Water, mineral nutrient and hormone flows and exchanges in the hemiparasitic association between root hemiparasite *Rhinanthus minor* and the host *Hordeum vulgare*

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1. Introduction

1.1 Parasitic plants: a general introduction

Within the angiosperms more than 3000 species in 17 plant families are parasites (Press et al., 1990), which constitutes about 1% of all flowering plants (Musselman and Press, 1995). Parasitism is thought to have arisen at least eight times during evolution and the plants occupy all global ranges from the poles to equator (Press, 1989). Parasitic angiosperms can be classified as either root or shoot parasites, depending on the site of attachment to the host. They can also be described as hemi- and holoparasites, with regards to the presence or absence of chlorophyll (Musselman and Press, 1995). Holoparasitic species are always obligate parasites, devoid of chlorophyll with little independent capacity to assimilate carbon and inorganic nitrogen. They receive all carbon from the host plants. Hemiparasites may be facultative or obligate; they are chlorophyllous and are traditionally thought to rely on their host only for water and minerals and synthesise carbon themselves (Press et al., 1990). Parasitic angiosperm can be obligate (e.g. *Striga, Alectra*), requiring host for survival, or facultative (e.g. *Rhinanthus, Euphrasia, Orthocarpus*) surviving also in the absence of the host.

Because of the serious damage on agricultural crops caused by some parasites, a lot of research has been performed in the past. The crops most affected are cereals and legumes and the parasites originate from a number of genera including the Scrophulariaceae, Orobanchaceae, Viscaceae, Loranthaceae and Convolvulaceae (Press et al., 1990). *Rhinanthus minor*, the facultative root hemiparasite, has been studied as the foe of the grassland during the first half of last century and the farmers have tried to get rid of it (Fig. 1.1 shows a grassland heavily infected with *Rhinanthus*). But recently more and more scientists have come to the conclusion that *Rhinanthus minor* can be used as a tool to increase the diversity of grasslands (Bullock, 2004; Matthies, 2004; Cameron and Seel, 2004). *Rhinanthus minor* may

play a role in shaping the structure of a community by suppressing grasses and favouring forb growth (Cameron and Seel, 2004). The benefits of infection for *Rhinanthus* on host grasses could be transfer of water, nutrients, assimilates and hormones after successful formation of haustoria.

Fig. 1.1 *Rhinanthus minor* in the meadow of an organically managed farm in north-east England.

1.2 Haustoria

Haustoria are a characteristic feature of all parasitic plants. It is the most common phenotypic expression of this highly diverse group of organisms. Originating in different species at either shoot or root positions, it is a remarkable unit, unique both in structure and function. They attach the parasite to the host and penetrate host tissue, thus enabling the transfer of water and solutes from host to parasite (Press et al., 1990; Stewart and Press, 1990). Mature haustoria often appear as swollen, round structures firmly attached to or clasping the host surface. The portion penetrating to the host vascular tissue is referred to as the endophyte.

Initiation of an haustorium is caused either by a mechanical signal (i.e. contact), a chemical signal, or both. The first haustorium inducers isolated were phenylpropanoids, named xenognosin A and B (7,2'-hydroxy-4'-methoxyisoflavone),

extracted from a nonhost plants, *Astragalus gummifer* (Lynn et al., 1981). A number of analogues were synthesised, which showed that *m*-methoxyphenol functionality was a strict requirement for haustorium-inducing activity in *Agalinis purpurea* (Steffens et al., 1982). Subsequently, a simple phenolic compound, 2,6-dimethoxy-*p*-benzoquinone (2,6-DMBQ) was identified and shown to be a haustorial-inducing factor for *Striga* and *Agalinis* (Chang, 1986; Chang and Lynn, 1986). It is important to emphasize that in addition to phenolic compounds, various cytokinins also induce haustoria (Worsham et al., 1959; Williams, 1961; Riopel and Timko, 1992).

The first response for haustorial initiation is a cortical enlargement and cell division of the root of the parasite generating a localised lateral or terminal protuberance (Riopel and Timko, 1995). After initiation, haustorial growth is rapid and there is a large variation in development, depending on species (Press et al., 1990).

The site of haustorial development in the Scrophulariaceae is usually at secondary or lateral positions along the root of the parasite. There are three characteristic regions within the haustoria of the Scrophulariaceae; the vascular core, the hydraline body (including the vessels) and endophyte (that portion of the haustorium contained within the host) (Riopel and Timko, 1995).

The existence of apoplastic continuity between host and parasite has led to the conclusion that the haustorium of hemiparasites has a passive role in solute transfer (Raven, 1983). Ultrastructural studies of the haustoria of several species indicate large numbers of parenchyma cells positioned at the endophyte interface. In haustoria of *Olax phyllanthi* (Olacaceae) Pate et al. (1990b) reported that the interface consists to more than 98.7% of parenchyma, only 0.01-0.11% of the total cell contacts between host and parasite involve direct apposition of conducting xylem elements. Several other studies, though not quantified, also report significant numbers of interfacial parenchyma. These include haustoria of the dwarf mistletoe *Arceuthobium* (Alosi and Calvin, 1985), the dwarf mistletoe *Korthalsella lindsayi* (Coetzee and Fineran, 1987), and the mistletoe *Ileostylus micranthus* (Condon and Kuijt, 1994). Histochemical

studies have shown the presence of high enzyme activities in the haustorial cells of *Striga hermonthica* (Ba and Kahlem, 1979). These features of haustorial ultrastructure imply an active metabolic role for the haustorium.

Host resistance mechanisms have been reported in several parasitic angiosperm-host associations. In the *Striga gesnerioides*-cowpea (Lane et al., 1993) and the *Orobanche aegyptiaca*-vetch association (Goldwasser et al., 2000), necrotic areas appeared at the site of parasite attachment due to localised cell death of the host tissue, resulting in degeneration of the parasite. Physical barriers to infection have been illustrated for the *Orobanche cumana*-sunflower/vetch association and for the *Striga hermonthica-Tripsacum dactyloides* association, where lignification of the host stele and tissues surrounding the penetrating endophyte was increased (Labrousse et al., 2001; Gurney et al., 2003). Chemical barriers to infection can be secreted at the host-parasite interface including phenolic compounds (Goldwasser et al., 1999) and induced phytoalexins (Wegmann et al., 1991; Jorrin et al., 1996).

1.3 Water relations

A noticeable physiological feature of most hemiparasites was their very high rates of transpiration, which exceeded those of their corresponding hosts by several fold. This maintains a gradient in leaf water potential towards the parasite and thus facilitates the flux of resources to the parasite. Early studies on mistletoe transpiration suggested little stomatal control over water loss in comparision with hosts (Härtel, 1956; Hellmuth, 1971), but recent evidence contradicts with this conclusion. Thus, the stomata of *Phoradendron villosum* have been shown to respond directly to vapour pressure deficit of the atmosphere (Hollinger, 1983). Stomatal closure occurs in *Loranthus europaeus* once leaf water content decreases (Glatzel, 1983), although lower leaf water potentials are required to induce stomatal closure in the parasite than in the host (Davidson et al., 1989; Glatzel, 1983). It is significant that night-time transpiration rates in parasites also exceed those of their hosts. While host stomata typically close at night, the stomata of annual and herbaceous root parasites in the

Scrophulariaceae often remain open at night, allowing transpiration to continue if leaf temperatures remain above the dew point (Shah et al., 1987; Press et al., 1988; Taylor and Seel, 1998). This phenomenon indicates that the parasite can extract water from its host throughout the night as well as during the day. It is surprising to find that stomatal of *Striga hermonthica* and *Striga asiatica* are less responsive to the water stress development, with stomata remaining open until the relative water content of the parasite leaves is reduced to about 70% (Shah et al., 1987). In line with these observations are reports that the stomata of *Striga* are much less sensitive to application of abscisic acid than those of *Antirrhinum majus*, a nonparasitic member of the Scrophulariaceae (Shah et al., 1987; Press et al., 1988).

The high rates of day and night transpiration and the weak responsiveness of stomata to water deficit and exogenous ABA may be general characteristics of leafy parasites. Nothing is known about the physiological background of this striking stomatal behaviour. Recent studies using epidermal strips prepared from *Striga hermonthica* leaves indicate that their high potassium content may modify their response to changes of irradiance, CO_2, and ABA (Smith, 1990).

High transpiration rates of parasites are generally interpreted as the principle factor responsible for maintaining a gradient in leaf water potential towards the parasites (Fisher, 1983; Schulze et al., 1984; Ehleringer et al., 1986). Comparisons of water potential between host and parasite for a wide range of species and enviromental conditions result in a more negative water potential of the parasite (Fisher, 1983; Scholander, 1965). Data collected from the mistletoes and their hosts have shown that there was a 1-2 MPa difference in the water potentials of mistletoes and the hosts to which they were attached (Scholander, 1965). Although the shoot water potential of *Rhinanthus serotinus* is lower before attachment, indicating a high resistance to water uptake from the roots, and becomes less negative after attachment, it is still more negative than that of the host plant, *Hordeum vulgare* (Klaren and Dijk, 1976).

The lower water potentials are accompanied by higher osmotic potentials. Indeed higher tissue concentrations of inorganic ions have been found in mistletoes (Popp,

1987; Pate et al., 1990), *Rhinanthus minor* (Klasen and Janseen. 1978; Seel and Jeschke, 1999) and in *Santalum acumimatum* (Loveys et al., 2001). The significant proportion of the osmotic potential was accounted for water-soluble ions. Higher concentrations of soluble carbohydrates such as mannitol has been found in the root parasites *Lathraea* (Stewart and Press, 1990), *Striga* (Simier et al., 1993) and *Santalum acumimatum* (Loveys et al., 2001), contributing substantially to osmotic potential.

Although there is little doubt that water potential differences between host and parasite are largely maintained by differential rates of transpiration, other factors such as the hydraulic resistance across the host-parasite interface play a role. Glatzel (1983) estimated haustorial resistances to be 2.6-4.2 times those generally found for plant stem. In experiment with *Amyema linophyllum* (Fenzl) Tieghem and *Casuarina obesa* Miq, Davidson et al. (1989) concluded that the haustorial junction was the principal site of resistance to transpiration-driven water flow into the parasite. Transpiration rates of *Amyema linophyllum* can decrease by a factor of 300 from a day's maximum to the following night's minimum, whereas water potential differences between host and parasite changes by a factor of two. So far no anatomical or physiological mechanisms have been investigated to account for variable resistance to flow, but it seems plausible that under conditions of high water stress during daytime parenchyma cells at the haustorial interface might contract as their relative water contents fell, thereby widening apoplastic pathway between haustorial cells and creating conditions of low hydraulic resistance at the interface. Conversely at night, when the water potential of the parenchyma cells recover, extracellular pathways would close and hydraulic resistances rise accordingly.

1.4 Nutritional relationships of parasites and their host

The most obvious change of parasites after attachment and formation of a functioning haustorium is a stimulation of growth (Klaren and Janssen, 1978; Seel et al., 1993a).

This appears to be related to an access to xylem sap including mineral elements and some organic substances (Seel and Jeschke, 1999; Seel and Press, 1993).

1.4.1 Nitrogen

Nitrogen is a limiting factor for growth of many plants. Parasitism can be considered as a strategy for acquiring more nitrogen. Nitrogen may be present in the transpiration stream in both organic and inorganic forms. Availability and utilisation of nitrate in parasites have been investigated by Mc Nally and Stewart (1987). They demonstrated that some root hemiparasites have low capacity to assimilate inorganic nitrogen. Nitrate reductase activity has been measured in some of the hemiparasitic Scrophulariaceae and activities range from below 1 µmol/h/g fresh weight in *Striga app.* to about 4 µmol/h/g in *Bartsia alpina*. However, some of those root hemiparasites appear to be able to take up inorganic nitrogen from the soil (*Rhinanthus minor*, Seel et al., 1993b) and show the ability of reducing NO_3^-. However, there is little evidence that the hemiparasites *Striga hermonthica* and *Striga asiatica* exhibit a reduced capacity for amino acid biosynthesis (Press et al., 1986). The root hemiparasite can reduce inorganic nitrogen to amino acids, and the nitrate reductase activities of the parasite differed between individuals parasitising on different hosts. Once *Rhinanthus* has attached to barley, 52% of nitrogen transported via the xylem became reduced and the percentage was 85% when *Trifolum* was the host (Seel and Jeschke, 1999). Asparagine (Asn) and aspartic acid (Asp) instead of glutamine (Gln) became the major amino acids in *Rhinanthus* xylem sap after parasitising on *Trifolum* and Asn instead of Gln was the principal amino acid in barley xylem sap after parasitism. These results show significant changes of compositions of amino acids in parasite xylem sap in response to attachment, also show changes in the host due to parasitism (Seel and Jeschke, 1999).

The extent to which growth is stimulated is much dependent on the nature of the host to which the parasite becomes attached, and biomass accumulation is much greater when the plants are parasitising a legume rather than a grass (Seel and Press,

1993; Cameron and Seel, 2004). Until now the reason is unclear, but we might expect that the plants show greater growth response to amino acids than to inorganic nitrogen.

1.4.2 Ion accumulation

The mineral relationships between host and hemiparasite in terms of elements composition have been studied by Klaren and Janssen (1978), Pate et al. (1990a) and Seel and Jeschke (1999). In most cases concentrations in dry matter of foliage or shoots of the parasites have been compared directly with those of the host. Virtually all studies record significantly higher concentrations of the major nutrients in parasites than in the host. The concentration ratios for elements in the hemiparasitic mistletoes to that in their hosts turn out to be by far the greatest for K^+ (maybe as much as 20 times those in their hosts) and then decrease in the order Mg^{2+}, Mn^{2+}, Na^+, Ca^{2+}, N and Fe^{2+} (Glatzel, 1983; Pate et al., 1990a), in the case of *Rhinanthus*/hosts associations the concentration ratios for elements in *Rhinanthus* to that in the hosts decreased in the order K^+, NO_3^-, $H_2PO_4^-$ (Seel and Jeschke, 1999). NO_3^- within the attached parasites is stored in the leaf tissue and in the stems, where it may serve as an osmoticum as NO_3^- is stored nearly exclusively in vacuoles (Martinoia et al., 1981). This vacular NO_3^- storage may contribute to the fleshy appearence of the leaves of *Rhinanthus* parasitising *Hordeum* (Seel and Jeschke, 1999).

If uptake occurred via the transpiration stream only, the relative proportions of ions in host and parasite leaves should be same. Differences in element ratios between host and parasite have promoted the suggestion that there is selective uptake of ions into parasite tissues via the haustorium (Lamont, 1983). This idea has been criticised on the grounds that no account is taken of the export of an ion such as potassium that occurs from host leaves via the phloem. High potassium concentrations in parasite leaves may simply reflect the absence of host-parasite phloem connections. The results of the study of *Dendrophthoe falcata* parasitising 48 different host species show a passive flux of ions from host to parasites (Glatzel, 1983). However, results

obtained with the *Odontites verna* parasiting on *Hordeum* and *Stellaria* (Govier et al., 1967), and with the *Striga-Sorghum* association (Mallaburn et al., 1990) show different compositions of xylem fluids of host and parasite.

Application of mineral nutrients to non-parasitising *Rhinanthus*, on the other hand showed growth stimulation only with added P but not with N or K^+, even though after addition particularly N was strongly accumulated in the parasite, suggesting that possibly nitrate reduction in unattached *Rhinanthus* was insufficient. The question what limits the growth of solitary *Rhinanthus*, therefore is still open.

1.4.3 Carbon assimilation

The hemiparasites are supposed to be able to assimilate carbon dioxide because of the presence of chlorophyll, but the rates of photosynthesis are low. The rates of photosynthesis in *Rhinanthus minor* plants grown in the absence of a host ranged from 0.6-2.3 μmol m^{-2} s^{-1}, depending on the nutrients supplied (Seel et al., 1993b). The highest rates of parasite photosynthesis were observed in those individuals attached to legumes. There was a strong positive relationship between the rates of photosynthesis in *Rhinanthus minor* and its growth on different hosts (Press and Seel, 1996). This relationship is at least partly a result of differences in the supply of organic nitrogen compounds from host to parasite, which may be used for a number of processes including the construction of photosynthetic machinery, as illustrated by higher concentrations of chlorophyll in the leaves of *Rhinanthus minor* associated with nitrogen-rich hosts (Seel et al., 1993a).

On a leaf area basis *Rhinanthus minor* has higher photosynthetic rates than *Striga* (Press et al., 1987b; Seel and Press, 1994). Low rates of photosynthesis in species of *Striga* are in part related to the relatively undifferentiated leaf mesophyll and the low number of plastids per mesophyll cell (Tuohy et al., 1986). Some studies have shown that *Striga* would not be able to maintain any appreciable positive carbon balance in the absence of carbon from the host (Graves et al., 1989; Graves et al., 1990). The extent to which hemiparasites are dependent on host carbon varies widely between

species, and also within species, again as a function of nitrogen supply. By comparison it has been shown that approximately 30% of carbon in the leaves of mature *Striga hermonthica* (Press et al., 1987a) or 5-21% of parasite carbon in eight Australian mistletoe associations (Marshall et al., 1994) and even 40% of the carbon in *Olax* parasitising *Acacia* (Tennakoon et al., 1997) were host derived. Whereas, the dependence of *Rhinanthus* on host C is smaller (only 10%) than in other hemiparasites like *Striga* and *Olax*.

The relationship between host and parasite is not a simple one. The photosynthesis of parasites is dependent on the different amount and composition of host-derived nitrogen, however, the parasitism also influenced the photosynthetic ability of their hosts. Lower rates of photosynthesis are frequently recorded in leaves of *Striga*-infected plants compared with uninfected controls (e.g. Press et al., 1987b; Graves, 1995). However, little is known about the primary mechanism by which photosynthesis is affected by *Striga hermonthica*. Although lower rates of CO_2 assimilation are commonly associated with lower rates of transpiration and stomatal conductance in the host, it is unclear whether stomatal conductance is a cause or a consequence of the observed effects on photosynthesis. Smith et al. (1995) have suggested that enhanced photorespiratory metabolism resulting from physical disruption of bundle sheath cells may contribute to the lower rates of photosynthesis.

Carbon assimilation has been studied in hemiparasites since several years. The work mainly focused on the specific trait of mannitol accumulation (Press, 1989; Fer et al., 1993; Press, 1995; Pageau et al., 1998). Different from the host, mannitol is a major carbohydrate in root parasites only. However, whether mannitol is essential to the survival of these species is still an open question. Investigations of mannitol have been mainly concentrated on characterising the mannitol biosynthetic pathway, and especially on elucidating the role of mannose 6-phosphate reductase and the corresponding gene(s) (Simier et al., 1994; Robert et al., 1999; Harloff and Wegmann, 1993). However, nothing has been done on the changes of mannitol in the parasites before and after a successful attachment to a host.

1.5 Phytohormones in hemiparasitic associations

Exploitation of xylem sap is enabled by establishing a continuously high leaf conductance (gs), also during the night (Taylor and Seel, 1998; Seel and Press, 1994, Seel et al., 1993a; Press et al., 1988). A possible physiological role of ABA in hemiparasitic associations has been studied by Taylor et al. (1996) and Frost et al. (1997) in the *Striga*/maize and *Striga/Sorghum* associations. Taylor et al. (1996) observed ABA to be higher by an order of magnitude in *Striga*, while it increased 60% in maize leaves after infection by the parasite. The authors suggested that localised water deficiency around the haustoria could stimulate ABA formation in the host roots. Drennan and Hiweris (1979) and Frost et al. (1997) investigated the *Striga / Sorghum* association and found increased xylem sap and leaf ABA concentrations in the parasitised host. It was suggested that this extra ABA could be involved in regulation of the host stomatal conductance, however, clear experimental data are still missing. Lechowski (1996) investigated the ABA relations in the epidermis of *Melampyrum arvense* parasitising on *Capsella bursa pastoris*. Before attachment both *Melampyrum* and *Capsella* showed a clear diurnal behaviour of epidermal ABA, with low concentrations during the night. After attachment, ABA of *Melampyrum* leaf epidermis remained continuously high, also during the night. ABA concentration in the xylem sap (ABA_{xyl}) of *Melampyrum* was 5-fold increased after attachment. But the changes in ABA flow, deposition and metabolism have not been detected within *Rhinanthus* before and after attachment to host barley.

Compared with the ABA studies, the knowledge about the cytokinins status of hemiparasites before and after attachment is small. Earlier reports suggested that only host-derived cytokinins (Cks) control changes in mistletoe leaf morphology (Atsatt, 1983; Hall et al., 1987). As it has been described by Seel et al. (1993a) that single *Rhinanthus* plants were less than 10 cm high, but after attachment the growth of *Rhinanthus* was stimulated dramatically. Cytokinins which stimulate plant cells division was expected to be a candidate to enhance the growth of *Rhinanthus* after the successful attachment. Lechowski and Bialczyk (1996) have investigated the

concentration of zeatin (Z) in *Melampyrum arvense* leaves and xylem sap increased about 100 times after attachment.

1.6 Project aims

The aims of this project are to investigate the physiological changes of *Rhinanthus minor* with or without an attachment to a host and the effects of parasitism of *Rhinanthus* on the host barley. The details are described as follows:

(1) to investigate the anatomical structure of haustoria attached to roots of different hosts.
(2) to quantify water flows between the rhizosphere and both partners, between the host and the parasite and between the different organs.
(3) to investigate the nutrient exchanges between host and parasite, and nutrients cycling within the unparasitised host, the unattached parasite and within the host/parasite system. Such a study provides information about the quantities of essential nutrients extracted from the host and hence may also give an insight into the reason for growth reductions seen in the host.
(4) the investigation of assimilate flows in the hemiparasitic association.
(5) to investigate ABA concentrations in the organs and transport fluids of *Rhinanthus minor*, *Hordeum vulgare* and of the *Rhinanthus minor* / *Hordeum vulgare* association, and to study the changes of ABA metabolism, deposition and flows in *Rhinanthus* and barley before and after attachment.
(6) to find the possible roles of cytokinins in the attached *Rhinanthus* and the changes of metabolism, deposition and flows of cytokinins in *Rhinanthus* and barley before and after attachment.

2. Materials and methods

2.1 Plant culture

Seeds of *Rhinanthus minor* obtained from Emorsgate Seed Suppliers (Kings Lynn, UK), were surface sterilised for 2-3 min in 6% sodium hypochlorite and germinated in petri dishes on a double layer of filter paper moistened with sterilised 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, however, without a positive effect on germination.

Caryopses of *Hordeum vulgare* were germinated on filter paper moistened with 0.5 mM $CaSO_4$ at 28°C for three days and transplanted into 1L 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 KNO_3, 0.5 NaH_2PO_4, 1.5 $MgSO_4$, 1.5 $Ca(NO_3)_2$, 0.1 $Na_2FeEDTA$, 0.05 'Fe-sequestrene' (Na_2Fe ethylene-diamine-o-hydroxy phenylacetate; Ciba-Geigy, Macclesfield, UK), 2.9×10^{-7} $CuCl_2\cdot2H_2O$, 2.1×10^{-7} $CoCl_2\cdot6H_2O$, 1.8×10^{-6} $MnCl_2\cdot4H_2O$, 1.0×10^{-7} $(NH4)_6Mo_7O_{24}\cdot4H_2O$, 0.8×10^{-7} $ZnSO_4\cdot7H_2O$, 4.6×10^{-5} H_3BO_3 (Seel and Jeschke, 1999). Three days later half-strength solution and after another three days full strength solution was supplied. Plants were cultivated in the greenhouse with a photoperiod of 12 h and a light intensity of 180-260µmol m^{-2} s^{-1}. Additionally, from July to September plants were cultivated under outside conditions (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), but protected with a glass roof to prevent input of rain water. These plants showed particularly good development and these were also used for measurements of nutrient, hormone and water flows.

The plants supplied with 1 mM NO_3^- and 1 mM NH_4^+ were cultivated and studied from March to June. The germination and transplanting occurred as described above.

The plants were watered daily, initially with a quarter-strength nutrient solution containing 5 mM NO_3^- (for the composition of the media, see description above).

Table 2.1 Compositions of nutrient solutions of 1 mM NO_3^- (I) and 1 mM NH_4^+ (II) supplies. (mM in full strength).

	I (mM)	II (mM)
KNO_3^-	0.4	
NH_4Cl		1
$NaH_2PO_4 \cdot H_2O$	0.5	0.5
$MgSO_4 \cdot 7H_2O$	1	1
K_2SO_4		0.2
$Ca(NO_3)_2 \cdot 4H_2O$	0.3	
$CaCl_2 \cdot 2H_2O$		0.3
$CaSO_4 \cdot 2H_2O$	0.5	0.5
Fe-EDTA	0.1	0.1
Fe-Sequeshen	0.05	0.05
$CuCl_2 \cdot 2H_2O$	2.9×10^{-7}	2.9×10^{-7}
$CoCl_2 \cdot 6H_2O$	2.1×10^{-7}	2.1×10^{-7}
$MnCl_2 \cdot 4H_2O$	1.8×10^{-6}	1.8×10^{-6}
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.0×10^{-7}	1.0×10^{-7}
$ZnSO_4 \cdot 7H_2O$	0.8×10^{-7}	0.8×10^{-7}
H_3BO_3	4.6×10^{-5}	4.6×10^{-5}

Fe-Sequestrene (Na_2Fe ethylene-diamine-o-hydroxy)

Three days later half-strength solution and after further three days full strength solution were supplied. At 32 days after transplanting (about 21 days after successful attachment), and 9 days before the first harvest, the pots with plants were washed several times with deionized water for removing of the nutrient solution containing 5 mM NO_3^-. Afterwards plants were supplied with solution I and II (see Tab. 2.1).

When the plants were supplied with 1 mM NO_3^- or 1 mM NH_4^+, they showed symptoms of N deficiency. It was not because 1 mM nitrogen was too low for barley growth, but because the pots containing the barley plants were only 1 liter and the capability of sustaining water of the sand was relative low. Therefore, N was not sufficient for barley growth when plants were supplied with a lower level of nitrogen.

2.2 Harvests of plant tissue

At 41d and 54d after planting corresponding to 30 and 43 d after attachment, five single unparasitised *Hordeum* plants, five unattached *Rhinanthus* plants and five *Hordeum /Rhinanthus* associations were harvested. Barley plants were separated into leaf laminae, leaf sheaths and roots, *Rhinanthus* plants were separated into leaves, the stem, lateral buds, the inflorescence and the root. For some analyses and for estimation of flows the shoot tissues were combined.

2.3 Collections of xylem sap and of phloem exudate

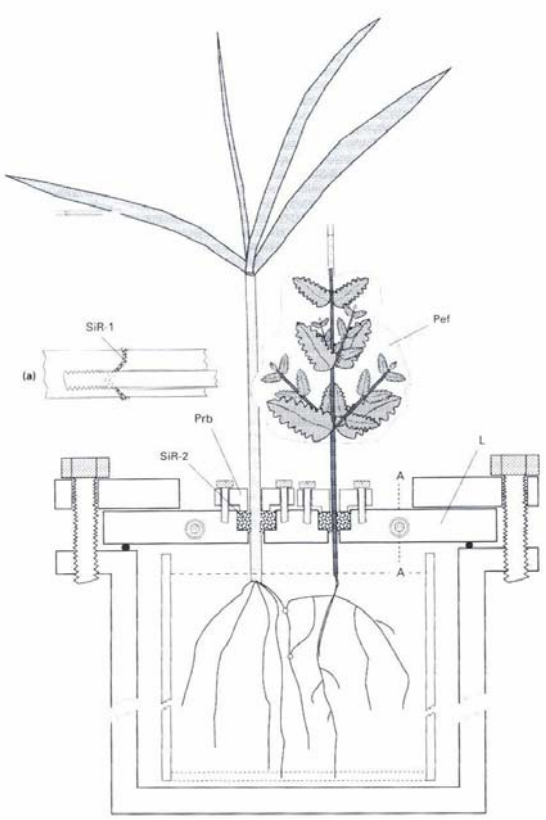


Fig. 2.1 Schematic representation of the pressure chamber (Seel and Jeschke, 1999).

In order to collect xylem sap from *Hordeum vulgare*, tillers were removed shortly after emergence to allow for successful sealing of the plants in the pressure pots (see Fig. 2.1, Seel and Jeschke, 1999). Xylem sap was collected between the two harvests

(time given above) by pressurising both the moistened sand substrate and the root system (Jeschke and Pate, 1991). Xylem sap was obtained from the main veins of the barley leaves and from the stem base of *Rhinanthus* plants after shoot excision (Seel and Jeschke, 1999). The cut surface was carefully washed and the first exudates discarded to avoid contamination from the cut surface. Phloem exudation was obtained by applying 1.5 ml of 5 mM Na_2EDTA to the base of excised barley leaves and *Rhinanthus* shoots, respectively. EDTA stimulates phloem exudation by chelating Ca^{2+} and thereby preventing the formation of callose, which seals sieve tubes after wounding (Wolf et al., 1990). Xylem sap and phloem sap samples were kept on ice during collection and stored at -25°C prior to analysis.

2.4 Modelling of water flows

For the 1st harvest, five unattached *Hordeum*, *Rhinanthus* and *Hordeum /Rhinanthus* association were used 41 days after planting, the second harvest occurred 13 days later. Barley plants were separated into leaf laminae, leaf sheaths and roots, *Rhinanthus* separated into leaves, the stem, lateral buds, the inflorescence and the root. For estimation of flows the shoot tissues were bulked. 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 also covered pots, however, without plants. The partitioning of transpiration between various plant parts was determined gravimetrically at harvest. This was done by measuring the water loss of a whole potted plant and then 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 (1991a).

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 occurring directly by the *Rhinanthus* roots of known fresh weight was the same as the uptake, that was 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 rather not be taken up from the host which contradicts the observed improved growth of the parasite. Assuming lower water uptake by the roots of the attached parasite there is no reason either. Moreover, if this were the case, then water and nutrient uptake from the host would be even higher than follows from the present modelling. 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 hours and that of barley over 14 hours (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).

2.5 Analysis of plant tissue and transport fluids for mineral elements, sugar, anions and amino acids

Elemental compositions of plant tissue and transport fluids were determined by ICP emission spectrometer (JY Plus, Division d'Instruments S.A., Jobin Yvon, France) (Homogenised tissue samples and transport fluids were taken up in 65% HNO_3 and kept for 10h at 170°C and 10 bar).

Plant dry matter was extracted with boiling water and the resulting extracts were analysed for sugar by HPLC (Dionex, series 4500i, America) and for anions by using an Anionenchromatograph IC 1000 (Biotronik, Maintal, Germany).

Amino acids in tissues and xylem sap were quantified by the Biochrom 20 Plus

Amino acid analyzer (Biochrom, Cambridge, UK). The preparations of the samples for amino acids occurred as follows: finely ground plant dry matter was extracted with boiling water and the extract was diluted with biochrom-dilution buffer; Xylem sap or phloem sap was extracted with Sulphosalicylic acid (12.5%) at 4℃ for 30 minutes, then diluted with biochrom-dilution buffer.

Total N and C were determined by oxidative combustion of 5-8 mg of homogenised, pulverised dry samples in a CHN-Analyser (Firma Elementar/Hanau). Concentrations of total N in xylem sap was calculated from the concentrations of amino acids and NO_3^-.

2.6 Estimation of net flows of mineral nutrients (N, P, K^+, Mg^{2+}) through xylem and phloem in unparasitised barley and unattached *Rhinanthus* and within the association of barley and *Rhinanthus*

The calculation of net flows of nutrients in the *Hordeum / Rhinanthus* association was based on the following three assumptions: (i) nutrient uptake occurring directly by the own roots of parasitising *Rhinanthus* having known fresh weight was the same as the uptake, that was measured for roots of unattached *Rhinanthus*, allowing for the root fresh weight; (ii) there are only direct xylem to xylem links between barley and the root hemiparasite *Rhinanthus* (Okonkwo 1966; Dobbins and Kuijt 1973); (iii) the quantities of mineral nutrients from barley to *Rhinanthus* was calculated from the nutrient depositions in the attached *Rhinanthus* tissue and minor uptake by its own roots. Net flows of nutrients within host and parasite plants were estimated using the method introduced by Hibberd et al. (1999) and Jiang et al. (2001). The calculations of nutrients flows were based on the water flows.

Based on the assumption that mass flow occurs in the xylem, net xylem flows of nutrients in mole per plant, from root to shoot, $J_{s,x}$ were calculated from the net xylem water flows, $J_{H2O,x}$ and the measured concentrations of nutrients $[S]_x$ in the xylem sap:

$$J_{s,x} = J_{H2O,x} * [S]_x \quad (1)$$

The net flows of nutrients in the phloem, $J_{s,p}$ were then calculated from the

difference between nutrient increments ΔS in each organ and the net xylem import to that organ.

$J_{s,p} = \Delta S - J_{s,x}$ (2)

The content of each element in the organs in moles per plant and increments in moles per plant over the study period were then calculated from the concentrations and the dry weights.

2.7 ABA analysis

2.7.1 Extraction of tissues

Freeze-dried tissue samples were homogenised and extracted in 80% aqueous methanol solution. Extracts were passed through Sep Pak C_{18}-cartridges. Methanol was removed under reduced pressure and ABA together with other organic solutes was extracted from the aqueous residue at pH 3 by three fold partitioning against ethylacetate. The ethyl acetate of the combined organic fractions was removed under reduced pressure. The newly obtained residue was taken up in TBS-buffer (Tris buffered saline; 150 mmol/l NaCl, 1 mmol/l $MgCl_2$ and 50 mmol/l Tris at pH 7.8). The aqueous fractions which contain ABA-conjugates were hydrolysed with NaOH (1 M) for 1 h, adjusted to pH 3.0 with HCl and partitioned against ethyl acetate as described above. ABA, released from ABA conjugates was determined by ELISA.

2.7.2 Principle of the analysis

There is a competition between (+)ABA in sample and RAMIG (rabbit anti-mouse immunoglobuline) which binding on the wall of microtitre plate for combining with the monoclonal ABA-antibody. After the compound of RAMIG binding with monoclonal ABA-antibody is combined with abscisic acid-alkaline phosphatase conjugate, *p*-nitrophenyl phosphate substrate reacts with the alkaline phosphatase forming a

yellow product. By detecting the quantity of the compound of RAMIG binding with monoclonal ABA-antibody combined on the wall, the concentrations of (+)ABA in sample is quantified. This technique is called ELISA (Enzyme Linked Immuno Sorbent Assay; Weiler, 1986).

2.7.3 Procedures of ELISA

Table 2.2 Pipette scheme in microtitre plates

B0: 50µl TBS (Tris buffered saline; 150 mmol/l NaCl, 1 mmol/l $MgCl_2$ and 50 mmol/l Tris at pH 7.8)

0.01; 0.02; 0.05; 0.1; 0.2; 0.5; 1; 2; 100 pmol ABA in 50µl TBS

1-32: 50µl sample solution

	1	2	3	4	5	6	7	8	9	10	11	12	
A		0.2	1	9	17	25	1	9	17	25	B0	0.2	A
B	B0	0.5	2	10	18	26	2	10	18	26	B0	0.5	B
C	B0	1.0	3	11	19	27	3	11	19	27	B0	1.0	C
D	B0	2.0	4	12	20	28	4	12	20	28	B0	2.0	D
E	0.01	100	5	13	21	29	5	13	21	29	0.01	100	E
F	0.02	B0	6	14	22	30	6	14	22	30	0.02	B0	F
G	0.05	B0	7	15	23	31	7	15	23	31	0.05	B0	G
H	0.1	B0	8	16	24	32	8	16	24	32	0.1	B0	H
	1	2	3	4	5	6	7	8	9	10	11	12	

Add 200 µl aliquot of RAMIG (rabbit anti-mouse immunoglobuline) to each well of a microtitre plate (96-well, MT-Platten F-Form Virion/Serion, Wuerzburg, Germany), and incubate plates overnight at 4^{o}C. Afterwards pour out RAMIG and wash the plate three times with water. Then add 200µl monoclonal ABA-antibody (Prof. Dr. E. Weiler, university Bochum, Weiler, 1986) to each well and incubate plates overnight at 4 ^{o}C. Before pipette 100µl TBS (Tris buffered saline: 50mM Tris, 150 mM NaCl, 1mM $MgCl_2$, pH 7.8) and 50 µl (+)ABA sample or standard to the wells, pour out antibody and wash the plate three times with water. Incubate plates at 4 ^{o}C for one hour. Then add 200µl aliquots of rabbit anti-mouse alkaline phosphatase conjugate (27µl tracer in 16.173 ml Tracer solution (TBS-Gelatine: 0.1% (w/v) gelation in TBS)) to each well

then keep the plate in the dark at 4°C for 3 h. Afterwards pour out the solution and wash the plate with water. Add 200µl aliquots of *p*-nitrophenyl phosphate substrate (1 M Diethanolamine, 0.5 mM $MgCl_2$, pH 9.8) to each well. Incubate 1-2 h at 37°C until the absorbance at 405 nm of control samples containing no ABA is approximately 1.0. Measure absorbance at 405 nm in an ELISA plate reader (BIO-RAD Microplate Reader Model 550; BIO-RAD Laboratory GmbH, München, FRG). Stop reaction by adding 50µl of 5 M KOH. During these procedures the first well of the microtitre plate was kept empty as a blank.

2.8 Cytokinins analysis

2.8.1 Extraction of tissue

Freeze-dried tissue samples were homogenised and extracted in 80% aqueous methanol solution and incubated 16-18 h at 4°C. After filtration, methanol was removed under reduced pressure and the aqueous residue was passed through Sep Pak C_{18}-cartridges. After washing of the loaded column with distilled water (20 ml), cytokinins were eluted with 80% (5 ml) methanol. The solvent was evaporated to dryness, the residues were dissolved in 0.02 ml of 80% methanol and applied to precoated silicagel 60 F-254 plates (Merck, Darmstadt, Germany) for thin layer chromatography (TLC). The solvent used for TLC was 2-butan-1-ol, 14 M NH_4OH and H_2O (6:1:2, v/v, upper phase). The zones of zeatin, dihydrozeatin, isopentenyladenine and their derivatives were eluted with 0.1 M phosphate buffer (pH 7.4) and added directly to the wells of the microtitre at several dilutions for immunoassay using antibodies against zeatin riboside, dihydrozeatin riboside and isopentenyladenosine (cross-reactivity of antiserum used is presented in the Table 2.3). The recovery of zeatin, dihydrozeatin, isopentenyladenine and its derivatives was above 90%. In case of xylem sap, samples were taken from refrigerator, melted and diluted in 2 ml of H_2O prior to purification. The antibodies were raised in the Institute

of Biology (Ufa, in Russia).

2.8.2 Procedures of ELISA

Hormone-ovalbumin conjugate was passively adsorbed to a 96-well polystyrene microtitre plate (MT-Platten F-Form Virion/Serion, Wuerzburg, Germany) in phosphate-buffered-saline (pH 7.2) at 37^{o}C for 1.5 h. The plate was washed 3 times with phosphate-buffered-saline containing 0.05% Tween 20, 0.9% NaCl and 2% 0.1 M phosphate buffer (pH 7.2).

Mixture of 10 µl of standard or sample, 90 µl of phosphate-buffered-saline plus 0.5% ovalbumin and 100 µl of antiserum was added to wells and incubated for 1 h at 37^{o}C. Unbound rabbit serum was washed away as described above and anti-rabbit IgG, conjugated to peroxidase, was incubated with the adsorbed antigen-antibody complex for 1 h at 37^{o}C. All wells were again washed and the substrate solution consisting of *o*-phenylenediamine (0.4 g l^{-1}), phosphate buffer (pH 5) and 30% hydrogen peroxide in the ratio of 16 ml:8 ml:10 µl was added. Colour development was stopped by adding 4N H_2SO_4 and quantified at 490 nm with a ELISA reader later.

Table 2.3 Cross-reactivity of various purine compounds with the antibodies against cytokinins; reciprocal molar concentrations of analysed compounds.

compounds	cross-reactivity of antibodies against		
	ZR	iPa	DHZR
Z	31	0.3	1.8
ZR	100	1.6	1.6
ZN	95	0.3	1.5
DHZ	8	1.9	40
DHZR	-	0.9	100
iP	0.9	63	0.1
iPa	0.6	100	0.2
BA	3	20	1.6
Ad	0	0	0

2.9 Modelling of ABA flows

Estimation of ABA flows was based on the assumptions, of which one assumption was the same as described above in the part "estimations of net flows of mineral nutrients" (assumption ii), additional assumptions were that a) the transfer of ABA from barley to *Rhinanthus* is given by the ABA concentration in barley xylem sap and the quantities of water transferred from barley to *Rhinanthus*; b) in the xylem and phloem mass flow occurs and hence solutes are translocated according to their relative concentrations. The basis for calculation of ABA flows were the water flows.

According with these assumptions net xylem flows of ABA ($J_{ABA,x}$), from host root to host shoot, or from host roots to the parasite, are given by the flow of water ($J_{H2O,x}$) and the concentration of ABA in xylem sap $[ABA]_x$:

$J_{ABA,x} = J_{H2O,x} * [ABA]_x$

The phloem flow of ABA ($J_{ABA,p}$) was estimated on the basis of the obtained flows of K as the product of the ratio of ABA to K^+ in phloem exudates $[ABA/ K^+]_p$ and the phloem flow of K^+ ($J_{K^+,p}$):

$J_{ABA,p} = [ABA/ K^+]_p * (J_{K^+,p})$

The differences between the estimated net flows of ABA going into or moving out of an organ and its increment (Δ ABA) in that organ yielded the net metabolic changes of ABA ($J_{ABA,met}$) either by degradation or by synthesis of ABA:

$J_{ABA,met} = \Delta ABA - J_{ABA,p} - J_{ABA,x}$

If the resulting metabolic changes were negative, then net degradation must have occurred, if they were positive then net synthesis was indicated.

Flow models of cytokinins and mannitol were obtained in the same way as described for abscisic acid.

2.10 Rough estimates of the movements of sucrose in barley

Sucrose proved undetectable in xylem sap, while it was the major sugar in EDTA-induced phloem exudates of barley. Carbon translocation from shoot to root

(J_{cp}) was estimated from the net increment of carbon in the root plus the amount of carbon respired during the experimental period, which was assumed to be a similar percentage (70%) as in castor bean roots (Jeschke et al., 1996). Assuming further, that in barley sucrose contributed 89% of the carbon in phloem sap, as in carbon bean (Jeschke et al., 1997) and taking into account that sucrose contains 12 C atoms, the estimated sucrose flow was obtained from:

$$(J_{sucrose,\ P})_{estimated} = (1.7 * \Delta C_{root}) * 0.89/12$$

Most of the sucrose imported into the root is converted into other compounds or respired and only a small amount is deposited in the form of sucrose. These quantities of metabolically converted sucrose were obtained from the difference between sucrose import and deposition.

2.11 Precision of models

The flow models presented in this paper depend on increments, i.e. differences in content, between first and second harvest, the standard errors of which have been presented in the tables corresponding with modelling figures. Because of a strong positive correlation in growing plants between first and second harvest, the errors of these differences are, as shown by Pate et al. (1979), see also Jeschke and Pate (1991b), not given by an addition of errors, but they are much smaller. Flows and depositions in the models are therefore within the limits of errors as given in the tables corresponding with modelling figures.

2.12 Leaf conductance

Leaf conductance was measured with 60-63 days 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*.

2.13 Scanning electron microscopy

Leaf samples were fixed in a mixture of ethanol/formalin/glacial acetic acid = 90/5/5 and the water was removed in an acetone series. After critical point drying samples were sputtered with gold and investigated in a Zeiss DSM 962 Scanning electron microscope. Acetone series: 30% Acetone (15 min); 50% Acetone (20 min); 75% Acetone (30 min); 90% Acetone (45 min); 100% Acetone (30 min) for 5 times.

