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Pathogen profile

Stem rust of small grains and grasses caused by *Puccinia graminis*

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SUMMARY

Stem rust has been a serious disease of wheat, barley, oat and rye, as well as various important grasses including timothy, tall fescue and perennial ryegrass. The stem rust fungus, *Puccinia graminis*, is functionally an obligate biotroph. Although the fungus can be cultured with difficulty on artificial media, cultures grow slowly and upon subculturing they develop abnormal ploidy levels and lose their ability to infect host plants [Bushnell and Bosacker (1982) *Can. J. Bot.* **60**, 1827–1836]. *P. graminis* is a typical heteroecious rust fungus with the full complement of five distinct spore stages that occur during asexual reproduction on its gramineous hosts and sexual reproduction that begins in the resting spore stage and culminates on the alternate host, barberry (*Berberis* spp.). There appears to be little polymorphism for resistance/susceptibility in *Berberis* species, but complex polymorphisms of resistance/susceptibility and matching virulence/avirulence exist in gene-for-gene relationships between small grain species and the forms of *P. graminis* that infect them.

Taxonomy: *Puccinia graminis* is a rust fungus in the phylum Basidiomycota, class Urediniomycetes, order Uredinales, and family Pucciniaceae, which contains 17 genera and approximately 4121 species, of which the majority are in the genus *Puccinia* [Kirk *et al.* (2001) *Ainsworth and Bisby's Dictionary of the Fungi*. Wallingford, UK: CAB International]. Various subdivisions of *P. graminis* into subspecies, varieties and formae speciales have been proposed based on spore size and host range. Crossing studies and DNA sequence comparisons support the separation of at least two subspecies, but not the proposed separation based on spore size.

Host range: The host range of *P. graminis* is very broad compared with that of most *Puccinia* spp.; it includes at least 365 species of cereals and grasses in 54 genera [Anikster (1984) *The Cereal Rusts*. Orlando, FL: Academic Press, pp. 115–130]. Wheat stem rust, *P. graminis* f. sp. *tritici*, was shown to infect 74 species in 34 genera in artificial inoculations of seedlings, but only 28 of those species belonging to eight genera were known to be natural hosts of the fungus. Other formae speciales of *P. graminis* have narrower host ranges than *P. graminis* f. sp. *tritici*.

Disease symptoms: Infections in cereals or grasses occur mainly on stems and leaf sheaths, but occasionally they may be found on leaf blades and glumes as well. The first macroscopic symptom is usually a small chlorotic fleck, which appears a few days after infection. About 8–10 days after infection, a pustule several millimetres long and a few millimetres wide is formed by rupture of the host epidermis from pressure of a mass of brick-red urediniospores produced in the infection. These uredinial pustules are generally linear or diamond shaped and may enlarge up to 10 mm long. The powdery masses of urediniospores appear similar to rust spots on a weathered iron surface. With age, the infection ceases production of brick-red urediniospores and produces a layer of black teliospores in their place, causing the stems of heavily infected plants to appear blackened late in the season.

ECONOMIC IMPORTANCE

Stem rust was once the most feared disease of wheat in most wheat-growing regions of the world. Indeed, several references in the Bible relate to epidemics of cereal rusts and smut inflicted upon the Israelites as punishment for their sins (Chester, 1946). Fragments of stem rust-infected wheat from the Bronze age have been discovered in Israel (Kislev, 1982). Numa Pompilius (715–672 BC) described the Roman festival of Robigalia that was established to protect cereal crops through prayer and sacrifice to the rust gods, but later Aristotle and Theophrastus associated cereal rust epidemics with warm, wet weather (Chester, 1946). Stem rust is primarily a warm weather disease, but it can cause great damage to susceptible wheat crops over broad geographical regions. A crop that appears healthy 3 weeks before harvest can be devastated by explosive buildup of stem rust if sufficient inoculum arrives from a heavily infected wheat crop in some distant region. Severe infection of stems interrupts nutrient flow to the developing heads, resulting in shrivelled grain. In addition, stems weakened by rust infection are prone to lodging and further loss of grain (Roelfs *et al.*, 1992).

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The most severe stem rust epidemics in Europe in the 20th century occurred in 1932 and 1951; national wheat yield losses of 9–33% occurred in Scandinavia in 1951, and losses of 5–20% were reported in eastern and central Europe in 1932 (Zadoks, 1963). In Australia stem rust epidemics occurred intermittently and mainly in the warmer areas of Queensland and northern New South Wales through the mid-20th century with occasional severe yield losses (Rees, 1972). In 1974 a serious epidemic of stem rust damaged wheat crops in the southern states of Australia (Watson, 1981). Stem rust of wheat in India is primarily a problem in the south where the growing season is warmer, but has not caused severe losses in the wheat belt of north-west India except in years with unusually warm weather in January and February (Joshi and Palmer, 1973). In China stem rust occurs mainly in the spring wheat area of northern China and Inner Mongolia. Severe epidemics occurred in 1948, 1951, 1952 and 1956 when higher than average temperatures and frequent rains favoured infection (Roelfs, 1977). In the United States stem rust was mainly a problem for spring wheat production in the northern Great Plains, but severe epidemics occurred occasionally in winter wheat crops in southern states (Leonard, 2001). In eight of the 40 years from 1920 to 1960 the spring wheat region of Minnesota, North Dakota and South Dakota suffered yield losses of greater than 10% as a result of stem rust. In five of those years the losses exceeded 20% of the US spring wheat crop; more than 50% of the wheat yield in North Dakota and Minnesota was lost to stem rust in the worst epidemic in 1935 (Leonard, 2001). Although there has not been a significant wheat stem rust epidemic anywhere in the US or Canada since 1974, the pathogen is still present and dangerous.

The current success in combating stem rust rests on continuing efforts to pyramid genes for race-specific resistance to stem rust in new wheat cultivars. Removal of barberry, the alternate host, in Europe and North America and effectiveness in suppressing the surviving populations of *P. graminis* in main wheat production areas of the world have made breeding efforts successful by limiting the genetic diversity and appearance of new virulent races in the *P. graminis* populations.

