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4 The Uredinales: Cytology, Biochemistry, and Molecular Biology

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CONTENTS

I. Introduction

Fungi belonging to the order *Uredinales* are commonly referred to as rust fungi. All members of the *Uredinales* are parasitic on plants, often causing dramatic losses in various important crop plants (Alexopoulos et al. 1996). Together with the powdery mildew fungi and the downy mildew-causing oomycetes, rust fungi form an extremely successful group of parasites, the obligate biotrophs. The term **obligate biotrophic** characterizes a specific lifestyle in which the pathogen is absolutely dependent on a living host to complete its life cycle. In turn, the host plant as a whole usually suffers only limited damage over an extended period of time (Staples 2000). By contrast, **necrotrophic parasites** kill their hosts quickly after infection and subsequently thrive on the dead plant material (Staples 2001). **Hemibiotrophic fungi**, such as *Colletotrichum* spp., are characterized by a more or less extended biotrophic phase before switching to necrotrophic growth and killing their host (Perfect and Green 2001). In order to separate the true obligate biotrophic pathogens from hemibiotrophs and necrotrophs we suggest the following six criteria:

1. Obligate biotrophs are not culturable in vitro (at least not to a point representing the parasitic phase)
2. They form highly differentiated infection structures (variations of the normally tubular cell shape, which are necessary for pathogenesis)
3. They have limited secretory activity
4. They establish a narrow contact zone separating fungal and plant plasma membranes
5. They engage in a long-term suppression of host defense responses
6. They form haustoria (specialized hyphae that penetrate host cells).

The peculiarities of the lifestyle of obligate biotrophs, paired with their huge economic impact, make rust fungi a versatile field of study at both the fundamental and the applied level. This chapter on *Uredinales* can by no means cover the complete literature on rust fungi. It is intended to summarize key references, review articles, and books to provide the interested reader with a gateway to more specialized literature on most aspects of research involving rust fungi. Readers new to the field are encouraged to consult the excellent textbooks by Alexopoulos et al. (1996) and Webster and Weber (2007) to gain easier access into the exciting field of mycology in general and obligate biotrophic plant parasites like the rust fungi in particular.

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II. A Brief History of Rust Fungi and Rust Research

There is evidence for a deep-rooted association of rust fungi with food and forage crops. For example, wheat leaf fragments infected with *Puccinia graminis*, the causative agent of stem rust of wheat, have been found in a storage jar from the Late Bronze Age (Kislev 1982). During the reign of the second Roman king Numa Pompilius, the festival of Robigalia was reported by Pliny the Elder to be introduced around 700 BC to appease the fertility god Robigus, god of rusts and mildews (Pliny 69). Thus it appears that rusts have plagued farmers around the globe throughout history. Many cereals and legumes, the two plant families most important for humans (Graham and Vance 2003), suffer from rust infection. Cereal rusts have been a recurring problem in many parts of the world, occasionally causing yield losses of sometimes more than 75% in some areas (Rapilly 1979; Eversmeyer and Kramer 2000; Long 2003). Cereal rusts have been under reasonable control for the past decades mainly through crop management and breeding of resistant wheat lines (see Sects. XII, XIII). However, a new hypervirulent strain of *P. graminis*, Ug99 or TTKS, which seems able to infect about 90% of 12 000 wheat lines tested, was recently found to spread from its original point of discovery in Africa, threatening the world's wheat production yet again (Stokstad 2007; see Sect. XIV). Legume rusts have so far prevailed in Africa, Asia, and Oceania. For example, yield losses of up to 50% have been reported due to infection of fava beans (*Vicia faba*) with *Uromyces fabae* (Tissera and Ayres 1986). Another legume rust, *Phakopsora pachyrhizi,* the causative agent of Asian soybean rust (ASR), has lately spread into the continental United States threatening soybean production there (Schneider et al. 2005; see Sect. XIV). This fact has made the United States Department of Agriculture (USDA) and soybean farmers go on high alert. The possible consequences of such a global spread of a pathogen are exemplified by another rust fungus, *Hemileia vastatrix*, the causative agent of coffee rust. After a first report of the fungus in Ceylon (formerly Sri Lanka) in 1869, it took less than three decades to annihilate the entire coffee production of the island, leaving the British society only tea as a social drink (Staples 2000).

Fontana (1767) was the first to link rust disease to a parasitic fungus. The first comprehensive description of rust fungi, comprising some 120 species, was published by Unger (1833). He found rust fungi on most plant families and correlated the extent of infection with humidity. He also studied cross-sections through infected leaves and noted the degradation of chlorophyll in diseased areas. De Bary (1853) was the first to notice the importance of the germ pore in urediospore walls for production of the germ tube. In addition, he discussed the significance of tip growth for the ability of a fungus to penetrate through stomatal openings. It was also de Bary (1863) who introduced the term haustorium to describe the only hyphae of obligate biotrophic parasites that invade plant host cells (see Sect. VIII). These structures were first described by Zanardini a decade earlier (von Mohl 1853). A few years later de Bary (1865) elucidated the life cycle of *P. graminis,* coined the term teleutospores as the final spore form in the life cycle of macrocyclic rusts, and defined the terms autoecious and heteroecious (see Sect. IV). Eriksson (1894) described the specialization of the rust fungi on cereals, and Stakman and Piemeisal (1917) identified different races of wheat stem rust (see Sect. III). This result was the basis for an effective breeding program for resistance in cereals and other plants (Kolmer 1996). In 1927, Craigie (1927) discovered heterothallism of *P. graminis* and revealed the function of the pycnia as sexual organs. This was the final step in the elucidation of the rust life cycle (see Sect. IV). Rusts, in particular *P. graminis*, gained notoriety through the attention paid to them by biological warfare researchers of both superpowers during the Cold War (Line and Griffith 2001). While biological warfare programs involving rust fungi were discontinued in the early 1970s, *P. graminis* today is considered one of the most important potential bio-terrorism threats to agriculture in the United States (Madden and Wheelis 2003). The "gene for gene" concept, describing the interaction between pathogenic microorganisms and their host plants, introduced by Flor (1955, 1956), resulted from experiments with the flax rust, *Melampsora lini,* and its host *Linum usitatissimum* (see Sect. XIII). Up to the middle of the past century, research involving rust fungi was mainly based on infection studies and cytological analyses using the light microscope. In the early 1960s, cytological analysis of the host–parasite interface was raised to a new level with the introduction of electron microscopy to the field (Moore and McAlear 1961; Keen 2000). Another significant event of this decade was the report of

the first axenic culture of a rust fungus (Williams et al. 1966; Keen 2000). However, in retrospect this method did not quite meet the expectations originally put into it. The 1960s were also characterized by a number of studies analyzing the physiology of host and parasite (for a review, see Bushnell 1972). These cytological and physiological studies continued through the 1970s and 1980s. Axenic cultures and the generation of infection structures by germinating spores on artificial surfaces such as collodion membranes (Dickinson 1949), polystyrene replicas of leaf surfaces (Wynn 1976), or structured polyethylene sheets (Staples et al. 1983) made biochemical analyses of proteins possible during the 1980s and 1990s (Mendgen et al. 1996). A significant event during that time was the finding that appressorium formation could be induced in vitro by simple topographic signals (Hoch et al. 1987; see Sect. VII). Another milestone in rust research was the introduction of a method to isolate rust haustoria from infected plant tissue (Hahn and Mendgen 1992). This work paved the way for more than a decade of molecular work mainly on *U. fabae* as a model organism (for a review, see Voegele 2006). The same period coincides with the molecular reconstruction of the gene for gene hypothesis fueled by the isolation and characterization of several rust resistance genes from flax and the corresponding avirulence genes from *M. lini* (Ellis et al. 2007a, b). Presently, new vistas are being opened to rust research, with the first rust genomes that are currently sequenced: (a) *Melampsora larici-populina*, the causative agent of poplar rust, and (b) *P. graminis* f. sp. *tritici*. The choice for *P. graminis* f. sp. *tritici* was based on its huge economic impact, whereas that for *M. larici-populina* was based on the fact that the host (*Populus trichocarpa*) genome has also been sequenced (Tuskan et al. 2006). In addition, the genomes of *P. trichocarpa* symbiotic fungal associates *Laccaria bicolor* and *Glomus intraradices* are also at or near completion. With the sequencing of the soybean pathogen *Puccinia pachyrhizi* in progress, three rust genomes will be available shortly. However, considering the phylogenic analysis by Maier and coworkers (2003) it would also be highly desirable to obtain genomic sequence information from a member of the genus *Uromyces*, the second largest genus among the rust fungi (see Sect. III).

Cytological, biochemical, and molecular work during the past five decades have mainly focused on five species of rust fungi: *P. graminis*, *P. triticina* (formerly *P. recondita* f. sp. *tritici*), *U. appendiculatus, U. fabae,* and *M. lini*. As already mentioned, *M. lini* and its host flax were used by Flor (1956) to demonstrate the gene for gene hypothesis. *U. appendiculatus* and *P. graminis* have been used in a number of cytological and physiological studies (Zhou et al. 1991; Leonard and Szabo 2005). Today molecular analyses of rust fungi mainly focus on *P. triticina* (Thara et al. 2003), *M. lini* (Catanzariti et al. 2006), and *U. fabae* (Jakupovic et al. 2006). Consequently, this chapter primarily focuses on work done using these organisms.

III. Phylogeny and Taxonomy

Like other man-made concepts of categorization, taxonomic placement and phylogenetic classification change over time as established methods improve and new methods are introduced. Traditionally, rust fungi are grouped together with smut fungi in the class *Teliomycetes* (Jülich 1981). However, recent molecular and ultrastructural data showed that rusts and smut fungi are only distantly related. Currently, a separation of three classes, namely *Urediniomycetes* (including the rust fungi), *Ustilaginomycetes*, and *Hymenomycetes* under the phylum *Basidiomycota* seems to be the best established classification (Swann et al. 1981; Cummins and Hiratsuka 2003). An important feature that distinguishes *Urediniomycetes* from other members of the *Basidiomycota* is the absence of the formation of clamp connections. Within this class of fungi nuclei in the growing hyphal tip divide conjugately, and as the daughter nuclei separate, a septum is formed to delimit two binucleate compartments, with the apical one continuing to elongate (Alexopoulos et al. 1996). Septum morphology is another characteristic to identify members of the *Urediniomycetes*. Septa are simple with a single open or plugged pore; a dolipore arrangement typical for other *Basidiomycota* is missing (Webster and Weber 2007). A third distinctive characteristic for members of the *Urediniomycetes* is the formation of transversely septated metabasidia from which basidiospores are formed laterally (Gäumann 1959).

