Rust Basidiospore Germlings and Disease Initiation

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1. INTRODUCTION

1.1. General

The rust fungi are potentially dangerous plant disease organisms. Infections caused by dikaryotic urediniospores play a major role in limiting agricultural production. Consequently, research has focused on infections of this type and has been the central theme of numerous reviews (Littlefield and Heath, 1979; Bushnell and Roelfs, 1984; Mendgen *et al.*, 1988; Hoch and Staples, this volume). Research on basidiospores and basidiospore-derived infections has received significantly less attention. This may be partially due to their lesser economic importance, but is also a result of the lack of reliable methods to activate teliospore germination, and therefore basidiospore production, of agriculturally important rusts (Petersen, 1974; Mendgen, 1984).

This chapter builds upon existing brief reviews of basidiospore germination (Petersen, 1974; Mendgen, 1984), host penetration (Littlefield and Heath, 1979), and the morphology of intracellular structures (Harder and Chong, 1984). Our approach relies on a comprehensive illustration of the events and structures important in the initiation of basidiospore-derived infections.

1.2. Terminology

The terminology suggested by Littlefield and Heath (1979) will be followed with the exception that intracellular infection structures, often designated as monokaryotic haustoria, will be called intracellular hyphae (Rijkenberg and Truter, 1972; Gold and Mendgen, 1984a). The extracellular, slimelike substance commonly associated with infection structures will be termed the extracellular matrix (see Gold and Mendgen, 1984b; Mims and Richardson, 1989; Nicholson and Epstein, this volume).

2. BASIDIOSPORE MORPHOLOGY AND GERMINATION

Knowledge of the morphology and germination of the rust basidiospores dates back to Gasparrini (1848) and the early accounts by Tulasne (1854), de Bary (1863, 1866), Reess (1865, 1870), and Sappin-Trouffy (1896). Basidiospores, also called sporidia in the earlier literature, are the only spore type in the rust life cycle that does not form after a preliminary infection event. Instead, they generally arise after the germination of diploid teliospores or, less commonly, in endocyclic species, after germination of aecoid teliospores (Petersen, 1974). The metabasidium generally gives rise to four, sometimes two, basidiospores on sterigmata (Figs. 1–3).

2.1. Morphology

2.1.1. Macroscopic

Masses of fresh basidiospores range from yellow to red (Reed and Crabill, 1915; Snow and Froelich, 1968) due to the presence of pigmented lipid droplets that may protect against UV radiation. Hyaline or white basidiospores have also been reported (Diner and Mott, 1982a; Gold and Mendgen, 1984c).

2.1.2. Microscopic

Mature basidiospores vary considerably in morphology, even within a single species. However, reniform (e.g., Fig. 4), round (e.g., Fig. 10), or ovate-elliptical (e.g., Fig. 2) are

Figure 1. Metabasidium (B) of *Uromyces appendiculatus*. Basidiospores (Bs) are borne at the tip of the sterigmata (arrow). (750 $\times$.)

Figure 2. Light (a) and fluorescence (b) micrographs of fixed, DAPI-stained basidiospores of *Uromyces appendiculatus* containing one or two nuclei (N) (620 $\times$). (From Gold and Mendgen, 1984c.)

Figure 3. Germinated teliospore (T) of *Gymnosporangium fuscum* with a septate metabasidium (B) and basidiospores (Bs). The labeled basidiospore is still attached to the sterigma. (1400 $\times$.) (Courtesy of B. Metzler, unpublished.)

Figure 4. Longitudinal section through a basidiospore of *Gymnosporangium juniperi-virginianae* prepared for TEM by freeze substitution. Nuclei (N), lipid bodies (L), mitochondria (Mi), microbodies (mb), and vacuoles (V) are shown. (12,070 $\times$.) (From Mims *et al.*, 1988.)

common shapes. Sizes range from $3 \times 4 \mu\text{m}$ (e.g., *Cronartium flaccidum*) to $14 \times 25 \mu\text{m}$ (e.g., *Coleosporium tussilaginis*). All basidiospores that have been examined by conventional SEM are smooth or slightly wrinkled (e.g., Fig. 25) and bear a prominent apiculus (Fig. 26).