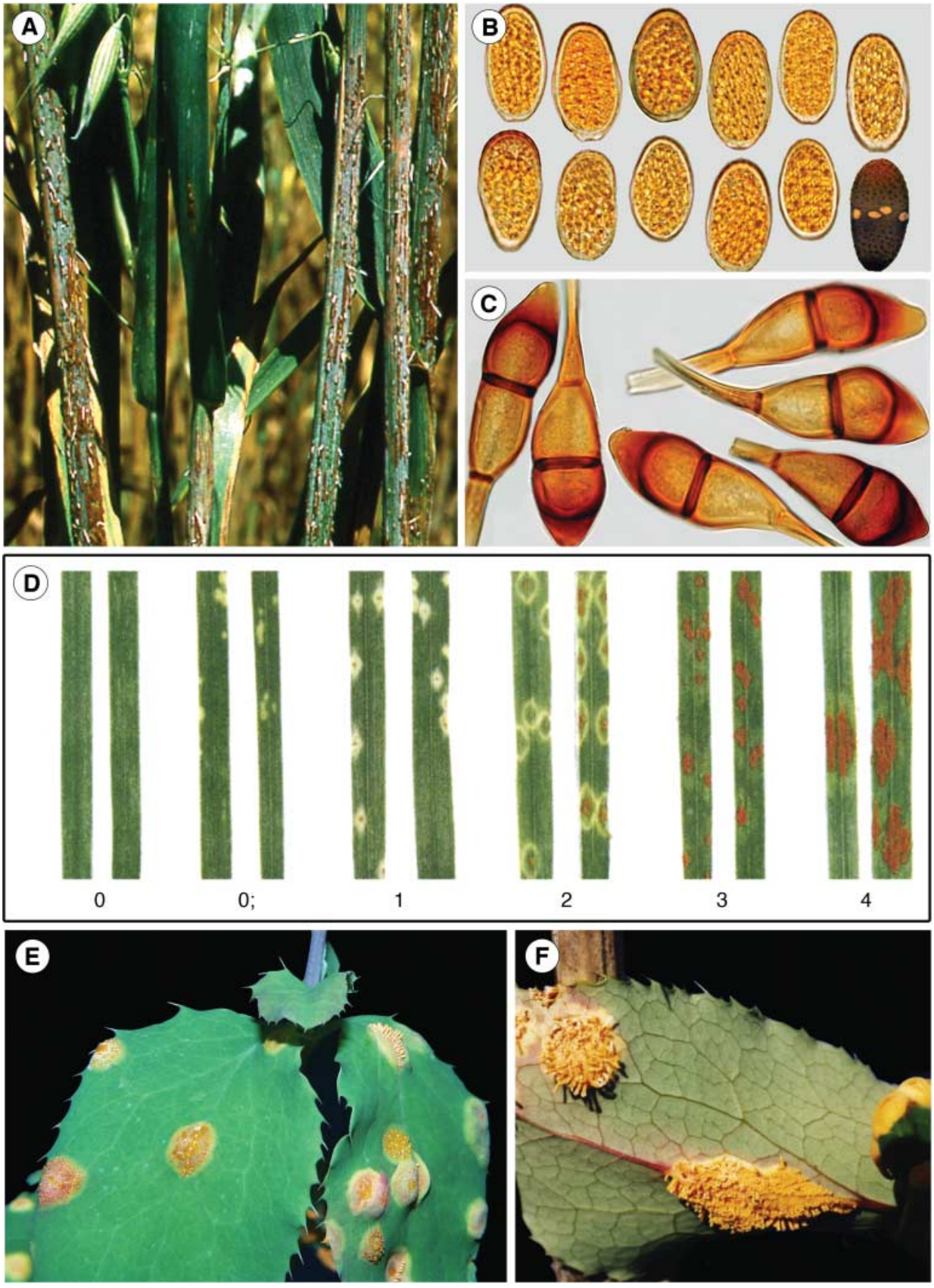
In addition to wheat stem rust, various formae speciales of *P. graminis* also cause potentially serious diseases of oat, barley and rye. In addition, stem rust is a serious problem for seed production of *Festuca* and *Lolium* spp. in the Pacific north-west region of the US (Pfender, 2001).

LIFE CYCLE

Puccinia graminis evolved as a full-cycle, heteroecious rust fungus. In temperate climates, *P. graminis* typically produces thick-walled, two-celled teliospores, the resting spore stage, near the end of the growing season of the gramineous host. Each teliospore cell contains two haploid nuclei (i.e. is dikaryotic) when first formed, but karyogamy occurs early in teliospore maturation (Fig. 1). Teliospore stalks remain intact and the spores are not dispersed from the telial pustule. Instead, they remain dormant in the infected straw until spring whereupon they germinate in synchrony with the onset of bud break and new leaf growth in the alternate host *Berberis* or *Mahonia* spp. (Roelfs, 1985; Roelfs and Groth, 1988). Meiosis begins shortly after karyogamy but is suspended at diplonema of the first meiotic division during the period of telisopore dormancy (Boehm *et al*., 1992). In the spring, one or both cells of the teliospore produces a hyphal protrusion called a promycelium or basidium. When meiosis is complete, the resulting four haploid nuclei are separated from each other in the promycelium by three transverse septa. A projecting sterigma forms on each promycelium cell, and the haploid nucleus migrates through the sterigma into the newly forming basidiospore as it expands at the tip of the sterigma (Roelfs, 1985). Mitosis results in two identical haploid nuclei per mature basidiospore (Fig. 1).

Mature basidiospores are ejected from the sterigmata and carried by air currents to infect alternative hosts in the genus *Berberis*, primarily common barberry (*B. vulgaris*). Barberry leaves become resistant as they age, presumably because basidiospore germ tubes are unable to penetrate directly through thick cuticles on the leaf surface. Infection results in the production of flask-shaped pycnia, usually on the upper surface of the barberry leaf (Figs 1 and 2E). Small, thin-walled pycniospores form within the pycnium and exude from the tip of the pycnium in a drop of pycnial nectar. The nectar is attractive to insects that, along with rain splashing, serve to disseminate pycniospores among pycnia. Pycniospores, which serve as the male gametes, consist mainly of a single haploid nucleus with little surrounding cytoplasm. Flexuous hyphae, which extend out of the top of the flask-shaped pycnia, serve as the female gametes. Two mating types, commonly designated + and –, have been identified and appear to be under monogenic control (Roelfs, 1985), although mating type alleles

Fig. 1 Life cycle of *Puccinia graminis*. The asexual uredinial stage is repeated on the grass host with a new generation of dikaryotic urediniospores every 14–20 days under favourable conditions. Teliospores, which begin the sexual stage of the life cycle, typically form as the grass host matures in late summer or autumn. Karyogamy occurs in maturing teliospores, and meiosis of the resulting diploid nucleus begins before the teliospore enters dormancy. In spring, meiosis is completed and teliospores germinate to produce four haploid basidiospores, two of each mating type (+ and –). Basidiospores infect barberry, the alternative host, on which the fungus produces haploid pycnia. Fertilization occurs with the fusion of a pycniopspore with a flexuous hypha of opposite mating type. Following fertilization, a dikaryotic aecium forms and begins producing dikaryotic aeciospores, which complete the life cycle by infecting the grass host. In regions with mild winters and adequate summer moisture, *P. graminis* may persist in the uredinial (asexual) stage on autumn-sown cereals in the winter and on volunteer cereal plants or susceptible wild grasses.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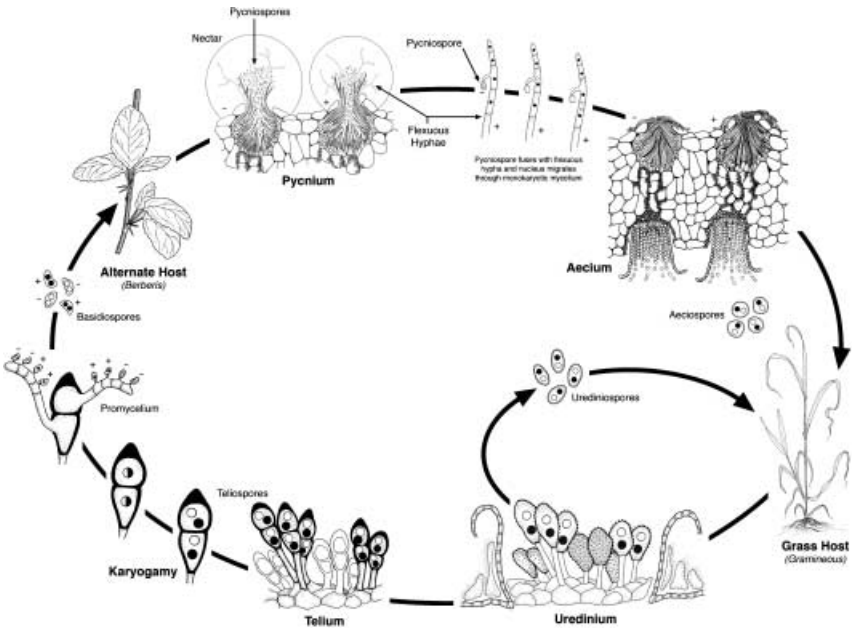

