Water flows in the parasitic association *Rhinanthus minor*/*Hordeum vulgare*

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Abstract

Using the facultative root hemiparasite *Rhinanthus minor* and its host *Hordeum vulgare* several aspects of water relations have been measured in this parasitic association. Extraction of xylem sap by the parasite from the host’s roots is facilitated by considerably higher transpiration per leaf area in the parasite than in the host and by the fact that stomata of attached *Rhinanthus* were open all day and night despite extremely high ABA concentrations in the leaves. By comparison, another root hemiparasite, *Melampyrum arvense*, parasitizing various grasses in the field, showed normal diurnal stomatal behaviour. The abnormal behaviour of *Rhinanthus* stomata was not due to anatomical reasons as closure could be induced by applying high external ABA concentrations. Remarkable differences have been detected between the hydraulic conductance of barley seminal roots showing relatively low values and that of *Rhinanthus* seminal roots showing very high values. The latter could be related to the observed high ABA concentrations in these roots. Whole plant water uptake, transpirational losses, growth-dependent deposition, and the flows of water within the plants have been measured in singly growing *Rhinanthus* and *Hordeum* plants and in the parasitic association between the two. Water uptake, deposition and transpiration in *Rhinanthus* were dramatically increased after attachment to the barley host; most of the water used by the parasite was extracted as xylem sap after penetrating the xylem vessels. In agriculturally used grassland even those facultative hemiparasites like *Rhinanthus minor* can cause significant damage (Parker and Riches, 1993). As shown previously with the host-specific, obligatory root hemiparasite *Striga hermonthica*, the harvesting of the host xylem sap is optimized by establishing a continuously high leaf conductance of the parasite (Taylor and Seel, 1998). Nothing, however, is known about the physiological background of this striking stomatal behaviour and the consequences for the water flows in the host/parasite association. Specifically, it is not known whether, in hemiparasites, the same mechanisms that limit transpiration in the case of soil water deficiencies, such as the action of ABA transmitted from the root to the leaves, also are operative in xylem-tapping root parasites. *Rhinanthus minor* as a hemiparasite is not host-specific and can grow on a wide range of hosts, albeit with a preference for legumes and grasses (Seel and Press, 1994). In the present study *Rhinanthus* has been cultivated on barley and a

Introduction

Xylem-tapping root hemiparasites, such as *Rhinanthus minor*, attach to the root systems of their hosts and extract xylem sap after penetrating the xylem vessels. In agriculturally used grassland even those facultative hemiparasites like *Rhinanthus minor* can cause significant damage (Parker and Riches, 1993). As shown previously with the host-specific, obligatory root hemiparasite *Striga hermonthica*, the harvesting of the host xylem sap is optimized by establishing a continuously high leaf conductance of the parasite (Taylor and Seel, 1998). Nothing, however, is known about the physiological background of this striking stomatal behaviour and the consequences for the water flows in the host/parasite association. Specifically, it is not known whether, in hemiparasites, the same mechanisms that limit transpiration in the case of soil water deficiencies, such as the action of ABA transmitted from the root to the leaves, also are operative in xylem-tapping root parasites. *Rhinanthus minor* as a hemiparasite is not host-specific and can grow on a wide range of hosts, albeit with a preference for legumes and grasses (Seel and Press, 1994). In the present study *Rhinanthus* has been cultivated on barley and a
technique of modelling water flows (Jiang et al., 2001; Jeschke et al., 1996) has been adapted for the *Rhinanthus Hordeum* association in order to describe quantitatively the water flows between the rhizosphere and both partners, between the host and the parasite and between the different organs. High leaf conductance has been shown to be a basis for successfully exploiting the host xylem sap, but a possible contribution of the hydraulic conductivity of host and parasite root systems has so far not been studied. Seel and Jeschke (1999) have shown that xylem transport in *R. minor* is markedly increased after attachment to barley, however, until now root hydraulic conductance in a hemiparasite has not been investigated. Some information about leaf conductance is available for *Striga* and some other hemiparasites (for references see the review article of Press et al., 1999), but *R. minor* has not been investigated in this respect except for some data published by Press et al. (1988). A possible role of the plant stress hormone abscisic acid (ABA) is of special interest in this system because it regulates both leaf conductance and root hydraulic conductance (Hose et al., 2000).