When examined by TEM, the spore wall appears thin ($0.05\text{--}0.25 \mu\text{m}$) in transverse section and consists of a single or a double layer. In the hilar region a thin, electron-dense outer layer is more pronounced in several species (Metzler, 1982; Bauer and Oberwinkler, 1988; Mims, 1977; Mims *et al.*, 1988). Preformed germ pores are not present. Basidiospores have a densely packed protoplasm and typically contain two nuclei, microtubules, and endoplasmic reticulum (ER) (Fig. 4). Filasomes and tubular-vesicular complexes (TVC) were detected in germinating basidiospores preserved by ultrarapid freezing and freeze substitution protocols (Mims and Richardson, 1989). TVC seem to include the Golgi apparatus of the cell (Welter *et al.*, 1988) and were tentatively distinguished as TVC 1 and TVC 2 (Figs. 5, 6). TVC 1 may be derived from the ER and has only been found in fungal structures growing within the leaf. TVC 1 seems to be involved in secretion (Knauf and Mendgen, 1988). TVC 2 resembles single cisternae of the dictyosomes. An obvious spatial relationship between vacuoles, lipid bodies, and microbodies interconnected by strands of ER has been observed (Fig. 4) (Gold and Mendgen, 1984a; Mims *et al.*, 1988). Such pronounced organization is presumably related to the metabolic activities of germination, particularly the utilization of lipid reserves (Mendgen, 1973).

2.2. Nuclear Condition

Basidiospores typically contain two haploid, homokaryotic nuclei at maturity. During teliospore germination the diploid nucleus undergoes meiosis, followed by a mitosis, usually after each daughter nucleus has migrated into a basidiospore initial (Fig. 2) (Gold and Mendgen, 1984c). Binucleate basidiospores were first observed by Poirault and Raciborski (1895) and Sappin-Trouffy (1896), a condition confirmed by subsequent reports (Hoffman, 1912; Ashworth,

Figures 5 and 6. Tubular-vesicular complexes (TVC) in intercellular hyphae of basidiospore-derived infections of *Uromyces appendiculatus*. **Figure 5.** TVC 1 may be part of the endoplasmic reticulum. ($35,000\times$.) **Figure 6.** TVC 2 appears similar to dictyosomes. ($60,000\times$.) (Courtesy of L. Brusciaglioni, unpublished.)

1935; Savile, 1939; Thirumalachar, 1939; Kapooria, 1971; Gopinathan Nair, 1972; Bauer, 1986; Mims *et al.*, 1988; see Allen, 1933; Petersen, 1974; Anikster, 1983; Gold and Mendgen, 1984c, for additional references). Other studies have described species with simplified two- or three-celled metabasidia (Petersen, 1974) and have also revealed unique alternative development patterns, including binucleate, heterokaryotic (self-fertile) (Lindfors, 1924; Thirumalachar, 1945; Anikster and Wahl, 1985) and quadranucleate (Olive, 1949; Kapooria, 1968; Kohno *et al.*, 1975) basidiospores. Anikster and co-workers (1980) and Petersen (1974) have summarized the numerous variations and consequences of functionally heterokaryotic basidiospores. Recent quantitative studies (Kapooria and Zadoks, 1973; Hansen and Patton, 1975; Anikster, 1983; Gold and Mendgen, 1984c) and numerous descriptive or semiquantitative reports confirm that binucleate spores are clearly the predominant form.

2.3. Germination

After being deposited on their host, basidiospores can germinate within a few hours. Germination may follow two characteristic forms (Fig. 7): either directly via germ tubes (Fig. 8) or indirectly via formation of secondary spores (Fig. 9). The pattern followed is dependent on environmental conditions and the host surface (Ragazzi and Dellavalle Fedi, 1982; Desprez-Loustau, 1986).

2.3.1. Influence of Water

Of the basic environmental factors influencing spore germination, water, in the form of humidity and surface moisture, appears to be the most crucial. High humidity is generally needed (Cotter, 1932; Hansen and Patton, 1975); however, the conditions required are difficult to generalize. For example, basidiospores of *Cronartium ribicola* (Hansen and Patton, 1975), *Puccinia horiana* (Kapooria and Zadoks, 1973), and *Puccinia malvacearum* (Ashworth, 1931) all germinate well on both water agar and dry glass slides enclosed in a moist chamber. In contrast, basidiospores of *Melampsora pinitorqua* on 1.5% water agar germinate equally well at humidities

Figure 7. Diagrammatic illustration of basidiospore germination and development on various substrata. Ap, appressorium; Bs, basidiospore; G, germ tube; IV, intraepidermal vesicle; P, primary hypha; S, septum. (Modified after Bauer, 1986.)

