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Knowing the Parasite: Biology and Genetics of *Orobanche*

Abstract: Due to their forms and colors, parasitic plants are most often considered to be botanical curiosities. However, in some cases, these are proved to be also deadly pests with the capacity to exploit other plants. Among the obligate root parasitic weeds, the holoparasites that are devoid of chlorophyll and thus unable to carry out photosynthesis totally rely on their hosts for their water, mineral, and carbohydrate supplies. Members of the genus *Orobanche* and *Phelipanche*, belonging to the Orobanchaceae family (the broomrape family), are thus the final result of this evolutionary transition from autotrophism to heterotrophism. The underlying process of this trophic exploitation, governed by a fine-tuned molecular dialogue between both partners, is an extraordinary example of adaptive plant biology operated by these parasitic organisms in the course of evolution. This transition is associated with remarkable morphological and physiological adaptations, such as the requirement for the seeds to germinate to perceive molecules produced by host roots, the development of a novel organ, the *haustorium*, which invades host tissues and establishes a physiological continuum between the parasite and the host, the establishment of a sink strength required for translocation of host resources, the loss of photosynthesis, and a reduced leaf and root architecture.

Keywords: conditioning, germination, *haustorium*, *Orobanche*, sink strength, tubercle

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Phylogenetic relationships

The scientific name *Orobanche* derives from the ancient Greek words ὅροβος (orobos, “bitter vetch”) and ἄγχω (ankhō, “to strangle”) with reference to the

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impact of the parasite on its host. From a systematic point of view, the Orobanchaceae family, formerly related to the Scrophulariaceae family, is a monophyletic group composed of 2,060 species distributed in 90 heterotrophic genera, except for a single autotrophic lineage, the genus *Lindenbergia* (Westwood *et al*., 2010). The parasitic way of life would have a unique origin and would arise from Laurasia in the northern Tethys Ocean (probably in East Asia) during the Tertiary period (Wolfe *et al*., 2005). Phylogenetic studies based on the association of several molecular markers propose a distribution in six major clades in which the transition from hemiparasitism to holoparasitism would have occurred three times in an independent manner (McNeal *et al*., 2013). The tribe *Orobancheae sensu stricto* is made of 170 herbaceous holoparasitic species from temperate to subtropical regions, mainly in the northern hemisphere. There is some confusion regarding the classification of the different species from this tribe due to the drastic reduction, induced by the parasitic way of life, of the number of phenotypic traits classically used in taxonomic identification. These species were traditionally grouped in four main sections (*Trionychon* Wallr., *Myzorrhiza* (Philippi), *Gymnocaulis* Nutt., and *Osproleon* Wallr. (=*Orobanche sensu stricto*)). Using plastid and nuclear (internal transcribed spacer, ITS) sequences, it has been possible to group the four sections into two phylogenetically distinct genera: the *Orobanche* genus (19 chromosomes) coupling the *Osproleon* section and the *Diphelypaea* genus, and the *Phelipanche* genus (12 chromosomes), including the sections *Gymnocaulis*, *Myzorrhiza*, and *Trionychon* (Joel, 2009). This dichotomy is also supported by studies based on LTR (long-terminal repeat) retrotransposon repertoire and repetitive DNA sequences (Piednoël *et al*., 2012; 2013). However, a degree of ambiguity exists in regard to the name of the *Phelipanche* genus because species were named for a long time *Orobanche* (i.e. *P. ramosa*, *P. aeagyptiaca*, *P. mutelii*, etc.). Nevertheless, the improvement of molecular techniques allowed, for example, the distinction between *O. cernua* and *O. cumana* which have long been considered as a single species (Delavault & Thalouarn, 2002), even though the debate is still open (Schneeweiss *et al*., 2004). Throughout the text the generic name *Orobanche* will be used when referring to broomrape species.

Among the different broomrape members, only about 20 species should be considered as harmful parasitic weeds in important crops, including *O. cumana* Wallr. on sunflower, *O. crenata* Forsk. and *O. foetida* Poir. on legumes, *P. ramosa* L. Pomel on oilseed rape, and *P. aegyptiaca* Pers. on tomato. They represent a major economical constraint as approximately 16 million hectares of arable land are threatened around the Mediterranean Sea and in West Asia.

An original life cycle

Common feature of all holoparasites is that the life cycle of *Orobanche* is atypical when compared with classical Angiosperms due to its high degree of trophic specialization and its synchronization with that of its host (Gibot-Leclerc *et al.*, 2012) (Figure 1). This cycle is divided into two distinct phases: the first one which is "independent," from the seed imbibition to radicle development, and occurs thanks to the seed storage reserves. This independency is only a matter of energy since a chemical signal produced by the host roots in the rhizosphere is required for seed germination. The "dependent" second phase starts with the *haustorium* formation and the connection of the parasite to the vascular system of the host. Survival and development of the parasite are then highly dependent