**Materials and methods**

**Plants**

Seeds of *Rhinanthus minor* from Emorsgate Seed Suppliers (Kings Lynn, UK) were surface-sterilized for 2–3 min in 6% sodium hypochlorite and germinated in petri dishes on a double layer of filter paper moistened with sterilized tap water at 4 °C. Two and a half months later radicles of 2–5 mm length emerged. An aqueous solution of 10−3 M gibberellic acid also has been tested, but without a positive effect on germination.

Caryopses of *Hordeum vulgare* were germinated on filter paper moistened with 0.5 mol m−3 CaSO₄ at 28 °C for 3 d and transplanted into 1.0 l pots containing washed sand. To obtain a *Rhinanthus* barley association *Rhinanthus* seedlings were placed into the sand at a distance of 1–1.5 cm from the *Hordeum* seedling. The plants were watered daily, initially with a quarter-strength nutrient solution containing (in mM for full strength): 2 KNO₃, 0.5 NaH₂PO₄, 1.5 MgSO₄, 1.5 Ca(NO₃)₂, 0.1 Na₂FeEDTA, 0.05 ‘Fe-sequestrene’ (Na₂ Fe ethylene-diamine-o-hydroxy phenylacetae; Ciba-Geigy, Macclesfield, UK), 2.9 × 10−7 CuCl₂·2H₂O, 2.1 × 10−7 CoCl₂·6H₂O, 1.8 × 10−6 MnCl₂·4H₂O, 1.0 × 10−7 (NH₄)₀.5MoO₃·4H₂O, 0.8 × 10−7 ZnSO₄·7H₂O, 4.6 × 10−5 H₂BO₃ (Seel and Jeschke, 1999). Three days later, half-strength solution was supplied and, after a further 3 d, full-strength solution was supplied. Plants were cultivated in the greenhouse with a photoperiod of 12 h and a light intensity of 180–260 μmol m−2 s−1. In addition, from July to September plants were cultivated under outside conditions, but protected with a glass roof to prevent the input of rain water. These plants showed particularly good development and were also used for measurements of nutrient and water flows.

**Leaf conductance**

Leaf conductance was measured with 60–63-d-old plants using a porometer (AP4, Delta-T Devices Ltd., Cambridge, UK). Leaves were treated for 3.5 h with abscisic acid. In microtome sections of *R. minor* leaves the cuticle proved to be very thin (pictures not shown).

Uptake of ABA over the pretreatment period does not seem to be a problem. Aqueous solutions were applied directly to the leaves using a soft brush. Controls were treated with water. Leaf conductance has also been measured in flowering plants of *Rhinanthus minor* and a close, hemiparasitic relative, *Melampyrum arvense*, both belonging to the Scrophulariaceae, both growing in the same natural grassland together with their likely hosts *Arenatherum elatius* and *Trisetum flavescens*.

**Modelling of water flows**

*Rhinanthus* and *Hordeum* were cultured under outside conditions (see above) with a light intensity of 850–1150 μmol m−2 s−1 on sunny days and 150–220 μmol m−2 s−1 on cloudy days. For the 1st harvest, five unattached *Hordeum* and five unattached *Rhinanthus*, and five *Hordeum/Rhinanthus* associations were used 41 d after planting, the second harvest occurred 13 d later. Barley plants were separated into leaf laminae, leaf sheath and roots, and *Rhinanthus* was separated into shoot and root. Fresh and dry weight was taken to determine the water content of the tissue.

Whole shoot transpiration was measured on a daily basis before and after the daily supply of nutrient solution and draining, by weighing pots containing solitary *Hordeum* or *Rhinanthus* plants or containing the *Rhinanthus/Hordeum* association. All pots were covered with a plastic film. Corrections were applied for the water loss from covered pots without plants. The partitioning of transpiration between various plant parts was determined gravimetrically at harvest. This was done by first measuring the water loss of a whole potted plant and then that of its separate, excised organs by a series of consecutive weighings over 5 min, immediately following detachment of each organ. The validity of the technique has been discussed and demonstrated previously by Jeschke and Pate (1991).