Figure 8. Basidiospore germlings of *Uromyces appendiculatus* on 2% water agar. Note long, thin, and wavy germ tubes (G). (290 $\times$.) (From Freytag *et al.*, 1988.)

Figure 9. Secondary basidiospore (2°Bs) of *Coleosporium tussilaginis* arises from the basidiospore (Bs) in a water droplet on 0.2% water agar. (540 $\times$.) (From Bauer, 1986.)

ranging from 35 to 100%, but fail to germinate under any humidity conditions when incubated on glass slides (Desprez-Loustau, 1986). Differing sensitivities to surface properties of glass and/or differences in spore water content may explain these results.

Basidiospores are extremely sensitive to desiccation. When exposed to unfavorable conditions, such as low humidity or direct sunlight, they quickly lose viability (Spaulding and Rathbun-Gravatt, 1926; MacLachlan, 1935; Blank and Leathers, 1963; Gould and Shaw, 1969; Snow, 1968a). Excessive free water is also generally inhibitory to germination (Desprez-Loustau, 1986) or leads to the formation of secondary basidiospores (Figs. 7, 9) (Hansen and Patton, 1975; Bauer, 1986; Mims and Richardson, 1989). A basidiospore can produce up to six successive secondary spores (Petersen, 1974; Bauer, 1986), all equally pathogenic (Roncadori, 1968).

It has long been proposed that secondary basidiospore formation is a survival mechanism for spores exposed to improper environmental conditions (Reess, 1870; Weimer, 1917; Hansen and Patton, 1975) or nonhost substrata (Bega, 1960; Van Sickle, 1975). Bauer (1986, 1987) reported that unfavorable positioning on the host, such as adhesion to leaf hairs, can also signal the formation of secondary spores, and demonstrated that one of the nuclei degenerates during this process (Fig. 10).

2.3.2. Influence of Temperature

Many *in vitro* studies have demonstrated that basidiospores can germinate over a wide temperature range. For example, Hansen and Patton (1975) germinated basidiospores of *Cronartium ribicola* between 5 and 24°C, with a maximum observed between 12 and 15°C. With the same species, Bega (1960) found germination on both water agar and host needles between 1 and 28°C, but with broad maxima between 5 and 24°C and 13 and 20°C, respectively. A similarly broad maximum between 8 and 20°C has been determined *in vitro* for basidiospores of *M. pinitorqua*, a species that may germinate and grow between 2 and 30°C (Desprez-Loustau, 1986). These results and others (Reed and Crabill, 1915; MacLachlan, 1935; Siggers, 1947; Yamada, 1956; Van Sickle, 1975; Ragazzi *et al.*, 1986) show that basidiospores have an enormous germination potential. Thermal death has been determined between 30 and 32°C for a few species (Reed and Crabill, 1915; MacLachlan, 1935; Desprez-Loustau, 1986).

Figure 10. Basidiospore of *Coleosporium asclepiadeum* with intact nucleus (N) and degenerated remnant nucleus (RN) prior to secondary spore formation. A, apiculus; L, lipid body; Mi, mitochondrion; mb, microbody. (11,500 $\times$) (From Bauer and Oberwinkler, 1988.)

2.3.3. Influence of Light

In order to avoid the effects of UV radiation and desiccation by direct sunlight, most basidiospores are formed and released rhythmically at night or early morning (see Gold and Mendgen, 1983). Darkness, however, is not necessary for germination. When other conditions are favorable, light has either no effect (Yamada, 1956; Bega, 1960; Diner and Mott, 1982a; Desprez-Loustau, 1986) or only a marginal negative effect (Gould and Shaw, 1969).

2.3.4. Influence of Host Substances

Fresh leaves of sagebrush inhibit the germination of *Cronartium comandrae* completely (Krebill, 1972). Leaf fragments with abundant glandular hairs (Robinson, 1914) or water-soluble exudates from *Pinus pinaster* inhibit germination in *Puccinia malvacearum* and *M. pinitorqua* (Desprez-Loustau, 1986), respectively. The inhibitory effect on *M. pinitorqua* is stronger with exudates from disease-resistant species. Water-insoluble monoterpenic compounds from epicuticular waxes are also inhibitory, although there are no differences between resistant and susceptible host species.