Today the *Uredinales* are thought to comprise more than 100 genera and around 7000 species (Maier et al. 2003). These numbers correspond to about 75% of the genera and even 95% of the species of the class *Urediniomycetes*. Based on

recent data the order *Uredinales* can be considered to be monophyletic (Swann et al. 1981). The order is divided into 13 families, each consisting of between three and 30 genera (Cummins and Hiratsuka 2003). Classic taxonomic classification is mainly based on spore and fruiting structure morphology, with a strong emphasis on teliospores morphology (usually two-celled for *Puccinia*, one-celled for *Uromyces* species; Cummins and Hiratsuka 2003). Some of these classifications, however, are controversial because the morphology of different spore types may leave some ambiguity with respect to final classification.

Rust fungi and their host plants are excellent examples of coevolution. Rusts infecting members of such old plant divisions as ferns or conifers are almost exclusively heteroecious and macrocyclic (see Sect. IV). Since the order *Uredinales* seems to be monophyletic (Swann et al. 1981), it can be inferred that the extraordinarily complex heteroecious macrocyclic life cycle evolved only once. Reductions seem to have occurred at many different stages of evolution (Laundon 1973). Wahl et al. (1984) discussed host–parasite coevolution for cereal rusts. In centers of coevolution, genes responsible for plant defense and genes for fungal virulence have accumulated. Redistribution of a host subsequently gave rise to independent evolution (Anikster 1984). This diversification may at least in part be responsible for some of the complications associated with rust taxonomy. It would therefore be highly desirable to scrutinize the classic taxonomical system based on morphological and physiological characters and amend/correct it using more DNA sequence data as they become available, in order to better define the phylogeny and taxonomy of rust fungi (Aime 2006).

In terms of number of species the *Pucciniaceae* are by far the largest among all rust families (Maier et al. 2007). Within this family, the genus *Puccinia*, with about 4000 species, and the genus *Uromyces*, with about 600 species, together represent almost two-thirds of all known rust species (Cummins and Hiratsuka 2003). While these two genera form a strongly supported group together with two more genera, the analysis by Maier and coworkers (2003) also suggests that these two genera are polyphyletic. This pioneering work on molecular phylogeny of rust fungi has lately been substantiated by two further molecular studies (Maier et al. 2007; van der Merwe et al. 2007). Findings from these studies indicate that some of the morphological characteristics, i.e. the number of cells per teliospore, may have arisen many times during evolution. Since certain of these characteristics were used in classic taxonomy, some species may have been mislabeled. However, more work on this topic is needed before any taxonomic and nomenclatural changes should be considered.

Using physiological characters for taxonomical classification often does not allow unambiguous resolution down to the species level. Such taxa consisting of clusters of closely related, but reproductively isolated individuals are usually referred to as a species complex. Species complexes are a common phenomenon among rust fungi (Gäumann 1959). Great caution has therefore to be taken, whether a rust fungus is named sensu strictu (in a strict sense) according to the classical concept of a species, or sensu lato (in a broader sense) describing a species complex.

Most rusts can attack more than one host. *P. graminis* for example can infect at least 365 species of cereals and grasses (Anikster 1984). Such rust species are sometimes subdivided into more specialized categories, each designated a *forma specialis* (f. sp.; variety, specialized form). There are virtually no distinctive morphological characteristics for the *formae speciales*, and they are identified by determination of the host species. This type of specialization was first described in the 1890s by Eriksson and Henning, working on cereal rusts (Eriksson 1894; Eriksson and Henning 1896). *P. graminis* f. sp. *tritici* for example exhibits a host preference for wheat and barley, while *P. graminis* f. sp. *avenae* shows a preference for oat.

Within rust species or *formae speciales*, a further specialization is commonly observed. It is known that certain genotypes of a pathogen are able to attack only certain host cultivars. Such races of the pathogen are typically assigned a number in the order of their identification. The race concept is tightly linked to the virulence/avirulence pattern of rust fungi and the susceptibility/resistance pattern of their respective host plants according to the gene for gene hypothesis introduced by Flor (1955). It was shown that broadly virulent pathogens occur more frequently in highly resistant host populations, whereas avirulent pathogens dominate susceptible populations (Thrall and Burdon 2003). The non-random spatial distribution maintained despite high pathogen mobility implies that selection favors virulent races in resistant hosts and avirulent races in susceptible hosts. Physiological races were first described by Stakman and Piemeisal (1917), who established a first set of wheat cultivar differentials which allowed the identification of different *P. graminis* f. sp. *tritici* races. Now extended and refined, this system still provides the basis for modern plant breeding (Kolmer 1996).

IV. Life Cycle

Rusts have one of the most complex life cycles of all fungi (Littlefield 1981). In its complete form the cycle includes five different spore forms. The already complex cycle also exhibits a high degree of plasticity, generating many different variations (see below). To make things even more complicated, there is also some ambiguity in the literature about the designation of the different spore types and fruiting structures (sori). Table 4.1 provides an overview of the terminology used for the different spore types and fruiting structures, with the most commonly used terms printed in bold. In addition to the morphological classification system, Table 4.1 also lists the Roman numerals assigned to the different developmental stages used in the ontogenic classification system (Littlefield 1981; Alexopoulos et al. 1996; Webster and Weber 2007).

Figure 4.1 depicts the life cycle of *U. fabae* and also indicates the nuclear condition during the different stages. 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 by the aid of a drop of liquid (Buller's drop; Webster et al. 1995), and after landing on the leaf surface of a host plant, they germinate and produce monokaryotic infection structures. Pycnia are produced on the upper surface of the

Table 4.1. Terminology (and synonyms) of spores and fruiting structures of rust fungi; morphologial terminology and developmental classification according to ontogenic terminology. The most commonly used terms are given in *bold*

Spore	Fruiting structure	Developmental stage
Pycniospore	**Pycnium**	0
Pycnospore	Spermogonium	
Spermatium		
Aeciospore	**Aecium**	I
Aecidiospore	Aecidium	
	Aecidiosorus	
Urediospore	**Uredium**	II
Urediniospore	Uredinium	
Uredospore	Uredosorus	
Teliospore	**Telium**	III
Teleutospore	Teleutosorus	
Basidiospore	**Metabasidium**	IV
Sporidium	Basidium	
	Promycelium	

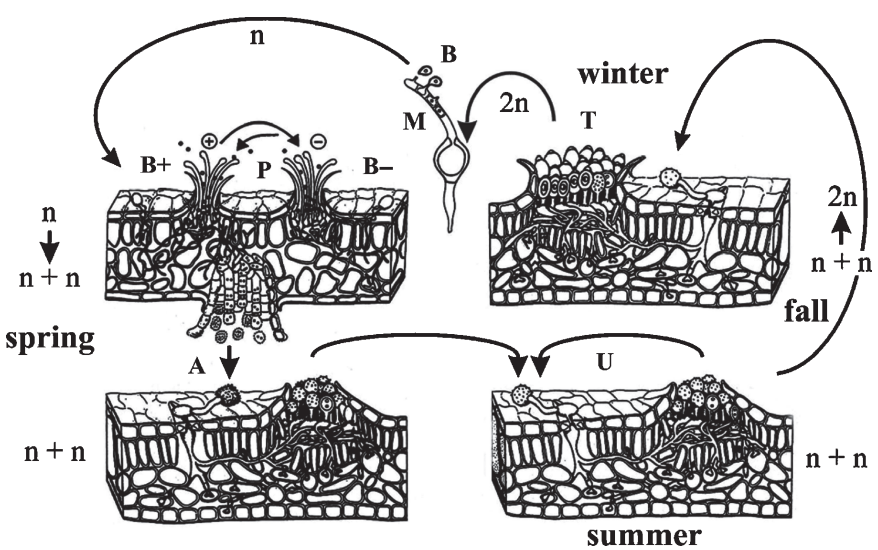

Fig. 4.1. Life cycle of *Uromyces fabae*. Overwintering diploid ($2n$) teliospores (T) germinate in the spring with a metabasidium (M) from which four haploid (n) basidiospores (B) of two mating types (+, −) are formed. Haploid pycniospores (P) are exchanged between pycnia of different mating types on 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 taken from Voegele (2006)

leaf, which contain pycniospores and receptive hyphae. Pycniospores are exchanged between pycnia of different mating types (heterothallism), and after spermatization, dikaryotization occurs in aecial primordia. An aecium differentiates at the lower side of the leaf and dikaryotic aeciospores are produced. After landing on a leaf surface, 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. In the fall, uredia differentiate into telia, the nuclei fuse during sporogenesis and single-celled, diploid teliospores develop for the winter, which closes the rust infection cycle.

Rusts capable of completing their entire life cycle on a single host species are called autoecious (de Bary 1865). Examples for such species are *U. fabae* on broad bean and *M. lini* on flax. Rust fungi requiring two host species in order to complete their life cycle are termed heteroecious (de Bary 1865). The two host species are typically well separated taxonomically. The classic example for an heteroecious rust is *P. graminis* which switches between cereals as main host and barberry as alternate host (Arthur 1962). Host alteration takes place after the aecial and telial stages. *P. graminis* occurs as pycnia and aecia on barberry and as uredia and telia on cereals. However, the term alternate host is used to denote either the pycnial/aecial host or the uredia/telia host and is usually applied to the host of lesser economic importance.

Not all rust fungi go through all five known spore forms. Rusts exhibiting all five spore forms are called macrocyclic. In so-called demicyclic rusts, the uredia stage (and sometimes the pycnial stage) is missing, and in microcyclic rust fungi usually only the pycnial and the telial stage are present (sometimes even only the telial stage). Since the aecial stage is missing in the latter case, all microcyclic rusts are necessarily autoecious. Macrocyclic and demicyclic rusts may be either autoecious or heteroecious. A more extensive description of the many variations of this topic can be found in the review by Petersen (1974) and the book by Cummins and Hiratsuka (2003).

V. Epidemiology

The different spore forms of rust fungi have different modes of dispersal. Pycniospores are released from their supporting cells into a viscous liquid and locally allocated by insects, splashing water, and contact among host plant organs (Littlefield 1981). Aeciospores are produced in tightly packed chains, released by the dissolution of intercalary cells, and aerially disseminated (Littlefield and Heath 1979). Teliospores may remain attached to the host organ they were produced on (Littlefield 1981). Alternatively, the pedicels on which teliospores are produced may break, and teliospores and attached pedicels are dispersed by the wind (Littlefield 1981). In any case, teliospores germinate to produce basidiospores, which are forcefully ejected from the metabasidium (involving Buller's drop; Webster et al. 1995) and then aerially dispersed (Littlefield 1981). Basidiospores are only suited for local dispersal since they desiccate rapidly.