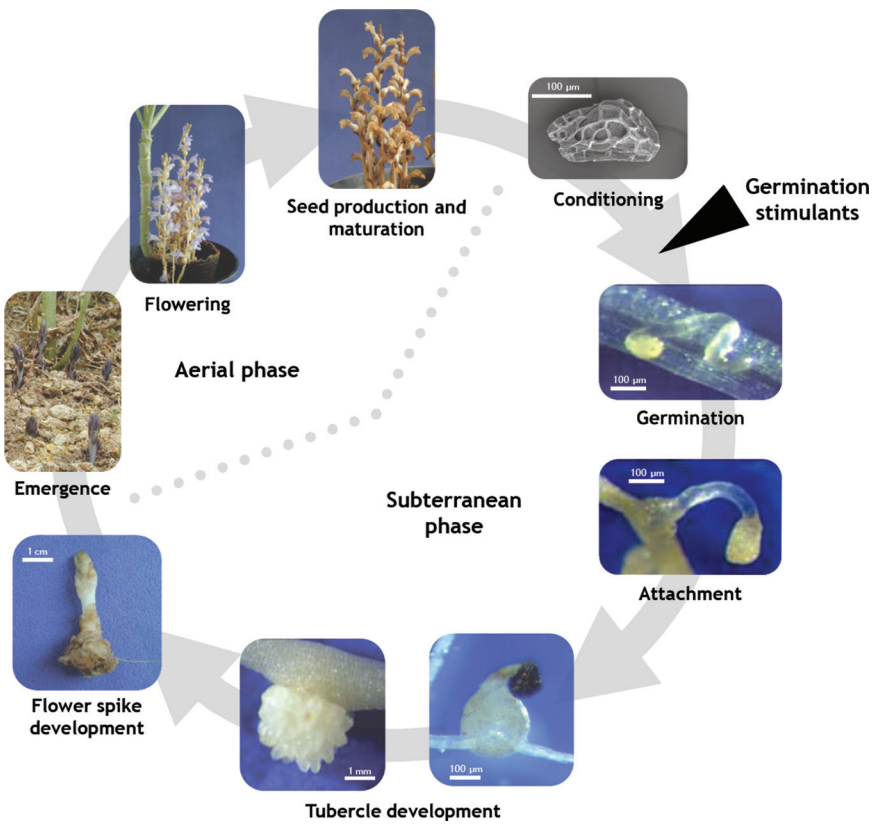

Figure 1: Life cycle of *Orobanche* (example of *Phelipanche ramosa*).

on this attachment step (Joel *et al.*, 2007). It is therefore the most susceptible step in the *Orobanche* life cycle. However, the quantity of seeds in the soil is so high that, despite a strong rate of unsuccessful germinations (no attachment to the host), the perennity of the parasitic species is not impacted. Broomrapes are mainly autogamous plants (Musselman *et al.*, 1982), with a strategy consisting in producing up to half a million extremely small seeds (200–300 µm) in order to maintain a high level of adaptability to environmental conditions (Irving & Cameron, 2009). Then, most of the life cycle occurs beneath the soil surface and only the inflorescence will end up emerging.

The seeds

Orobanche seed coat is characterized by a honeycomb pattern resulting from the dehydration of a maternal cell layer (only the cell walls remain visible). These patterns are species specific and could then be used for species identification. Ovoid seeds are composed of lipids, with oleic and linoleic acids as main storage material in *P. aegyptiaca* (Bar-Nun & Mayer, 2002). The endosperm is composed of three to four cell layers and contains several oil bodies and starch grains. It surrounds a reduced embryo that is composed of a spherical body without a plumule, a radicle, or cotyledons. Usually considered as an undifferentiated body, recent works indicated that two distinct regions may be present in the embryo of *P. aegyptiaca* (Joel *et al.*, 2011). It is suggested that the two perisperm cells located between the embryo and the micropyle may contain the putative receptors of germination stimulants (GSs) (Lechat *et al.*, 2012). The *Orobanche* tiny seeds may easily spread to other fields and can persist in soil for decades (Prider *et al.*, 2013), leading to an accelerated increase of contamination in the infested areas in which susceptible crops might be affected. Broomrape seed dispersal is facilitated by wind, water, humans and their agricultural tools, and animal. They are also suspected of being dispersed by forage and contaminated crop seed lots (Dongo *et al.*, 2011). This last mode of dissemination and the resulting contamination of new areas are of special relevance for seed companies and other institutions supplying crop seeds, as well as for seed transfer regulation.

The conditioning period

After maturation and desiccation, *Orobanche* seeds are more or less buried into the soil where they enter dormancy, a waiting period for environmental

conditions suitable for their germination. The dormancy discussed here seems to be specific to broomrape species because some recent works suggest that *P. ramosa* seeds do not have classical physical dormancy (Lechat *et al.*, 2012). On the contrary, these seeds require a conditioning period that would correspond to a moist environment and suitable temperatures and which is specific to each broomrape species. It is suggested that this period corresponds to physiological processes resulting in the setup of the machinery needed for GS perception. Seed hydration and major metabolic pathways are initiated during seed conditioning. However, some broomrape species may not require this conditioning phase (Plakhine *et al.*, 2009) but it is still a matter of debate. In *P. ramosa*, it has been shown that seeds require a minimum of 4 days of conditioning to allow optimal germination in response to GSs (Figure 2). The conditioning period starts with seed imbibition which takes around 1 h. This rapid imbibition is obtained by water entering in the seed through the micropyle which opens after 30 min (Joel *et al.*, 2011). Then several physiological process occur, indicating a rapid metabolic reactivation. First, an optimal adenylate energy charge (AEC = 0.9) is reached as of first day of conditioning. A strong decrease in abscisic acid (ABA) seed content occurred during the same period of conditioning in *P. ramosa* (Lechat *et al.*, 2012) along with a high release in the medium in the case of *O. minor* (Chae *et al.*, 2004). A characteristic pattern of respiration, protein synthesis, and utilization of reducing sugars (Bar-Nun & Mayer, 1993; 2002) occurs as well as a strong alternative oxidase activity. Uematsu *et al.* (2007) also demonstrated an accumulation of cyclic adenosine monophosphate (cAMP) associated with a gibberellin

