First publ. in: FEMS Microbiol Letters 259 (2006) pp. 165–173

Uromyces fabae: development, metabolism, and interactions with its host *Vicia faba*

Ralf T. Voegele

Lehrstuhl Phytopathologie, Fachbereich Biologie, Universität Konstanz, Konstanz, Germany

Correspondence: Ralf T. Voegele, Lehrstuhl Phytopathologie, Fachbereich Biologie, Universität Konstanz, Universitätsstr. 10, 78457 Konstanz, Germany. Tel.: +49 7531 88 4305; fax: +49 7531 88 3035; e-mail: ralf.voegele@uni-konstanz.de

Received 31 January 2006; revised 7 March 2006; accepted 17 March 2006.
First published online 19 April 2006.

doi:10.1111/j.1574-6968.2006.00248.x

Editor: Richard Staples

Keywords
Uromyces fabae; *Vicia faba*; rust fungus; obligate biotrophy; haustorium.

Abstract

This MiniReview is intended to provide an overview of the current knowledge regarding cytological, physiological, and molecular aspects of *Uromyces fabae*. For almost five decades this rust fungus has served as a model system to gain insight into the features characterizing an obligate biotrophic parasite. While earlier studies mostly focused on cytological aspects, later studies were concerned with biochemical and molecular characteristics. Despite the fact that there is still no stable transformation system available for any obligate biotroph, recent molecular analyses have provided new insights into this highly sophisticated interaction of a fungus with its host.

Obligate biotrophic parasites

Some of the most devastating plant pathogens are obligate biotrophic parasites (Brown & Hovmøller, 2002). This term characterizes a lifestyle in which the host suffers only minor damage over a longer period of time. The pathogen in turn depends on a living host to complete its life cycle (Staples, 2000). This form of parasitism stands in contrast to necrotrophic pathogens, which kill their hosts quickly and subsequently thrive on the dead plant material (Mendgen & Hahn, 2002). In order to mark off the true obligate biotrophic pathogens from hemibiotrophs or necrotrophs, the following criteria were suggested: (a) highly differentiated infection structures; (b) limited secretory activity; (c) a narrow contact zone separating fungal and plant plasma membranes; (d) long term suppression of host defense responses; (e) the formation of haustoria (Mendgen & Hahn, 2002). Accordingly the true obligate biotrophs comprise the Downey Mildews, the Powdery Mildews, and the Rusts.

Rust fungi

Rusts have plagued farmers around the globe throughout history. Many cereals and legumes, the two plant families most important for humans (Graham & Vance, 2003), suffer from rust infections. Cereal rusts have been a recurring problem in Northern America, occasionally causing huge losses (Long, 2003). Legume rusts prevail in Africa, Asia, and Oceania.

The rust fungi comprise more than 100 genera and around 7000 species (Maier *et al*., 2003). *Puccinia* represents the largest genus with about 4000 species, followed by the genus *Uromyces* with about 600 species (Maier *et al*., 2003). The analysis by Maier *et al*. (2003) also suggests that these two genera are polyphyletic. This implies that although morphologically similar there might be differences at the molecular level.

Mainly five species of rust fungi have served as model organisms in the laboratory. *Melampsora lini* and its host *Linum usitatissimum*, for example, were used by Flor (1956) to demonstrate the gene-for-gene hypothesis. *Uromyces appendiculatus*, and *Puccinia graminis* have been used in a number of cytological and physiological studies (Zhou *et al*., 1991; Leonard & Szabo, 2005). Today molecular analyses of rust fungi mainly focus on *P. triticina* (Thara *et al*., 2003), *M. lini* (Catanzariti *et al*., 2006), and *Uromyces fabae* (Jakupovic *et al*., 2006).

Uromyces fabae

Uromyces fabae (*U. vicia-fabae*) attacks several important crop species such as broad bean (*Vicia faba*), pea, and lentil,

as well as more than 50 other *Vicia* species, and about 20 *Lathyrus* species (Conner & Bernier, 1982). However, it is most commonly referred to as rust of faba bean where yield losses of up to 50% have been reported (Tissera & Ayres, 1986). The pathogen has served as a model organism for almost half a century. It started in the 1960s and 1970s with physiological and cytological investigations (Thrower & Thrower, 1966; Abu Zinada *et al.*, 1975). These studies were continued in the 1980s (Kapooria & Mendgen, 1985), before biochemical aspects became a new focus in the early 1990s (Deising *et al.*, 1991). Work on molecular aspects of biotrophy became possible with the advent of a procedure to isolate haustoria (Hahn & Mendgen, 1992). While much progress has been made during the last couple of years, two facts still impede research with obligate biotrophs: first, none of the true obligate biotrophs can be grown in culture (at least not to a point reflecting the 'parasitic phase'), and second there is currently still no stable transformation system available.

Life cycle

Uromyces fabae is a macrocyclic rust fungus, it exhibits all five spore forms known for the Uredinales. It is also autoecious, as all spore forms are produced on a single host (Mendgen, 1997). Figure 1 depicts the lifecycle of *U. fabae*. After overwintering on residual plant material, diploid teliospores germinate in the spring with a metabasidium. After meiosis, the latter produces four haploid basidiospores with two different mating types. These are ejected from the metabasidium and after landing on a leaf of a host germinate and produce infection structures. Pycnia are produced which contain pycniospores and receptive hyphae. Pycniospores are exchanged between pycnia of different mating types and after spermatization, dikaryotization occurs in aecial primordia. An aecium differentiates and dikaryotic aeciospores are produced. These aeciospores germinate and form infection structures from which uredia which produce urediospores are formed. Urediospores are the major asexual spore form of rust fungi produced in massive amounts through repeated infection of host plants during the summer. Urediospores are aerially dispersed and can travel thousands of kilometers carried by the wind (Brown & Hovmøller, 2002). In the fall, uredia differentiate into telia, the nuclei fuse during sporogenesis and single-celled diploid teliospores develop for the winter.