Urediospores are the most important asexual spore form of most rust fungi. They are produced in enormous numbers through repeated infection of host plants for short- and long-range distribution during the vegetation period. Rust fungi are therefore typical "*r*-strategists" (Deising et al. 2002). For *P. graminis* f. sp. *tritici* for example, it was determined that a single uredium can produce about 600 urediospores day^{-1} (Eversmeyer and Kramer 2000). Even moderate infection can thus easily result in the production of 10^{12}–10^{13} urediospores day^{-1} ha^{-1} (Deising et al. 2002). While it is generally accepted that the spores are dry-dispersed by wind (Littlefield 1981) or carried by vectors (Wandeler and Bacher 2006), rain also seems to have a dramatic effect at least for local dissemination (Geagea et al. 1999). Spores can also be carried over distances of several hundreds or even thousands of kilometers by winds causing dissemination across or even between continents (Nagarajan and Singh 1990; Eversmeyer and Kramer 2000; Brown and Hovmøller 2002; Kolmer 2005). Annual long-distance transport of *P. graminis* occurs across the Great Plains in North America along the "Puccinia Path". Meteorological data support the idea that urediospores of *H. vastatrix* produced during a coffee rust epidemic in Angola in 1966 were carried across the Atlantic at an altitude of 1500–2000 m and deposited 5–7 days later over the coffee estates of Bahia, Brazil (Bowden et al. 1971; n.b. urediospores show a significant loss of viability only after five days; Nagarajan and Singh 1990). Similarly, urediospores could easily spread from South Africa to Australia in less then 5 days traveling at an altitude of 12 000 m (Nagarajan

and Singh 1990). Deposition of spores may occur simply by sedimentation caused by gravity, or spores may be washed from the air during rainfall. Recently it was shown that, at least in the case of *P. pachyrhizi*, a rain event seems to be necessary to wash the spores from the air and cause infection (Barnes et al. 2006a; Krupa et al. 2006).

Upon successful infection the fungus colonizes the host tissue inter- and intracellularly (Fig. 4.2). However, symptoms do not become visible until several days thereafter (*sporulation phase* in Fig. 4.2). Typical symptoms include the spore-releasing fruiting structures breaking through the epidermis of the host plant. These structures and the released spores usually have a yellow, orange, red, or brownish coloration, eponymous for the disease. However, there are a number of other symptoms, like dwarfing and various tissue and organ malformations related to rust disease (Littlefield 1981). These are observed mainly when rust fungi spread systemically through the plant, which usually occurs after infection with monokaryotic basidiospores (Larous and Lösel 1993). Noteworthy examples of tissue malformation are the pseudoflower-inducing rust fungi (Roy 1993). Here the plant is inhibited from flowering. Instead, the host is induced by the fungus to form pseudoflowers which resemble true flowers in color and shape (Pfunder et al. 2001). Pseudoflower-inducing rust fungi are also a good example for the role insects play, at least in short-distance allocation (Pfunder and Roy 2000).
Quantification of the pathogen within the infected host plant during the *parasitic phase* and *sporulation phase* (Fig. 4.2) proves quite difficult. Traditional methods mostly rely on visual methods, either scoring symptoms according to a macroscopically visible phenotype, or by using microscopical methods in order to estimate the fungal contribution to the total biomass of an infected plant (Winton et al. 2003; Mendgen, unpublished data). However, these methods cannot give an accurate measure of the fungal fraction at a given point of infection. Biochemical methods are mostly based on the quantification of the fungus-specific sterol ergosterol (Winton et al. 2003). Yet, ergosterol determination cannot discriminate between different fungi and its content may vary between different species and even between the different developmental stages of a single organism (Zhao et al. 2005). Another biochemical method based on the quantification of chitin also has its limitations (Mayama et al. 1975). A versatile tool which allows species specific quantification is real time PCR (Higuchi et al. 1992). Using one of several modifications of the original method, Boyle et al. (2005) were able to quantify poplar rust caused by *Melampsora medusae* f. sp. *deltoidae* and *M. larici-populina*. Their estimates based on DNA as a template were in the order of 20% fungal contribution to the total DNA sample. However, estimates by Jakupovic et al. (2006) and our own quantifications (Voegele and Schmid, unpublished data) based on mRNA provided a considerably higher value, between 40% and 50% fungal contribution.

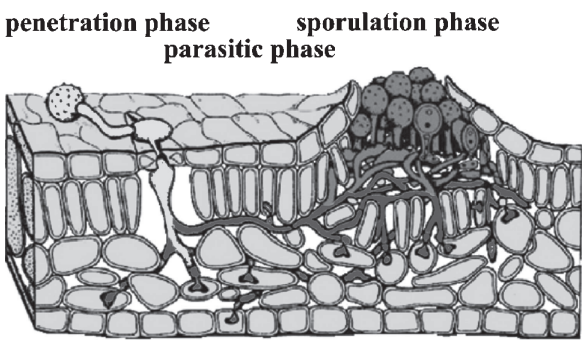

Fig. 4.2. Developmental phases of urediospore infection. Early infection structures of the *penetration phase*, structures of the *parasitic phase*, and structures of the *sporulation phase*. Drawing taken from Voegele (2006)

VI. Spore Germination and the Formation of Infection Structures

Telio- and pycniospores do not infect plants, whereas basidio-, aecio-, and urediospores do. Teliospores represent the final spore form of rust fungi and provide the main basis for their nomenclature (Mendgen 1984). They have mostly been studied using cytological techniques (Gold and Littlefield 1979; Mims and Thurston 1979; Mims 1981a; Anikster 1986). Pycniospores have also been studied primarily on an ultrastructural basis (Gold and Littlefield 1979; Gold et al. 1979). However, noteworthy are the measurements of nuclear DNA content in the early 1990s (Eilam et al. 1994). Pycniospores are important for the sexual reproduction of rust fungi (Craigie 1927). Aeciospores were also the subject of ultrastructural analysis (Mims 1981b). However, what is more important is the finding that aeciospores behave similar to urediospores, at least with respect to germination and response to topographical stimuli (Stark-Urnau and Mendgen 1993). Some studies have been performed regarding the nuclear DNA content of basidiospores (Eilam et al. 1992), their ultrastructure (Mims 1981a), and their derived infection structures (Kapooria 1971; Freytag et al. 1988; Gold and Mendgen 1991). Yet, the best studied rust spore form is the urediospore (Staples and Macko 1984, Deising et al. 1992). Almost all biochemical and all recent molecular studies are based on infection structures derived from urediospores (Mendgen et al. 1996, 2000; Hahn et al. 1997a; Hahn 2000; Voegele and Mendgen 2003; Struck et al. 2004a; Voegele 2006). The fact that infection structures from both basidio- and urediospores of *U. fabae*

have been analyzed morphologically allows a comparison between mono- and dikaryotic infection structures on the same host plant (Fig. 4.3; Mendgen et al. 1996). Thick-walled, darkly pigmented and ornamented urediospores (Fig. 4.3A) germinate with a germ tube which differentiates into a well defined appressorium upon contact of the germ tube with a topographic signal of the correct magnitude (Hoch and Staples 1987; Hoch et al. 1987). A penetration hypha is formed at the base of the appressorium, 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 is differentiated from which a haustorium is formed. Basidiospores (Fig. 4.3B) by contrast are smooth and thin-walled. There is no evidence for topographical signals involved in surface recognition, and infection structures like appressorium, vesicle, and haustorium are noticeably less differentiated. Moreover, the penetration mechanism seems to be completely different, since in this developmental stage the fungus enters the plant by direct penetration into epidermal cells. Further studies at the molecular level are needed to determine the causes and consequences of these differences. It is noteworthy to mention that, in contrast to other rusts, urediospores of some *Phakopsora* species produce infection structures which penetrate the leaf surface directly (Bonde et al. 1976; Hoppe and Koch 1989).

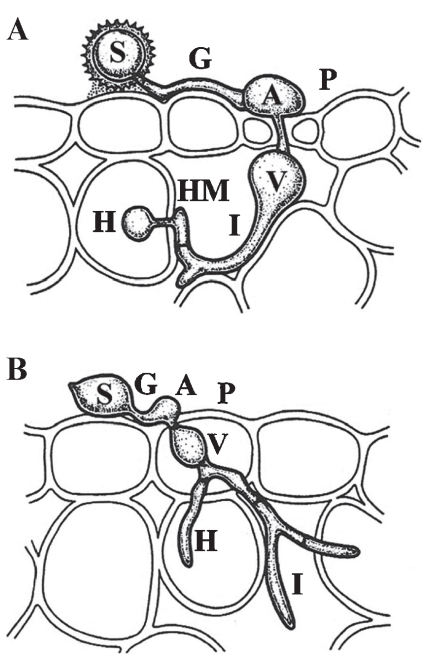

Fig. 4.3. Infection structures derived from: urediospores (**A**) and basidiospores (**B**). *A* appressorium, *G* germ tube, *H* haustorium, *HM* haustorial mother cell, *I* infection hypha, *P* penetration hypha, *S* spore, *V* vesicle. Drawing taken from Voegele (2006)

VII. Features of Urediospore Infection

Urediospores are single-celled, thick-walled, hydrophobic, usually darkly pigmented, and carry spines on their surface (Woods and Beckett 1987). An important morphological feature used to distinguish different rust species is the number and position of germ pores on the surface (Gäumann 1959). Premature germination of spores, for example within uredia, is prevented by the presence of germination inhibitors (Wolf 1982). While methyl *cis*-3,4-dimethoxycinnamate has been identified in some rust species (Macko et al. 1970, 1971), there is also a large number of rusts where self-inhibitors were reported but the compounds could not be identified (Marte 1971; Macko et al. 1976). The time-frame within which these inhibitors are effective is restricted to the first 30 min after the initiation of hydration of the spore (Wolf 1982). There also seem to be endogenous germination stimulators. One of the first stimulators to be identified and one of the most widely distributed is pelargonaldehyde (*n*-nonanal; French and Weintraub 1957). However, many more chemically unrelated compounds were also shown to have stimulatory effects (French 1992). There is also evidence for exogenous stimulators and inhibitors produced by the host plant which might contribute to the regulation of germination (Gold and Mendgen 1983; Staples and Hoch 1997). Mendgen et al. (2006) were able to show that *U. fabae* stimulates the emission of specific volatiles by its host which in turn control the differentiation of infection structures (Fig. 4.4).

