceae, orobanche, root hemiparasite, transpiration, *Triticum aestivum*, water regime, water secretion, xylem.

INTRODUCTION

About 1 % of flowering plants corresponding to 4500 species parasitize other plants by specialized organs called haustoria to acquire essential resources (Heide-Jørgensen, 2008). The majority of parasitic plant species are hemiparasites, green photosynthetic plants acquiring water, mineral nutrients and a certain amount of heterotrophic carbon from the host xylem (Press, 1989; Irving and Cameron, 2009; Těšitel *et al.*, 2010a; Heide-Jørgensen, 2013). In contrast, holoparasites completely lack photosynthetic ability and thus acquire all essential resources heterotrophically from the host (Hibberd and Jeschke, 2001; Irving and Cameron, 2009).

Holoparasites are generally thought to have evolved repeatedly from hemiparasites (Westwood *et al.*, 2010; McNeal *et al.*, 2013; Naumann *et al.*, 2013), but such an evolutionary transition can rarely be documented or studied due to the extinction of assumed hemiparasitic ancestors (Nickrent and Duff, 1996;

Nickrent *et al.*, 1998; Naumann *et al.*, 2013). However, the family Orobanchaceae provides an opportunity to study the macroevolutionary transition between the trophic strategies of parasitic plants as it encompasses closely related non-parasitic, hemiparasitic and holoparasitic species (Bennett and Mathews, 2006; Heide-Jørgensen, 2008; Westwood *et al.*, 2010; McNeal *et al.*, 2013; Naumann *et al.*, 2013). This is the case of the sister genera *Rhinanthus* and *Lathraea*, and closely related *Rhynchosorys* which form a separate sub-clade within the Rhinanthoid clade of Orobanchaceae (Těšitel *et al.*, 2010c). Moreover, *Tozzia alpina*, another related Rhinanthoid species, displays a parallel evolutionary tendency towards holoparasitism (Těšitel *et al.*, 2010c).

Rhinanthus species are hemiparasitic annuals possessing a highly efficient resource acquisition strategy based on an open vascular connection with the host xylem (Cameron *et al.*, 2006) and a high transpiration rate directing the xylem stream from the

host (Klaren and Janssen, 1978; Stewart and Press, 1990; Jiang *et al.*, 2010). Despite the acquisition of substantial amount of carbon from the host in the form of xylem-mobile organic elements (Těšitel *et al.*, 2010a, 2011), the hemiparasite's own photosynthesis plays a crucial role in realization of its fitness (Těšitel *et al.*, 2015). Most of the species of the Rhinanthoid clade are in principal physiologically similar to *Rhinanthus*, i.e. they are photosynthetic root hemiparasites acquiring resources from the host root xylem (Těšitel *et al.*, 2010a; McNeal *et al.*, 2013).

In contrast, *Lathraea*, *T. alpina* and the perennial species *Rhynchosorys* are holoparasitic, at least in early ontogenic stages of underground individuals, but unlike most other holoparasitic species (Irving and Cameron, 2009) they do not feature a connection to the host phloem in their haustoria. *Lathraea* species are characterized by extensive perennial underground rhizomes covered by fleshy scales of leaf origin (Ziegler, 1955; Renaudin, 1966). Shoots are short lived and their only function is flowering and seed production. The third genus of the sub-clade, *Rhynchosorys*, contains both species which are morphologically similar to *Lathraea* (rhizomes with scales, e.g. *R. elephas*), but retain photosynthetic activity in their green above-ground shoots (Kubat and Weber, 1987), and annual species which are closely similar to *Rhinanthus* (e.g. *R. orientalis*) (Těšitel *et al.*, 2010c). The plant architecture and physiological functioning of the more distantly related *T. alpina* are closely similar to those of perennial *Rhynchosorys* species and the species is also known to have only a xylem connection in its haustoria (Weber, 1973). As a result of the underground growth habit, these species cannot transpire to discharge excess water taken up from the host xylem, which requires an alternative mechanism of water secretion for their physiological functioning.