2.3.5. Influence of Massing

Massing of basidiospores has been reported to inhibit germination (Hansen and Patton, 1975). Spaulding and Rathbun-Gravatt (1926) attributed the effect to insufficient oxygen or secretion of toxic substances. Self-inhibitors of basidiospore germination *per se* have not been reported.

3. BASIDIOSPORE GERMLINGS IN VITRO

Physiological studies of germ tube development have been limited by difficulties in obtaining sufficient spore material. Furthermore, the limited life span of basidiospores and their restricted growth pattern in axenic culture have also hindered such research.

3.1. Germ Tube Differentiation

3.1.1. Influence of Environmental Conditions

C. ribicola germ tubes grow best at 12–15°C; however, most intraepidermal vesicles are formed between 19 and 26°C. Incubation at 16°C followed by treatment at 24 or 28°C for 1–2 hr increases the incidence of vesicle formation considerably (Hansen and Patton, 1975), indicating that basidiospore germ tubes can react to temperature shocks as do those of urediniospores (see Hoch and Staples, this volume).

Robinson (1914) studied the tropic effect of light on germ tube growth in *Puccinia malvacearum*. Freshly cast spores were illuminated unilaterally with incident light from a window. In this simple experiment the germ tubes emerged predominantly from the shadowed side of the spores, and when these were subsequently rotated through 90°, exhibited a marked negative phototropic response.

3.1.2. Influence of Artificial Substrata

The induction of infection structures *in vitro* depends greatly on the hardness of the substratum. For example, in water or on 0.2–0.7% water agar, spores of *Gymnosporangium clavariiformae* germinate with a broad germ tube and subsequently form a secondary spore (Fig. 9) (Bauer, 1987). On 1–2% water agar, a thin, unbranched, and undifferentiated nonseptate germ tube is formed (Fig. 8). Agar concentrations around 4–5% induce the formation of short germ tubes with appressoria (Bauer, 1986; Freytag *et al.*, 1988). With *Uromyces viciae-fabae*, *U. vignae*, and *U. appendiculatus*, infection structure development may proceed as far as a vesicle (Fig. 11) or a primary hypha (Freytag *et al.*, 1988).

Artificial membranes on agar, such as nitrocellulose (Figs. 12, 19), collodion (Fig. 13), polyethylene (Fig. 15), and dialysis tubing (Figs. 16, 17), can induce infection structures from basidiospores (Hansen and Patton, 1975; Freytag *et al.*, 1988; Mims and Richardson, 1989). The hard cuticular surface of barley coleoptiles (R. E. Gold, unpublished) and even glass (Fig. 14) (Bauer, 1986; Freytag *et al.*, 1988) induce the formation of appressoria, suggesting that surface recognition in the haploid stage is not very specific. In all these cases the surface hardness and the amount of water available determine whether (1) a germ tube forms, (2) a secondary spore is produced, or (3) germ tube growth continues to produce normal infection structures (see Fig. 7).

Isolated cuticles of *Vicia faba*, *Vigna sinensis*, and *Phaseolus vulgaris* induce the formation of infection structures of *U. viciae-fabae*, *U. vignae*, and *U. appendiculatus*, respectively (Freytag *et al.*, 1988). Cross-inoculations show this response to be nonhost specific (S. Freytag and L. Bruscaaglioni, unpublished). On leaf cuticles mounted on 2% agar, basidiospores germinate, form short germ tubes, differentiate appressoria and intraepidermal vesicles either on the surface of the cuticle (Fig. 18) or below the cuticle in the agar (S. Freytag, unpublished). Under natural conditions, such vesicles develop within host epidermal cells (Figs. 22, 23a).

3.2. Surface Carbohydrates

In vitro, basidiospore germ tubes may be copiously covered with an extracellular matrix (Figs. 16, 17) (Mims and Richardson, 1989). Basidiospores and germ tubes of *U. viciae-fabae*

Figure 11. Basidiospore germlings of *Uromyces vignae* on 5% water agar. Appressoria (Ap) of the basidiospores (Bs) are visible on the agar surface. Vesicles (IV, not in focus) form within the agar. (640 $\times$.) (From Freytag *et al.*, 1988.)