Fully developed urediospores are almost completely dehydrated upon release from uredia, which gives them an irregular shape (Clement et al. 1998). Only upon hydration do spores adopt their typically round to ellipsoid form. Although dry urediospores hydrate rapidly, their surface is non-wettable (Clement et al. 1994). It is this hydrophobicity which seems to be responsible for the initial adhesion of spores to the host surface (Clement et al. 1993b). This interaction also seems to involve the spines. The initial contact is quickly

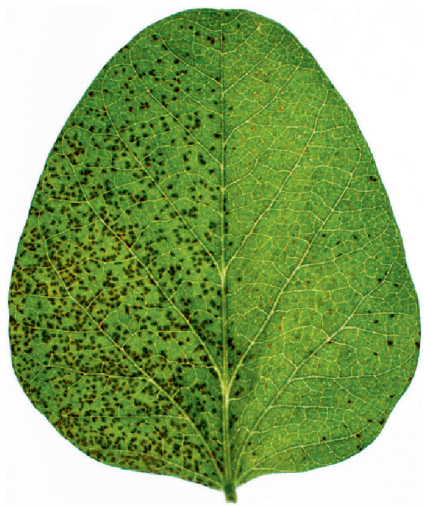

Fig. 4.4. Control of rust disease by leaf fragrances. Leaf fragrances can induce or suppress haustorium differentiation and may be used to control rust disease. Here, soybean was inoculated with *P. pachyrhizi*. The right half of the leaf was treated with 10 µl farnesyl acetate according to Mendgen et al. (2006)

followed by the production of an extracellular matrix consisting of low-molecular-weight carbohydrates and glycosylated polypeptides (Clement et al. 1993a). This matrix seems to originate from solubilization of surface components and lysis of the germ pore plug. The next step is the formation of an adhesion pad underneath the attached spore. Both seem to be exclusively of fungal origin, since they are also formed on artificial surfaces (Deising et al. 1992). Cutinases and esterases seem to be involved in the adhesion process, since autoclaved spores or spores treated with esterase inhibitors form an adhesion pad but fail to adhere (Deising et al. 1992).

Aside from liquid water or high humidity (Clement et al. 1997), light and temperature are also important for germination. For *U. fabae* a period of at least 40 min of darkness is required to induce germination (Joseph and Hering 1997). Especially harmful to germination seem to be wavelengths in the far red. Urediospores of *U. fabae* germinate in a range between 5 °C and 26 °C, with the optimal germination temperature being 20 °C (Joseph and Hering 1997). Given the correct physical parameters the spore germinates on almost any surface, indicating that no additional signals are needed to induce germination. Spores even germinate on a water surface or submerged in water if proper aeration is provided (Struck et al. 1996). However, no infection structures are formed in the absence of a structured surface. The cytoplasm of the spore moves into the growing germ tube as the developing germ tube meanders across the surface attached to it via matrix-like material (Hoch et al. 1987; Clement et al. 1994). Germ tubes are tube-like structures with a hemispherical or hemi-ellipsoidal apical region at which growth occurs (Wessels 1993). Vesicles originating from the Golgi migrate to the apex and accumulate in the so-called Spitzenkörper (Mendgen et al. 1996). Within the apex, the Spitzenkörper is shifted towards the substrate, which results in a sort of "nose down" growth of the hypha, which might aid in the recognition of physical stimuli (Hoffmann and Mendgen 1998).

In order to produce infection structures further signals are needed for differentiation. It was shown for a number of rust species that a topographical signal is needed for the differentiation of an appressorium (Wynn 1976; Hoch et al. 1987; Allen et al. 1991; Read et al. 1997). *U. appendiculatus* and *Uromyces vignae* were found to form appressoria if a ridge of 0.4–0.8 µm in height is provided (Allen et al. 1991). The values determined roughly correspond to the height of the stomatal guard cell lips of the respective host plants. Other studies involving *Puccinia hordei* and different accessions of the host *Hordeum chinense* showed that a failure to differentiate appressoria was due to the presence of a prominent layer of wax over the guard cells of particular lines probably obscuring the relevant topographic signals (Vaz Patto and Niks 2001). Studies involving *Uromyces striatus* on artificial surfaces indicated a range of 0.1–1.2 µm ridge height as capable of inducing appressorium formation (Kemen et al. 2005a). This wider range might be correlated with the broad host range of *U. striatus*, which comprises at least 141 species and subspecies from the tribes Trifolieae, Cicereae, and Vicieae. In the membrane fraction derived from *U. appendiculatus* germ tubes a mechanosensitive channel was identified which may be involved in the transduction of the topographic signal into a differentiation response (Zhou et al. 1991). There is also some data available about the involvement of the cytoskeleton in thigmotropic signaling and appressorium associated differentiation processes (Bourett et al. 1987; Kwon et al. 1991). The cytoplasm is transferred to the growing appressorium and the vacuolated germ tube is separated from the newly developed structure by a septum. The differentiation of the appressorium coincides with the release of a number of lytic enzymes (Fig. 4.5; Deising et al. 1995b). 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 is considerably

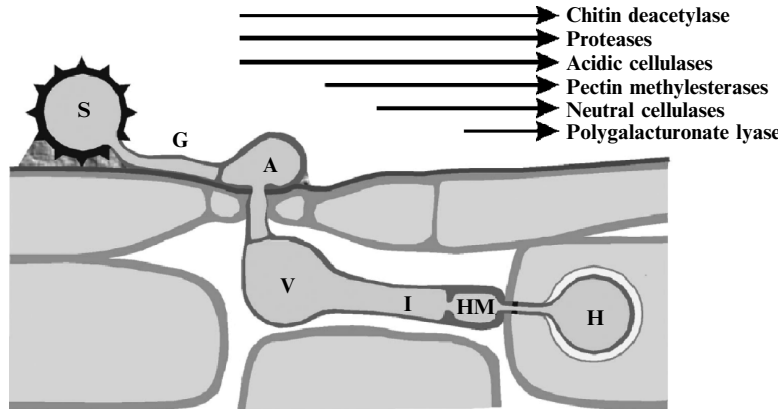

Fig. 4.5. Lytic enzymes in early dikaryotic infection structures. *A* appressorium, *G* germ tube, *H* haustorium, *HM* haustorial mother cell, *I* infection hypha, *P* penetration hypha, *S* spore, *V* vesicle. Drawing modified from Mendgen and Deising (1993)

less than the turgor pressure reported for example for appressoria of *Magnaporthe grisea*, but still 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 appressorium and the penetration hypha by a septum (Kapooria and Mendgen 1985). Only upon contact with a leaf mesophyll cell a haustorial mother cell is differentiated, which is again separated from the infection hypha by a septum. Again most of the cytoplasm moves into the differentiating haustorial mother cell and earlier structures become more or less vacuolated. Similar to appressoria, haustorial mother cells have a thick, multilayered wall that attaches firmly to the host cell wall and forms a penetration hypha to invade the host cell (Heath 1997). The haustorial mother cell therefore functionally resembles an appressorium. However, it remains to be elucidated whether the functional similarity extends to the molecular level. Results from research on the penetration process support the idea that pressure and the controlled secretion of lytic enzymes act together to prepare successful penetration of the host cell wall (Hahn et al. 1997a).

During the penetration phase (Fig. 4.2), infection structures up to the haustorial mother cell can be induced in vitro by germinating spores on artificial surfaces such as collodion membranes (Dickinson 1949), or on structured polyethylene sheets (Staples et al. 1983). While high humidity and the correct topographical signal seem to be sufficient for legume rusts to efficiently produce infection structures in vitro, the situation seems to be more complex for rust fungi infecting monocotyledonous host plants (Wiethölter et al. 2003). A number of physical and chemical stimuli such as a mild heat shock (Maheshwari et al. 1967), organic compounds (Macko et al. 1978), host epicuticular waxes (Grambow 1977), leaf volatiles (Grambow 1977), or combinations thereof (Collins et al. 2001; Wiethölter et al. 2003) have been reported to trigger the sequential in vitro development of appressoria, substomatal vesicles, infection hyphae, and haustorial mother cells of *P. graminis*. Although there are some reports about the formation of haustoria in vitro (Heath 1989, 1990a, b; Mendgen et al. 2006), true functional haustoria and structures of the parasitic phase and sporulation phase (Fig. 4.2) are only formed in planta.

The fact that haustoria and structures of the parasitic phase and sporulation phase (Fig. 4.2) are only formed in planta makes it extremely difficult to analyze processes involving these structures at a molecular level. Although conditions for axenic cultures have been established for some biotrophic fungi (Maclean 1982; Fasters et al. 1993), most of the economically important biotrophic parasites remain non-culturable, at least not to a point equivalent to the biotrophic phase (Mendgen and Hahn 2002). Studies by Heath (1990b) indicated that it is not a mere lack of specific nutrients that prevent haustoria to be formed in vitro, but rather a lack of appropriate signals from the host plant. The pathogen and the host together seem to form a new entity, the aegricorpus (disease body; Loeghering 1984). A study of the pathogen (axenic culture) or the host alone can therefore not explain the physiology of the diseased plant.

VIII. Structural Aspects of the Dikaryotic Haustorium

The haustorium represents one of the hallmarks of obligate biotrophic parasites. These structures

have generated the interest of plant pathologists ever since their first description by Zanardini about 150 years ago (von Mohl 1853). When naming these structures [Latin: *haurire* (*haurio*, *hausi*, *haustum*), to drink, to draw] de Bary (1863) proposed one of the possible functions for haustoria – the uptake of nutrients from the host. However, until recently there was evidence for an involvement of haustoria in nutrient uptake for powdery mildew fungi (Ascomycota) only (for a review, see Hall and Williams 2000).

The dikaryotic rust haustorium develops from the haustorial mother cell with a slender neck and a haustorial body that forms distally to the neck (Heath and Skalamera 1997). During formation of the haustorium the cell wall of the host cell is breeched. The expanding haustorium invaginates the host plasma membrane and new membrane is probably synthesized. There is some evidence that the membrane of the host enclosing the haustorial body, the so-called extrahaustorial membrane, is modified and therefore no longer resembles a conventional plant plasma membrane. 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 and Mendgen 1982). Cytochemical studies on powdery mildew haustoria (Gay et al. 1987; Manners 1989) and later work on rust haustoria (Baka et al. 1995) 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 apparently joining the two plasma membranes of host and parasite (Harder and Chong 1984). This "neckband" (Fig. 4.6) seals the extrahaustorial matrix against the bulk apoplast, not unlike the Casparian strip in the endodermis (Heath 1976). The haustorium is therefore not truly intracellular, it remains outside the physiological barrier of the host cell (Fig. 4.6). With the development of the haustorial body, a zone of separation between the plasma membranes of parasite and host is formed. It is composed of the fungal cell wall and the extrahaustorial matrix (Hahn et al. 1997a). It seems noteworthy to mention that, while normally a cell wall is formed from the plant cell cytoplasmic membrane, no such structure is formed from the extrahaustorial membrane.