Water flows were calculated as described earlier by Jiang et al. (2001) and Hibberd et al. (1999). The calculation of net water flows in the *Hordeum/Rhinanthus* association was based on the assumption that water uptake by the attached *Rhinanthus* roots of known fresh weight was the same as the uptake measured simultaneously for roots of unattached *Rhinanthus*, allowing for the root fresh weight. A higher water uptake by attached *Rhinanthus* roots appears unlikely, since haustoria are likely to provide the water pathway of lowest resistance. Otherwise, xylem sap would not be taken up from the host, which contradicts the observed improved growth of the parasite. There is no reason either for assuming a lower water uptake by the roots of the attached parasite. If this were the case, then water and nutrient uptake from the host would be even higher than follow from the present modelling. In this context the anatomy of the roots of attached *Rhinanthus* above and below the haustoria needs to be investigated. These studies are in progress. The water loss by the shoot of the attached *Rhinanthus* was calculated from the partitioning of transpiration between *Hordeum* and *Rhinanthus* and the total water loss from the association. Because of the light-insensitive *Rhinanthus* stomata, the partitioning of transpiration between *Hordeum* and *Rhinanthus* was obtained using the water loss data of *Rhinanthus* over 24 h and that of barley over 14 h (the day length during the time of experiment). The estimation of the water uptake and water flow in xylem and phloem was outlined by Jiang et al. (2001).

**Scanning electron microscopy**

Leaf samples were fixed in a mixture of ethanol/formalin/glacial acetic acid (90/5/5 by vol) and the water was removed in an aceton series. After critical point drying samples were sputtered with gold and investigated in a Zeiss DSM 962 scanning electron microscope.

**Analysis of ABA**

Freeze-dried tissue samples were homogenized and extracted in 80% methanol. Extracts were passed through a Sep-Pak C₁₈-cartridge. Methanol was removed under reduced pressure and the aqueous residue partitioned three times against ethyl acetate at pH 3.0. The
ethyl acetate of the combined organic fractions was removed under reduced pressure. The residue was taken up in TBS-buffer (TRIS-buffered saline; 150 mmol l⁻¹ NaCl 1 mmol l⁻¹ MgCl₂ and 50 mmol l⁻¹ TRIS; pH 7.8) and subjected to an immunological ABA assay (ELISA) as described earlier (Peuke et al., 1994; Mertens et al., 1985). The accuracy of the ELISA was verified in earlier investigations (Hartung et al., 1994). Recoveries of ABA during purification procedures were checked routinely using radioactive ABA and found to be more than 95%. The immunochemicals were generously supplied by Professor Weiler, Ruhr Universität Bochum (Germany).

Hydraulic conductivity of roots
A root pressure probe has been used to determine the root hydraulic conductivity of roots of Rhinanthus and of barley seedlings. Rhinanthus roots 5–8 cm in length and excised seminal roots of 14-d-old barley plants 11–12 cm in length were used. Measurements have been performed as described earlier by Steudle and Jeschke (1983), Steudle (1993) and Hose et al. (2001). Experiments have been performed at least four times. Standard errors are given in the figures and tables.

Results
SEM micrographs of the surface of fully differentiated leaves of Rhinanthus and Melampyrum arvense are shown in Fig. 1. Whereas stomata of unattached R. minor plants appeared to be closed (Fig. 1c), even in the light, those of

Fig. 1. Scanning electron micrographs (SEM) of the surface of leaves of attached Rhinanthus in the light (a) or in dark (b) and of unattached Rhinanthus minor leaves in the light (c). SEM pictures of Melampyrum arvense (d in the dark, e in the light) are also presented. Leaves of Rhinanthus minor were from plants growing on barley, M. arvense was from the field.
attached *R. minor* were always open, even in darkness (Fig. 1a, b). In darkness, stomata seemed to be even wider open than in the light. *Melampyrum arvense* exhibited more normal daily changes. Their stomata were open in daylight and closed in the dark (Fig. 1d, e).

