Chapter 6
The Physiology of the Established Parasite–Host Association

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6.1 General Physiological Considerations

The connection between parasite and host lies at the core of parasitism. The haustorium is the feature that defines parasitism (see Chap. 2), and it forms a unique point of interaction between a parasitic plant and its environment. The haustorium shares some functional characteristics with roots in that it absorbs water and minerals, but it also acts as an organ for carbon acquisition as it absorbs sugars and other organic molecules similar to the way a minor leaf vein uploads the products of photosynthesis. Depending on the parasitic species under consideration, the materials obtained from the host may include part or all of the resources needed for parasite growth. This chapter concentrates on the physiological processes associated with the mature haustorium, the transfer of materials and the relevant metabolic capacity needed to integrate with overall parasite metabolism.

Given the central importance of haustorial function in resource acquisition, it is surprising that many details of haustorium physiology remain poorly characterized. What types of materials are translocated from host to parasite? By what mechanisms do these transfers occur? Is the parasite selective in extracting certain compounds and excluding others? Do parasites induce changes in the host that increase the flow of nutrients? And how does the parasite use the acquired material?

The answers to these questions are complicated by the fact that parasite species may have widely differing nutritional dependencies on their hosts. The Orobanchaceae is remarkable in that the full trophic spectrum of parasitism is represented among its various species (Westwood et al. 2010). Levels of host dependence range from facultative hemiparasites to obligate hemiparasites and obligate holoparasites. Each of these has specific requirements of their hosts and

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hence different strategies for resource acquisition. When considering parasites, the specific nature of vascular continuity (xylem and phloem connections, see Sect. 3.9) is more important than whether the parasite attaches to shoot or root, so care must be taken in generalizing trends across different parasites (Hibberd and Jeschke 2001; Irving and Cameron 2009). With this in mind, it is useful to consider key features associated with xylem and phloem feeding.

6.1.1 Xylem Feeders

All parasites have xylem connections that take up water and minerals from their hosts, so a major difference among parasites lies in the extent to which they also have phloem or other symplastic connections. Parasites that rely on xylem connections as the primary route of transfer from hosts are considered to be xylem feeders. Among xylem-feeding parasites, further distinctions are made between facultative hemiparasites that have a significant functional root system of their own (and thus have a viable alternative to haustorial connections) and obligate hemiparasites that are more dependent on the haustorium for capturing host resources (see Chap. 2). The xylem-feeding facultative parasites that have been the subjects of most research into haustorium physiology and metabolism are *Rhinanthus* and *Triphysaria* spp., while studies of obligate hemiparasites have focused almost exclusively on *Striga* spp. Anatomical studies, which are described in Sect. 2.1.5.1, demonstrate direct xylem connections (including oscula), e.g. between *Rhinanthus* and its hosts (Cameron et al. 2006), with similar structures also being observed in *Triphysaria* (Heide-Jørgensen and Kuijt 1995), some *Striga* species (Dörr 1997) and other parasites. Indeed, even obligate holoparasites appear to have luminal contact with the xylem of their hosts (Dörr 1996), so there is a universal ability among parasites to acquire resources from the host xylem.

Dissolved nutrients moving from host to parasite in the xylem stream appear to be assimilated in the parasite haustorium. High metabolic capacity in this region is suggested by the hyaline tissue anatomy (see Sect. 3.12.1) present in the haustoria of many hemiparasites such as *Striga* (Dörr 1997; Neumann et al. 1999) and *Rhinanthus* (Jiang et al. 2010). In *Triphysaria*, which lacks a distinctive hyaline tissue, the cells of the haustorium adjacent to the xylem bridge nevertheless have densely staining cytoplasm and invaginated plasma membranes characteristic of hyaline tissue cells (Heide-Jørgensen and Kuijt 1995). These cells are similar to xylem parenchyma cells of non-parasitic plants that efficiently import solutes from xylem into neighbouring tissues. The transfer of sugars from the xylem would provide metabolic energy to cells of the haustorial region, permit sugar conversion into parasite-specific compounds, and avoid potentially unfavourable accumulation of solutes in the xylem.

Parasite maintenance of a distinct metabolic profile, which generates a favourable osmotic gradient relative to the host and drives the uptake of water, is an important requirement for parasite competitiveness with respect to the host. It is

therefore reasonable to hypothesize that an important role of the haustorium is the initial metabolism of host-derived resources.

6.1.2 Phloem Feeders

The phloem-feeding holoparasites that have received the most research attention among Orobanchaceae are species of the genera *Orobanche* and *Phelipanche*. These parasites have direct symplastic connections between their own cells and the sieve elements of their hosts as observed by electron microscopy (Dörr and Kollmann 1995). The discovery of such symplastic connections is supported by physiological and tracer studies that indicate direct phloem transmission of dyes, proteins (Aly et al. 2011) and even viruses (Gal-On et al. 2009). A potential addition to this category could include *Striga gesnerioides*, which has chimeric plasmodesmata bridging its cells with those of the host rather than direct phloem connections (Dörr 1996). The precise role of this latter symplastic feeding is uncertain because the plasmodesmatal connections may be short lived (Reiss and Bailey 1998), but measures of carbon gain indicate that *S. gesnerioides* obtains nearly all of its photosynthates from hosts, which is similar to known phloem feeders (Press and Graves 1991).

Symplastic continuity has important implications for host–parasite transfer of materials. Rather than relying on extraction of nutrients from the host xylem stream, symplast-feeding parasites may absorb a potentially wider range of photo-assimilates directly from the host phloem. These parasites have much lower water needs because they grow underground for a prolonged period of time before their shoots emerge from the soil, and even then they lack transpiring expanded leaves. This suggests a different type of metabolic interaction of holoparasites with their hosts as compared to hemiparasites, a difference that is reflected in anatomy (see Sect. 3.8). The haustoria of *Orobanche* and *Phelipanche* lack the hyaline tissue that is typical of some hemiparasites. This may indicate a lower rate of nutrient uptake from the xylem stream, although it is also possible that the function of the hyaline tissue is accomplished in the tubercle (see Sect. 3.14). No physiological studies have directly addressed this issue.

6.1.3 Apoplastic vs. Symplastic Phloem Loading

The parallels between haustorium feeding strategies described above and physiological mechanisms of phloem loading are informative. Phloem loading has typically been studied in relation to sugar movement from mesophyll cells into phloem cells of minor veins in leaves for subsequent translocation to other parts of the plant. However, loading is also important in the context of parasitism, because the parasite haustorium functions in acquiring carbon from the host analogously to the leaf

minor veins. Furthermore, variations in phloem loading also correspond with the different parasite feeding strategies, so the general concept has potential application to all parasites.

Two broad categories of phloem loading, symplastic and apoplastic, are recognized in plants, although gradations exist between these two and some species may not fall clearly into either camp (Gamalei 1989; Rennie and Turgeon 2009). Symplastic loading takes place when sugars are transported into phloem companion cells from surrounding cells via plasmodesmata. This process is passive and based on diffusion along concentration gradients. Apoplastic loading, in contrast, involves exporting sugars from cells around the vein into the apoplastic space and then rapidly re-importing them into the phloem companion cells. This process requires active transport of sugars and carries an energetic cost. Symplastic- and apoplastic-transporting plants differ anatomically in the number of plasmodesmatal connections between companion cells and surrounding cells. Symplastic loaders have many more connections, as would be required for the transport of sugars via plasmodesmata. Apoplastic loading species have phloem that is more isolated symplastically, with fewer plasmodesmatal connections to neighbouring cells. There is also a biochemical difference, as all apoplastic loaders transport sucrose whereas symplastic loading species typically translocate raffinose series oligosaccharides (raffinose, stachyose, verbascose and galactinol). The use of these sugars enables 'polymer trapping', whereby simple sugars move from one cell to another where they are polymerized into higher-order polysaccharides that do not readily re-diffuse out of the cell, thereby creating a concentration gradient that facilitates further movement. The principles of how apoplastic and symplastic loading could be applied to the xylem- and phloem-feeding strategies of parasites are illustrated in Fig. 6.1.