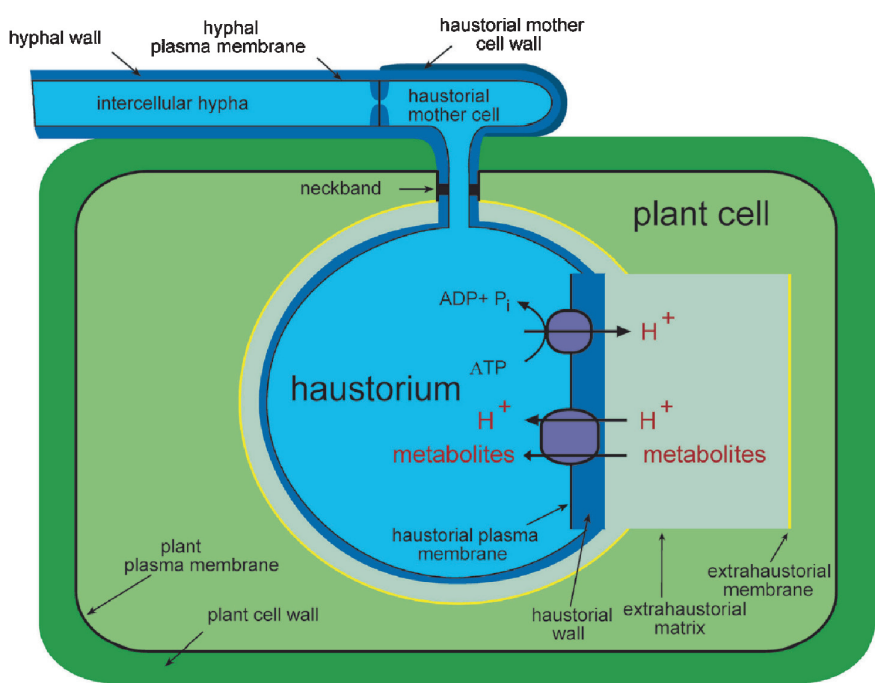

Fig. 4.6. Schematic representation of a dikaryotic rust haustorium. Structures derived from the fungus are depicted in *blue*, structures contributed by the plant are shown in *green*. The extrahaustorial matrix is shown in *light blue* and the extrahaustorial membrane in *yellow*. Drawing taken from Voegele (2006)

The extrahaustorial matrix resembles an amorphous mixture of components, mainly carbohydrates and proteins, partly of fungal but primarily of plant origin (Harder and Chong 1991), and provides the most intimate contact between host and parasite. This view is supported by the cytological analysis of hemibiotrophic parasites. The initial biotrophic phase of some hemibiotrophs, like for example *Colletotrichum* spp., is also characterized by the presence of a narrow contact zone between host and parasite (Perfect and Green 2001; Mendgen and Hahn 2002). Upon switching to necrotrophic growth the host plasma membrane surrounding the hyphae disintegrates and parasitic growth continues with narrower unsheathed hyphae. It therefore seems likely that this zone of separation plays an important role in the maintenance of the biotrophic lifestyle. Undoubtedly, the extrahaustorial matrix represents a formidable trading place for the exchange of nutrients and information between the host and the fungus (Heath and Skalamera 1997). In a recent study on *Puccinia hemerocallidinis* Mims and coworkers (2002) showed long tubular extensions of the extrahaustorial membrane contiguous with the extrahaustorial matrix. Similar structures were already described by Stark-Urnau and Mendgen (1995) for monokaryotic haustoria (haustoria derived from basidiospore infection) of *U. vignae*. These structures reach far into the host cytoplasm and exhibit coated vesicles at their tip. However, it remains to be shown whether there is any kind of trafficking linked to these structures.

Based on the seal made by the neckband and the presence of the plant plasma membrane surrounding the whole structure, it was suggested that the extrahaustorial matrix should be considered a symplastic compartment (Heath and 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. The neckband does not seem to be only a line of demarcation for the extrahaustorial matrix. Using GFP-tagged plasma membrane markers and laser scanning microscopy in the pathosystem *Erysiphe cichoracearum/Arabidopsis thaliana*, Koh et al. (2005) were able to show that these membrane proteins were excluded from the extrahaustorial membrane and accumulated in rings around the neckband. Although done on a different system, this work corroborates earlier findings that the composition of the extrahaustorial membrane seems to be different from that of a conventional plasma membrane.

A further special feature of the haustorium is a highly dilated ER, which exhibits a shift from mostly parallel sheets to a predominantly tubular-vesicular network (Welter et al. 1988; Mims et al. 2002). Based on the distribution of ER markers such as BIP- and HDEL-containing proteins, this network appears to be a functional sub-compartment of the ER (Bachem and Mendgen 1995). The highly increased ER may indicate an enhanced synthesis of secreted proteins. In addition, haustoria may also contain more than two nuclei (Chong et al. 1992).

Analysis of the potential role(s) of rust haustoria has been hampered by the fact that haustoria are exclusively formed in planta and that their isolation encountered numerous problems (Bushnell 1972). As a result, haustoria have been mostly studied using cytological techniques (Harder and Chong 1991). The introduction of biochemistry and molecular biology into the field of phytopathology opened up a new dimension to investigate the role(s) of haustoria (see below). A picture is beginning to emerge indicating that haustoria do not serve only in nutrient uptake – the task postulated for these structures ever since their discovery. In fact, they seem to perform enormous biosynthetic duties and are thought to be engaged in the suppression of host defense responses and in redirecting and/or reprogramming the host's metabolic flow.

IX. Biochemical and Molecular Analyses of Rust Fungi

The early years of basic research involving rust fungi were dominated by physiological analyses of metabolites and their changes in the course of infection. One aspect analyzed was the metabolism of germinating spores. Unhydrated spores are metabolically largely inactive. However, upon hydration, both the Emden–Mayerhof–Parnas pathway and the pentose phosphate pathway seem to be active (Shu and Ledingham 1956). Apparently, early infection structures of the penetration phase are not capable of taking up considerable amounts of nutrients (Staples and Macko 1984). Therefore, the fungus relies largely on metabolites stored within the urediospore until the first haustorium is formed and contact is made to the rich resources of the host plant. This manifests itself in the fact that the

cytoplasm and all its content is always kept close to the growing hyphal tip, while older structures are more or less vacuolated and separated by septum formation. Some studies indicate that it is mainly lipids which are utilized as nutrients during the germination process (Langenbach and Knoche 1971a,b). Re-synthesis of these and further compounds occurs after a period of degradation. By contrast, using *P. graminis* as a model, Daly and coworkers (1967) found that lipids and carbohydrate, mainly in the form of polyols, were the metabolites primarily used during germination. Accumulation of acyclic polyols is a common phenomenon among fungi (Lewis and Smith 1967; Jennings 1984), and there are a number of studies indicating that mannitol, D-arabitol, and/or D-sorbitol are present in urediospores of rusts (Lewis and Smith 1967; Maclean and Scott 1976; Maclean 1982; Manners et al. 1982, 1984). Recent research on this topic sheds a new light on the roles these polyols may play in pathogenesis (Link et al. 2005; Voegele et al. 2005; Voegele 2006; see below). Another aspect of this early research was the analyses of nutrient fluxes between host and parasite. These approaches were based on feeding experiments involving radioactive tracer substances. Mendgen (1979, 1981) for example employed ^{3}H-labeled amino acids using *Uromyces* spp. These experiments gave indirect evidence for a role of haustoria in nutrient uptake without providing conclusive proof.

Biochemical analyses initially focused on structures that could be generated in vitro and almost exclusively specialized on *U. fabae* as a model (Deising et al. 1991, 1995b). Acidic and neutral cellulase (Heiler et al. 1993), extracellular protease (Rauscher et al. 1995), chitin deacetylase (Deising and Siegrist 1995), pectin esterase (Frittrang et al. 1992), pectin methylesterase (Deising et al. 1995a), neutral cellulase (Heiler et al. 1993), and polygalacturonate lyase (Deising et al. 1995a) activities were found (Fig. 4.5). No significant protein secretion could be found before the onset of appressorium formation, protein secretion continuously increased thereafter, and reached a maximum upon the differentiation of infection hyphae and haustorial mother cells (Hahn et al. 1997a). Based on the biochemical characteristics of the enzymes identified, a model was suggested for obligate biotrophs explaining the highly coordinate action of these enzymes in a localized breakdown of the plant cell wall (Deising et al. 1995b). In addition to host cell wall-degrading enzymes, two proteinaceous elicitors of plant defense responses were purified and characterized from *U. vignae* (D'Silva and Heath 1997). Molecular approaches analyzing differentiation regulated gene expression started as early as 1989 with the isolation and characterization of *INF24* from *U. appendiculatus* (Bhairi et al. 1989). This work was soon followed by the characterization of another differentiation-specific gene, *INF56* (Xuei et al. 1992, 1993), and the analysis of a larger set of stage specifically regulated genes from *P. graminis* (Liu et al. 1993).

Much of the recent biochemical and molecular work on rust fungi involves haustoria. However, biochemical and molecular work on haustoria is greatly hindered by the fact that haustoria are only formed in planta. During the early 1990s several methods were introduced to isolate haustoria from infected plant tissue for further analysis (Hahn and Mendgen 1992; Tiburzy et al. 1992; Cantrill and Deverall 1993). While some of these methods were too laborious and inefficient, the chromatographic method developed by Hahn and Mendgen (1992) proved a milestone in the research involving rust haustoria. The method is based on a selective binding of oligosaccharides present in the haustorial wall to the lectin concanavalin A immobilized on a Sepharose 6MB backbone. Repeated cycling of cell extracts of infected leaves yielded considerable quantities of highly enriched haustoria. This method provided the basis for biochemical and molecular analyses of rust haustoria.

Using this method, a number of genes preferentially or exclusively expressed in haustoria, so-called in planta induced genes (*PIG*s), were identified (Hahn and Mendgen 1997). Two of the most abundant genes in a haustorial cDNA library encode enzymes involved in vitamin B1 synthesis (Hahn and Mendgen 1997). *THI1* and *THI2* together make up about 5% of the total transcripts in haustoria. Vitamin B1 is a co-factor 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.