Figures 2 and 3 show daily courses of transpiration of *Rhinanthus* and its host grown in the field in its natural habitat and in the greenhouse. In both cases, an unusual diurnal pattern of leaf conductance could be observed. The leaf conductance of *R. minor* in the field was extremely high (1200 mmol m⁻² s⁻¹) during the morning hours and decreased slightly down to values of approximately 600 mmol m⁻² s⁻¹ in the afternoon. By the end of the light period, transpiration measured in darkness, however, had increased again, reaching levels similar to those earlier in the day. The leaf conductance of the host *Arenatherum elatius* and *Trisetum flavescens* in the field were always clearly lower than in *Rhinanthus minor*. They showed the normal diurnal pattern (Fig. 2).

In the greenhouse (Fig. 3) the overall leaf conductance of *Rhinanthus* was clearly lower (290 mmol m⁻² s⁻¹). Although the experimental set-ups were different, the diurnal pattern was similar to the pattern that had been observed in the field. As shown by Fig. 3, the leaf conductance of *Rhinanthus* was highest when measured in the dark in the early morning and decreased during the day, however, it increased again overnight. When *Rhinanthus* leaves were treated with 10⁻⁵ M ABA, leaf conductance remained unaffected, whereas in barley 10⁻⁶ M ABA significantly reduced leaf conductance. Treatment of *Rhinanthus* leaves with 10⁻⁴ M ABA was needed to induce a closing response in *Rhinanthus* stomata to ABA (Table 1). In fact, endogenous ABA in leaves of *Rhinanthus* plants proved to be 53 times higher than in leaves of the parasite-infected barley host (Fig. 4).

Hydraulic conductivity of roots of *Rhinanthus* and *Hordeum vulgare*

Hydraulic conductivity of seedling roots of barley and *R. minor* have been measured using a root pressure probe (Hose et al., 2000), which allows the estimation of the conductivity of the apoplastic and symplastic transport (Table 2). Both conductivities were clearly higher in *Rhinanthus*, *Lpr* (symplastic) being ten times and *Lpr* (apoplastic) even 100 times higher than in barley. Light microscopy of roots of *Rhinanthus* did not clearly reveal the existence of any visible and stainable apoplastic barrier such as Casparian bands in the hypodermis or endodermis (Fig. 5). The concentrations of endogenous ABA in seedlings’ roots of *Rhinanthus* and of barley have been compared, because ABA has been shown to regulate the symplastic *Lpr* in maize (Hose et al., 2001). Endogenous ABA of *Rhinanthus* roots was higher than in barley by a factor of up to 3.7 (Fig. 6).

Flows of water

Water flow models in unattached *Rhinanthus*, in non-parasitized barley and in the parasitic association barley/ *Rhinanthus* are shown in Figs 7 and 8. Almost all the water taken up by unattached *Rhinanthus* was released by the leaves to the atmosphere. Due to the very slow growth only marginal quantities of cell water were incorporated into the roots and shoots of *Rhinanthus* (Fig. 7). After attachment to barley, total water uptake into *Rhinanthus* was increased nearly 5-fold, the largest portion of which was extracted from the host roots. About 18% of the water taken up by the barley roots was diverted to the parasite. Water uptake by incorporation to the roots of *Rhinanthus* was doubled after attachment, as was the incorporation into root tissues,
whilst water incorporation into shoot tissues increased 14-fold (Fig. 8), reflecting the substantial increase in the parasite growth and in its shoot-to-root ratio. The growth-dependent incorporation of water into roots of parasitized barley was not affected by the parasite, whereas incorporation into growing shoot tissues was substantially decreased in parasitized barley, compared to uninfected barley, i.e. in leaf lamina by 23% and in the leaf sheath fraction by 43% (Figs 7, 8). The marked effect in the leaf sheath fraction was due to the fact that it also contained the growing stem and the apical bud.