Fig. 2 Symptoms of *Puccinia graminis* on primary and alternative hosts, and morphology of urediniospores and teliospores. (A) Uredinia of *P. graminis* f. sp. *avenae* on oat plants. (B) Urediniospores of *P. graminis*, ×625; one stained spore shows the characteristic equatorial position of germ pores. (C) Teliospores of *P. graminis*, ×625. (D) Infection types of *P. graminis* f. sp. *tritici* on seedlings of differential wheat cultivars; types 0–2 are considered resistant, and types 3 and 4 are considered susceptible reactions (adapted from Stakman *et al.*, 1962). (E) Clusters of pycnia of *P. graminis* on the upper surface of *Berberis vulgaris* leaves. (F) Clusters of aecia of *P. graminis* on the lower surface of a *B. vulgaris* leaf.

have not been cloned in *P. graminis*. Pycniospores of *P. graminis* are apparently preconditioned for mating by a protein complex in pycnial nectar of the opposite mating type. This protein complex induces production of a cap, which may consist of glycoproteins, on the surface of pycniospores of opposite mating types but not on pycniospores of the same mating type as the pycnium that produced the nectar (Anikster *et al.*, 1999). When a pycniospore of one mating type contacts a flexuous hypha in a pycnium of the other mating type, fusion occurs and the haploid nucleus from the pycniospore migrates through a fusion tube into the flexuous hypha and through the monokaryotic hyphae until it reaches the cells of the protoaecium at the base of the pycnium (Johnson and Newton, 1946). With nuclear division and paired association of + and – mating type nuclei the dikaryotic state is established. New growth commences and a cup-shaped, dikaryotic aecium is formed below the pycnium, eventually rupturing the lower epidermis of the barberry leaf (Figs 1 and 2F). Chains of single-celled, dikaryotic aeciospores are produced, which can infect the gramineous host but not barberry (Roelfs, 1985).

When an aeciospore successfully infects its gramineous host, the fungus produces a dense mat of hyphae beneath the host epidermis. Sporophores grow from the mat and produce masses of single-celled dikaryotic urediniospores (Fig. 2B) that rupture the host epidermis producing a pustule known as a uredinium. Urediniospores are dispersed by wind and can reinfect the gramineous host (Fig. 1). Infections typically occur on stems and leaf sheaths of gramineous hosts, although leaf blades may also be infected to some extent, especially when seedlings of a susceptible host are inoculated. On maturing hosts, uredinia eventually cease production of urediniospores and begin to produce two-celled teliospores (Fig. 2C). At that stage the infection structure is called a telium (Cummins and Hiratsuka, 1983). In the central region of the United States, where common barberry has been largely eradicated, *P. graminis* can persist in the uredinial stage on wheat throughout the year, moving from autumn-sown winter wheat in the southern Great Plains north to infect developing winter wheat crops in the central Great Plains and, eventually, the spring-sown wheat in the northern Great Plains. Volunteer wheat plants from seed that sprout in fields and along roadsides after harvest serve as a 'green bridge' to sustain the uredinial stage of *P. graminis* on wheat in the central or southern Great Plains until the next winter wheat crop is sown in the autumn. A similar sequence of winter wheat crops and summer volunteer wheat plants accounts for the persistence of the wheat stem rust fungus in the uredinial stage in Australia and India. Wheat stem rust is no longer a major problem in Europe, and its ability to survive the winter in northern Europe or summer in southern Europe is not well known (Zadoks and Bouwman, 1985).

INFECTION PROCESS

Nearly all research on infection processes of *P. graminis* has focused on the uredinial stage because of its obvious economic importance. Infection of cereal or grass plants by germinating urediniospores follows an intricate series of stages and structures, which apparently are essential for the establishment of a successful parasitic relationship of the stem rust fungus with its host (Staples and Macko, 1984; Wiethölter *et al.*, 2003b). Urediniospore germination occurs if the spore is in contact with a film of water on the stem or leaf surface. The germinating urediniospore produces a germ tube that typically orientates its growth on the host leaf or stem surface perpendicular to the long axis of the

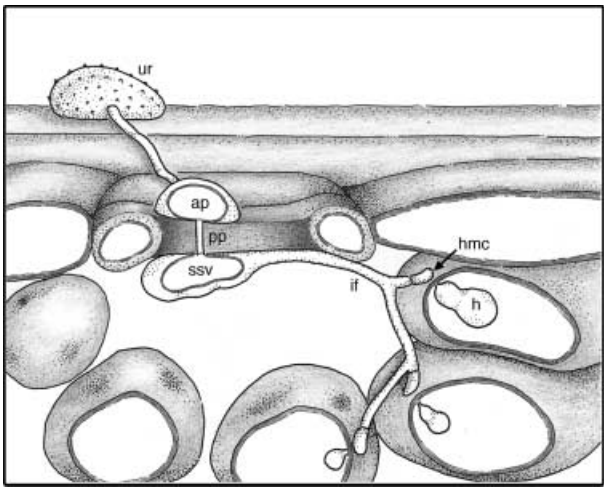

Fig. 3 Infection structures formed during the early stages of uredinial development by *P. graminis*: ur = urediniospore, ap = appressorium, pp = penetration peg, which passes between the guard cells and through the stoma, s = stoma, ssv = substomatal vesicle, ih = infection hypha, hmc = haustorial mother cell, h = haustorium.