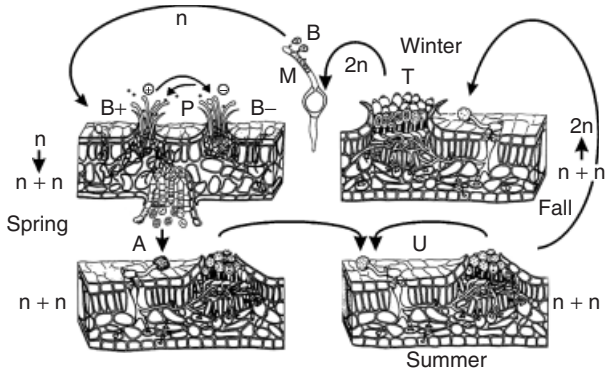

Fig. 1. Life cycle of *Uromyces fabae*. Overwintering diploid (2n) teliospores (T) germinate in the spring with a metabasidium (M) from which four haploid (n) basidiospores (B) of two mating types (+, −) are formed. Haploid pycniospores (P) are exchanged between pycnia of different mating types on the upper surface of a leaf. After spermatization dikaryotic (n+n) aeciospores (A) are formed in aecia at the lower surface of the leaf. Infecting aeciospores produce uredia from which dikaryotic urediospores (U) are formed. At the end of summer uredia differentiate into telia from which teliospores are formed and the cycle closes. Drawing modified from Mendgen (1997).

Spore germination and the formation of infection structures

Telio- and pycniospores do not infect plant material, whereas basidio-, aecio- and urediospores do. Teliospores represent the final spore form of rust fungi and provide the basis for their nomenclature (Mendgen, 1984). Aside from a few morphological descriptions not much is known about this spore form. Even less is known about aecio- or pycniospores, except that the latter enable sexual reproduction of rust fungi. Some studies have been performed with basidiospore derived infection structures (Kapooria, 1971; Gold & Mendgen, 1991). Almost all biochemical and later all molecular studies are based on infection structures derived from urediospores. The fact that infection structures from both basidio- and urediospores of *Uromyces fabae* have been analyzed morphologically allows a comparison between mono- and dikaryotic infection structures on the same host plant (Fig. 2). Thick-walled, darkly pigmented and ornamented urediospores (Fig. 2a) germinate with a germ tube which differentiates into a well defined appressorium. A penetration hypha is formed, which enters the leaf through the stomatal opening. A vesicle is formed within the stomatal cavity from which an infection hypha emerges. Upon contact with a mesophyll cell a haustorial mother cell

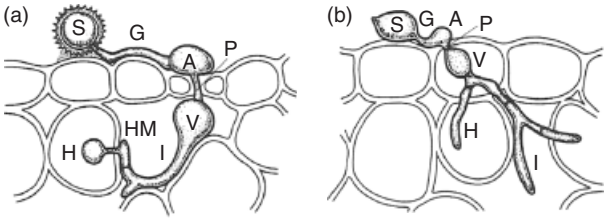

Fig. 2. Infection structures derived from uredio- (a) and basidiospores (b). S, spore; G, germ tube; A, appressorium; P, penetration hypha; V, vesicle; I, infection hypha, HM, haustorial mother cell; H, haustorium. Drawing modified from Mendgen (1997).

is differentiated from which a haustorium is formed. Basidiospores (Fig. 2b) by contrast are smooth and thin-walled. Infection structures like appressorium, vesicle and haustorium are noticeably less differentiated. Moreover, the penetration mechanism seems to be completely different, as in this developmental stage the fungus enters the plant by direct penetration.

Features of urediospore infection

Urediospores are single-celled, hydrophobic, and carry spines on their surface (Woods & Beckett, 1987). An important morphological feature used to distinguish different rust species is the number and position of germ pores. Three to four germ pores in an equatorial or near equatorial position are typical for *Uromyces fabae* (Emeran *et al.*, 2005). Premature germination of urediospores is prevented by the presence of germination inhibitors. However, the nature of the germination inhibitor(s) in *U. fabae* is unknown (Marte, 1971). Fully developed urediospores are almost completely dehydrated which gives them an irregular shape (Clement *et al.*, 1998). Only upon hydration do spores adopt an ellipsoid form. Although dry urediospores hydrate rapidly, their surface is nonwettable (Clement *et al.*, 1994). This hydrophobicity is responsible for the initial adhesion to the host surface (Clement *et al.*, 1993b). The initial contact is quickly followed by production of an extracellular matrix consisting of low-molecular-weight carbohydrates and glycosylated polypeptides (Clement *et al.*, 1993a). This matrix originates from solubilization of the surface matrix and lysis of the germ pore plug. The next step is the formation of an adhesion pad. The composition of the pad is similar to the composition of the matrix. Both seem to be exclusively of fungal origin, as they are also formed on artificial surfaces (Deising *et al.*, 1992). Cutinases and esterases are involved in the adhesion process because while spores treated with esterase inhibitors do form an adhesion pad, they fail to adhere (Deising *et al.*, 1992). Besides hydration (Clement *et al.*, 1997), two other factors have been found to have an influence on germination. One of these factors is light. A period of at least 40 min of darkness is required to induce germination (Joseph & Hering, 1997). The other parameter is temperature. Urediospores will germinate in a range between 5 and 26 °C with the optimal germination temperature being 20 °C (Joseph & Hering, 1997). Given the correct physical parameters the spore will germinate on almost any surface, even when completely submerged in water, indicating that no additional signals are needed to induce germination (Struck *et al.*, 1996). The cytoplasm moves into the germ tube as the developing germ tube meanders across the surface attached to it via matrix like material (Clement *et al.*, 1994). In order to produce infection structures downstream of the germ tube further signals are required. It has been shown that a topographical signal is needed for the differentiation of an appressorium (Allen *et al.*, 1991). *Uromyces appendiculatus* and *U. vignae* have been found to induce appressorium differentiation if a ridge of 0.4–0.8 µm in height is provided (Allen *et al.*, 1991). The values correspond to the height of the stomatal guard cell lips. In *U. appendiculatus* a mechanosensitive channel has been identified which might be involved in the transduction of the topographic signal into a differentiation response (Zhou *et al.*, 1991). The cytoplasm transfers to the appressorium and the vacuolated germ tube is separated by a septum. Differentiation of the appressorium coincides with the detection of a number of lytic enzymes (Fig. 3) (Deising *et al.*, 1995b). Acidic cellulases (Heiler *et al.*, 1993), extracellular proteases (Rauscher *et al.*, 1995), and chitin deacetylase (Deising & Siegrist, 1995) were found. At the base of the appressorium a penetration hypha is formed (Terhune *et al.*, 1993). For *U. appendiculatus* a turgor pressure of 0.35 MPa has been reported. This pressure is high enough to distort artificial surfaces or stomatal guard cell lips (Terhune *et al.*, 1993). Within the stomatal cavity a substomatal vesicle is formed which is separated from the penetration hypha by a septum. The vesicle is a stretched cylindrical structure which narrows into an infection hypha (Kapooria & Mendgen, 1985). More enzymes can be detected which may have a role in the local breakdown of the host cell wall (Deising *et al.*, 1995b). Pectin esterases (Frittrang *et al.*, 1992), pectin methylesterases (Deising *et al.*, 1995a), and neutral cellulases (Heiler *et al.*, 1993) can be found (Fig. 3). A haustorial mother cell is differentiated upon contact with a mesophyll cell, and it is separated from the infection hypha by a septum. The cytoplasm moves into the haustorial mother cell and earlier structures are vacuolated. Formation of the haustorial mother cell coincides with the onset of polygalacturonate lyase activity (Fig. 3) (Deising *et al.*, 1995a). Up to the haustorial mother cell, infection structures of this 'penetration phase' (Fig. 4) can be generated *in vitro* by