Discussion

Although as a facultative parasite Rhinanthus is able to survive, to grow slowly and reproduce without a host, for normal leaf and stem development it needs to find and successfully attach to the roots of a suitable host (Seel et al., 1993). Unattached seedlings of Rhinanthus minor reached a height of approximately 10 cm only within three months and its leaves showed all the symptoms of a cytokinin and nitrogen deficiency. Indeed the cytokinins content of the zeatin type in roots of unattached Rhinanthus proved to be significantly lower than those in barley roots (zeatin 20% and zeatin riboside 40% of barley roots: F Jiang, unpublished data). Both hormones were required for an undisturbed leaf development, auxin for the vascular system and cytokinins for the mesophyll (Wareing and Phillips, 1981). The zeatin concentration in the xylem sap of barley was found to be in the range of 400–500 nM and that of auxin 500 nM (F Jiang, unpublished data), hence these hormones together with mineral nutrients could be exploited easily by the parasite Rhinanthus after forming an haustorium and penetrating into the xylem vessels of the barley host root. This exploitation of xylem sap was facilitated by the high leaf conductance and transpiration of Rhinanthus. High transpiration rates of hemiparasites as a means of exploiting the host xylem have long been noted in mistletoe species like Amyema nestor (Gill and Hawksworth, 1961) and in Striga hermonthica (Taylor and Seel, 1998). Press et al. (1988) reported for Rhinanthus minor a night/day transpiration ratio of 0.84 indicating a slightly reduced stomatal aperture during the night. The experiments of this paper gave, for the first time, daily courses of Rhinanthus minor leaf conductance growing in the field on Arenatherum elatius and Trisetum flavescens and in the greenhouse parasitizing on barley. The leaf conductance of attached Rhinanthus grown in their natural habitat was extremely high (1200 mmol m⁻² s⁻¹) during the morning. It is decreased during the day to values around 600 mmol m⁻² s⁻¹, whereas the likely hosts exhibited much lower leaf conductance and showed normal diurnal patterns of leaf conductance (Fig. 2). The decrease in transpiration during the day may have resulted from the very high prevailing temperatures at the particular time of the year (up to 38 °C). When the light intensity decreased during the afternoon, stomata of the host began to close, whereas those of Rhinanthus minor opened again resulting in values above 1000 mmol m⁻² s⁻¹, whereas the likely hosts exhibited much lower leaf conductance and showed normal diurnal patterns of leaf conductance (Fig. 2). The decrease in transpiration during the day may have resulted from the very high prevailing temperatures at the particular time of the year (up to 38 °C). When the light intensity decreased during the afternoon, stomata of the host began to close, whereas those of Rhinanthus minor opened again resulting in values above 1000 mmol m⁻² s⁻¹, whereas the likely hosts exhibited much lower leaf conductance and showed normal diurnal patterns of leaf conductance (Fig. 2). The decrease in transpiration during the day may have resulted from the very high prevailing temperatures at the particular time of the year (up to 38 °C). When the light intensity decreased during the afternoon, stomata of the host began to close, whereas those of Rhinanthus minor opened again resulting in values above 1000 mmol m⁻² s⁻¹, whereas the likely hosts exhibited much lower leaf conductance and showed normal diurnal patterns of leaf conductance (Fig. 2). The decrease in transpiration during the day may have resulted from the very high prevailing temperatures at the particular time of the year (up to 38 °C). When the light intensity decreased during the afternoon, stomata of the host began to close, whereas those of Rhinanthus minor opened again resulting in values above 1000 mmol m⁻² s⁻¹, whereas the likely hosts exhibited much lower leaf conductance and showed normal diurnal patterns of leaf conductance (Fig. 2).

Fig. 4. The concentrations of endogenous ABA in leaves of infected and uninfected plants of Rhinanthus minor and Hordeum vulgare. Means ± SE, n=12.