The ability to load phloem via apoplastic vs. symplastic mechanisms could have been a pre-adaption in the evolution of parasitism. The loading method tends to be a characteristic feature of plant families, with most members of a family usually having the same mechanism of phloem loading. Unfortunately, a comprehensive study of loading in the Orobanchaceae has not been completed and the literature contains conflicting reports. The only study of phloem loading mechanisms in the Orobanchaceae reported that the leaves of the hemiparasites *Castilleja* and *Pedicularis* have the symplastic type, while the hemiparasites *Cymbaria* and *Melampyrum* (which belong to different clades of the Orobanchaceae; see Sect. 14.2.2 and Fig. 14.2) have variations of apoplastic loading (Gamalei 1989). Study of three non-parasitic members of the related family Scrophulariaceae concluded that they use a combination of symplastic loading with polymer trapping (Rennie and Turgeon 2009). Finding raffinose series oligosaccharides in parasites would be an indirect indicator of symplastic loading style, but the only report of stachyose or related sugars in parasitic Orobanchaceae was in *Orobanche foetida* growing on faba bean where it seems that these carbohydrates are derived from the host rather than synthesized by the parasite (Abbes et al. 2009).

An alternate hypothesis is that parasite success is more related to the phloem loading mechanism of the hosts than to that of the parasites themselves. After all,

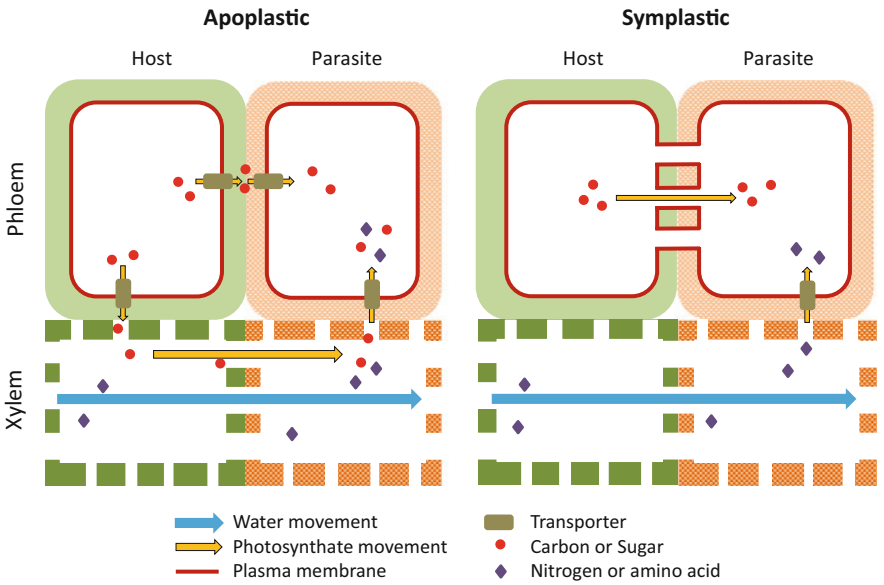

Fig. 6.1 Pathways of nutrient transfer from host to parasite. Schematic diagram of apoplastic and symplastic pathways of nutrient transfer from host to parasite. Apoplastic movement requires unloading sugars into the apoplast and then uptake into the parasite cell. Uptake may occur directly in adjacent cells or after some distance of travel in xylem. Symplastic transfer of sugars occurs through plasmodesmata or sieve pore connections between host and parasite. Uptake of water and nutrients from host xylem may still be important for phloem-feeding parasites

host resources must be available for uptake by the parasite, and in the case of xylem-feeding parasites, there would be clear advantages to attacking hosts that are genetically preprogrammed to export resources into the apoplastic space (as in Fig. 6.1). Whether by coincidence or evolution, this appears to hold true for many hosts of xylem-feeding parasites. Apoplastic loading is regarded as a more evolutionarily advanced development and is characteristic of grasses and herbaceous plants (Gamalei 1989). In barley (and apparently also in maize and other Poaceae) the loading and unloading of phloem appears to be apoplastic (Evert et al. 1996). In certain parasitic plant interactions with hosts, the host becomes enriched in levels of specific nitrogenous compounds needed by the parasite (Pageau et al. 2003), suggesting that part of the parasite effect is the reprogramming of host metabolism in a way that promotes export into the apoplast of resources needed by the parasite.

Maintaining a high concentration of osmotically active solutes in the parasite relative to the host is evolutionarily advantageous for the parasite. In this sense the use of apoplastic pathways of resource capture seems to maintain membrane separation between parasite and host. A membrane barrier is necessary for maintaining osmotic differentials to create a driving force for water and solutes into the parasite. The barrier may also help protect the parasite from risks associated with open connections with the host. Unregulated connections could result in

accumulation of unwanted materials from the host, including salts, phytoalexins, and even pathogens detrimental to parasite health (see Sects. 3.9.1 and 3.13). Such separation also seems to maintain metabolic autonomy. Viewed in this light, the evolution of phloem feeding (symplastic loading) in holoparasites is all the more remarkable.

There is no evidence that parasitic Orobanchaceae evolved from a progenitor having a specific style of phloem loading. However, it is perhaps not surprising that core functionalities of the haustorium have features known from other plant physiological processes. The mechanisms of nutrient transfer are part of the basic repertoire of all plant organs. What is special about parasitism—and the haustorium—is the evolutionary reconfiguring of many diverse processes into a new organ.

6.2 Nutrient Acquisition and Transport

6.2.1 Water and Mineral Nutrients

Water and mineral nutrients move from host to parasite in what appears to be a continuous xylem pathway. Electron micrographs of the host–parasite junction reveal parasite xylem elements in open connection with their hosts (Dörr 1996) that facilitate transmission of macromolecules of at least up to 70 kDa (Aly et al. 2011). Therefore, the point of regulation for uptake of xylem contents exists not at the host–parasite boundary, but within the parasite system at the interface of all cells where there is unloading of xylem contents into the symplast. The water potential of parasite cells in the haustorium, stem and leaves (in hemiparasites) determines the flow.

Important aspects of xylem feeding by parasites include the types of solutes available in the host xylem stream, how the solutes are accumulated by the parasite and how the overall movement of water is regulated. In general, the flow of mineral nutrients into the parasite reflects the content of the host xylem (Irving and Cameron 2009). Nevertheless, parasites selectively accumulate certain cations and potassium is foremost among these, always occurring in concentrations higher than those recorded in corresponding host tissues (Table 6.1). The facultative parasite *Odontites lutea* accumulates 1.5 times more potassium than its host, while obligate parasites have four- to fivefold higher concentrations than their hosts. The obligate parasites appear to reach this same level of potassium accumulation despite different rates of xylem utilization between various species, e.g. between *Striga* and *Orobanche* (Hibberd et al. 1999). Calcium, magnesium and sodium are also among the minerals with highest concentrations in parasites (Abbes et al. 2009), but these rarely accumulate to levels higher than those found in host tissues.