Hemiparasites of the Rhinanthoid clade of Orobanchaceae were shown to have glandular trichomes on the abaxial side of their leaves (Fedorowicz, 1915; Kaplan and Inceoglu, 2003; Těšitel and Tesařová, 2013), frequently located close to leaf veins (Govier *et al.*, 1968). Anatomically identical trichomes were also revealed on the scales of the below-ground rhizomes of *Lathraea* and *Rhynchosorys* (Groom, 1897; Ziegler, 1955; Renaudin, 1966; Kubat and Weber, 1987). The ultrastructure of these trichomes revealed numerous mitochondria, labyrinthine cell walls and plasmodesmata, structures suggesting their high metabolic activity (Schnepf, 1964; Renaudin and Garrigues, 1967; Těšitel and Tesařová, 2013). Govier *et al.* (1968) suggested a function of the trichomes as hydathode trichomes actively secreting water based on their observation of guttation from the leaves of hemiparasitic *Odontites vernus* Dumort. and a radioisotope tracing experiment. Moreover, extensive water secretion was also observed from the underground scale-like leaves of *Lathraea*. First reported by Darwin (1880), the secretion was later suggested to be associated with the glandular trichomes (Renaudin and Garrigues, 1967). To sum up, there is convincing evidence of the presence of metabolically active glandular trichomes in the Rhinanthoid Orobanchaceae and of an intense water secretion from the leaves of these parasitic plants. However, direct evidence relating the trichomes to water secretion and the carbon budget of the assumed, energy-demanding water secretion is yet to be revealed.

In this study, we aim to present conclusive direct evidence on the physiological role of the assumed hydathode trichomes and integrate their function into the physiology of hemiparasites.

Macroscopic and microscopic observations were combined with gas exchange measurements to capture the physiological activity of the trichomes on the leaves of hemiparasitic *Rhinanthus alectorolophus*. Using the gas exchange measurements, we were able to estimate the carbon budget of the hydathode trichome activity. Moreover, our experimental set-up allowed testing of the effects of the hemiparasite developmental stage and availability of below-ground abiotic resources on the hydathode trichome activity.

MATERIALS AND METHODS

Plant material

Seeds of *Rhinanthus alectorolophus* (Scop.) Pollich were collected from the natural population near Zechovice, Czech Republic (49°09'28"N, 13°52'13"E; 510 m a.s.l.). Seeds of wheat (*Triticum aestivum* L.) used as a host species were obtained from the school farm of the Faculty of Agriculture, University of South Bohemia.

Experimental design and conditions

The experiment was carried out in a growth chamber at the Faculty of Science, University of South Bohemia from December 2013 to March 2014. Three-day-old seedlings of wheat germinated on a Petri dish with moist filter paper were sown to 0.8 L pots (one seedling per pot) filled with a mixture of sand and peat (1:1, v/v ratio). Half of the pots received 1 g of Osmocote Exact Standard 5–6 M fertilizer (Scotts Miracle-Gro Company, UK) per litre of substrate (high nutrient treatment, N+). According to the manufacturer's specifications, the fertilizer contains 150 mg N g⁻¹, 90 mg P g⁻¹ and 120 mg K g⁻¹. The other half of the pots did not receive any additional nutrients (low nutrient treatment, N-). All pots ($n=98$) were well watered and maintained in the growth chamber with a 12 h light/12 h dark cycle and temperature regime of 20–22 °C (light):17–18 °C (dark). The photosynthetically active radiation (PAR) intensity during the day period was from 400 to 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The pots were randomized once a week to filter out possible heterogeneity in non-treatment cultivation conditions (mainly PAR intensity). Seedlings of *R. alectorolophus*, pre-germinated on moist filter paper at 4 °C after approx. 8 weeks, were added to the pots (two seedlings per pot) 1 d after wheat sowing. The hemiparasite seedlings were thinned to one per pot, and two contrasting water regimes were established 27 d after *Rhinanthus* sowing (DAS). High irrigation pots (W+) and low irrigation pots (W-) received 150 and 100 mL of tap water every fourth day, respectively. The nutrient and watering treatments were established in a full factorial design. The purpose of the nutrient and water treatments was to create certain environmental variability since hemiparasite physiology is known to be profoundly affected by the availability of these abiotic resources (Těšitel *et al.*, 2015). However, the length of the simulated environmental gradients was much shorter than in the study of Těšitel *et al.* (2015) and was not of primary interest in our study.