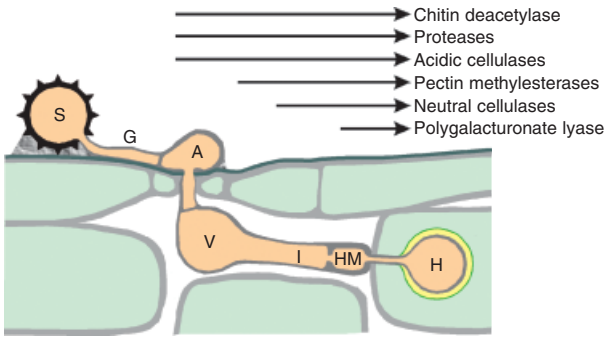

Fig. 3. Lytic enzymes in early dikaryotic infection structures. S, spore; G, germ tube; A, appressorium; P, penetration hypha; V, vesicle; I, infection hypha; HM, haustorial mother cell; H. haustorium. Drawing modified from Mendgen & Deising (1993).

germinating spores on colloidon membranes (Kapooria & Mendgen, 1985) or on structured polyethylene sheets (Deising *et al*., 1991). Haustoria, and structures of the 'parasitic phase' and the 'sporulation phase' are only formed *in planta* (Fig. 4). Haustorial mother cells have a thick, multilayered wall that attaches firmly to the host wall and forms a penetration hypha to invade the host cell (Heath, 1997). One or more signals of the host are needed to complete the differentiation of the haustorium (Heath, 1990). The haustorium develops from the haustorial mother cell with a slender neck and a haustorial body (Heath & Skalamera, 1997). During the formation of the haustorium, the cell wall of the host cell is breeched. The expanding haustorium invaginates the host plasma membrane. Therefore, the haustorium is not truly intracellular, it remains outside the physiological barrier of the host cell (Fig. 5). With development of the haustorium, a zone of separation is formed between the plasma membranes of parasite and host. It is composed of the fungal cell wall and the extrahaustorial matrix (Hahn *et al*., 1997a). The extrahaustorial matrix resembles an amorphous mixture of components, mainly carbohydrates and proteins, partly of fungal but primarily of plant origin (Harder & Chong, 1991). It seems likely that this zone plays an important role in maintaining the biotrophic life style. Undoubtedly the extrahaustorial matrix represents a formidable trading place for the exchange of nutrients and information (Heath & Skalamera, 1997). There is some evidence that the cytoplasmic membrane of the host enclosing the haustorial body, the so-called extrahaustorial membrane, is modified. Harder and Chong (1991) summarized results obtained by freeze fracture electron microscopy with bean rust and oat crown rust. In both interactions the extrahaustorial membrane lacks intramembranous particles, and exhibits a dramatic reduction of sterols (Harder & Mendgen, 1982). Cytochemical studies on powdery mildew haustoria (Gay *et al*., 1987; Manners, 1989) and later work by Baka *et al*. (1995) on rust haustoria suggested that the extrahaustorial membrane lacks ATPase activity. This implies that there is no control over solute fluxes from the host cell. The neck region of the haustorium is characterized by electron-dense material joining the plasma membranes of host and parasite (Harder & Chong, 1984). This 'neckband' seals the extrahaustorial matrix against the bulk apoplast, not unlike the Casparian strip in the endodermis (Heath, 1976). Based on the sealing by the neckband and the presence of the plant plasma membrane surrounding the whole structure it was suggested that the

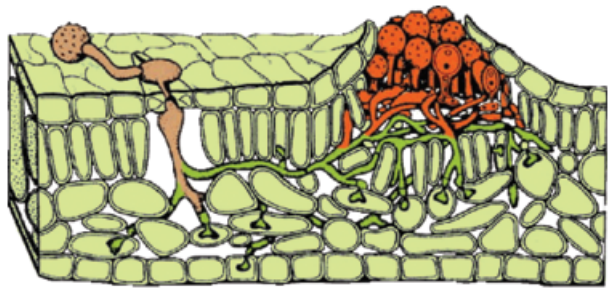

Fig. 4. Developmental phases of urediospore infection. Early infection structures of the 'penetration phase' are depicted in brown, structures of the 'parasitic phase' are shown in green and structures of the 'sporulation phase' are depicted in red. Drawing modified from Hahn (2000).