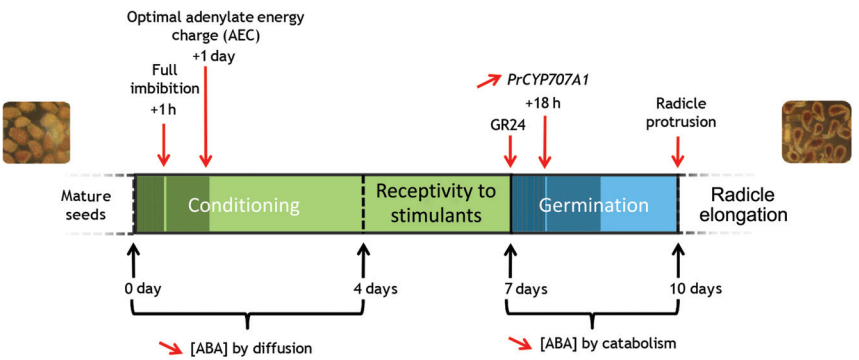

Figure 2: Molecular mechanisms in conditioning and germination of *P. ramosa* seeds (Lechat *et al.*, 2012). ABA, abscisic acid; *PrCYP707A1*, an ABA 8′-hydroxylase encoding gene.

synthesis in *O. minor* seeds while a decreased quantity of cAMP was observed in *P. ramosa* (Personal communication, MM. Lechat). All these events are potentially involved in the setup of the mechanisms needed for stimulant perception or signal translocation and thus for broomrape seed germination, without however knowing the underlying mechanism. However, recent works indicate that an epigenetic mechanism, a DNA demethylation, may occur during the conditioning phase and would explain the non-responsiveness of seeds to GSs during this period (see below).

It is noteworthy that the overall phenomenon of conditioning is reversible. Indeed, in the absence of a host in the immediate vicinity, seeds enter a secondary dormancy (Kebreab & Murdoch, 1999). This mechanism enables the seeds to face unfavorable environmental conditions or a time lag with the development cycle of the host plant. Seeds have to go through a new cycle of desiccation and conditioning to be again perceptive to the GS produced by another host plant (Matusova *et al.*, 2004). This phenomenon appears to be a key to longevity of broomrape seed (Joel *et al.*, 2007).

Germination: the stimulants and their mode of action

Unlike most angiosperms, seeds of broomrape species are unable to germinate without stimulation by a chemical stimulus, the GS, produced and exuded in the rhizosphere by surrounding host roots. Most GSs identified thus far belong to the strigolactone (SL) family (Yoneyama *et al.*, 2010). The first SL, strigol, was discovered in 1966 by Cook *et al.* It was exuded by cotton roots and induced at a very low concentration (1×10^{-11} M) for the germination of *Striga* seeds. Since then, more than 15 structural variants of strigol have been discovered and all derive from the carotenoid biosynthesis pathway (Matusova *et al.*, 2005). Interestingly, SL also act as host recognition signals for symbiotic arbuscular mycorrhizal fungi (Besserer *et al.*, 2006) and are considered to be a novel class of plant hormones involved in controlling shoot branching inhibition (Gomez-Roldan *et al.*, 2008). Several studies have also investigated the SL signaling pathway in plants as well as the relationships between SLs and other phytohormones during the control of plant architecture. SLs interact with auxin and cytokinins (CK) in bud outgrowth control, during adventitious root initiation or in nutrient–stress responses. In addition, cross-talk can occur between SLs, auxin, and ethylene in the control of root hair elongation.