Two sets consisting of 20 plants (i.e. five individual plants per each treatment combination, [Supplementary Data Table S1](#))

were selected for observations and physiological measurements conducted before and during the peak flowering period (55 and 73 DAS, [Supplementary Data Fig. S1A, B](#)). The plants were watered (following the watering protocol) several hours before the measurements. Repeated measurements on individual plants usually could not be performed due to frequent mortality of plants that had been subjected to the first measurement. Elevated plant mortality was probably caused by accidental mechanic damage.

Macroscopic and microscopic observations

The leaf surface of plants to be measured by gas exchange (see ‘Gas exchange measurements’) was examined for the density and size of guttation drops immediately before the measurements. Drops were classified on an ordinal scale (0, no drops; 0.5, small drops, i.e. <25 % leaf area covered by guttation drops; and 1, large drops, i.e. >25 % leaf area covered by guttation drops; [Fig. 1](#)). Leaves of *R. alectorolophus* were detached from some of the young non-flowering plants cultivated under each treatment combination and cut with a razor blade into thin sections. These sections were placed in either water or mineral oil as mounting media and subsequently subjected to light microscopy using an Olympus CX41 Microscope (Olympus Imaging America Inc., Center Valley, PA, USA) and INFINITY1-3 C 3.1 MP CMOS Color Camera (Lumenera Corp., Ottawa, Canada).

Gas exchange measurements

Night-time rates of respiration ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and transpiration ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$) were measured on intact leaves with a Li-6400 Portable Photosynthetic System (Li-Cor, Lincoln, NE, USA) coupled to a 2 cm^2 circular leaf chamber. Each measurement was done between 0200 and 0900 h at ambient temperature and an air relative humidity of 65–70 % in the dark. Air relative humidity inside the measurement chamber and ambient CO_2 concentration were controlled at 60–75 % and $400 \mu\text{mol mol}^{-1}$, respectively. The surface of the leaves subjected to measurements had been dried by filter paper prior to the gas exchange measurements. Dark respiration and transpiration rates were recorded in 5 s intervals for approx. 3 min

after a steady-state gas exchange rate was achieved. The surface of the measured leaves was dry before and after the gas exchange measurements. Mean values of these measurement series were then used in the data analysis as respiration and transpiration rates of the corresponding plants.

In addition, the relative water content (RWC) of substrate was measured in the pots used in the gas exchange measurements with an HH2 Moisture Meter with an SM200 sensor (Delta-T Devices Ltd, Cambridge, UK).

Carbon budget calculations

Gas exchange measurements allowed us to estimate the concentration of organic carbon in the xylem sap of the hemiparasite necessary to compensate the carbon loss through respiration. Since no studies on the efficiency of carbon filtering from the xylem sap of hemiparasites were available, we assumed only the concentration of organic carbon in the xylem sap (i.e. filtering efficiency of 100 %) in the calculation of the carbon budget of the hydathode trichome activity ([Supplementary Data Methods](#)). Therefore, our carbon budget calculation indicates the maximal possible carbon acquisition from the xylem sap. In reality this might be lower, which is reflected in the discussion.

Data analysis

Linear (LM) and generalized linear models (GLM) were used to analyse the effect of developmental stage and water and nutrient treatments on the physiological parameters of *Rhinanthus* plants. Respiration and transpiration rates were analysed by LMs, while binomial GLM was used to analyse the presence and size of guttation drops, which was allowed by the quasi-binomial coding. The correlation between night-time transpiration and respiration rates was analysed as a linear regression (respiration–transpiration), which produces numerical results identical to Pearson correlation. All analyses were conducted in R, version 3.0.1 ([R Core Team, 2013](#)). The relationships among all treatments and parameters monitored were summarized by principal component analyses (one analysis for each of the two developmental stages) included as [Supplementary Data Fig. S3](#). These analyses were based on the