Table 6.1 Concentrations of mineral nutrients in some Orobanchaceae parasites

Mineral	*Odontites lutea* leaf[a] (μmol g^{-1} DW)	*Striga hermonthica* leaf[b] (μmol g^{-1} DW)	*Orobanche cernua* xylem sap[c] (mM)	*O. fasciculate* tissue[d] (mM)
K^+	75 (1.50)	1,381 (5.35)	19.4 (4.04)	501.3 (4.45)
Ca^{2+}	146 (0.49)	45 (1.38)	1.85 (1.54)	331.8 (0.47)
Mg^{2+}	46 (0.66)	617 (2.94)	1.78 (2.44)	148.1 (0.49)
Na^+	–	–	0.41 (1.05)	69.6 (1.60)
Fe^{2+}	0.99 (0.14)	5 (0.75)	–	21.4 (0.53)
Zn^{2+}	0.54 (0.93)	1 (0.32)	–	0.3 (0.75)
Cu^{2+}	0.16 (1.53)	–	–	0.2 (0.59)
Mn^{2+}	–	3 (1.08)	–	0.8 (0.65)

The number in parentheses is the ratio of cation concentration in parasites divided by the concentration in the corresponding host tissue. Values from references b and c were converted to mM

O. fasciculate = *Aphyllon fasciculatum*

[a]Llugany et al. (2009)

[b]Stewart et al. (1984)

[c]Hibberd et al. (1999)

[d]Brotherson et al. (2005)

The driving force for water flow is the gradient of water potential between host and parasite (Ehleringer and Marshall 1995). Parasites maintain lower water potentials relative to hosts through accumulation of solutes, open stomata or a combination of these, depending on the type of parasite. Parasites lower their water potentials by accumulating high levels of osmotically active compounds such as mineral ions (e.g. potassium), sugars and sugar alcohols (Sect. 6.2.2.2). In hemiparasites water potentials are decreased by maintaining open leaf stomata even when the host is under severe water stress (Smith and Stewart 1990). For example, *Rhinanthus* and *S. hermonthica* plants that are attached to hosts keep open stomata (Jiang et al. 2003), resulting in a high rate of transpiration that preserves high flow of xylem contents into the parasite. It is notable that the stomata in free-living *Rhinanthus* are regulated in a more usual manner, being closed much of the time, but after *Rhinanthus* attaches to a host, the leaf stomata remain continuously open (Jiang et al. 2003).[1] Models of host resource acquisition by *Rhinanthus* indicate that water use by the parasite is a relatively minor fraction of the total water flux of the host (Fig. 6.2). Holoparasites have lower transpiration rates than hemiparasites because holoparasites are underground for much of their lives and even after emergence lack the expanded leaves necessary for effective transpiration.

The flow of water from host to parasite could be limited by the number of haustorial attachments (Cameron et al. 2005) and the size of the connections. The cross-sectional area for xylem transport of a lateral haustorium is small, measured

[1] Guttation through glandular hydathodes may also contribute to xylem flow, as suggested by Renaudin and Garrigues (1967, Sur l'ultrastructure des glandes en bouclier de *Lathraea clandestina* L. et leur role physiologique. C R Acad Sci Paris 264:1984–1987).

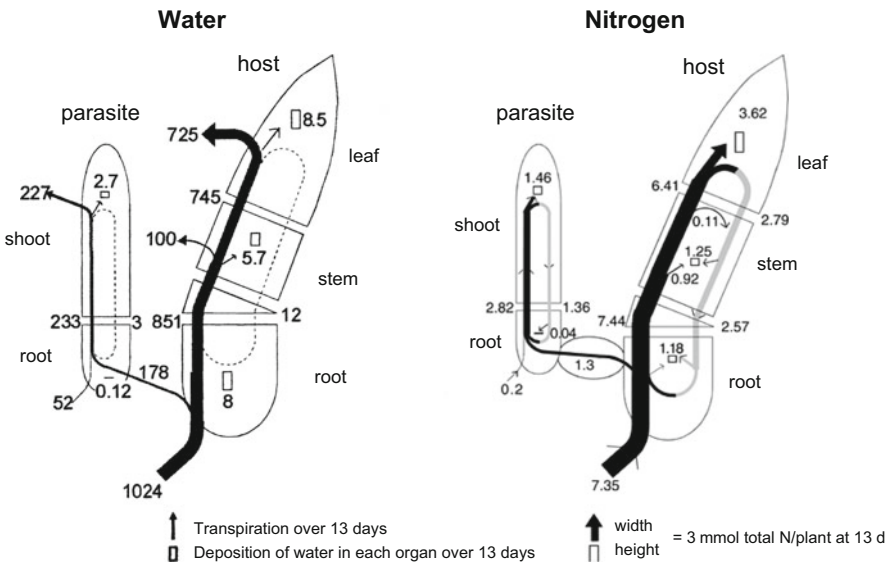

Fig. 6.2 Water and nitrogen transfer from host to parasite. Diagrams for flow of water and nitrogen from barley to *Rhinanthus minor*. Width of the arrows represents flow for xylem (*black arrows*) and phloem (*dashed*/*grey arrows*). The numbers indicate the water transpiration (ml) or nitrogen flux (mmol) in the respective figures (figure is compiled from Jiang et al. (2003) with permission of Oxford University Press and Jiang et al. (2004a) with permission from CSIRO Publishing)

at a tenth of the xylem area of a host stem in one case (Hibberd et al. 1999). Even a mature parasite with multiple haustoria has much less physical capacity for translocation compared to the vascular system of an autotrophic plant. The small haustorial cross section is compensated by a decrease of the hydraulic conductivity of the parasite tissues, which could be attributed in part to anatomical adaptations. *Rhinanthus* roots apparently lack Casparian strips in the endodermis and hypodermis (Jiang et al. 2003) that would restrict apoplastic water movement. Also, the high abscisic acid (ABA) concentrations typical of parasites (see Sect. 6.4.1) are correlated with increased hydraulic conductivity of cell membranes (Hose et al. 2001), which may further facilitate efficient water uptake through the haustoria.

6.2.2 Carbon

6.2.2.1 Carbon Assimilation

The proportion of organic carbon and nitrogen derived from hosts is directly related to the level of parasite dependence on hosts (Irving and Cameron 2009). Hemiparasites are able to fix some or all their carbon through photosynthesis so

are primarily parasitic for nitrogen (inorganic or organic forms), while holoparasites require both nitrogen and carbon from their hosts. In terms of carbon, a rule of thumb is that facultative parasites obtain 10 % of their carbon from hosts, obligate hemiparasites such as *Striga* (emerged) take about 30 % and holoparasites such as *Orobanche* take 100 % (Irving and Cameron 2009). Of course, the exact proportion of carbon acquired depends on many factors including growth stage of the parasite (e.g. underground *Striga* is 100 % dependent on hosts), host species and environmental availability of resources for host and parasite. This may explain the wide ranges of host carbon assimilation that are documented in the literature.

Estimated carbon budgets for host–parasite interactions highlight differences among parasite species and the pronounced influence of host species on parasite metabolism. The heterotrophic carbon gain for the hemiparasites *Euphrasia rostkoviana* and *R. minor* was estimated at 25 and 50 %, respectively, with the greater carbon gain by *R. minor* being consistent with its greater negative impact on hosts (Těšitel et al. 2010). The estimates of photosynthesis rates for *Striga* species suggest that the parasites are not capable of fixing enough carbon themselves to meet their own respiration and growth needs, thus being dependent on their hosts for most carbon (Press et al. 1991). Specifically, the percent of carbon *S. hermonthica* gained from hosts was estimated to be around 80 % when growing on millet, 66 % on maize and 40 % on sorghum. *S. gesnerioides* parasitizing cowpea gained greater than 99 % of its carbon from cowpea, placing it at the extreme of carbon dependence for these genera (Press and Graves 1991).

Holoparasites, by definition, derive all of their carbon needs from the host, with various species taking up and accumulating different sugars. For example, it is estimated that greater than 99 % of *O. cernua* carbon is taken from tobacco phloem (Hibberd et al. 1999). The question of which form of carbon is acquired by the parasites is more difficult to determine because the parasites rapidly convert host compounds into parasite metabolites. The main host-derived compound in *O. crenata* growing on $^{14}CO_2$-fed faba beans was sucrose, followed by glucose and fructose (Aber et al. 1983). The concentrations of all sugars in parasite tubercles were between six- and eightfold higher than concentrations of the same compounds in the host roots, indicating a strong accumulation by the parasite. Sucrose is not accumulated in *P. ramosa* but rather is metabolized to other compounds such as hexoses, mannitol and starch. It was hypothesized that cleavage of sucrose into glucose and fructose by invertases and the reverse action of sucrose synthases improve the osmotic potential of the parasite. Indeed, at least one invertase gene from *P. ramosa* was expressed at high levels during seed germination and in all subsequent growth stages (Draie et al. 2011). Ultimately, sugars are converted into storage products, and holoparasites such as *O. foetida* parasitizing faba bean accumulate starch, especially in the tubercles as compared to shoots (Abbes et al. 2009). *Orobanche foetida* growing on faba bean substantially changed the carbohydrate profile. Whereas the faba bean phloem sap contained primarily raffinose and stachyose, with significant levels of fructose, glucose and sucrose, tubercles and shoots of *O. foetida* contained highest levels of glucose and fructose, followed by stachyose, mannitol and sucrose (Abbes et al. 2009).