Although most of the GSs identified so far belong to the SL family and correspond to butenolides signaling molecules, other molecules have also been identified such as sesquiterpene lactones, polyphenols, and isothiocyanates involved in the germination of *O. cumana* (Raupp & Spring, 2013), *O. foetida* (Evidente *et al.*, 2010), and *P. ramosa* (Auger *et al.*, 2012), respectively. In contrast, although the key role of SLs as GSs has been known for several decades, almost nothing is known about the early molecular events governing the germination of root parasitic plants in response to SLs, nor about how SLs interact with parasitic phytohormones during this process. For a long time, identification of SL receptors has remained elusive and their subcellular location was under debate despite several structure–activity studies. However, recent works have allowed significant progress in understanding the components of this key step in the *Orobanche* biology. First, using a transcriptomic approach, Lechat *et al.* (2012) highlighted the major role of *PrCYP707A1*, an ABA catabolic gene, in the germination of *P. ramosa* seeds in response to the SL analogue GR24 (Figure 2). *PrCYP707A1* is expressed at low levels during conditioning during which an initial decline in ABA levels was recorded. GR24 application after conditioning triggered a strong upregulation of *PrCYP707A1* during the first 18 h, followed by an eight-fold decrease in ABA levels detectable 3 days after treatment. Concomitant treatments of conditioned seeds with GR24 and exogenous ABA, or Abz-E2B, a specific inhibitor of CYP707A, caused inhibition of germination. These results demonstrated that germination occurs after a dormancy release of the seeds by ABA catabolism mediated by the GR24-dependent activation of *PrCYP707A* gene. Responses of *P. ramosa*, *O. cumana*, *O. minor*, and *S. hermonthica* seeds to different GS – the synthetic SL GR24, the sunflower sesquiterpene lactone dehydrocostus lactone (DCL), and the 2-phenylethyl isothiocyanate (ITC) present in the rhizosphere of oilseed rape – were also analyzed (Delavault *et al.*, 2013). The seeds displayed differential response patterns according to the species, the stimulants, and the applied concentration. Thus, the four species germinated in response to GR24, only the three broomrape species responded to DCL, and only *P. ramosa* germinated in response to ITC. Whatever the GS and the species, when germination was triggered, a *CYP707A* upregulation was observed. These results revealed the ubiquitous key role of *CYP707A* in parasitic plant seed germination triggered by GS.

In situ hybridization experiments on GR24-treated seeds revealed a specific *PrCYP707A1* mRNA accumulation in the perisperm cells located between the embryo and the micropyle, suggesting that it could be the location of SL receptors in *P. ramosa* (Lechat *et al.*, 2012). The mechanisms involved in perception and signal transduction of GSs upstream of *PrCYP707A1* still remain unknown. However, the group of David Nelson working on karrikins, molecules

present in smoke and known to trigger the germination of non-parasitic plant seeds, strongly suggests that the perception system in Orobanchaceae employs a mechanism involving KAI2, an α/β-fold hydrolase, as the receptor of SL, and an F-box protein MAX2 as the mediator of the response (Conn *et al.*, 2013). It has been shown also that *P. ramosa* seeds require a conditioning period of at least 4 days to be receptive to the GS and that, during this 4-day period, *PrCYP707A1* is not expressed. Interestingly, a global DNA demethylation process was shown to occur progressively during the conditioning period. DNA methylation is known as an epigenetic modification that affects gene expression in plants, with a high level of cytosine methylation strongly correlated with gene silencing. In the case of *PrCYP707A*, its repression during the minimal 4-day conditioning period seems to be associated with a high degree of DNA methylation in its promoter sequence (Lechat *et al.*, unpublished results).

The *haustorium*, a key organ

A common feature of all parasitic plants is their capacity to attach to a host plant, thanks to a specialized endophytic organ called *haustorium* (from the Latin word *haurire* meaning "to draw up"). Once they are stimulated, *Orobanche* seeds produce a germ tube considered as a radicle and called *procaulôme*. The germ tube grows by cell elongation toward the host roots probably guided by a positive chemotropism related to an increasing concentration gradient of stimulating molecules produced by the host (Bouwmeester *et al.*, 2003). Once the seedlings reach a host root, radicle elongation ends while processes of radial expansion are initiated and apical cells differentiate into papillae secreting a mucilaginous substance that promotes adhesion to the host. The radicle apex starts then to swell and develops a structure called *appressorium*, allowing anchoring to the host root surface and also penetration into the root cortex via intrusive cells. Progression of *appressorium* cells into the root tissues occurs through mechanical pressure facilitated by the secretion of enzymes with pectin methylesterase and polygalacturonase activities (Véronési *et al.*, 2007). These enzymes would adapt the chemical composition and the physical properties of host cell walls to the *haustorium* development, without the involvement of degrading processes that would generate strong defense responses by the host. Endodermis is the last physical barrier that the *appressorium* should pass through to reach the central cylinder containing the host vascular system. This last step would be facilitated by secretion of enzyme with a cutinase activity. After penetration through host endodermis, parasite cells initiate the most

important organ of the interaction, the *haustorium sensu stricto*, which "serves as the structural and physiological bridge that allows the parasites to withdraw water and nutrients from the conductive systems of living host plants." Apparition of the invasive *haustorium* in plants was the major event that permits the evolutionary transition toward a parasitic lifestyle. Orobanchaceae have two kinds of *haustoria*, lateral *haustoria*, which develop as lateral extensions of parasitic roots, and terminal *haustoria* which develop at the apex of the radicle. The terminal *haustorium* is a characteristic feature of the holoparasitic *Orobanche* clade of Orobanchaceae. As mentioned earlier, seed germination of obligate root parasitic plant is triggered by chemical compounds produced by the host. In the case of the *haustorium* formation, these are also chemical signals derived from host which initiate its development in Orobanchaceae. These signals are called xenognosins or haustorium-inducing factors (HIFs). However, among the Orobanchaceae, there are notable exceptions, the *Phelipanche* and *Orobanche* species for which no host factors for *haustorium* development have been to date discovered.