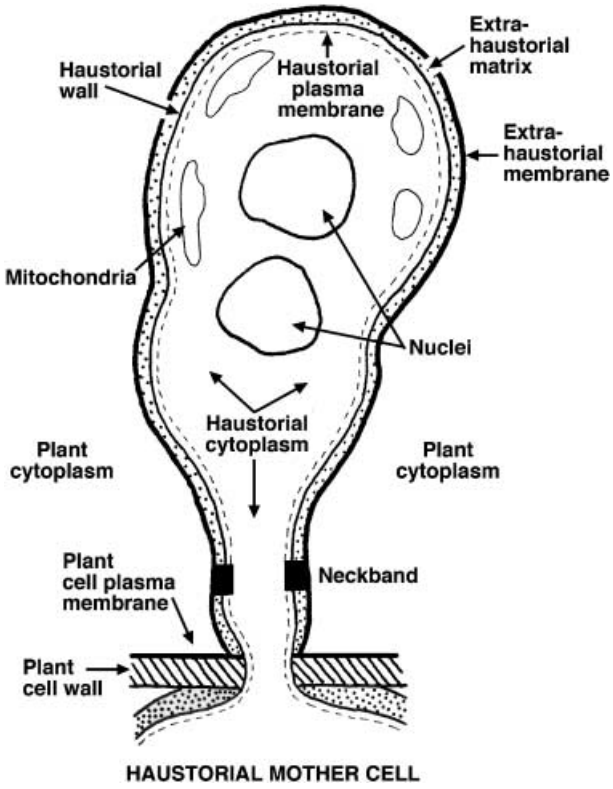

Fig. 4 Diagram of a typical haustorium of *P. graminis*. The interface between the plant and fungal cytoplasm comprises haustorial plasma membrane, haustorial wall, extrahaustorial matrix (a gel-like layer enriched in carbohydrates) and the extrahaustorial membrane (derived from the invaginated plant plasma membrane). A neckband seals the extrahaustorial matrix from the plant cell wall region so that the matrix is an isolated, apoplastic-like compartment. The haustorium contains cytoplasm, two nuclei and mitochondria, as well as other cellular components, and is directly connected to the haustorial mother cell through which nutrients are transported to the developing fungal hyphae.

epidermal cells. Proper orientation, which requires that the germ tube be appressed to the waxy cuticle on the epidermis, maximizes the chances that the germ tube will encounter a stoma through which penetration of the host may occur. At the stoma the germ tube stops elongating and forms an appressorium over the stomatal opening (Fig. 3). The two nuclei, which migrated from the urediniospore into the tip of the germ tube, enter the appressorium and undergo mitosis after they are cut off from the now empty germ tube by a septum. Germination and appressorium formation usually occur at night when dew is present on the plant surface. Development in *P. graminis* typically stops after formation of the appressorium until dawn when fungal growth resumes. This resumption of development probably depends only indirectly on light through its stimulation of photosynthesis and reduction of CO_2 concentration at stomata in the host plant. Yirgou and Caldwell (1968) showed that penetration of the host by *P. graminis* is inhibited by CO_2 but will occur nearly equally well in light or dark in CO_2-free air. Penetration by appressoria of *P. graminis* did not occur on non-photosynthetic albino wheat plants or etiolated plants in 1% ambient CO_2.

Under conducive conditions, a narrow penetration peg grows out from the lower surface of the appressorium, through the stoma, and into the substomatal cavity below the epidermis (Fig. 3). There it produces an elongate substomatal vesicle in which a second round of synchronous mitoses occurs. From each end of the vesicle an infection hypha may be produced, and a pair of nuclei migrate into the developing infection hypha. When the tip of the infection hypha contacts a host cell, it differentiates a haustorial mother cell that is separated from the hypha by a septum (Fig. 3). At this stage, the haustorial mother cell typically contains 2–4 nuclei. The haustorial mother cell produces a narrow peg that penetrates the host cell wall, apparently by enzymatic dissolution (Harder and Chong, 1984) as well as pressure. The haustorial mother cell contains a specialized cell wall that includes two additional layers not found in the intercellular hyhae (Chong *et al*., 1985). Upon penetrating the plant cell wall, a specialized fungal hypha expands to form an enlarged haustorium in the periplasmic space of the host cell (Figs 3 and 4). While the haustorium is forming, the infection hypha may produce a new branch just proximal to the first haustorial mother cell, resume growth and form a new haustorial mother cell and haustorium when it contacts another host cell. The process may be repeated to produce a third haustorium in yet another host cell, but by this time the nutrient reserves of the original urediniospore have been exhausted and further development depends upon the success of

the haustoria in extracting nutrients from the host without inducing a resistant response (Staples and Macko, 1984; Wiethölter *et al*., 2003a). An amino acid transporter gene (Hahn *et al*., 1997) and a hexose transporter gene (Voegele *et al*., 2001) have been shown to be specifically activated in haustoria of broad bean rust, *Uromyces fabae*, providing molecular evidence that the haustorium plays a special role in the uptake of nutrients from host cells. Furthermore, Struck *et al*. (1996) demonstrated high activity of H^+-ATPase, which plays a key role in active nutrient uptake, associated with membrane proteins from isolated haustoria of *U. fabae*. These observations are consistent with the earlier observation (Jäger and Reisener, 1969) that *P. graminis* f. sp. *tritici* derives amino acids mainly from the host plant and exhibits only limited biosynthesis of amino acids.

The infection structures of *P. graminis* can be induced *in vitro* by a combination of chemical and physical stimuli. For example, appressoria form at high frequencies on polystyrene surfaces with closely spaced ridges 1.5 μm apart and 2.0 μm high, which mimic the close spacing of cell junctions adjacent to stomata on cereal leaves (Read *et al*., 1997). In addition, appressoria, vesicles and haustorial mother cells can be induced by *trans*-2-hexen-1-ol, one of several six-carbon derivatives of the plant lipoxygenase (LOX) pathway that has been shown to be induced by pathogen attack (Collins *et al*., 2001; Wiethölter *et al*., 2003b). Interestingly, appressorium formation can be induced also by acrolein, a compound extracted from viable urediniospores of *P. graminis*. It is possible that hexanols produced in plants may mimic the activity of acrolein or that both may mimic the activity of a critical part of a much larger unknown molecule (Collins *et al*., 2001). The topographical stimulus of contact with a plastic replica of the host epidermis with small, closely spaced ridges synergistically increases the *in vitro* production of infection structures in response to *trans*-2-hexen-1-ol as does mild heat shock (Collins *et al*., 2001; Festers *et al*., 1993). It is significant that axenic cultures of *P. graminis* are unable to infect the host unless the host epidermis is removed to allow direct contact of hyphae with the host mesophyll cells (Festers *et al*., 1993; Williams *et al*., 1967).

Once a compatible relationship has been established in a rust-infected host, the normal direction of phloem transport is altered to divert nutrients to the infected tissue at the expense of actively growing plant tissue (Mendgen, 1981). This change is characterized by massive increases of respiration and accumulation of cytokinins in the infected area. Sugars accumulate in the lesion area, and an invertase provides hexoses used by the fungus for growth and sporulation. The basic needs of the rust fungus, as shown by culture on artificial media, are various sugars, a balanced mixture of amino acids including a sulphur amino acid as a source of reduced sulphur, and basic inorganic nutrients (Mendgen, 1981). The infection process by which *P. graminis* meets these needs is a highly regulated response system that involves signalling and response in both host and pathogen.