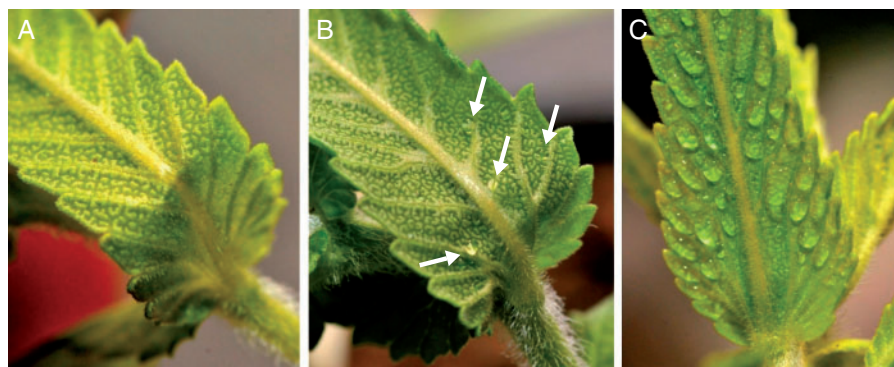


FIG. 1. The density and size of drops on the leaves of *Rhinanthus alectorolophus* (55 d after sowing) classified on an ordinal scale: (A) no drops (0), (B) small drops (0.5), (C) large drops (1). The plant was cultivated under (A) low irrigation and nutrient treatment, (B) low irrigation and high nutrient treatment, and (C) high irrigation and nutrient treatment. Images were taken immediately before the physiological measurement.

TABLE 1. Summary of (generalized) linear models testing the effects of developmental stage, water and nutrient treatment on the presence and size of guttation drops, respiration and transpiration rates in *R. alectorolophus*

Effect	Drops			Respiration			Transpiration		
	d.f.	Deviance	<i>P</i>	Sum Sq.	<i>F</i>	<i>P</i>	Sum Sq.	<i>F</i>	<i>P</i>
Nutrients	1	4.39	0.0362	3.17	10.23	0.0031	0.36	0.51	0.48
Water	1	0.04	0.84	0.03	0.10	0.76	0.60	0.84	0.37
Stage	1	7.08	0.0078	0.0002	0.0005	0.98	9.28	13.02	0.0010
Nutrients × Water	1	0.65	0.42	0.16	0.52	0.48	0.76	1.07	0.31
Nutrients × Stage	1	0.50	0.48	0.29	0.95	0.34	0.01	0.01	0.93
Water × Stage	1	0.13	0.72	1.02	3.30	0.08	1.99	2.79	0.10
Nutrients × Water × Stage	1	0.00	1.00	0.56	1.79	0.19	0.50	0.70	0.41
Residuals	32	21.37		9.92			22.80		

Statistically significant results ($P < 0.05$) are highlighted in bold.

Non-significant terms ($P > 0.05$) were omitted from the final models.

variables centred by mean subtraction and standardized by dividing by the standard deviation, and were performed in Canoco for Windows, version 5 (ter Braak and Šmilauer, 2012).

RESULTS

Macroscopic and microscopic observations

Guttation drops were observed on the abaxial leaf surface of 50 % of non-flowering plants (55 DAS) and 15 % of flowering plants (73 DAS). The presence and size of drops were significantly ($P < 0.05$) affected by the developmental stage of a plant and nutrient treatment (Table 1). The presence of large drops was significantly higher under the N+ treatment ($z = 2.076$, $P = 0.038$) and lower in flowering plants ($z = -2.311$, $P = 0.021$). No large drops were found on flowering plants (Supplementary Data Table S1). Both stalked and sessile hydathode trichomes were observed on the abaxial leaf surface of examined plants of all treatments. They were omnipresent on the abaxial surface, but sporadically occurred also on the adaxial surface. Microscopic observation in mineral oil revealed drops of liquid secreted from both trichome types (Figs 2A–D and 3A–F). No drops of liquid were observed in water as the mounting medium (Supplementary Data Fig. S2).