The nature of the conductive system between the *haustorium* and the host vascular system diverges depending on the parasite species. Indeed, obligate hemiparasites such as *Striga* species are xylem feeders because they produce direct connections with the host xylem via a specific organ called *osculum*, but little or no phloem connections. By contrast, *Orobanche* are "phloem feeders" because there are clear indications for both phloem and xylem continuums as they have been demonstrated by using specific xylem- and phloem-mobile dyes.

Post-attachment development of the parasite

Phloem connections provide *Orobanche* with the ability to extract nitrogen and carbohydrate compounds from their host. Once the connections are established, the *haustorium* grows, distends host root tissues, and rapidly becomes a small tubercle. As it grows, the tubercle produces short adventitious roots with a rudimentary cap but without root hair zone, making them unable to uptake water and mineral compounds from the soil. Nevertheless, each of these roots is a growing organ connected to host phloem that contributes to increase the sink strength of the tubercle. This tuberous cell mass, with a not yet defined morphology, constitutes then a storage organ accumulating temporarily mainly hexoses, polyols (mannitol, inositol, etc.), amino acids, and starch (Abbes *et al.*, 2009; Delavault *et al.*, 2002; Draie *et al.*, 2011). The tubercle exhibits one

or more shoot apical meristems that, when the parasite will perceive appropriate host-derived signals (nutrients, hormones…), will produce achlorophyllous scaly stems. After the stems emerge from the soil, they develop one or more flower spikes supporting several flowers. The rate of stem branching and the number of flowers per spike depend on the species. Every flower, after (self)-fertilization, gives a capsule containing thousands of seeds. It is noteworthy that the life cycle of *Orobanche* is synchronized with that of the host plant. Every step (bud burst of apical meristem, flowering, and seed production) is then realized simultaneously with those of the host (Gibot-Leclerc *et al*., 2012).

Source–sink relationship, sink strength, and host–parasite transfers

All plant organs require a constant supply in photoassimilates and reduced nitrogen compounds. Autotrophic plants should then proceed to a partitioning of nutrients in order to supply organs unable to produce such compounds. This is referred to as source–sink relationships with, for example, the relation between mature source leaves, which are highly photosynthetic with then an excess of nutritive compounds, and sink organs such as young leaves, buds, or roots. The term sink strength can be defined as the competitive ability of an organ to attract assimilates and water coming from conducting tissues. Thus, holoparasites act like supernumerary sink organs that are highly dependent on carbohydrate and nitrogen compounds remobilized from the different source organs of host (Figure 3). Because *Orobanche* are achlorophyllous holoparasites lacking functional roots, their carbon and nitrogen nutrition relies totally on an acquisition from the host. In plants, nitrogen assimilation is a process highly dependent on carbon and energy. Because of a lack of reducing power and carbon skeletons (usually provided by photosynthesis) and a low nitrate reductase activity in broomrape species, the amino acid uptake from the host seems to be the most efficient nitrogen acquisition in terms of energy (Irving & Cameron, 2009). Moreover, because their transpiration rate is very low, it is almost impossible for *Orobanche* to obtain nutrients by diverting the flow of xylem content of the host, as it is the case in the hemiparasite species (Hibberd *et al*., 1999). Thus, *Orobanche* derives all of its nutrients needed for its development from the host phloem (“phloem feeder”). This is done by reducing its osmotic potential thanks to an accumulating high levels of osmotically active compounds such as cations, sugars, amino acids, and polyols, and also to the establishment of a decreasing concentration

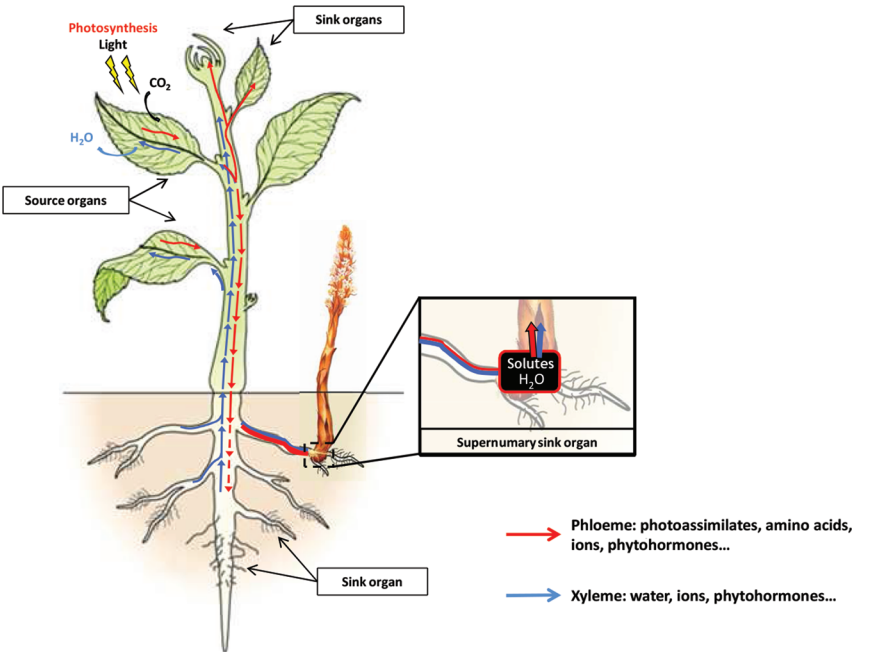

Figure 3: Source–sink relationship in a host plant–*Orobanche* interaction.

gradient between the *haustorium* tissues close to the connections and the other organs.