2.14 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. *Rhinanthu*s roots of 5-8 cm length and excised seminal roots of 14 days old barley plants of 11-12 cm length were used. The root segment is tightly connected to the root pressure probe by silicone seals with the aid of a screw so that the root pressure develops in the measuring system which consists of the root, the seal, a measuring capillary, and a small pressure chamber. Half of the system is filled with silicone oil and the other by water so that a meniscus forms in the measuring capillary. This meniscus is used as a point of reference during the measurements. When a steady-state root pressure (Pro) is established, water flows can be either induced by changing the pressure in the system using a metal rod (hydrostatic experiments) or by changing the osmotic pressure of the medium osmotic experiments. After each experiment with a given root, the integrity of the seal was

tested by cutting off the root at the seal and measuring the decrease in the time constants of pressure relaxation (Hose et al., 2000). When the root xylem remained open, there was a drastic decrease in the half-times after the cut. If this was not the case, the experiment was discarded. Root Lpr was evaluated from root pressure relaxations. Water flows were induced by changing the root pressure with the aid of a movable metal rod in the equipment. Root pressures (Pr in MPa) were measured with a pressure transducer, which converted the signal into a proportional voltage. Pressure-time curves were recorded on a chart recorder. Hydrostatic relaxation curves were composed of two distinct regions brought about by different rates of changes of root pressure corresponding to time: the initial very rapid phase, immediately following the change in Pr (half-time $T^{w}_{1/2}A$) and finally a slow component (half-time $T^{w}_{1/2}B$). For calculation of half-times $T^{w}_{1/2}$, chart recorder strips were digitised on digitising tables. Curves were analysed using exponential fits. Root hydraulic conductivity (Lpr) was calculated for $T^{w}_{1/2}A$ and $T^{w}_{1/2}B$ according to Eq.2

$$Lp_r = \frac{\ln(2)}{Ar \cdot T^{w}_{1/2} \cdot \frac{\Delta p}{\Delta V}}$$

Ar: root surface

$\frac{\Delta p}{\Delta V}$: elastic coefficient (measured by rapidly changing the position of meniscus in the measuring capillary (ΔVs) and recording the response in pressure (ΔPr))

2.15 Collection of root exudates from barley and *Rhinanthus* seedlings

Caryopses of *Hordeum vulgare* were germinated on filter paper moistened with 0.5 mM $CaSO_4$ at 28 ℃ for three days. Seeds of *Rhinanthus minor* were germinated in the same way as described above. After 5 months, the root of the *Rhinanthus* seedlings was about 1.5-4.0 cm. By using the method developed by Neumann and Römheld (1999), for collecting root exudates, the roots of barley or *Rhinanthus* seedling were washed with deionized water for 2 or 3 minutes and then spread on a clean and dry glass Petri dish. The roots were placed between small disks (Ø: 5 mm)

of filter paper (Schleicher & Schüll 2992, Dassel, Germany) moistened with deionized water. The small pieces of filter paper were previously washed with absolute methanol. The remaining root and shoot were covered with moistened filter paper. After 1h, 2h, and 3h, the filter papers were collected, extracted and analysed for ABA, mannitol and sucrose.

2.16 Stain for haustoria sections

Sections were dewaxed in Xylene and a series of ethanol and stained in 0.2% astra blue (dissolved in 2% acetic acid) for 7 minutes. After rinsed well in aqua dest, the sections were stained in 0.5% safranin (dissolved in 50% alcohol) for 7 minutes. After washing with aqua dest, sections were put in 70-80% alcohol and then dehydrated in 100% isopropanol. The slides were covered with deckglass. Lignified cell walls are in red and cellulose cell walls are in blue.

2.17 Immunolocalisation of ABA

2.17.1 Tissue preparation

Haustorial were fixed with 3% (w/v) paraformaldehyde in 4% (w/v) 1-Ethyl-3-(3-Dimethylamino-propyl)carbodiimide (EDAC) and 0.1% (v/v) Triton X-100 at 4℃ to immobilise ABA. After washing the fixed samples with stabilising buffer (MTBS) and PBS (phosphate buffered saline) to remove the fixative, the samples were dehydrated with a graded series of ethanol/PBS. Tissues were then infiltrated with increasing concentration of Steedman's wax (polyester with lower melting point; polyethyleneglycol-distearate in 1-Hexadecanol 9:1; Vitha et al., 1997). Sections of 11 μm were made with a microtome (Schlittenmikrotom HN 40, Jung, Heidelberg, Germany) and collected on poly-L-lysine-coated slides.

2.17.2 Immunocytochemistry

Sections were dewaxed in a series of ethanol (technical grade ethanol because it increases the autofluorescence) and rinsed with MTBS (5 mM EGTA, 50 mM HEPE, 5 mM $MgSO_4 \cdot 7H_2O$, pH 6.9), methanol (-20 ℃) and PBS/1%Tween 20. Before incubating with the primary monoclonal ABA antibody (Linaris, Wertheim-Bettingen, Germany) overnight, the sections were pretreated with rabbit serum for 1h to reduce unspecific binding. After washing with PBS/Tween 20 sections were incubated with red Alexa conjugate 568 (568 goat anti-mouse IgG, H+L, Molecular Probes, Göttingen, Germany; excitation 568 nm, emission 598-608 nm) as a secondary antibody for 2 h. After washing with PBS and staining with toluidine blue (to quench the autofluorescence of the lignified cell walls; Peterson 1988) and with aniline blue, sections were embedded in 1,4-diazabicyclo-(2,2,2)octane (DABCO; 25mg/ml in PBS/glycerol 1+9). The covered slides were sealed with nail varnish. Sections were viewed with a laser scanning microscope (Zeiss LSM 5 Pascal 5 (Axioskop 2 mot plus), excitation 543, HFT 543, NFT 645, emission (BP) 560-615). Images were converted to a false color glow mode to improve the contrasts. Control sections were treated with neither ABA antibody nor Alexa.

3. Results

3.1 Growth of single and parasitising *Rhinanthus*

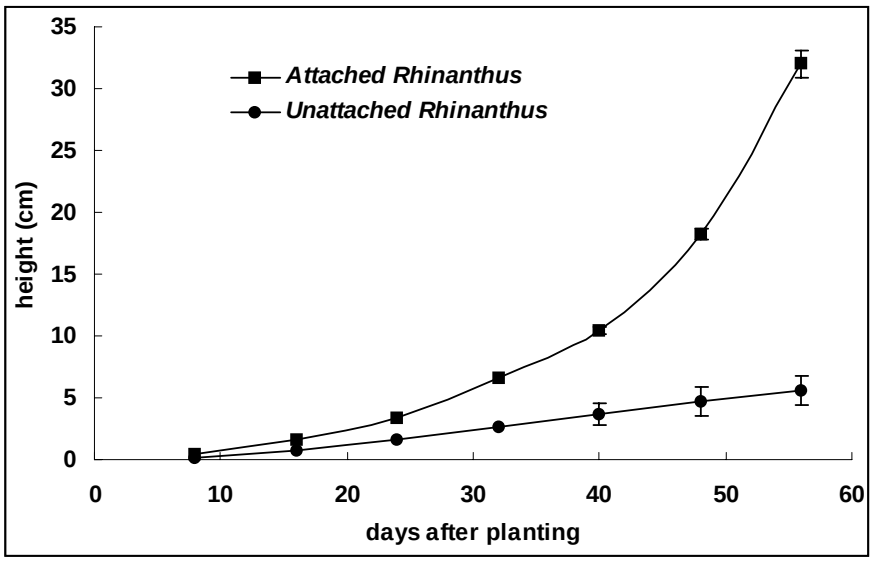


Fig. 3.1 Growth of single *Rhinanthus* and *Rhinanthus* parasitising on barley over 56 days (n=3-5).

Single *Rhinanthus minor* grows very slowly and only reaches a height of less than 10 cm within two months. After attachment, however, the growth of *Rhinanthus* is significantly stimulated (Fig. 3.1).

In Fig. 3.2a it is shown that single *Rhinanthus* are less than 10 cm high and the leaves and flowers are very tiny. The leaves of young single *Rhinanthus* appear dark green, later when they are mature, the lower stratum leaves are yellow and the plants look pale. After attachment to host barley, *Rhinanthus* reaches a height of about 30 cm with bright green, sharply toothed leaves about 4 cm long and with bright yellow flowers of about 1.5 cm long (Fig. 3.2b).

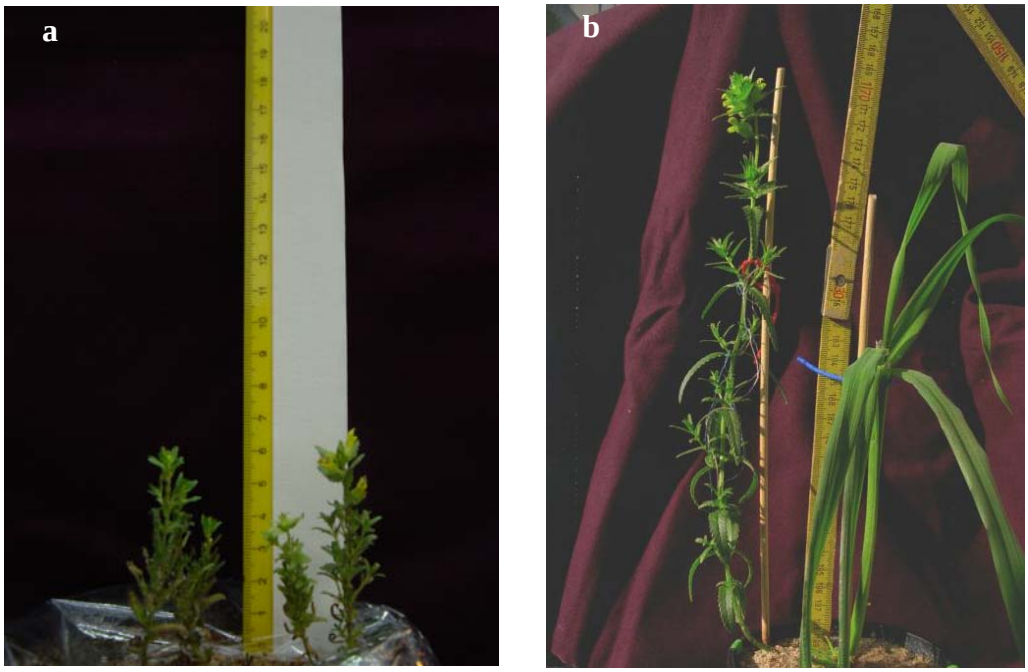


Fig. 3.2 Single (a) and parasitising *Rhinanthus minor* (b) in the pots.

3.2 Haustorium

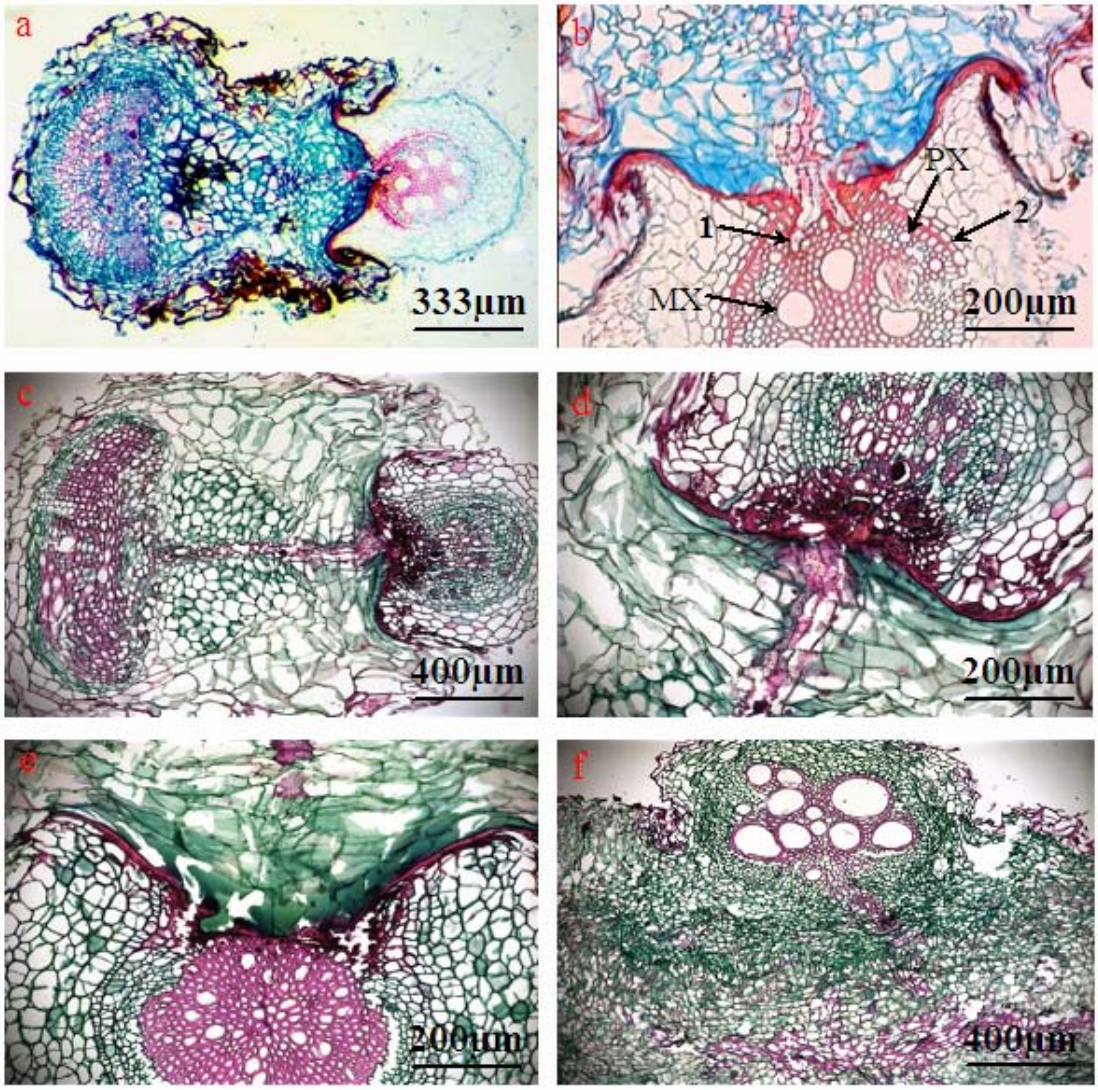


Fig. 3.3 Haustorium of *Rhinanthus* attaches to barley (a, b), to *Vicia cracca* (c, d) to *Plantago lanceolata* (e) and *Striga gesneroides* attaches to *Ipomea hackeliana* (f).

Fig. 3.3 shows sections of haustoria of *Rhinanthus* attached to barley (a, b). First, the penetrating cells, the oscula, enter the phloem. The phloem degrades before a xylem vessel is punctured (arrow 1). The endodermis, close to the site of penetration, prematurely reaches a tertiary state (arrow 2). *Vicia cracca*, a good host for *Rhinanthus*, forms strongly thickened and lignified cells in the stele of the host roots (c, d). In *Plantago lanceolata* the cortex only is penetrated. A layer of dead host root cells prevents further penetration into the stele (e). In the haustorium of the *Striga*

gesneroides/*Ipomea hackeliana* association (f) none of these structural changes can be observed. In this case the host will not survive infection.

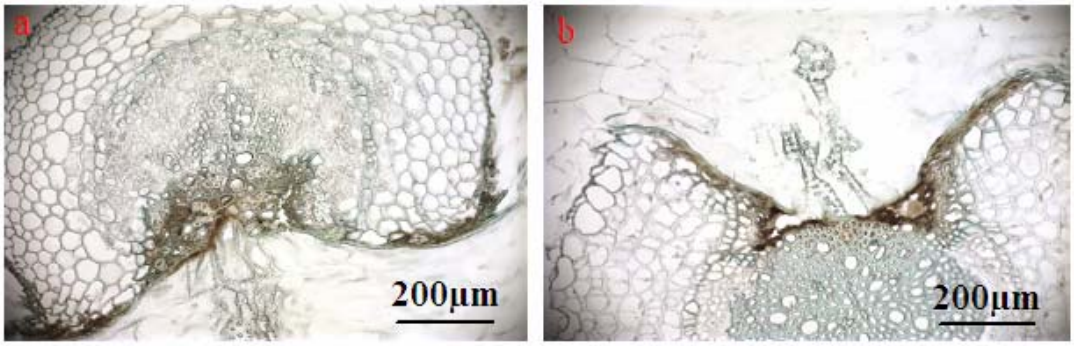


Fig. 3.4 Cross sections of haustoria attached to roots of *Vicia cracca* (a) and *Plantago lanceolata* (b) have been stained with the lipophilic dye Sudan blue (= oil blue N).

In Fig. 3.4a a lipophilic layer, presumably suberin can be detected in the interface between the host root and the haustorium. In *Vicia cracca* the endodermis shows strong suberin depositions (Fig 3.4a). In *Plantago lanceolata* the interface consists of dead cell material (Fig 3.4b). The oscula are prevented to penetrate the xylem. Suberin has been analysed in this interface in the meantime by Cameron (personal communication).

3.3 Water flows in the parasitic associaton *Rhinanthus minor/Hordeum vulgare*

3.3.1 The stomatal movements of *Rhinanthus* and *Melampyrum arvense*

Scanning electron micrographs of the surface of fully differentiated leaves of *Rhinanthus* and *Melampyrum arvense* are shown in Fig. 3.5. Whereas stomata of unattached *R. minor* plants appeared to be closed (Fig. 3.5c), even in the light, those of attached *R. minor* were always open, also in darkness (Fig. 3.5a,b). In darkness stomata seemed to be even wider open than in the light. *Melampyrum arvense* exhibited normal daily changes. Their stomata were open at day time and closed in the dark (Fig. 3.5d, e).

Figures 3.6 and 3.7 show daily courses of transpiration of *Rhinanthus* and its host grown in the field in its natural habitat and in the green house. 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 decreases 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 similar levels as 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. 3.6).

In the greenhouse (Fig. 3.7) the overall leaf conductance of *Rhinanthus* was clearly lower (290 mmol $m^{-2}s^{-1}$). Although the experimental setups were different, the diurnal pattern, however, was similar to that of the field grown plants. As shown by Fig. 3.7, the leaf conductance of *Rhinanthus* was highest when measured in the dark in early morning and decreased during the day, however, it went up over night again. When *Rhinanthus* leaves were treated with 10^{-5} M ABA, leaf conductance remained unaffected, whereas in barley already 10^{-6} M ABA significantly reduced leaf conductance. A treatment of *Rhinanthus* leaves with 10^{-4} M ABA was needed to induce a closing response in *Rhinanthus* stomata to ABA (Table 3.1). 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. 3.8).

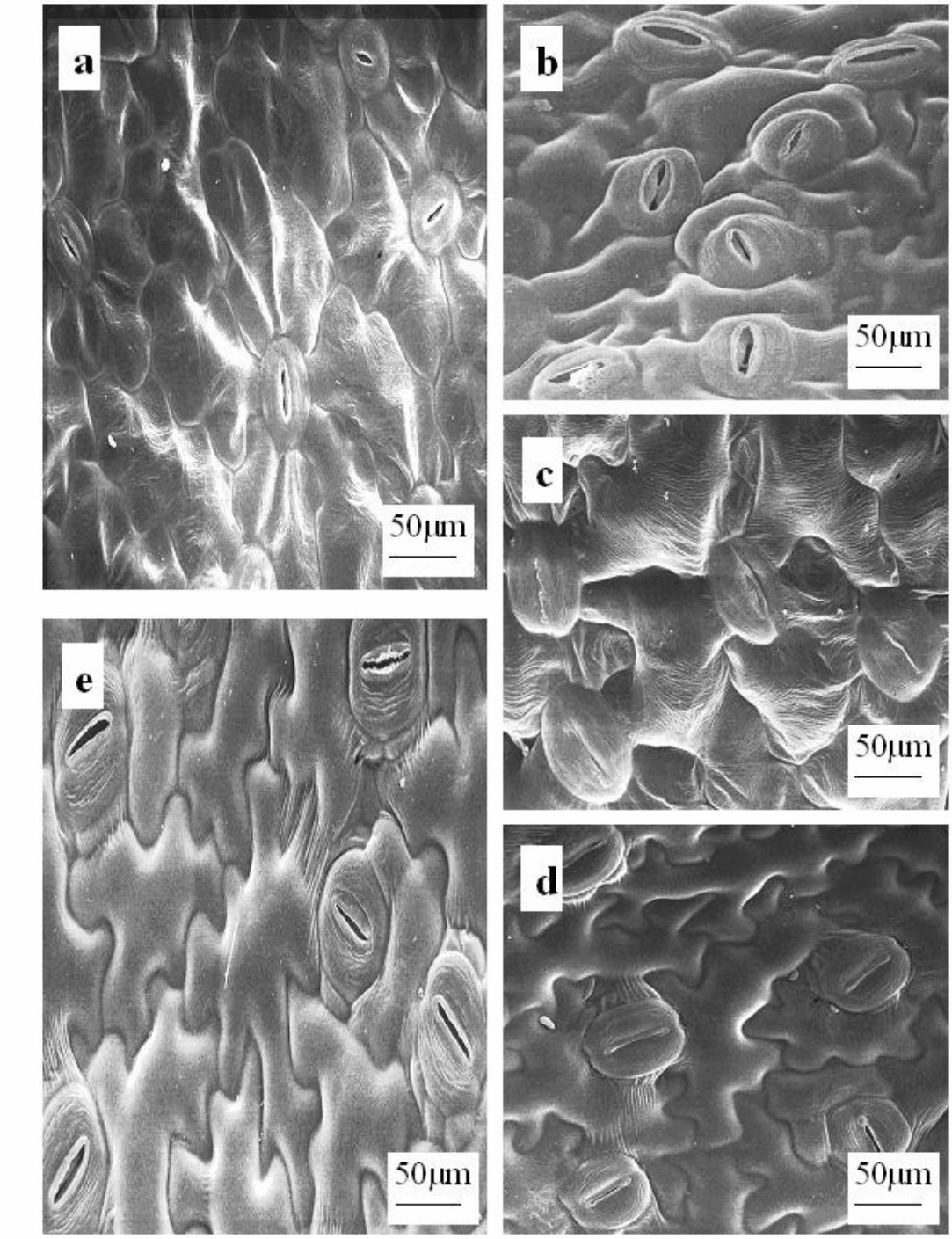


Fig. 3.5 Scanning electron micrographs (SEM) of the surface of leaves of attached *Rhinanthus* in the light (a) or in dark (b) growing on barley and of unattached *Rhinanthus minor* leaves in the light (c). SEM pictures of *Melampyrum arvense* (d, in the dark; e, in the light) harvested from the field.

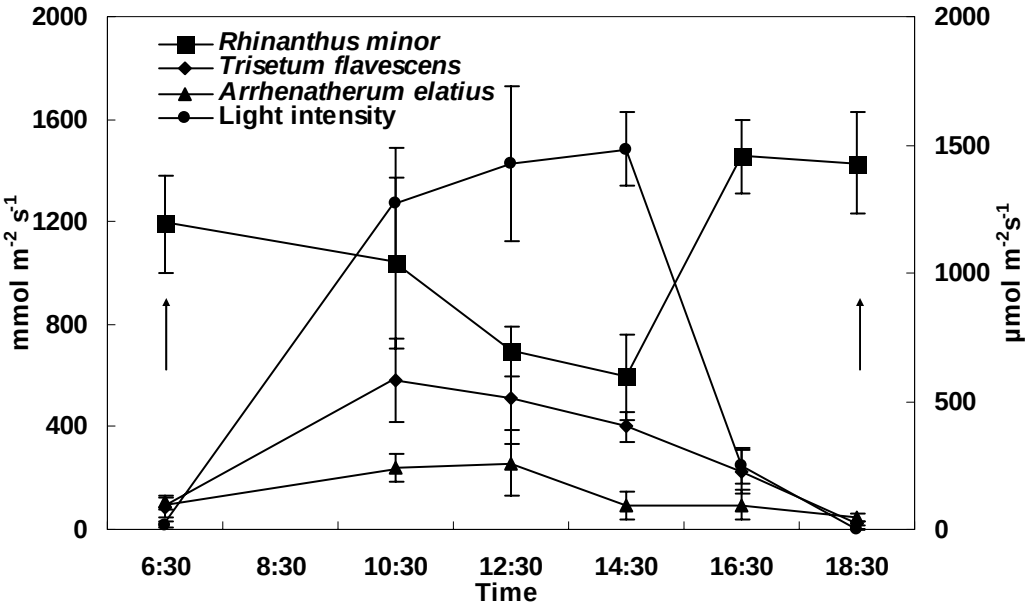


Fig. 3.6 Diurnal changes of leaf conductance of *Rhinanthus minor*, *Arenatherum elatius* and *Trisetum flavescens* and the light intensity on its natural habitat. The maximum temperature: 38°C and the maximum light intensity: 1450 µmol $m^{-2}s^{-1}$. The arrows indicate measurements at darkness. Means ± SE, n = 7-16.

Table 3.1 Effect of ABA applied directly to leaves of the *Rhinanthus*/*Hordeum* association on leaf conductance. Means ± SE, n=7.

	Leaf conductance(mmol s^{-1} m^{-2})			
	Attached *Rhinanthus*		Attached *Hordeum*	
Treatment	Control	ABA Treatment	Control	ABA Treatment
10^{-4} M ABA	463 ± 44	242 ± 29		
10^{-5} M ABA	451 ± 60	450 ± 51	665 ± 49	273 ± 45
10^{-6} M ABA			539 ± 47	155 ± 28

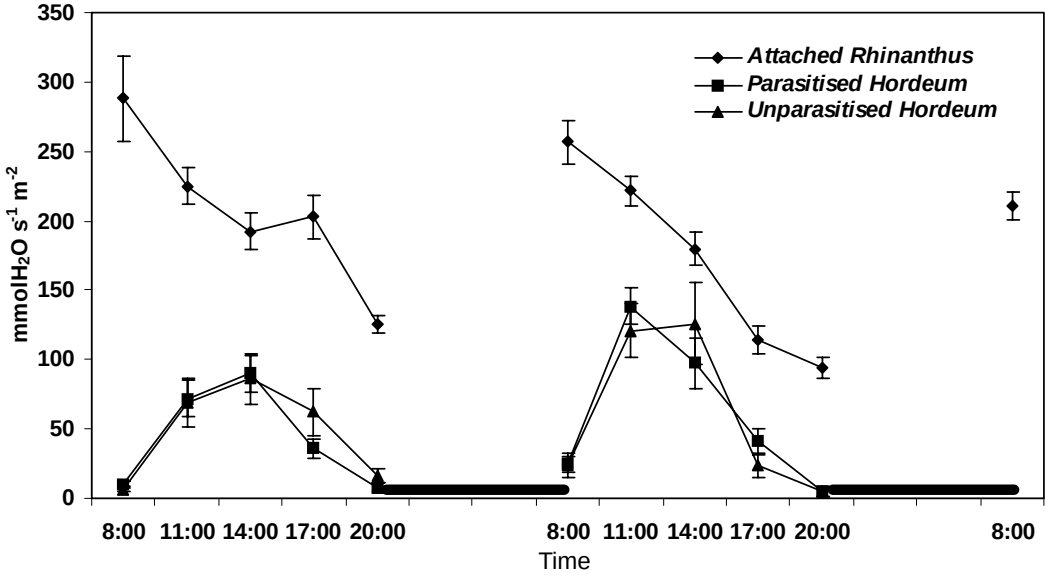


Fig. 3.7 Leaf conductance of fully expanded leaves of parasitising *Rhinanthus minor* and of leaves of infected and uninfected *Hordeum vulgare* plants measured over two diurnal courses in the greenhouse. In order to obtain unequivocal data for the night period, the plants were darkened 1 hour before the 8 pm until after the 8 am measurements. Light intensity: 0-215 µmol $m^{-2}s^{-1}$, temperature: 17-26 °C, CO_2 concentration: 500 ppm, age of the plants: 60-62 days after planting. Each point represents the mean ± SE n= 8-14 (infected *Rhinanthus*), 11-20 (parasitised or unparasitised *Hordeum*).

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

Hydraulic conductivity of seedling roots of barely 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 way (Table 3.2). Both conductivities were clearly higher in *Rhinanthus*, Lpr (symplastic) being ten times, the 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 barriers such as Casparian bands in the hypodermis or endodermis (Fig. 3.9). 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. 3.10).

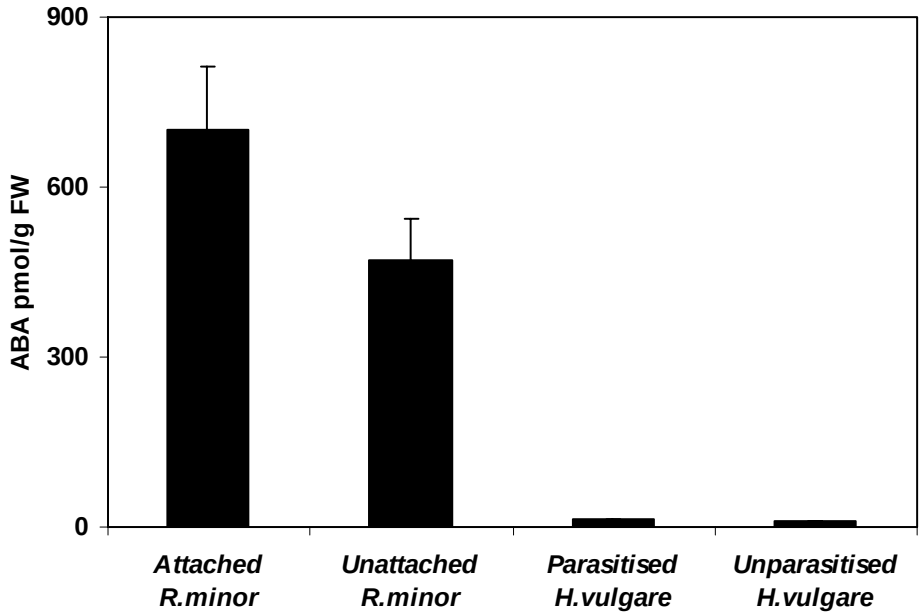


Fig. 3.8 The concentrations of endogenous ABA in leaves of infected and uninfected plants of *Rhinanthus minor* and *Hordeum vulgare*. Means ± SE, n=12.

Table 3.2 Apoplastic and symplastic hydraulic conductivities (Lpr) of roots of seedlings of *Rhinanthus* and barley . *For comparison, data of Steudle and Jeschke (1983) are given. Means ± SE, n= 4.

Rhinanthus		Barley	
Lpr (apoplastic)×10^7	Lpr (symplastic)×10^9	Lpr (apoplastic)×10^7	Lpr (symplastic)×10^9
($ms^{-1}MPa^{-1}$)		($ms^{-1}MPa^{-1}$)	
120 ± 0.2	46 ± 2.7	1.2 ± 0.3	4.4 ± 0.9
		0.8 ± 0.4*	

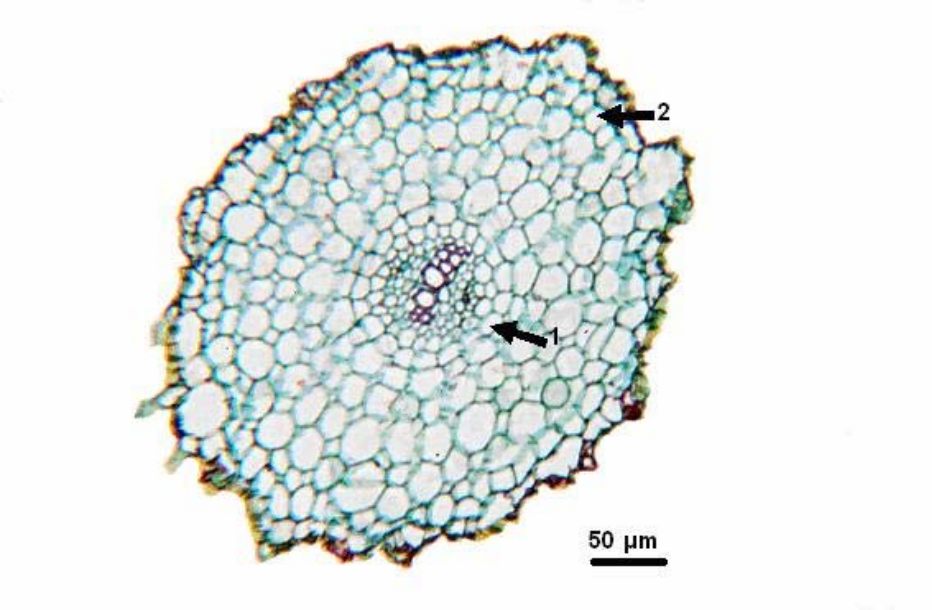


Fig. 3.9 Cross section of a seedling root of unattached *Rhinanthus minor*. 11 µm paraffin sections after fixation with 70% ethanol/40% formalin/glacial acetic acid (90/5/5 by vol.) were stained using the W-3A technique (Wacker unpublished). The arrows indicate the position of the hypodermis (2) and the endodermis (1), the horizontal bar the magnification.

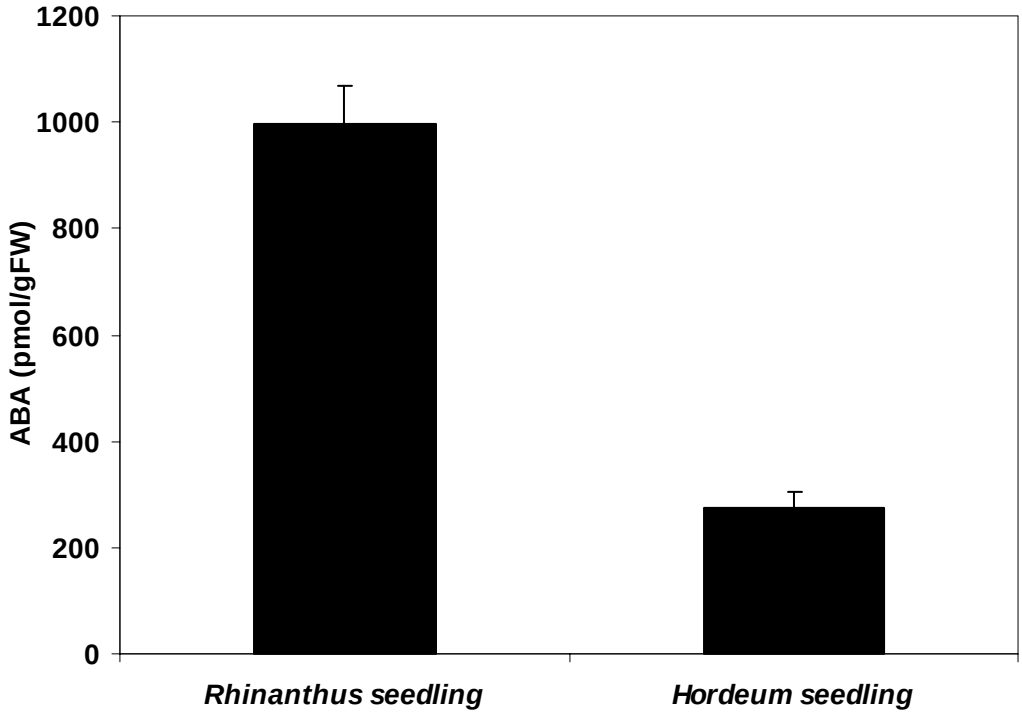


Fig. 3.10 Endogenous ABA concentrations in roots of unparasitised barely and unattached *Rhinanthus minor* seedlings. Age of *Rhinanthus* seedlings : 90 days, age of barley seedlings: 3 days; Means ± SE, n=5.

3.3.3 Water flows in the parasitic associaton *Rhinanthus minor/Hordeum vulgare* supplied with 5 mM NO_3^-

Water flow models in unattached *Rhinanthus*, in non-parasitised barley and in the parasitic association barley/*Rhinanthus* are shown in Figures 3.11 and 3.12. 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. 3.11). After attachment to barley, total water uptake into *Rhinanthus* was nearly 9-fold increased, 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 incorporation to the roots of *Rhinanthus* was doubled after attachment, whilst water incorporation into shoot tissues increased 14-fold (Fig. 3.11 and Fig. 3.12), reflecting the substantial increase in the parasite growth and in its shoot to root ratio. Growth-dependent incorporation of water into roots of parasitised barley was not affected by the parasite, whereas incorporation into growing shoot tissues was substantially decreased in parasitised barley, compared to uninfected barley, i.e. in leaf lamina by 23% and in the leaf sheath fraction by 43% (Fig. 3.11 and Fig. 3.12). The strong effect in the leaf sheath fraction was due to the fact, that it contained also the growing stem and the apical bud.

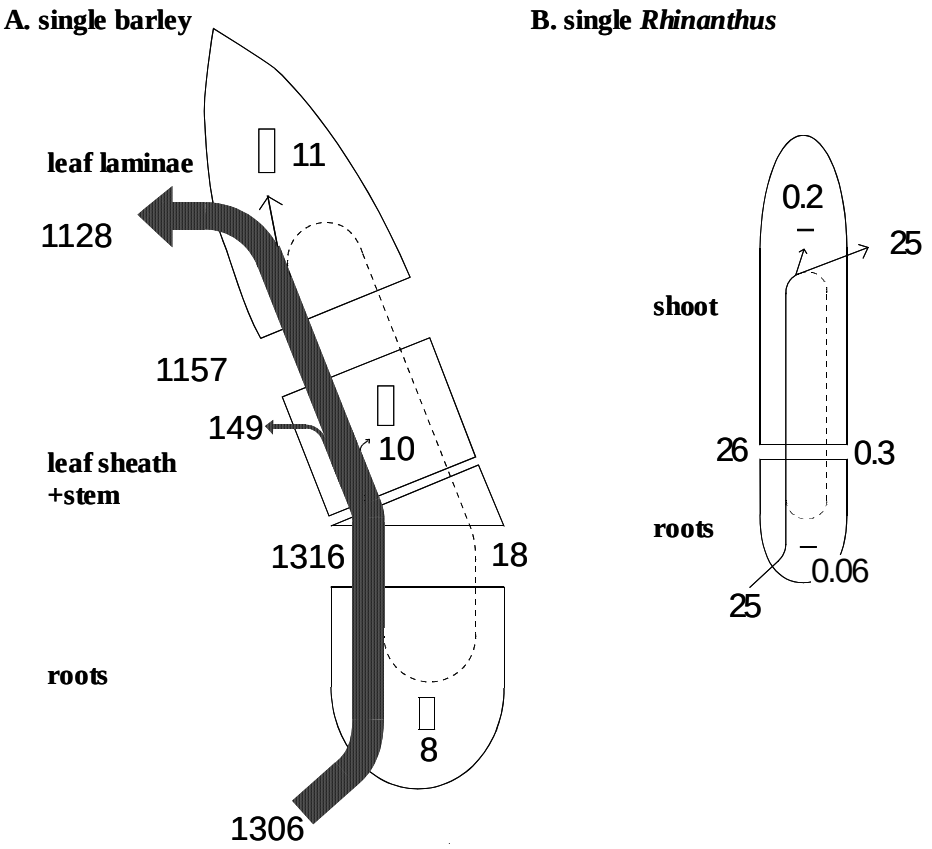


Fig. 3.11 Flow profiles for uptake, transport, utilisation, and transpirational loss of H_2O in single barley (A) and *Rhinanthus* (B) supplied with 5 mM NO_3^- over 13 d experimental period, starting 41 d after sowing. The height of vertical histograms (H_2O incorporation) and the width of oblique histograms with arrows (transpiration) are drawn in proportion to the absolute rates of water flow, of water use and transpiration. Solid lines represent water uptake, flow in the xylem and transpiration, the dotted line indicates the water flow in the phloem. The numbers indicate the values of uptake, transport, utilisation and transpiration (g H_2O per plants over the study period).

According to Parker and Riches (1993), growth of *Rhinanthus* can be controlled in the field by manipulating the N-nutrition. Therefore plants have been cultivated at low NO_3^- supply (1mM). For a group of plants, NO_3^- has been replaced by 1mM NH_4^+.

3.3.4 Water flows in the parasitic association *Rhinanthus minor/Hordeum vulgare* supplied with 1 mM NO_3^- or 1 mM NH_4^+

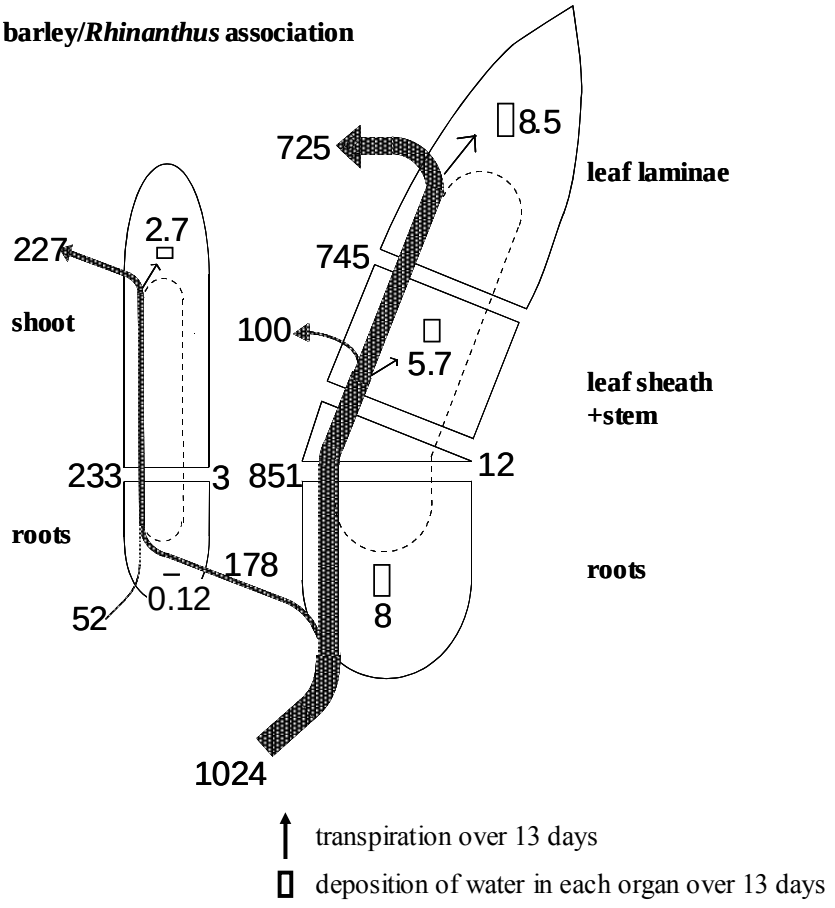


Fig. 3.12 Flow profiles for uptake, transport, utilisation, and transpirational loss of H_2O in *R.minor/H.vulgare* association supplied with 5 mM NO_3^- over 13 d experimental period, starting 41 d after sowing. Further details as in Fig. 3.11.

Water flow models in single *Rhinanthus*, non-parasitised barley and in the parasitic association *Rhinanthus minor/barley* supplied with 1 mM NO_3^- or 1 mM NH_4^+ are presented in Fig. 3.13, Fig. 3.14, Fig. 3.15 and Fig. 3.16. Under both conditions, single *Rhinanthus* plants behaves similar as described above for single *Rhinanthus* supplied 5 mM NO_3^-. Almost all the water taken up by single *Rhinanthus* was released by the leaves to the atmosphere. After a successful attachment to barley, total water uptake into *Rhinanthus* was increased nearly 14-fold (1 mM NO_3^- supply, see Fig. 3.13 and Fig. 3.14) and 13-fold (1 mM NH_4^+ supply, see Fig. 3.15 and Fig. 3.16), the largest portion of which was extracted from the host roots. About 22% of the water taken up by the barley roots was diverted to the parasite under both of these two kinds

of nutrient supply. Water uptake by incorporation to the roots of *Rhinanthus* (1 mM NO_3^- supply) was 4 times increased (Fig. 3.13 and Fig. 3.14), 3 times increased (1 mM NH_4^+ supply) (Fig. 3.15 and Fig. 3.16) after attachment, while water incorporation into shoot tissues (1 mM NO_3^-) increased 31 times, and 27 times increased when plants were supplied with 1 mM NH_4^+ (Fig. 3.13, Fig. 3.14, Fig. 3.15 and Fig. 3.16). When plants were supplied with 1 mM NH_4^+, water uptake by both unparasitised and parasitised barley roots was reduced by 13% compared with 1 mM NO_3^- supply, and transpiration in leaf laminae was reduced by 16-18%.