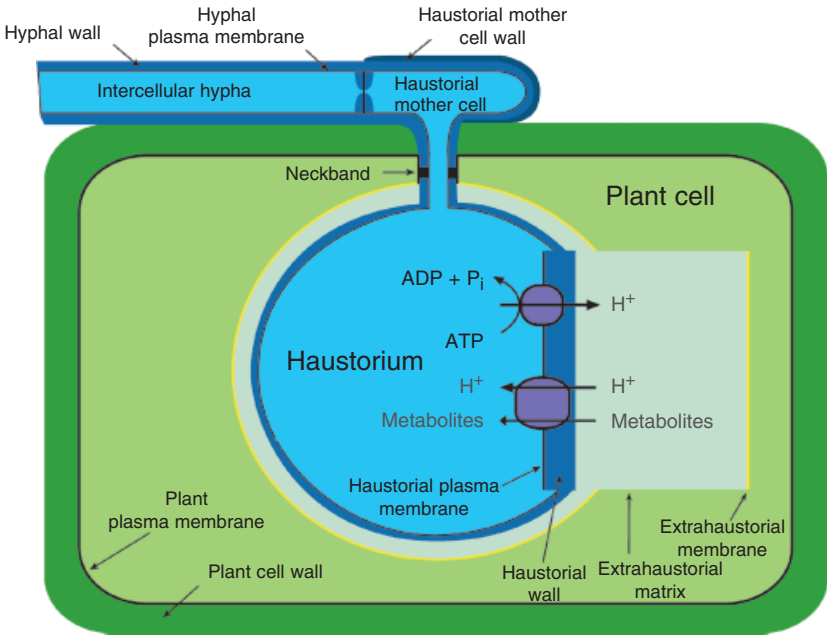

Fig. 5. Schematic representation of a dikaryotic rust haustorium. Structures derived from the fungus are depicted in blue, structures contributed by the plant are shown in green. The extrahaustorial matrix is shown in light blue and the extrahaustorial membrane in yellow. Drawing modified from Hahn *et al*. (1997a).

extrahaustorial matrix should be considered a symplastic compartment (Heath & Skalamera, 1997). However, it might also be regarded as a highly specialized portion of the apoplast, providing conditions different from those present in the bulk apoplast.

Metabolism of *U. fabae*

Most biochemical or molecular work on *Uromyces fabae* involves haustoria. This may come as no surprise since haustoria are one of the hallmarks of biotrophy and therefore have drawn the interest of scientists ever since their discovery more than 150 years ago (von Mohl, 1853). Work on haustoria has been greatly facilitated with the introduction of a method to isolate haustoria from infected plant material (Hahn & Mendgen, 1992). Soon after, a number of genes preferentially or exclusively expressed in haustoria, so-called *in planta*-induced genes (*PIG*s), were identified (Hahn & Mendgen, 1997). Two of the most abundant genes in a haustorial cDNA library code for enzymes involved in vitamin B_1 synthesis (Hahn & Mendgen, 1997). *THI1* and *THI2* together make up about 5% of the total transcripts in haustoria. Vitamin B_1 is a cofactor required for the activity of several enzymes of the central carbon metabolism (Sohn *et al.*, 2000). Therefore, haustoria can be considered as power plants providing essential nutrients through *de novo* synthesis.

In naming these structures [fr. L. haustor: the pail], de Bary (1863) suggested another possible function for haustoria: the uptake of nutrients. Early studies on nutrient uptake in rust fungi involved feeding experiments (Mendgen, 1979, 1981). These experiments gave indirect evidence for a role of haustoria in nutrient uptake without providing conclusive proof. Later work revealed an increased plasma membrane H^+-ATPase activity for haustorial membranes (Struck *et al.*, 1996, 1998). The proton gradient generated by this ATPase was suggested to drive secondary active transport systems engaged in nutrient uptake (Hahn *et al.*, 1997a).

Among the *PIG*s, putative secondary transporters for amino acids were identified (Hahn & Mendgen, 1997; Hahn *et al.*, 1997b). These findings strengthened the potential role of rust haustoria in nutrient uptake (Hahn *et al.*, 1997a). However, while an exclusive localization of AAT2p in haustoria could be shown, no transport activity could be detected (Mendgen *et al.*, 2000). AAT1p was recently characterized as a broad specificity amino-acid secondary active transporter with a specificity for L-histidine, and L-lysine (Struck *et al.*, 2002). However, the transporter has yet to be localized. AAT3p, another amino-acid secondary active transporter, exhibits a substrate preference for L-leucine, L-methionine, and L-cysteine (Struck *et al.*, 2004). Taken together it seems that amino acid uptake in *U. fabae* may not be limited to haustoria. The transporters that have been characterized are clearly energized by the proton-motive force, and show a preference for amino acids present in low abundance in infected leaves (Struck *et al.*, 2004).

Sugar uptake on the other hand seems to proceed exclusively via haustoria (Voegele *et al.*, 2001). HXT1p was localized preferentially at the tip of monokaryotic haustoria (Voegele & Mendgen, 2003), and in the periphery of the body of dikaryotic haustoria (Voegele *et al.*, 2001). No specific labeling was found in intercellular hyphae. Neither nested PCR, nor genomic Southern Blot analyses yielded evidence for additional hexose transporters present in *U. fabae* (Voegele *et al.*, 2001). Heterologous expression of *HXT1* revealed that HXT1p is a proton-motive-force driven monosaccharide transporter. It exhibits specificity for D-glucose, and D-fructose (Voegele *et al.*, 2001). This work provided the first conclusive proof that haustoria are indeed nutrient uptake organs. Overall a picture is starting to emerge indicating that *U. fabae* makes use of several strategies to cover its nutritional demands. Uptake of amino acids seems to occur via haustoria and also via intercellular hyphae. Uptake of carbohydrates seems to be limited to haustoria. Substrate translocation is executed by secondary active transport systems which allow direct coupling of transport to the proton gradient established by the H^+-ATPase (Fig. 5).

Elucidating the mechanism and specificity of carbohydrate uptake in *U. fabae* provided an important advance in understanding the biotrophic relationship (Szabo & Bushnell, 2001). Focusing on carbohydrate metabolism, we were recently able to identify a β-glucosidase (Haerter & Voegele, 2004) and an invertase (Voegele *et al.*, 2006). Both enzymes could contribute substrate for HXT1p. However, other roles for these enzymes are also discussed (see below). In the lumen of haustoria we identified two alcohol dehydrogenases. One NADP-dependent mannitol dehydrogenase (MAD1p) (Voegele *et al.*, 2005), and a NADP-dependent D-arabitol dehydrogenase (ARD1p) (Link *et al.*, 2005). MAD1p seems to be responsible for the formation of mannitol from D-fructose in haustoria. Although apparently not made in urediospores the enzyme seems to be deposited there together with large amounts of mannitol. Assuming a water content of spores of 20%, the concentration of mannitol in spores is close to its solubility level. The polyol disappeared rapidly during germination indicating a role in carbon storage. While there is evidence from other systems that lipids and proteins constitute the major substrates during spore germination (Shu *et al.*, 1954; Solomon *et al.*, 2003) utilizing the pool of mannitol first would enable a quick start of glycolysis, as the conversion of mannitol to D-fructose is a single enzyme step. At the same time, oxidation of mannitol to D-fructose would provide reducing power for anabolic processes. D-arabitol is produced in haustoria by the action of ARD1p from

D-ribulose and D-xylulose in a NADP-dependent reaction (Link *et al.*, 2005). The coupling of NADP reduction to D-arabitol oxidation constitutes a novel enzymatic mechanism. Although D-arabitol is also deposited in spores and rapidly consumed during germination, no ARD1p could be detected in spores. Most likely utilization of D-arabitol in spores occurs via other enzymatic pathways.