Gas exchange measurements

Dark respiration and transpiration rates were affected by the nutrient treatment and developmental stage, respectively (Table 1). Flowering *Rhinanthus* plants had lower transpiration rates than those measured before flowering ($t_{38} = -3.613$, $P < 0.001$). *Rhinanthus* cultivated under the N+ treatment displayed a higher dark respiration rate ($t_{38} = 3.172$, $P = 0.003$). Regardless of the significant effect of the water treatment on the RWC in pots (Welch two sample t-test: $t_{32.3} = 3.005$, $P = 0.005$), it did not have any significant effect on the gas exchange parameters (Table 1).

The gas exchange measurements revealed a strong positive relationship between night-time respiration and transpiration rates in non-flowering *R. alectorolophus* (Fig. 4A). The regression slope estimate was 0.55, which corresponds to 0.55 μmol respired carbon for the release of 1 mmol water in the form of

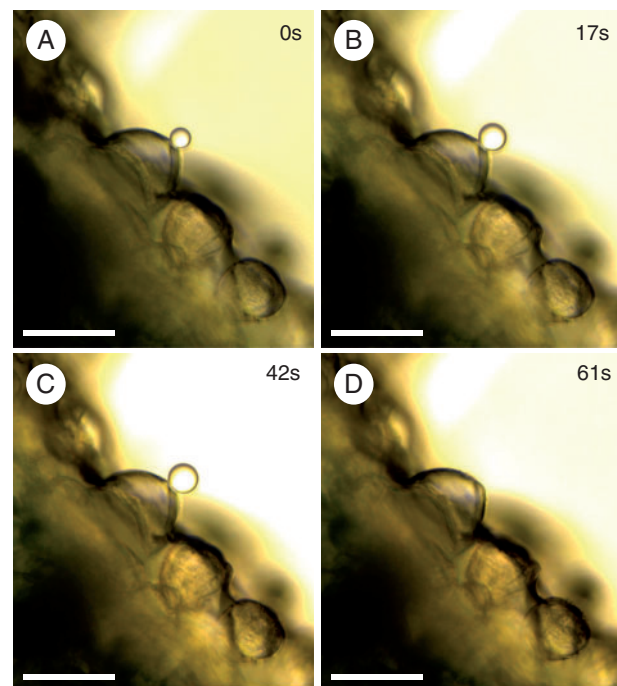


Fig. 2. Micrographs showing secretion from sessile hydathode trichomes on the abaxial leaf surface of *Rhinanthus alectorolophus*. The secretion was observed in oil shortly after immersion of the sample (0s, A) and in the time series as indicated (B–D). The drop of liquid finally detached from the trichome and moved out of view (D). The scale bars indicate 50 μm .

guttation drops and stomatal transpiration. Moreover, both processes were also positively associated with the presence and size of guttation drops (Figs 4A and 5). The positive correlation among transpiration, respiration and size of the guttation drops is also demonstrated by the principal component analysis (Supplementary Data Fig. S3). In contrast, flowering hemiparasites exhibited no such relationship between the gas exchange physiological processes (Fig. 4B; Table S1; Fig. S3).

DISCUSSION

The combination of macroscopic and microscopic observations with the gas exchange measurements of *Rhinanthus* leaves