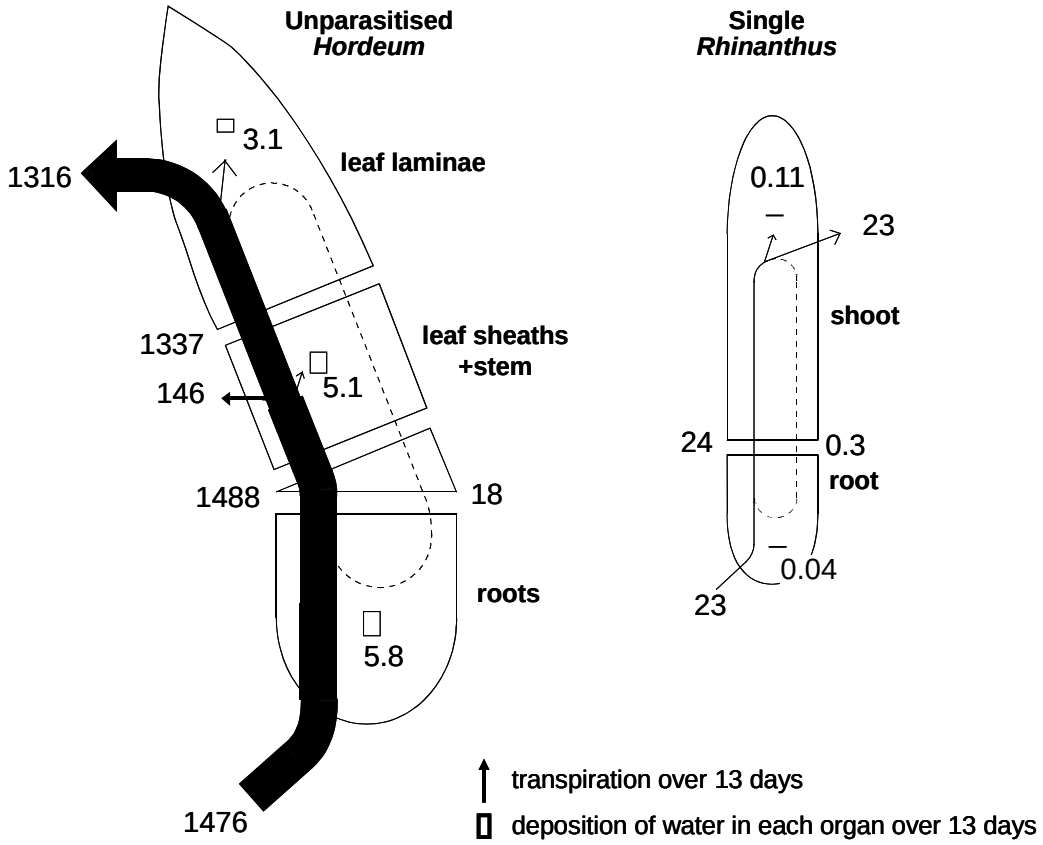


Fig. 3.13 Flow profiles for uptake, transport, utilisation, and transpirational loss of H_2O in single barley (A) and *Rhinanthus* (B) supplied with 1 mM NO_3^- over 13 d experimental period, starting 41 d after sowing. Further details as in Fig. 3.11.

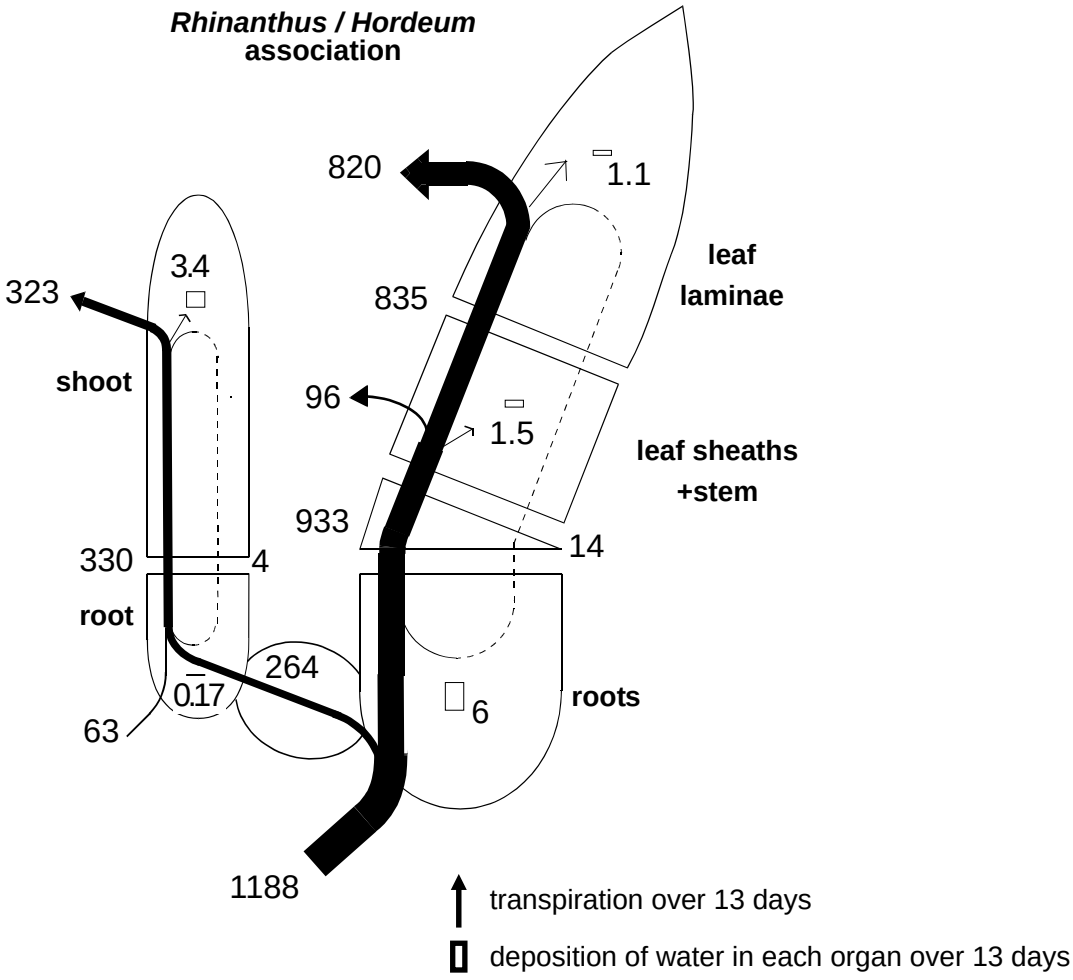


Fig. 3.14 Flow profiles for uptake, transport, utilisation, and transpirational loss of H_2O in *R.minor/H.vulgare* association supplied with 1 mM NO_3^- over 13 d experimental period, starting 41 d after sowing. Further details as in Fig. 3.11.

The growth-dependent incorporation of water into roots of parasitised barley was not affected by the parasite, whereas incorporation into growing shoot tissues was substantially decreased in parasitised barley, compared to unparasitised barley, i.e. in leaf laminae by 65% and in the leaf sheath fraction by 71% for the barley plants supplied with 1 mM NO_3^- (Fig. 3.13 and Fig. 3.14). For barley plants supplied with 1 mM NH_4^+, water incorporation in leaf laminae decreased by 59% and in leaf sheath by 67% (Fig. 3.15 and Fig. 3.16). The ratio of root to shoot xylem transport to uptake by the roots decreased from 101% in uninfected barley plants with 1 mM NO_3^- or 1 mM NH_4^+ supplies to 79% (1 mM NO_3^- supply) (Fig. 3.13 and Fig. 3.14) and 78% (1

mM NH_4^+) (Fig. 3.15 and Fig. 3.16) in infected barley plants.

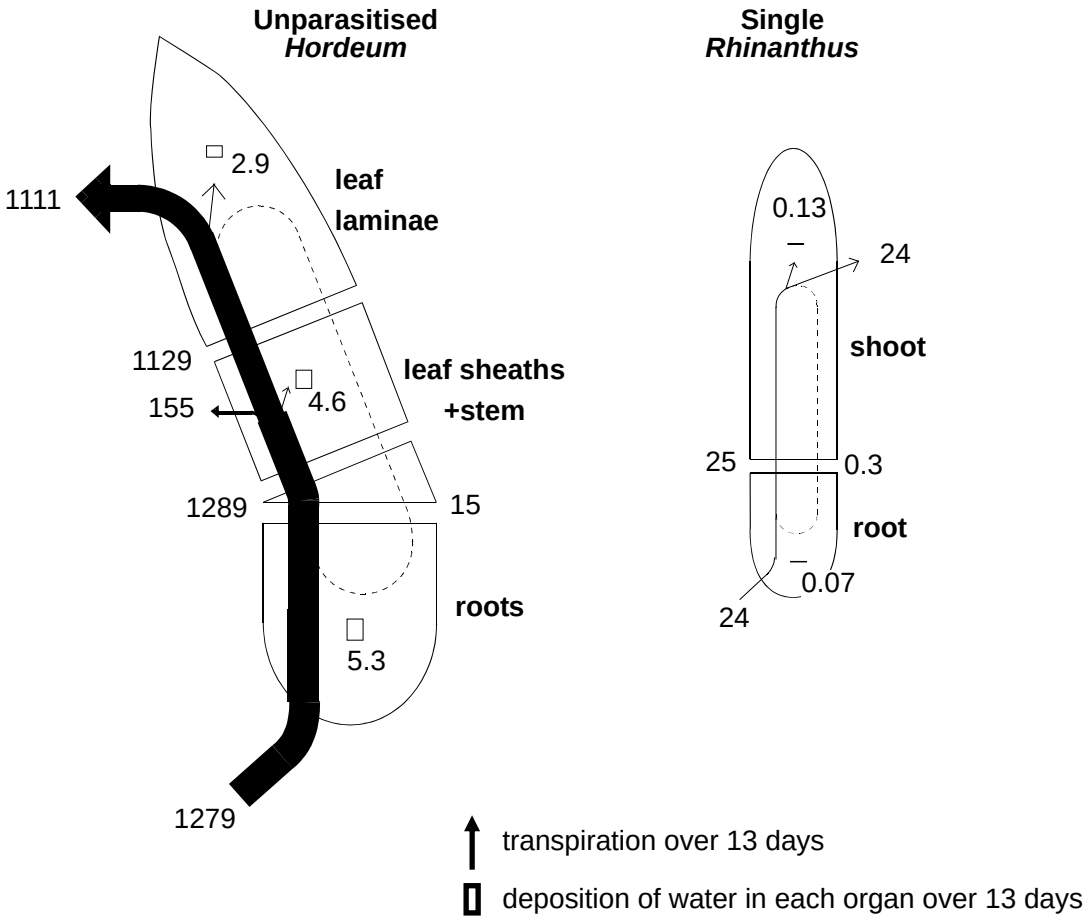


Fig. 3.15 Flow profiles for uptake, transport, utilisation, and transpirational loss of H_2O in single barley (A) and *Rhinanthus* (B) supplied with 1 mM NH_4^+ over 13 d experimental period, starting 41 d after sowing. Further details as in Fig. 3.11.

3.3.5 Comparision of water flows in the parasitic association *Rhinanthus minor/Hordeum vulgare* supplied with 5 mM NO_3^- or 1 mM NO_3^-

In contrast to the plants with 5 mM NO_3^- supply, deposition of water in shoot and root of single *Rhinanthus* did not change much; in attached *Rhinanthus* deposition of water in shoot increased about 15-26% and 42-92% increased in root after the plants were supplied with a lower level of nitrogen (Fig. 3.11, Fig. 3.12, Fig. 3.13, Fig. 3.14, Fig.

3.15, Fig. 3.16). However, when the plants were supplied with 1 mM NO_3^- in single barley water deposition decreased by 72% in leaf laminae, by 49% in leaf sheath and by 28% in root (Fig. 3.11, Fig. 3.13); In parasitised barley 1 mM NO_3^- caused a decrease of 87% in leaf laminae, 74% in leaf sheaths and 25% in roots (Fig. 3.12, Fig. 3.14). The water deposition into organs of 1 mM NH_4^+ - supplied plants was similar as in the case of 1 mM NO_3^- - supplied plants.

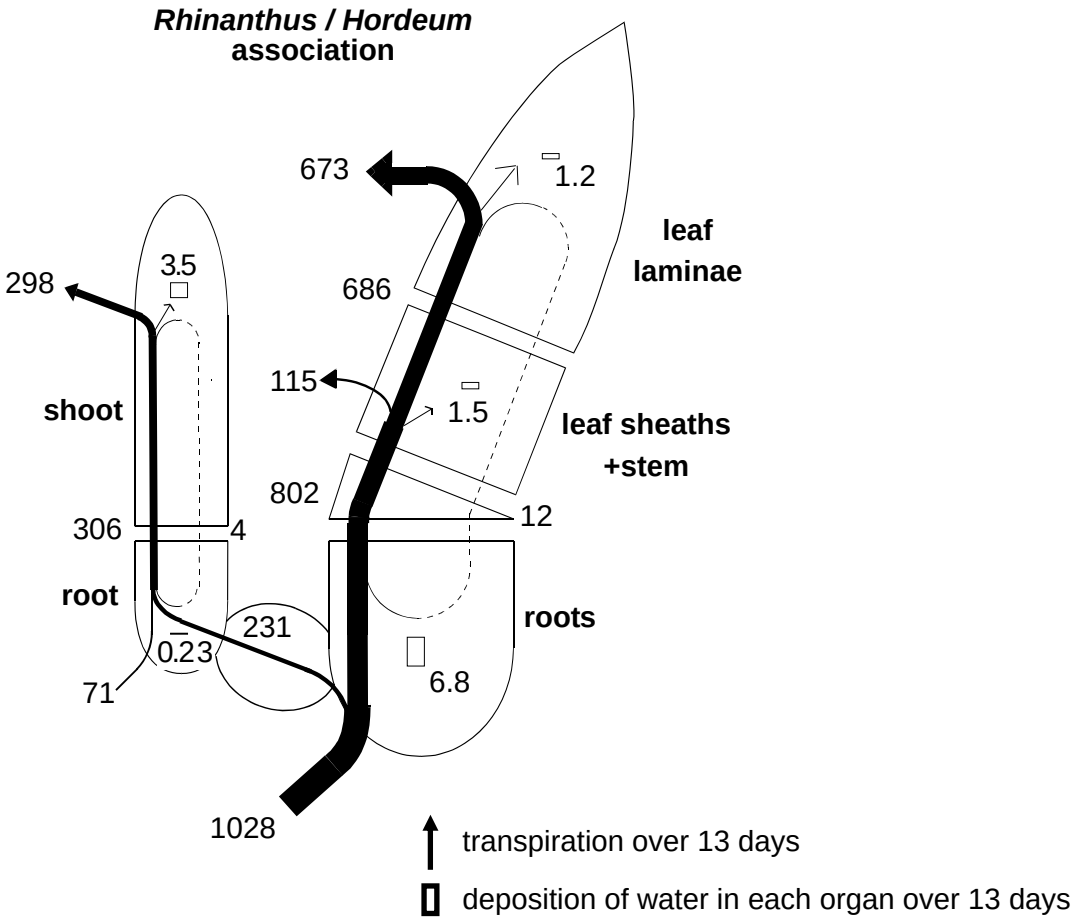


Fig. 3.16 Flow profiles for uptake, transport, utilisation, and transpirational loss of H_2O in *R.minor/H.vulgare* association supplied with 1 mM NH_4^+ over 13 d experimental period, starting 41 d after sowing. Further details as in Fig. 3.11.

3.4 Nutritional flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite nutrient relations

3.4.1 Plants supplied with 5 mM NO_3^-

3.4.1.1 Biomass accumulation

Rhinanthus minor had substantially negative effects on the biomass accumulation of parasitised *Hordeum* leaf laminae and leaf sheaths and had a relatively small negative influence on *Hordeum* roots, as similarly found for *Poa alpina* by Seel and Press (1996). Compared with unparasitised *Hordeum* the reductions in biomass accumulation of host *Hordeum* leaf laminae, leaf sheaths and roots over 13 days were 33%, 52%, 20% respectively (Table 3.3). As a consequence, at first and second harvest the ratio of shoot to root of parasitised *Hordeum* was much lower than that of unparasitised *Hordeum* (Table 3.5). On the other hand, when the *Rhinanthus* had attached to *Hordeum*, its shoot dry matter gain was strongly improved, and was 12 times higher than that of solitary plants (Table 3.4). However, the dry matter gain of attached *Rhinanthus* roots was only two times that of plants without a host (Table 3.4). Consequently, the shoot to root ratio in parasitising *Rhinanthus* was 4.6 fold higher than that of single *Rhinanthus* and at the same time 8.4 fold or 6.7 fold higher than that of parasitised or unparasitised *Hordeum* (Table 3.5), respectively.

3.4.1.2 Mineral nutrients partitioning in control barley, in solitary *Rhinanthus* and in the association between barley and *Rhinanthus*

The contents of mineral elements are presented in Table 3.3. For all elements the contents per plant in leaf laminae and sheaths in general were somewhat lower in parasitised than in unparasitised *Hordeum* at first harvest, i.e. around 30 days after parasite attachment. The differences were clearly higher at second harvest after

further 13 days of parasitism. Consequently, the nutrient increments within 13 days were substantially decreased by 31%, 23%, 26%, 19%, 16%, 33% respectively for N, P, K^+, Ca^{2+}, Mg^{2+}, and C in the leaf laminae. By comparison, the effects on nutrient increments in leaf sheaths were much stronger and resulted in decreases of 49% for N, 52% for P, 46% for K^+, 45% for Ca^{2+}, 47% for Mg^{2+} and 52% for C (Table 3.3).

Comparing attached *Rhinanthus* plants with unattached individuals, increments in the mineral nutrients (N, P, K^+), however, were as 18, 42, 28 times increased, i.e. more than dry matter growth (12-fold). Increments of Ca^{2+} and Mg^{2+} in attached *Rhinanthus* shoot were not as dramatically (4.9 or 7.1-fold) enhanced (Table 3.4). As far as nutrient increments in the roots of attached and single *Rhinanthus* plants are concerned, those of N, P, K^+, Ca^{2+}, Mg^{2+} in parasitsing plant roots were 2.2, 2.6, 0.9, 3.8 and 2.7 times, respectively higher than in unattached *Rhinanthus* roots, similar to the two-fold increase in root dry matter gain (Table 3.4).

3.4.1.3 Concentrations of mineral nutrients, amides and amino acid-N in xylem sap

Concentrations of K^+, P, Mg^{2+} and NO_3^- proved to be higher in parasitised *Hordeum* than in unparasitised *Hordeum,* but this was within the statistical error. Concentrations of K^+, P and Mg^{2+} in attached *Rhinanthus* xylem sap were higher than those in both, unparasitised and parasitised *Hordeum*. Compared with single *Rhinanthus*, K^+, P and NO_3^- concentrations in parasitising *Rhinanthus* were rather higher, however, the Mg^{2+} concentration was much lower. Total N was calculated from the sum of NO_3^-, amides and amino acids. The highest total N concentrations were found in xylem sap of attached *Rhinanthus* (12.1 mM), 36% occurred as NO_3^-, and 64% as amino acids. In parasitised *Hordeum* xylem sap, 51% of total N was NO_3^- and 49% were amino acids, while 41% NO_3^- and 59% amino acids were found in unparasitised *Hordeum* xylem sap (Table. 3.6).

Table 3.3 Biomass (g/plant) and elemental content (mmol/plant) and their increment in *Hordeum vulgare* supplied with 5 mM NO_3^- (control or parasitised by *Rhinanthus minor* at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment). Data are shown as means ± SE; n = 5. Note: higher scale compared to Tab. 3.4.

		Unparasitised Barley			Parasitised Barley		
	Harvest	Leaf laminae	Leaf sheaths	Roots	Leaf laminae	Leaf sheaths	Roots
Dry matter	1	1.57 ± 0.22	1.03 ± 0.17	0.86 ± 0.14	1.40 ± 0.25	0.79 ± 0.19	0.83 ± 0.16
	2	3.81 ± 0.29	2.76 ± 0.28	1.89 ± 0.14	2.91 ± 0.19	1.62 ± 0.16	1.65 ± 0.14
	increment	2.24	1.73	1.03	1.51	0.83	0.82
N	1	4.01 ± 0.31	1.68 ± 0.15	1.17 ± 0.11	4.11 ± 0.48	1.50 ± 0.20	1.34 ± 0.17
	2	9.27 ± 0.39	4.15 ± 0.34	2.43 ± 0.10	7.73 ± 0.44	2.75 ± 0.21	2.52 ± 0.17
	increment	5.26	2.47	1.26	3.62	1.25	1.18
P	1	0.21 ± 0.01	0.16 ± 0.02	0.09 ± 0.01	0.23 ± 0.03	0.15 ± 0.03	0.11 ± 0.01
	2	0.52 ± 0.02	0.47 ± 0.03	0.17 ± 0.01	0.47 ± 0.03	0.30 ± 0.02	0.21 ±0.02
	increment	0.31	0.31	0.08	0.24	0.15	0.10
K^+	1	1.71 ± 0.11	1.02 ± 0.09	0.24 ± 0.02	1.64 ± 0.16	0.95 ± 0.14	0.29±0.02
	2	3.84 ± 0.18	2.57 ± 0.17	0.33 ± 0.01	3.22 ± 0.16	1.78 ± 0.16	0.35±0.02
	increment	2.13	1.55	0.09	1.58	0.83	0.06
Ca^{2+}	1	0.14 ± 0.02	0.05 ± 0.01	0.08 ± 0.01	0.16 ± 0.02	0.05 ± 0.01	0.08 ± 0.02
	2	0.37 ± 0.03	0.15 ± 0.02	0.18 ± 0.03	0.36 ± 0.03	0.11 ± 0.02	0.14 ± 0.02
	increment	0.23	0.10	0.10	0.20	0.06	0.06
Mg^{2+}	1	0.16 ± 0.02	0.08 ± 0.01	0.14 ± 0.03	0.17 ± 0.02	0.07 ± 0.01	0.12 ± 0.03
	2	0.47 ± 0.05	0.25 ± 0.03	0.29 ± 0.02	0.43 ± 0.03	0.16 ± 0.01	0.26 ± 0.03
	increment	0.31	0.17	0.15	0.26	0.09	0.14
C	1	57.2 ± 8.3	34.1 ± 5.7	28.7 ± 4.5	49.7 ± 9.4	25.4 ± 6.4	28.3 ± 5.4
	2	140 ± 11.1	93.5 ± 10.1	67.2 ± 4.6	105 ± 6.3	53.8 ± 4.9	58.2 ± 5.3
	increment	82.8	59.4	38.5	55.3	28.4	29.9

Table 3.4 Biomass (mg/plant) and elemental content (µmol/plant) and their increment in *Rhinanthus minor* plants parasitising (or not) on *Hordeum vulgare* (plants supplied with 5 mM NO_3^-) at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment. Data are shown as means ± SE; n = 5. Note: lower scale compared to Tab.3.3.

		Unattached *Rhinanthus*		Attached *Rhinanthus*	
	Harvest	Shoot	Root	Shoot	Root
Dry matter	1	25.3 ± 3.24	5.99 ± 0.63	152 ± 11.6	11.7 ± 2.07
	2	64.7 ± 18.7	14.8 ± 4.28	625 ± 76.9	27.0 ± 3.22
	increment	39.4	8.81	473	15.3
N	1	52.2 ± 6.68	12.2 ± 1.3	635 ± 47.8	30.6 ± 5.41
	2	133 ± 16.5	30.3 ± 4.3	2094 ± 262	70.7 ± 8.42
	increment	81.1	18.1	1459	40.1
P	1	2.36 ± 0.30	1.55 ± 0.16	50.2 ± 3.89	3.47 ± 0.61
	2	6.01 ± 0.74	3.84 ± 0.53	203 ± 25	9.50 ± 1.13
	increment	3.66	2.29	153	6.04
K^+	1	15.5 ± 2.05	7.72 ± 0.84	271 ± 21.7	8 ± 1
	2	39.5 ± 4.88	19.1 ± 2.61	942 ± 115	18 ± 3
	increment	24	11.4	671	10
Ca^{2+}	1	8.84 ± 1.13	1.40 ± 0.15	23.4 ± 1.89	4.83 ± 0.85
	2	22.6 ± 2.80	3.47 ± 0.48	86.5 ± 10.7	13.3 ± 1.58
	increment	13.7	2.07	63.1	8.48
Mg^{2+}	1	8.22 ± 1.05	0.92 ± 0.09	27.6 ± 2.17	2.68 ± 0.48
	2	21.0 ± 2.60	2.27 ± 0.31	119 ± 14.8	6.36 ± 0.76
	increment	12.8	1.35	91.4	3.68
C	1	873 ± 112	194 ± 20.5	4463 ± 332	384 ± 67.8
	2	2228 ± 276	479 ± 68.6	19241 ± 2374	886 ± 105
	increment	1356	285	14778	502

Table 3.5 Shoot to root ratios of unparasitised and parasitised barley and parasitising and solitary *Rhinanthus minor* plants (supplied with 5 mM NO_3^-) at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment. (n = 5).

	Harvest 1	Harvest 2
Unparasitised barley	3.1 ± 0.2	3.5 ± 0.1
Parasitised barley	2.7 ± 0.2	2.8 ± 0.3
Unattached *Rhinanthus*	4.0 ± 0.1	5.1 ± 0.9
Attached *Rhinanthus*	15.2 ± 4.2	23.4 ± 1.8

Table 3.6 Concentrations (mM) of K^+, Mg^{2+}, total P, NO_3^-, amide and amino acid-N in xylem sap of unparasitised and parasitised barley and single and attached *Rhinanthus* (supplied with 5 mM NO_3^-). (n = 3-12). ∗Data from Seel and Jeschke (1999).

	Unparasitised barley	Parasitised barley	∗Single *Rhinanthus*	Attached *Rhinanthus*
K^+	4.35 ± 0.80	5.30 ± 0.84	3.37 ± 1.28	6.85 ± 1.33
Total P	0.66 ± 0.12	0.71 ± 0.16	0.41 ± 0.12	1.24 ± 0.27
Mg^{2+}	0.43 ± 0.09	0.50 ± 0.08	1.55 ± 0.44	0.65 ± 0.12
NO_3^-	3.49 ± 0.58	4.46 ± 0.54	2.26 ± 0.77	4.39 ± 1.48
Amide-N	2.63 ± 0.81	2.44 ± 0.43	3.01 ± 0.98	4.89 ± 0.72
Amino acid-N	2.43 ± 0.44	1.84 ± 0.24	1.18 ± 0.27	2.84 ± 0.46

3.4.1.4 Net flows of mineral nutrients within uninfected barley, unattached *Rhinanthus* and in the association between barley and *Rhinanthus*

Fig. 3.17 depicts the flow models for nitrogen in single *Hordeum*, left, and in unattached *Rhinanthus*, center left. Flows have been depicted between the root, leaf sheaths and laminae only. Major sinks for nitrogen in *Hordeum* were the laminae, attracting 59% of total nitrogen. There was substantial N retranslocation in the phloem (31% of xylem flow), which exceeded N deposition in the root (14% of total

N) and hence led to a recirculation of N towards the shoot. Consequently, xylem transport to the shoot exceeded the uptake of N from the soil by 25%.

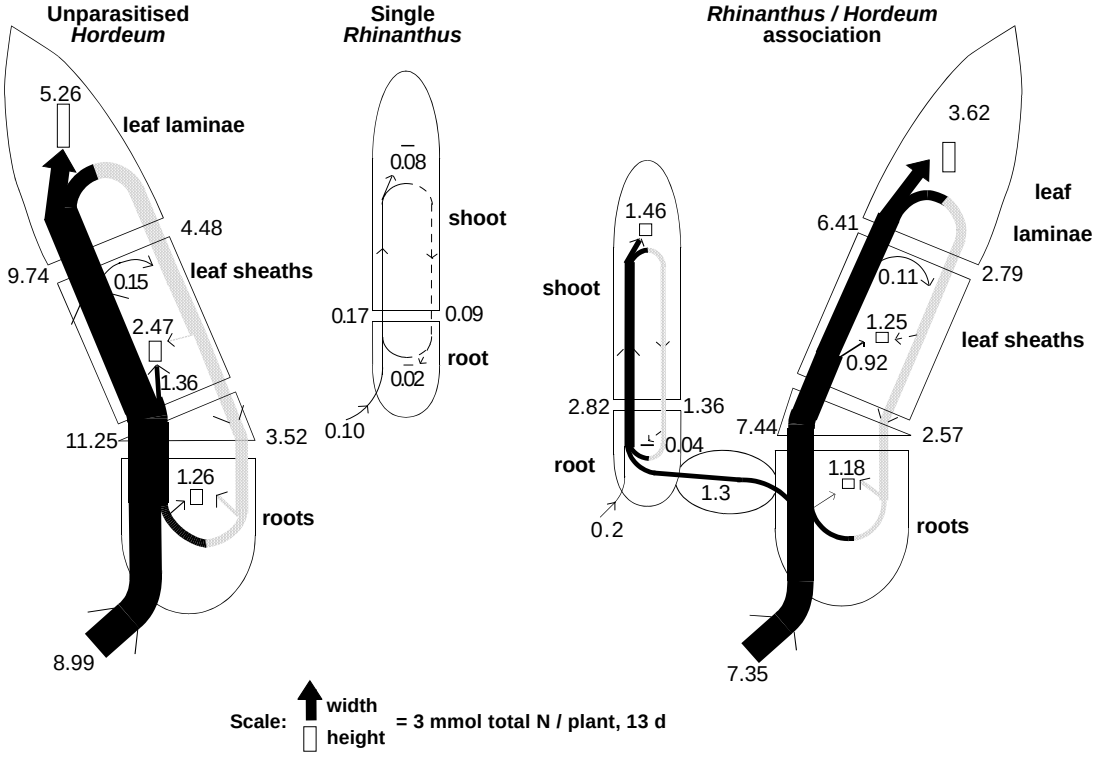


Fig. 3.17 Empirical models of the uptake of nitrate and the transport and utilisation of total nitrogen in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Numbers are presented in mmoles N per plant over 13 days. Plants were supplied with 5 mM NO_3^-. The width of arrows (net flows in xylem (black) or phloem (dotted)) and the height of histograms (deposition of total N in each organ) are drawn in proportion to the rates of flows or to the magnitude of deposition. The triangle between root and leaf sheaths symbolises the stem.

By comparison, in the small, unattached *Rhinanthus*, N uptake and flows were extremely small, N uptake was only 1.4% of that in *Hordeum*. Merely phloem retranslocation of nitrogen appeared to be comparatively high (53% of xylem N flow).

After successful attachment to its *Hordeum* host (Fig. 3.17, right hand side), the most dramatic changes in nitrogen flows were seen in the parasite *Rhinanthus*: total

nitrogen uptake then was 15-fold enlarged, 87% of this nitrogen being withdrawn from the *Hordeum* host. By far most of the nitrogen taken up (97%) was used for shoot growth and development of the inflorescence. Also in parasitising *Rhinanthus* there was substantial shoot to root retranslocation of nitrogen (48% of xylem transport), most of which was recirculated through the root to the shoot with the result that xylem N flow almost twofold exceeded total N uptake from host plus from the soil.

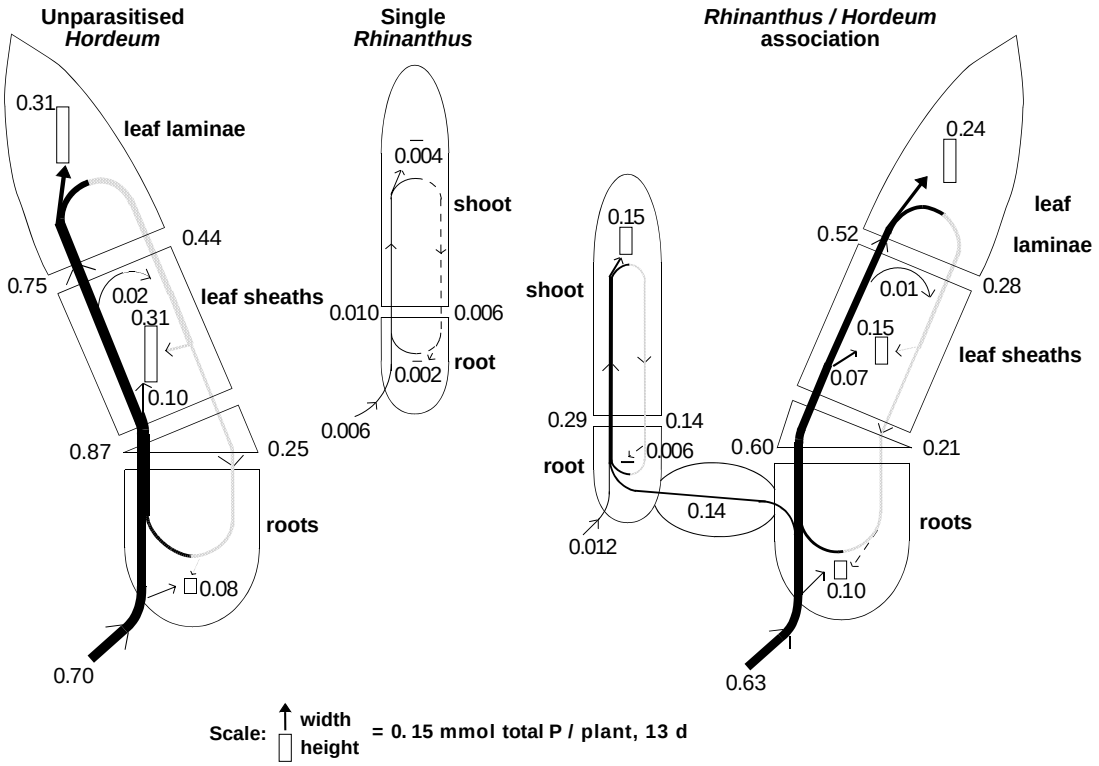


Fig. 3.18 Empirical models of the uptake of phosphate and the transport and utilisation of total phosphorus in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were supplied with 5 mM NO_3^-. Numbers are presented in mmoles P per plant over 13 days. Further details as in Fig.3.17.

Concomitant with these dramatic changes of N utilisation in the parasite, there were substantial influences on the host:

(i) *Rhinanthus* scavenged almost one fifth (18%) of the host's nitrogen uptake (51% as nitrate and 49% as amino acids, Table. 3.6), which itself was 18% decreased compared with the control.

(ii) Within the host the mentioned 18% of the root xylem flow were redirected towards the parasite and hence less nitrogen was available for xylem flow to the shoot (66% of the control), with the consequence that xylem N transport was smaller than total uptake, rather than larger than in the control.

(iii) The effects on N incorporation were different: in the laminae it was 69%, in the sheaths 51%, but in the roots it remained at 94% of the control.

(iv) Remarkably, phloem retranslocation of N was relatively increased in response to parasitism (34.5% vs. 31.3% of xylem transport in the control).

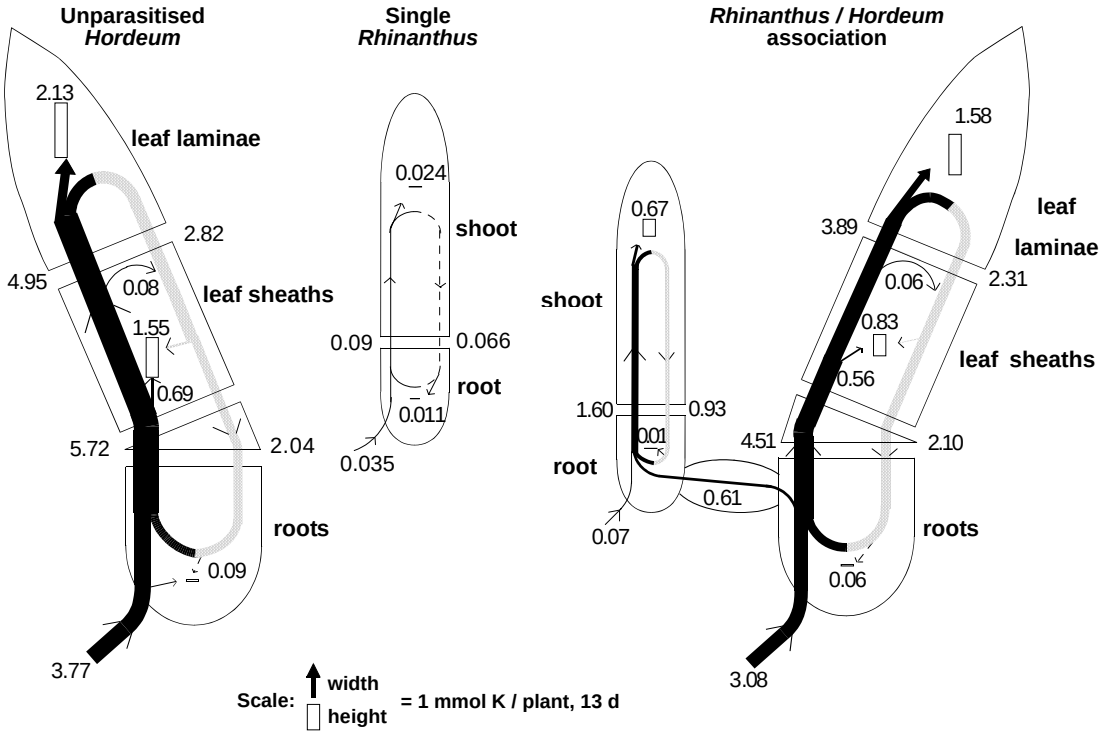


Fig. 3.19 Empirical models of the uptake of K^+ and the transport and utilisation of K^+ in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were irrigated with 5 mM NO_3^-. Numbers are presented in mmoles K per plant over 13 days. Further details as in Fig.3.17.

In Figs. 3.18 – 3.20 flows and partitioning of P, K^+ and Mg^{2+} are presented and as is clearly seen, the general patterns are similar as in the case of nitrogen, although the magnitude of flows declined substantially in the order N > K^+ > P > Mg^{2+}, the flows of Mg amounting to only 5% of the nitrogen flows. Only a few items shall be mentioned here:

(v) There was a conspicuous difference in the relative deposition of nutrients in *Hordeum* shoot and root: whereas 6.1 – fold more nitrogen was incorporated in the shoot of control *Hordeum* (4.1 – fold in parasitised plants), it was 7.8 – fold, control (3.9 – fold, parasitised *Hordeum*) for P, 41 – fold, control (40 – fold, parasitised *Hordeum*) for K^+ and only 3.2 – fold, control (2.5 – fold, parasitised *Hordeum*) for Mg^{2+}, indicating higher root deposition for Mg^{2+} and low root utilization of K^+.

(vi) Attachment to the host *Hordeum* enabled *Rhinanthus* to acquire 15 times the nitrogen taken up by solitary *Rhinanthus*; this factor was 25 in the case of P, 19 for K^+ and only 7 for Mg^{2+}.

(vii) By contrast to these large differences between particular nutrients, their relative withdrawal from the host xylem flow was comparatively similar and amounted to 18% of total N uptake by the host, 22% of total P, 20% of total K^+ and 13% of total Mg^{2+} uptake.

(viii) For all mineral nutrients parasitism by *Rhinanthus* led to an increase in the proportionate retranslocation from shoot to root, as seen for N (see item iv): in the case of P retranslocation increased to 35% of xylem transport (29% in the control), for K^+ it was 47% in parasitised *Hordeum* (36%, control) and for Mg^{2+} 19% (16%), indicating a relative increase in root utilisation of minerals in response to the attachment of the root parasite.

(ix) For all nutrients parasitism led to decreased uptake, which was lower by 18% for N, 18% for K^+, 10% for P, and 11% for Mg^{2+} compared with the control *Hordeum* plants.

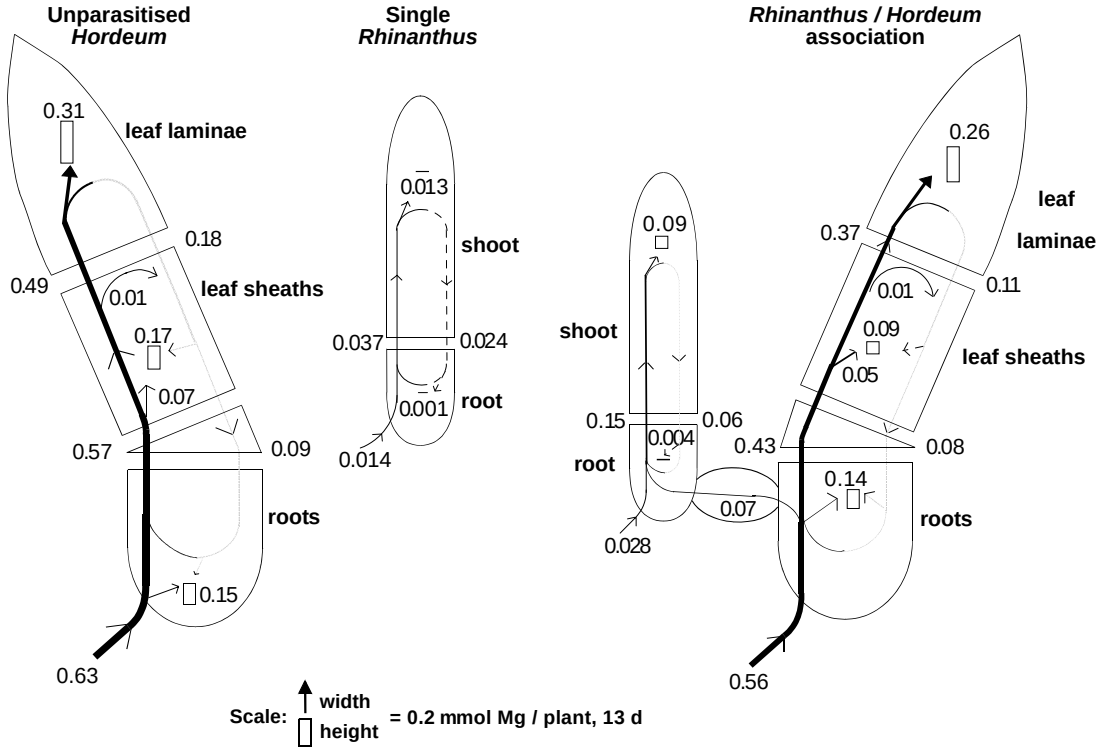


Fig. 3.20 Empirical models of the uptake of Mg^{2+} and the transport and utilisation of Mg in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were supplied with 5 mM NO_3^-. Numbers are presented in mmoles Mg per plant over 13 days. Further details as in Fig. 3.17.

3.4.2 Plants supplied with 1 mM NO_3^- and 1 mM NH_4^+

3.4.2.1 Biomass accumulation

When the plants were supplied with 1 mM NO_3^- and 1 mM NH_4^+, *Rhinanthus minor* had more negative effects on the biomass accumulation of parasitised *Hordeum* leaf laminae and leaf sheaths compared with 5 mM NO_3^- and had a relatively small negative influence on *Hordeum* roots. Compared with unparasitised *Hordeum* the reductions in biomass accumulation of host *Hordeum* (supplied with 1 mM NO_3^-) leaf laminae, leaf sheaths and roots over 13 days were 48%, 53%, 1.7% and 53%, 49% in

Hordeum (supplied with 1 mM NH_4^+) leaf laminae and leaf sheaths respectively, whereas 6.7% increased in 1 mM NH_4^+ supplied barley roots (Table 3.7 and Table 3.9). As a consequence, at first and second harvest the ratio of shoot to root of parasitised *Hordeum* was lower than that of unparasitised *Hordeum* (Table 3.11).

On the other hand, when the *Rhinanthus* had attached to *Hordeum*, its shoot dry matter gain was strongly improved, and was 19 times higher than that of solitary plants when supplied with 1 mM NO_3^- (Table 3.8) and 15 times higher than that of solitary plants when supplied with 1 mM NH_4^+ (Table 3.10). However, the dry matter gain of attached *Rhinanthus* roots was only two times that of plants without a host when supplied with 1 mM NO_3^- and 2.9 times that of single *Rhinanthus* supplied with 1 mM NH_4^+. (Table 3.8 and Table 3.10). Consequently, the shoot to root ratio in parasitising *Rhinanthus* was 4.5 fold higher than that of single *Rhinanthus* and at the same time 7.8 fold or 4.5 fold higher than that of parasitised or unparasitised *Hordeum* when plants were supplied with 1 mM NO_3^-. When the plants were supplied with 1 mM NH_4^+, the values were 3.5, 8.3 and 6.0 (Table 3.11), respectively.

3.4.2.2 Mineral nutrients partitioning in control barley, in solitary *Rhinanthus* and in the association between barley and *Rhinanthus*

1 mM NO_3^- supply

The contents of mineral elements are presented in Table 3.7. For all elements the contents per plant in leaf laminae and sheaths in general were somewhat lower in parasitised than in unparasitised *Hordeum* at first harvest, i.e. around 30 days after parasite attachment. The differences were clearly higher at second harvest after further 13 days of parasitism. Consequently, the nutrient increments within 13 days were substantially decreased by 94%, 30%, 39%, 19%, 31%, 50% respectively for N, P, K^+, Ca^{2+}, Mg^{2+}, and C in the leaf laminae. By comparison, except for N (77%), the effects on other nutrients increments in leaf sheaths were much stronger and resulted

in decreases of 65% for P, 86% for K^+, 57% for Ca^{2+}, 57% for Mg^{2+} and 54% for C (Table 3.7).