Given the central importance of the *haustorium* function in resource acquisition, it is surprising that the molecular mechanisms involved in the physiology of this organ remain poorly characterized. Concerning carbon, Draie *et al.* (2011) demonstrated the major role of PrSAI1, a *P. ramosa* vacuolar soluble acid invertase, in the sink strength of the flowering shoot. PrSAI1 would act in the phloem unloading of sucrose from the host and then in the subsequent accumulation of hexoses. Péron *et al.* (2012) highlighted the role of *PrSus1*, a *P. ramosa* sucrose synthase encoding gene, in the utilization of host-derived sucrose in meristematic areas and in cellulose biosynthesis in differentiating vascular elements. Importance of accumulating mannitol has been long demonstrated in *Orobanche* because it is a useful solute that functions as an osmoticum involved in reducing the osmotic potential, a storage form for reduced carbon, an osmoprotectant, and a scavenger of reactive oxygen species (Delavault *et al.*, 2002). Regarding nitrogen, few studies have been conducted to examine the actors of the sink strength as well as of the nitrogen metabolism in *Orobanche*. Glutamine synthetase 2 (GS2) and nitrate reductase have been shown to be

missing or reduced in activity in several *Orobanche* species. The fact that herbicides, targeting specifically the amino acid biosynthesis, have a major impact on broomrapes tends to prove that these plants have their own machinery for amino acid metabolism (Eizenberg *et al*., 2012). In *O. foetida*, tubercles accumulate preferentially soluble amino acids, especially aspartate and asparagine, suggesting an important role for a glutamine-dependent asparagine synthetase in the N metabolism of the parasite (Abbes *et al*., 2009). To illustrate the tremendous sink strength developed by the parasite, an example is *O. cernua* that gained 99% of its carbon and 95% of its nitrogen from tobacco phloem (Hibberd *et al*., 1999).

Conclusion and perspectives

The trophic exploitation developed by *Orobanche* impacts the host plant physiology at several levels. From a global point of view, the infection strongly reduces the host plant biomass and its fertility. At the field level, this results in a yield loss in economically important crops that could be total depending on the broomrape species and the host genotype. It is worth noting that the subterranean phase of the parasite development is most detrimental for the crop. Thus, when the infection becomes visible with the parasite emerging from the soil, it is already too late for the crop because the parasite would have caused irreversible damage that will reduce crop yield. To date, in spite of intense efforts, means to selectively control the various broomrape species are still scarce and inefficient in terms of sustainability. Given the alarming impact of *Orobanche* species on world agriculture, deciphering the physiological and molecular events governing the parasite development and the establishment of the interaction with its host is then a necessary step toward the development of targeted control methods. Indeed, every developmental or metabolic particularity of the parasite might be considered as a point of vulnerability that could be potentially exploited. Unfortunately, when compared with other plant–pathogen interactions, our knowledge is far too scarce. Progress in understanding the unique biology of broomrapes has been hampered by the lack of genomic resources in *Orobanche* and the lack of protocols allowing gene modification (knock-out or expression modulation) in these parasites. However, most of these barriers have been very recently alleviated with the availability of transcriptomic data for *P. aegyptiaca* (Parasitic Plant genome Project) and *P. ramosa* (P. Delavault, University of Nantes), the possibility of manipulating *P. aegyptiaca* gene expression via hairpin RNAi through the genetic transformation of the host plant and

the systemic movement of the silencing signal from host to parasite (Aly *et al.*, 2009), and the development of a robust system for *Agrobacterium rhizogenes*-mediated transformation and subsequent regeneration of the *P. aegyptiaca* holoparasitic plant (Fernandez-Aparicio *et al.*, 2011). In any case, the road ahead is still long – "development of effective and low-cost control measures for *Orobanche* remains the "holy grail" for plant pathologists, agronomists, and biotechnologists" (Nickrent, Southern Illinois University).

References

Abbes, Z., Kharrat, M., Delavault, P., Chaïbi, W., Simier, P., 2009. Nitrogen and carbon relationships between the parasitic weed *Orobanche foetida* and susceptible and tolerant faba bean lines. Plant Physiology and Biochemistry 47: 153–159.

Aly, R., Cholakh, H., Joel, D.M., Leibman, D., Steinitz, B., Zelcer, A., Naglis, A., Yarden, O., Gal-On, A., 2009. Gene silencing of mannose 6-phosphate reductase in the parasitic weed *Orobanche aegyptiaca* through the production of homologous dsRNA sequences in the host plant. Plant Biotechnology Journal 7: 487–498.