The recent analysis of genes expressed in haustoria by Jakupovic & coworkers (2006) extended the earlier analysis by Hahn & Mendgen (1997) considerably. The authors found very strong *in planta* expression for two *PIG*s encoding putative metallothioneins. Furthermore, several genes involved in ribosome biogenesis and translation, glycolysis, amino-acid metabolism, stress response, and detoxification showed an increased expression in the parasitic mycelium. These data indicate a strong shift in gene expression in *U. fabae* between the 'penetration phase' and the 'parasitic phase', and provide the basis for future analyses of the metabolism of *U. fabae*.

Suppression of host defenses and influence on host metabolism

The establishment of biotrophy requires the evasion or suppression of host defenses. *Uromyces fabae* seems to have evolved a number of mechanisms to avoid recognition through host surveillance systems. Analyses of early infection structures, for example, indicated the most obvious differences between infection structures produced outside the leaf, and those produced inside (Kapooria & Mendgen, 1985; Freytag & Mendgen, 1991b). One possibility would be the masking of chitin contained in rust infection structures through the action of acidic cellulases and proteases (Freytag & Mendgen, 1991a). Another explanation could be the direct conversion of chitin to chitosan by the action of chitin deacetylase (El Gueddari *et al.*, 2002). The β-glucosidase identified might also play a role in the suppression of host defenses. The protein shows high homology to other fungal β-glucosidases involved in the detoxification of saponins (Haerter & Voegele, 2004). It is therefore possible that BGL1p has additional or alternative functions than providing substrate for HXT1p. There is also evidence that mannitol and D-arabitol are released from the fungal mycelium into the apoplast (Link *et al.*, 2005; Voegele *et al.*, 2005). Results from mammalian (Chaturvedi *et al.*, 1996) and from plant pathosystems (Jennings *et al.*, 2002) indicate that at least mannitol can be effectively used to suppress host defense responses involving reactive oxygen species. The concentrations of mannitol and D-arabitol found in infected *Vicia faba* tissue have been shown to be sufficient to effectively quench reactive oxygen species (Link *et al.*, 2005; Voegele *et al.*, 2005). Differences in the morphology of extrahaustorial membranes produced by *Puccinia graminis* or *P. coronata* on oat suggest that formation of the fine structure of the haustorial host–parasite interface is under the control of species-specific signals from the fungus (Harder & Chong, 1991). Such signals may include suppressors, which have been implicated in maintaining basic compatibility between the parasite and its host plants (Bushnell & Rowell, 1981). Evidence for such suppressors comes from a phenomenon called induced susceptibility. French bean tissue already infected by *U. vignae* supported additional infections by several nonhost pathogens (Fernandez & Heath, 1991). Suppressors for plant defense responses have been described, but they are either poorly characterized or nonproteinaceous (Basse *et al.*, 1992; Knogge, 1997; Moerschbacher *et al.*, 1999). Nevertheless, it is reasonable to assume that fungi, like their bacterial counterparts, have evolved mechanisms to deliver proteins as effectors to take control of host metabolism. Recently, Kemen *et al.* (2005) were able to show that one of the *PIG*s identified by Hahn & Mendgen (1997) is actually not only secreted into the extrahaustorial matrix as expected from its targeting sequences, but it is further transferred to the host cell cytoplasm and nucleus (Fig. 6). It remains to be shown if Rust Transferred Protein 1 (RTP1p) acts as a suppressor or has other functions. However, RTP1p distribution seems to be limited to the infected host cell (Fig. 6). That infection with *U. fabae* has far reaching effects on the host metabolism exceeding the boundary of the infected cell has been shown by expression analysis of *V. faba* genes in response to attack by the pathogen (Wirsel *et al.*, 2001). Several of the analyzed genes showed altered expression patterns in the infected organ. However, some of the analyzed genes also showed alterations in expression in far remote organs, such as stems and roots. This work clearly shows that the influence on host

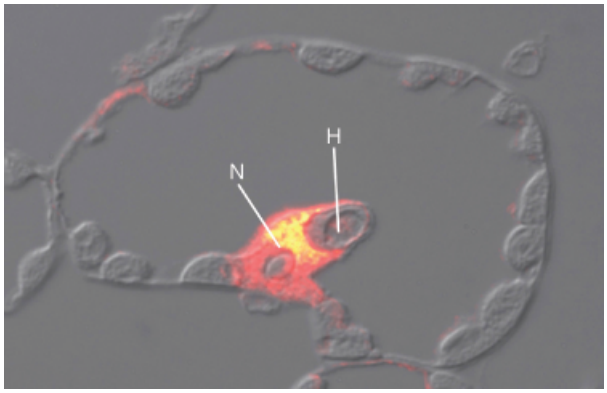

Fig. 6. Localization of RTP1p in *Vicia faba*. In infected cells the area around the haustorial body is labeled. The nucleus closely associated to the haustorium exhibits strong fluorescence. Much weaker signals are obtained in areas away from the haustorium. Overlay of differential interference contrast and epifluorescence images using S746p as primary antibody, and Cy3-labeled goat anti-guinea pig secondary antibody. H, haustorium; N, plant nucleus.

metabolism by a leaf pathogen is not limited to the infected organ alone. Our results regarding the expression of host and pathogen invertases also show far reaching effects on host metabolism (Voegele *et al*., 2006). Alterations in the expression level of plant invertases indicate systemic effects of infection. An attractive explanation for the observed expression of the fungal invertase INV1p in early infection structures, where no fungal uptake system for monosaccharides is detectable, stems from the role insoluble acid plant invertases have in the determination of the sink strength of a plant organ. Apoplastic hydrolysis of sucrose would limit export of carbohydrates from the infected tissue via the phloem and therefore would condition the infected organ for conversion from a source tissue to a sink tissue. This new parasitic sink would then stand in competition with naturally occurring sinks (Voegele *et al*., 2006).