Other work on *U. fabae* revealed an increased plasma membrane H^{+}-ATPase activity for haustorial membranes compared to membranes isolated from spores and germlings (Struck et al. 1996, 1998). The proton gradient generated by this ATPase was suggested to drive secondary active transport systems engaged in nutrient uptake by the parasite (Hahn et al. 1997a). The finding that some of the *PIG*s encoded putative secondary transporters for

amino acids was further evidence for a special role of haustoria in nutrient uptake (Hahn and Mendgen 1997; Hahn et al. 1997a, b). However, while an exclusive localization of AAT2p in haustoria could be shown, no transport activity could be detected (Mendgen et al. 2000). AAT1p was characterized as a broad-specificity amino acid secondary active transporter with a main specificity for L-histidine and L-lysine, but immunolocalization data could not be provided (Struck et al. 2002). AAT3p was shown to exhibit substrate preference for L-leucine and the sulfur-containing amino acids L-methionine and L-cysteine (Struck et al. 2004b). AAT1p and AAT3p 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. 2004b). Taken together, it seems that amino acid uptake in *U. fabae* may not be limited to haustoria. By contrast, hexose uptake seems to proceed exclusively via haustoria (Voegele et al. 2001). HXT1p was localized preferentially at the tip of monokaryotic haustoria (Voegele and 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 under low stringency conditions yielded evidence for additional hexose transporters present in *U. fabae* in any of the developmental stages tested (Voegele et al. 2001). Heterologous expression in yeast and *Xenopus* oocytes revealed that HXT1p is a proton-motive force-driven monosaccharide transporter. The transporter exhibits specificity for D-glucose, 2-deoxy-D-glucose, D-fructose and D-mannose, with increasing K_m values in this order (Voegele et al. 2001). This work provided the first conclusive evidence that rust haustoria are indeed nutrient uptake organs. Taken together, these data indicate that *U. fabae* makes use of several strategies to cover its nutritional demands. While amino acids seem to be taken up via both haustoria and 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. 4.6).

Elucidating the mechanism and specificity of carbohydrate uptake in *U. fabae* provided an important advance in understanding the biotrophic relationship, but at the same time put forward a series of new challenging questions (Szabo and Bushnell 2001). Focusing on carbohydrate metabolism, we identified a β-glucosidase (EC 3.2.1.21; Haerter and Voegele 2004) and an invertase (EC 3.2.1.26; Voegele et al. 2006) in *U. fabae*. Both enzymes could contribute substrates for the hexose transporter; however, other roles are also possible (see below). In the lumen of haustoria we identified two alcohol dehydrogenases. One NADP-dependent mannitol dehydrogenase (MAD1p; EC 1.1.1.138; Voegele et al. 2005), and a novel enzyme, an NADP-dependent D-arabitol dehydrogenase (ARD1p; EC 1.1.1.287; 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, MAD1p seems to be deposited there together with large amounts of mannitol. Assuming spores have a water content of 20%, the concentration of mannitol found in spores is close to its solubility level. The polyol disappeared rapidly from spores during germination indicating a role of this polyol 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, since 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 most likely produced in haustoria by the action of ARD1p from D-ribulose and D-xylulose in an 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 another enzymatic pathway. Aside from serving as carbohydrate storage compounds, there is evidence that both polyols have a role in the suppression of host defenses (see below).

The original analysis of *U. fabae PIG*s by Hahn and Mendgen (1997) was considerably extended by further haustorial EST – and microarray analysis (Jakupovic et al. 2006). 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 parasitic phase (Fig. 4.2) and provide the basis for future analyses of the metabolism of *U. fabae*. Similar analyses involving both host and parasite genes were performed using barley plants infected and *P. triticina*. While Zhang and coworkers (2003) used the AFLP technique and Northern blot and RT-PCR, Thara and coworkers (2003) used a suppression subtractive hybridization approach and differential expression analysis. Analysis of the

M.larici-populina/poplar interaction also included a comparison of compatible and incompatible host–parasite interactions (Rinaldi et al. 2007). A different, yet interesting approach is the EST analysis of germinating urediospores from *P. pachyrhizi* (Posada-Buitrago and Frederick 2005). The molecular response of soybean to *P. pachyrhizi* infection was recently analyzed by the groups of Whitham and Baum (van de Mortel et al. 2007). These data will add considerably to the information which will become available upon completion of the *P. pachyrhizi* genome sequence. Genomic sequencing projects for *M. larici-populina* and *P. graminis* f. sp. *tritici* have already been completed. The sequence data and the possibility of comparing the different genomes will add a new impetus to rust research.

Recently, proteomics approaches were also introduced to rust fungi. Cooper et al. (2006) analyzed proteins from urediospores of *U. appendiculatus* and indicated similar upcoming analyses for the germ tube stage and infection structures. Rampitsch et al. (2006) have started to analyze the proteome of *P. triticina* during its interaction with its host. Integration of results from such approaches with array, EST, and genomic data will provide useful information to understand the molecular details of obligate biotrophy.

A serious drawback in research involving rust fungi remains the lack of a system for the stable transformation of obligate biotrophs. There are a number of reports on the transient transformation of rust fungi involving either microinjection of antisense oligonucleotides (Barja et al. 1998) or particle bombardment using the β-glucoronidase (GUS) gene as a color marker (Bhairi and Staples 1992; Li et al. 1993; Schillberg et al. 2000). However, all these studies used infection structures grown in vitro, and although in the latter cases stable transformation may have been achieved, no propagules could be recovered due to the failure of rust fungi to reach the sporulation phase in vitro. Currently, there is a promising approach using insertional mutagenesis into avirulence genes and selection for infection of *P. triticina* using wheat lines resistant to the wild type (Webb et al. 2006). Yet, a convincing demonstration of the stability of transformation through repeated spore cycling and a recovery of the plasmid marker remains to be provided. Based on the initial work by Wirsel et al. (2004), we recently started a different approach using the introduction of single point mutations in the genes encoding β-tubulin (*TBB1*) and the Fe-S-subunit of succinate dehydrogenase (*SucDH1*) and in planta selection of potential transformants using the fungicides benomyl and carboxin (Voegele et al, unpublished data). In addition, a variety of different color markers were introduced into the plasmids used for transformation in order to visualize successful transformation events. Expression of the transgenes is driven by regulatory elements derived from *U. fabae* and DNA delivery is either accomplished using biolistics or *Agrobacterium tumefaciens*-mediated transformation.

The increasing availability of sequence data paired with the prospects of a stable transformation system will once again open new vistas to research focusing on the molecular principles underlying the obligate biotrophic lifestyle.

X. Suppression of Host Defenses

The establishment of biotrophy requires the evasion or suppression of host defense reactions. Rust fungi seem to have evolved a number of mechanisms to avoid recognition through host surveillance systems. Analyses of cell wall components of early infection structures, for example, indicated the most obvious differences between germlings and appressoria, which are outside the plant tissue and stained by the chitin-specific lectin WGA, and infection structures produced inside, which are not or only weakly stained by WGA (Kapooria and Mendgen 1985; Freytag and Mendgen 1991a). This observation can be explained either by the masking of chitin (Freytag and Mendgen 1991b) or by the conversion of chitin to chitosan via chitin deacetylase (Deising and Siegrist 1995; 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 and Voegele 2004). It is therefore possible that BGL1p has additional or alternative functions other than providing substrate for HXT1p. There is also evidence that mannitol and D-arabitol are released from the fungal mycelium into the apoplast in significant amounts (Link et al. 2005; Voegele et al. 2005). Results from mammalian (Chaturvedi et al. 1996) and plant pathosystems (Jennings et al. 2002) indicate that polyols, especially mannitol, can effectively suppress host defense responses involving reactive oxygen species. The concentrations of mannitol and D-arabitol found in infected *V. 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 *P. graminis* or *Puccinia 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 and Chong 1991). Such signals may include suppressors which have been implicated in maintaining basic compatibility between the parasite and its host plants (Bushnell and 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 non-host pathogens (Fernandez and Heath 1991). Suppressors for plant defense responses have been described, but they are either poorly characterized or non-proteinaceous (Basse et al. 1992; Knogge 1997; Moerschbacher et al. 1999).

A very active field of research today is the analysis of proteins secreted by a pathogen. These proteins include virulence factors, toxins, or avirulence gene products, which are now combined under the term effectors (Kamoun 2006). Such effector molecules are thought to manipulate host cell structure and/or function thereby facilitating infection and/or triggering defense responses. They might be released by the parasite into the apoplast, or alternatively they might be transferred into the host cell. Transfer of effectors into the host cell is especially interesting in the view of taking direct influence on host metabolism. Because of the intimate contact between host and parasite around haustoria (Fig. 4.7), these structures resemble the ideal location for such a transfer. Recent work on the *M. lini/L. usitatissimum* pathosystem by the groups of Jeff Ellis and Peter Dodds identified a number of haustorium-specific secreted proteins for some of which a direct interaction with the corresponding host resistance gene products could be shown (for a review, see Ellis et al. 2007a; see Sect. XIII). This work nicely confirms the gene for gene hypothesis put forward by Flor (1955, 1956) more than 60 years ago at the molecular level. However, it has to be kept in mind that the interaction of avirulence gene products and resistance gene products results in an incompatible interaction, in other words a failure of the pathogen to establish an infection. While this is certainly an interesting aspect with respect to the basic understanding of resistance reactions and the identification of new avirulence gene/resistance gene combinations and therefore also advantageous for breeders, this situation does not reflect the true obligate biotrophic lifestyle, which is based on a long-lasting interaction of host and parasite. Recently, Kemen and coworkers (2005b) were able to show that one of the *PIG*s identified by Hahn and Mendgen (1997) is not only secreted into the extrahaustorial matrix as expected from its targeting sequences, but is further transferred to the host cell cytoplasm. However, it remains to be shown what the functions and targets of rust transferred protein 1 (RTP1p) are and how this and other effectors are translocated into the host cell.

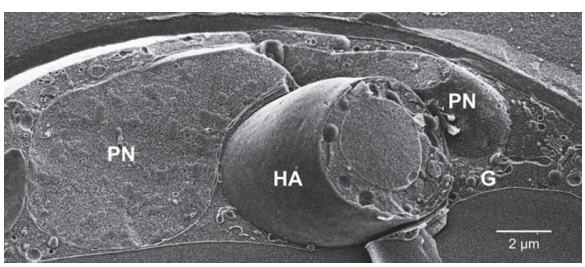

Fig. 4.7. Tight association of haustorium and plant cell nucleus. Fracture through a high-pressure frozen broad bean cell (*V. faba*) revealing the haustorium (*HA*) of *U. fabae* as seen with a cryo-scanning electron microscope. The haustorium is closely surrounded by the plant nucleus (*PN*) and Golgi bodies (*G*) of the host cell (Kemen and Mendgen, unpublished data).

It now seems well established that in eukaryotes, like in their bacterial counterparts, effectors can be directly transferred into the targeted host cell (Catanzariti et al. 2006, 2007). However, the mechanism by which this transfer is achieved still remains enigmatic (Ellis et al. 2006). For bacterial pathogens, specialized type III secretion systems (T3SS) have been shown to be implicated in the transfer of effectors directly into the target cells (Ghosh 2004; Mota et al. 2005). Yet, in eukaryotic pathogens no such "molecular syringes" could be identified. A clue as to how such a transfer could be accomplished comes from the identification of specific targeting signals in oomycete plant pathogens (Rehmany et al. 2005). This RXLR motif is conserved in all known avirulence proteins of oomycetes (Kamoun 2006) and is reminiscent of a host-targeting signal in malaria parasites (*Plasmodium* sp.) that is required for translocation of proteins into the cytoplasm of host cells (Hiller et al. 2004). However, such a consensus translocation signal has so far not been identified in rust effectors. Furthermore,

the mode of translocation of any of the known effectors is still enigmatic. There are a couple of possible routes an effector molecule may take when secreted from the haustorium. These include: *I* direct membrane transfer, *II* protein-mediated translocation,*III* endosomal transfer (possibly involving the tubular vesicular structures), and *IV* retrograde transport through the host Golgi system (Fig. 4.8). Thus not only will it be interesting to determine the function and potential targets of these effectors, but it will also be motivating to clarify the transfer mechanisms for these proteins.