Additional insights into parasite carbon acquisition have come from experimental manipulations of atmospheric CO_2 levels. For the facultative parasite *R. minor* growing on *Poa pratensis*, elevated CO_2 concentrations (650 ppm compared to 350 ppm) led to greater growth of the parasite, but did not significantly alter the percentage of total carbon or nitrogen in the parasite tissue, indicating that the parasite was able to correspondingly increase its level of nitrogen along with carbon (Hwangbo et al. 2003). Raising CO_2 levels in this case did not benefit the host, which decreased in biomass in response to parasitism regardless of CO_2 availability. Raising CO_2 levels from 350 to 700 ppm in the *S. hermonthica* interaction with a C_4 grass, *Eragrostis pilosa*, led to an increase in parasite photosynthesis rate and increased soluble sugar content, but did not translate into an increase in parasite biomass (Watling and Press 1998). In this case *Striga* derived 27 % of its carbon from the host under ambient CO_2, and this increased to 39 % under high CO_2, suggesting that the *Striga* benefitted more from increases in host photosynthesis than from changes in its own photosynthesis. Again, the host did not benefit from higher CO_2 in terms of growth as its biomass accumulation was reduced by half by *Striga* parasitism under either CO_2 regime. In contrast to these observations, elevated CO_2 levels (550 ppm) did not significantly affect growth of the holoparasite *O. minor* on *Trifolium repens*, but did produce greater biomass accumulation by the host, which largely alleviated the negative impact of parasitism on the host (Dale and Press 1998). In sum, the impacts of changes in CO_2 levels on host–parasite interactions will vary depending on the parasite capacity for photosynthesis. Rising atmospheric CO_2 levels are likely to benefit hemiparasites that are able to take advantage of gains in photosynthesis, while holoparasites may not benefit.

6.2.2.2 Mannitol Metabolism

The capacity to biosynthesize the polyol mannitol has long been recognized in parasitic plants and has been detected widely in Orobanchaceae genera, including *Orobanche* and *Phelipanche* (Harloff and Wegmann 1993), *Striga* (Robert et al. 1999), *Euphrasia*, *Lathraea*, *Orthocarpus*, *Odontites*, *Parentucellia*, *Pedicularis* and *Rhinanthus* (Press 1995). Mannitol has also been reported in other parasitic lineages such as *Thesium humile* (Santalaceae) (Simier et al. 1994). Other polyols such as galactitol may be accumulated in addition to mannitol or may even be the principal polyol, as is the case for *Melampyrum* (Press 1995).

While these sugar alcohols provide a strong osmoticum that drives the flow of water into the parasite (Ehleringer and Marshall 1995), polyols are not limited to parasitic plants. Mannitol is the most common sugar alcohol in nature and has been found in over 100 species of non-parasitic plants besides the Orobanchaceae, including some host plants (Stoop et al. 1996). Nevertheless, mannitol can be a useful solute for plants because of its many physiological functions. In addition to acting as a storage form for reduced carbon, it functions as a compatible solute that can be accumulated to high physiological concentrations without damaging the cell.

Mannitol has features of an osmoprotectant, reducing cellular stress by substituting for water in coating macromolecules, thereby preserving their activity and function. Finally, mannitol can scavenge reactive oxygen species. These same benefits of mannitol are thought to play an important role in the success of pathogenic fungi (Vélëz et al. 2007), so it appears that mannitol is an especially advantageous chemical for pathogens of many types, including parasitic plants.

Mannitol is commonly synthesized as a product of photosynthesis in source leaves and transported to sink tissues where it may be converted back to mannose and related sugars such as fructose (Noiraud et al. 2001; Stoop et al. 1996). The site of mannitol synthesis in parasitic plants has not been demonstrated, but *S. hermonthica* sap contained high levels of mannitol (58 % of soluble sugar) whereas no mannitol was detected in sap of the sorghum host (Press et al. 1991). Mannitol represented up to 77 % of total soluble sugars in *S. asiatica* leaves, and although *Orobanche hederae* stems contained a lower concentration of just 34 %, this is still a substantial portion of the carbon reserve. *Orobanche foetida* also synthesizes mannitol as an important component of the carbohydrate profile (Abbes et al. 2009).

The key metabolic step in mannitol synthesis in plants is the conversion of mannose-6-P to mannitol-1-P by the enzyme mannose 6-phosphate reductase (M6PR). The importance of mannitol to parasites has prompted suggestions that M6PR would be an ideal target for selectively disrupting parasite growth (Robert et al. 1999). This was tested using trans-specific gene silencing (see Sect. 6.5.1) to specifically reduce the levels of M6PR gene expression in *P. aegyptiaca* attached to transgenic tomato hosts (Aly et al. 2009). The experiment caused a decrease in M6PR transcript levels in the parasite and reduced the percentage of mannitol in the tubercles by up to 50 % in the best case. The silencing appeared to lead to increased mortality of tubercles, suggesting that mannitol accumulation is in fact a vital process for parasites. The fact that less than complete parasite death resulted from the decreased mannitol may be due to inefficiencies in the transmission of gene silencing from the host plant such that M6PR enzyme production was not completely shut off or alternatively that mannitol accumulation is not of vital importance for the parasite. It will be interesting to know how parasites would survive if mannitol synthesis was completely disrupted.

6.2.3 Nitrogen

6.2.3.1 Nitrogen Assimilation

The acquisition of fixed nitrogen from another plant is perhaps the greatest benefit of parasitism for hemiparasites. By tapping into a host, the parasite gains access to an expanded root system and a direct supply of fixed inorganic nitrogen. Considering that nitrogen is the limiting nutrient in many ecosystems, this is an important advantage in conditions of high competition and nutrient scarcity. An additional

gain is that the parasite can accomplish this with relatively low investment in its own root system and assimilation machinery. Prior to emergence from the ground *S. hermonthica* depended on its host for 100 % of its carbon but only up to 59 % of its nitrogen (Aflakpui et al. 2005), with the remainder perhaps coming directly from the soil through *Striga* roots (Igbinnosa and Thalouarn 1996). In contrast, the emerged parasite was more self-sufficient for carbon, with mature plants taking only about 35 % of their carbon from the host, but the reverse was true for nitrogen. As the parasite became older, its dependency on host inorganic nitrogen increased until it was nearly 100 % in the mature parasite (Aflakpui et al. 2005).

In plants, the first two major steps in nitrogen assimilation are the reduction of nitrate to nitrite by the enzyme nitrate reductase and the conversion of nitrite to ammonium by nitrite reductase. Nitrate can be taken up by cells of roots or leaves, stored in vacuoles and transported in xylem from root to shoot. Nitrate reductase can occur in roots or shoots, depending on the species, but is always located in the cytoplasm of cells. The nitrite reductase enzyme functions in plastids but is coordinated with nitrate reductase ensuring that all phytotoxic nitrite is converted to ammonium. Nitrate reductase activity is substrate-inducible by nitrate and is co-regulated with photosynthesis (light) and carbon metabolism, thereby ensuring sufficient energy for the reduction reactions.

Parasites have less capacity for nitrate reduction than fully autotrophic plants. For example, the *S. hermonthica* nitrate reductase activity is much lower than that of host plants and the enzyme is not inducible by addition of nitrate (Press et al. 1986). *O. crenata* has no detectable nitrate reductase activity (Press et al. 1986; Stewart et al. 1984). The finding that nitrate reductase is low in hemiparasites and absent in holoparasites is consistent with parasites being able to obtain from their hosts all or a significant proportion of their nitrogen in fully reduced forms such as ammonium or amino acids.