Conclusion

During the last decade much progress has been made in determining some of the aspects of obligate biotrophic growth. One of the organisms that has contributed most considerably to our current understanding of biotrophy is *Uromyces fabae*. While we are far from establishing culture conditions to produce 'parasitic phase' infection structures *in vitro*, stable transformation has drawn a step closer (Wirsel *et al*., 2004). Such a system would greatly facilitate future work on *U. fabae*. Hopefully this review has created enough interest among young scientists to join research involving this challenging but most interesting pathogen.

Acknowledgements

I would like to apologize to all researchers whose work could not be cited in this review for spatial reasons. I would like to thank all colleagues who have contributed to our current understanding of *U. fabae* over the past. And, last but not least, I would like to thank Dr K. Mendgen for providing Fig. 6 and for critically reading the manuscript.

References

Abu Zinada AAH, Cobb A & Boulter D (1975) An electron-microscopic study of the effects of parasite interaction between *Vicia faba* L. and *Uromyces fabae*. *Physiol Plant Pathol* **5**: 113–118.

Allen EA, Hazen BE, Hoch HC, *et al*. (1991) Appressorium formation in response to topographical signals by 27 rust species. *Phytopathology* **81**: 323–331.

Baka ZA, Larous L & Losel DM (1995) Distribution of ATPase activity at the host–pathogen interfaces of rust infections. *Physiol Mol Plant Pathol* **47**: 67–82.

Basse CW, Bock K & Boller T (1992) Elicitors and suppressors of the defense response in tomato cells. Purification and characterization of glycopeptide elicitors and glycan suppressors generated by enzymatic cleavage of yeast invertase. *J Biol Chem* **267**: 10258–10265.

Brown JK & Hovmøller MS (2002) Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**: 537–541.

Bushnell WR & Rowell JB (1981) Suppressors of defense reactions: a model for roles in specificity. *Phytopathology* **71**: 1012–1014.

Catanzariti AM, Dodds PN, Lawrence GJ, Ayliffe MA & Ellis JG (2006) Haustorially expressed secreted proteins from flax rust are highly enriched for avirulence elicitors. *Plant Cell* **18**: 243–256.

Chaturvedi V, Wong B & Newman SL (1996) Oxidative killing of *Cryptococcus neoformans* by human neutrophils. Evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. *J Immunol* **156**: 3836–3840.

Clement JA, Butt TM & Beckett A (1993a) Characterization of the extracellular matrix produced in vitro by urediniospores and sporelings of *Uromyces viciae-fabae*. *Mycol Res* **97**: 594–602.

Clement JA, Martin SG, Porter R, Butt TM & Beckett A (1993b) Germination and the role of extracellular matrix in adhesion of urediniospores of *Uromyces viciae-fabae* to synthetic surfaces. *Mycol Res* **97**: 585–593.

Clement JA, Porter R, Butt TM & Beckett A (1994) The role of hydrophobicity in attachment of urediniospores and sporelings of *Uromyces viciae-fabae*. *Mycol Res* **98**: 1217–1228.

Clement JA, Porter R, Butt TM & Beckett A (1997) Characteristics of adhesion pads formed during imbibition and germination of urediniospores of *Uromyces viciae-fabae*. *Mycol Res* **101**: 1445–1458.

Clement JA, Porter R & Beckett A (1998) The orientation of urediniospores of *Uromyces viciae-fabae* during fall and after landing. *Mycol Res* **102**: 907–913.

Conner RL & Bernier CC (1982) Host range of *Uromyces viciae-fabae*. *Phytopathology* **72**: 687–689.

de Bary A (1863) Recherches sur le developpement de quelques champignons parasites. *Ann Sci Nat, Part Bot* **20**: 5–148.

Deising H & Siegrist J (1995) Chitin deacetylase activity of the rust *Uromyces viciae-fabae* is controlled by fungal morphogenesis. *FEMS Microbiol Lett* **127**: 207–211.

Deising H, Jungblut PR & Mendgen K (1991) Differentiation-related proteins of the broad bean rust fungus *Uromyces viciae-fabae*, as revealed by high resolution two-dimensional polyacrylamide gel electrophoresis. *Arch Microbiol* **155**: 191–198.

Deising H, Nicholson RL, Haug M, Howard RJ & Mendgen K (1992) Adhesion pad formation and the involvement of cutinase and esterases in the attachment of uredospores to the host cuticle. *Plant Cell* **4**: 1101–1111.

Deising H, Frittrang AK, Kunz S & Mendgen K (1995a) Regulation of pectin methylesterase and polygalacturonate lyase activity during differentiation of infection structures in *Uromyces viciae-fabae*. *Microbiology* **141**: 561–571.

Deising H, Rauscher M, Haug M & Heiler S (1995b) Differentiation and cell wall degrading enzymes in the