EVOLUTION AND SUBSPECIFIC VARIATION

Leppik (1959) proposed that early ancestors of *Puccinia graminis* on species of the Berberidaceae originated in central Asia and spread through the northern hemisphere with the wide expansion of grassland vegetation during the Tertiary. Some rusts on hosts in the Berberidaceae transferred their telial stage to grasses that were closely associated with their aecial host. Differentiation of *P. graminis* must have occurred at a time when the ancestral Berberidaceae had already split into recent genera but before *Mahonia* and *Berberis* were genetically separated (Leppik, 1959, 1961). The host range for its telial stage supports the central Asian origin of *P. graminis*; 90% of its gramineous hosts are in the subfamily Festucoideae, which occur mainly in the northern hemisphere. This also suggests that the family Poaceae had split into subfamilies before the Festucoideae encountered rust from barberry (Leppik, 1961). With the advent of agriculture, *P. graminis* was spread by humans throughout Asia and through the Mediterranean countries to Europe, Africa, the Americas and eventually Australia.

Puccinia graminis has been subdivided into distinct types based on host range or morphology, primarily size and shape of urediniospores. The first subdivision was into six formae speciales, *tritici* (wheat), *secalis* (rye), *avenae* (oat), *agrostidis* (*Agrostis* spp.), *poae* (bluegrass) and *airae* (*Aira caespitosa*), based on the most prominent cereal or common grass host of each type. Additional formae speciales such as *epigaei* (*Calamagrostis* spp.) were added as new host specificities were found. In addition, the closely related species *P. phlei-pratensis*, found primarily on timothy grass, was sometimes considered a forma specialis of *P. graminis* (Anikster, 1984; Johnson, 1949). Urban (1967) recognized two subspecies of *P. graminis*, ssp. *graminis* and ssp. *graminicola*, in Europe based on characteristics of the urediniospores. *P. graminis* ssp. *graminis* was further subdivided into var. *graminis* and var. *stakmanii*. Urediniospores of ssp. *graminis* are long-ellipsoidal to subcylindrical and 1.5–1.8 times larger than the broadly ellipsoidal to ovoid urediniospores of ssp. *graminicola*. Urediniospores of var. *graminis* in ssp. *graminis* are somewhat larger than those of var. *stakmanii*. The wheat stem rust fungus is included in *P. graminis* ssp. *graminis* var. *graminis*; the oat stem rust and rye stem rust fungi are included in *P. graminis* ssp. *graminis* var. *stakmanii*; and the stem rust fungi of most of the festucoid grasses are included in *P. graminis* ssp. *graminicola* (Savile, 1984; Savile and Urban, 1982; Urban, 1967). *P. graminis* ssp. *graminicola* is regarded as typical of the ancestral rust from which the stem rust fungi of cultivated cereals evolved.

The genetic basis for differences in spore morphology and hence the subspecific classification of *P. graminis* has not been determined. In fact, some results have called this separation of subspecies and varieties into question. For example, Johnson (1949) found high levels of interfertility in crosses between the wheat and rye stem rust fungi (ssp. *graminis* var. *graminis* × ssp.

graminis var. *stakmanii*) and between the oat stem rust fungus and stem rust fungi of *Agrostidis* or *Poa* (ssp. *graminis* var. *stakmanii* × ssp. *graminicola*) but little or no fertility between the wheat and oat stem rust fungi (ssp. *graminis* var. *graminis* × ssp. *graminis* var. *stakmanii*), the rye and oat stem rust fungi (ssp. *graminis* var. *stakmanii* × ssp. *graminis* var. *stakmanii*), or between the rye stem rust fungus and the stem rust fungus of *Poa* (ssp. *graminis* var. *stakmanii* × ssp. *graminicola*). Furthermore, Zambino and Szabo (1993) found that DNA sequences in the internal transcribed spacer regions (ITS) of the nuclear rDNA were identical for isolates of ff. spp. *avenae*, *dactylis*, *lolii* and *poae* but different from the DNA sequences of ff. spp. *tritici* and *secalis*. Thus, members of *P. graminis* that were placed in different subspecies, f. sp. *avenae* and f. sp. *lolii*, appeared to be closely related, but other members placed in the same subspecies and variety, f. sp. *avenae* and f. sp. *secalis*, appeared to be more distantly related. In this regard, it is interesting that Johnson (1949) found that when barberry was infected with basidiospores from the teliospores of an F_1 hybrid between the wheat stem rust and oat stem rust fungi the few pycnia that were produced did not develop any nectar, and no aecia were formed from attempted fertilizations. Eventually, however, a few urediniospores and teliospores were observed in old pycnial infections. Thus it appears that genetic control of the normal life cycle of *P. graminis* was severely disrupted in the hybrid between the wheat stem rust and oat stem rust fungi. The same disruption was also observed in the F_1 of a cross between the rye stem rust and oat stem rust fungi, which, presumably, both belong to *P. graminis* ssp. *graminis* var. *stakmanii*. Johnson (1949) also found that in successful crosses between what he considered closely related formae speciales, the level of pathogenicity (fitness) of the F_1 progeny on a given host was lower than that of the more pathogenic parent on that host. He concluded that two factors tend to limit natural hybridization between formae speciales of *P. graminis*. One is sterility between forms that are not closely related and the other is reduced fitness of the hybrids.

GENOME CHARACTERISTICS

From DNA reassociation kinetics, Backlund and Szabo (1993) determined the size of the *P. graminis* f. sp. *tritici* genome to be 67 Mbp, with approximately 64% unique (single copy) sequences, 30% repetitive sequences and 4% foldback sequences. The mitochondrial genome, which is approximately 80.2 kb (Sock *et al.*, 1994), is included in this total. The repetitive sequences have about 390 kb total complexity with an average of 52 copies. Backlund and Szabo (1993) estimated that the ribosomal repeat accounts for about 5% of the repetitive fraction. The copy number of the mitochondrial DNA was 19–29 per germinating urediniospore (Sock *et al.*, 1994). The G+C base composition of the total DNA was 45.3%.

Table 1 Chromosome numbers and estimated genome size in selected rust species.

Rust species	Host	Genome size* (mb)	Chromosome number†
Puccinia graminis	*Triticum aestivum*	67	18‡
P. coronata	*Avena sativa*	78	17§
P. triticina	*T. aestivum*	89	16–18¶
P. sorghi	*Zea mays*	101	nd**
P. hordei	*Hordeum vulgare*	122	nd
P. recondita	*Secale cereale*	128	nd
P. recondita	*Aegilops variabilis*	154	18–20¶
Melampsora lini	*Linum usitatissimum*	170	18††
Uromyces vignae	*Vigna unguiculata*	402	nd
U. appendiculata	*Phaseolus vulgaris*	414	nd

*Genome size of *P. graminis* was determined by reassociation kinetics (Backlund and Szabo, 1991). Genome sizes of other species relative to *P. graminis* were determined by fluorescence values of DNA relative to that of *P. graminis* for pycniospores measured by flow cytometry (Eilam *et al.*, 1994).
†Haploid chromosome numbers determined by reconstruction of serially sectioned pachytene nuclei, e.g. Boehm *et al.* (1992).
‡Boehm *et al.* (1992).
§Boehm (1992) and D.E. Krueger *et al.* (unpublished data).
¶Preliminary results (D.E. Krueger and W. R. Bushnell, unpublished data).
**nd = not determined.
††Boehm and Bushnell (1992).