Comparing attached *Rhinanthus* plants with unattached individuals, increments in the mineral nutrients (N, P, K^+), however, were as 20, 53, 77 times increased, i.e. more than dry matter growth (19-fold). Increments of Ca^{2+} and Mg^{2+} in attached *Rhinanthus* shoot were not as dramatically (6.6 or 9.1-fold) enhanced (Table 3.8). As far as nutrient increments in the roots of attached and single *Rhinanthus* plants are concerned, those of N, P, K^+, Ca^{2+}, Mg^{2+} in parasitsing plant roots were 2.1, 5.1, 8.8, 2.5 and 1.8 times, respectively higher than in unattached *Rhinanthus* roots (Table 3.8).

1 mM NH_4^+ supply

At the second harvest, except for N, all the other elements had larger reductions in leaf sheath of parasitised barley than in leaf laminae compared with unparasitised barley. They were 61%, 94%, 50%, 46%, 48% for P, K^+, Ca^{2+}, Mg^{2+} and C in leaf sheaths, and 58%, 58%, 17%, 27%, 32% for P, K^+, Ca^{2+}, Mg^{2+} and C in leaf laminae. N decreased much stronger in leaf laminae (151%) than in leaf sheaths (75%) (Table 3.9).

Comparing attached *Rhinanthus* plants with unattached individuals, increments in the mineral nutrients (N, P, K^+, Ca^{2+}, Mg^{2+}), however, were as 18, 51, 33, 17, 19 times increased, i.e. more than dry matter growth (15-fold). As far as nutrient increments in the roots of attached and single *Rhinanthus* plants are concerned, those of N, P, K^+, Ca^{2+}, Mg^{2+} in parasitsing plant roots were 2.6, 4.4, 3.0, 0.98 and 1.7 times, respectively higher than in unattached *Rhinanthus* roots (Table 3.10).

Table 3.7 Biomass (g/plant) and elemental content (mmol/plant) and their increment in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor* at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment). Plants were supplied with 1 mM NO_3^-. Data are shown as means ± SE; n = 5. Note: higher scale compared to Tab. 3.8.

		Unparasitised Barley			Parasitised Barley		
	Harvest	Leaf laminae	Leaf sheaths	Roots	Leaf laminae	Leaf sheaths	Roots
Dry matter	1	1.83 ± 0.07	1.04 ± 0.04	0.93 ± 0.06	1.76 ± 0.05	0.99 ± 0.02	0.93 ± 0.09
	2	3.75 ± 0.12	2.84 ± 0.09	2.12 ± 0.13	2.76 ± 0.13	1.83 ± 0.06	2.10 ± 0.08
	increment	1.92	1.8	1.19	1	0.84	1.17
N	1	4.09 ± 0.23	1.39 ± 0.07	1.28 ± 0.05	3.73 ± 0.17	1.21 ± 0.06	1.26 ± 0.66
	2	5.08 ± 0.09	2.33 ± 0.10	1.84 ± 0.05	3.79 ± 0.11	1.43 ± 0.05	1.92 ± 0.10
	increment	0.99	0.94	0.56	0.06	0.22	0.66
P	1	0.31 ± 0.03	0.19 ± 0.02	0.14 ± 0.01	0.29 ± 0.03	0.15 ± 0.01	0.13 ± 0.01
	2	0.61 ± 0.03	0.39 ± 0.02	0.20 ± 0.01	0.50 ± 0.05	0.22 ± 0.03	0.18 ±0.01
	increment	0.3	0.2	0.06	0.21	0.07	0.05
K^+	1	1.83 ± 0.14	1.00 ± 0.07	0.27 ± 0.05	1.67 ± 0.14	0.81 ± 0.04	0.25±0.04
	2	2.66 ± 0.05	1.44 ± 0.05	0.25 ± 0.03	2.18 ± 0.11	0.87 ± 0.05	0.22±0.01
	increment	0.83	0.44	-0.02	0.51	0.06	-0.03
Ca^{2+}	1	0.18 ± 0.01	0.06 ± 0.01	0.06 ± 0.005	0.18 ± 0.01	0.06 ± 0.003	0.05 ± 0.003
	2	0.39 ± 0.01	0.13 ± 0.03	0.10 ± 0.01	0.35 ± 0.03	0.09 ± 0.01	0.10 ± 0.01
	increment	0.21	0.07	0.04	0.17	0.03	0.05
Mg^{2+}	1	0.20 ± 0.02	0.09 ± 0.004	0.13 ± 0.02	0.20 ± 0.03	0.08 ± 0.01	0.12 ± 0.02
	2	0.52 ± 0.02	0.23 ± 0.01	0.21 ± 0.03	0.42 ± 0.06	0.14 ± 0.02	0.15 ± 0.01
	increment	0.32	0.14	0.08	0.22	0.06	0.03
C	1	65.2 ± 2.34	34.4 ± 1.46	31.5 ± 2.46	63.0 ± 1.92	33.8 ± 0.98	32.2 ± 3.51
	2	136 ± 4.54	99.3 ± 3.57	75.4 ± 3.99	98.7 ± 4.81	63.4 ± 2.03	70.8 ± 2.6
	increment	70.8	64.9	43.9	35.7	29.6	38.6

Table 3.8 Biomass (mg/plant) and elemental content (µmol/plant) and their increment in *Rhinanthus minor* plants parasitising (or not) on *Hordeum vulgare* at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment. Plants were supplied with 1 mM NO_3^-. Data are shown as means ± SE; n = 5. Note: lower scale compared to Tab.3.7.

		Unattached *Rhinanthus*		Attached *Rhinanthus*	
	Harvest	Shoot	Roots	Shoot	Roots
Dry matter	1	26.7 ± 2.29	7.50 ± 0.82	172 ± 39.9	13.9 ± 1.89
	2	68.3 ± 8.48	15.8 ± 2.10	957 ± 70.9	31.1 ± 6.70
	increment	41.6	8.3	785	17.2
N	1	79.4 ± 5.60	16.1 ± 2.1	653 ± 111	32.9 ± 5.03
	2	156 ± 13.0	27.3 ± 1.81	2198 ± 195	56.6 ± 12.2
	increment	76.6	11.2	1545	23.7
P	1	3.70 ± 0.31	2.44 ± 0.31	54.8 ± 12.9	3.70 ± 0.67
	2	9.15 ± 0.89	3.92 ± 0.50	342 ± 21.5	11.2 ± 2.54
	increment	5.45	1.48	287	7.5
K^+	1	17.3 ± 1.45	10.6 ± 1.38	274 ± 64.8	7.73 ± 1.40
	2	28.3 ± 2.76	12.4 ± 1.58	1118 ± 71.1	23.6 ± 6.21
	increment	11	1.8	844	15.8
Ca^{2+}	1	10.7 ± 0.90	1.11 ± 0.14	26.3 ± 6.05	2.18 ± 0.39
	2	29.8 ± 2.90	3.08 ± 0.40	152 ± 10.9	7.19 ± 1.63
	increment	19.1	1.97	126	5.01
Mg^{2+}	1	10.5 ± 0.88	1.49 ± 0.19	39.9 ± 9.51	3.48 ± 0.63
	2	31.1 ± 3.02	4.08 ± 0.52	227 ± 18.6	8.05 ± 1.83
	increment	20.6	2.59	187	4.57
C	1	996 ± 70.3	265 ± 27.6	5591 ± 1059	484 ± 73.9
	2	2278 ± 189	540 ± 69.1	31674 ± 2476	1121 ± 242
	increment	1282	275	26083	637

Table 3.9 Biomass (g/plant) and elemental content (mmol/plant) and their increment in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor* at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment). Plants were supplied with 1 mM NH_4^+. Data are shown as means ± SE; n = 5. Note: higher scale compared to Tab. 3.10.

		Unparasitised Barley			Parasitised Barley		
	Harvest	Leaf laminae	Leaf sheaths	Roots	Leaf laminae	Leaf sheaths	Roots
Dry matter	1	1.99 ± 0.04	1.19 ± 0.04	0.96 ± 0.07	1.65 ± 0.15	0.92 ± 0.10	0.92 ± 0.11
	2	3.72 ± 0.21	2.79 ± 0.15	2.01 ± 0.06	2.47 ± 0.11	1.73 ± 0.15	2.04 ± 0.10
	increment	1.73	1.6	1.05	0.82	0.81	1.12
N	1	4.38 ± 0.10	1.47 ± 0.04	1.21 ± 0.03	3.73 ± 0.47	1.24 ± 0.17	1.17 ± 0.07
	2	5.03 ± 0.21	2.14 ± 0.06	1.79 ± 0.11	3.40 ± 0.19	1.42 ± 0.10	1.88 ± 0.01
	increment	0.65	0.67	0.58	-0.33	0.17	0.71
P	1	0.30 ± 0.03	0.17 ± 0.01	0.14 ± 0.01	0.26 ± 0.04	0.14 ± 0.02	0.13 ± 0.01
	2	0.61 ± 0.03	0.34 ± 0.02	0.20 ± 0.01	0.39 ± 0.02	0.21 ± 0.01	0.20 ±0.01
	increment	0.31	0.18	0.06	0.13	0.07	0.07
K^+	1	1.85 ± 0.14	0.95 ± 0.05	0.24 ± 0.05	1.67 ± 0.14	0.86 ± 0.08	0.23±0.04
	2	2.62 ± 0.06	1.29 ± 0.06	0.22 ± 0.03	1.99 ± 0.07	0.88 ± 0.04	0.28±0.03
	increment	0.77	0.34	-0.02	0.32	0.02	0.04
Ca^{2+}	1	0.20 ± 0.01	0.09 ± 0.01	0.06 ± 0.005	0.19 ± 0.02	0.07 ± 0.01	0.05 ± 0.003
	2	0.43 ± 0.02	0.17 ± 0.01	0.09 ± 0.01	0.37 ± 0.02	0.11 ± 0.005	0.10 ± 0.01
	increment	0.23	0.08	0.03	0.19	0.04	0.05
Mg^{2+}	1	0.21 ± 0.03	0.11 ± 0.01	0.16 ± 0.02	0.19 ± 0.02	0.08 ± 0.01	0.13 ± 0.01
	2	0.48 ± 0.04	0.23 ± 0.01	0.23 ± 0.03	0.37 ± 0.02	0.15 ± 0.01	0.27 ± 0.03
	increment	0.26	0.13	0.07	0.19	0.07	0.14
C	1	71 ± 1.24	39.3 ± 1.40	32.6 ± 2.49	58.5 ± 5.48	30.2 ± 3.38	30.3 ± 3.50
	2	131 ± 8.11	95.8 ± 5.52	69.4 ± 2.13	86.6 ± 8.43	59.7 ± 5.29	70.4 ± 3.87
	increment	60	56.5	36.8	28.1	29.5	40.1

Table 3.10 Biomass (mg/plant) and elemental content (µmol/plant) and their increment in *Rhinanthus minor* plants parasitising (or not) on *Hordeum vulgare* at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment. Plants were supplied with 1 mM NH_4^+. Data are shown as means ± SE; n = 5. Note: lower scale compared to Tab.3.9.

		Unattached *Rhinanthus*		Attached *Rhinanthus*	
	Harvest	Shoot	Roots	Shoot	Roots
Dry matter	1	20.8 ± 2.29	6.16 ± 0.59	165 ± 49.6	16.1 ± 4.58
	2	62.7 ± 7.89	13.3 ± 1.79	798 ± 159	36.8 ± 5.50
	increment	41.9	7.14	633	20.7
N	1	59.3 ± 6.55	14.5 ± 1.01	695 ± 205	39.8 ± 11.3
	2	121.4 ± 9.52	23.0 ± 2.77	1831 ± 411	61.9 ± 9.2
	increment	62.1	8.58	1136	22.1
P	1	2.88 ± 0.33	2.12 ± 0.19	55.0 ± 16.5	4.35 ± 1.24
	2	8.05 ± 0.69	3.58 ± 0.54	320 ± 60.9	10.7 ± 1.83
	increment	5.17	1.46	265	6.38
K^+	1	13.6 ± 1.57	7.73 ± 0.68	282 ± 83.3	12.7 ± 3.61
	2	34.1 ± 2.93	10.5 ± 1.59	959 ± 185	20.9 ± 3.56
	increment	20.5	2.75	677	8.18
Ca^{2+}	1	6.55 ± 0.76	0.86 ± 0.08	29.8 ± 8.62	2.32 ± 0.66
	2	18.6 ± 1.59	3.21 ± 0.49	238 ± 48.4	4.62 ± 0.79
	increment	12	2.35	208	2.3
Mg^{2+}	1	6.38 ± 0.74	1.28 ± 0.11	38.9 ± 12.6	4.98 ± 1.42
	2	17.1 ± 1.47	3.09 ± 0.47	244 ± 50.8	8.02 ± 1.37
	increment	10.8	1.81	206	3.04
C	1	776 ± 85.7	218 ± 15.2	5428 ± 1575	552 ± 157
	2	2170 ± 170	418 ± 59.3	24424 ± 5024	1175 ± 183
	increment	1394	200	18995	623

Table 3.11 Shoot to root ratios of unparasitised and parasitised barley and parasitising and solitary *Rhinanthus minor* plants (supplied with 1 mM NO_3^- or 1 mM NH_4^+) at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 5±SE).

	1 mM NO_3^-		**1 mM NH_4^+**	
	Harvest 1	Harvest 2	Harvest 1	Harvest 2
Unparasitised barley	2.91 ± 0.26	2.95 ± 0.20	3.13 ± 0.30	2.84 ± 0.21
Parasitised barley	2.84 ± 0.34	2.04 ± 0.15	2.83 ± 0.29	2.06 ± 0.06
Unattached *Rhinanthus*	3.47 ± 0.18	3.54 ± 0.58	4.51 ± 0.47	4.87 ± 0.54
Attached *Rhinanthus*	11.8 ± 1.58	16.0 ± 4.1	10.69 ± 4.16	17.0 ± 2.64

Table 3.12 Concentrations (mM) of K^+, Mg^{2+}, total P, NO_3^-, amide and amino acid-N in xylem sap of unparasitised and parasitised barley and single and attached *Rhinanthus* (supplied with 1 mM NO_3^-) (n = 3-12±SE).

	Unparasitised barley	Parasitised barley	Single *Rhinanthus*	Attached *Rhinanthus*
K^+	2.3 ± 0.3	2.6 ± 0.5	1.5 ± 0.2	3.7 ± 0.3
Total P	0.6 ± 0.03	0.5 ± 0.2	0.5 ± 0.3	0.9 ± 0.05
Mg^{2+}	0.4 ± 0.1	0.4 ± 0.1	1.3 ± 0.5	1.0 ± 0.1
NO_3^-	0.16 ± 0.03	0.29 ± 0.07	1.19 ± 0.30	0.5 ± 0.3
Amide-N	1.52 ± 0.34	0.75 ± 0.16	1.75 ± 0.18	4.95 ± 0.35
Amino acid-N	0.58 ± 0.15	0.13 ± 0.30	0.94 ± 0.08	2.05 ± 0.35

3.4.2.3 Concentrations of mineral nutrients, amides and amino acid-N in xylem sap

1 mM NO_3^- supply

Concentrations of K^+, P and Mg^{2+} in attached *Rhinanthus* xylem sap were higher than those in both, unparasitised and parasitised *Hordeum*. Compared with single *Rhinanthus*, K^+, P concentrations in parasitising *Rhinanthus* were rather higher, however, the Mg^{2+} and NO_3^- concentration was much lower. The highest total N

concentrations were found in xylem sap of attached *Rhinanthus* (7.5 mM), 25% occurred as NO_3^-, and 75% as amino acids. In parasitised *Hordeum* xylem sap, 25% of total N was NO_3^- and 75% were amino acids, while 7% NO_3^- and 93% amino acids were found in unparasitised *Hordeum* xylem sap (Tab. 3.12).

1 mM NH_4^+ supply

Concentrations of K^+, P, Mg^{2+} in parasitised *Hordeum* proved to be similar as those in unparasitised *Hordeum,* but NO_3^- in barley xylem sap was not decetable. Concentrations of K^+, P and Mg^{2+} in attached *Rhinanthus* xylem sap were higher than those in both, unparasitised and parasitised *Hordeum*. Compared with single *Rhinanthus*, K^+, P and Mg^{2+} concentrations in parasitising *Rhinanthus* were rather higher. Total N was calculated from the sum of NO_3^-, amides and amino acids. The highest total N concentrations were found in xylem sap of attached *Rhinanthus* (9.3 mM), all as amino acids. In unparasitised barley xylem sap total N was 2.4 times higher than that in parasitised barley (Tab. 3.13).

Table 3.13 Concentrations (mM) of K^+, Mg^{2+}, total P, NO_3^-, amide and amino acid-N in xylem sap of unparasitised and parasitised barley and single and attached *Rhinanthus* (supplied with 1 mM NH_4^+) (n = 3-12±SE).

	Unparasitised barley	Parasitised barley	Single *Rhinanthus*	Attached *Rhinanthus*
K^+	2.1 ± 0.1	2.1 ± 0.4	2.2 ± 0.5	4.7 ± 1.3
Total P	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	1.0 ± 0.3
Mg^{2+}	0.4 ± 0.05	0.4 ± 0.04	0.7 ± 0.2	0.9 ± 0.2
NO_3^-	not detectable	not detectable	0.44 ± 0.10	not detectable
Amide-N	2.10 ± 0.85	1.00 ± 0.42	4.28 ± 0.88	7.38 ± 1.71
Amino acid-N	0.55 ± 0.12	0.11 ± 0.04	0.94 ± 0.26	1.88 ± 0.37

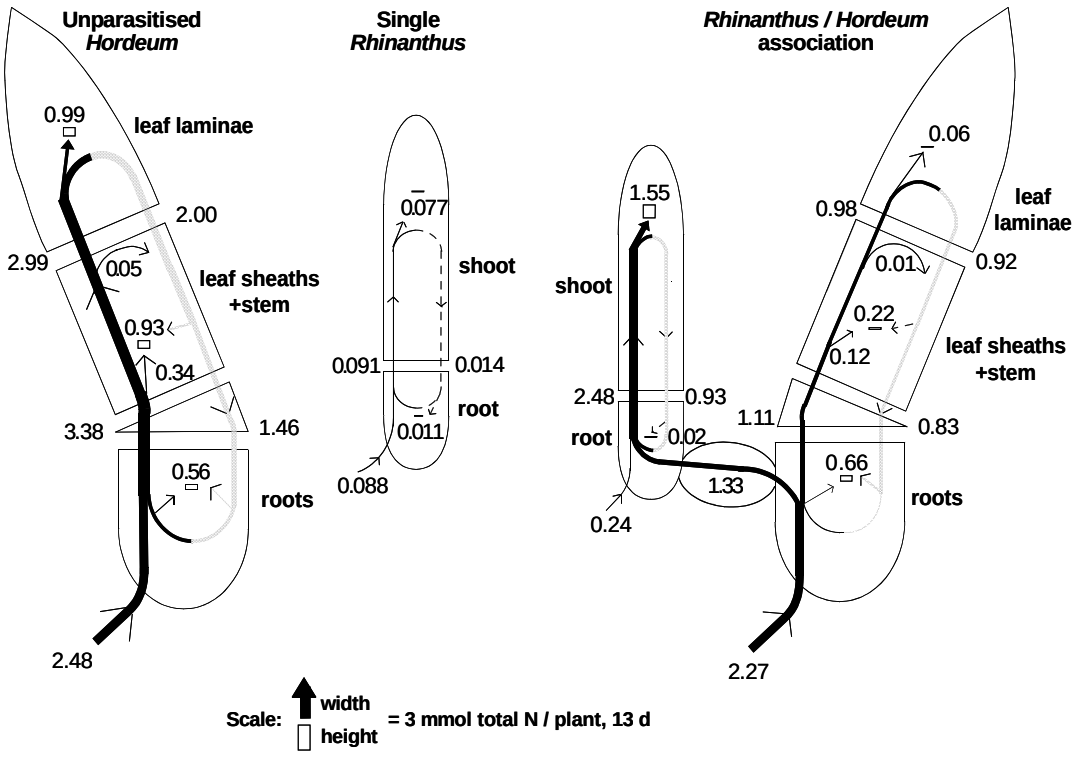


Fig. 3.21 Empirical models of the uptake of nitrogen and the transport and utilisation of total nitrogen in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Numbers are presented in mmoles N per plant over 13 days. Plants were supplied with 1 mM NO_3^-. Further details as in Fig. 3.17.

3.4.2.4 Net flows of mineral nutrients within uninfected barley, unattached *Rhinanthus* and in the association between barley and *Rhinanthus*

1 mM NO_3^- supply

Under 1 mM NO_3^- supply, in unparasitised barley, there was substantial N retranslocation in the phloem (43% of xylem flow), which exceeded N deposition in the root (23% of total N) and hence led to a recirculation of N towards the shoot. Consequently, xylem transport to the shoot exceeded the uptake of N from the soil by 36% (Fig. 3.21).

By comparison, in the small, unattached *Rhinanthus* N uptake and flows were

extremely small, N uptake was only 3.9% of that in *Hordeum*. After successful attachment to its *Hordeum* host (Fig. 3.21, right hand side), the most dramatic changes in nitrogen flows were seen in the parasite *Rhinanthus*: total nitrogen uptake then was 18-fold enlarged, 85% of this nitrogen being withdrawn from the *Hordeum* host. By far most of the nitrogen taken up (99%) was used for shoot growth and development of the inflorescence. Also in parasitising *Rhinanthus* N transported in the xylem increased 27 times.

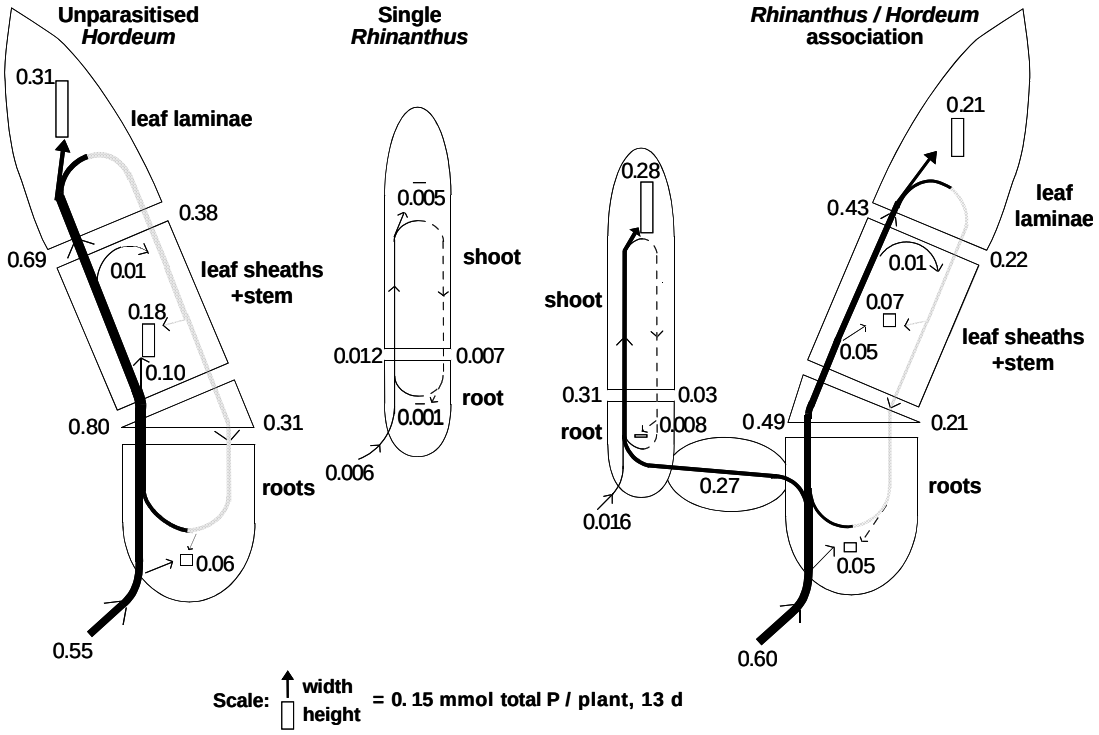


Fig. 3.22 Empirical models of the uptake of phosphate and the transport and utilisation of total phosphorus in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were supplied with 1 mM NO_3^-. Numbers are presented in mmoles P per plant over 13 days. Further details as in Fig.3.17.

Rhinanthus scavenged almost 59% of the host's nitrogen uptake (25% as nitrate and 75% as amino acids, Table. 3.12).

(x) Within the host the mentioned 59% of the root xylem flow were redirected towards the parasite and hence less nitrogen was available for xylem flow to the shoot (33% of the control), with the consequence that xylem N transport was smaller than total uptake, rather larger than in the control.

(xi) The effects on N incorporation were different: in the laminae it was 6%, in the sheaths 24%, but in the roots it remained at 118% of the control.

(xii) Remarkably, phloem retranslocation of N was increased in response to parasitism and lower N supply (75% vs. 43% of xylem transport in the control).

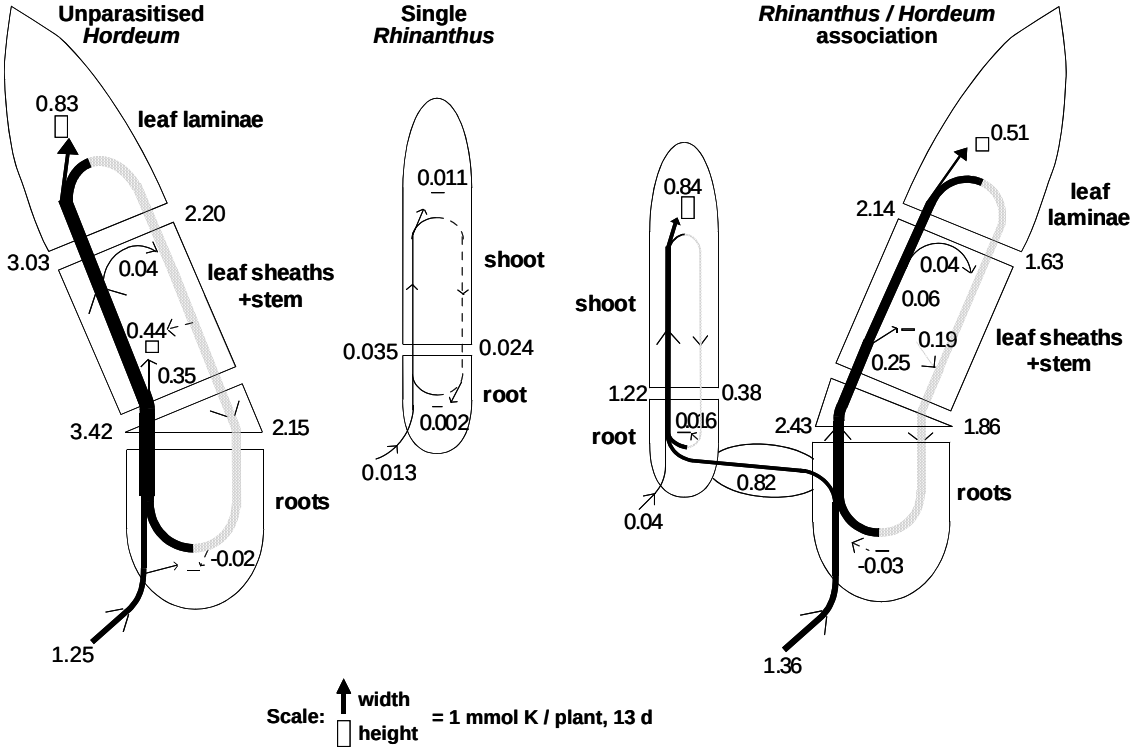


Fig. 3.23 Empirical models of the uptake of K^+ and the transport and utilisation of K^+ in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were irrigated with 1 mM NO_3^-. Numbers are presented in mmoles K per plant over 13 days. Further details as in Fig.3.17.

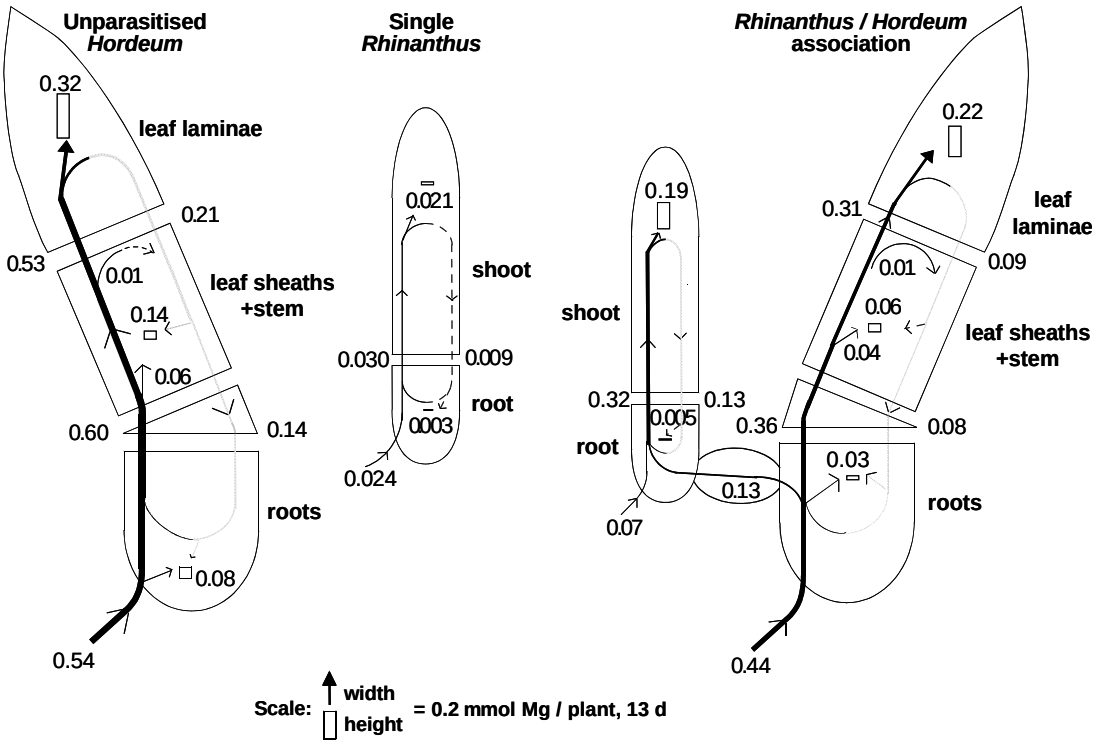


Fig. 3.24 Empirical models of the uptake of Mg^{2+} and the transport and utilisation of Mg in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were supplied with 1 mM NO_3^-. Numbers are presented in mmoles Mg per plant over 13 days. Further details as in Fig. 3.17.

In Fig. 3.22 – Fig. 3.24, flows and partitioning of P, K^+ and Mg^{2+} are presented and as is clearly seen, the general patterns are similar as in the case of nitrogen, although the magnitude of flows declined substantially in the order N > K^+ > P > Mg^{2+}. Only a few items shall be mentioned here:

(xiii) Under 1 mM NO_3^- and 0.4 mM K^+ supply, less N deposited in barley shoot and the deposition of N in shoot and root: whereas 3.4 – fold nitrogen was incorporated in the shoot of control *Hordeum* (0.4 – fold in parasitised plants). Negative deposition of K^+ was found in barley root. It has shown that more K^+ was mobilised from roots back to the xylem.

(xiv) Attachment to the host *Hordeum* enabled *Rhinanthus* to acquire 18 times the nitrogen taken up by solitary *Rhinanthus*; this factor was 48 in the case of P, 66 for K^+ and only 8 for Mg^{2+}.

(xv) By contrast to the plants with 5 mM NO_3^- supply, attached *Rhinanthus* was a stronger sink, their relative withdrawal from the host xylem flow was much higher and amounted to 59% of total N uptake by the host, 45% of total P, 60% of total K^+ and 30% of total Mg^{2+} uptake.

For all mineral nutrients except for Mg^{2+}, parasitism by *Rhinanthus* led to an increase in the proportionate retranslocation from shoot to root, as seen for N (see item xii): in the case of P retranslocation increased to 43% of xylem transport (39% in the control), for K^+ it was 77% in parasitised *Hordeum* (60%, control) indicating a relative increase in root utilisation of minerals in response to the attachment of the root parasite.

1 mM NH_4^+ supply

When 1 mM NO_3^- has been replaced by 1 mM NH_4^+, the N uptake by barley roots decreased 23% in single barley and 33% in parasitised barley. There was substantial higher N retranslocation in the phloem (62% of xylem flow, control; 117%, parasitised barley) (Fig. 3.25).

By comparison, in the small, unattached *Rhinanthus* N uptake and flows were extremely small, N uptake was only 4.7% of that in *Hordeum*. After successful attachment to its *Hordeum* host (Fig. 3.25, right hand side), the most dramatic changes in nitrogen flows were seen in the parasite *Rhinanthus*: total nitrogen uptake then was 16-fold enlarged, 83% of this nitrogen being withdrawn from the *Hordeum* host. By far most of the nitrogen taken up (98%) was used for shoot growth and development of the inflorescence. Also in parasitising *Rhinanthus* N transported in the xylem increased 21 times.

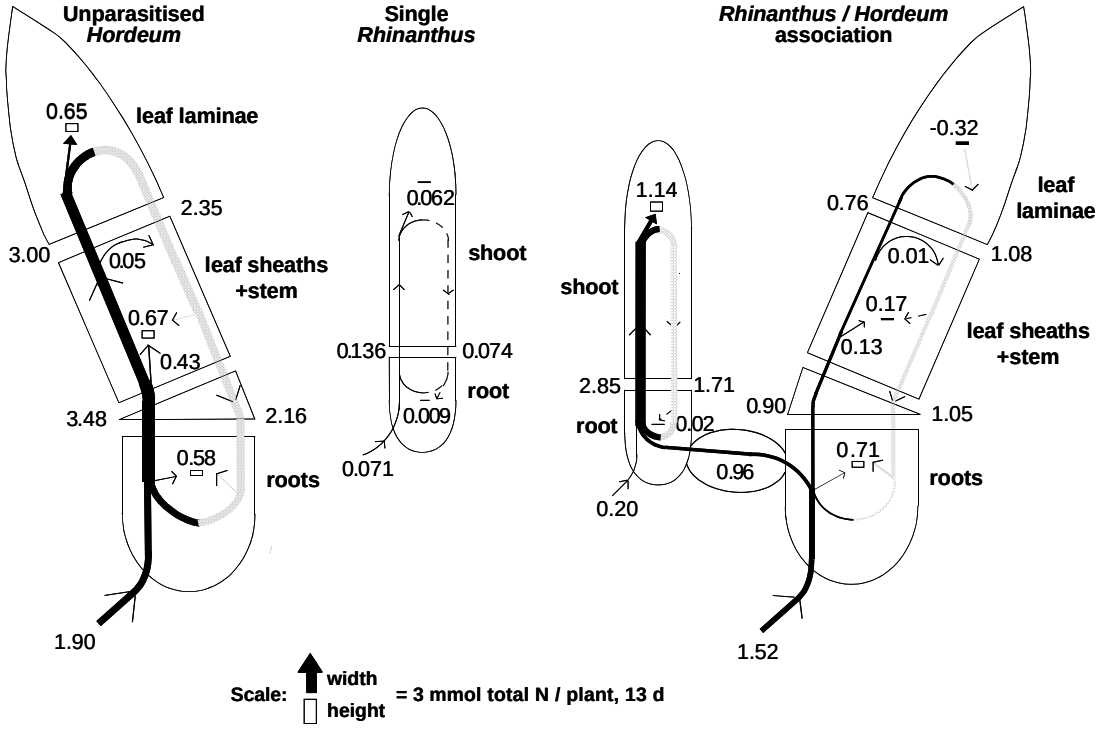


Fig. 3.25 Empirical models of the uptake of nitrogen and the transport and utilisation of total nitrogen in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Numbers are presented in mmoles N per plant over 13 days. Plants were supplied with 1 mM NH_4^+. Further details as in Fig. 3.17.

Together with these changes of N utilisation in the parasite, there were important influences on the host:

(xvi) *Rhinanthus* scavenged almost 63% of the host's nitrogen uptake (all as amino acids, Table. 3.13).

(xvii) Within the host the mentioned 63% of the root xylem flow were redirected towards the parasite and hence less nitrogen was available for xylem flow to the shoot (26% of the control), with the consequence that xylem N transport was smaller than total uptake, rather larger than in the control.

(xviii) The effects on N incorporation were different: in the laminae N was mobilised and exported to phloem, in the sheaths 25% of the control, but in the roots it increased 22%.

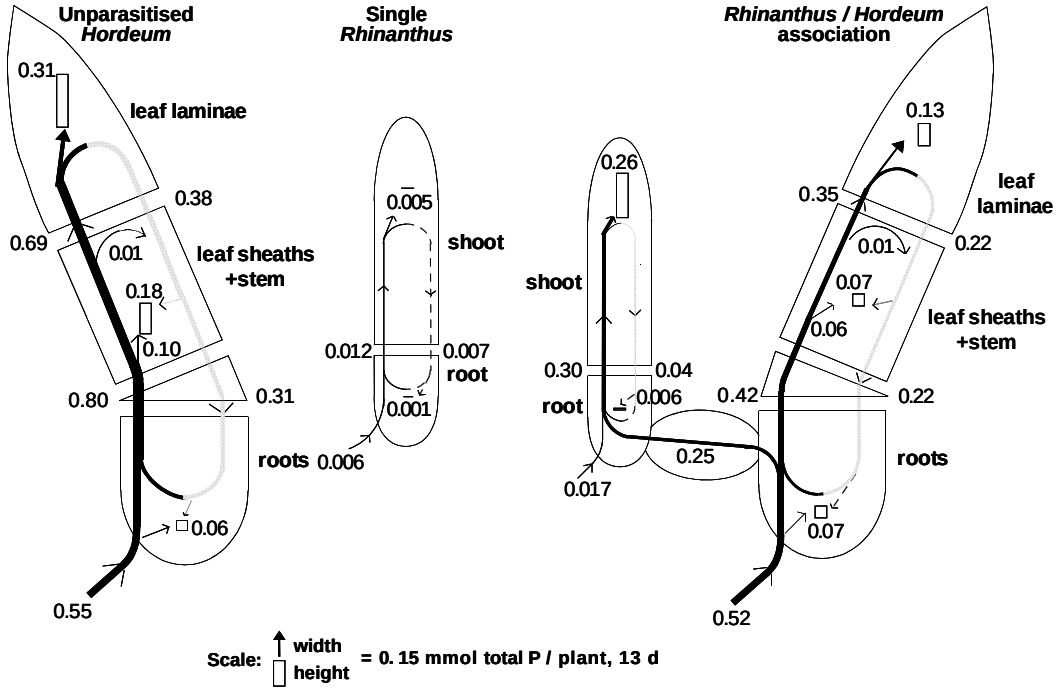


Fig. 3.26 Empirical models of the uptake of phosphate and the transport and utilisation of total phosphorus in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were supplied with 1 mM NH_4^+. Numbers are presented in mmoles P per plant over 13 days. Further details as in Fig.3.17.

(xix) Remarkably, phloem retranslocation of N was increased in response to parasitism and 1 mM NH_4^+ supply (117% vs. 62% of xylem transport in the control).

In Figs. 3.26 – Fig. 3.28 flows and partitioning of P, K^+ and Mg^{2+} are presented and the general patterns are similar as in the case of nitrogen. A few items are mentioned here:

(xx) Under 1 mM NH_4^+ supply, less N is deposited in barley shoot and the deposition of N in shoot and root: whereas 1.9 – fold nitrogen was incorporated in the shoot of control *Hordeum* (-0.2 – fold in parasitised plants). Negative deposition of K^+ was found in barley root. It has shown that more K^+ was recirculated back to the xylem.

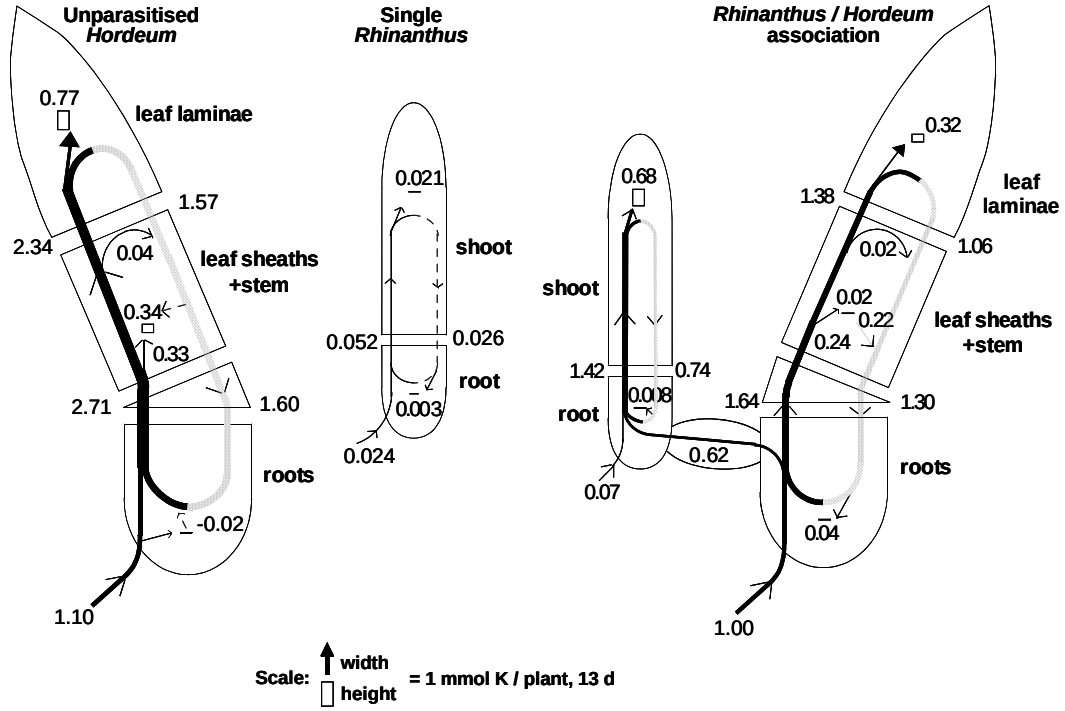


Fig. 3.27 Empirical models of the uptake of K^+ and the transport and utilisation of K^+ in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were nourished with 1 mM NH_4^+. Numbers are presented in mmoles K per plant over 13 days. Further details as in Fig.3.17.

(xxi) Attachment to the host *Hordeum* enabled *Rhinanthus* to acquire 16 times the nitrogen taken up by solitary *Rhinanthus*; this factor was 44 in the case of P, 29 for K^+ and only 16 for Mg^{2+}.

(xxii) By contrast to the plants with 5 mM NO_3^- supply, attached *Rhinanthus* was a stronger sink, their relative withdrawal from the host xylem flow was much higher and amounted to 63% of total N uptake by the host, 48% of total P, 62% of total K^+ and 30% of total Mg^{2+} uptake.

For N, P, K^+, parasitism by *Rhinanthus* led to an increase in the proportionate retranslocation from shoot to root, as seen for N (see item xix): in the case of P retranslocation increased to 52% of xylem transport (39% in the control), for K^+ it was 79% in parasitised *Hordeum* (59%, control) indicating a relative increase in root utilisation of minerals in response to the attachment of the root parasite.

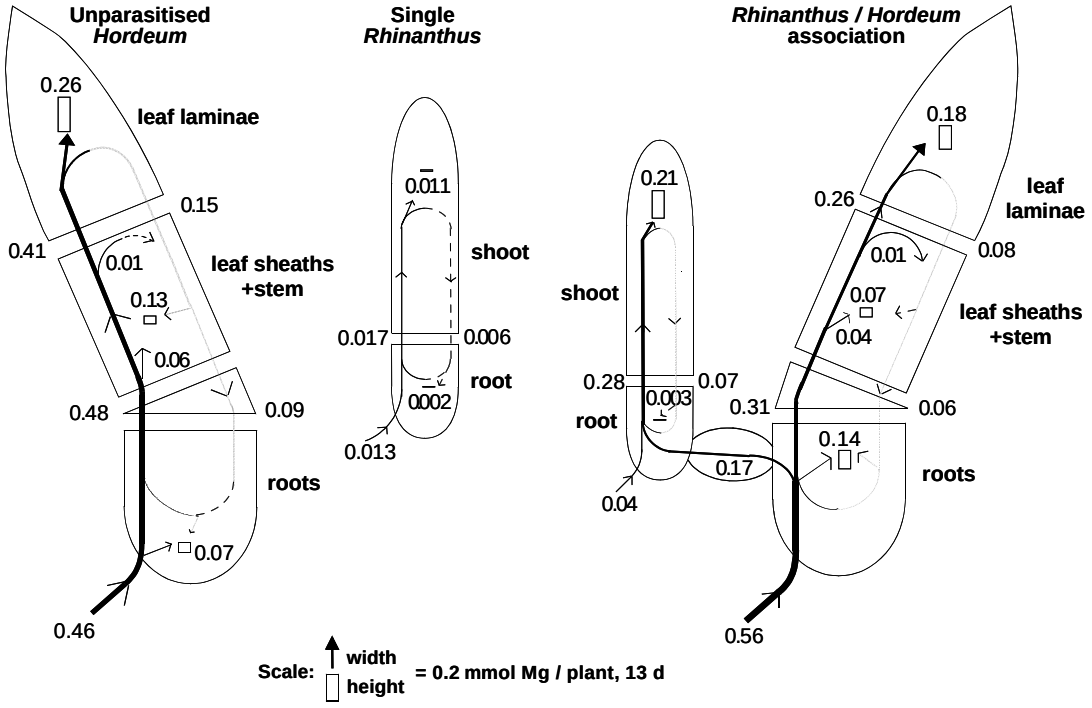


Fig. 3.28 Empirical models of the uptake of Mg^{2+} and the transport and utilisation of Mg in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13 days study period starting 41 days after sowing or about 30 days after attachment of the parasite. Plants were supplied with 1 mM NH_4^+. Numbers are presented in mmoles Mg per plant over 13 days. Further details as in Fig. 3.17.