XI. Host Responses to Rust Infection

Successful rust infection leads to profound changes in host plant metabolism. Alterations in host physiology after rust infection have been described in detail in a number of studies (for summaries, see Farrar and Lewis 1987; Hahn 2000).

Rust-infected plant tissue shows a general decrease in chlorophyll content and photosynthesis, in a complex spatial and temporal fashion (Scholes and Farrar 1985). Marked differences are observed between colonized and non-colonized tissue of the same leaf. Photosynthesis and chlorophyll are often kept at higher levels for longer times in infected tissue regions than in surrounding non-infected tissue (Scholes and Farrar 1985 1986). This fact became generally known as the "green island" phenomenon (Scott 1972; Bushnell 1984; Walters and McRoberts 2006). While chlorophyll and photosynthetic activity are retained in infected regions, the surrounding tissue shows premature senescence and chlorosis. Video-based quantitative imaging of chlorophyll fluorescence was used for sensitive, high-resolution measurement of photosynthesis in living leaf tissue. By using this technique, changes in photosynthesis of rust-infected bean and crown rust-infected oat plants were found to follow a complex spatial and temporal pattern during disease development (Peterson and Aylor 1995; Scholes and Rolfe 1996).

Infection-related changes in carbon metabolism are considered to be of central importance during rust infection. Rust infection leads to a massive relocation of carbohydrates within the plant. In rust infection sites, starch accumulation is observed, and carbohydrate export from the leaf is

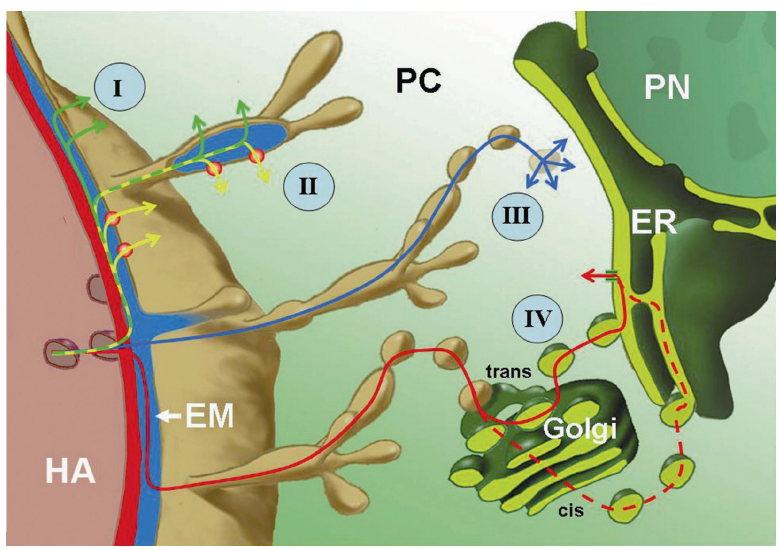

Fig. 4.8. Possible routes for effector proteins transferred from the haustorium into the host cell. *I* Direct transfer of the effector across the extrahaustorial membrane due to special characteristics of the protein. *II* Transfer mediated through specific transporters. *III* Participation of the tubular extensions in endosomal transfer. Budding vesicles fuse with lytic vesicles and become early endosomes. *IV* Retrograde transfer of effectors with participation of the complete Golgi apparatus (*dotted line*) or of the trans-Golgi network only (*solid line*). *EM* extrahaustorial matrix, *ER* endoplasmic reticulum, *HA* haustorium, *PC* plant cytoplasm, *PN* plant nucleus (Kemen and Mendgen, unpublished data)

progressively reduced (Bushnell 1984; Scholes and Farrar 1987). At later stages of infection, systemic effects become increasingly evident. Rust-infected leaves can develop into particularly effective sinks for carbohydrates. This again can be related to the well known phenomenon of "green island" formation (see above). Increased activities of apoplastic invertases at the sites of infection have been measured in a variety of pathosystems with biotrophic fungi. They are likely to be involved in the accumulation of soluble hexoses and formation of a sink tissue. Evidence for the plant or fungal origin of these activities was provided for the interactions between *A. thaliana* with white blister rust and powdery mildew, respectively (Chou et al. 2000; Fotopoulos et al. 2003). Increased expression of a host cell wall invertase gene was also observed in rust-infected *V. faba* leaves, in addition to the INV1p invertase that is secreted by the rust fungus (Voegele et al. 2006). Interestingly, induction of the host cell wall invertase gene was also observed in root tissue, which confirms the systemic effects of rust infection on the host plant. The expression of the fungal invertase INV1p in early infection structures, in which no uptake system for the produced monosaccharides is detectable, could play a role in increasing the sink strength of the invaded tissue. 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 which competes with naturally occurring sinks (Voegele et al. 2006). Wirsel et al. (2001) have shown that infection with a rust fungus can have far-reaching effects on host metabolism, exceeding the boundary of the infected cell. As expected, several of the genes analyzed by RT-PCR showed altered expression patterns in the infected organ. However, some of the analyzed genes also showed alterations in gene expression in far remote organs, such as stems and roots.

The search for flax genes that are induced by rust infection resulted in the identification of the *fis1* gene that is likely to encode Δ-1-pyrroline-5-carboxylate dehydrogenase, an enzyme involved in proline degradation (Roberts and Pryor 1995; Ayliffe et al. 2002). The induction was found to occur only after rust infection but not after infection with other pathogens or after wounding. Disruption of *fis1*, however, did not alter the response of the plant to rust infection (Mitchell et al. 2006). Thus, its role during the interaction remains unclear. In *Festuca rubra* leaves infected with *Puccinia* spp., several plant genes were found to be up-regulated, including a MAP kinase gene, a putative resistance gene, and a gene encoding a Hsp70 protein (Ergen et al. 2007). In the cowpea/*U. vignae* interaction, infection related changes of gene expression were studied by extracting RNA isolated from individual epidermal cells in which the cell walls but not yet the cell lumen were penetrated by the fungal hypha (Mould et al. 2003). More than 24 genes were found to be up-regulated both in susceptible and resistant cells. Few genes were found to be specifically induced in resistant cells during rust penetration; mentionable are two encoding a PR10 pathogenesis-related protein and a phenylalanine ammonia lyase (Mould et al. 2003). A comprehensive genome-wide study of rust-induced changes in gene expression during several stages of infection was performed with resistant and susceptible soybean varieties (van de Mortel et al. 2007). In both the compatible and incompatible interaction, a biphasic pattern of differential gene expression response to rust infection was observed. Significant changes in gene expression were detected within the first 12 h after inoculation, but thereafter most of the differentially expressed genes returned to normal levels. A second wave of rust-induced up-regulation of several hundred genes was observed earlier in the incompatible (starting at 48 h) than in the compatible (72 h) interaction. The early changes in host gene expression might reflect the unusual direct mode of penetration of the soybean rust fungus. The subsequent down-regulation of early-induced genes might be interpreted as the ability of the rust fungus to suppress host defense gene induction, even when this has already been turned on (van de Mortel et al. 2007).

Taken together, changes in plant gene expression induced by rust infection seem to be both pathogen- and host-specific and follow a complex kinetic pattern, which makes them difficult to interpret in terms of molecular signals and responses.

XII. Control of Rust Disease

The first effective procedures to control rust disease were mandated by law in Rouen, France, as early as 1660. The law called for the destruction of barberry bushes, the alternate host of *P. graminis*, in the vicinity of grain fields in order to control cereal rust epidemics. Elimination of the alternate host disrupts the life cycle of the pathogen and thus causes a reduction of initial inoculum, along with a decrease in pathogen genetic variability (Roelfs 1982). The Connecticut barberry law of 1726, together with subsequent barberry laws in other states, continues to be an effective control mechanism today (Peterson et al. 2005). However, federal funding for the Barberry Eradication Program was discontinued in 1980. While no effects were detectable until 2002, it remains to be seen what consequences the discontinuation of the program will bring in the future (Peterson et al. 2005).

Another important means of controlling rust disease is the use of fungicides. The latest rust specific compilation of fungicides dates back to the early 1980s (Buchenauer 1982). However, azole- and dithiocarbamate-based formulations are still among the most effective fungicides since they exhibit high biological activity at low application rates. Moreover, the resistance risks associated with these fungicides used on cereal rusts is considered medium or even low (Brent 1995).

Biological control of rusts with hyperparasitic fungi has been described (Kranz 1981; Buchenauer and Leinhos 1982; Sharma and Sankaran 1988). However, no real breakthrough has been achieved to date. One possible explanation might be the hyperparasites' requirement for high humidity. These control methods can therefore not be very effective in temperate or arid regions (Grabski and Mendgen 1986). However, better results may be obtained in the tropics (Saksirirat and Hoppe 1990). A different, more successful story is the use of rust fungi as a biological control agent of invasive plant species (Wandeler and Bacher 2006; Fisher et al. 2007; Wood and Morris 2007).

Genetic resistance remains the most economic and environmentally friendly method to minimize yield losses due to rust fungi (Deising et al. 2002; Webb and Fellers 2006). Most commercial cereal cultivars remain resistant to rust infection for less than a decade, which is about the life span of an active breeding program (Roelfs et al. 1992). Others remain resistant for many years. The *Rpg1* gene, for example, provided North American barley cultivars with resistance to the stem rust fungus *P. graminis* f. sp. *tritici* for more than six decades (Staples 2003). A more detailed description of genetic resistance to rust fungi is provided in Sect. XIII.

XIII. Genetics and Molecular Biology of Rust Resistance

Because of the obligate biotrophic mode of rust infection, it is not surprising that successful defense of resistant plants against rust fungi is usually coupled to programmed cell death of the infected cells, the so-called hypersensitive response (HR). In most cases, HR is the consequence of genetic interactions between resistance (R) genes in the host plants and so-called avirulence (Avr) genes in certain races of the pathogen. Remarkably, the gene for gene hypothesis that appropriately describes such interactions in a variety of different plant-pathosystems was developed by detailed studies on the flax/flax rust interaction (Flor 1955, 1956). A flax plant is resistant to rust infection when it carries at least one R gene that corresponds to or matches a specific avirulence gene present in the attacking rust strain. The simplest interpretation in molecular terms for this situation is that the R genes encode specific receptors for the Avr gene products. Molecular studies on a variety of gene for gene systems revealed that direct interactions between R proteins and Avr proteins can occur, but also showed that R proteins can recognize Avr proteins indirectly, e.g. by monitoring the integrity of host cellular targets of effector action (Jones and Dangl 2006). This "guard hypothesis" implies that R proteins recognize pathogen effectors only indirectly (see Chaps. 17, 18).