Figure 12. Germinated basidiospore (Bs) of *Uromyces vignae* on nitrocellulose membrane filter showing vesicle (IV). (640 $\times$.) (From Freytag *et al.*, 1988.)

Figure 13. Basidiospore germling of *Cronartium ribicola* on collodion membrane with four germ tubes (G) and pyriform vesicle (SV). Septa (S) are visible proximal to the vesicle. (420 $\times$, estimated.) (From Hansen and Patton, 1975.)

Figure 14. Basidiospore germlings of *Gymnosporangium clavariiformae* on glass. The appressorial ring (arrow) is clearly visible. Bs, basidiospore. (1060 $\times$.) (Courtesy of R. Bauer, unpublished.)

Figure 15. Basidiospore germlings of *Uromyces appendiculatus* on polyethylene sheet. One basidiospore (Bs) is shown with appressorium (Ap) and vesicle (IV). (730 $\times$.)

Figures 16 and 17. Basidiospore germlings of *Gymnosporangium juniperi-virginianae* differentiate infection structures *in vitro* on dialysis tubing membrane (DM). **Figure 16.** Longitudinal section of a germinated basidiospore (Bs) showing one of its nuclei (N), germ tube (G), and prominent appressorium (Ap). The electron-dense extracellular matrix (arrows) is abundant and attaches the germling to membrane. (6500 $\times$.) (From Mims and Richardson, 1989.) **Figure 17.** Appressorium with appressorial ring (AR) outside plasma membrane (PM) and in close contact with membrane. The extracellular matrix (EM) covers appressorium. Note microtubules (mt) and tubular-vesicular complex (TVC2). (20,000 $\times$.) (Courtesy of C. W. Mims and E. A. Richardson, unpublished.)

Figure 18. Germinated basidiospore (Bs) of *Uromyces appendiculatus* on isolated host bean leaf cuticle. The vesicle (IV) and septate hypha (P) formed on the surface of the cuticle. S, septum. (960 $\times$.) (From Freytag *et al.*, 1988.)

Figure 19. Basidiospore germling of *Uromyces viciae-fabae* on nitrocellulose filter after staining with DAPI/calcofluor. The hypha contains two nuclei (N) separated by a septum (S). Bs, basidiospore. (500 $\times$.) (Courtesy of S. Freytag, unpublished.)

strongly bind lectins of *Triticum vulgaris* and *Canavalia ensiformis*, and weakly those of *Lens culinaris*, *Tetragonolobus purpureas*, and *Ricinus communis* (Fig. 20). These results suggest that they are covered with carbohydrates or glycoproteins that consist mainly of chitin, α -D-mannose, α -D-glucose, and some α -L-fucose and β -D-galactose. In comparison to urediniospore-derived infection structures, which appear to cover its chitin with other carbohydrates as soon as it penetrates the plant (Mendgen *et al.*, 1985), the basidiospore-derived infection structure reduces chitin while the affinity to other lectins remains at the same level (Fig. 20).

Figure 20. Affinity of some lectins to infection structures of *Uromyces viciae-fabae* *in vitro*. The lectins were labeled with FITC and the fluorescence was measured with a microscope photometer. B, basidiospore; G, germ tube; V, intraepidermal vesicle. WGA, wheat germ agglutinin; Con A, *Canavalia ensiformis* agglutinin; LCA, *Lens culinaris* agglutinin; LTA, *Tetragonolobus purpureas* agglutinin; RCA I, *Ricinus communis* agglutinin. (Courtesy of S. Freytag, unpublished.)

4. BASIDIOSPORE GERMLINGS IN VIVO

4.1. Historical Perspective

Much of the early rust work focused on basidiospore-derived infections of stem rust of cereals and coniferous tree rust diseases. de Bary (1865) demonstrated the relationship between infections of *Puccinia graminis* on wheat and barberry, which stimulated numerous subsequent studies on host penetration and infection (de Bary, 1866; Reess, 1870; Ráthay, 1881; Fischer, 1898, 1901; Eriksson, 1911; Waterhouse, 1921; Allen, 1930, 1932, 1934, 1935; Nusbaum, 1935). These initial studies, often containing accurate light microscopic observations (see Figs. 21, 22), gave way to studies on the epidemiologically more important urediniospore, which dominate the literature today.