3.4.2.5 Comparision of mineral relations in the parasitic association *Rhinanthus minor/Hordeum vulgare* supplied with 5 or 1 mM NO_3^-

The net flows and circulations of nutrient elements N, P, K^+ and Mg^{2+} have also been investigated in solitary *Hordeum* and *Rhinanthus* plants and within the *Rhinanthus*/*Hordeum* association under a lower level of nitrogen. Because there was no obvious difference of all nutrients deposition in barley between 1 mM NO_3^- and 1 mM NH_4^+ supply, the following comparision will be done only between 1 mM NO_3^- and 5 mM NO_3^- supply. Uptake and flows in xylem of nitrogen and potassium by both unparasitised and parasitised barley roots decreased dramatically after the plants were

supplied with 1 mM NO_3^-, whereas uptake and xylem flows of phosphorus and Mg^{2+} were reduced slightly. In the case of nitrogen, uptake and xylem flows in single barley decreased by 72%, 70%, in parasitised barley decreased 69%, 85% (Fig. 3.17, Fig. 3.21). For K^+, uptake and flows in xylem of single barley reduced by 67%, 40%, in parasitised barley they reduced by 56%, 46% (Fig. 3.19, Fig. 3.23). In contrast to the plants with 5 mM NO_3^- supply, significant reductions also appear in the depositions of nitrogen and K^+ in barley shoot and root when the plants were fed with a lower level of nitrogen. Nitrogen deposition in leaf laminae, leaf sheath and roots of single barley decreased 81%, 62%, 56% respectively; in parasitised barley, the reduction proportions were 98%, 82%, 44% in leaf laminae, leaf sheaths and roots respectively when plants were supplied with 1 mM NO_3^- (Tab. 3.3, Tab. 3.7). For K^+, in leaf laminae and leaf sheaths of unparasitised barley, deposition decreased 61% and 72% respectively; they were 68%, 93% in parasitised barley (Tab. 3.3, Tab. 3.7). In both uninfected and infected barley roots, K^+ increment was negative, because its high ability of mobilisation and low contribution to the roots growth. In the case of P, deposition decreased 42%, 25% in leaf sheaths and roots of single barley respectively; in parasitised barley leaf sheaths and roots, 53% and 50% respectively. In leaf laminae of both single and parasitised barley, it remained unchanged.

Compared with the retranslocation of elements in the plants supplied with 5 mM NO_3^-, the extent of scavenging xylem-borne nutrients by the parasite from the host amounted to substantially high percentages of nutrients taken up by host roots (59% for N; 45% for P; 60% for K^+) (Fig. 3.21, Fig. 3.22, Fig. 3.23). Under lower nitrogen supply, retranslocation of N, P, K^+ increased compared with those in the plants supplied with 5 mM NO_3^-. Total N transported from shoot to root in the plants supplied with 1 mM NO_3^- increased 39% (single barley) and 117% (parasitised barley) (Fig. 3.17 and Fig. 3.21), P (34%, single; 23% parasitised) (Fig. 3.18 and Fig. 3.22), K^+ (69%, single; 66%, parasitised) (Fig. 3.19 and Fig. 3.23).

The growth of attached *Rhinanthus* has not been reduced by the 1 mM NO_3^- or 1 mM NH_4^+ supply. Data show that the dry matter of *Rhinanthus* shoot even increased after supply with 1 mM NO_3^- (Tab. 3.4, Tab. 3.8, Tab. 3.10). The deposition of

mineral elements including N, P, K^+, Mg^{2+} was also higher compared with the plants supplied with 5 mM NO_3^-. These results show that *Rhinanthus* can grow very well and extract as much nutrients as they need from host, independent on external nutrient amounts.

Manipulation of N-supply had no effects on the uptake of nitrogen and phosphorus by the single *Rhinanthus* roots (Fig. 3.17, Fig. 3.18, Fig. 3.21, Fig. 3.22, Fig. 3.25, Fig. 3.26).

3.5 Contents and flows of assimilates (mannitol and sucrose) in the hemiparasitic *Rhinanthus minor/Hordeum vulgare* association

3.5.1 Contents and flows of mannitol and sucrose

Mannitol concentrations in shoots from single *Rhinanthus* supplied with 5 mM nitrate were 35 mmol kg^{-1}_{FW} and 14 mmol kg^{-1}_{FW} in roots. These concentrations increased substantially when plants were supplied with 1 mM nitrate or when the nitrate was replaced by 1 mM ammonium. Attachment of *Rhinanthus* to barley tended to decrease the mannitol concentrations in the organs; however, the differences were not statistically significant (Fig. 3.29).

Mannitol is the major soluble carbohydrate of *Rhinanthus* in both, shoot and roots, and ranged between 45 and 50%. This percentage increased to 55-75% under conditions of reduced nitrate or ammonium supply (Fig. 3.30).

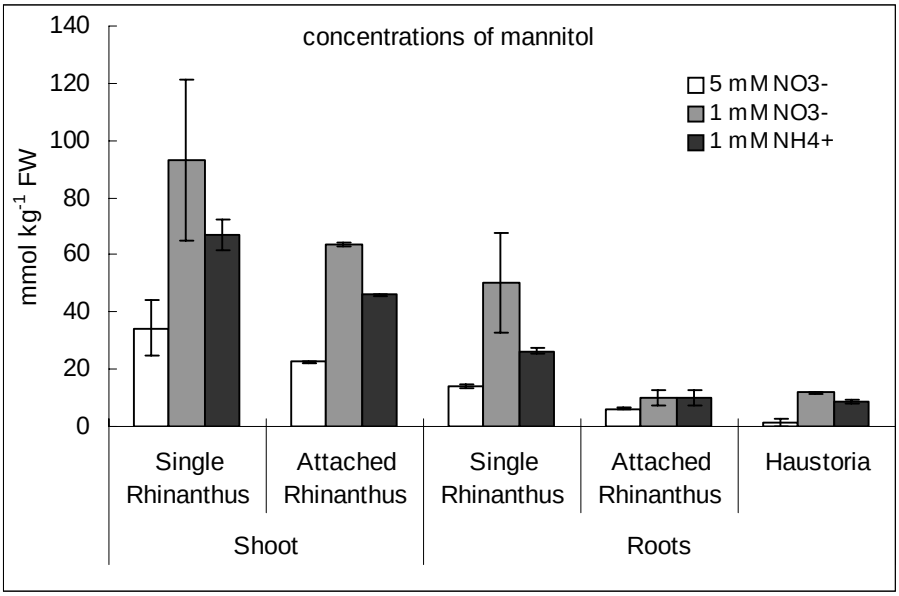


Fig. 3.29 Mannitol concentrations (mmol kg^{-1} FW) in shoot and root of single and attached *Rhinanthus* (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively (n = 5-15 ±SE).

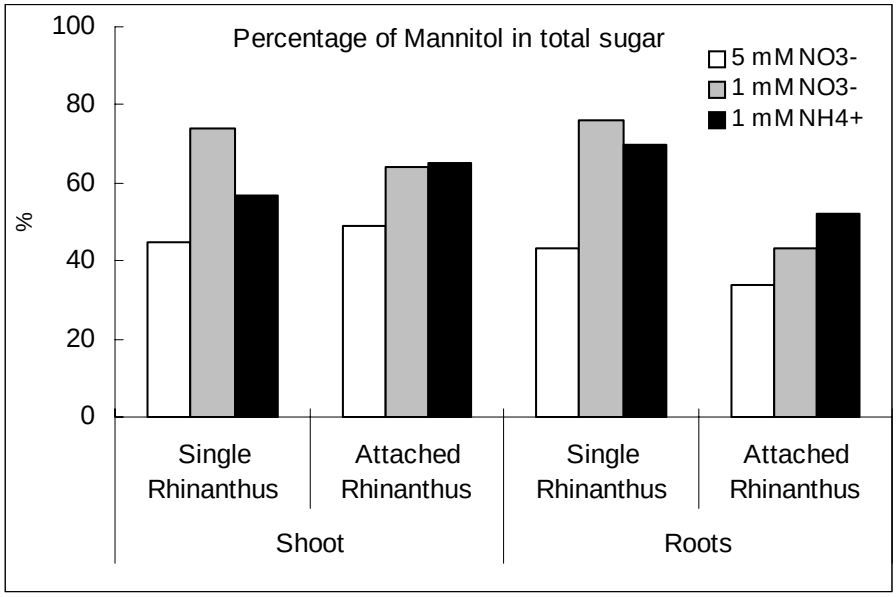


Fig. 3.30 Percentage of mannitol in total sugar in shoot and root of single and attached *Rhinanthus* (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively (n = 5-15 ± SE).

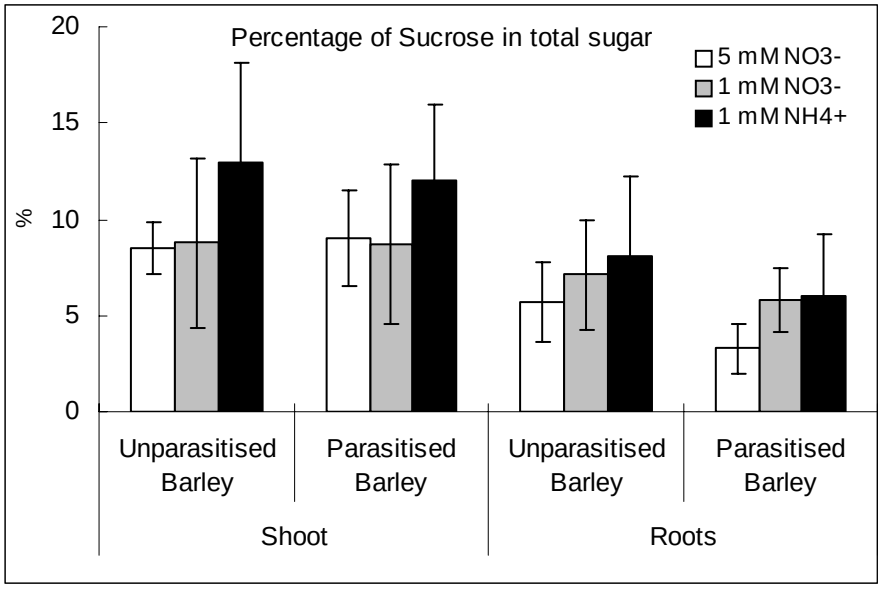


Fig. 3.31 Percentage of sucrose in total sugar in shoot and root of unparasitised and parasitised barley (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively (n = 5-15 ± SE).

Compared with mannitol, percentage of sucrose in total sugar was much lower. Under

the different nitrogen supplies, the percentage of sucrose of total sugars ranged from 3% to 13% (Fig. 3.31).

3.5.2 Mannitol in xylem sap and mannitol/K in phloem sap

The xylem sap concentration of mannitol was around 0.35 mM under all conditions applied, both in single and parasitising plants. The mannitol/potassium ratios of phloem exudates were also similar in all plants tested (approximately 0.3) except ammonium supplied single *Rhinanthus* plants where this ratio reached a value of 0.85 (Table 3.14).

Table 3.14 Mannitol in xylem sap (mM) and Mannitol/K in phloem sap of single and attached *Rhinanthus* (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively (n = 5 ±SE).

		5 mM NO_3^-	1 mM NO_3^-	1 mM NH_4^+
Xylem sap	Single *Rhinanthus*	0.32 ± 0.13	0.31 ± 0.13	0.24 ± 0.08
	Attached *Rhinanthus*	0.35 ± 0.07	0.38 ± 0.10	0.49 ± 0.23
Mannitol/K in phloem sap	Single *Rhinanthus*	0.36 ± 0.08	0.28 ± 0.02	0.85 ± 0.57
	Attached *Rhinanthus*	0.24 ± 0.12	0.31 ± 0.08	0.31 ± 0.21

3.5.3 Flows of mannitol and sucrose

5 mM NO_3^-

Table 3.15 and Table 3.16 show mannitol and sucrose contents in roots and shoots of *Rhinanthus* and *Hordeum* respectively under different nitrogen supplies. The data of two harvests of 41 and 54 d after planting, corresponding to about 30 to 43 days after parasite attachment, are given as well as the increments within the two harvests. These data have been used to estimate the flows of mannitol in *Rhinanthus* and of

sucrose in *Hordeum* according to the technique of Hibberd et al. (1999) and the assumptions described in Materials and Methods.

Table 3.15 Mannitol content (µmol) and its increment (µmol) in single or attached *Rhinanthus* and haustoria. Plants were supplied with 5 mM NO_3^-, 1 mM NO_3^- and 1mM NH_4^+ and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 5 ± SE).

plants supplied with 5 mM NO_3^-				
	Harvest	Shoot	Root	Haustoria
Unattached *Rhinanthus*	1	4.7 ± 0.6	0.9 ± 0.1	
	2	12 ± 0.1	2.2 ± 0.3	
	increment	7.3	1.3	
Attached *Rhinanthus*	1	26 ± 2	0.7 ± 0.1	0.018 ± 0.003
	2	100 ± 12	1.7 ± 0.2	0.079 ± 0.026
	increment	74	1	0.06

plants supplied with 1 mM NO_3^-				
	Harvest	Shoot	Root	Haustoria
Unattached *Rhinanthus*	1	7.7 ± 0.6	2.9 ± 0.4	
	2	29 ± 2.8	6.7 ± 0.9	
	increment	22	3.8	
Attached *Rhinanthus*	1	39 ± 16	1.0 ± 0.2	0.185 ± 0.037
	2	337 ± 26	6.0 ± 1.7	0.768 ± 0.155
	increment	298	5	0.58

plants supplied with 1 mM NH_4^+				
	Harvest	Shoot	Root	Haustoria
Unattached *Rhinanthus*	1	6.6 ± 0.7	2.2 ± 0.2	
	2	20 ± 1.7	3.5 ± 0.5	
	increment	13	1.3	
Attached *Rhinanthus*	1	44 ± 12	2.1 ± 0.9	0.148 ± 0.045
	2	256 ± 53	5.6 ± 1.5	0.768 ± 0.155
	increment	212	3.5	0.62

Table 3.16 Sucrose content (µmol) and its increment (µmol) in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor*). Plants were supplied with 5 mM NO_3^-, 1 mM NO_3^- and 1 mM NH_4^+ and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 5 ± SE).

plants supplied with 5 mM NO_3^-			
	Harvest	Shoot	Roots
Unparasitised Barley	1	136 ± 51	16 ± 5
	2	320 ± 60	43 ± 17
	increment	184	27
Parasitised Barley	1	101 ± 62	3 ± 0.3
	2	161 ± 35	23 ± 11
	increment	60	20

plants supplied with 1 mM NO_3^-			
	Harvest	Shoot	Roots
Unparasitised Barley	1	106 ± 15	6 ± 0.6
	2	228 ± 94	55 ± 26
	increment	122	49
Parasitised Barley	1	128 ± 18	6 ± 2
	2	208 ± 47	36 ± 12
	increment	80	30

plants supplied with 1 mM NH_4^+			
	Harvest	Shoot	Roots
Unparasitised Barley	1	191 ± 29	6 ± 0.6
	2	318 ± 101	52 ± 20
	increment	127	46
Parasitised Barley	1	117 ± 24	8 ± 2
	2	202 ± 73	51 ± 24
	increment	85	43

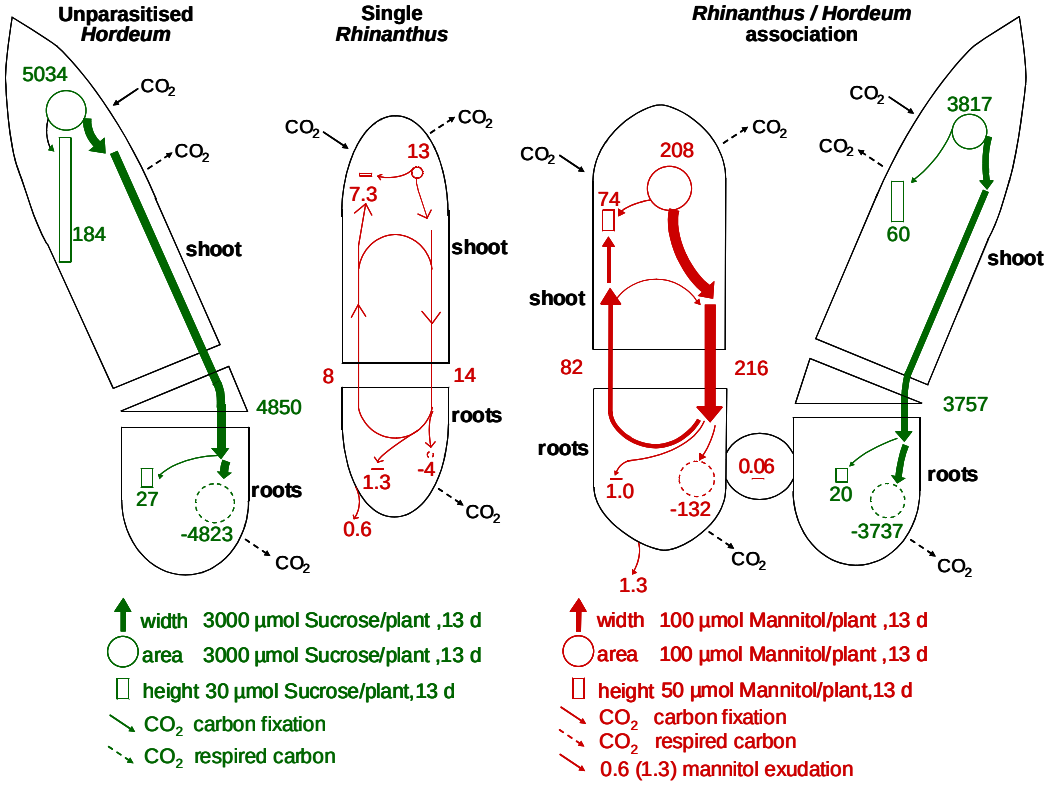


Fig. 3.32 Flow profiles for metabolism, transport and deposition of sucrose (in green colour) in whole plants of unparasitised and parasitised *Hordeum vulgare*, of mannitol (in red colour) in whole plants of solitary *Rhinanthus minor* and attached *Rhinanthus* supplied with 5 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Numbers are presented in µmoles per plant over 13 days. The width of arrows and the height of histograms (deposition of sucrose or mannitol in each organ) are drawn in proportion to the rates of flows or to the magnitude of deposition. The triangle between root and leaf sheaths symbolises the stem.

In response to a successful attachment to the host, dramatic changes in mannitol flows on a per plant basis, and in metabolism and deposition have been observed in *Rhinanthus*. Biosynthesis in the shoot was 16-fold higher after attachment resulting in a 15-fold higher mannitol flow in the phloem and in a 10-fold higher deposition (Fig. 3.32). A large portion of this mannitol was metabolised or respired in the root (nearly 33 fold increase in mannitol metabolism compared to single *Rhinanthus*). Xylem flows of mannitol were 10-fold increased after attachment. A substantial portion of mannitol translocated from shoot to root in the phloem was recirculated back to the

shoot via xylem. Using the technique of Neumann & Römheld (1999) an exudation of mannitol from *Rhinanthus* roots to the rhizosphere has been detected (0.02 µmol $g^{-1}h^{-1}$). By comparison, the major assimilate of barley, sucrose, was not released from the roots. Mannitol was also deposited in the haustoria of the *Rhinanthus*/barley association, but no mannitol could be found in barley roots even in the direct vicinity of the haustoria (Fig. 3.34).

In parasitised compared to unparasitised barley the deposition of sucrose in shoot was reduced by 67% and net sucrose synthesis that was decreased by 24%. As a result also the phloem transport of sucrose was reduced by 23%. No sucrose could be detected in barley xylem sap and there also was no indication of a sucrose transfer from the host to the parasite.

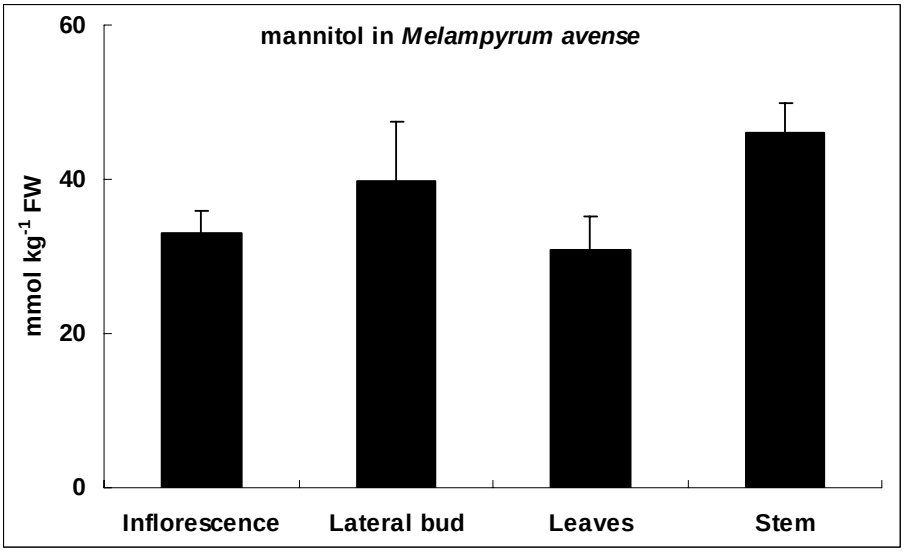


Fig 3.33 Mannitol concentrations (mmol kg^{-1} FW) in *Melampyrum avense* harvested from the field in the middle of June (n=4 ± SE).

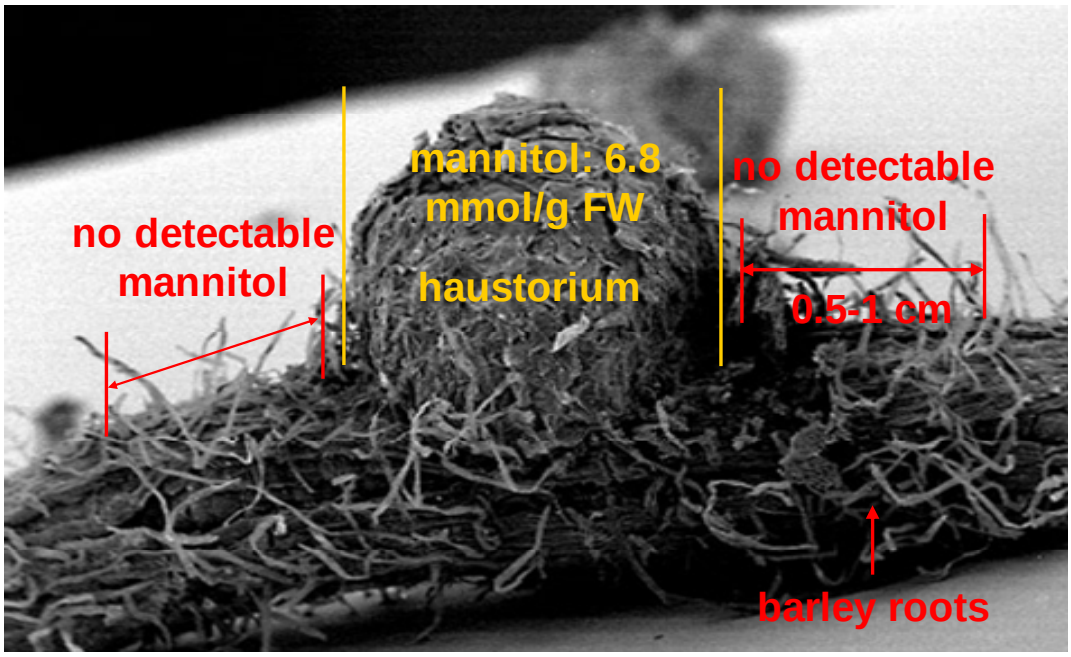


Fig. 3.34 The distribution of mannitol between a haustorium and the adjacent barley root tissues.

1 mM NO_3^-

Compared with the plants supplied with 5 mM NO_3^-, deposition of mannitol in shoot and root of single *Rhinanthus* increased 3 times; in attached *Rhinanthus* deposition of shoot increased 4-fold and 5-fold in roots after the plants were supplied with 1 mM NO_3^-. Also the net synthesis of mannitol increased 2 times in shoot of single and attached *Rhinanthus*. As it has been decribed above for Fig. 3.32, in response to a successful attachment to the host, dramatic changes in mannitol flows on a per plant basis, and in metabolism and deposition have been observed in *Rhinanthus*. Biosynthesis in the shoot was 13-fold higher after attachment resulting in a 15-fold higher mannitol flow in the phloem and in a 10-fold higher deposition (Fig. 3.35). A large portion of this mannitol was metabolised or respired in the root (nearly 23 fold increase in mannitol metabolism compared to single *Rhinanthus*). Xylem flows of mannitol were 18-fold increased after attachment. A substantial portion of mannitol translocated from shoot to root in the phloem was recirculated back to the shoot via

xylem. Mannitol was also deposited in the haustoria of the *Rhinanthus*/barley association, but no mannitol could be found in barley roots even in the direct vicinity of the haustoria (Fig. 3.34).

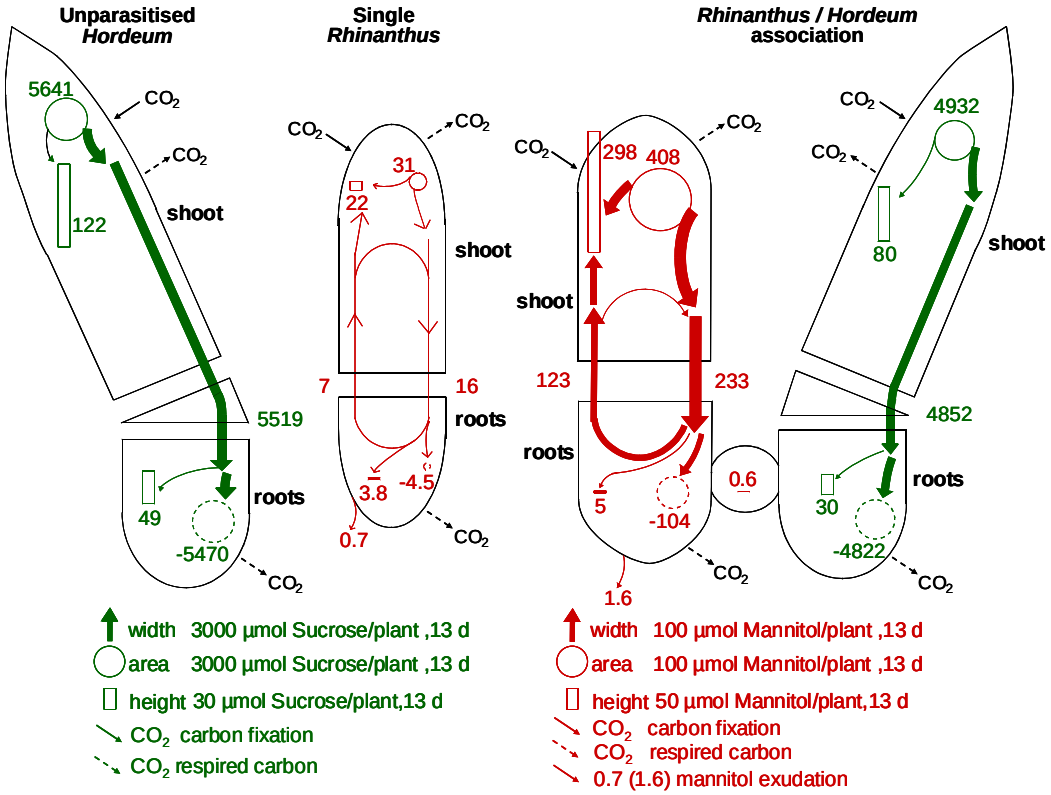


Fig. 3.35 Flow profiles for metabolism, transport and deposition of sucrose (in green colour) in whole plants of unparasitised and parasitised *Hordeum vulgare*, of mannitol (in red colour) in whole plants of solitary *Rhinanthus minor* and attached *Rhinanthus* supplied with 1 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details as in Fig. 3.32.

In parasitised compared to unparasitised barley the deposition of sucrose in shoot was reduced by 34% and net sucrose synthesis that was decreased by 13%. As a result also the phloem transport of sucrose was reduced by 12%. No sucrose could be detected in barley xylem sap and there also was no indication of a sucrose transfer from the host to the parasite.

Similar changes of mannitol in *Rhinanthus* and sucrose in barley before and after attachment have been found in the plants supplied with 1 mM NH_4^+ instead of nitrate, for details see Fig. 3.36.

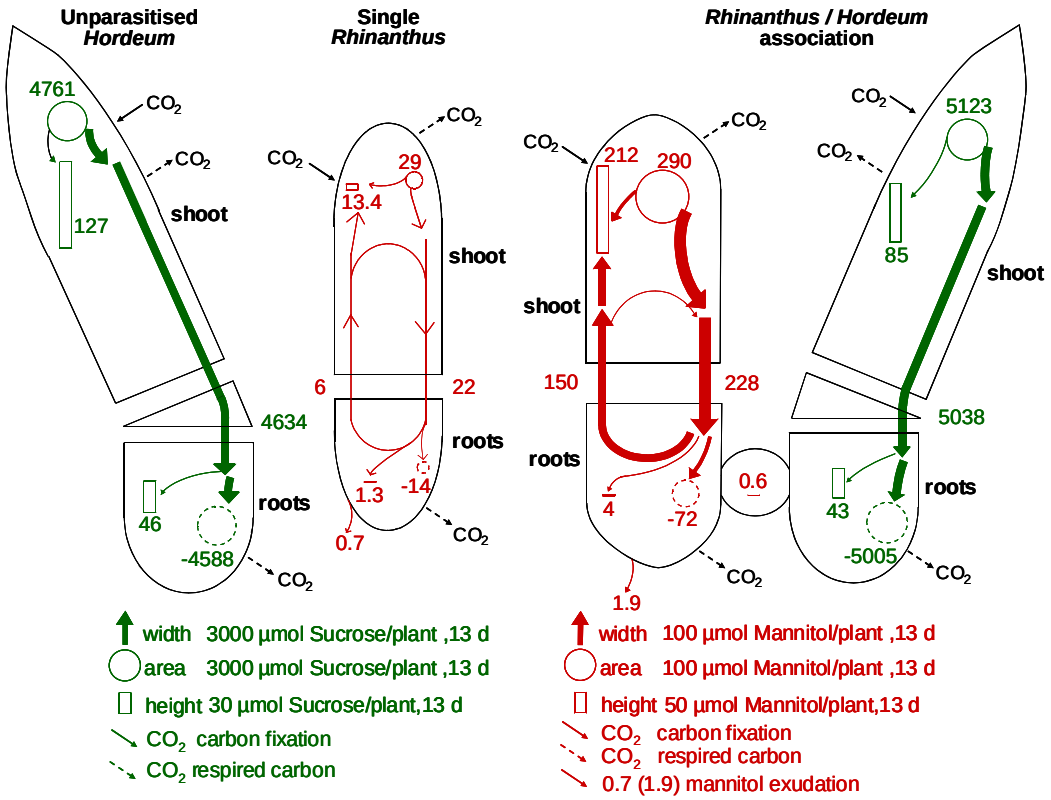


Fig. 3.36 Flow profiles for metabolism, transport and deposition of sucrose (in green colour) in whole plants of unparasitised and parasitised *Hordeum vulgare*, of mannitol (in red colour) in whole plants of solitary *Rhinanthus minor* and attached *Rhinanthus* supplied with 1 mM NH_4^+ over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details as in Fig. 3.32.

3.6 Abscisic acid (ABA) flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite abscisic acid relations

3.6.1 Abscisic acid content in single and attached barley and *Rhinanthus minor*

For unparasitised barley plants supplied with 5 mM NO_3^- solution, the highest concentrations of ABA have been observed in the leaf laminae, those in leaf sheaths being slightly lower. No significant differences between single and parasitised barley were observed (Fig. 3.37a, b). Compared with unparasitised barley, the ABA concentrations in single, non-parasitising *Rhinanthus minor* were higher by a factor of 5.7 in roots and 12.7 than in barley leaf laminae (Fig. 3.37a, Fig. 3.38). An obvious increase of ABA was seen in shoots of parasitised *Rhinanthus* supplied with 5 or 1 mM NO_3^- after attachment to barley (Fig. 3.38). The only clear significant effects of N supply can be seen in the ABA concentrations of leaves (Fig. 3.37a,b): both, reduced NO_3^- and replacement of lower NO_3^- by NH_4^+, caused two-fold increases in ABA in parasitised barley leaf laminae. Small but insignificant differences were seen in leaf sheaths and roots (Fig. 3.37a,b). Lower levels of nitrogen supply doubled the ABA concentrations in attached *Rhinanthus* leaves (Fig. 3.39). When plants were supplied with 5 mM NO_3^-, higher ABA concentrations were found in inflorescence and later bud.

3.6.2 ABA in the xylem sap

When plants were supplied with 5 mM NO_3^-, xylem sap ABA concentrations of attached *Rhinanthus* were up to 10.5-fold higher than those of parasitised barley. In barley, the xylem sap ABA was nearly doubled after attachment (Table 3.17). Compared to 5 mM NO_3^-, low NO_3^- or 1 mM NH_4^+ ABA concentrations in barley were slightly increased and nearly halved in attached *Rhinanthus* (Fig. 3.40a,b). Independent of different N supplies there was no difference between ABA

concentrations in xylem sap in single and attached *Rhinanthus*.

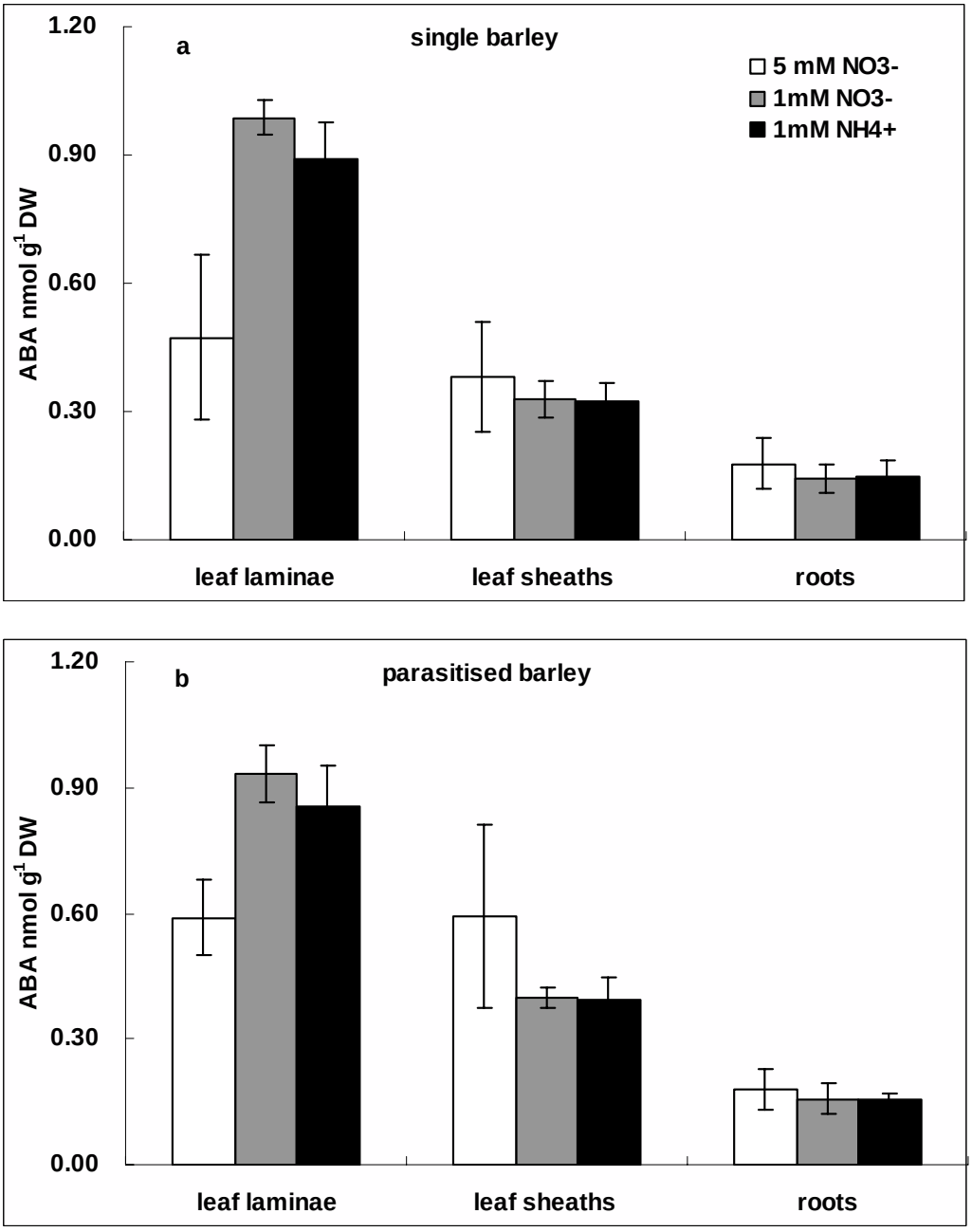


Fig. 3.37 ABA concentrations (nmol·g^{-1}DW) in leaf laminae, leaf sheaths and roots of single *Hordeum vulgare* (a) (54 days after planting) and of parasitised *Hordeum vulgare* (b) (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively (n = 5 ± SE).

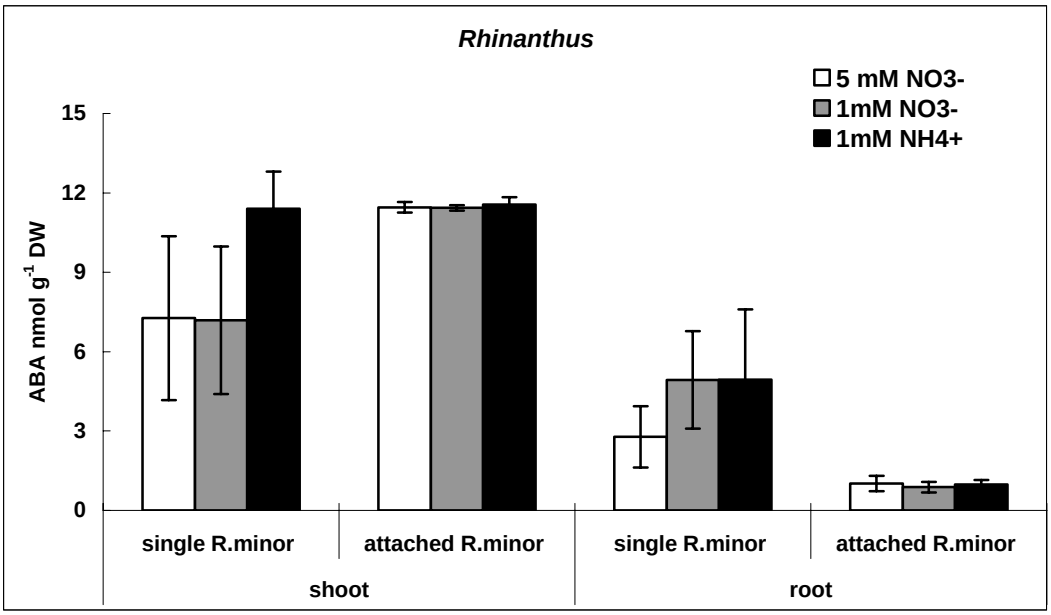


Fig. 3.38 ABA concentrations (nmol·g^{-1}DW) in shoot and roots of single *Rhinanthus* and attached *Rhinanthus* (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively **(**n = 5-12 ± SE).

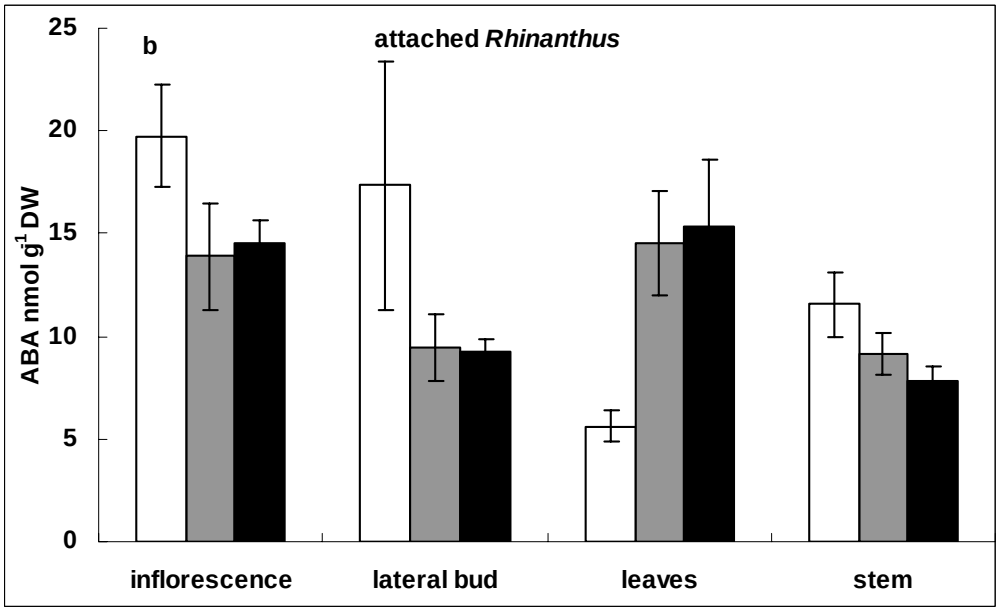


Fig. 3. 39 ABA concentrations (nmol·g^{-1}DW) in each part of attached *Rhinanthus* shoot (54 days after planting, about 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively **(**n =4-5 ± SE).

3.6.3 ABA flows

5 mM NO_3^- supply

The ABA flows have been calculated from the data shown in Table 3.17 and table 3.18. For barley, the xylem flows of ABA, on a per plant basis, were somewhat (13%) increased after infection by *Rhinanthus* (Fig. 3.41); the estimated ABA synthesis in the root was increased (61%) or slightly increased (17%) in leaf sheaths, while the resulting ABA degradation in leaf laminae was somewhat decreased (-10%). Deposition was significantly affected in the leaf laminae (3 fold) and in leaf sheaths (2.4 fold), but not in roots (Fig. 3.41).

In response to attachment to the host, dramatic changes in ABA flows on a per plant basis, and in metabolism and deposition, however, have been observed in *Rhinanthus*. Biosynthesis in the roots was 12-fold higher after attachment resulting in a 14-fold higher ABA flow in the xylem. A large portion of this ABA was metabolised in the shoot (nearly 12.5 fold increase in ABA degradation compared to single *Rhinanthus*), and a fraction of this was deposited (even this fraction was 17.9 times larger than in the unattached controls). Phloem flows of ABA were increased 13 fold after attachment. A significant deposition of ABA also was detected in the haustoria of the *Rhinanthus*/barley association, which was slightly higher than in the root systems of single and parasitising *Rhinanthus* (Fig. 3.41).

Table 3.17 The ratio of ABA to K in phloem exudates obtained with the EDTA-technique and ABA concentrations (nM) in the xylem sap of *Hordeum vulgare* (saps were collected from control or parasitised barley between the beginning and the end of the study period 41 to 54 days after planting, about from 30 to 43 days after attachment), of attached *Rhinanthus* (from 41 to 54 days after planting, from 30 to 43 days after attachment) and of single *Rhinanthus* (from 41 to 54 days after planting). Plants were supplied with 5 mM NO_3^- (n = 4-8 ± SE).