The next step in assimilating inorganic nitrogen into organic compounds is the incorporation of ammonium into the amino acid glutamine by glutamine synthetase (GS). Glutamine synthetase generally occurs in two forms in plants, with each form localized to distinct regions of the cell and having a specialized role in metabolism (McNally et al. 1983). GS1 is located in the cytosol of cells and is expressed more prominently in roots where it functions in primary assimilation of ammonium. GS2 is located in chloroplasts, is the most abundant form in leaves and is thought to have a major role in re-assimilation of ammonium from photorespiration. Whereas the expression of GS2 is associated with photosynthetic cells of leaves and stems, GS1 expression is associated with phloem. Non-parasitic plants generally have two to four genes for GS1 and one gene for GS2, but parasites are unusual in that the GS2 form is generally missing or reduced in activity. That parasites would contain primarily GS1 is consistent with this form of glutamine synthetase being more closely associated with the phloem and with expression in non-photosynthetic tissues such as etiolated tissues or roots.

S. asiatica, *S. hermonthica* and *S. gesnerioides* contain both GS1 and GS2 forms, with GS1accounting for 80–90 % of their total glutamine synthetase activity (Press et al. 1986; Stewart et al. 1984). Parasites that entirely lack GS2 include the

holoparasites *O. cernua*, *O. hederae*, *O. minor* and *P. ramosa* (McNally et al. 1983). The holoparasite *Lathraea clandestina* was initially reported to be among those species that lack GS2, but subsequent studies detected low levels of the enzyme activity, with highest activity in the scale leaves (Thalouarn et al. 1987). These authors used antibodies to identify the two forms of glutamine synthetase and found GS1 in the cytosol and a form weakly corresponding to GS2 in the stroma of amyloplasts. Further investigation of this species demonstrated the presence of several other nitrogen metabolic enzymes including nitrate reductase, nitrite reductase, glutamate synthase, glutamate oxoglutarate aminotransferase and glutamate dehydrogenase, suggesting that the parasite is equipped to deal with a variety of nitrogenous compounds coming from the host (Thalouarn et al. 1988).

Taken together, the reports of missing or reduced activity of nitrogen assimilation machinery could imply that some parasites—and especially holoparasites—have undergone an evolutionary reduction that leaves them dependent on their hosts for nitrogen that has already been converted to reduced and organic forms, such as ammonium and amino acids. The main argument against this is the ability for parasites such as *Striga* and *Phelipanche* to grow and develop on minimal media culture (Deeks et al. 1999; Zhou et al. 2004). While all basic culture media contain ammonium, the fact that parasites growing in such conditions can produce recognizable structures such as infective roots, shoots and in some cases even flowers indicates that they are able to process inorganic nitrogen with their own metabolic systems. The fact that herbicidal inhibitors of amino acid synthesis (e.g. glyphosate and acetolactate synthase inhibitors; see Sect. 23.2.1) kill attached parasites provides further evidence that they rely substantially on their own enzymes for amino acid synthesis (Eizenberg et al. 2012; Gressel 2009).

Asparagine synthase is another important enzyme in nitrogen assimilation, transferring ammonium from glutamine to asparagine for subsequent storage, transport and metabolism and also playing an important role in parasite metabolism (Pageau et al. 2003). Asparagine has the second highest N:C ratio of any amino acid, which may explain why it evolved to be a storage amino acid. The expression of asparagine synthase is suppressed by light and enhanced in darkness and is thus most abundant in etiolated tissues. Asparagine synthase of *S. hermonthica* is encoded by a small gene family of at least two genes, one of which is not suppressed in light (Simier et al. 2005). The *Triphysaria versicolor* enzyme is also encoded by a small gene family of 2–3 genes, and asparagine synthase gene expression in the roots was induced by host root exudates, suggesting that it is induced in the parasite prior to host contact (Delavault et al. 1998). This asparagine synthase was not induced by the haustorium-inducing quinone DMBQ, so its expression pattern is distinct from the haustorial induction pathway (see Sect. 4.3).

6.2.3.2 Nitrogen Uptake and Translocation

Accessing nitrogen from the vascular system of a host plant presents ‘challenges’ for a parasite given that the form of translocated nitrogen varies considerably

depending which host species is encountered. For example, some plant species translocate nitrate from roots to shoots, while other species directly metabolize nitrate to amino acids or other organic forms in root cells prior to transport to the shoots (Pate 1973). Most plants use some combination of these approaches, so parasites drawing from the host xylem stream encounter compounds ranging from nitrate and amino acids to ammonium, amides and ureides (Miller et al. 2009; Pate 1973; Schjoerring et al. 2002; Xu et al. 2012). Parasites would be expected to have the ability to adapt to nutrient content of their hosts, and as discussed above, the xylem parenchyma cells of the haustorium are likely geared for the rapid extraction of host nitrogenous compounds. In principle, absorption of translocated nitrogen into parasite cells could take place at any point along the parasite continuum from the haustorium to the shoot tip. This would not require unusual metabolic capacity on part of the parasite because nitrate and amino acid transporters are known to occur in all parts of plants to regulate nitrogen uptake, metabolism and regeneration (Miller et al. 2009).

Facultative hemiparasites can absorb mineral nitrogen from soil using their own roots. Nevertheless, haustoria provide a significant supplement and it was estimated that *R. minor* acquired 17 % of its mineral nitrogen from the xylem of a grass host *Cynosurus cristatus* (Cameron and Seel 2007). The parasites appear to have no selective mechanism to accomplish this uptake from the host, as the proportion of mineral nitrogen taken into the parasite from xylem of barley roots was roughly the same as that of phosphate and potassium (~20 % for each) and water (Jiang et al. 2003, 2004a) (Fig. 6.2). This is consistent with an unregulated exchange between continuous xylem cells of the two species.

To answer the question of whether host transport of different nitrogen forms is a factor in parasite success, *R. minor* was grown on *Vicia faba* plants that obtained their nitrogen from either roots containing N_2-fixing nodules or roots lacking nodules but fed a complete nutrient solution. The rationale was that nodules produce organic forms of nitrogen whereas nutrient solution supplies inorganic forms. No differences in parasite growth were attributable to differences in host nitrogen composition, although the overall greater nitrogen levels in the fertilized system resulted in higher nitrogen content in both hosts and parasites (Jiang et al. 2008). It is noteworthy that although *R. minor* takes a relatively small fraction of the host mineral nitrogen, this is still a detriment to the host and the competition for nitrogen between *R. minor* and its host *Poa pratensis* appears to be more important than competition for carbon (Hwangbo et al. 2003).

Nitrogen uptake by obligate hemiparasites also occurs primarily through xylem. Rapid transfer of host root-applied ^{15}N-nitrate occurred between *Sorghum bicolor* and *Striga hermonthica* (Pageau et al. 2003). The labelled nitrogen in the *Sorghum* xylem sap was distributed about evenly among nitrate and free amino acids, indicating *Sorghum* ability to fix some nitrogen in the roots and to translocate both inorganic and organic forms within the plant. The parasite appeared to take up the nitrogen in approximately the same ratios as present in the host xylem, although nitrate and amino acid concentrations in *S. hermonthica* xylem were several times higher than those of the host (Pageau et al. 2003) (Fig. 6.3). The labelled nitrate was