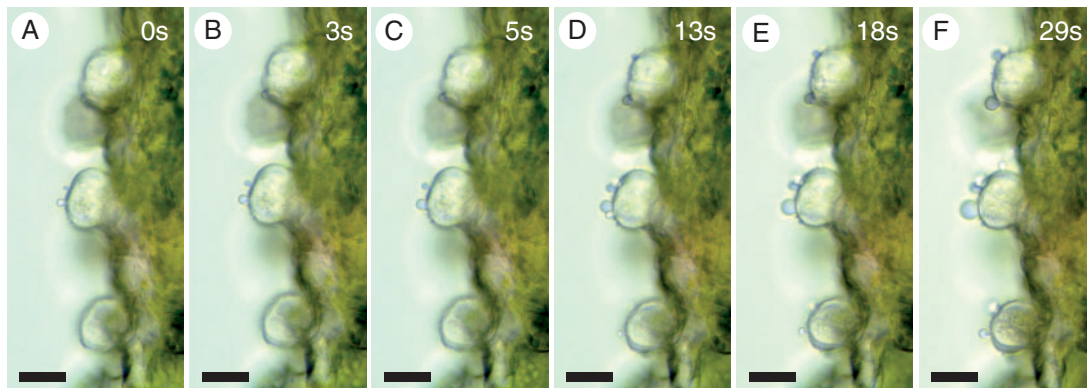


FIG. 3. Micrographs showing secretion from stalked hydathode trichomes on the abaxial leaf surface of *Rhinanthus alectorolophus*. The secretion was observed in oil shortly after immersion of the sample (0s, A) and in the time series as indicated (B–F). The scale bars indicate 25 μm .

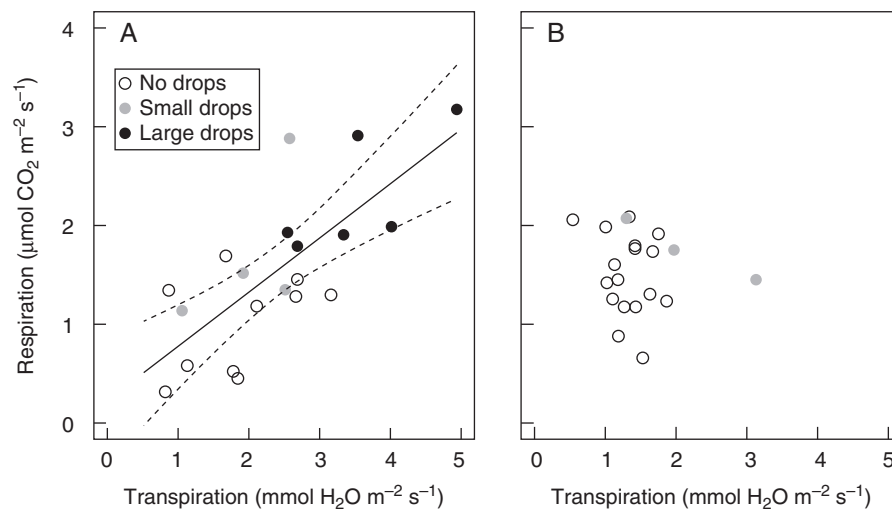


FIG. 4. The relationship between the night-time rates of respiration and transpiration in (A) non-flowering and (B) flowering *Rhinanthus alectorolophus*. Each circle relates to one individual plant. The size of drops observed on the leaves of examined plants immediately before the physiological measurement is indicated in the key. Linear regression ($r^2 = 0.55$, $F_{1,18} = 22.31$, $P < 0.001$) and the 95 % confidence interval are presented by solid and dashed lines, respectively. No large drops were observed on the leaves of flowering plants.

provided the first unequivocal direct evidence on the physiological role of hydathode trichomes in water secretion in the Rhinanthoid Orobanchaceae. Their role is further supported by their ultrastructure (Schnepf, 1964; Renaudin and Garrigues, 1967; Těšitel and Tesařová, 2013) and explains earlier field measurements documenting an elevated night-time respiration and its correlation with night-time transpiration in multiple young hemiparasitic species (Press et al., 1988; Press, 1989). A similar relationship was found here in young leaves of *R. alectorolophus* and it was correlated with the presence and size of guttation drops secreted from hydathode trichomes.

The observed effects of developmental stage (young vs. flowering plants) and nutrient availability on the hydathode trichome activity provide a partial explanation of the high variability in the respiration rate and net photosynthesis reported in the Rhinanthoid hemiparasites (Press et al., 1988; Press, 1989; Seel and Press, 1993; Lechowski, 1996; Těšitel et al., 2011). The other part of the explanation lies in well-known effects of host species and nutrient availability on the photosynthetic

efficiency and growth of hemiparasites (van Hulst et al., 1987; Seel et al., 1993; Cameron and Seel, 2007; Mudrák and Lepš, 2010; Těšitel et al., 2013, 2015). Thus, the physiological functioning of attached hemiparasites is highly plastic, depending not only on the host quality and environmental conditions, but also on the developmental stage. This should be considered in all ecophysiological studies focusing on the Rhinanthoid hemiparasites as it is unlikely to capture the activity of hydathode trichomes during standard photosynthetic measurements (e.g. light response curves) of flowering specimens.