4.2. Penetration

The anatomy of basidiospore germination, host penetration, and the development of characteristic inter- and intracellular structures is shown diagrammatically in Figs. 23 and 28. Penetration directly through the epidermis has been observed more often than indirect, stomatal penetration. Indirect penetration is common for species that attack gymnosperms, although exceptions do occur (Figs. 24, 42). Gymnosperms have a thick-walled epidermis covered by a thick cuticle. The extreme rigidity of even young needles presents a tough physical barrier and rusts may have adapted to stomatal penetration as a response to the impenetrable host epidermis (Fischer, 1898; Patton and Johnson, 1970; Grill *et al.*, 1980; Bauer, 1986).

4.2.1. Development on Host Epidermis

Basidiospores of many rusts produce a single, unbranched germ tube (Fig. 33) (Van Sickle, 1975; Kohno *et al.*, 1977b; Jacobi *et al.*, 1982; Metzler, 1982; Gold and Mendgen, 1984a) that generally emerges opposite the apiculus (Fig. 24) or laterally (Fig. 26). In *Cronartium ribicola* and *C. asclepiadeum*, several germ tubes may develop (Figs. 34, 35), which can become highly branched (Bega, 1960; Hansen and Patton, 1975; Bauer, 1986; Ragazzi *et al.*, 1987). Gold and

Figures 21 and 22. Epidermal infection structures. **Figure 21.** Diagrammatic representation. Bs, basidiospore; G, germ tube; IV, intraepidermal vesicle; P, primary hypha. (Modified after de Bary, 1863.) **Figure 22.** Ovate intraepidermal vesicle (IV) and primary hypha (P) of *Uromyces appendiculatus* are visible in leaf epidermis. Note the two nuclei (N). (830 $\times$.) (From Gold and Mendgen, 1984a.)

Figure 23. Diagrammatic representation of a direct-penetrating (a) and an indirect-penetrating (b) basidiospore germling.

Littlefield (1979) described branched germ tubes in *Melampsora lini* associated with septum formation.

In contrast to urediniospore germ tubes, which tend to grow at right angles to the epidermal ridges (Hoch and Staples, this volume), basidiospore germ tubes grow more randomly on the epidermal surface (Hansen and Patton, 1977; Gray *et al.*, 1983). The amount of germ tube growth prior to appressorium formation varies considerably and is dependent on the selection of an appropriate penetration site (Gold and Mendgen, 1984a).

The extracellular matrix, a common feature of direct-penetrating rusts, accumulates along

Figure 24. Sugar pine hypocotyl inoculated with vegetative hyphae of an axenic basidiospore subculture of *Cronartium ribicola*. Note apparent direct penetration of epidermal cell by the appressorium-like hyphal tips (arrow) closely appressed to the epidermis. (3450 $\times$.) (From Diner and Mott, 1982c.)

Figure 25. Germinated basidiospore (Bs) of *Gymnosporangium fuscum* on pear leaf. Note extracellular matrix material (arrow) near appressorium (Ap) at the penetration site. (2620 $\times$.) (From Metzler, 1982.)

Figure 26. Direct penetration by basidiospore germling of *Uromyces appendiculatus* near the junction of three epidermal cells. Note the fibrouslike extracellular matrix (arrows) along the germ tube (G) and at the base of the appressorium (Ap). A, apiculus; Bs, basidiospore. (4020 $\times$.) (From Gold and Mendgen, 1984a.)

all areas of contact between the epidermis and fungus (Figs. 25, 26), especially at the periphery of the appressorium (Fig. 27) (Waterhouse, 1921; Littlefield and Heath, 1979; Metzler, 1982; Gold and Mendgen, 1984a). Jacobi and co-workers (1982) also showed that it occurs at contact points between basidiospores, suggesting a nonspecific contact response. It appears to attach the germ tube and appressorium to the plant surface, protect it from desiccation and UV radiation, and serve as a reservoir for "penetration enzymes" (see Nicholson and Epstein, this volume). The enzymes necessary for penetration may arise from the appressorial ring (Metzler, 1982), a tubular, collarlike zone that flanks the periphery of the penetration pore (Figs. 14, 28, 32) (Gold and Mendgen, 1984a; Mims and Richardson, 1989). The appressorial ring may also be important for attachment.