Auger, B., Pouvreau, J.B., Pouponneau, K., Yoneyama, K., Montiel, G., Le, B. Bizec, Yoneyama, K., Delavault, P., Delourme, R., Simier, P., 2012. Germination stimulants of *Phelipanche ramose* in the rhizosphere of Brassica napus are derived from the glucosinolate pathway. Molecular Plant-Microbe Interactions 25: 993–1004.

Bar-Nun, N., Mayer, A.M., 1993. Preconditioning and germination of *Orobanche seeds*: respiration and protein synthesis. Phytochemisty 34: 39–45.

Bar-Nun, N., Mayer, A.M., 2002. Composition of and changes in storage compounds in *Orobanche aegyptiaca* seeds during preconditioning. Israel Journal of Plant Sciences 50: 278–279.

Besserer, A., Puech-Pagès, V., Kiefer, P., Gomez-Roldan, V., Jauneau, A., Roy, S., Portais, J.C., Roux, C., Bécard, G., Séjalon-Delmas, N. 2006. Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biology 4:e226.

Bouwmeester, H.J., Matusova, R., Zhongkui, S., Beale, M.H., 2003. Secondary metabolite signalling in host–parasitic plant interactions. Current Opinion in Plant Biology 6: 358–364.

Chae, S.H., Yoneyama, K., Takeuchi, Y., Joel, D.M., 2004. Fluridone and norflurazon, carotenoid-biosynthesis inhibitors, promote seed conditioning and germination of the holoparasite *Orobanche minor*. Physiologia Plantarum 120: 328–337

Conn, C.E., Neumann, D., Dyer, K.A., and Nelson, D.C., 2013. An investigation of the genetic basis for strigolactone perception in parasitic plant germination. P. 28. In: Proc. 12th World Congress on Parasitic Plants, Sheffield, UK.

Cook, C.E., Whichard, L.P., Turner, B., Wall, M.E., and Egley, G.H., 1966. Germination of Witchweed (*Striga lutea Lour.*): isolation and properties of a potent stimulant. Science 154: 1189–1190.

Delavault, P., Thalouarn, P., 2002. The obligate root parasite *Orobanche cumana* exhibits several *rbcL* sequences. Gene 297: 85–92.

Delavault, P., Lechat, M.M., Pouvreau, J. B., Montiel, G., Véronési, C., and Simier, P., 2013. *CYP707A1*, an ABA catabolic gene, is ubiquitous component of parasitic plant seed germination in response to various germination stimulants. In: Proc. 1st Stream Meeting, Jerusalem, Israel.

Delavault, P., Simier, P., Thoiron, S., Véronési, C., Fer, A., Thalouarn, P., 2002. Isolation of mannose 6-phosphate reductase cDNA, changes in enzyme activity and mannitol content in broomrape (*Orobanche ramosa*) parasitic on tomato roots. Physiologia Plantarum 115: 48–55.

Dongo, A., Leflon, M., Simier, P., Delavault, P., 2011. Development of a high-throughput real-time quantitative PCR method to detect and quantify contaminating seeds of *Phelipanche ramosa* and *Orobanche cumana* in crop seed lots. Weed Research 52: 34–41.

Draie, R., Péron, T., Pouvreau, J.B., Véronési, C., Jégou, S., Delavault, P., Thoiron, S., Simier, P., 2011. Invertases involved in the development of the parasitic plant *Phelipanche ramosa*: characterization of the dominant soluble acid isoform, PrSAI1. Molecular Plant Pathology 12: 638–652.

Eizenberg, H., Aly, R., Cohen, Y., 2012. Technologies for Smart Chemical Control of Broomrape (*Orobanche* spp. and *Phelipanche* spp.). Weed Science 60: 316–323.

Evidente, A., Cimmino, A., Fernández-Aparicio, M., Andolfi, A., Rubiales, D., Motta, A., 2010. Polyphenols, including the new peapolyphenols A–C, from pea root exudates stimulate *Orobanche foetida* seed germination. Journal of Agricultural and Food Chemistry 58: 2902–2907.

Fernandez-Aparicio, M., Rubiales, D., Bandaranayake, P.C.G., Yoder, J.I., Westwood, J.H. 2011. Transformation and regeneration of the holoparasitic plant *Phelipanche aegyptiaca*. Plant Methods 7: 36.

Gibot-Leclerc, S., Sallé, G., Reboud, X., Moreau, D., 2012. What are the traits of *Phelipanche ramosa* (L.) Pomel that contribute to the success of its biological cycle on its host *Brassica napus* L.? Flora – Morphology, Distribution, Functional Ecology of Plants 207: 512–521.

Gomez-Roldan, V., Fermas, S., Brewer, P.B. et al., 2008. Strigolactone inhibition of shoot branching. Nature 455: 189–194.

Hibberd, J.M., Quick, W.P., Press, M.C., Scholes, J.D., Jeschke, W.D., 1999. Solute fluxes from tobacco to the parasitic angiosperm *Orobanche cernua* and the influence of infection on host carbon and nitrogen relations. Plant, Cell & Environment 22: 937–947.

Irving, L.J., Cameron, D.D., 2009. You are what you eat: interactions between root parasitic plants and their hosts. Advances in Botanical Research 50: 87–138.