Resource acquisition from the host is driven by the water potential difference between the host and parasites in xylem-feeding parasitic plants (Ehleringer and Marshall, 1995; Seel and Jeschke, 1999; Hibberd and Jeschke, 2001). A strongly negative water potential is maintained by the high content of osmotically active compounds (such as sugar alcohols) and the elevated transpiration rate, physiological traits shared by many Rhinanthoid Orobanchaceae (Hodgson, 1973; Press et al., 1988; Ehleringer and Marshall, 1995; Jiang et al., 2003;

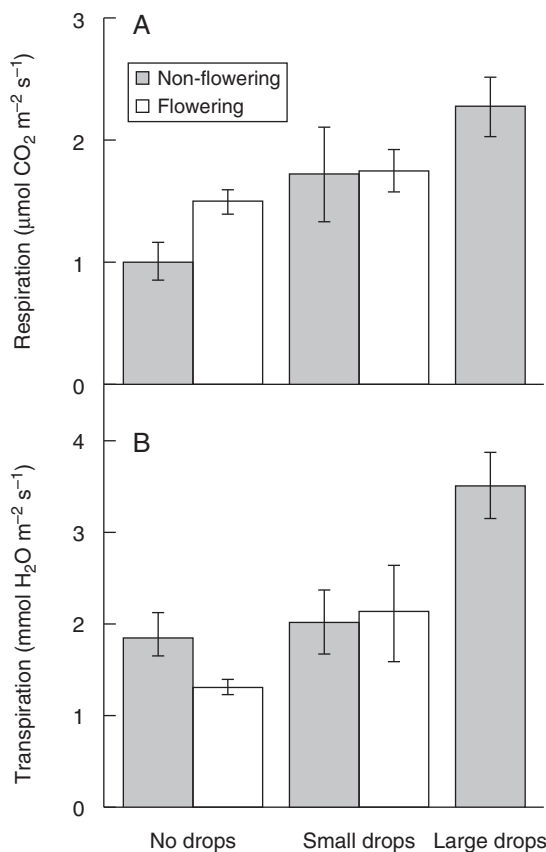


Fig. 5. Rates of respiration (A) and transpiration (B) measured on the leaves of *Rhinanthus alectorolophus* with various sizes of water drops at the two developmental stages of the plants. Means and standard errors are presented. Non-flowering plants (55 d after sowing) and flowering plants (73 d after sowing) are indicated in the key. No flowering plants with large drops were recorded.

Phoenix and Press, 2004). Stomata of some hemiparasitic species including *Rhinanthus* spp. are insensitive to abscisic acid and remain open even at night or under water stress (Smith and Stewart, 1990; Jiang *et al.*, 2003). Still, the hemiparasite's night transpiration rate is very low due to high ambient relative air humidity. Driving the xylem stream during night-time independently of air humidity, the active water secretion by hydathode trichomes can play a crucial role of an additional mechanism decreasing the water potential. The hemiparasite does not compete with the host shoot for the host xylem stream under these conditions, which results in an exclusive flow of the xylem sap to the hemiparasite strongly facilitating resource acquisition. Such a role for hydathode trichomes in plant mineral nutrition and water balance is not unique to the (hemi)parasitic plants discussed here. These structures were suggested to play a similar role in young leaves of some non-parasitic plants, in particular under the conditions when transpiration is low (Frey-Wyssling, 1941; Höhn 1950; Klepper and Kaufmann, 1966; Heide-Jørgensen, 1980). The mechanism of active water secretion from hydathode trichomes, when water is transported through the cell wall against its osmotic potential, is not known yet. Nevertheless, recent studies suggest that water secretion may be driven by a co-transport of water and ions through

specialized protein co-transporters (Zeuthen and MacAulay, 2012; Wegner, 2014).