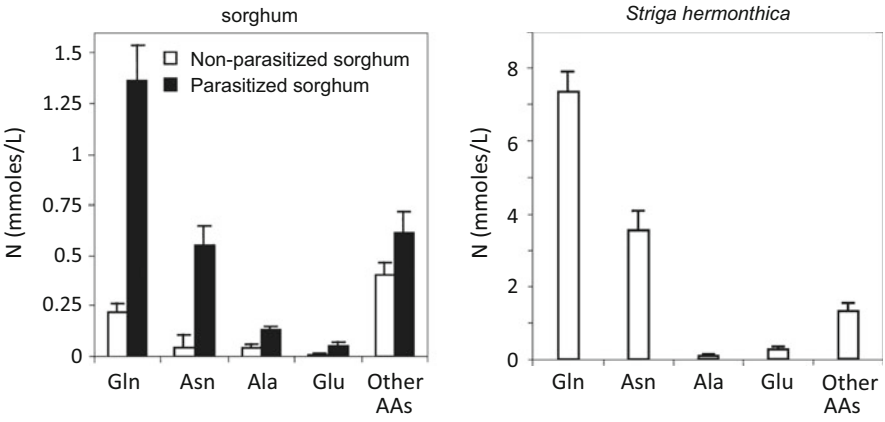

Fig. 6.3 Free amino acids in xylem sap of host and parasite. Nitrogen content of the major free amino acids (mmol N L^{-1}) in xylem sap from non-parasitized and parasitized sorghum (*left*) and from *Striga hermonthica* parasitizing sorghum (*right*) (figure is from Pageau et al. (2003) with permission of Oxford University Press)

assimilated in the parasite into predominantly glutamine and asparagine. These amino acids are the first products in the process of assimilating ammonium (more on this below) and are important transport forms of nitrogen, so it is not surprising that they are the first to appear in the parasite. This study also measured accumulation of ^{15}N-labelled compounds in different organs and suggests distinct patterns of metabolism. Specifically, the haustorium had a tenfold higher concentration of nitrate than parasite roots or shoots or even host roots (Pageau et al. 2003). In contrast, free amino acids occurred at low concentrations in the haustorium, but much higher concentrations in the shoots. A striking example is asparagine, which was measured at 0.05 μmol g^{-1} FW in haustoria but more than two orders of magnitude higher in *S. hermonthica* roots and three orders of magnitude in shoots. Such differences argue for a distinct set of metabolic functions between the haustorium and the rest of the parasite.

The profiles of amino acids are parasite species specific, reflecting different metabolic requirements and resources available from hosts. However, in general parasites tend to accumulate aspartate, asparagine, glutamate and glutamine. Other accumulated amino acids include alanine and arginine, depending on the parasite in question (Nandula et al. 2000; Press et al. 1986). The levels for selected amino acids found in *Striga*, *Orobanche* and *Phelipanche* are shown in Table 6.2. Although the data reflect various parasites, hosts and experimental conditions, the broad trends are similar and show common metabolic equilibrium points for these parasites.

Many amino acids, including aspartate and glutamate, accumulate in xylem sap of plants under stress, which would suit the needs of parasites (Nemec 1995). Parasites can thus influence the quality of resources coming from the host. *S. hermonthica* parasitism induced an increase in both nitrate and free amino acid levels in xylem sap of sorghum (Pageau et al. 2003). Furthermore, the

Table 6.2 Examples of free amino acid content reported for parasites

Amino acid	*Striga hermonthica* xylem sap[a] (nmol cm^{-3})	*S. hermonthica* shoot tissue[b] (μmol g^{-1} FW)	*Orobanche foetida* tubercle tissue[c] (mM)	*Phelipanche aegyptiaca* tubercle tissue[d] (μmol g^{-1} DW)
Aspartate		4.6	11.7	3.3[e]
Asparagine	25–50	81.4	17.1	
Glutamate	500–1,500	3.5	2.5	3.4[e]
Glutamine	50–750	2.4	0.2	
Alanine	10–20	2.0	0.4	3.9
Arginine	–	–	1.0	6.7
Glycine	Trace	–	0.1	0.3
Isoleucine	Trace	–	0.3	0.9
Leucine	10–20	–	0.4	1.1
Serine	Trace	–	0.5	2.4
Threonine	Trace	–	0.36	1.3
Valine	5–30	–	0.56	1.5

[a]Stewart et al. (1984)
[b]Pageau et al. (2003)
[c]Abbes et al. (2009)
[d]Nandula et al. (2000)
[e]Combined asp + asn and glu + gln

concentrations of xylem glutamine and asparagine increased dramatically (Fig. 6.3). Carrot leaves had similar or higher amino acid levels when parasitized by *P. aegyptiaca* compared to those of non-parasitized plants (Nandula et al. 2000). In contrast, roots of parasitized carrots had similar or lower levels than non-parasitized plants. The parasite tubercles were at the same time higher in asparagine/aspartate and glutamine/glutamate than associated host roots, and this held true for most amino acids, indicating preferential accumulation in the parasite. The differences in parasite effect on host amino acid composition between *Striga* and *Phelipanche* may reflect the different feeding styles of the parasites and hence different stresses placed on the host.

Changes in the nutritional status of the host would be expected to affect the parasite since parasites are linked directly to their hosts for their nutritional supply (see Sect. 22.3.3). Several experiments have looked at this possibility by varying nitrogen or carbon status of the host. Increasing nitrogen fertilization in a sorghum-*S. hermonthica* association led to greater nitrogen incorporation into parasite leaves compared to hosts, demonstrating the proficiency of these parasites at extracting nitrogen (Cechin and Press 1993). The photosynthesis rate of *S. hermonthica* also increased with higher nitrogen supply, but the overall effect of high nitrogen was that the parasites suffered. *S. hermonthica* grew well at lower rates of nitrogen, but its ability to gain carbon decreased from 27 % to just 6 % as nitrogen rates increased (Cechin and Press 1993). Another study failed to find this nitrogen effect in the interaction between *S. hermonthica* and maize, but the concentrations of N used were lower and may not have caused the same level of stress in the parasite (Aflakpui et al. 2005).

The fact that these parasites combine high capacity for nitrogen uptake with a reduced capacity for nitrogen metabolism may explain why they generally do not grow well under conditions of high soil nitrogen fertility (Igbinnosa and Thalouarn 1996). For example, high rates of ammonium nitrate applied to sorghum decreased biomass accumulation of attached *S. hermonthica* (Cechin and Press 1993). Toxicity in this case may be due to elevated uptake of nitrogen from the host and accumulation in parasite leaves, leading to an overload of the parasite's metabolic system. These parasites are especially well adapted to conditions of low nitrogen and seem to have limited capacity to deal with excess nitrate and ammonium. This may be particularly true of certain obligate parasites, which are especially susceptible in the seedling stage when they have limited biomass and metabolic reserves. This could amount to a counter-adaptation for dealing with the metabolic demands of transporting and metabolizing unusually high levels of nitrogen (Britto and Kronzucker 2002). The concept of fertilizing to reduce parasitic weeds has been demonstrated repeatedly, although many conflicting reports also exist (e.g. Kamara et al. 2007) and various factors, such as soil types, nitrogen forms and weather, likely confound the effect. It is also possible that soil fertility affects parasite success through its impact on germination signalling more than on later metabolic interactions with the host (Fernández-Aparicio et al. 2011). Nevertheless, adequate fertility can be part of an integrated approach to control of certain parasitic weeds (Hearne 2009; Tesso and Ejeta 2011) (see Sect. 22.3.3 for discussion of agronomic aspects).

6.3 Direction of Movement

Most of the literature on parasite nutrition assumes that movement occurs as a rule from host to parasite. Indeed, the strong translocation of materials from host to parasite is well documented in the literature, but it is not clear whether lack of evidence for parasite-to-host movement is due to actual negative results or because the question is rarely addressed. Considering the physiology of vascular connections, the most likely cases of parasite-to-host translocation should be observed in holoparasites where phloem connections may allow for bidirectional flow of materials. In fact, some movement from parasite to host has been demonstrated for both xylem and phloem feeders. One example of 'reverse flow' comes from an experiment in which radiolabelled CO_2, urea and sulphur were used to trace translocation between sorghum and *S. senegalensis* (Okonkwo 1966). Low amounts of radiolabelled material applied to the parasite also moved into the host, with above-background levels of photo-assimilate detected in host tissues and in other non-labelled parasites attached to the same host. A similar study in which ^{14}C-urea or $^{14}CO_2$ were applied to the hemiparasite *Odontites verna* detected low levels of radiolabelled products in the host (Govier et al. 1967).