	ABA(nM) / K(mM) in phloem sap	ABA concentrations in xylem sap (nM)
unparasitised barley	0.7 ± 0.1	4.9 ± 0.4
parasitised barley	0.7 ± 0.2	8.7 ± 0.9
single *Rhinanthus*	10 ± 1.9	62 ± 12
attached *Rhinanthus*	5.9 ± 0.7	91 ± 21

Table 3.18 ABA content (nmol) and its increment (nmol) in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor*), in single or attached *Rhinanthus* and haustoria. Plants were supplied with 5 mM NO_3^- and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 5 ± SE).

	harvest	leaf laminae	leaf sheaths	roots
unparasitised Barley	1	0.50 ± 0.04	1.18 ± 0.2	0.18 ± 0.02
	2	1.00 ± 0.2	1.32 ± 0.43	0.32 ± 0.09
	increment	0.50	0.14	0.14
parasitised Barley	1	0.17 ± 0.02	1.05 ± 0.13	0.34 ± 0.09
	2	1.69 ± 0.31	1.38 ± 0.27	0.46 ± 0.06
	increment	1.52	0.33	0.12

	harvest	shoot	root
unattached *Rhinanthus*	1	0.18 ± 0.02	0.02 ± 0.002
	2	0.47 ± 0.06	0.04 ± 0.006
	increment	0.29	0.02
attached *Rhinanthus*	1	2.26 ± 0.26	0.012 ± 0.002
	2	7.46 ± 0.91	0.028 ± 0.008
	increment	5.2	0.016

	harvest	haustoria
	1	0.008 ± 0.002
	2	0.04 ± 0.01
	increment	0.032

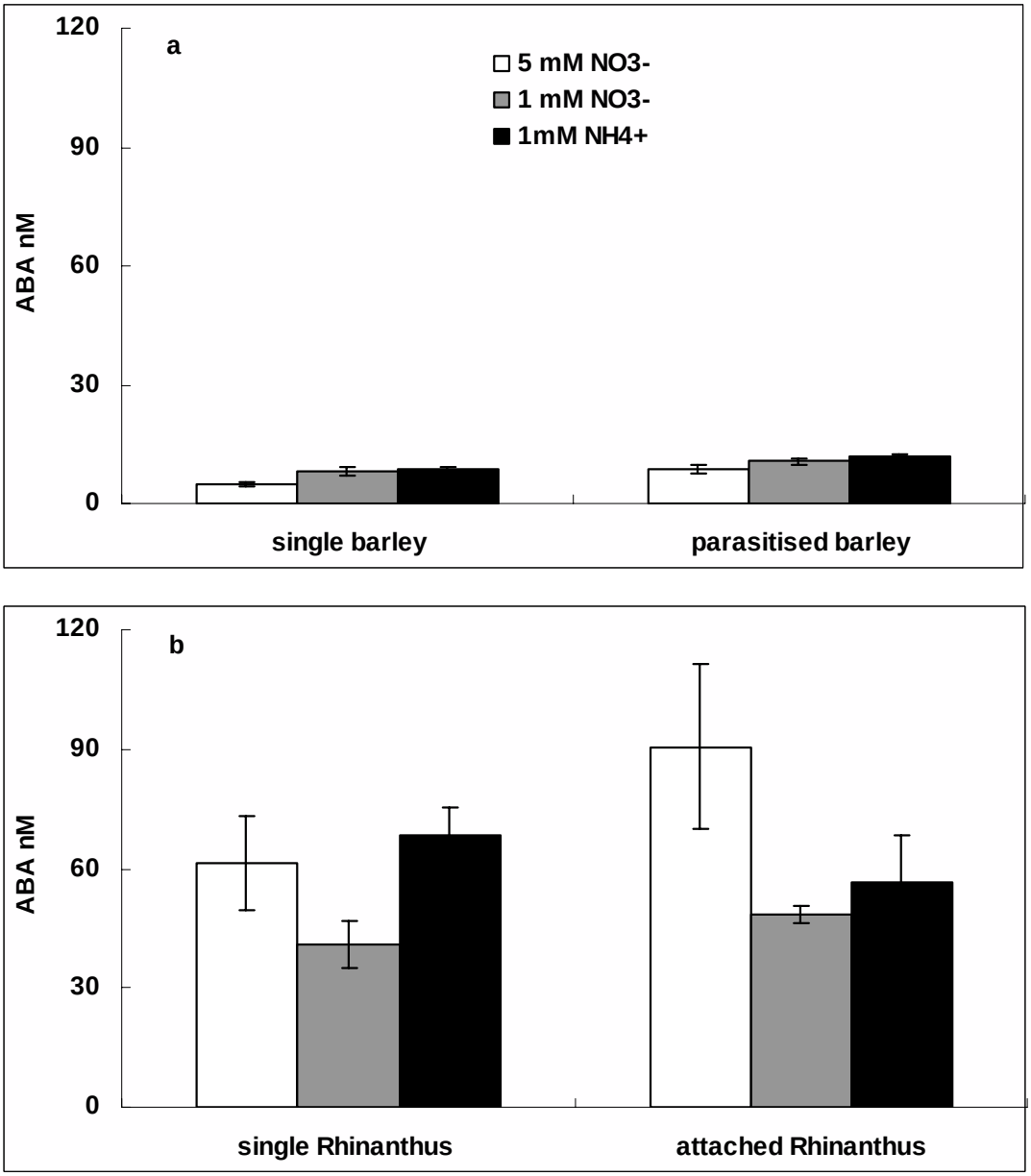


Fig. 3.40 ABA concentrations (nM) in the xylem sap of *Hordeum vulgare* (a) (saps were collected from unparasitised or parasitised *Hordeum vulgare* between the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment) and of *Rhinanthus minor* (b) (xylem sap was harvested from single *Rhinanthus* from 41 to 54 days after planting and from attached *Rhinanthus*, correspondingly from about 30 to 43 days after attachment). Plants were supplied with 5 mM NO_3^-, 1mM NO_3^- and 1 mM NH_4^+ respectively (n = 5-8 ± SE).

1 mM NO_3^- supply

For barley (Tab. 3.19 and Tab. 3.20), the xylem flows of ABA, on a per plant basis,

were somewhat (20%) decreased after infection by *Rhinanthus* (Fig. 3.42); the estimated ABA synthesis appeared in the root of parasitised barley and ABA degradation appeared in the root of single barley. ABA deposition in the leaf laminae and in leaf sheaths decreased 41% and 53% respectively (Fig. 3.42) after barley was infected by *Rhinanthus*.

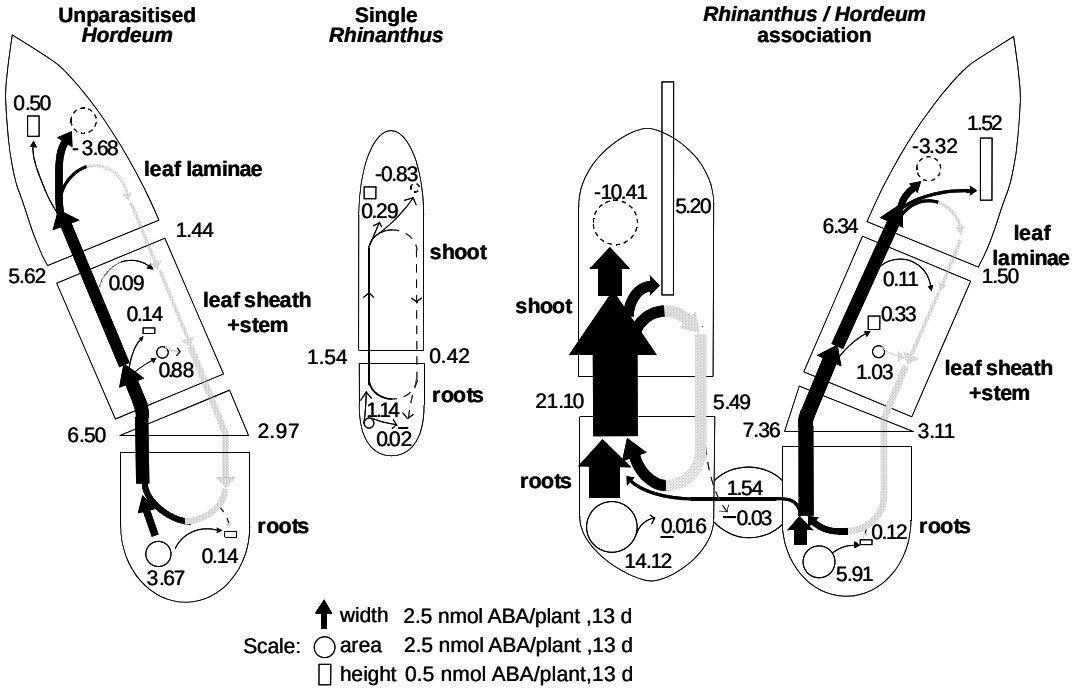


Fig. 3.41 Flow profiles for metabolism, transport and deposition of ABA in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 5 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Numbers are presented in ABA nmoles per plant over 13 days. The width of arrows (net flows in xylem (black) or phloem (dotted)) and the height of histograms (deposition of ABA in each organ) are drawn in proportion to the rates of flows or to the magnitude of deposition. The triangle between root and leaf sheaths symbolises the stem.

After attachment to the host, dramatic changes in ABA flows on a per plant basis, and in metabolism and deposition, however, have been observed in *Rhinanthus*. Biosynthesis in the roots was 12-fold higher after attachment resulting in a 16-fold higher ABA flow in the xylem. A large portion of this ABA was metabolised in the shoot (nearly 14 fold increase in ABA degradation compared to single *Rhinanthus*),

and a fraction of this was deposited (even this fraction was 16.3 times larger than in the unattached controls). Phloem flows of ABA were increased 34 fold after attachment. A significant deposition of ABA also was detected in the haustoria of the *Rhinanthus*/barley association (Fig. 3.42).

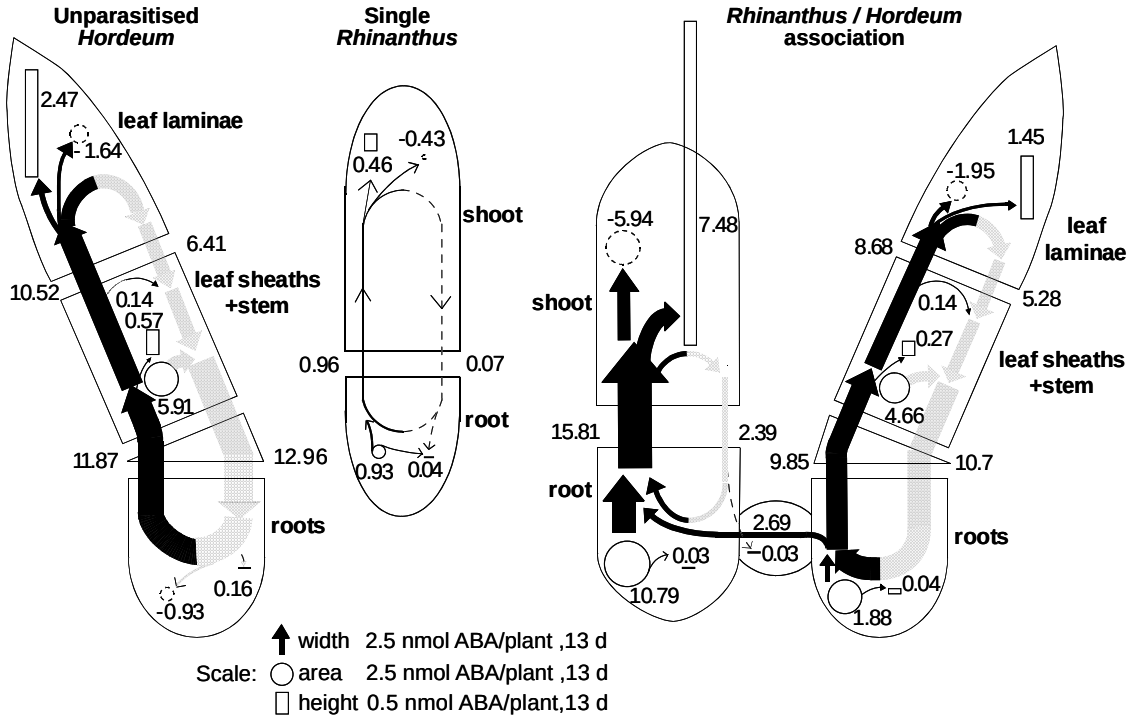


Fig. 3.42 Flow profiles for metabolism, transport and deposition of ABA in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 1 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details see Fig. 3.41.

1 mM NH_4^+ supply

When the plants were supplied with 1 mM NH_4^+ solution, barley plants have shown the symptom of N deficiency, for reasons as described earlier. For barley, the xylem flows of ABA, on a per plant basis, were somewhat (14%) decreased after infection by *Rhinanthus* (Fig. 3.43); the ABA synthesis in the root was increased 38%, similar as in leaf sheaths. ABA deposition in the leaf laminae and in leaf sheaths decreased

46% and 44% respectively (Fig. 3.43) after infection.

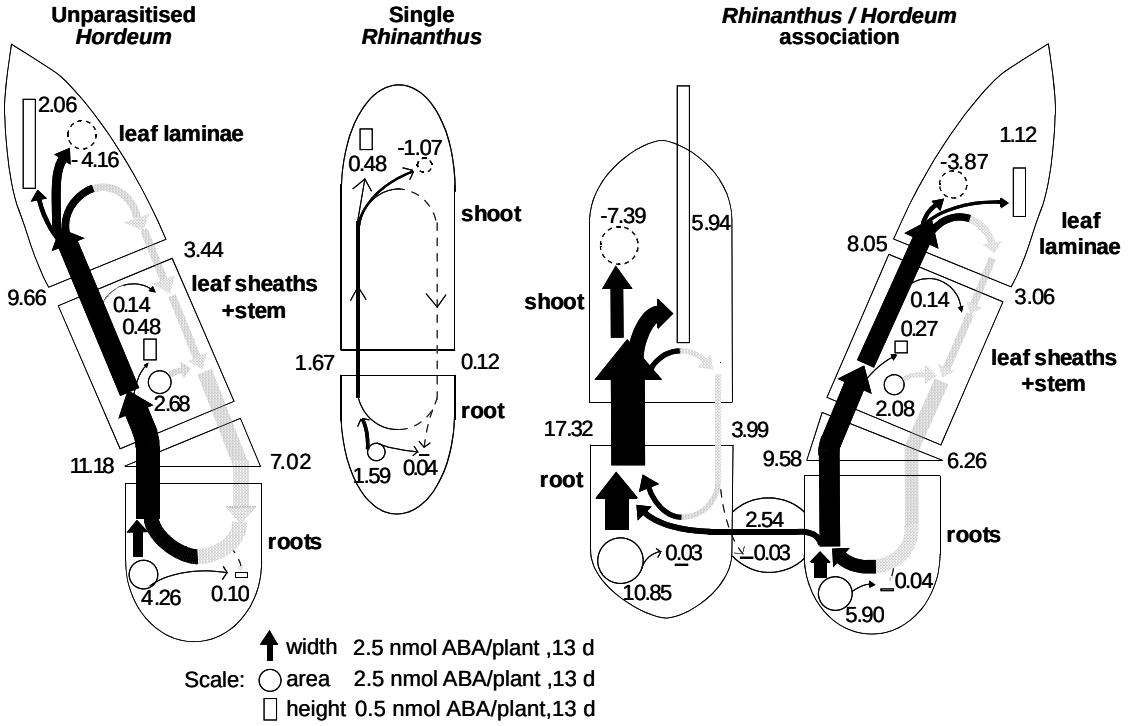


Fig. 3.43 Flow profiles for metabolism, transport and deposition of ABA in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 1 mM NH_4^+over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details see Fig. 3.41.

In response to attachment to the host, dramatic changes in ABA flows on a per plant basis, and in metabolism and deposition, however, have been observed in *Rhinanthus*. Biosynthesis in the roots was 7-fold higher after attachment resulting in a 10-fold higher ABA flow in the xylem. A large portion of this ABA was metabolised in the shoot (nearly 7 fold increase in ABA degradation compared to single *Rhinanthus*), and a fraction of this was deposited (even this fraction was 12 times larger than in the unattached controls). Phloem flows of ABA were increased 33 fold after attachment. A significant deposition of ABA also was detected in the haustoria of the *Rhinanthus*/barley association (Fig. 3.43).

3.6.4 Comparision of ABA in *Rhinanthus/Hordeum* associations cultivated with 1 or 5 mM NO_3^-

When the plants were supplied with 1 mM NO_3^- or when this nitrate was replaced by 1 mM NH_4^+ (as compared to 5 mM NO_3^-) only the ABA relations of barley were affected. Deposition of ABA in barley leaf sheaths and laminae were increased by lower NO_3^- supply, and to a weaker extent also in 1 mM NH_4^+ - plants. ABA concentrations and flows in parasitising *Rhinanthus*, however, were hardly affected by altered N supply. Independent of variations in ABA accumulation of the host plants, in response to altered N-nutrition, the parasitising *Rhinanthus* maintained its ABA relations at comparatively unaltered levels (Fig. 3.41, Fig. 3.42, Fig. 3.43).

3.6.5 Effects of Tetcyclacis

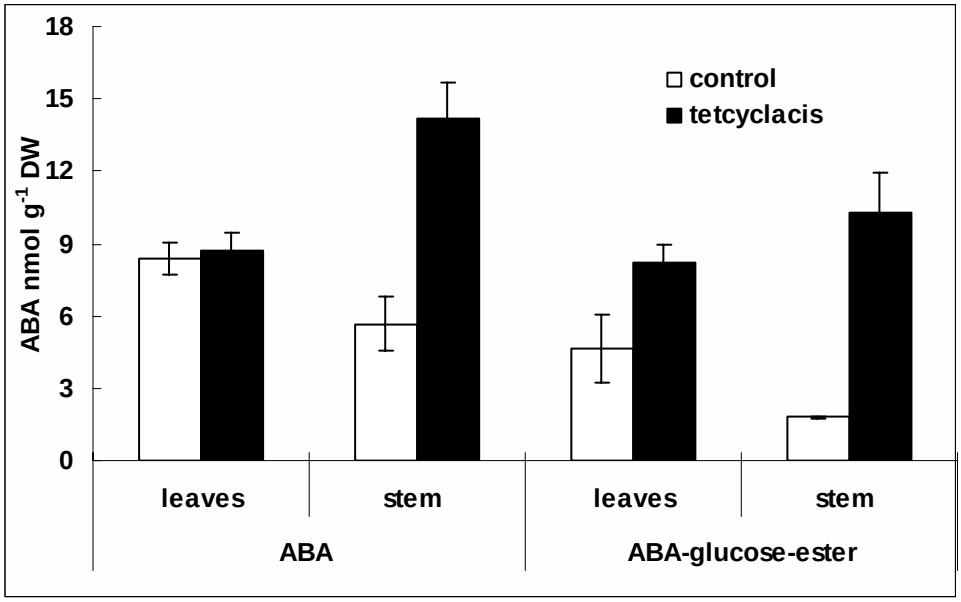


Fig. 3.44 The concentrations of ABA and ABA-glucose-ester in leaves and stems of attached *Rhinanthus* growing in the field (control plants and plants painted with 10^{-5} M tetcyclacis were harvested in June, n = 4-5 ± SE).

When leaves and stem of parasitising *Rhinanthus* were treated with 10^{-5} M tetcyclacis for 4 days the concentrations of ABA-glucose ester were significantly increased in leaves (77%) and the stem (471%). Free ABA in stems rose by 249% and remained

unchanged in leaves (Fig. 3.44).

3.6.6 Effects of water deficiency on ABA concentrations in attached *Rhinanthus* and host barley

To apply water deficiency, nutrient medium has been removed from the pots under reduced pressure and plants have not been irrigated for the two following days. The water content of the sand was determinated by Time-Domain-Reflectrometry (TDR). as shown in Fig 3.45 water deficiency caused clear 8-fold increased in the ABA of shoot and root of host barley, however, only 2-3 fold increased in leaves and stems of attached *Rhinanthus*.

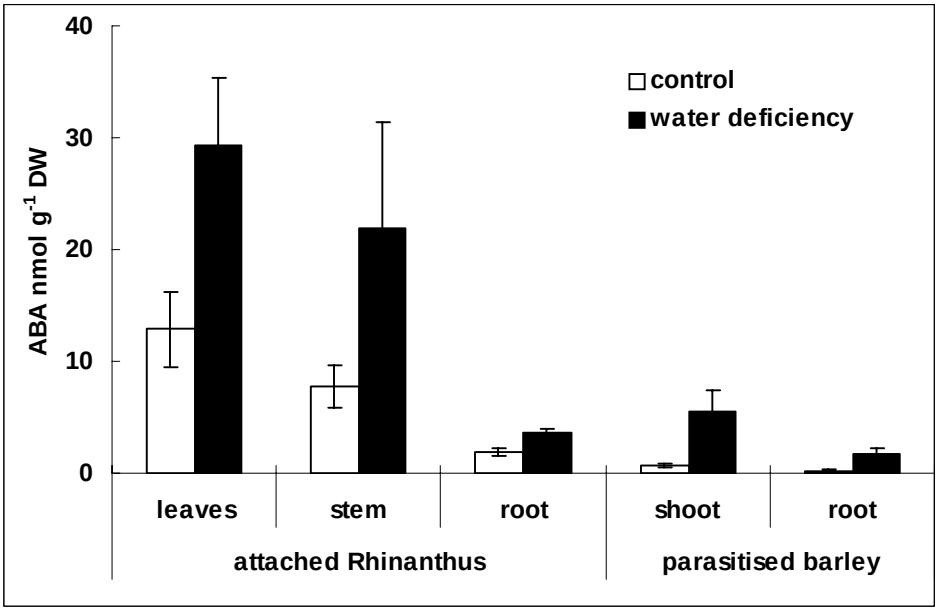


Fig. 3.45 Effects of water deficiency on ABA concentrations (nmol g^{-1} DW) in attached *Rhinanthus* and host barley (55 days old). Plants were supplied with 5 mM NO_3^- (n=4-10±SE).

Table 3.19 ABA content (nmol) and its increment (nmol) in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor*), in single or attached *Rhinanthus* and haustoria. Plants were supplied with 1 mM NO_3^- and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 5 ± SE).

	Harvest	Leaf laminae	Leaf sheaths	Roots
Unparasitised Barley	1	0.95 ± 0.09	0.36 ± 0.04	0.11 ± 0.01
	2	3.42 ± 0.31	0.92 ± 0.10	0.27 ± 0.05
	increment	2.47	0.57	0.16
Parasitised Barley	1	0.75 ± 0.06	0.34 ± 0.05	0.22 ± 0.03
	2	2.20 ± 0.33	0.61 ± 0.10	0.26 ± 0.06
	increment	1.45	0.27	0.04

	Harvest	Shoot	Root
Unattached *Rhinanthus*	1	0.30 ± 0.02	0.04 ± 0.004
	2	0.76 ± 0.07	0.08 ± 0.008
	increment	0.46	0.04
Attached *Rhinanthus*	1	3.45 ± 0.61	0.01 ± 0.002
	2	10.9 ± 0.75	0.04 ± 0.01
	increment	7.45	0.03

	Harvest	Haustoria
	1	0.008 ± 0.002
	2	0.033 ± 0.006
	increment	0.025

Table 3.20 The ratio of ABA to K in phloem exudates obtained with the EDTA-technique and ABA concentrations (nM) in the xylem sap of *Hordeum vulgare*. Further details see table 3.17. Plants were supplied with 1 mM NO_3^- (n = 4-8 ± SE).

	ABA(nM) / K(mM) in phloem sap	ABA concentrations in xylem sap (nM)
Unparasitised barley	2.98 ± 0.48	7.98 ± 0.97
Parasitised barley	2.84 ± 0.63	10.57 ± 0.59
Single *Rhinanthus*	3.42 ± 0.42	40.83 ± 6.08
Attached *Rhinanthus*	6.45 ± 2.87	48.36 ± 2.35

Table 3.21 ABA content (nmol) and its increment (nmol) in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor*), in single or attached *Rhinanthus* and haustoria. Plants were supplied with 1 mM NH_4^+ and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 5 ± SE).

	Harvest	Leaf laminae	Leaf sheaths	Roots
Unparasitised Barley	1	1.08 ± 0.10	0.36 ± 0.05	0.13 ± 0.008
	2	3.14 ± 0.22	0.84 ± 0.09	0.23 ± 0.07
	increment	2.06	0.48	0.1
Parasitised Barley	1	0.86 ± 0.08	0.37 ± 0.05	0.21 ± 0.04
	2	1.98 ± 0.20	0.63 ± 0.07	0.25 ± 0.04
	increment	1.12	0.27	0.04

	Harvest	Shoot	Root
Unattached *Rhinanthus*	1	0.24 ± 0.02	0.02 ± 0.002
	2	0.72 ± 0.06	0.06 ± 0.006
	increment	0.48	0.04
Attached *Rhinanthus*	1	3.17 ± 0.87	0.01 ± 0.004
	2	9.10 ± 1.65	0.04 ± 0.01
	increment	5.93	0.03

	Harvest	Haustoria
	1	0.007 ± 0.002
	2	0.04 ± 0.007
	increment	0.03

Table 3.22 The ratio of ABA to K in phloem exudates obtained with the EDTA-technique and ABA concentrations (nM) in the xylem sap of *Hordeum vulgare*. Further details see table 3.17. Plants were supplied with 1 mM NH_4^+ (n = 4-8 ± SE).

	ABA(nM) / K(mM) in phloem sap	ABA concentrations in xylem sap (nM)
Unparasitised barley	2.15 ± 0.26	8.67 ± 0.60
Parasitised barley	2.35 ± 0.37	11.94 ± 0.55
Single *Rhinanthus*	4.48 ± 1.34	68.21 ± 7.03
Attached *Rhinanthus*	5.54 ± 2.41	56.6 ± 11.8

3.7 Cytokinins flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite cytokinins relations

3.7.1 Cytokinins in roots of *Rhinanthus* and of different potential hosts

Table 3.23 Cytokinins (pmol g^{-1} FW) in the seedling roots of *Rhinanthus*, barley, wheat and maize (n=4±SE).

	Rhinanthus	Barley	Wheat	Maize
Zeatin (Z)	32±11	110±6	166±7	185±6
Zeatin riboside (ZR)	38±11	72±6	86±11	62±7
Dihydrozeatin (DHZ)	29±4	24±2	59±3	37±5
Isopentenyladenine (IP)	32±3	23±1	34±5	24±3
Isopentenyl adenosine (IPA)	5±1	6±3	5±2	5±1

Five cytokinin derivatives (For chemical structures, see appendix) in seedling roots of *Rhinanthus*, barley, wheat and maize have been analysed with ELSA. Z and ZR as the active forms in the seedling root of *Rhinanthus* were much lower than those in the other crops seedling roots. Compared with those in barley, wheat and maize seedling roots, Z concentrations were 71-83% lower in *Rhinanthus* seedling roots, and ZR concentration was halved. DHZ concentration in wheat seedling root was twice of that in *Rhinanthus* root. No significant difference has been observed in IP and IPA between *Rhinanthus* and the other three crops (Tab. 3.23).

3.7.2 Cytokinins content in single and attached barley and *Rhinanthus minor*

ZR concentrations in shoot and root of *Rhinanthus* were doubled after attachment. Compared with single *Rhinanthus*, the Z concentration in attached *Rhinanthus* shoot increased without statistic significance; but in *Rhinanthus* roots Z increased 4-fold after the parasitism. There were no obvious differences in ZN in shoot and root between single and parasitising *Rhinanthus*. The substantial amount of cytokinins could be detected in haustoria (Fig. 3.46).

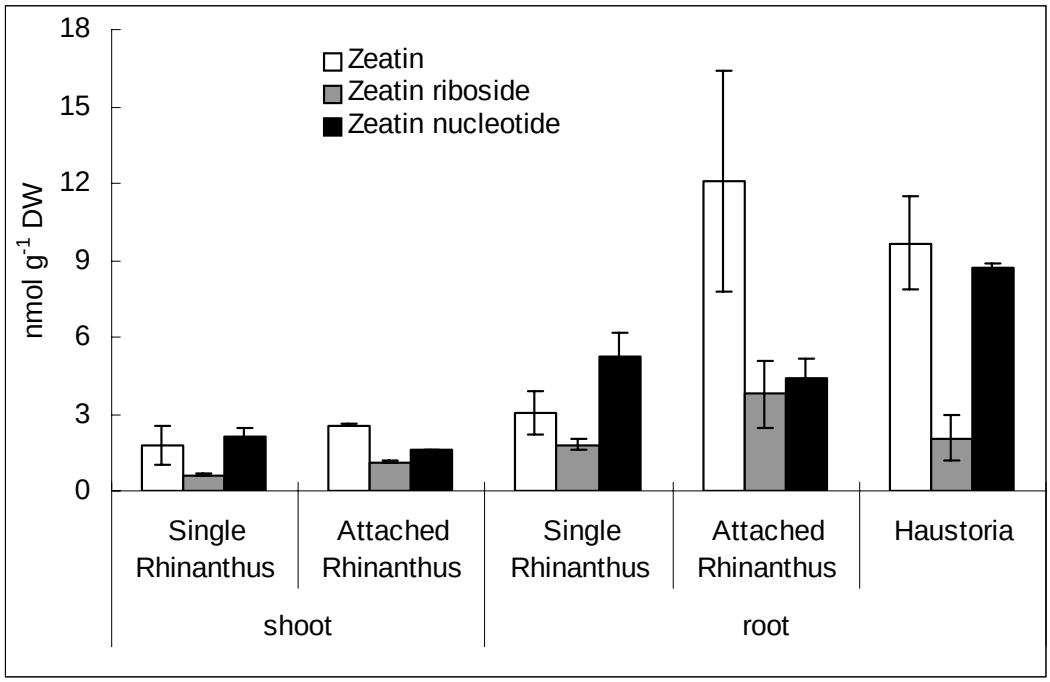


Fig. 3.46 The concentrations of three cytokinin derivatives (Z, ZR, ZN) (nmol·g^{-1}DW) in shoot and roots of single *Rhinanthus* and attached *Rhinanthus* (54 days after planting, about 43 days after attachment). Plants were supplied with 1mM NO_3^- **(**n = 4 ± SE).

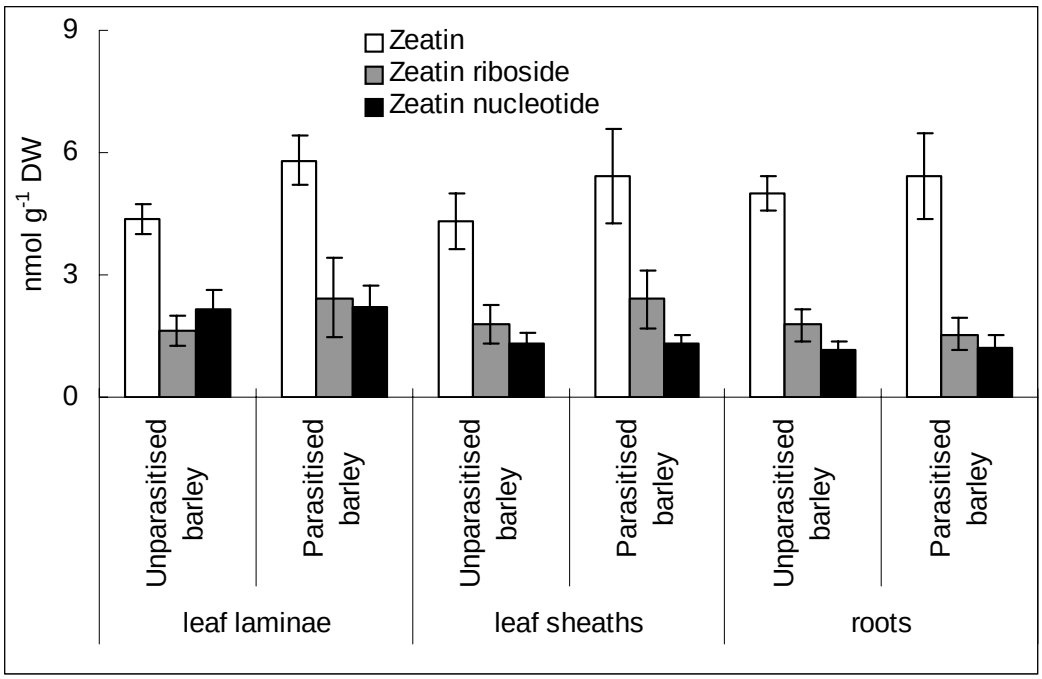


Fig. 3.47 The concentrations of three cytokinin derivatives (Z, ZR, ZN) (nmol·g^{-1}DW) in leaf laminae, leaf sheaths and roots of single *Hordeum vulgare* (54 days after planting) and of parasitised *Hordeum vulgare* (54 days after planting, about 43 days after attachment). Plants were supplied with 1mM NO_3^- (n = 4 ± SE).

No significant differences of these three cytokinin derivatives (Z, ZR, ZN) concentrations have been found in the leaf laminae, leaf sheaths and roots of barley before and after attachment. Zeatin was always the major cytokinin in each part of barley (Fig. 3.47). Concentrations of Z and ZR in leaf laminae and leaf sheaths of single and parasitised barley were in the ranges of 4.3-5.9 and 1.6-2.4 (nmol g^{-1} DW) respectively, which were much higher than those in single and attached *Rhinanthus* shoot (Fig. 3.46, Fig. 3.47).

3.7.3 Cytokinins flows

Total zeatin type cytokinins

For barley, the xylem flows of cytokinins, on a per plant basis, were dramatically (57%) decreased after infection by *Rhinanthus* (Fig. 3.48); the phloem flows of cytokinins slightly increased (12%); remarkably, phloem retranslocation of cytokinins was significantly increased in response to parasitism (48% vs. 18% of xylem transport in the control); the estimated cytokinins synthesis in the root was reduced clearly (57%) , while the resulting cytokinin degradation in leaf laminae was significantly decreased (73%). Deposition was not significantly affected in the leaf laminae, in leaf sheaths and roots (Fig. 3.48).

In response to attachment to the host, dramatic changes in cytokinins flows on a per plant basis, and in metabolism and deposition, however, have also been observed in *Rhinanthus*. Net degradation of cytokinins in the attached *Rhinanthus* roots has been observed, however, net synthesis of cytokinins has been found in single *Rhinanthus* roots. The cytokinins flow in xylem increased 13-fold after parasitising the host barley and a large portion (65%) of these cytokinins was extracted from the host. The phloem flow of cytokinins increased 50 times after attachment and phloem translocation of cytokinins also increased (44% vs. 11% of xylem transport in the single *Rhinanthus*). Depositions of cytokinins increased 9-fold and 12-fold in shoot

and root respectively. A significant deposition of cytokinins also was detected in the haustoria of the *Rhinanthus*/barley association (Fig. 3.48).

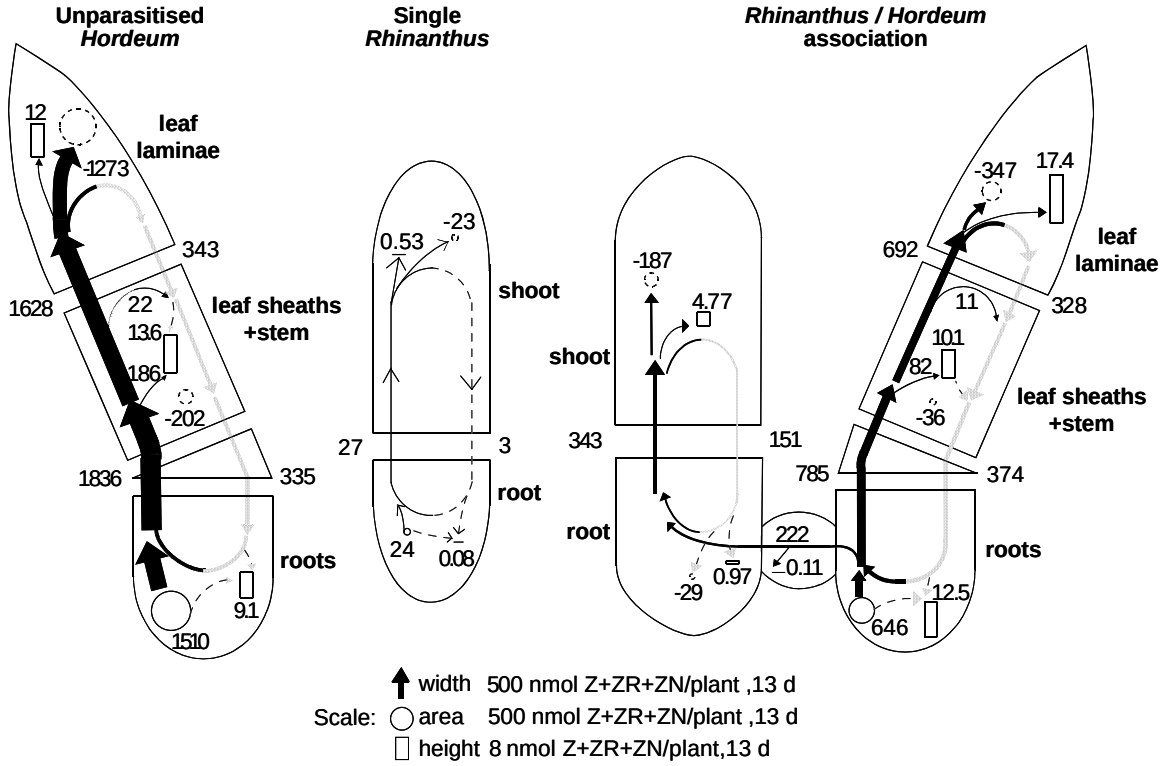


Fig. 3.48 Flow profiles for metabolism, transport and deposition of total zeatin cytokinins (Z+ZR+ZN) in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 1 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Numbers are presented in cytokinins nmoles per plant over 13 days. The width of arrows (net flows in xylem (black) or phloem (dotted)) and the height of histograms (deposition of cytokinins in each organ) are drawn in proportion to the rates of flows or to the magnitude of deposition. The triangle between root and leaf sheaths symbolises the stem.

Zeatin

For barley, the xylem flows of Z, on a per plant basis, were dramatically (56%) decreased after infection by *Rhinanthus* (Fig. 3.49); the phloem flows of zeatin massively increased (3-fold); remarkably, phloem retranslocation of zeatin was significantly increased in response to parasitism (35% vs. 5% of xylem transport in

the control); the estimated zeatin synthesis in the root was reduced (57%) , as well as the resulting zeatin degradation in leaf laminae (71%). Deposition has not been significantly affected in the leaf laminae, in leaf sheaths and roots (Fig. 3.49).

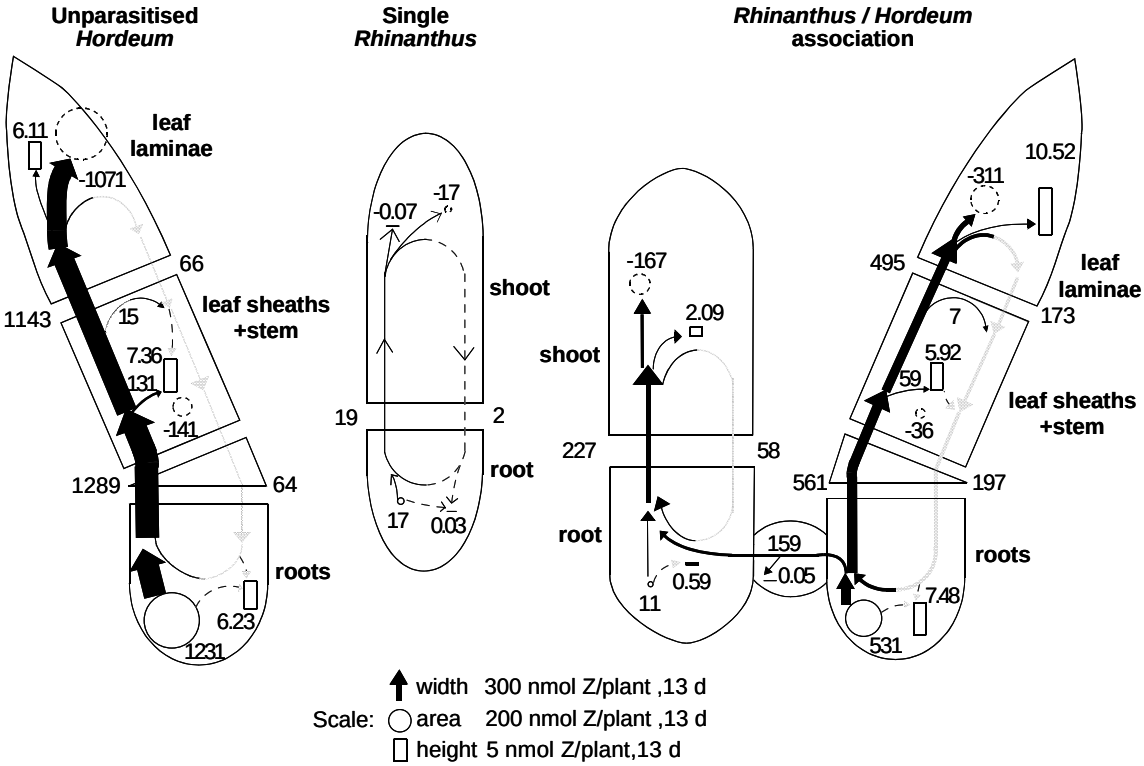


Fig. 3.49 Flow profiles for metabolism, transport and deposition of zeatin (Z) in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 1 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details as in Fig. 3.48.

In response to attachment to the host, dramatic changes in zeatin flows on a per plant basis, and in metabolism and deposition, however, have also been observed in *Rhinanthus*. Net biosynthesis of zeatin in *Rhinanthus* roots decreased by 39% after infection. The zeatin flows in *Rhinanthus* xylem via root to shoot increased 12-fold after parasitising the host barley and 70% of zeatin flowing in xylem from root to shoot were extracted from the host. The phloem flow of zeatin increased 29 times after attachment and phloem translocation of zeatin also increased (26% vs. 10% of xylem transport in the single *Rhinanthus*). Depositions of zeatin increased 24-fold in

root. In single *Rhinanthus* shoot zeatin increment was negative and zeatin was retranslocated back to the phloem or degraded in shoot. A significant deposition of zeatin also was detected in the haustoria of the *Rhinanthus*/barley association (Fig. 3.49).

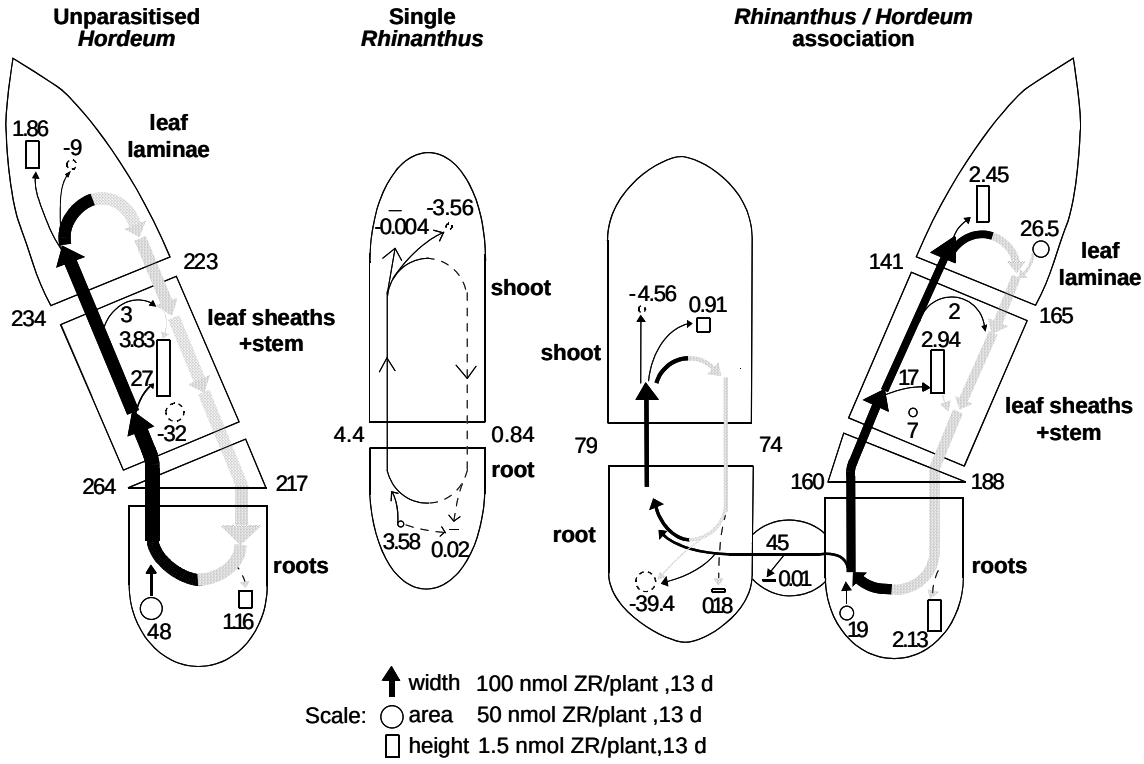


Fig. 3.50 Flow profiles for metabolism, transport and deposition of zeatin riboside (ZR) in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 1 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details as in Fig. 3.48.