Relative DNA contents of nuclei of other rust fungi have been determined from fluorescence intensity of propidium-iodide-stained nuclei both by microphotometry (Eilam *et al.*, 1992) and by flow cytometry (Eilam *et al.*, 1994). The genome size of *P. graminis* was among the smallest of six cereal and grass rust species tested, which all were much smaller than the genomes of common bean rust (*Uromyces appendiculata*) and cowpea rust (*U. vignae*) (Table 1).

Reconstructed images from transmission electron microscopy of serial sections through meiotic nuclei at first pachytene in teliospores revealed that the haploid chromosome number for *P. graminis* is 18, the same as that of *Melampsora lini* (Boehm and Bushnell, 1992; Boehm *et al.*, 1992). Preliminary information indicates that the cereal leaf rust fungi, *P. coronata*, *P. triticina* and *P. recondita*, have in the range of 16–20 chromosomes (Boehm, 1992; W.R. Bushnell, personal communication). These limited data do not show a strong connection between haploid chromosome number and genome size in the rust fungi (Table 1).

CLASSICAL GENETICS OF *P. GRAMINIS*

Three features of *P. graminis* f. sp. *tritici* have made routine genetic studies difficult. First, most of the commonly found genotypes of the wheat stem rust fungus have adapted to survival in the uredinial stage in the absence of barberry, the alternative host. In making this adaptation, they have largely lost their ability

to produce teliospores, which are essential for meiosis. Second, even when teliospores are produced, they must be induced to break dormancy before the sexual stage of the fungus can be completed. The first difficulty is best overcome by selecting isolates for study that come from regions of the world where *P. graminis* f. sp. *tritici* is still associated with barberry and dependent on it for overwinter survival (Zambino *et al*., 2000). Teliospore dormancy can be broken by simply leaving telia outside during the winter in cold climates and bringing them into the laboratory before the spring rains begin. Other methods include cycles of alternate freezing and thawing or alternate spraying or soaking in cold water and drying until germinating teliospores are seen. Zambino *et al*. (2000) combined alternate freezing and thawing of teliospores as well as wetting and drying. Once dormancy has been broken, teliospores can be stored frozen for years without loss of germination (Roelfs and Groth, 1988). The third difficulty in classical genetic work with *P. graminis* is that crosses can be made only on susceptible barberry leaves. This means that the barberry plants to be used as hosts for crossing must be manipulated to produce a flush of new leaves at just the time that germinating teliospores are available.

Early research by Johnson and Newton (1946) and Loegering and Powers (1962) established that virulence/avirulence in the wheat and oat stem rust fungi is inherited in Mendelian ratios and fit the gene-for-gene relationship between host resistance and pathogen avirulence proposed by Flor (1971) for flax rust. However, in two cases, avirulence in *P. gramins* f. sp. *tritici* to 'Marquis' wheat and avirulence in *P. graminis* f. sp. *avenae* to 'Sevonthree' oat, Johnson and Newton (1946) found maternal inheritance of avirulence. That is, avirulence of all progeny reflected that of the parent fertilized by pycnispores and not the pycniospore donor parent. Sock *et al*. (1994) were unsuccessful in attempts to identify the gene for avirulence to 'Sevonthree' oat in mitochondrial DNA from *P. graminis* f. sp. *avenae*.

Johnson and Newton (1946) determined that two genes control urediniospore colour in *P. graminis*. Urediniospores normally are brick red, but loss of the dominant gene for spore wall pigment results in orange urediniospores due to substantial quantities of β-carotene, γ-carotene and lycopene in the cytoplasm (Bush, 1967). Loss of the dominant gene for the orange carotenoid pigments of the cytoplasm results in grey–brown urediniospores due to the presence of melanin or a melanin-like substance in the spore walls (J.E. Backlund and L.J.S., unpublished observations). Bush (1967) found that urediniospores of the grey–brown mutant contained unusually high quantities of phytoene, a colourless precursor in the carotenoid biosynthetic pathway, which suggests the loss of a phytoene-specific enzyme in the mutant.

Johnson and Newton (1946) also identified a culture of *P. graminis* f. sp. *tritici* in which loss of ability to produce aecia segregated as a single gene. The mutant culture could function as female parent in crosses with wild-type isolates, but fertilized pycnia formed small numbers of urediniospores and teliospores and did not develop aecia. A similar phenomenon was observed in F_2 plants from an f. sp. *tritici* × f. sp. *avenae* cross (Johnson, 1949), perhaps indicating loss of part of a chromosome in the hybrid due to incomplete pairing of homologous chromosomes. By selfing more than 42 cultures of *P. graminis* f. sp. *tritici* cultures, Johnson (1954) showed that heterozygosity for virulence and avirulence was common in field isolates tested on ten differential lines of wheat. Johnson and Newton (1946) also observed that repeated selfing of *P. graminis* cultures often led to decreased sporulation, loss of pathogenic vigour and greater sensitivity to loss of virulence at high temperatures.

From the mid-1960s there was little or no additional genetic research on *P. graminis* until Zambino *et al*. (2000) published a detailed analysis of segregation for avirulence to ten wheat differential lines with known genes for stem rust resistance. They reported evidence of eight single dominant genes, *AvrT6*, *AvrT8a*, *AvrT9a*, *AvrT10*, *AvrT21*, *AvrT28*, *AvrT30* and *AvrTU* for avirulence on differential lines with resistance genes *Sr6*, *Sr8a*, *Sr9a*, *Sr10*, *Sr21*, *Sr28*, *Sr30* and *SrU*, respectively (*T* is used to indicate avirulence specifically to resistance genes in cultivated *Triticum*, because other genes for race-specific resistance to *P. graminis* f. sp. *tritici* also exist in related cereal or grass species). Avirulence to wheat line 8N221Sr; RHR with the resistance gene provisionally designated *Sr*; was inherited in a 15 : 1 ratio, indicating two dominant genes for avirulence with epistatic interaction. Avirulence to the line with *Sr9d* segregated in a ratio more similar to 3 : 13 than to 1 : 3, which may indicate segregation at two loci: one for dominant avirulence and one for dominant inhibition of avirulence. In an earlier cross, Green (1964) found a ratio of ten avirulent to 16 virulent F_2 progeny on the durum cultivars 'Arnautka' and 'Mindum', which have *Sr9d*. In Green's F_2 population of *P. graminis* f. sp. *tritici*, avirulence to *Sr9d* appeared to be linked to grey–brown colour of the urediniospores.

Zambino *et al*. (2000) also analysed segregation for 970 RAPD and AFLP markers in their cross, and found DNA markers linked to each of the eight single avirulence loci. Two of the eight single avirulence loci, *AvrT10* and *AvrTU*, are linked (9 cM). Based on the 970 DNA markers, 56 linkage groups were found in the partial map for *P. graminis*, indicating that many, if not all, of the 18 chromosomes identified in TEM reconstructions (Boehm *et al*., 1992) are still incompletely mapped. Developing a complete genetic map with dominant markers such as RAPDs and AFLPs is difficult because *P. graminis* is dikaryotic and because teliospore dormancy makes backcrossing or selfing *P. graminis* cultures a tedious process.