The above results require that xylem flow be reversed in the host–parasite interaction, at least temporarily. This could be possible if the osmotic pull of the

parasite were overcome by the host or otherwise interrupted (see Chap. 3 for structural aspects). An experiment using dye loaded into the cut root tip of *P. aegyptiaca* showed rapid movement of the dye into the host, ultimately appearing in the host leaves (Aly et al. 2011). The experiment required cutting the parasite root, which disrupted the integrity of the parasite system, but nevertheless it demonstrated that open flow is possible between host and parasite and suggested that the direction of flow can be reversed by physical damage to the parasite.

RNAs appear to move bidirectionally between parasites and hosts (see Sect. 6.5.1). The evidence to date strongly suggests that RNAs move both into and out of *Triphysaria*. *Triphysaria* (Tomilov et al. 2008) can bridge two different host plants and transmit RNAi signals across a span of parasite tissue (similar to the Convolvulaceae parasite *Cuscuta*; Birschwilks et al. 2006), demonstrating both entry and exit from the parasite. Only host-to-parasite movement of viruses has been indicated for *Orobanche* (Gal-On et al. 2009), but the possibility of transmission in the reverse direction has not been tested.

Taken together, these data support a model of parasite physiology in which the haustorial connections allow bidirectional flow of materials, but the dominant flow occurs towards the parasite. A reversal of flow could occur as part of the tension between host and parasite or any environmental or physiological conditions that temporarily weaken the osmotic advantage of the parasite.

It is also possible that parasites have a mechanism that allows flow of compounds such as toxins or effectors into the host that facilitate parasitism. It has long been speculated that parasites such as *Striga* secrete substances into their hosts that cause stunting or otherwise reconfigure host metabolism (Musselman 1980), and backflow could be one mechanism to accomplish that goal.

6.4 Hormone Interactions

6.4.1 Abscisic Acid and Cytokinin

A general feature of the parasitic Orobanchaceae is the accumulation of ABA and cytokinin in the parasites following attachment to hosts. Cytokinin levels are low in unattached parasites but increase nearly 100-fold once the parasite has attached to the host (Lechowski and Bialczyk 1996). ABA levels follow a similar trend, increasing dramatically after attachment to the host. For example, the hemiparasite *R. minor* has about 35 times more ABA than its barley host even while growing autotrophically, but after attachment the parasite ABA concentration increases to 53 times higher than its host (Fig. 6.4) (Jiang et al. 2003). Xylem sap of the hemiparasite *Melampyrum arvense* has about a third of the ABA level of its *Capsella* host when growing autotrophically, but the ABA concentration of attached parasites exceeds that of the host by 50 % (Lechowski 1996). Pre- and

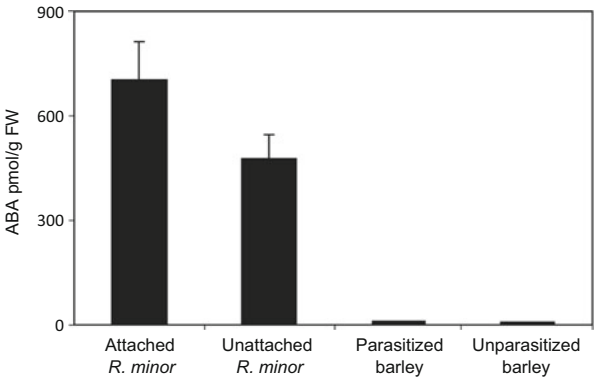

Fig. 6.4 ABA status in host–parasite association. ABA concentrations in leaves of *Rhinanthus minor* and barley living in parasitic association and independently (figure is from Jiang et al. (2003) with permission of Oxford University Press)

post-attachment ABA levels are not documented for obligate parasites, but the approximately tenfold higher ABA levels in *S. hermonthica* leaves as compared to its maize host suggest that elevated ABA levels in parasites are common (Taylor et al. 1996).

The reports of high ABA levels have led to much speculation about the mechanisms of ABA accumulation and its role in the life of the parasite. The high ABA concentration is primarily due to increased ABA synthesis in the parasites, although there is certainly a contribution from host xylem. In fact, many parasites stimulate their hosts to increase synthesis of ABA, which is then available for uptake into the parasite or may directly affect host physiology to the benefit of the parasite (Lechowski 1996). The ABA levels in sorghum leaf tissue and xylem sap were approximately doubled following parasitism by *S. hermonthica* (Frost et al. 1997). ABA levels were also higher in maize tissues parasitized by *Striga* (Taylor et al. 1996). This does not seem to hold true for barley (*Hordeum vulgare*), which maintained ABA levels following parasitism by *R. minor* (Jiang et al. 2004b). It is possible that low water potentials in the parasite stimulate the increase in ABA concentrations due to the role of ABA as a drought-associated hormone, but no clear explanation for high ABA levels in the host is currently available (Jiang et al. 2010).

The functions of elevated ABA concentrations in the host–parasite association are not certain, although effects in host and parasite are likely different. In host plants, high ABA may have a role in reducing host stomatal apertures and lead to reduced photosynthesis and plant growth (Frost et al. 1997). While detrimental to host growth, this can lead to a shift in water usage from the host to the parasite, thereby ensuring sufficient water flow into the parasite. ABA also suppresses the salicylic acid-mediated defence response in plants, and increasing ABA levels in host tissues—through either direct synthesis by the pathogen or induction of host biosynthesis—is a common theme in fungal and bacterial pathogenesis (Cao et al. 2011). The increase in host ABA concentrations could contribute to the observed lack of salicylic acid associated defence responses in parasitized host roots (Griffitts et al. 2004; Vieira Dos Santos et al. 2003).

In most plants ABA acts to induce closing of stomata, but parasite stomata remain open almost constantly despite exceptionally high ABA concentrations. Parasites are less sensitive to ABA than non-parasites as evidenced by higher concentrations of ABA needed to induce closing of *R. minor* stomata compared with host stomata (Jiang et al. 2003). Another possible explanation for open stomata in parasites is high cytokinin levels, which may antagonize the ABA effect and force open the stomata. Stomata of *Melampyrum arvense* are closed in darkness when this hemiparasite is growing without a host but open wide and become insensitive to cytokinins after the parasite attaches to a host (Lechowski 1997).

Root hydraulic conductivity is also regulated by ABA in plants (Markhart et al. 1979) and high ABA levels in the parasite could contribute to greater water flow into the parasite (Jiang et al. 2004b). The parasite–host connections are not limited by physical barriers such as the Casparian strips (see Sect. 3.16) and the effect of ABA on increasing membrane permeability would be advantageous to the parasite.

6.4.2 Auxin

The role of IAA in established parasites has received less attention than ABA or cytokinin. The central role of auxin in plant growth and development suggests that this hormone must also be important to parasites, but no role has been documented in the mature haustorium. Auxin is important in the development of vascular tissue in the haustorium and may be important in establishing the directionality of xylem differentiation between host and parasite (Bar-Nun et al. 2008). Auxin is also an important component for regulation of haustorial formation in *Triphysaria* (Tomilov et al. 2005). *Triphysaria* forms haustoria more readily when exposed to IAA (Tomilov et al. 2004), and inhibitors of auxin and ethylene reduced the number of haustoria in *Triphysaria* (Tomilov et al. 2005).

6.5 Macromolecules

6.5.1 Proteins and RNA

In addition to translocating small molecules from host to parasite, the haustorium may also transmit large molecules such as proteins and nucleic acids. As discussed above for small molecules, the type of molecules transmitted may depend on the anatomy of the specific parasite and the metabolic needs of that parasite. Many reports of macromolecular trafficking between hosts and parasites come from *Cuscuta*, which unlike the Orobanchaceae appears to have exceptionally open connections to host vascular tissues. *Cuscuta* takes up many substances from the host phloem, including dye tracers (Birschwilks et al. 2006), soluble proteins

(Haupt et al. 2001), mRNA (Roney et al. 2007) and viruses (Birschwilks et al. 2006). It appears that these same compounds are also mobile into at least certain holoparasitic members of the Orobanchaceae.