Joel, D., Hershenhorn, J., Eizenberg, H., Aly, R., Ejeta, G., Rich, P.J., Ransom, J.K., Sauerborn, J., Rubiales, D. 2007. Biology and management of weedy root parasites. Revue Horticole 33: 267–349.

Joel, D.M. 2009. The new nomenclature of *Orobanche* and *Phelipanche*. Weed Research 49: 6–7.

Joel, D.M., Bar, H., Mayer, A.M., Plakhine, D., Ziadne, H., Westwood, J.H., and G.E Welbaum. 2011. Seed ultrastructure and water absorption pathway of the root-parasitic plant *Phelipanche aegyptiaca (Orobanchaceae)*. Annals of Botany 109: 181–195.

Kebreab, E., Murdoch, A.J., 1999. A quantitative model for loss of primary dormancy and induction of secondary dormancy in imbibed seeds of *Orobanche* spp. Journal of Experimental Botany 50: 211–219.

Lechat, M.M., Pouvreau, J.B., Peron, T., Gauthier, M., Montiel, G., Veronesi, C., Todoroki, Y., Le Bizec, B., Monteau, F., Macherel, D., Simier, P., Thoiron, S., Delavault, P., 2012.

PrCYP707A1, an ABA catabolic gene, is a key component of *Phelipanche ramosa* seed germination in response to the strigolactone analogue GR24. Journal of Experimental Botany 63: 5311–5322.

Matusova, R., Rani, K., Verstappen, F.W., Franssen, M.C., Beale, M.H., Bouwmeester, H.J., 2005. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. Plant Physiology 139: 920–934.

Matusova, R., Mourik, T., Bouwmeester, H.J., 2004. Changes in the sensitivity of parasitic weed seeds to germination stimulants. Seed Science Research 14: 335–344.

McNeal, J.R., Bennett, J.R., Wolfe, A.D., and Mathews, S., 2013. Phylogeny and origins of holoparasitism in *Orobanchaceae*. American Journal of Botany 100: 971–983.

Musselman, L.J., Parker, C., Dixon, N., 1982. Notes on autogamy and flower structure in agronomically important species of *Striga* (*Scrophulariaceae*) and *Orobanche* (*Orobanchaceae*). Beitrage zur Biologie der Pflanzen 56: 329–343.

Péron, T., Véronési, C., Mortreau, E., Pouvreau, J.B., Thoiron, S., Leduc, N., Delavault, P., Simier, P., 2012. Role of the sucrose synthase encoding *PrSus1* gene in the development of the parasitic plant *Phelipanche ramosa* L.(Pomel). Molecular Plant-Microbe Interactions 25: 402–411.

Piednoël, M., Aberer, A.J., Schneeweiss, G.M., Macas, J., Novak, P., Gundlach, H., Temsch, E.M., Renner, S.S., 2012. Next-generation sequencing reveals the impact of repetitive DNA across phylogenetically closely related genomes of *Orobanchaceae*. Molecular Biology and Evolution 29: 3601–3611.

Piednoël, M., Carrete-Vega, G., Renner, S.S., 2013. Characterization of the LTR retrotransposon repertoire of a plant clade of six diploid and one tetraploid species. Plant Journal 75: 699–709.

Plakhine, D., Ziadna, K., Joel, D.M., 2009. Is seed conditioning essential for Orobanche germination? Management Science 65: 492–496.

Prider, J., Craig, A., Matthew, J., Correll, R., 2013. Natural seed bank decline of *Phelipanche mutelii* in South Australia. P. 59. In: Proc. 12th World Congress on Parasitic Plants, Sheffield, UK.

Raupp, F.M., Spring, O., 2013. New sesquiterpene lactones from sunflower root exudate as germination stimulants for *Orobanche cumana*. Journal of Agricultural and Food Chemistry 61: 10481–10487.

Schneeweiss, G.M., Colwell, A., Park, J.M., Jang, C.G., Stuessy, T.F., 2004. Phylogeny of holoparasitic *Orobanche* (*Orobanchaceae*) inferred from nuclear ITS sequences. Molecular Phylogenetics and Evolution 30: 465–478.

Uematsu, K., Nakajima, M., Yamaguchi, I., Yoneyama, K., Fukui, Y., 2007. Role of cAMP in gibberellin promotion of seed germination in *Orobanche minor* Smith. Journal of Plant Growth Regulation 26: 245–254.

Véronési, C., Bonnin, E., Calvez, S., Thalouarn, P., Simier, P., 2007. Activity of secreted cell wall modifying enzymes and expression of peroxidase-encoding gene following germination of *Orobanche ramosa*. Biologia Plantarum 51: 391–394.

Westwood, J.H., Yoder, J.I., Timko, M.P., C.W. dePamphilis. 2010. The evolution of parasitism in plants. Trends in Plant Science 15: 227–235.

Wolfe, A.D., Randle, C.P., Liu, L., and Steiner, K.E., 2005. Phylogeny and biogeography of *Orobanchaceae*. Folia Geobotanica 40: 115–134.

Yoneyama, K., Awad, A.A., Xie, X., Yoneyama, K., Takeuchi, Y., 2010. Strigolactones as germination stimulants for root parasitic plants. Plant & Cell Physiology 51: 1095–1103.