GENETICS OF HOST–PATHOGEN RELATIONS

More than 40 distinct genes for resistance to various races of wheat stem rust are known. For each resistance gene there is a

matching avirulence gene in the pathogen that is required for recognition by the host and initiation of the resistant response. Stem rust resistance genes in wheat are officially designated when the chromosomal arm on which they are located has been determined and when it has been established that the new gene is distinct from other known stem rust resistance genes on that chromosomal arm. Among the 46 officially designated stem rust resistance genes (McIntosh *et al*., 1995), only 20 were found originally in hexaploid bread wheat (*Triticum aestivum*); nine were transferred to bread wheat from tetraploid *T. turgicum* (either wild or cultivated durum wheat), three were from diploid *T. monococcum*, two were from cultivated rye, and 11 other stem rust resistance genes were transferred to bread wheat from various wild relatives (*Triticum comosum*, *T. speltoides*, *T. tauschii*, *T. timopheevii*, *T. ventricosum* and *Thinopyrum ponticum*). In addition, 24 other presumably distinct resistance genes have received provisional designations pending further characterization; 11 of these genes were found in bread wheat, one is from durum and two are from *Thinopyrum* species. Except for *Sr2*, which has long provided stable partial resistance in the adult plant stage over widespread use in cultivars grown throughout the world (McIntosh *et al*., 1995), the officially and provisionally designated stem rust resistance genes are known or suspected to be race-specific. Virulence has not been confirmed for *Sr26*, *Sr32*, *Sr33*, *Sr37* or *Sr39*, but these genes have not been tested extensively (McIntosh *et al*., 1995).

Generally, when two or more genes for race-specific resistance are effective against a race of *P. graminis* f. sp. *tritici* the gene conditioning the highest level of resistance is epistatic to the gene(s) conditioning lower levels of resistance (Roelfs, 1988). For example, avirulent races of *P. graminis* f. sp. *tritici* induce only a small chlorotic fleck at infection sites on wheat plants homozygous for resistance gene *Sr6*, whereas avirulent races develop small uredinia with limited sporulation and surrounded by a chlorotic halo at infection sites on wheat plants homozygous for resistance gene *Sr8a*. On plants with both *Sr6* and *Sr8a* a race with matching avirulence genes to both will induce only the flecks typical of the resistance of *Sr6*. With several resistance genes in durum wheat (*Triticum turgidum*), however, additive interactions have been observed. Individually, these resistance genes typically condition resistance characterized by small uredinia with limited sporulation when each is matched by its corresponding avirulence gene in the pathogen. Combinations of two or more of these resistance genes (with matching avirulence genes in the pathogen) result in a higher level of resistance than is seen with either resistance gene singly. That is, the uredinia are smaller and sporulation is reduced more or there may be no sporulation at all when both resistance genes are present and matched by the corresponding avirulence gene in the pathogen (Roelfs, 1988).

Both resistance in wheat and avirulence in *P. graminis* f. sp. *tritici* are often incompletely dominant (Roelfs, 1988; Roelfs and Groth, 1988). Wheat is a self-pollinated crop, so commercially grown cultivars are highly homozygous. Thus, the incomplete dominance of resistance has little or no practical significance. *Puccinia graminis* f. sp. *tritici*, by contrast, reproduces asexually in most regions of the world where wheat stem rust is a problem disease, and most clones of the pathogen are highly heterozygous. This often results in two levels of resistance being expressed on wheat cultivars with effective *Sr* genes: a high level of resistance against clones of *P. graminis* f. sp. *tritici* homozygous for the corresponding avirulence gene and an intermediate level of resistance against clones heterozygous for the corresponding avirulence gene (Roelfs and Groth, 1988; Roelfs, 1988).

Expression of many of the *Sr* genes in wheat is temperature sensitive, usually with greater resistance being expressed at low temperatures and a tendency for the resistance to break down at high temperatures. For example, wheat plants with *Sr6* develop only small chlorotic flecks when infected with an avirulent race of *P. graminis* f. sp. *tritici* at 15 °C, but plants with *Sr6* develop large, nearly fully susceptible type uredinia when kept at temperatures above 24 °C after inoculation with the same avirulent race. The gene *Sr15* is the most temperature sensitive; reactions can change from resistant at 18 °C to nearly fully susceptible at 20 °C (Roelfs, 1988). Resistance often shows greater temperature sensitivity when challenged by a *P. graminis* f. sp. *tritici* clone that is heterozygous for the corresponding avirulence gene rather than by one that is homozygous avirulent.

RACE-SPECIFIC RESISTANCE

Levels of race-specific resistance in wheat stem rust vary for different resistance gene/avirulence gene combinations. In the type 0 reaction (Fig. 2D), often referred to as immunity, no macroscopically visible symptoms are seen at infection sites. For example, plants with the resistance gene *Sr5* limit the growth of avirulent races of *P. graminis* f. sp. *tritici* so quickly that only one or two haustorium mother cells are produced per fungus colony and there is no further fungal growth beyond 24 h after development of the appressorium. This inhibition is present even before necrosis occurs in the one or two cells where attempted haustorial penetration occurred. It is significant that the resistance of *Sr5* is expressed in both mesophyll and epidermal cells of wheat leaves (Rohringer and Heitefuss, 1984), because more than 30% of the first haustoria (Skipp and Samborski, 1974), and possibly as many as 95% of first haustoria (Tiburzy *et al*., 1990), are formed in epidermal cells of wheat leaves rather than in the mesophyll.

Plants with *Sr22* allow greater development of avirulent races of *P. graminis* f. sp. *tritici*. In the type 2 reaction typical of *Sr22* (Fig. 2D) a small uredinium forms that is surrounded by a halo of chlorotic tissue in the host leaf. Sporulation is greater than in type 1 uredinia but much less than in fully susceptible reactions. The

resistant response associated with *Sr22* is delayed, although it is not known whether this is due to delayed expression of *Sr22* or delayed expression of the corresponding avirulence gene. Few necrotic host cells are seen until 72 h after inoculation, and reduction in growth rate of the fungal colonies does not occur until 24 h later (Rohringer and Heitefuss, 1984). This indicates that before host cells penetrated by haustoria collapse, the pathogen is able to obtain sufficient nutrition from them to maintain limited growth and to support some sporulation. It also indicates that antifungal compounds produced in the host defence responses to the infection are not released quickly enough or in sufficient quantity to suppress further growth of the fungus completely. Bushnell (1970) showed that colonies of *P. graminis* f. sp. *tritici* in type 2 reactions in leaves of wheat seedlings continue to grow slowly until the leaves begin to senesce.