Zeatin riboside

For barley, the xylem flows of ZR, on a per plant basis, were dramatically decreased (by 39%) after infection by *Rhinanthus* (Fig. 3.50); the phloem flows of zeatin riboside slightly decreased (13%); phloem retranslocation of zeatin riboside was slightly increased in response to parasitism (118% vs. 82% of xylem transport in the

control); the estimated zeatin riboside synthesis in the root was also reduced (60%), while net ZR synthesis was found in leaf laminae of parasitised barley and net degradation of ZR was found in single barley leaf laminae. Deposition has not been significantly affected in the leaf laminae, in leaf sheaths and roots (Fig. 3.50).

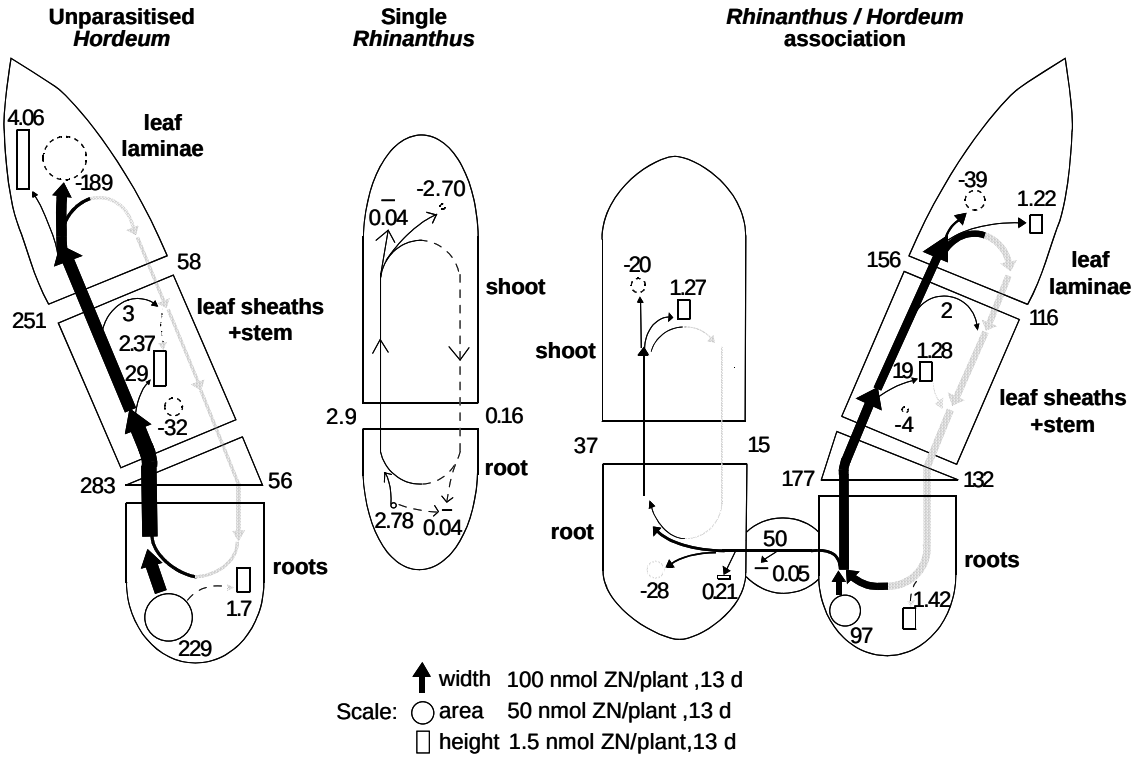


Fig. 3.51 Flow profiles for metabolism, transport and deposition of zeatin nucleotide ZN in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* supplied with 1 mM NO_3^- over the 13 days of study period starting 41 days after sowing or about 30 days after attachment of the parasite. Further details as in Fig. 3.48.

In response to attachment to the host, dramatic changes in ZR flows on a per plant basis, and in metabolism and deposition, however, have been observed in *Rhinanthus*. Net degradation of zeatin riboside in attached *Rhinanthus* root was found. The ZR flowing in xylem via root to shoot increased 18-fold after parasitising on the host barley and a large portion (57%) of xylem flow of ZR was extracted from the host. The phloem flow of zeatin riboside increased 88 times after attachment and phloem translocation of zeatin riboside also increased (93% vs. 19% of xylem

transport in the single *Rhinanthus*). Depositions of ZR increased 12-fold in root. In single *Rhinanthus* shoot zeatin riboside increment was negative and a substantial portion was degraded in shoot and the rest was retranslocated back to the root in the phloem. A significant deposition of ZR also was detected in the haustoria of the *Rhinanthus*/barley association (Fig. 3.50).

Table 3.24 Contents of total zeatin type cytokinins (Z+ZR+ZN), zeatin (Z), zeatin riboside (ZR), and zeatin nucleotide (ZN) (nmol) and their increments (nmol) in *Hordeum vulgare* (control or parasitised by *Rhinanthus minor*). Plants were supplied with 1 mM NO_3^- and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 4 ± SE).

		Unparasitised Barley			Parasitised Barley		
	Harvest	Leaf laminae	Leaf sheaths	Roots	Leaf laminae	Leaf sheaths	Roots
Z+ZR+ZN	1	18.6 ± 1.24	7.53 ± 0.83	7.82 ± 1.01	11.9 ± 1.57	6.75 ± 0.72	6.20 ± 0.52
	2	30.6 ± 3.28	21.1 ± 2.75	16.9 ± 2.00	29.2 ± 4.94	16.9 ± 3.71	18.7 ± 3.65
	increment	12	13.6	9.09	17.4	10.1	12.5
Z	1	10.3 ± 0.76	5.00 ± 0.56	4.49 ± 0.85	5.73 ± 0.96	4.09 ± 0.26	3.93 ± 0.16
	2	16.4 ± 1.63	12.4 ± 2.31	10.7 ± 1.54	16.2 ± 2.44	10.0 ± 2.45	11.4 ± 2.26
	increment	6.11	7.36	6.23	10.5	5.92	7.48
ZR	1	4.37 ± 1.33	1.22 ± 0.27	2.59 ± 0.58	1.42 ± 0.06	1.52 ± 0.46	1.11 ± 0.13
	2	6.23 ± 1.48	5.05 ± 1.36	3.75 ± 0.91	3.87 ± 0.59	4.46 ± 1.43	3.24 ± 0.88
	increment	1.86	3.83	1.16	2.45	2.94	2.13
ZN	1	3.95 ± 0.63	1.31 ± 0.40	0.74 ± 0.17	4.70 ± 0.78	1.13 ± 0.30	1.15 ± 0.37
	2	8.01 ± 1.50	3.67 ± 0.73	2.44 ± 0.35	5.92 ± 1.40	2.41 ± 0.30	2.58 ± 0.78
	increment	4.06	2.37	1.7	1.22	1.28	1.42

Zeatin nucleotide

For barley, the xylem flows of ZN, on a per plant basis, were dramatically (by 37%) decreased after infection by *Rhinanthus* (Fig. 3.51); the phloem flows of ZN, however, were increased (2.3-fold); as well as the phloem retranslocation (75% vs. 20% of

xylem transport in the control). The estimated ZN synthesis was reduced in roots by 58%. In the leaf laminae a net ZN metabolism was observed which was reduced by 79% after attachment. Depositions of ZN in leaf laminae and leaf sheaths have been halved after attachment (Fig. 3.51).

Table 3.25 Contents of total zeatin type cytokinins (Z+ZR+ZN), zeatin (Z), zeatin riboside (ZR), and zeatin nucleotide (ZN) (nmol) and their increments (nmol) in single or attached *Rhinanthus* and haustoria. Plants were supplied with 1 mM NO_3^- and harvested at the beginning and end of the study period 41 and 54 days after planting, about 30 and 43 days after attachment (n = 4 ± SE).

		Unattached *Rhinanthus*		Attached *Rhinanthus*		
	Harvest	Shoot	Roots	Shoot	Roots	Haustoria
Z+ZR+ZN	1	0.34 ± 0.03	0.08 ± 0.01	0.92 ± 0.17	0.25 ± 0.04	0.03 ± 0.007
	2	0.87 ± 0.08	0.16 ± 0.02	5.69 ± 0.19	1.22 ± 0.30	0.14 ± 0.03
	increment	0.53	0.08	4.77	0.97	0.11
Z	1	0.19 ± 0.02	0.02 ± 0.003	0.36 ± 0.09	0.15 ± 0.03	0.02 ± 0.003
	2	0.12 ± 0.01	0.05 ± 0.006	2.45 ± 0.21	0.74 ± 0.18	0.07 ± 0.01
	increment	-0.07	0.03	2.09	0.59	0.05
ZR	1	0.05 ± 0.004	0.01 ± 0.002	0.17 ± 0.04	0.05 ± 0.01	0.003 ± 0.0007
	2	0.04 ± 0.004	0.03 ± 0.004	1.08 ± 0.10	0.23 ± 0.06	0.014 ± 0.003
	increment	-0.004	0.02	0.91	0.18	0.01
ZN	1	0.10 ± 0.01	0.04 ± 0.005	0.24 ± 0.06	0.05 ± 0.01	0.01 ± 0.003
	2	0.14 ± 0.01	0.08 ± 0.01	1.51 ± 0.14	0.26 ± 0.07	0.06 ± 0.01
	increment	0.04	0.04	1.27	0.21	0.05

In response to attachment to the host, dramatic changes in ZN flows on a per plant basis, and in metabolism and deposition, however, have been observed in *Rhinanthus*. Net degradation of ZN in attached *Rhinanthus* roots was found. The ZN flowing in xylem via root to shoot increased 18-fold after parasitising the host barley and a large portion (135%) of xylem flow of ZN was extracted from the host. The phloem flow of ZN increased 95 times after attachment and phloem retranslocation of ZN also increased (41% vs. 6% of xylem transport in the single *Rhinanthus*). Depositions of ZN increased 5-fold in root and 31-fold in shoot. A significant

deposition of ZN also was detected in the haustoria of the *Rhinanthus*/barley association (Fig. 3.51).

Table 3.26 The ratio of zeatin (Z) to K, zeatin riboside (ZR)/K, zeatin nucleotide (ZN)/K and total zeatin type cytokinin derivatives (Z+ZR+ZN)/K in phloem exudates obtained with the EDTA-technique and concentrations of zeatin (Z), zeatin riboside (ZR), zeatin nucleotide (ZN) and total zeatin type cytokinins (Z+ZR+ZN) (nM) in the xylem sap of *Hordeum vulgare*, of attached *Rhinanthus*, of single *Rhinanthus*. Saps were collected from control or parasitised barley between the beginning and the end of the study period 41 to 54 days after planting, about from 30 to 43 days after attachment. Plants were supplied with 1 mM NO_3^- (n = 4-8 ± SE).

	Z+ZR+ZN(nM) / K(mM) in phloem sap	Z+ZR+ZN concentrations in xylem sap (nM)
Unparasitised barley	156 ± 37	1234 ± 220
Parasitised barley	201 ± 25	843 ± 36
Single *Rhinanthus*	144 ± 28	1150 ± 313
Attached *Rhinanthus*	409 ± 69	1037 ± 245
	Z(nM) / K(mM) in phloem sap	Z concentrations in xylem sap (nM)
Unparasitised barley	30 ± 6	866 ± 183
Parasitised barley	106 ± 22	602 ± 70
Single *Rhinanthus*	98 ± 20	834 ± 255
Attached *Rhinanthus*	157 ± 32	688 ± 188
	ZR(nM) / K(mM) in phloem sap	ZR concentrations in xylem sap (nM)
Unparasitised barley	101 ± 31	177 ± 49
Parasitised barley	101 ± 35	171 ± 18
Single *Rhinanthus*	40 ± 15	193 ± 71
Attached *Rhinanthus*	199 ± 46	238 ± 56
	ZN(nM) / K(mM) in phloem sap	ZN concentrations in xylem sap (nM)
Unparasitised barley	26 ± 7	190 ± 26
Parasitised barley	71 ± 24	190 ± 14
Single *Rhinanthus*	8 ± 3	124 ± 12
Attached *Rhinanthus*	41 ± 18	112 ± 12

3.8 ABA and cytokinins in haustoria

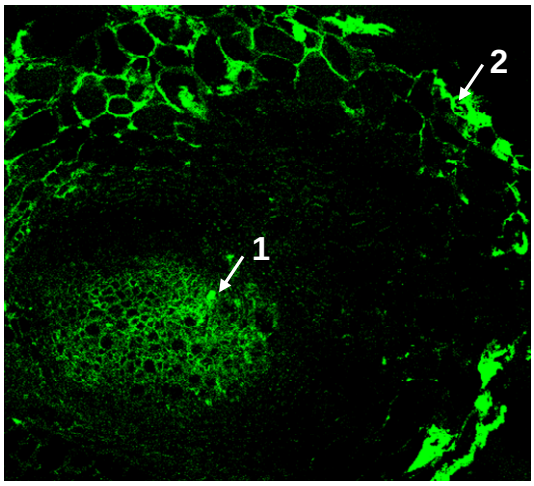


Fig. 3.52 Immunolocalisation of ABA in the haustorium of *Rhinanthus minor*.

The distribution of ABA within a haustorium has been visualised using the technique of immunolocalisation as described by Veselov et al. (2002).The strongest signals are emitted from the marginal cell layer (2) and the lignified (xylem-like) cells (1) of the haustorium (Fig. 3.52).

Table 3.27 The concentrations of ABA, and cytokinins (zeatin Z, zeatin riboside ZR, zeatin nucleotide ZN; nmol/l cell water) in parasitised barley roots, single and attached *Rhinanthus* roots and haustoria (n=5±SE).

	ABA	Z	ZR	ZN
Parasitised barley roots	22±5	728±134	216±54	162±40
Attached *Rhinanthus* roots	86±20	1173±420	371±127	430±78
Single *Rhinanthus* roots	512±242	418±121	243±27	715±121
Haustoria	597±292	1232±229	267±114	1105±25

ABA concentrations in the haustoria were nearly 27 or 7 times higher than in parasitised barley or attached *Rhinanthus* roots respectively (Table 3.27). Haustoria of other associations accumulated even more ABA, 42 times higher in those attached to *Vicia cracca*, 480 times in those attached to *Plantago lanceolata* (data are not shown). Cytokinins concentrations of haustoria were also higher than in barley root tissues. However, the differences are by far not as distinct as with ABA. Cytokinins may be less important for haustorium formation than ABA.

4. Discussion

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 these facultative hemiparasites like *Rhinanthus minor* can cause damage (Parker and Riches, 1993). The studies on *Rhinanthus minor* have been carried since many years, however, nothing has been reported systematically of the water, nutrients, hormones, assimilates relations and the formation of haustorium in the parasitic association *Rhinanthus minor/Hordeum vulgare*. In this study, *Rhinanthus minor/Hordeum vulgare* association has been investigated from these aspects and the possible physiological role of nutrients, hormones and assimilates have been disscussed.

4.1 Haustorium

Potential host plants of *Rhinanthus minor* show different defence strategies against infection and haustorium formation. This includes an increased lignin and suberin formation in the interface between haustorium and host, a suberin deposition in the endodermis and lignification of stelar tissues of the host root (Fig. 3.4). Abscisic acid accumulates dramatically in the haustoria and may be involved in suberin deposition and formation of small stelar cells with lignified and thickened cell walls (Fig. 3.52, Tab. 3.27). Similar ABA effects have been reported previously (Cottle and Kolatukutty, 1982; Pharis et al., 1981). The accumulation of cytokinins is much weaker. They obviously play a more important physiological role in the parasite shoot than in the haustorium. From the distribution of mannitol (the major assimilate of *Rhinanthus* that does not occur in barley) between the haustorium and the adjacent barley root tissues (Fig. 3.34) it is concluded that there is no reverse transfer of substances from the haustorium to the host. Similar conclusions have been drawn by Pate et al. (1994) in the case of *Olax phyllanthi* haustoria.

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

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., 1993a). Unattached seedlings of *Rhinanthus minor* reach a height of approximately 10 cm only within three months and its leaves showed all symptoms of a cytokinin and nitrogen deficiency (Fig. 3.1, Fig. 3.2). Indeed content of the cytokinins of the zeatin type in roots of unattached *Rhinanthus* proved to be significantly lower than those in barley roots (zeatin 30% and zeatine riboside 50% of barley roots (Tab. 3.23). Both hormones were required for an undisturbed leaf development, auxin for the vascular system and cytokinins for the mesophyll (Dore and Champion, cited after Wareing Phillips, 1981). The zeatin concentration in the xylem sap of barley was found in the range of 400-500 nM and that of auxin 500 nM (data not shown), hence these hormones together with mineral nutrients could be exploited easily by the parasite *Rhinanthus* after forming a 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 mean for exploiting the host xylem have early been noted in mistletoe species like *Amyema nestor* (Gill and Hawksworthy, 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 night. The experiments of this study 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 parasitising 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 a much lower leaf conductance and showed normal diurnal patterns of leaf conductance (Fig. 3.6). 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 $m^{-2}s^{-1}$.

In the greenhouse the leaf conductance of *R.minor* parasitising on barley showed a similar diurnal pattern, however, on a clearly lower level (Fig. 3.7). 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. 3.5); it is more likely, that the high CO_2 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* exhibited clearly higher leaf conductance than those of the hosts (Fig. 3.6). The facultative hemiparasite *Rhinanthus* thus clearly appears to optimise 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 CO_2 exchange (Jeschke et al., 1994b; 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 parasitised host barley (Fig. 3.8). A similar situation has been observed in the *Striga/Zea* association by Taylor and Seel (1998). Despite of the 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 (Tab. 3.1). These findings show that guard cells obviously can react to internal and external factors such as CO_2 and ABA. The leaf conductance of *Rhinanthus* normally was above that of the host, except for a few occasions in the greenhouse. The extreme insensitivity to internal and external factors apparently was

not a result of structural defects of the guard cells as it 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 probably is the result of special biochemical features (receptors?), or another reason which will be discussed later.

At present we cannot explain 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 the zeatin type may be good candidates. The existence of other unknown substances also 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 therefore has been used to determine apoplastic and symplastic root hydraulic conductivity of barley and *Rhinanthus* similarly 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 (Tab. 3.2). 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. 3.9, indeed 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. 3.10).

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 when the plants were supplied with 5 mM NO_3^-. Most of the water taken up by roots of unattached *R. minor* was released by the

leaves to the atmosphere (Fig. 3.11). This could either only happen by cuticular transpiration or by a residual transpiration via closed stomata, because in unattached *Rhinanthus* the stomata always are tightly closed. Since the unattached *Rhinanthus* show some growth and their photosystem 2 is clearly operative (unpublished data), some CO_2 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 3.11 and 3.12 and their changes in barley, due to parasitic infection, and in *Rhinanthus* in response to successful attachment to a host are 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. 3.11 and 3.12 also reflect the impact of parasite infection in the host. Growth-dependent water deposition in the host was decreased, by 23% in the leaf lamina and by 43% in the leaf sheath fraction (which contained also the growing stem and apical bud), but water deposition in the root was as high as in the non-parasitised barley. This points to a decreased shoot to root ratio, which actually decreased from 3.5 to 2.8 (Tab. 3.5), 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, these changes reflect, however, 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 parasitising just one barley plant.

When plants were supplied with 1 mM NO_3^- or 1 mM NH_4^+, the response of the host to attachment was similar. The growth of attached and unattached barley, however, was lower under these changed nutritional conditions. This also resulted in a reduced water incorporation into the tissue. Ammonium additionally also reduced the transpiration of barley, similar as described previously by Raab and Terry (1994), Adler et al. (1996).

4.3 Nutrient flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite nutrient relations

4.3.1 Plants supplied with 5 mM NO_3^-

This study reveals substantial growth reduction of *Hordeum* parasitised by *Rhinanthus*, even though *Rhinanthus* is a hemiparasite which withdraws nutrients from the host xylem only and does not take photosynthates from the phloem. However, it takes up a certain amount of organic carbon from the xylem. Using the C/N-ratio (1.8) in the host xylem sap, the N flow from host to parasite and the total C deposition in the parasite (Tab. 3.4), and assuming that 50% of photosynthesis is lost in respiration, it was estimated that total C intake together with the xylem sap amounted to 10% of the total C uptake by *Rhinanthus*. By comparison it has been shown that approximately 30% of carbon in the leaves of mature *Striga hermonthica* (Press et al., 1987) or 5-21% of parasite carbon in eight Australian mistletoe associations (Marshall et al., 1994) and even 40% of the carbon in *Olax* parasitising *Acacia* (Tennakoon et al., 1997)

were host derived. This suggests that the dependence of *Rhinanthus* on host C is smaller than in other hemiparasites like *Striga* and *Olax*. *Rhinanthus* altered the biomass partitioning of the *Hordeum* host, more severely by reducing shoot growth and relatively favoring root development. Prior to discussing in detail possible reasons for these changes, the flows of nutrient in host and parasite should be addressed.

4.3.1.1 Mineral nutrient flows in the host

In the present study the net flows and circulation of nutrient elements including the macronutrients N, P and K^+ and one of the minor essential nutrients Mg^{2+} have been investigated in solitary *Hordeum* and *Rhinanthus* plants and within the *Rhinanthus*/ *Hordeum* association. K^+ flows within *Hordeum* have previously been measured (Wolf and Jeschke, 1991), albeit at an earlier developmental stage (38-45 days). Therefore the net flows on a per plant basis could not be compared. However there is fair comparison between the recirculation of K^+ (53% in presence of salt) in the phloem. For all nutrients studied here there was significant translocation via phloem from shoot to root as has previously been found in *Lupinus albus* (Pate and Layzell 1981, Jeschke et al., 1987) *Ricinus* (Jeschke and Pate, 1991a,b, Jeschke et al., 1997) *Triticum* (for nitrogen, Cooper and Clarkson, 1989) and in *Nicotiana tabacum* (Hibberd et al., 1999, Jiang et al., 2001). In the unparasitised *Hordeum* the recirculation was highest for K^+ and lowest for Mg^{2+}. As has been shown here and in these previous studies, the quantities of phloem-retranslocated nutrients to a varying degree exceeded the quantities of nutrients deposited in the roots during the study period. As a consequence there was a circulation of the nutrients from shoot to root and back to the shoot again. The extent of this circulation varied between elements and was - as measured by the ratio of root to shoot xylem transport to uptake by the roots - highest for K^+ (152%), followed by N (125%) and P (124%) and lowest for Mg^{2+} (90%). This sequence compares well with *Ricinus*, where circulation was high for K and low for Mg^{2+}. The reason for low circulation of Mg compared with other elements was relatively high investment of Mg^{2+} for root growth (see item viii in the

results and compare Fig. 3.20 with Figs. 3.17-3.19), as it was also found in *Ricinus*. Similarly, the high recirculation of K^+ can be attributed to an exceedingly low deposition of K^+ in the root, see item IX in the results.

4.3.1.2 Flows in the association *Rhinanthus/ Hordeum*

When looking at the flows of nutrients within the host/parasite association between *Hordeum* and *Rhinanthus*, the first and principal observation is a substantial withdrawal of elements from the host xylem. Interestingly, the extent of scavenging xylem-borne nutrients by the parasite from the host amounted to virtually the same proportion of uptake (around 20%) for all nutrients studied here (see item vii in the results). Exactly this would have been anticipated, if xylem to xylem transfer from host to parasite occurred apoplastically, either via open vessel to vessel connections between the two xylem strands, or through dead walls of adjacent xylem vessels or tracheids. Therefore, the very similar percentage of removal of nutrients from the host xylem argues against the presence of living transfer cells between host and parasite xylem elements. This is a difference to the situation in the hemiparasite *Olax phyllanthi*, as studied by Pate and coworkers (Pate et al., 1990a), where no open xylem to xylem connections between various hosts and *Olax* have been found (Pate et al., 1990b). The even withdrawal of ions by *Rhinanthus* agrees with similar increases in the concentration of various ions in *Rhinanthus* after attachment to *Hordeum* as found by Klaren and Janssen (1978).

4.3.1.3 Benefits of attachment to the host for the parasite and possible causes for its improved growth

Independent of the apparently nonspecific withdrawal of nutrients from the host xylem, the impact of various nutrients on *Rhinanthus* appeared to be quite different. This can for instance be seen, when the quantities of nutrients withdrawn from the host are compared to those taken up by the parasite's own roots. The quantities

withdrawn varied from 2.5 fold the total uptake in the case of Mg^{2+}, to 8.7 fold for K^{+}, 12 fold for P and 6.5 fold for N. Particularly this enormous, 12 fold increase in phosphorus supply improved growth after attachment and this corresponds with results of Seel et al. (1993b) showing improved growth of unattached *Rhinanthus* with added P nutrition. On the other hand, in agreement with Seel and Jeschke (1999), nitrogen could be a second candidate which is mainly responsible for the improvement in growth of *Rhinanthus* after successful attachment to the *Hordeum* host.

The enlarged supply of P could be a possible reason for the stimulation of shoot growth in attached *Rhinanthus*. Phosphorus is an essential element for higher plants and required in substantial concentrations in plant tissues. When P concentrations in dry matter fall below about 0.1-0.2% typical deficiency symptoms usually occur, including a marked reduction in leaf expansion and leaf surface area (Fredeen et al., 1989). Cell and leaf surface expansion are retarded to a greater extent than chloroplast and chlorophyll formation (Hecht-Buchholz, 1967) resulting a darker green leaf colour under P deficiency (Rao and Terry, 1989). In unattached *Rhinanthus* leaves the concentration of P in dry matter was 0.28% (1.2% in attached *Rhinanthus* leaves). Compared with attached *Rhinanthus*, the colour of single *Rhinanthus* leaves was darker and the surface area was much smaller (own observations and from Klaren and Janssen, 1978). It seems that P in the culture substrate was not sufficient for the single *Rhinanthus* but enough for the host *Hordeum*. These phenomena imply that dwarfed growth of single *Rhinanthus* is possibly partly due to limitation of P uptake.

The improved nitrogen supply in parasitising *Rhinanthus* could also be a reason for the massive increase in shoot growth (12 – fold) and much less increased root growth (less than twofold compared to unattached *Rhinanthus*), because the shoot is the primary destination of the xylem-borne nitrogen obtained from the host, and because of the observation that luxuriant N-supply favors shoot over root development (Marschner et al., 1996). An ample N supply was shown by the substantial decrease in the C/N ratio after attachment of *Rhinanthus* to its host (from 16.6 in single *Rhinanthus* to 9.3 in attached *Rhinanthus*). This then implies that

unattached *Rhinanthus* plants exhibit dwarfed development as a consequence of limited N uptake. It is interesting to ask why N uptake is limited, since other Scrophulariaceae grow well without the need for parasitism, and other plants including the potential hosts growing in the same soil as the *Rhinanthus* show ample development on the basis of the same N resources. One reason might be low efficiency of N uptake systems in *Rhinanthus* roots, which need to be studied, but insufficient nitrate reduction, as suggested by Seel et al. (1993a) is another possibility.

4.3.1.4 Effects of parasitism on the host

So far the impact of parasitism on nutrient supply for the parasite has been considered. The next question is the extent to which withdrawal of nutrients from the host affects the nutrient flows and circulation within the parasitised host. Since *Rhinanthus* exploits nutrients from the host xylem, the effect of the parasite on the xylem supply from root to shoot in the host first ought to be considered. When different nutrient elements are compared, the ratios of root to shoot xylem transport to nutrient uptake, due to recirculation in unparasitised *Hordeum* were 1.52 for K^+, 1.25 for N, 1.24 for P and 0.90 for Mg^{2+}. After the parasite had attached, these values were slightly decreased to 1.46 in the case of K^+, to 1.01 for N, 0.95 for P, and 0.77 for Mg^{2+}, showing that the supplies of nitrogen and of phosphorus to the shoot were most strongly decreased. This may suggest that the observed decrease in *Hordeum* shoot growth after infection by the parasite could be related to decreased N and P supply to the host. In *Hordeum* shoot growth was more affected than root growth, as has similarly been found in *Poa alpina* (Seel and Press, 1996), and this agrees with a shortage in nitrogen and phosphorus, which both lead to decreased shoot and relatively favored root growth (Marschner, 1995).

4.3.1.5 Comparison with flows of nutrients between host and parasite in other parasitic associations

Throughout the four parasitic associations studied so far, including two hemiparasites, *Olax* (I) (Tennakoon et al., 1997,) and *Rhinanthus* (this study), and two holoparasites *Cuscuta* (II) (Jeschke et al., 1994b) and *Orobanche* (III) (Hibbered et al., 1999) only nitrogen exchanges have been studied in all of the four papers and may be compared. In the two associations *Olax/Acacia* (I) and *Cuscuta/Lupinus* (II) symbiotically-fed hosts were parasitised and here the largest shares of N were 'stolen' from the host: 214% (II) or 56% (I) of the current N-fixation. The holoparasite *Cuscuta* (II) extracted by far more N than the hemiparasite *Olax* (I), and the extreme N-robbery exerted by *Cuscuta* was accompanied by massive N-mobilisation in the host and eventually lead to its death. In the other two associations *Orobanche/Nicotiana* (III) and *Rhinanthus/Hordeum* the hosts were nitrate-fed, and again N-deprival by the holoparasite *Orobanche*, 28% was larger than by the hemiparasite *Rhinanthus*, 18% of NO_3^- uptake. The smaller N-deprival by hemiparasites clearly can be related to generally lower damages exerted by hemi- than by holoparasites, but see the hemiparasite *Striga* (Parker and Riches, 1993).

For potassium a comparison of the deprival from different hosts also shows considerable differences, *Cuscuta* (II) removing 68% (Jeschke et al., 1995), *Orobanche* (III) 16% and *Rhinanthus* 20% of total K^+ uptake from the host. The much lower percentages of K^+- compared to N- removal in the holoparasites II and III indicates rather selective withdrawal by these parasites, enabled by withdrawal from phloem and xylem, whereas the similar K^+- and N- removal by *Rhinanthus* indicates more or less non-selective access to the host xylem. This is also shown by a comparable N/K^+ ratio in the parasitising *Rhinanthus* (1.77) and parasitised *Hordeum* (1.65) xylem sap. Whether mineral nutrient withdrawal from the xylem in hemiparasites is selective or not, has been discussed extensively, as reviewed by Stewart and Press (1990), and this certainly depends on the structure of the haustorium and on the xylem to xylem interface. When living cells between host and parasite xylem strands are present as in *Olax* symbioses (Pate et al., 1990b), selective exchanges are likely. The presently found low selectivity of withdrawal suggests purely apoplastic connections between host (*Hordeum*) and parasite (*Rhinanthus*)

xylem vessels.

The low selectivity found here at first sight appears to be at variance with the data of xylem sap composition (Seel and Jeschke, 1999), showing for example higher K^+ concentrations (9.5 mM) in *Rhinanthus* xylem sap than in that of the host (7.4 mM). However, the relative abundance of various nutrients in the two xylem fluids was similar, again arguing for low selectivity. The question of selectivity of transfer has been discussed particularly in relation to nitrogen nutrition (Stewart and Press, 1990), since hemiparasites like *Rhinanthus minor* have been suggested to be unable to reduce nitrate (Seel and Press, 1993; Seel et al., 1993a). This suggestion, however, is not supported by the present results, showing that *Rhinanthus* retrieved nitrogen from the host xylem at a ratio of 51% nitrate and 49% amino acids (Tab. 3.6) and that in leaf tissues nitrate was only 20% of total nitrogen, showing clearly that parts of the nitrate have been reduced and converted in amino acids, proteins etc. Nevertheless, nitrate concentration in *Rhinanthus* leaf tissues (100 mM vs, 16 mM in *Hordeum* leaves) was relatively high, supporting the notion, that *Rhinanthus* parasitising *Hordeum* contains luxuriant levels of nitrogen and *Hordeum* suffers from incipient N deficiency.

4.3.2 Plants supplied with 1 mM NO_3^- or 1 mM NH_4^+

Parker and Riches (1993) suggested that *Rhinanthus* could easily be controlled by nitrogen fertilisation in the grassland. Therefore, the net flows and circulation of nutrient elements including the macronutrients N, P and K^+ and Mg^{2+} have also been investigated in solitary *Hordeum* and *Rhinanthus* plants and within the *Rhinanthus*/ *Hordeum* association under a lower level of nitrogen (1 mM NO_3^- or 1 mM NH_4^+) supply. In the unparasitised *Hordeum* the recirculation was highest for K^+ and lowest for Mg^{2+}, similar as described as for the plants supplied with 5 mM NO_3^-. Uptake and flows in xylem of nitrogen and potassium by both unparasitised and parasitised barley roots decreased dramatically after the plants were supplied with 1 mM NO_3^- or with 1 mM NH_4^+ (as compared with the 5 mM NO_3^-), whereas uptake and xylem flows of phosphorus and Mg^{2+} were reduced slightly. When the plants were supplied with

lower nitrogen, the extent of scavenging xylem-borne nutrients by the parasite from the host amounted to substantially high percentage of uptake for all nutrients by host roots (59% for N; 45% for P; 60% for K^+), although the parasite only extract 22% of water uptake by host. It is therefore concluded that under these conditions, in xylem sap, the concentrations of these nutrients is increased. This may be a result of stimulated recirculation.

Under lower nitrogen supply, the host was affected more seriously by parasitism than under 5 mM NO_3^- supply. In the case of 1 mM NO_3^- supply, biomass in leaf laminae and leaf sheath reduced by 48% and 53% respectively compared to the control after attachment. When the plants were given lower nitrogen, the effect of the parasite on the xylem supply from root to shoot became more serious. For example, under 1 mM NO_3^- supply, when different nutrient elements are compared, the ratios of root to shoot xylem transport to nutrient uptake were 2.7 for K^+, 1.5 for P, 1.4 for N and 1.11 for Mg^{2+}. After the parasite had attached, these values were slightly decreased to 1.8 in the case of K^+, to 0.82 for P, 0.49 for N and 0.81 for Mg^{2+}, showing that the supplies of nitrogen and of phosphorus to the shoot were most strongly decreased. This may suggest that the observed decrease in *Hordeum* shoot growth after infection by the parasite could be related to decreased N and P supply to the host. The similar results were also found in the plants with 1 mM NH_4^+ supply.

The growth of attached *Rhinanthus* has not been reduced by the 1 mM NO_3^- or 1 mM NH_4^+ supply. Data have shown that the dry matter of *Rhinanthus* shoot even increased after supply with lower nitrogen. The depositions of mineral elements including N, P, K^+, Mg^{2+} were also higher compared with 5 mM NO_3^-. This showes that *Rhinanthus* plants can grow very well and extract as much nutrients as they need from the host, independent on the nutrient supply. Different from Park and Riches (1993), it is concluded that to control the *Rhinanthus* in the field is not possible by changing the N-fertilisation.

4.4 Contents and flows of assimilates (mannitol and sucrose) in the hemiparasitic *Rhinanthus minor/Hordeum vulgare* association

One of the peculiarities of hemiparasitic Scrophulariaceae is the occurrence of mannitol as major assimilate. Already Hodgson (1973) has followed the $^{14}CO_2$ labelling pattern in different hemiparasites and found an incorporation of label into mannitol in leaves of *Rhinantus minor*, *Euphrasia nemorosa*, *Pedicularis sylvatica*, *Orthocarpus faucibarbatus*, *Parentucellia viscosa* and *Lathraea squamaria*. In all the species, except for *Rhinanthus*, Hodgson (1973) found also galacticol. Hodgson (1973) could not detect mannitol in *Melapyrum pratense*. In *Melampyrum arvense*, used in this study, it was found in the range of 30-40 mmol kg^{-1}_{FW} (Fig. 3.33), which is comparable to *Rhinanthus minor* (Fig. 3.29).

Mannitol was also detected in *Striga hermonthica* (Pageau et al. 2000) in *Orobanche ramosa* (Pageau et al. 2000), in qandong (*Santalum acuminatum*; Loveys et al. 2001) and in *Thesium humile* (*Santalaceae*) (Simier et al. 1993).

In the *Rhinanthus* plants of this study the mannitol content of the shoot was in the range of 25-90 mmol kg^{-1}_{FW} and in the root of 6-50 mmol kg^{-1}_{FW} (Fig. 3.29). Under reduced nitrogen supply and with ammonium as the only N-form the concentrations tended to be increased by approximately 2-fold. In root tips of attached *Rhinanthus* of the above plants the osmotic potential (π) was 0.5 MPa. It is concluded that this π of the cell sap of *Rhinanthus* roots is a result of the high mannitol concentration. *Rhinanthus* roots form haustoria after contact with the surface of the host root. Such a contact should cause a localised dehydration of the host root tissue with the result of an increased ABA biosynthesis in this part. Abscisic acid, however, seems to play an important role for the formation and differentiation of the haustoria as concluded from the extremely high ABA concentrations in *Rhinanthus* haustoria (Tab. 3.27).

The mannitol and sucrose data of this study have been used to create a quantitative model of assimilate flows of the single growing parasite and host and its association. Both flows are strictly separated and no mannitol passes the haustorium to the host and no sucrose is present in the barley xylem sap to be taken up by the

parasite. A similar conclusion was drawn by Pate et al. (1994) who could not find the passage of a parasite specific amino acid (S-ethenyl cysteine) from *Olax phyllanthi* to its host. Attachment of *Rhinanthus* to the host reduced the sucrose biosynthesis by 24% which also resulted in a 67% lower deposition in the leaves and a 23% reduced phloem transport. This is consistent with chlorophyll fluorescence data of Seel et al. (personal communication) which show a clear reduction of photosynthesis in host leaves after infection.

In the parasite, however, mannitol formation was increased 16-fold on a per plant basis after infection resulting in a 10-fold deposition in the shoot and a 15-fold more intensive phloem transport to the root (Fig. 3.32). Phloem import of mannitol into the *Rhinanthus* roots obviously was sufficiently strong that besides a substantial net metabolism and respiration a significant portion of the mannitol was recirculated via xylem back to the shoot or released from the roots to the rhizosphere. Thus roots of *Rhinanthus* produce a special chemical composition of their surface and their rhizosphere which again may be important for the belowground signalling from *Rhinanthus* roots to potential host roots. A sucrose exudation from barley roots could not be detected (Fig. 3.32).

When the plants were supplied with 1 mM NO_3^- or when this nitrate was replaced by 1 mM NH_4^+ (as compared to 5 mM NO_3^-), deposition of mannitol in shoot and root of single *Rhinanthus* increased 2-3 times; in attached *Rhinanthus* deposition of shoot increased 4-fold and 4-5-fold increased in root. Also the net synthesis of mannitol increased 2 times in shoot of single and attached *Rhinanthus* (Fig. 3.35 and Fig. 3.36).

With exudation and recirculation of the transport assimilate mannitol, *Rhinanthus* shows two peculiarities that may be characteristic for hemiparasites.

High mannitol concentration in tissues of *Rhinanthus* also may explain the high amounts of the plant stress hormone abscisic acid in all organs of single and parasitising *Rhinanthus*, although they have not been exposed to environmental stresses. It is suggested that a high osmotic potential caused by high mannitol concentrations may be responsible for this remarkable ABA accumulation. In Fig. 4.1 all ABA data (excluding data from inflorescences and lateral buds) have been

plotted against the ABA concentration in these samples. Indeed a positive correlation (Spearman rank correlation, r_s=0.367, n=60, p<0.02) can be observed between mannitol and ABA accumulation which may explain the special ABA relations in single and attached *Rhinanthus* plants. This may have positive consequences for the drought tolerance of *Rhinanthus minor* when growing under dry and hot conditions.

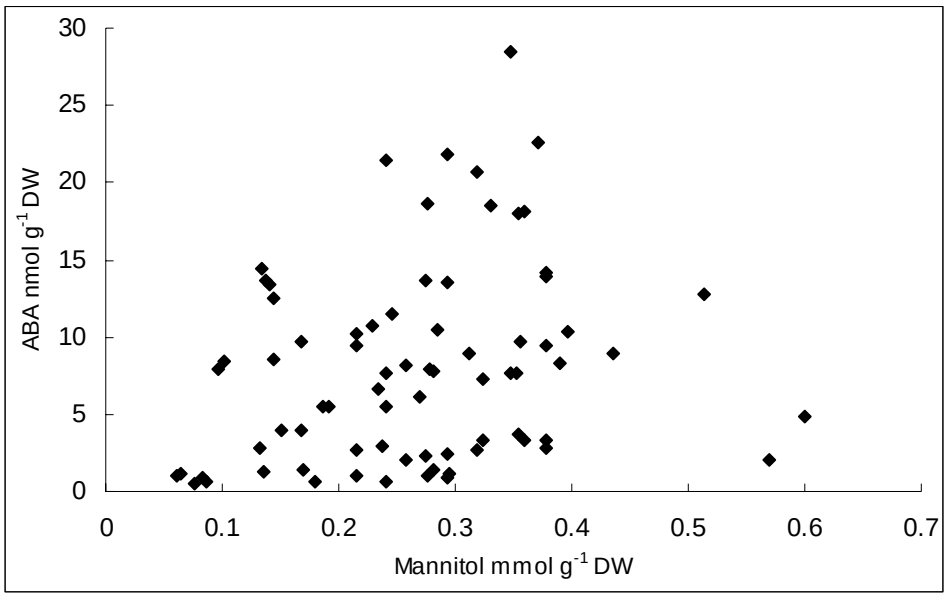


Fig. 4.1 The relation between the concentration of mannitol and abscisic acid in roots, shoots, stems and leaves of single and attached *Rhinantus minor*. The ABA data were obtained from Fig. 3.38 and Fig. 3.39.

4.5 Abscisic acid (ABA) flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite abscisic acid relations

Abscisic acid concentrations in shoots and roots of single *Rhinanthus* proved to be higher than in the unparasitised, potential host plant barley by a factor of 15 (Fig. 37a, Fig. 3.38). Xylem sap ABA of single *Rhinanthus minor* was also up to 10 times higher than that of unparasitised barley (Fig. 3.40a, b). In the *Rhinanthus*/barley association leaf ABA of the parasite *Rhinanthus* was higher by an order of magnitude than in the

host, which resembles the situation in the *Striga*/maize association (Taylor et al., 1996). In the *Striga/Sorghum* association, however, Frost et al (1997) found leaf ABA of the parasite only 1.5-fold and xylem sap ABA 2-fold higher than in the host.

In barley the ABA concentration was only slightly affected by parasitism without statistical significance (Fig. 3.37a, b). This is different from the *Striga*/maize system where at least in the case of one maize cultivar ABA was increased by 60% in leaves, while in other cultivars ABA was not affected (Taylor et al., 1996). Compared to plants, supplied with 5 mM NO_3^-, leaf ABA of single and parasitised barley was stimulated when 1mM NO_3^- or 1mM NH_4^+ was given. Altered N supplies had no effects on ABA concentrations in shoots and roots of single and attached *Rhinanthus*.

Water deficiency caused clear additional 2-3 fold increased in the ABA of leaves and stems of attached *Rhinanthus* (Fig. 3.45).

The data of the present experiments as they are shown in Tab. 3.17 and Tab. 3.18 have been used to calculate ABA flows within the parasite, the host and within the parasitic association. As Fig. 3.41 shows, in single barley net ABA biosynthesis was observed in the roots, a big portion of which was indicated to be fed into the xylem, whereas a small part only was deposited in the roots. Most of the ABA arriving in the leaf laminae was metabolised, approximately 25.6% were retranslocated in the phloem to the roots and the smallest part was deposited. A similar situation was detected in single *Rhinanthus*, however, with much lower flows and rates of deposition and metabolism. One should not forget that in *Rhinanthus* these weak dynamics happen upon high levels of tissue-, xylem and phloem sap-ABA concentrations. Parasite attachment had only weak consequences for the ABA-flows and -deposition in the host. ABA net synthesis in roots is increased by 61%, ABA flows in the xylem and phloem remained more or less unchanged. The clearest effects were observed in the leaf sheaths and leaf laminae where ABA deposition increased 2-3 fold. Dramatic changes, however, happened after attachment in tissues of the parasite *Rhinanthus*. Net synthesis in roots, xylem flow to the shoot, net metabolism in the shoot and phloem transport on a per plant basis were higher by a factor of 12-14, and ABA deposition in the shoot was increased 18 fold. It is also noteworthy, that

ABA deposition in the haustoria was higher than net ABA deposition in the whole *Rhinanthus* root system. ABA extracted from the host contributed by only 7 % to the ABA following via xylem from the *Rhinanthus* root to the shoot. Nearly 67% of this ABA_{xyl} originated from biosysthesis and 26% were recirculated in the root from the phloem to the xylem.