Many R genes against rust fungi were identified a long time ago by classic genetics; and their introgression into commercial cultivars of wheat, barley, oat and other crops remains a major task of agricultural resistance breeding today. A catalogue of resistance genes for different crop plants is available from the USDA (http://www.ars.usda.gov). This catalogue lists, for example, more than 45 wheat R genes against *P. graminis* f. sp. *tritici*. For the same host plant, more than 56 R genes against *P. triticina* are described. At least 19 R genes are identified in barley against *P. hordei*, and in oat more than 96 R genes against *P. coronata*.

During the past decade, a number of R genes have been cloned. One of the best characterized plants is flax, in which several members and alleles from all of the five R gene loci were sequenced (Dodds et al. 2001; Catanzariti et al. 2006, 2007). They encode typical R proteins of the Toll/interleukin resistance (TIR)–nucleotide binding site (NBS)–leucine-rich repeats (LRR) type (Ellis et al. 2007a). Comparative sequence analyses of the flax R gene alleles and artificially created hybrid genes reveal that the LRR domains are the major determinants of Avr protein recognition specificity (Ellis et al. 1999, 2007b; Dodds et al. 2001). In cereal crops, the cloning of several agronomically important R genes has been achieved. A prominent example is the *Rpg1* gene from barley which provides resistance to most pathotypes of *P. graminis* f. sp. *tritici*. The gene is incorporated into all major North American barley cultivars and has protected them from significant stem rust losses for more than 60 years (Staples 2003). As in other plants, cloning of *Rpg1* was achieved by a map-based approach (Brueggeman et al. 2002). The Rpg1 protein contains two tandem serine/threonine kinase motifs which are both required for Rpg1-mediated resistance (Nirmala et al. 2006). The known rust-specific R genes from grasses encode either NBS-LRR-type R proteins without an N-terminal TIR domain (barley *Lr1*, *Lr10*, *Lr21*, maize *Rp1*), or in the case

of barley *Rpg1*, a serine/threonine protein kinase (Feuillet et al. 2003; Huang et al. 2003; Nirmala et al. 2006; Cloutier et al. 2007). Transfer of *Rpg1* into a susceptible commercial barley variety by transformation rendered it highly resistant against stem rust infection, illustrating the potential for transformation-assisted breeding (Horvath et al. 2003).

The sequencing of R alleles from resistant and susceptible plants provided insights into the evolution of rust resistance genes. Evidence for diversifying selection was obtained for different alleles of R genes in the L and P locus of flax (Ellis et al. 2007a). In the complex *Rp1* locus of maize, more than 30 similar R-like genes exist in the vicinity of the active *Rp1* gene. Unequal intragenic recombination between members of this gene cluster was shown to be an important source for the generation of novel resistance specificities towards different races of the maize rust *Puccinia sorghi* (Smith and Hulbert 2005).

The cloning of both R genes and their corresponding Avr genes, in the flax/flax rust pathosystem provided the opportunity to study the molecular interaction between R proteins and effector/avirulence proteins. The predicted amino acid sequences of the flax R proteins suggest that they are located in the cytoplasm, the L and M proteins possibly including N terminal membrane anchors (Ellis et al. 2007a). Transient expression of the *M. lini* Avr proteins in flax cells, using *A. tumefaciens*-mediated transformation, led to an HR in plants carrying the corresponding R genes (Dodds et al. 2004). For the L6 protein, a direct binding to the cognate AvrL567 protein was shown using yeast-two-hybrid interaction studies (Dodds et al. 2006).

Genes involved in non-host resistance against rust fungi are of interest for breeders because, despite their usually lower efficiency against pathogens compared to R genes, they are thought to confer greater durability under field conditions. Compared to R-gene-dependent resistance, non-host resistance is less frequently correlated with an HR. Instead, common observations include failure of germ tubes to invade non-host plants via stomata (possibly due to the absence of the correct topographical cues), abortive growth and premature death of the infection hypha before formation of the first haustorium, or rapid encasement of the haustorium (Heath and Skalamera 1997). A cross between a barley cultivar that is hypersusceptible to several non-host rust fungi and a normal barley cultivar revealed a number of quantitative trait-like loci that condition the defense against different rust fungi (Jafary et al. 2006). In a similar study, the progeny of a cross between two barley lines with different degrees of resistance to wheat leaf rust revealed that both pre- and post-haustorial mechanisms are involved in non-host rust resistance (Neu et al. 2003). In both cases, R-type genes were found to be possibly involved in certain types of non-host resistance. The model plant *A. thaliana* was described to be infected by the rust fungus *Puccinia thlaspeos* (Gäumann 1959). However, host invasion occurs by monokaryotic basidiospores in roots and gives rise to systemic infections, which is hardly reproducible in the laboratory (K. Mendgen, unpublished data). Nevertheless, *A. thaliana* with its large collection of mutants is a useful model for the analysis of non-host resistance against rust fungi. *A. thaliana* mutants defective in salicylic acid-dependent defense signaling were found to allow an increased development of heterologous rust fungi, in particular *U. vignae*, up to the establishment of functional biotrophic relationships (Mellersh and Heath 2003).

Resistance to rust fungi can be induced in susceptible plants by chemical treatments, leading to systemic acquired resistance (SAR). In *V. faba*, SAR induced by treatment with salicylic acid or 2,6-dichloro-isonicotinic acid leads to significant inhibition of the invading rust fungus *U. fabae* (Rauscher et al. 1999). The inhibition correlated with the inhibitory activity of apoplastic fluids obtained from induced resistant plants and was proposed to be due to the increased expression of the pathogenesis-related protein PR-1.

XIV. Imminent Threats: The Cases of *P. pachyrhizi* and *P. graminis* Ug99

The effectiveness of fungicide treatment and genetic and molecular breeding programs has successfully protected farmers from rust infection for more than half a century. However, this success may lead to a false sense of security. A possible scenario is that relaxed efforts to generate new resistant cultivars, the discontinuation of eradication programs, and a lack of funding for basic research engaged to clarify the molecular nature of biotrophic infections may lead to dramatic consequences in the future. The recent emergence of a new race of *P. graminis* f. sp. *tritici*, UG99 or TTKS, and the spread of Asian soybean rust (ASR) into

the continental United States illustrate the danger that obligate biotrophs still pose to agriculture.

Apart from the introduction of new rust pathogens, the boom and bust cycle poses a special threat to the farmers. In the boom, a resistant cultivar with single, major resistance is introduced. As a result of its good agronomic qualities, it may be widely accepted and planted over large areas. If the pathogen population is exposed to this resistance gene, races with a mutation from avirulence to virulence have a better chance to occur. In the bust part of this cycle, the virulent pathotypes spread, infect fields with the resistant cultivar, and cause an epidemic. The cycle begins again with the introduction of a new resistant cultivar (McDonald 2004). Typical examples are leaf rust of wheat caused by *P. triticina* and stem rust of wheat and barley caused by *P. graminis* f. sp. *tritici*. Some 40–50 races of leaf rust are identified annually in the United States. This high degree of variability has allowed the fungus to adapt to new resistant wheat cultivars, very often within a short period of time (Kolmer et al. 2007). By contrast, only three to five races of stem rust are found annually in the United States. This is most likely a result of the eradication of the alternate host barberry (Roelfs et al. 1992). However, many of the currently used wheat cultivars, albeit not all, are susceptible to race UG99 detected in Uganda in 1999 (Pretorius et al. 2000). This new race is spreading rapidly in the Eastern African highlands and poses a new threat to wheat production wherever the *Sr31* resistance gene is used (Wanyera et al. 2006). However, several lines of resistance genes appear to be effective against Ug99, both at the seedling and adult plant stages (Jin et al. 2007). A rapid introduction of such genes into cultivars used throughout Eastern Africa and Asia is now in progress. Wheat and barley breeders in other areas where wheat and barley are major crops should be prepared to prevent a major outbreak of Ug99.

The spread of ASR into the United States is a typical example for the introduction of an old foe into a previously pathogen-free region. In the past few years, the disease has spread from Asia to Hawaii in 1994, Africa in 1996, and South America in 2001 (Pivonia and Yang 2004). Aerial transport seems crucial for the recent transport of the disease to Florida. Model simulations suggest a transport of soybean rust spores from South America north of the equator into the United States with a tropical cyclone in August 2003 (Isard et al. 2005). Recently, soybean rust reached the eastern and central United States (Barnes et al. 2006b; Krupa et al. 2006). Sensitive PCR assays have been developed in order to detect and differentiate both *P. pachyrhizi* and the less aggressive *Phakopsora meibomiae* (Frederick et al. 2002). This technology is now widely used to trace the pathogen in rain samples (Barnes et al. 2006b; Krupa et al. 2006). The USDA has introduced an online Pest Information Platform for Extension and Education (PIPE), which allows farmers to continuously update on disease progress (http://www.sbrusa.net/). The pathogen overwinters on Kudzu (*Pueraria lobata*) in parts of Florida, Georgia, and Alabama, where leaves stay green during the winter and urediospores can survive and start a new epidemic the next year. Although teliospores are produced and may develop basidiospores, no alternate host has yet been found (Saksiriat and Hoppe 1991). Therefore, it seems unlikely that teliospores are responsible for spread of the pathogen. However, the broad host range, which comprises 31 species in 17 genera of legumes, including common bean cultivars and the widespread Kudzu, favor spread of the pathogen and makes it difficult to breed for resistance (Bromfield 1984; Miles 2007). At the moment it is therefore only the climatic conditions that prevent a massive ASR outbreak in the soybean growing areas of the United States.

XV. Conclusions and Perspectives

During the past decade much progress has been made in determining some of the aspects of obligate biotrophic growth. The increasing availability of sequence data, especially through genomic sequencing projects, paired with expression analysis in macro- and microarray format will further our understanding of the molecular details underlying this intricate plant–parasite interaction. While we are far from establishing culture conditions to produce "parasitic phase" infection structures in vitro, functional, stable transformation has drawn a step closer (Wirsel et al. 2004; Webb et al. 2006). Combined with gene expression and protein localization studies, the availability of transgenic rust fungi would greatly facilitate future molecular and biochemical work on rust fungi. For instance, gene silencing methodology paired with the identification of stage-specific promoter sequences could help to identify elements crucial

for the establishment and maintenance of the obligate biotrophic lifestyle.

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