The formation of a slightly swollen, terminal appressorium (Figs. 24, 25, 32) by direct-penetrating species occurs preferentially at or near epidermal cell junctions (Fischer, 1898; Eriksson, 1911; Melhus *et al.*, 1920; Allen, 1932, 1935; Gold and Mendgen, 1984a). This site preference may be related to the greater availability of nutrients and moisture due to a higher rate of exosmosis in these areas and/or the chemical composition and physical structure of the wax layer that affects both pH and wettability of the leaf surface (Cutler *et al.*, 1982). This characteristic appears to be independent of nuclear condition, as evidenced by direct-penetrating urediniospore germings (see Koch and Hoppe, 1988). Appressoria do not form in indirect-penetrating species (Figs. 33–35).

Figure 27. Apparent direct penetration near junction of two epidermal cells by basidiospore germling of *Uromyces appendiculatus*; note the copious deposition of the extracellular matrix (arrows). Ap, appressorium. (12,120 $\times$.)

Figure 28. Detailed diagrammatic representation of basidiospore germling and epidermal penetration. (Modified after Metzler, 1982.) ER, endoplasmic reticulum; F, filasomes; L, lipid bodies; mb, microbodies; Mi, mitochondrion; mt, microtubules; mivb, multivesicular bodies; N, nucleus; RER, rough endoplasmic reticulum; TVC, tubular-vesicular complex; V, vacuoles.

4.2.2. Mode of Ingress

The earliest stages of direct penetration are presumably effected by a narrow (diam. = ca. $0.5\ \mu\text{m}$) peg that has only been observed by light microscopy (Fig. 29). Once through the outer region, the peg expands in diameter (Metzler, 1982; Gray *et al.*, 1983; Gold and Mendgen, 1984a), breaches the epidermal wall, and invaginates the host plasmalemma. After a brief period of apical growth, it enlarges distally to form an ovate to oblong intraepidermal vesicle (Fig. 30). At this stage the nuclei migrate from the spore to the vesicle (see Figs. 30, 32).

Figures 29–31. Penetration and infection of bean epidermis by *Uromyces appendiculatus*. **Figure 29.** Initial stage of penetration. Binucleate basidiospore germling with appressorium (Ap) and phase-dark penetration peg (arrow). N, nucleus. ($1500\times$.) (From Gold and Mendgen, 1984a.) **Figure 30.** The two nuclei (N) and cytoplasm have migrated from the basidiospore (Bs) through the narrow penetration peg (arrow) into the intraepidermal vesicle (IV). Note aggregation of host cytoplasm (HC) in the invaded epidermal cell. ($1390\times$.) (From Gold and Mendgen, 1984a.) **Figure 31.** TEM of penetration site showing appressorium (Ap), intraepidermal vesicle (IV) with two nuclei (N) and primary hypha (P), separated by a septum (arrow). Note aggregation of host cytoplasm (HC) and chloroplast (ch) below primary hypha. ($3540\times$.)

Figure 32. *Gymnosporangium juniperi-virginianae* on susceptible apple leaf prepared for TEM by freeze substitution. Penetration peg (pp) from appressorium (Ap) has grown through epidermal wall (EW). The appressorial ring (arrows) is visible at the point of penetration. Only one of the two nuclei (N) is clearly visible in the basidiospore (Bs). (8000 $\times$.) (From Mims and Richardson, 1989.)

de Bary (1884) reported that the penetration pore becomes plugged after migration of the spore protoplast into the intraepidermal vesicle. Ultrastructural evidence of this was presented by Metzler (1982) as a septumlike structure in the neck region of the vesicle and by Gold and Mendgen (1984a) as an electron-dense occlusion of the penetration pore. Normal septum formation has been shown in *Cronartium fusiforme* (Fig. 43) (Gray *et al.*, 1983).

The average minimum time for penetration and formation of a vesicle is 4–6 hr (Weimer, 1917; Waterhouse, 1921; Snow, 1968b; Van Sickle, 1975; Gold and Mendgen, 1984a), although periods of up to 24 hr have also been reported (Allen, 1932, 1935; Nusbaum, 1935; Kohno *et al.*, 1977a). Light has either no influence (Hansen and Patton, 1975; Gold and Mendgen, 1984a) or a small inhibitory effect (Cotter, 1932).