Despite requiring energy, the water secretion from the hydathode trichomes is highly efficient according to our gas exchange measurements (1 mmol water release per the loss of 0.55 µmol C) (Fig. 4A). The effect of water secretion on the carbon balance of hemiparasites depends on the concentration of carbon in the xylem sap (Těšitel *et al.*, 2010b, 2011; Bell and Adams, 2011) and the efficiency of its filtering from the sap on its way to the guttation fluid (Govier *et al.*, 1968). The organic carbon is contained in the xylem sap mostly in the form of organic acids, amino acids and sugars (Canny and McCully, 1988). The concentration of organic carbon (in terms of organic C atoms) in the xylem sap necessary to compensate the carbon loss through respiration is 31 mM (Supplementary Data Methods). Taking this concentration into account and considering the filtering efficiency of <100 %, we expect that the direct effect of water secretion on carbon balance would be close to neutral (Govier *et al.*, 1967; Seel and Jeschke, 1999; Alvarez *et al.*, 2008) to positive (Canny and McCully, 1988) in hemiparasites growing on grass species. Although the amount of organic carbon in the xylem sap of trees varied significantly between seasons, the effect of water secretion on carbon balance in holoparasitic *Lathraea* growing on tree species would be positive [Schill *et al.*, 1996; Heizmann *et al.*, 2001; Escher *et al.*, 2004; but not in all cases, see Furukawa *et al.* (2011); Supplementary Data Methods]. The positive carbon balance of the active water secretion by hydathode trichomes might be crucial for the evolution of the xylem-only feeding holoparasitic strategy of *Lathraea* (Ziegler, 1955) and early developmental stages of *Rhynchosorys* and *Tozzia* species (Weber, 1973; Kubat and Weber, 1987), which would not be able to compensate the negative carbon balance of the active water secretion by their own photosynthesis.

The increased activity of the hemiparasite hydathode trichomes under the N+ conditions probably reflects a generally better physiological performance of hemiparasitic plants. However, the host may also perform better under the N+ conditions and its competitive ability (in terms of competition for light) may increase. This can reduce the fitness of hemiparasites which are in general poor competitors (Matthies, 1995; Lepš, 1999; Mudrák and Lepš, 2010; Fibich *et al.*, 2010; Těšitel *et al.*, 2013) and decrease the effect of parasitism (Těšitel *et al.*, 2015). The increased activity of the hydathode trichomes might thus partially compensate this negative effect by facilitating host-derived carbon acquisition and also inflicting more harm to the host. Both of these effects would decrease the competitive ability of the host and shift the hemiparasite–host fitness balance in favour of the hemiparasite.

Conclusion

Hydathode trichomes might be seen as an evolutionary innovation facilitating the resource acquisition of hemiparasitic Rhinanthoid Orobanchaceae and decreasing the adverse effects of the competitive pressure from the host community. Given their ubiquity among the Rhinanthoid Orobanchaceae (Fedorowicz, 1915; Kaplan and Inceoglu, 2003), they might also be considered a physiological pre-adaptation allowing the

evolution of the xylem-only feeding holoparasitic strategy. This xylem-only feeding holoparasitic strategy evolved two or three times independently within the Rhinanthoid clade, and the incomplete and complete transitions from hemiparasitism to holoparasitism in the Rhinanthoid clade represent relatively recent evolutionary events (Těšitel *et al.*, 2010c; Scheunert *et al.*, 2012; McNeal *et al.*, 2013). The knowledge of the evolutionary mechanism of these transitions together with well-resolved phylogenetic relationships thus make the Rhinanthoid clade an ideal model group for studying the macroevolution of trophic strategies in parasitic plants.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournaljournal.org and consist of the following. **Figure S1**: images of hemiparasitic *Rhinanthus alectorolophus* before and during the peak flowering period. **Figure S2**: image of stalked and sessile hydathode trichomes on the abaxial leaf surface of *R. alectorolophus* in water as mounting medium. **Figure S3**: ordination diagrams correlating response data and environmental variables in non-flowering and flowering plants. **Table S1**: guttation, respiration, transpiration and relative water content data recorded in the study. Methods: carbon budget calculations regarding the activity of hydathode trichomes.

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