The phloem-localized dye carboxyfluorescein and the green fluorescent protein (GFP) are both readily mobile into *P. aegyptiaca* (Aly et al. 2011). Importantly, the GFP had to be expressed in host phloem cells and in a form soluble in the cytoplasm in order to move to the parasite. Parallel host transformants containing GFP with an endoplasmic reticulum targeting signal did not move. The GFP was localized to the parasite phloem, suggesting that it was translocated through phloem connections.

Single-stranded RNA and DNA viruses can also translocate from hosts to *P. aegyptiaca* (Gal-On et al. 2009). The question of whether mRNAs are mobile between hosts and Orobanchaceae has not been satisfactorily addressed, but there is evidence for movement of small RNAs associated with gene silencing. The best example to date is from *T. versicolor* plants that were transformed to constitutively express the *GUS* (β-glucuronidase) reporter gene, the expression of which was shut down following parasitism of lettuce that expressed a silencing construct for *GUS* (Tomilov et al. 2008). Both the *GUS* mRNA levels and the activity of the GUS enzyme were suppressed in the parasite near the point of contact and in young root tissues that developed after parasitizing the host, indicative of host-to-parasite transport of the silencing signal. Although the GUS silencing was strongest immediately around the point of haustorial connection to the host, silencing also occurred away from the haustorium, indicating that the signal was transmitted some distance through the parasite tissue.

A second example of this trans-specific gene silencing process comes from a study aimed at suppressing mannose 6-phosphate reductase (M6PR) expression in *P. aegyptiaca* (Aly 2007). In this case, tomato was transformed with a silencing construct targeting the parasite version of M6PR, which was discussed above for its role in mannitol metabolism and osmotic regulation in the parasite (Sect. 6.2.2.2). Parasite M6PR transcript levels were reduced in the tubercles of plants parasitizing the transgenic tomato, but no information is available on the spatial distribution of the silencing effect in the parasite.

In contrast to the situation in *Triphysaria* and *Phelipanche*, the trans-specific gene silencing approach has not yet succeeded in silencing *Striga asiatica* (L. Kuntze) *genes*. RNA interference constructs were generated for five essential *S. asiatica* genes and were transformed into maize (*Zea mays*), but parasites grown on these hosts showed no measurable effect (de Framond et al. 2007). Although this report was from a work in progress and several experimental factors could have contributed to the results, it suggests that caution is required in generalizing about uptake dynamics of different parasites. It is reasonable to hypothesize that *Triphysaria* and *Striga* are equivalent in their mechanisms of xylem feeding, but the details of RNAi signal transmission and function in host–parasite interactions need further study to understand the apparent differences among parasite species.

Beyond the demonstration of macromolecule movement between hosts and parasites, the biological function of such exchange is unknown. The *Triphysaria* and *Phelipanche* experiments with gene silencing provide a strong indication that

gene silencing signals not only move but may function in the parasites. Post-transcriptional gene silencing in plants takes many forms but involves the generation of 21–24 bp double-stranded RNA by a dicer-like enzyme, leading in many cases to a systemic signal that can propagate throughout a plant. At the core of this signal is a single-stranded RNA about 21 nucleotides long (Kehr and Buhtz 2008). In order for this to have practical significance in altering parasite gene expression, the host must express a silencing RNA that has high homology to a corresponding section of parasite RNA (see Sect. 24.4.2). The extent to which this occurs will become clearer as more parasite gene sequences become available.

6.5.2 DNA

Horizontal gene transfer between parasitic plants and their hosts is another indicator of nucleic acid transfer. In these cases, horizontal gene transfer is usually discovered as part of phylogenetic studies in which some genes align with greater homology to counterparts in distantly related species rather than to close relative species as judged by traditional phylogenetic arrangements (see Sect. 15.5). This indicates the evolutionary transfer of a gene through a means other than typical vertical inheritance from parent to progeny. The process of nucleic acid movement between species required for horizontal gene transfer is facilitated by direct haustorial connections between donor and recipient plants, so it is not surprising that parasitic plants are well represented among cases of horizontal gene transfer.

Orobanchaceae are involved in several examples of horizontal gene transfer that involve genes from mitochondrial (Mower et al. 2004), plastid (Park et al. 2007) and nuclear genomes (Yoshida et al. 2010). At this time little is known about the mechanisms of horizontal gene transfer or movement of RNAs and protein in plants, so it is only possible to speculate about the mechanisms involved in haustorial transfer of these materials. The movement of large sections of DNA between plants has been proposed as one mechanism for horizontal gene transfer (Mower et al. 2010), although the movement of a gene into *Striga* carries evidence of missing introns and a polyadenylated tail sequence that suggests the transfer was mediated by an mRNA molecule. To the extent that the haustorium functions as a continuation element of host and parasite vascular systems, it should be expected that any macromolecules capable of moving systemically in the host are equally capable of moving into the parasite. Messenger RNAs are known to move systemically in phloem (Lough and Lucas 2006), but no such evidence exists for movement of DNA. Large sections of DNA may transfer short distances in the area of a graft junction (Stegemann and Bock 2009) and haustoria share some features with grafts (Kuijt 1983), including inter-specific symplastic connections (Sect. 2.1.5.1), so it would be interesting to know whether DNA exchange can take place in the haustorial region. Stable incorporation of foreign DNA into a plant under this scenario would require that cells of the haustorial junction give rise to adventitious shoots that could flower and transmit the new gene(s). So far parasite regeneration

from a haustorium was not described for the Orobanchaceae. Considering the current interest in horizontal gene transfer, parasitic plants are sure to receive additional attention.

6.6 Conclusions

The mature haustorium is the central feature in the interactions between host and parasite, yet many aspects of haustorium physiology and function remain unknown. Questions surround the precise mechanisms of uptake of the diverse range of materials transferred from host to parasite, as well as the metabolic fate of host molecules within the parasite. Complicating the subject are the significant biological differences represented in the range of host dependences encompassed by species of the Orobanchaceae. The haustoria of holoparasites that make both xylem and phloem connections to the host represent a different evolutionary direction than haustoria of hemiparasites that connect to just the xylem. These basic differences in function are compounded by other variations among parasitic species and the fact that parasite interactions may be influenced by specific hosts or environmental conditions, resulting in a diverse body of literature on the physiological interactions of various parasites and hosts.

Despite differences in parasite haustoria, some common themes span all parasites and point to the core functions of haustoria. Both hemi- and holoparasites have evolved mechanisms of nutrient uptake and transport to enable their growth. Foremost, haustoria are anatomical connections that enable exchange of resources between hosts and parasites. Regardless of whether parasites form direct connection with host symplast, all appear to have some ability to absorb reduced carbon compounds from hosts. Furthermore, haustoria appear to be the first line of parasite metabolism, actively converting host resources into chemicals used by the parasite. These include osmotic compounds that contribute to the process of drawing water and dissolved solutes into the parasite. The accumulation of osmotic solutes is important even in hemiparasite species in which water potential differentials are aided by transpiring leaves, as all Orobanchaceae appear to accumulate ions (primarily potassium) and sugar alcohols (mannitol), and have elevated synthesis and accumulation of ABA. Nitrogen (in either mineral or reduced forms) is an absolute requirement of parasites, and reliance on hosts for this nutrient is reflected in their diminished capacity to incorporate nitrogen via nitrate reductase and glutamine synthetase. The haustorium appears to be active in converting nitrogenous compounds to preferred amino acids (glutamine and asparagine) and in processing host-derived sugars into preferred simple sugars, sugar alcohols and starch.

Much of the research on physiological interactions between parasites and hosts has focused on large organs such as stems, leaves and roots. This is understandable because the haustorium is small and embedded in host tissues underground, so it presents a challenge to study. Nevertheless, this structure holds key insights into

understanding parasitism and the tools of modern biology will allow detailed investigation into the metabolism and gene expression of tissues and cells within the haustorium to understand how it regulates interactions between host and parasite. Most transport and metabolic functions of the haustorium have parallels in other tissues or stages of plant development but have likely been recruited through evolution to function in the haustorium. It will be interesting to learn more about how these functions have been combined and coordinated in this remarkable structure.

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