Table 1. Effect of ABA applied directly to leaves of the Rhinanthus/Hordeum association on leaf conductance

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf conductance (mmol s⁻¹ m⁻²)</th>
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<tbody>
<tr>
<td></td>
<td>Attached Rhinanthus</td>
</tr>
<tr>
<td></td>
<td>Control ABA treatment</td>
</tr>
<tr>
<td>10⁻¹ M ABA</td>
<td>463±44</td>
</tr>
<tr>
<td>10⁻² M ABA</td>
<td>451±60</td>
</tr>
<tr>
<td>10⁻³ M ABA</td>
<td>450±51</td>
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</tbody>
</table>

In the greenhouse the leaf conductance of R. minor parasitizing on barley showed a similar diurnal pattern, however, on a clearly lower level (Fig. 3). This is not very likely to have resulted from the lower light intensity, because stomata of Rhinanthus were shown to be open in the dark (Fig. 1); it is more likely that the high CO₂
concentration within the greenhouse, reaching values up to 500 ppm, was the more decisive external factor. As in the field, however, leaves of *Rhinanthus minor* clearly exhibited higher leaf conductance than those of the hosts (Fig. 2). The facultative hemiparasite *Rhinanthus* thus clearly appears to optimize xylem sap extraction from its hosts in the same way as the obligate hemiparasite *Striga hermonthica* (Taylor and Seel, 1998) by rates of transpiration higher than in the host. By contrast, holoparasites such as *Cuscuta* and *Orobanche*, which exploit both phloem and xylem, maintain low rates of transpiration and CO₂ exchange (Jeschke *et al.*, 1994; Ehleringer and Marshall, 1995). These parasites in this way avoid excessive extraction of minerals from their hosts, which might lead to osmotic stress, excessive nitrogen nutrition or even toxic effects.

Abscisic acid, the universal plant stress hormone that regulates water relations of the plants on the stomatal level, was very high in the leaves of attached *Rhinanthus minor* compared to those of the parasitized host barley (Fig. 4). A similar situation has been observed in the *Striga/Zea* association by Taylor and Seel (1998). Despite high endogenous ABA levels in *Rhinanthus* leaves, their stomata were fully open. Closure could only be achieved after the leaves had been painted with 10⁻⁴ M ABA, a concentration which is two orders of magnitude higher than that required to close stomata of the host (Table 1). These findings show that guard cells can obviously react to internal and external factors such as CO₂ and ABA. The leaf conductance of *Rhinanthus* was normally above that of the host, except for a few occasions in the greenhouse. The extreme insensitivity to internal and external factors was apparently not a result of structural defects of the guard cells as can be the case in the stomata of floating organism (Lemnaceae, Landolt and Kandeler, 1987) or of tobacco plants with disturbed ABA relations (Wigger *et al.*, 2002).

Light microscopy of cross-sections and scanning electron microscopy of *Rhinanthus minor* stomata did not indicate that the stomata may be locked open by anatomical features. The remarkable insensitivity is probably the result of special biochemical features (receptors?). At present it cannot be explained which constituents of the host xylem sap, after successful attachment, cause the previously closed stomata of *Rhinanthus* to open. Of all compounds detected in the xylem of barley, cytokinins of

### Table 2. Apoplastic and symplastic hydraulic conductivities (Lpr) of roots of seedlings of *Rhinanthus* and barley

<table>
<thead>
<tr>
<th></th>
<th><em>Rhinanthus</em> (ms⁻¹ MPa⁻¹)</th>
<th><em>Barley</em> (ms⁻¹ MPa⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lpr (apoplastic)×10⁷</td>
<td>120±0.2</td>
<td>1.2±0.3</td>
</tr>
<tr>
<td>Lpr (symplastic)×10⁹</td>
<td>46±2.7</td>
<td>0.8±0.4*</td>
</tr>
</tbody>
</table>

* For comparison, data of Steudle and Jeschke (1983) are given. Means ±SE, n=4.
the zeatin type may be good candidates. The existence of other unknown substances cannot be excluded.