Although none of the 40+ genes for stem rust resistance in wheat has been cloned yet, a gene *Rpg1* for stem rust resistance in barley was cloned recently. The *Rpg1* gene, which provided stable resistance in barley to the wheat stem rust fungus *P. graminis* f. sp. *tritici* for 50 years, was mapped to the short arm of barley chromosome 1 (Jin *et al.*, 1993; Kilian *et al.*, 1994), and was recently cloned (Brueggeman *et al.*, 2002). The encoded protein is unique among products of plant resistance genes in that it contains a receptor kinase-like protein with two tandem protein kinase domains. The identity of *Rpg1* was confirmed by genetic transformation, which converted the highly susceptible barley cultivar Golden Promise into a highly resistant transformant (Horvath *et al.*, 2003). The structure of the *Rpg1* protein is similar to that of the protein product of the *Pto* gene in tomato for resistance to a bacterial disease (*Pseudomonas syringae* pv. *tomato*) in that it encodes a cytoplasmically located serine/threonine kinase. This is in contrast to the other characterized rust resistance genes, including *Lr10* and *Lr21* for leaf rust resistance in wheat (Feuillet *et al.*, 2003; Huang *et al.*, 2003), *Rp1* and *Rp3* for common rust resistance in corn (Collins *et al.*, 1999; Webb *et al.*, 2002), and *L6*, *M*, *N* and *P* for rust resistance in flax (Anderson *et al.*, 1997; Dodds *et al.*, 2001a,b; Lawrence *et al.*, 1995). All of these rust resistance genes are members of the NBS-LRR class of plant disease resistance genes. Like *Rpg1* in barley, the protein products of these rust resistance genes appear to be located in the cytoplasm, indicating that the interaction between the resistance protein complex and the fungal elicitor (avirulence gene product) occurs inside the host cell.

HOST–PARASITE SPECIFICITY

The hypersensitive response is doubly effective against biotrophs such as rust fungi because of the accompanying up-regulation of a multitude of defence genes (Bohland *et al.*, 1997; Heath, 1997; Li *et al.*, 2001; Lin *et al.*, 1998; Münch-Garthoff *et al.*, 1997) as well as the loss of an essential nutritional base when host cells bearing fungal haustoria collapse and die. Thus, the hypersensitive response to pathogen invasion is one of the most important natural defences that *P. graminis* must overcome to establish a successful parasitic relationship. Germ tube walls of *P. graminis* f. sp. *tritici* contain a glycoprotein (molecular mass = 67 000) that induces a hypersensitive lignification response when applied to healthy wheat leaves of both resistant and susceptible genotypes. A similar or identical elicitor was found in apoplastic fluids of stem rust-infected leaves (Beissmann *et al.*, 1992). Therefore, it appears that for a successful parasitic relationship to be established, it is essential that the rust fungus must also produce an endogeneous suppressor of the disease resistance elicitation (Beissmann *et al.*, 1992; Heath, 1997; Wiethölter *et al.*, 2003a). Little is known of the suppressor(s) of the general elicitation, and even less is known of the specific molecular interactions that determine race-specificity in the gene-for-gene relationship between wheat and *P. graminis* f. sp. *tritici*. As Heath (1997) pointed out, the most interesting interactions between host plant and rust fungus may be those that keep the invaded host cells alive and, thus, condition susceptibility of the plant to the rust fungus. Yet we know almost nothing about these interactions, although data suggest that rust haustoria play a key role in regulating the host–parasite interaction by suppressing plant defence responses in compatible combinations (Mendgen *et al.*, 2000).

GENETIC TRANSFORMATION

Recently, transient transformation of *P. graminis* f. sp. *tritici* was achieved by particle bombardment of urediniospores during germination on agar (Schillberg *et al.*, 2000). In the transformation, the promoter from *P. graminis* f. sp. *tritici* translation elongation factor 1α gene (*EF-1*α) was used to drive the bacterial marker genes hygromycin B phosphotransferase (*hpt*) and β-glucuronidase (*GUS*) to confirm the activity of inserted genes. Suitable markers and procedures for stable transformation of rust mycelia in uredinia within host tissue have yet to be developed. Additional genes from *P. graminis* f. sp. *tritici* have been isolated as candidates for promoters that may be useful in genetic transformation. These include the gene *Hss1* for the 70-kDa heat shock protein (L.J.S. and R. J. Staples, unpublished data) and a gene (*uam*) of unknown function, which is highly expressed in hyphae of *P. graminis* f. sp. *tritici* (Liu *et al.*, 1993). Promoters from *Hss1* and the actin gene of *P. graminis* showed stronger activity than the *EF-1*α promoter in transient expression assays (C.A. Webb *et al.*, unpublished).

The greatest impediment to development of a stable transformation system for *P. graminis* is the lack of a suitable selective marker for use *in vivo* in infected wheat leaves. Attempts to use current antibiotic resistance and colour selection have not been successful. A marker that could knock out (homologous recombination) or inhibit the expression of avirulence would be ideal for

this purpose, especially if the avirulent infection type is 0 or 0;. The recent success of Dodds *et al*. (2004) in cloning avirulence genes from flax rust, *Melampsoara lini*, has brought this prospect much closer. The *AvrL*567 genes of *M. lini* are expressed in the haustoria and code for secreted proteins, implying that they are delivered across the haustorial and host cell membranes. When transformed into flax plants, *AvrL*567-A and *AvrL*567-B induced hypersensitive resistance-like necrosis when the corresponding resistance genes were present. An important goal of current mapping (Zambino *et al*., 2000) and genome sequencing (L.J.S. *et al.* unpublished data) is cloning of avirulence genes and avirulence inhibitor genes for possible use in developing an efficient selective marker for *P. graminis* transformation.

CONCLUSIONS AND PROSPECTS

Because of its destructiveness and the economic importance of its cereal hosts, *Puccinia graminis* is one of the most widely studied of all plant pathogens. Thousands of papers have been published on the ecology, epidemiology and host–parasite interactions of wheat stem rust from research conducted throughout the world. Much remains to be learned, however, about the specific mechanisms by which this fungus is able to cause disease. The complex, developmentally regulated infection process of *P. graminis* includes eight distinct structures. We know that leaf surface topography and carbon dioxide concentration stimulate production of the early infection structures, but are there other signals (both plant and fungal) that are involved? What regulates the development of a specialized feeding structure (haustorium) in the periplasmic space of the host? How is the obligate–parasitic relationship established? How does the virulent pathogen elude or suppress the host defence system? What are the elicitors (avirulence gene products) that trigger the specific host resistance response in the gene-for-gene relationship between resistance and avirulence? Does recognition of these elicitors occur within the host cell as predicted by the resistance gene structure? What genes and what signals control the cessation of spread of the pathogen by asexual reproduction and the shift to production of teliospores and initiation of sexual reproduction? Does *P. graminis* have a simple bipolar mating system as crossing frequency data suggest or is it more complex?

The development of an annotated genome sequence for *P. graminis* within the next few years will provide a wealth of information and we hope lead to a renaissance in the study of *P. graminis*. Successful exploitation of this sequence information will require developing or adapting methods for functional analysis, more expeditious genetic routines and stable transformation designed specifically for *P. graminis*, as well as multifaceted research programmes combining biochemistry, histology, genetics and genomics to explore the remaining unanswered questions about this important pathogen.

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