obligately biotrophic rust fungus *Uromyces viciae-fabae*. *Can J Bot* **73**: S624–S631.
El Gueddari NE, Rauchhaus U, Moerschbacher BM & Deising HB (2002) Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi. *New Phytol* **156**: 103–112.
Emeran AA, Sillero JC, Niks RE & Rubiales D (2005) Infection structures of host-specialized isolates of *Uromyces viciae-fabae* and of other species of *Uromyces* infecting leguminous crops. *Plant Dis* **89**: 17–22.
Fernandez MR & Heath MC (1991) Interactions of the nonhost French bean plant (*Phaseolus vulgaris*) parasitic and saprophytic fungi. IV. Effect of preinoculation with the bean rust fungus on growth of parasitic fungi nonpathogenic on beans. *Can J Bot* **69**: 1642–1646.
Flor HH (1956) The complementary genetic systems in flax and flax rust. *Adv Gen* **8**: 29–54.
Freytag S & Mendgen K (1991a) Carbohydrates on the surface of urediniospore- and basidiospore-derived infection structures of heteroecious and autoecious rust fungi. *New Phytol* **119**: 527–534.
Freytag S & Mendgen K (1991b) Surface carbohydrates and cell wall structure of *in vitro*-induced uredospore infection structures of *Uromyces viciae-fabae* before and after treatment with enzymes and alkali. *Protoplasma* **161**: 94–103.
Frittrang AK, Deising H & Mendgen K (1992) Characterization and partial purification of pectinesterase, a differentiation-specific enzyme of *Uromyces viciae-fabae*. *J Gen Microbiol* **138**: 2213–2218.
Gay JL, Salzberg A & Woods AM (1987) Dynamic experimental evidence for the plasma membrane ATPase domain hypothesis of haustorial transport and for ionic coupling of the haustorium of *Erysiphe graminis* to the host cell (*Hordeum vulgare*). *New Phytol* **107**: 541–548.
Gold RE & Mendgen K (1991) Rust basidiospore germlings and disease initiation. *The Fungal Spore and Disease Initiation in Plants and Animals* (Cole GT & Hoch HC, eds), pp. 67–99. Plenum Press, New York.
Graham PH & Vance CP (2003) Legumes: importance and constraints to greater use. *Plant Physiol* **131**: 872–877.
Haerter AC & Voegele RT (2004) A novel β-glucosidase in *Uromyces fabae*: feast or fight? *Curr Genet* **45**: 96–103.
Hahn M (2000) The rust fungi: cytology, physiology and molecular biology of infection. *Fungal Pathology* (Kronstad J, ed), pp. 267–306. Kluwer Academic Publishers, Dordrecht.
Hahn M & Mendgen K (1992) Isolation of ConA binding haustoria from different rust fungi and comparison of their surface qualities. *Protoplasma* **170**: 95–103.
Hahn M & Mendgen K (1997) Characterization of in planta-induced rust genes isolated from a haustorium-specific cDNA library. *Mol Plant-Microbe Interact* **10**: 427–437.
Hahn M, Deising H, Struck C & Mendgen K (1997a) Fungal morphogenesis and enzyme secretion during pathogenesis. *Resistance of Crop Plants against Fungi* (Hartleb H, Heitefuss R & Hoppe H-H, eds), pp. 33–57. Gustav Fischer, Jena.
Hahn M, Neef U, Struck C, Göttfert M & Mendgen K (1997b) A putative amino acid transporter is specifically expressed in haustoria of the rust fungus *Uromyces fabae*. *Mol Plant-Microbe Interact* **10**: 438–445.
Harder DE & Chong J (1984) Structure and physiology of haustoria. *The Cereal Rusts Origins, Specificity, Structure, and Physiology, Vol. I*. (Bushnell WR & Roelfs AP, eds), pp. 431–476. Academic Press Inc., Orlando.
Harder DE & Chong J (1991) Rust haustoria. *Electron Microscopy of Plant Pathogens* (Mendgen K & Lesemann D-E, eds), pp. 235–250. Springer, Berlin.
Harder DE & Mendgen K (1982) Filipin-sterol complexes in bean rust- and oat crown rust-fungal/plant interactions: freeze-etch electron microscopy *Uromyces appendiculatus*. *Protoplasma* **112**: 46–54.
Heath MC (1976) Ultrastructural and functional similarity of the haustorial neckband of rust fungi and the Casparian strip of vascular plants. *Can J Bot* **54**: 2484–2489.
Heath MC (1990) Influence of carbohydrates on the induction of haustoria of the cowpea rust fungus in vitro. *Exp Mycol* **14**: 84–88.
Heath MC (1997) Signalling between pathogenic rust fungi and resistant or susceptible host plants. *Ann Bot* **80**: 713–720.
Heath MC & Skalamera D (1997) Cellular interactions between plants and biotrophic fungal parasites. *Adv Bot Res* **24**: 195–225.
Heiler S, Mendgen K & Deising H (1993) Cellulolytic enzymes of the obligated biotrophic rust fungus *Uromyces viciae-fabae* are regulated differentiation-specifically. *Mycol Res* **97**: 77–85.
Jakupovic M, Heintz M, Reichmann P, Mendgen K & Hahn M (2006) Microarray analysis of expressed sequence tags from haustoria of the rust fungus *Uromyces fabae*. *Fungal Genet Biol* **43**: 8–19.
Jennings DB, Daub ME, Pharr DM & Williamson JD (2002) Constitutive expression of a celery mannitol dehydrogenase in tobacco enhances resistance to the mannitol-secreting fungal pathogen *Alternaria alternata*. *Plant J* **32**: 41–49.
Joseph ME & Hering TF (1997) Effects of environment on spore germination and infection by broad bean rust (*Uromyces viciae-fabae*). *J Agric Sci* **128**: 73–78.
Kapooria RG (1971) A cytological study of promycelia and basidiospores and the chromosome number in *Uromyces fabae*. *Neth J Plant Pathol* **77**: 91–96.
Kapooria RG & Mendgen K (1985) Infection structures and their surface changes during differentiation in *Uromyces fabae*. *J Phytopathol* **113**: 317–323.
Kemen E, Kemen AC, Rafiqi M, Hempel U, Mendgen K, Hahn M & Voegele RT (2005) Identification of a protein from rust fungi transferred from haustoria into infected plant cells. *Mol Plant-Microbe Interact* **18**: 1130–1139.
Knogge W (1997) Elicitors and suppressors of the resistance response. *Resistance of Crop Plants against Fungi* (Hartleb H, Heitefuss R & Hoppe H-H, eds), pp. 159–182. Gustav Fischer, Jena.
Leonard KJ & Szabo LJ (2005) Stem rust of small grains and grasses caused by *Puccinia graminis*. *Mol Plant Pathol* **6**: 99–111.
Link T, Lohaus G, Heiser I, Mendgen K, Hahn M & Voegele RT (2005) Characterization of a novel $NADP^+$-dependent