Besides stomatal conductance the root hydraulic conductivity also plays an important role for water relations and water flows within a plant especially under transpiring conditions. The root pressure probe has, therefore, been used to determine apoplastic and symplastic root hydraulic conductivity of barley and *Rhinanthus*, as has been described earlier by Steudle and Jeschke (1983), Steudle (1993) and Hose et al. (2001). The apoplastic component of root hydraulic conductivity of unattached *Rhinanthus* roots proved to be 100 times higher than in barley and the symplastic component of *Rhinanthus* roots was still 10 times higher than in the host plant (Table 2). The very high apoplastic Lpr of *Rhinanthus* raises the question about properties of apoplastic transport barriers in *Rhinanthus* roots. As can be seen in the micrograph of Fig. 5, no stainable structures that resemble Casparian bands, neither in the endodermis nor in the hypodermis can be seen. As in the case of stomata, root hydraulic conductivity is also regulated by ABA (Hose et al., 2001). The high levels of endogenous ABA of *R. minor* roots (3.7 times higher than in barley) corresponds with such a role of this hormone (Fig. 6).

The water flow models, which were obtained according to the technique of Jiang et al. (2001) and Jeschke et al. (1996), indicated how the phenomena described above are integrated in the intact system. Most of the water taken up by roots of unattached *R. minor* was released by the leaves to the atmosphere (Fig. 7). This could only happen either by cuticular transpiration or by a residual transpiration via closed stomata, because in unattached *Rhinanthus* the stomata are always tightly closed. Since the unattached *Rhinanthus* show some growth and their photosystem II is clearly operative (F Jiang., unpublished data), some CO₂ exchange also occurred. Indeed, the tightly closed stomata and restricted water uptake by solitary, unattached *Rhinanthus* is likely to be a precaution against excessive uptake of mineral salts and nutrients, which, due to the so far unexplained restriction of leaf and shoot growth, cannot be used but rather could lead to some salt damage. In this respect the closed stomata appear to be a ‘strategic’ precaution. As evidenced by the high hydraulic conductance of *Rhinanthus* roots water uptake was clearly not restricted by the roots.

The data of water incorporation in Figs 7 and 8 and their changes in barley due to parasitic infection and in *Rhinanthus* in response to successful attachment to a
host are indicative and the result of growth, i.e. primarily elongation growth. Water incorporation in unattached *Rhinanthus* was extremely small, reflecting the poor growth. After attachment, water incorporation into the *Rhinanthus* shoot was 14-fold increased, but in the root it was only doubled, together reflecting an enormous increase in the shoot-to-root ratio of the parasite.

Uptake of water by *Rhinanthus* roots was also doubled, but the largest proportion of water used by the parasite was derived from the barley host, a quantity which amounted to nearly 20% of the total water taken up by the host. The water flow models of Figs 7 and 8 also reflect the impact of parasite infection in the host: as indicated by the water deposition. Growth-dependent water deposition in the host was decreased by 23% in the leaf lamina and by 43% in the leaf sheath fraction (which also contained the growing stem and apical bud), but water deposition in the root was as high as in the non-parasitized barley. This points to a decreased shoot-to-root ratio, which actually decreased from 3.5 to 2.8, and to relatively favoured root growth in response to parasite infection, as has similarly but much more dramatically been observed after the infection of *Sorghum* by the root hemiparasite *Striga hermonthica* (Parker and Riches, 1993).

Even though in the host the reduction in total water uptake (by 28%), in growth-dependent water deposition (by 33%) or in transpiration (by 36%) was not that dramatic, however, these changes reflect the impact of the relatively small xylem-tapping hemiparasite *Rhinanthus* on the host and agrees with the significant damage *Rhinanthus* can cause in agriculturally used grassland (Parker and Riches, 1993), although this effect again is much smaller than the damage caused by *Striga hermonthica* (Parker and Riches, 1993). However, the reduction in the growth of barley was much more severe, when more than one *Rhinanthus* plants were parasitizing just one barley plant.

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We are grateful to Dr Wendy Seel (Aberdeen) and Professor Steudle (Bayreuth) for helpful discussions and experimental help, to Dr Markus Woitke for measuring the atmospheric CO2 in the greenhouse, to Mr Robin Wacker (Würzburg) for help with microtomy, and to Mrs Bianca Röger for skilful technical help. The financial support of Deutsche Forschungsgemeinschaft (SFB 567, TPC1) is gratefully acknowledged.

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