When the plants were supplied with 1 mM NO_3^- or when this nitrate was replaced by 1 mM NH_4^+ (as compared to 5 mM NO_3^-) only the ABA relations of barley were affected. Deposition of ABA in barley leaf sheaths and laminae were increased by lower NO_3^- supply, and to a weaker extent also in 1 mM NH_4^+ - plants. ABA concentrations and flows in parasitising *Rhinanthus*, however, were hardly affected by altered N supply. Independent of variations in ABA accumulation of the host plants, in response to altered N-nutrition, the parasitising *Rhinanthus* maintained its ABA relations at comparatively unaltered levels (Fig. 3.41, Fig. 3.42, Fig. 3.43).

Most remarkably, roots of *Rhinanthus minor* plants increased ABA biosynthesis dramatically on a per plant basis after attachment to a host (Fig. 3.41), although they are not exposed to external stress conditions. *Rhinanthus minor*, as many other Scrophulariaceae parasites (Hodgson, 1973, Pageau et al, 2000) form large amounts of mannitol as the major assimilate resulting in a high osmotic potential of the cell sap of *Rhinanthus* tissues. Tissues with high concentrations of an osmoticum such as mannitol may also synthesis and accumulate high concentrations of ABA.

Abscisic acid metabolism is an important mechanism to avoid extremely high ABA concentrations in tissues. In *Rhinanthus* shoots ABA metabolism was therefore increased 12.5 fold after attachment (Fig. 3.41). Leaves and stems of parasitising *Rhinanthus* (growing in the botanical garden in Würzburg, parasitising various hosts), were treated with 10^{-5} M tetcylacis (for chemical structure, see appendix), a norbornanodiacetine derivate that inhibits hydroxylation of the 8' – C- methyl group of ABA and the formation of phaseic acid (PA). Inhibition of the oxidative ABA degradation further doubled ABA in stems, whereas the amount of ABA-glucose ester (ABA-GE) rose 5.7 fold (Fig. 3.44). In the leaves the inhibition of PA-formation doubled ABA-GE. The ABA concentration remained unaffected (Fig. 3.44). A

diversion of ABA metabolism to conjugation together with a further accumulation of ABA, has been observed earlier by Zeevaart et al. (1990). To avoid an increase significantly above 10-12000 pmol gDW^{-1} in absence of PA formation, conjugation becomes an important mechanism of ABA homeostasis in attached *Rhinanthus*.

Exudation of ABA from roots also could contribute to control an ABA accumulation. Indeed, using the technique of Neumann and Römheld (1999) ABA exudation rates from *Rhinanthus* seedling roots (2-3.5 cm long) and barley seedlings roots (3.5-4.5cm long) into an ABA free surrounding medium have been detected (*Rhinanthus*: 0.033 $nmol \cdot g^{-1} \cdot h^{-1}$; barley: 0.0012 $nmol \cdot g^{-1} \cdot h^{-1}$). However, ABA exudation has not been included into the flow models of Fig. 3.41-Fig. 3.43, because it is not possible to check the ABA exudation of the adult root system of attached *Rhinanthus*. Additionally, under natural conditions the soil solution under different plants contains ABA in the low nM range (Hartung et al., 1996) which may significantly reduce exudation (Slovik et al, 1995). Therefore ABA exudation rates must be lower than observed in ABA free medium. Exactly this could be shown in a small series of preliminary experiments. We can not exclude a regulation of the *Rhinanthus* ABA content in the roots by exudation. However, to include exudation data to the ABA models more experimental data are required.

At present no clear explanations for the function of the extremely high ABA concentrations in attached *Rhinanthus* can be given. In leaves and roots of single *Rhinanthus minor* ABA could keep stomata closed and the hydraulic conductivity of roots high (described above). In shoots of attached *Rhinanthus*, however, where stomata are continuously open, even during the night, a stomatal function of ABA seems to be extremely unlikely.

In the field, attached *Rhinanthus* wilted severely under conditions of serious water shortage and high air temperatures, whereas host plants which could close their stomata still exhibited high turgor. The wilted *Rhinanthus* plants, however, recovered completely and rapidly without any symptoms of damage when the external conditions improved slightly. This lack of damage could be a result of the action of dehydrins or other protective proteins whose formation is regulated under stress by

high ABA concentrations. Similar mechanisms have been observed in poikilohydric angiosperms which also do not show anatomical and morphological adaptions to external stress, which are, however, protected biochemically by proteins such as dehydrins and others (Hartung et al., 1998).

Putative physiological roles of the high ABA concentrations in haustoria are at present under investigation. It seems to be unlikely that ABA facilitates solute and water tapping. In *Rhinanthus* haustoria no barriers, whose permeability could be increased by ABA, have to be crossed. A regulation of the formation of suberinised and lignified cells, as shown earlier by Cottle and Kolatukutty (1982) and Pharis et al. (1981) seems to be more likely.

4.6 Cytokinins flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite cytokinins relations

The possible reasons for the dwarfism of the unattached hemiparasite *Rhinanthus minor* has been discussed above, including the limited uptake of nitrogen and phosphus by *Rhinanthus* roots and the lower capability of nitrogenous metabolism. Although nutrients play an important role in the growth of plants, the importance of growth regulating phytohormones for the development of plants should not be neglected, since hormones together with mineral nutrients can be exploited easily by the parasite *Rhinanthus* after forming the haustoria and penetrating into the xylem vessels of barley host root. Cytokinins which stimulate the division of plant cells, nutrient mobilisation and the formation and activity of shoot apical meristems have been, therefore, studied in *Rhinanthus*/barley association.

Table 3.23 shows the cytokinins composition of roots of unattached *Rhinanthus* seedlings. The zeatin cytokinin proved to be significantly lower than those in barley, wheat and maize seedling roots (zeatin 30% and zeatin riboside 50% of barley roots) (For chemical structures of the zeatin type cytokinins, see appendix). Moreover in mature single *Rhinanthus* shoot and root, Z concentrations were about 65% and 33% lower than those respectively in barley leaf laminae, leaf sheath and roots. After

parasitising on barley, Z concentration in *Rhinanthus* shoot increased without statistical significance, but in root it increased 4-fold, and ZR concentrations in shoot and root of *Rhinanthus* were doubled (Fig. 3.46). The changes of Z concentrations in *Rhinanthus* shoot after a successful attachment were not as distinct as those in *Melampyrum arvense* leaves, which increased nearly 100 times (Lechowski and Bialczyk, 1996). Drennan and Hiweris (1979) compared the cytokinins of *Sorghum* with and without *Striga hermonthica* as parasite. Attachment of *Striga* to three varieties of *Sorghum* strongly reduced the cytokinins in the xylem sap by 91-97% and in shoot by 70-99%. Different from this study they did not analysed the different cytokinins. Their analysis of total cytokinins was done using a bioassay.

The data of the present experiments as they are shown in Table 3.24 and Table 3.25 have been used to calculate Z and ZR flows within the parasite, the host and within the parasitic association.

As Fig. 3.49 shows, in single barley net Z biosynthesis was observed in the roots, a big portion of which was fed into the xylem whereas a small part only was deposited in the roots. Most of the Z arriving in the leaf laminae was metabolised, approximately 6% were retranslocated in the phloem to the roots and the smallest part was deposited. A similar situation was detected in single *Rhinanthus*, however, with much lower flows and rates of deposition and metabolism. Parasite attachment had very strong consequences for the Z-flows and -metabolism in the host. Z net synthesis in roots was decreased by 57%, Z flows in the xylem decreased by 56%, phloem flows, however, increased 3-fold. Dramatic changes also happened after attachment in tissues of the parasite *Rhinanthus*. Net deposition in roots, xylem flow to the shoot, net metabolism in the shoot and phloem transport on a per plant basis were higher by a factor of 24, 12, 10, 29 respectively, but net synthesis of Z in attached *Rhinanthus* root decreased 39%. It is noteworthy that the Z increment in single *Rhinanthus* shoots was negative. Different from ABA, 70% of zeatin in the xylem of attached *Rhinanthus minor* originated from the host. Only 5% of this xylem flow originated from biosysthesis in the root and 25% were recirculated from the phloem to the xylem.

The tendency of ZR changes in *Rhinanthus* shoot and root was similar as that of Z before and after attachment. Parasitism also had very strong effects for the ZR-flows and -metabolism in the host. Net degredation of ZR was found in the roots and net synthesis of ZR was found in leaf sheath (Fig. 3.50).

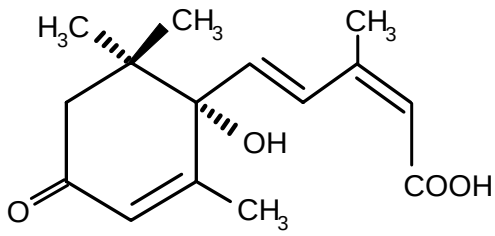
Cytokinins are believed to be transported preferentially in the xylem. In the *Rhinanthus*/barley system, however, all the cytokinins analysed, occured also in the phloem exudates resulting in remarkably strong phloem flows. Cytokinins have been detected also in phloem exudates of tree species (Weiler and Ziegler, 1981), *Ricinus communis* (Komor et al., 1993; Kamboj et al., 1998) and in white lupin (Taylor et al., 1990). According to Komboj et al. (1998) the major xylem sap cytokinin is ZR, whereas in the phloem mainly Z is transported. In this study, however, zeatin was also detected in the xylem in high concentrations as well as the zeatin riboside in the phloem (Tab. 3.26, Fig. 3.50). ZN (zeatin nucleotide) have been not investigated until now in transport fluids. In the *Rhinanthus*/barley system they can be found in the phloem and in the xylem (Fig. 3.51, Tab. 3.26).

Concerning the physiological functions of cytokinins the following points shall be discussed more in detail: (a) Cytokinins may act as antagonists of ABA on stomatal movement. It has been shown that stomata of *Rhinanthus* stay widely open although leaves have accumulated extremely high amounts of ABA. The significantly increased concentrations of cytokinins in *Rhinanthus* leaves after attachment may act as antagonists for ABA and keep the stomatal open. Such antagonistic effects of cytokinins on stomatal have been described previously by (Jewer and Incoll, 1980; Jewer and Incoll, 1981; Blackman and Davies 1984, Blackman and Davies, 1985; Chapin, 1991; Manfield and McAinsh, 1995). (b) Besides the role of cytokinins as cell division hormones, they also have been shown to regulate the differentiation and development of leaves. It has been suggested that cytokinins control mesophyll growth. This has been shown for the horseradish plant (*Amoracea lapathifolia*) where normal development of the interveinal tissue fails to occur when cytokinins transport from the root to shoot is impaired (Dore and Champion, cited after Wareing and Phillips, 1981). This would be consistent with the characteristic changes of leaf

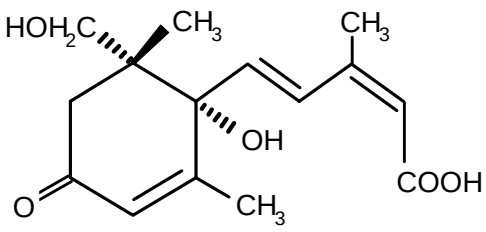
development of *Rhinanthus* after attachment.

The experiments of this work have shown that flows of nutrients and hormones created important signals within an hemiparasitic association. In the case of the *Rhinanthus minor* these signals show a subtle balance. That allows the potential host to survive the parasitic attack with relatively small damage. Thus an optimal development of *Rhinanthus* is guaranteed.

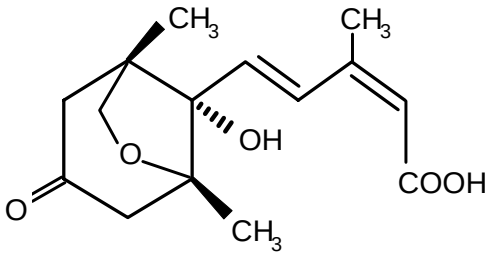
5. **Appendix**



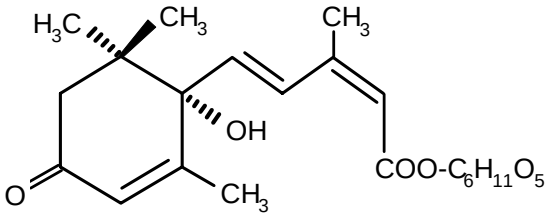
(+) – *cis* –abscisic acid



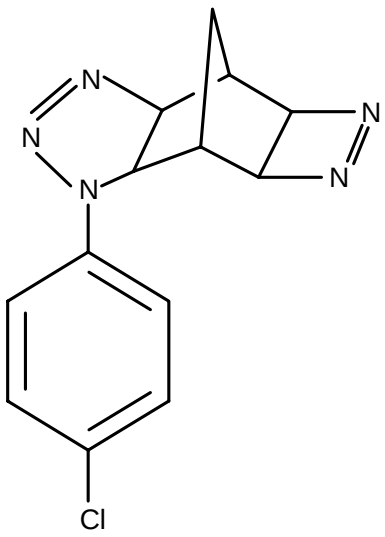
(+)-8'-Hydroxy-ABA



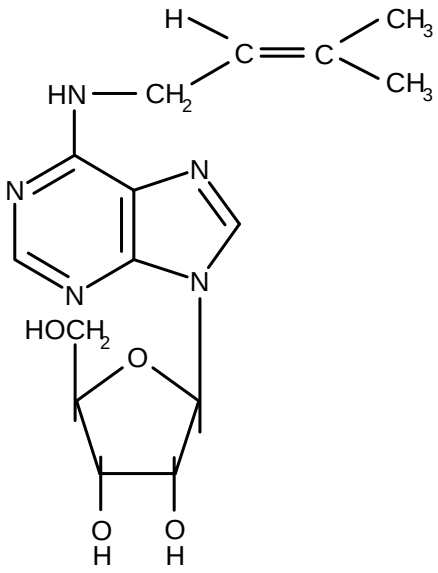
Phaseic acid



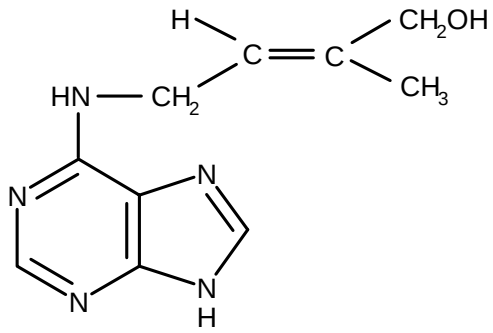
(+)-ABA-glucose ester



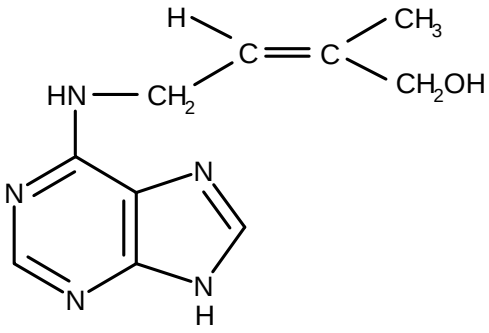
Tetcyclacis



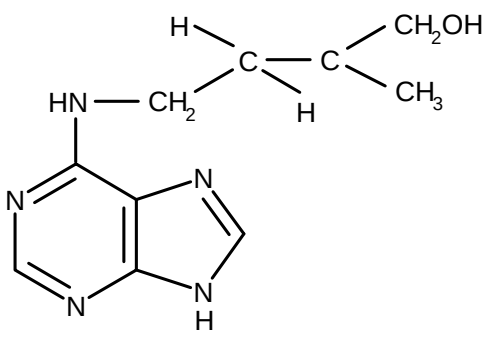
Isopentenyl-adenosine



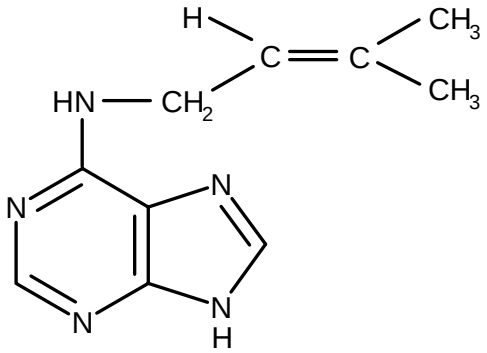
***trans* – Zeatin**



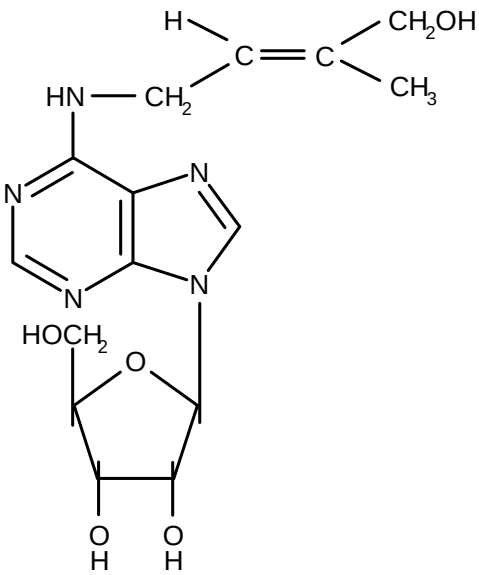
***cis* - Zeatin**



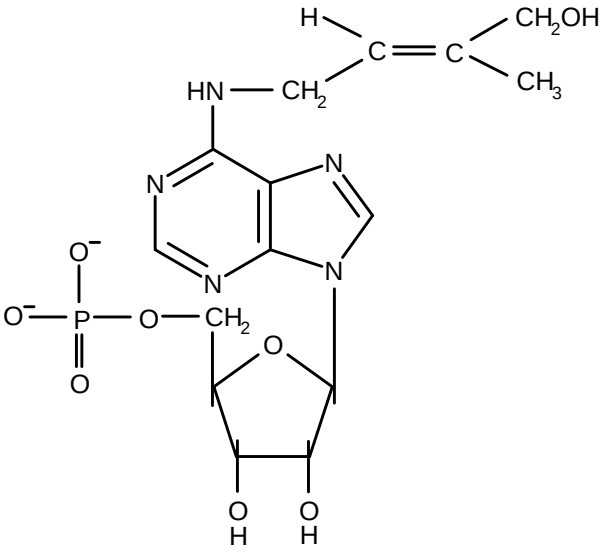
Dihydrozeatin



Isopentenyl adenine



Zeatin riboside



Zeatin nucleotide (Zeatin ribotide)

6. Zusammenfassung

An dem fakultativen Hemiparasiten *Rhinanthus minor*, einem Wurzelparasiten, und Gerste (*Hordeum vulgare*) als Wirtspflanze wurden verschiedene Aspekte des Wasserhaushaltes sowie die Flüsse von mineralischen Nährstoffen in den Leitbahnen von Wirt und Parasit, die Verteilung der Nährstoffe in den ganzen Pflanzen sowie deren Transport vom Wirt zum Parasiten untersucht. Auch für die pflanzlichen Hormone Abszisinsäure (ABA) und die Cytokinine vom Zeatintyp - Zeatin (Z), Zeatinribosid (ZR) und Zeatinnukleotid (ZN) - und die hauptsächlichen Transportmetabolite Mannit (im Parasiten) und Saccharose (in der Wirtspflanze) wurden deren Flüsse in den Leitbahnen der Pflanzen, ihre Verteilung in den Pflanzen, ihre metabolischen Umwandlungen und der mögliche Austausch zwischen Wirt und Parasit untersucht. Der Unersuchungszeitraum lag zwischen 41 und 54 Tagen nach der Aussaat und das war zugleich zwischen ca. 30 und 43 Tage nach dem erfolgreichen Befall des Wirtes durch den Parasiten.

Wasserhaushalt.

Ein Entzug von Xylemsaft durch die Haustorien des Parasiten aus den Wurzeln des Wirtes wird dadurch bewirkt, dass der Parasit eine wesentlich höhere Transpiration pro Blattfläche aufweist als die Wirtspflanze, sowie im speziellen Fall dadurch, dass bei parasitierenden *Rhinanthus*-Pflanzen trotz sehr hoher ABA-Konzentration in den Blättern die Stomata Tag und Nacht weit geöffnet sind. Bei einem verwandten Wurzel-Hemiparasiten, *Melampyrum arvense*, der im botanischen Garten auf verschiedenen Gräsern parasitierte, wurde anders als bei *Rhinanthus* im Freiland ein normales diurnales Öffnen und Schließen der Stomata beobachtet. Das anomale Spaltöffnungsverhalten bei *Rhinanthus* beruht freilich nicht auf anatomischen Besonderheiten, denn ein Schließen der Stomata ließ sich durch Applikation extrem hoher ABA – Konzentrationen induzieren. Bemerkenswerte Unterschiede zwischen

der Wirtspflanze und dem Parasiten wurden hinsichtlich der hydraulischen Leitfähigkeit der Keimwurzeln von Gerste mit vergleichsweise niedrigen Werten und derjenigen der Keimwurzel von *Rhinanthus* mit sehr hohen Werten gemessen. Letzteres könnte mit den beobachteten hohen ABA Konzentrationen in den Wurzeln des Parasiten zusammenhängen. Die Wasseraufnahme durch die ganze Pflanze, die Transpiration, die Deposition von Zellwasser in den Organen und die Wasserflüsse in den Leitbahnen innerhalb der Pflanze wurden in unabhängig wachsenden *Rhinanthus*- und in *Hordeum*- Pflanzen sowie innerhalb der parasitischen Assoziation zwischen beiden gemessen. Die Aufnahme von Wasser, seine Einlagerung und die Transpiration waren bei *Rhinanthus* nach erfolgreichem Befall einer Gersten – Wirtspflanze drastisch erhöht, wobei der Hauptanteil des vom Parasiten genutzten Wassers vom Wirt stammte und wodurch 20% der gesamten Wasseraufnahme der Gerstenwurzel entzogen wurde. In der vom Parasiten befallenen Gerste wurde dadurch jedoch die Wasseraufnahme im Vergleich zu einer unbefallenen Pflanze um 22% erniedrigt und zugleich auch das Wachstum behindert. Insgesamt kam es in der Wirtspflanze Gerste zu vermindertem Spross- und relativ gefördertem Wurzelwachstum, während im Parasiten *Rhinanthus* besonders das Sprosswachstum und weniger das Wurzelwachstum gesteigert wurde. Diese Veränderungen in der Wirtspflanze wurden deutlich intensiviert, wenn die Gerste von mehr als einem *Rhinanthus*-Parasiten befallen wurde.

Mineralstoffwechsel.

Düngung mit 5 mM NO_3^-

In parasitierenden *Rhinanthus*-Pflanzen war das Sprosswachstum 12 – fach, das der Wurzel aber nur zweifach im Vergleich zu selbstständig wachsenden (sehr kleinen) Pflanzen erhöht. Andererseits war in der vom Parasiten befallenen Gerste das Sprosswachstum – gemessen als Trockenmassezunahme – deutlich erniedrigt, und zwar um 33% in Blattspreiten und um 52% in Blattscheiden, während das

Wurzelwachstum nur geringfügig im Vergleich zur unbefallenen Kontrolle vermindert war. Die mit dem Wachstum verbundenen Inkorporationen von N, von P, von K sowie von Ca und Mg waren im Spross von parasitisch ernährtem *Rhinanthus* beträchtlich erhöht, besonders die von N und von P, die 18- oder sogar 42-fach im Vergleich zu den selbständig wachsenden Pflänzchen erhöht waren. Andererseits waren in der Wirtspflanze infolge des Parasitenbefalles die Inkorporationen der genannten Mineralstoffe in den Blattscheiden stärker als in den Blattspreiten erniedrigt. Eine Berechnung der Nährstoffflüsse zeigte, dass *Rhinanthus* dem Xylemsaft des Wirtes jeweils ähnliche Anteile der Nährstoffe entzog: 18% des insgesamt aufgenommenen N, 22% des P und 20% des K. Innerhalb der Wirtspflanze Gerste waren die Nettoflüsse fast aller mineralischen Nährstoff-Ionen in den Leitbahnen infolge des Parasitenbefalles erniedrigt, aber die Retranslokation im Phloem vom Spross zur Wurzel war für alle Nährstoffe – in Bezug auf den Xylemtransport - geringfügig erhöht. Quantitative Daten deuten an, dass das drastisch gesteigerte Sprosswachstum in parasitisch ernährtem *Rhinanthus* sowie das vermindere Sprosswachstum bei der Gerste nach dem Parasitenbefall kausal einerseits mit der beträchtlich erhöhten Zufuhr von gebundenem Stickstoff und Phosphor an den Parasiten und andererseits mit einem einsetzenden Mangel an diesen Nährstoffen in der befallenen Wirtspflanze verbunden ist. Insgesamt werden die Nährstoff-Flüsse von der Wirtspflanze zum Parasiten im Lichte einer nur geringfügigen Selektivität des Entzuges von Nährstoff-Ionen zusammen mit dem Xylemsaft aus dem Xylem des Wirtes durch die Haustorien des Wurzelparasiten *Rhinanthus minor* diskutiert.

Düngung mit 1 mM NO_3^- oder 1 mM NH_4^+

Das als Trockenmassezunahme gemessene Sprosswachstum von parasitisch ernährtem *Rhinanthus* war 19-fach (1 mM NO_3^-) bzw. 15-fach (1 mM NH_4^+) , das Wurzelwachstum aber nur 2-fach (1 mM NO_3^-) bzw. 2.9-fach (1 mM NH_4^+) im Vergleich zur selbständig wachsenden Kontrolle erhöht. In der Wirtspflanze Gerste war das Sprosswachstum deutlich vermindert, während das Wurzelwachstum

nur wenig beeinflusst war. Die mit dem Wachstum verbunden Zunahme an gebundenem N und P sowie an K, Ca und Mg waren im Spross von parasitisch ernährtem *Rhinanthus* stark vermehrt, und zwar besonders die Zunahme an gebundenem N bzw. P, die 20- bzw. 53-fach (bei 1 mM NO_3^-) und 18- bzw. 51-fach (bei 1 mM NH_4^+) im Vergleich zu selbständig wachsenden *Rhinanthus*- Pflänzchen erhöht waren. Innerhalb der Wirtspflanze Gerste waren fast alle Nettoflüsse der verschiedenen Nährstoff-Ionen infolge des Parasitenbefalles erniedrigt.

Flüsse von Mannit im Parasiten *Rhinanthus* und von Saccharose in der Wirtspflanze Gerste.

Im Falle einer Stickstoffernährung mit 5 mM NO_3^- war die Biosynthese von Mannit in den Blättern von parasitisch ernährtem *Rhinanthus* 16-fach im Vergleich zum selbständig wachsenden Pflänzchen erhöht und das führte zu einem 15-fach gesteigerten Phloemtransport zur Wurzel und einer 10-fach erhöhten Einlagerung von Mannit im Spross. Auch der Rücktransport von Mannit im Xylem von der Wurzel zum Spross war 10-fach erhöht. Im Falle einer Stickstoffernährung mit geringerer Konzentration war die Einlagerung von Mannit im Spross und in der Wurzel sowohl bei selbständig wachsendem als auch bei parasitisch ernährtem *Rhinanthus* im Vergleich zu 5 mM NO_3^- erhöht. In der Wirtspflanze Gerste konnte kein Mannit detektiert werden, auch nicht in den Wurzeln von *Rhinanthus*-befallenen Pflanzen, selbst nicht in direkter Nachbarschaft zu den Haustorien des Parasiten; das bedeutet, es erfolgte kein Rücktransport von Xylemsaft vom Parasiten zur Wirtspflanze. In den Gerstenpflanzen – bei Ernährung mit 5 mM oder mit 1 mM NO_3^- – wurde die Nettosynthese von Saccharose sowie ihre Einlagerung im Spross und ihr Transport im Phloem zur Wurzel erheblich infolge eines Befalles mit *Rhinanthus* erniedrigt. Im Xylemsaft von Gerste war die Konzentration von Saccharose unterhalb der Nachweisgrenze und folglich gab es keinen Hinweis auf einen Transfer von Saccharose aus dem Wirt in den Parasiten. Eine mögliche Bedeutung von Mannit für die hohen ABA-Konzentrationen in *Rhinanthus* wird diskutiert.

Konzentrationen und Flüsse von ABA.

Bei einer Stickstoffernährung mit 5 mM NO_3^- hatte ein parasitischer Befall mit *Rhinanthus* nur geringen oder keinen Einfluss auf die Flüsse, die Biosynthese oder den metabolischen Abbau von ABA in der Gerstenpflanze. Wohl aber wurde die wachstumsbedingte Einlagerung von ABA in den Blattspreiten (3-fach) und den Blattscheiden (2.4-fach) vermehrt, diejenige in der Wurzel blieb aber unverändert. In *Rhinanthus* dagegen führte der erfolgreiche Befall einer Wirtspflanze zu drastischen Veränderungen der Flüsse, des Metabolismus und der Einlagerung von ABA in die Gewebe, jeweils gemessen im Bezug auf eine ganze Pflanze. Auf dieser Basis war die ABA-Biosynthese in den Wurzeln nach erfolgreichem Befall eines Wirtes 12-fach höher und dies führte zu 14-fach höherem ABA-Transport im Xylem. Ein großer Anteil dieser ABA wurde im Spross metabolisiert, ein kleiner Anteil wurde in die Gewebe eingelagert. Auch die ABA-Flüsse im Phloem wurden 13-fach erhöht. Gleichzeitig waren die ABA-Konzentrationen in den Geweben und im Xylemsaft der parasitierenden *Rhinanthus*-Pflanze um eine Größenordnung höher als in den Geweben und im Xylemsaft der Wirtspflanze. Dasselbe gilt auch für einen Vergleich zwischen den hohen ABA-Konzentrationen im Xylemsaft von selbstständig wachsendem *Rhinanthus* und den niedrigen in nicht vom Parasiten befallener Gerste. Im Vergleich zur Ernährung mit 5 mM NO_3^- führte 1 mM NO_3^- oder auch 1 mM NH_4^+ zu einer Verdoppelung der ABA-Konzentrationen in den Blattspreiten von Gerste, hatte aber nur geringe oder keine Wirkung in den anderen Organen. Mögliche spezielle Funktionen von ABA für den Parasiten werden diskutiert.

Cytokinine vom Zeatin-Typ

Ein parasitischer Befall verminderte bei Zeatin (Z) seine Synthese in der Wurzel der Wirtspflanze Gerste (um 57%), den Xylemtransport (um 56%) und den

metabolischen Umbau in den Blattspreiten (um 71%), jedoch vergrößerte er zugleich substantiell (3-fach) den Phloemtransport von Zeatin in der Gerste. In *Rhinanthus* wurde die Einlagerung von Zeatin in der Wurzel und seine auf eine ganze Pflanze bezogenen Flüsse im Xylem und im Phloem 24-, 12- bzw. 29-fach nach erfolgreichem Befall von Gerste erhöht. Jedoch die Netto-Biosynthese von Zeatin in der Wurzel von *Rhinanthus* verminderte sich um 39% nach erfolgreichem Befall des Wirtes. Das bedeutet, dass ein großer Anteil (70 %) des im Xylem von *Rhinanthus* transportierten Zeatin zuvor dem Xylem der Wirtspflanze Gerste entzogen wurde. Im Spross selbständig wachsender *Rhinanthus*-Pflänzchen war die Einlagerung von Zeatin in die Sprossgewebe in der Bilanz negativ, d.h. Zeatin wurde abgebaut bzw. im Phloem abtransportiert.

Die Flüsse von Zeatinribosid (ZR) im Xylem von Gerste wurden nach einem Befall durch *Rhinanthus* um 39% erniedrigt, aber seine Retranslokation vom Spross zur Wurzel im Phloem, die 117% des Xylemtransportes betrug, wurde nur schwach (um 13%) nach dem Befall erniedrigt. Die Einlagerung von ZR in die Gewebe wurde in den Blattspreiten, den Blattscheiden und den Wurzeln nicht signifikant beeinflusst. Andererseits waren nach einem erfolgreichen Befall von Gerste in *Rhinanthus* die Einlagerung von ZR in Wurzelgewebe und seine Flüsse im Xylem und im Phloem, alle im Bezug auf die ganze *Rhinanthus*-Pflanze, 12-, 18- bzw. 88-fach erhöht. Ein beträchtlicher Anteil (57%) des im Xylem von *Rhinanthus* transportierten ZR entstammte freilich wiederum dem Xylem der Wurzel der Wirtspflanze Gerste. Im Spross selbständig wachsender *Rhinanthus*-Pflänzchen nahm die Menge an ZR (wie die von Zeatin) im Laufe des Untersuchungs-Intervalles ab, wobei ein beträchtlicher Anteil im Spross metabolisiert wurde und der Rest mit dem Phloem in die Wurzel transportiert. In die an den Gerstenwurzeln sitzenden Haustorien des Parasiten *Rhinanthus* wurden beträchtliche Mengen an Zeatin und an ZR eingelagert. Auch die Flüsse und die Einlagerung der Zeatinnukleotide in die Gewebe sind untersucht worden. Eine mögliche physiologische Funktion der aus dem Wirt stammenden großen Mengen an Zeatin und an ZR für das gesteigerte Wachstum und die Stomata-Öffnung im parasitierenden *Rhinanthus* wird diskutiert.

7. Summary

Using the facultative root hemiparasite *Rhinanthus minor* and *Hordeum vulgare* as a host, several aspects of water relations, the flows and partitioning of mineral nutrients, the flows, depositions and metabolism of abscisic acid (ABA) and zeatin type cytokinins (zeatin Z, zeatin riboside ZR, zeatin nucleotide ZN) within the host, the parasite and between host and parasite and the flows and partitioning of the transport metabolites mannitol in the parasite, and of sucrose in the host, have been studied during the study period 41 to 54 days after planting, i.e about 30 to 43 days after successful attachment of the parasite to the host.

Water relations

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 wide open all day and night despite extremely high ABA concentrations in the leaves. By comparison, another related root hemiparasite, *Melampyrum arvense*, parasitising on various grasses in the field (botanic garden), 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* the seminal root 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 from the host, thereby scavenging 20% of the total water taken up by the host's roots. This water uptake by the parasitised host, however, due to a parasite induced reduction in the hosts growth, was decreased by 22% as compared to non- parasitised barley. The overall changes in growth-related water deposition in host and parasite pointed to decreased shoot and relatively favoured root growth in the host and to strongly favoured shoot growth and less strongly increased root growth only in the parasite. These changes in the host became more severe, when more than one *Rhinanthus* was parasitising one barley plant.

Mineral nutrients relations

5 mM NO_3^- supply

In parasitising *Rhinanthus* shoot growth was 12-fold, but root growth only twofold increased compared to the non-parasitising (very small) plants. On the other hand, in the *Hordeum* host, shoot dry matter growth was clearly reduced, by 33% in leaf laminae and by 52% in leaf sheaths, whereas root growth was only slightly reduced as a consequence of parasitism. Growth-dependent increments of total N and P and of K, Ca and Mg in parasitising *Rhinanthus* shoot were strongly increased, particularly increments of total N and P, which were 18 and 42 times, respectively, higher than in the small solitary *Rhinanthus*. On the other hand, increments of the above mineral nutrients in leaf sheaths of parasitised *Hordeum vulgare* were more strongly decreased than in leaf laminae in response to parasitic attack. Estimation of the flows of nutrients revealed that *Rhinanthus* withdrew from the host xylem sap about the same percentage of each nutrients: 18% of total N, 22% of P and 20% of K. Within the host almost all net flows of nutrient ions were decreased due to parasitism, but retranslocation from shoot to root-as related to xylem flow-was somewhat increased for all nutrients. Quantitative information is provided to show that the substantially increased growth in the shoot of attached *Rhinanthus* and the observed decrease in

Hordeum shoot growth after infection were related to strongly elevated supply of nitrogen and phosphorus in the parasite and to incipient deficiency of these nutrients in the parasitised host. The flows of nutrients between host and parasite are discussed in terms of low selectivity of nutrient abstraction from the host xylem by the hemiparasite *Rhinanthus minor*.

1 mM NO_3^- or 1 mM NH_4^+ supply

Rhinanthus shoot growth as measured by dry matter increase, was 19-fold (1 mM NO_3^-) and 15-fold (1 mM NH_4^+), but root growth only twofold (1 mM NO_3^-) and 2.9-fold (1 mM NH_4^+) increased-relative to singly growing *Rhinanthus*-when parasitising on host barley. In the *Hordeum* host, shoot dry matter growth was clearly reduced, whereas root growth was only slightly affected. Growth-dependent increments of total N and P and of K, Ca and Mg in parasitising *Rhinanthus* shoot were strongly increased, particularly increments of total N or of P, which were 20 or 53 times (1 mM NO_3^-) and 18 or 51 times (1 mM NH_4^+) , respectively, higher than those in solitary *Rhinanthus*. Within the host almost all net flows of nutrient ions were decreased due to parasitism.

Flows of mannitol in parasite and sucrose flows in host barley

When the plants were supplied with 5 mM NO_3^-, the biosynthesis of mannitol in *Rhinanthus* shoots increased 16-fold by parasitism, resulting in a 15-fold higher mannitol flow in the phloem and a 10-fold higher deposition in the shoot. Also the backward transport of mannitol in the xylem were increased 10-fold after attachment. Lower level nitrogen supply increased the deposition of mannitol in both single and attached *Rhinanthus* shoot and root. No mannitol was found in barley roots even in the direct vicinity of the haustoria. This indicates there are no backward transport of xylem sap from parasite to host. Compared to unparasitised barley, the net biosynthesis and deposition of sucrose in the shoot and the phloem flow was

decreased substantially when plants were supplied with 5 mM NO_3^- or 1 mM NO_3^-. No sucrose has been detected in barley xylem sap and consequently there was no indication of a sucrose transfer from the host to the parasite. A possible involvement of mannitol in the abscisic acid relations of the parasite is discussed.

ABA relations

When the plants were supplied with 5 mM NO_3^-, there were weak or no effects of parasitism on ABA flows, biosynthesis and ABA degradation in barley. However, ABA growth-dependent deposition was significantly increased in the leaf laminae (3 fold) and in leaf sheath (2.4 fold), but not in roots. Dramatic changes in ABA flows, metabolism and deposition on a per plant basis, however, have been observed in *Rhinanthus*. Biosynthesis in the roots was 12-fold higher after attachment resulting in 14-fold higher ABA flows in the xylem. A large portion of this ABA was metabolised, a small portion was deposited. Phloem flows of ABA were increased 13-fold after attachment. The concentrations of ABA in tissues and xylem sap were higher in attached *Rhinanthus* by an order of magnitude than in host tissues and xylem sap. Similar dramatic difference existed when comparing the high concentrations in the xylem sap of single *Rhinanthus* with unparasitised barley. As compared to 5 mM NO_3^-, lower NO_3^- or 1 mM NH_4^+ supply doubled the ABA concentrations in barley leaf laminae, while having only small or no significant effects in the other organs. The possible special functions of ABA for the parasite are discussed.

Zeatin type cytokinins relations

Parasitism decreased, in the case of zeatin (Z), the synthesis (by 57%) in the root, xylem flows (by 56%) and metabolism (by 71%) in leaf laminae, however, increased the phloem flows of zeatin massively (3-fold) in host barley. The deposition of zeatin in the root of *Rhinanthus* and the flowing in xylem and phloem were 24, 12, 29-fold, respectively, increased after successfully attaching to the host barley. However, net

biosynthesis of zeatin in *Rhinanthus* roots decreased by 39% after attachment. This indicates that a large portion (70%) of xylem flow of zeatin in attached *Rhinanthus* was extracted from the host. In singly growing *Rhinanthus* plants, the balance of zeatin deposition in the shoot was negative, i.e. zeatin was metabolised and exported back to root in the phloem.

The xylem flows of zeatin riboside (ZR) in barley decreased by 39% after infected by *Rhinanthus*; phloem flow, which was 117% relative to xylem flow was less decreased (by 13%) after infection. Deposition of ZR has not been significantly affected in the leaf laminae, in leaf sheaths and roots. After parasitising on the host barley depositions in root, xylem flow and phloem flow increased 12, 18, 88–fold respectively in *Rhinanthus*. A large portion (57%) of xylem flow of ZR in attached *Rhinanthus* was extracted from the host. In single *Rhinanthus* increament of shoot zeatin riboside was negative and a substantial portion was degraded in shoot and the rest was retranslocated back to the root in the phloem. A significant depositions of Z and ZR were detected in the haustoria of the *Rhinanthus*/barley association. Flows and deposition of zeatin nucleotides also have been investigated. The possible physiological functions of the large quantities of Z and ZR derived from the host barley, for the improved growth and the stomatal opening in the parasitising *Rhinanthus* are discussed.

8. Abbreviations

ABA	abscisic acid
ABA_{xyl}	ABA flows in xylem
Ad	adenine
Asn	asparagine
Asp	aspartic acid
BA	benzyladenine
DABCO	1,4-diazabicyclo-(2,2,2)octane
DHZ	dihydrozeatin
DHZR	dihydrozeatin riboside
DW	dry weight
EDAC	1-Ethyl-3-(3-Dimethylamino-propyl)carbodiimide
EGTA	Ethylenedioxy-bis-(ethylenenitrilo)-tetraacetic acid
ELISA	Enzyme Linked Immuno Sorbent Assay
Fig.	Figure
FW	fresh weight
Gln	glutamine
HEPE	N-(2-Hydroxyethyl) piperazine-N'-2-ethanesulfonic acid
iP	isopentenyl adenine
iPa	isopentenyladenosine
J_{cp}	carbon translocation from shoot to root
$J_{H2O,X}$	net xylem water flows
Js,p	net flows of nutrients in phloem
$J_{s,x}$	nutrient flows in xylem
Lpr	root hydraulic conductivity
Na_2EDTA	ethylenediaminetetraacetic acid disodium salt
PA	phaseic acid
PBS	phosphate buffered saline
PEG	Polyethylenglycoldistearat
pH	hydrogen ion concentration, negative logarithm
P_r	root pressure
RAMIG	rabbit anti-mouse immunoglobuline
Tab.	Table
TBS-buffer	Tris buffered saline
Tet	tetcyclacis
TLC	thin layer chromatography
Tris	Tris hydroxymethy amino methane
Z	zeatin
ZN	zeatin nucleotide
ZR	zeatin riboside

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Publication List

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Jiang F, Jeschke WD, Hartung W (2004c) Contents and flows of assimilates (mannitol and sucrose) in the hemiparasitic *Rhinanthus minor/Hordeum vulgare* association. *Folia Geobotanica*. submitted.

Presentations

Hartung W, **Jiang F** (2002) Water flows in the parasitic association *Rhinanthus minor/Hordeum vulgare*. 1st Symposium Sonderforschungsbereich 567, Mechanismen der interspezifischen Interaction von Organismen, Retzbach, Germany, December 4-6, 2002.

Jiang F, Jeschke WD, Hartung W (2004) The abscisic acid (ABA) relations of the parasitic association *Rhinanthus minor/Hordeum vulgare*. Comparative Biochemistry and Physiology 137, (Suppl.) S205.

Jiang F, Jeschke WD, Hartung W (2004) Contents and flows of mannitol in the hemiparasite *Rhinanthus minor* before and after attachment to *Hordeum vulgare*. 1st International symposium on the biology of non-weedy hemiparasitic (ex-)Scrophulareaceae. Wageningen, the Netherlands 15-16 April 2004.

Poster presentations

Jiang F, Jeschke WD, Hartung W (2002) Haustorium of the *Hordeum/Rhinanthus* association. 1st Symposium Sonderforschungsbereich 567, Mechanismen der interspezifischen Interaction von Organismen, Retzbach, Germany, December 4-6, 2002.

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ERKLÄRUNG

Hiermit erkläre ich, die vorliegende Dissertation selbst angefertigt und nur die angegebenen Quellen und Hilfsmittel verwendet zu haben.

Weiterhin erkläre ich, dass die vorliegende Dissertation weder in gleicher noch ähnlicher Form einem anderen Prüfungsverfahren vorgelegt wurde.

Hiermit bewerbe ich mich erstmals um den Doktorgrad der Naturwissenschaften der Bayerischen Julius-Maximilians-Universität Würzburg.

Würzburg, 2004

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