D-arabitol dehydrogenase from the plant pathogen *Uromyces fabae*. *Biochem J* **389**: 289–295.
Long DL (2003) Cereal Rust Bulletin: Final Report August 7, 2003. Cereal Disease Laboratory, U.S. Department of Agriculture.
Maier W, Begerow D, Weiß M & Oberwinkler F (2003) Phylogeny of the rust fungi: an approach using nuclear large subunit ribosomal DNA sequences. *Can J Bot* **81**: 12–23.
Manners JM (1989) The host–haustorium interface in powdery mildews. *Aust J Plant Physiol* **16**: 45–52.
Marte M (1971) Studies on self-inhibition of *Uromyces fabae* (Pers.) De Bary. *J Phytopathol* **72**: 335–343.
Mendgen K (1979) Microautoradiographic studies on host–parasite interactions. II. The exchange of ^{3}H-lysine between *Uromyces phaseoli* and *Phaseolus vulgaris*. *Arch Microbiol* **123**: 129–135.
Mendgen K (1981) Nutrient uptake in rust fungi. *Phytopathology* **71**: 983–989.
Mendgen K (1984) Development and physiology of teliospores. *The Cereal Rusts*, *Vol. I*. (Bushnell WR & Roelfs AP, eds), pp. 375–398. Academic Press, Orlando.
Mendgen K (1997) The Uredinales. *The Mycota Vol. V Plant Relationships Part B* (Esser K & Lemke PA, eds), pp. 79–94. Springer-Verlag, Berlin.
Mendgen K & Deising H (1993) Infection structures of fungal plant pathogens – a cytological and physiological evaluation. *New Phytol* **124**: 193–213.
Mendgen K & Hahn M (2002) Plant infection and the establishment of fungal biotrophy. *Trends Plant Sci* **7**: 352–356.
Mendgen K, Struck C, Voegele RT & Hahn M (2000) Biotrophy and rust haustoria. *Physiol Mol Plant Pathol* **56**: 141–145.
Moerschbacher BM, Mierau M, Graessner B, Noll U & Mort AJ (1999) Small oligomers of galacturonic acid are endogenous suppressors of disease resistance reactions in wheat leaves. *J Exp Bot* **50**: 605–612.
Rauscher M, Mendgen K & Deising H (1995) Extracellular proteases of the rust fungus *Uromyces viciae-fabae*. *Exp Mycol* **19**: 26–34.
Shu P, Tanner KG & Ledingham GA (1954) Studies on the respiration of resting and germinating uredospores of wheat stem rust. *Can J Bot* **32**: 16–23.
Sohn J, Voegele RT, Mendgen K & Hahn M (2000) High level activation of vitamin B1 biosynthesis genes in haustoria of the rust fungus *Uromyces fabae*. *Mol Plant-Microbe Interact* **13**: 629–636.
Solomon PS, Tan K-C & Oliver RP (2003) The nutrient supply of pathogenic fungi; a fertile field for study. *Mol Plant Pathol* **4**: 203–210.
Staples RC (2000) Research on the rust fungi during the twentieth century. *Annu Rev Phytopathol* **38**: 49–69.
Struck C, Hahn M & Mendgen K (1996) Plasma membrane H^{+}-ATPase activity in spores, germ tubes, and haustoria of the rust fungus *Uromyces viciae-fabae*. *Fungal Genet Biol* **20**: 30–35.
Struck C, Siebels C, Rommel O, Wernitz M & Hahn M (1998) The plasma membrane H^{+}-ATPase from the biotrophic rust fungus *Uromyces fabae*: molecular characterization of the gene (*PMA1*) and functional expression of the enzyme in yeast. *Mol Plant-Microbe Interact* **11**: 458–465.
Struck C, Ernst M & Hahn M (2002) Characterization of a developmentally regulated amino acid transporter (AAT1p) of the rust fungus *Uromyces fabae*. *Mol Plant Pathol* **3**: 23–30.
Struck C, Müller E, Martin H & Lohaus G (2004) The *Uromyces fabae UfAAT3* gene encodes a general amino acid permease that prefers uptake of *in planta* scarce amino acids. *Mol Plant Pathol* **5**: 183–189.
Szabo LJ & Bushnell WR (2001) Hidden robbers: the role of fungal haustoria in parasitism of plants. *Proc Natl Acad Sci USA* **98**: 7654–7655.
Terhune BT, Bojko RJ & Hoch HC (1993) Deformation of stomatal guard cell lips and microfabricated artificial topographies during appressorium formation. *Exp Mycol* **17**: 70–78.
Thara VK, Fellers JP & Zhou JM (2003) In planta induced genes of *Puccinia triticina*. *Mol Plant Pathol* **4**: 51–56.
Thrower LB & Thrower SL (1966) The effect of infection with *Uromyces fabae* on translocation in Broad Bean. *J Phytopathol* **57**: 267–276.
Tissera P & Ayres PG (1986) Transpiration and the water relations of faba bean (*Vicia faba*) infected by rust (*Uromyces viciae-fabae*). *New Phytol* **102**: 385–395.
Voegele RT & Mendgen K (2003) Rust haustoria: nutrient uptake and beyond. *New Phytol* **159**: 93–100.
Voegele RT, Struck C, Hahn M & Mendgen K (2001) The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proc Natl Acad Sci USA* **98**: 8133–8138.
Voegele RT, Hahn M, Lohaus G, Link T, Heiser I & Mendgen K (2005) Possible roles for mannitol and mannitol dehydrogenase in the biotrophic plant pathogen *Uromyces fabae*. *Plant Physiol* **137**: 190–198.
Voegele RT, Wirsel S, Möll U, Lechner M & Mendgen K (2006) Cloning and characterization of a novel invertase from the obligate biotroph *Uromyces fabae* and analysis of expression patterns of host and pathogen invertases in the course of infection. *Mol Plant-Microbe Interact*, in press.
von Mohl H (1853) Ueber die Traubenkrankheit. *Bot Zeitung* **11**: 585–590.
Wirsel SG, Voegele RT & Mendgen KW (2001) Differential regulation of gene expression in the obligate biotrophic interaction of *Uromyces fabae* with its host *Vicia faba*. *Mol Plant-Microbe Interact* **14**: 1319–1326.
Wirsel SGR, Voegele RT, Bänninger R & Mendgen KW (2004) Cloning of β-tubulin and succinate dehydrogenase genes from *Uromyces fabae* and establishing selection conditions for their use in transformation. *Eur J Plant Pathol* **110**: 767–777.
Woods AM & Beckett A (1987) Wall structure and ornamentation of the urediniospores of *Uromyces viciae-fabae*. *Can J Bot* **65**: 2007–2016.
Zhou XL, Stumpf MA, Hoch HC & Kung C (1991) A mechanosensitive channel in whole cells and in membrane patches of the fungus *Uromyces*. *Science* **253**: 1415–1417.