4.3. Differentiation of Infection Structures

In direct-penetrating species, the vesicle forms within an epidermal cell and gives rise via apical growth to the primary hypha (Figs. 31, 37, 43) (Allen, 1983; Gold and Mendgen, 1984a). The primary hypha develops rapidly into a branched, multicellular network of mycelia (Fig. 41). Growth directly into neighboring epidermal cells (Figs. 37, 38, 41) and subtending palisade parenchyma is common (Figs. 39, 40) as is excellular growth into intercellular spaces (Figs. 42, 43). Here, the invaginated plasmalemma of the epidermal cell becomes reunited with the native plasmalemma at points directly adjacent to the exit site (Fig. 43) (Gray *et al.*, 1983). Once the hyphae reach the intercellular spaces, extensive ramification of the tissue occurs (Fig. 44) (Gold *et al.*, 1979; Grill *et al.*, 1980).

Figure 33. Indirect penetration of *Pinus pinaster* needle by germ tubes (G) of basidiospores of *Cronartium flaccidum*. Bs, basidiospore. St, stoma. (625 $\times$.) (From Ragazzi *et al.*, 1987.)

Figure 34. Basidiospore germling of *Coleosporium tussilaginis* produces a highly branched germ tube on a needle of Scotch pine. Penetration of host through host stoma (arrow). Bs, basidiospore. (610 $\times$.) (From Bauer, 1986.)

In indirect-penetrating species, intercellular hyphae are derived from a vesicle that forms in the substomatal chamber (Fig. 36). Further development occurs via infection structures equivalent to those of direct-penetrating species.

The bi- or multinucleate condition remains until development of the primary hypha and subsequent infection structures. Thereafter, many rusts revert to the mononucleate condition (Eriksson, 1911; Colley, 1918; Allen, 1930, 1935; Ashworth, 1935; Nusbaum, 1935; Metzler, 1980; Freytag *et al.*, 1988), but the binucleate (Lindfors, 1924; Thirumalachar, 1939; Kohno *et al.*, 1977b) or multinucleate (Allen, 1934; B. Müller, unpublished) condition persists in others.

Ultrastructural studies often refer to an amorphous, electron-opaque extracellular matrix between the host and the intercellular hyphae (Fig. 45) (Harder, 1978; Borland and Mims, 1980; Al-Khesraji and Lösel, 1981; Longo *et al.*, 1982; Gray *et al.*, 1983; Hopkin and Reid, 1988). The origin and function of this material are uncertain, but many authors assume it to be of fungal origin and to act as an aid to attachment (Littlefield and Heath, 1979; Harder and Chong, 1984; Hopkin and Reid, 1988). It may also be involved in signal exchange between host and parasite (Mendgen *et al.*, 1988).

Intercellular hyphae grow in contact with mesophyll cells and enter them to form intracellular hyphae (Fig. 48). The morphology of intracellular hyphae has been described in detail recently by Littlefield and Heath (1979), Harder and Chong (1984), and Hopkin and Reid (1988) and thus need not be repeated here. A characteristic of intracellular hyphae is the septum near the site of penetration either just outside (Figs. 46, 48) or inside (Figs. 49, 50) the host cell wall. Septum formation appears associated with a differentiation of intracellular infection structures. A highly conserved feature is the fingerlike or filamentous growth habit (Figs. 46–51). Conventional TEM shows no evidence of a definable neck ring (Harder and Chong, 1984). The extrahyphal

Figures 35 and 36. Development of *Cronartium ribicola* before and after stomatal penetration of needles of eastern white pine. **Figure 35.** Penetration by basidiospore germling through host stoma (arrow). Note branched germ tube (G) and lack of prominent appressorium. (600 $\times$.) (From Patton and Spear, 1980.) **Figure 36.** Cross section of stomatal penetration of secondary needle. Germ tube of the basidiospore penetrated between guard cells (arrow) to form substomatal vesicle (SV) and intercellular hypha (IH). (900 $\times$, estimated.) (From Patton and Johnson, 1970.)

membrane formed by the host usually stains weakly with PACP (a stain for plasma membranes) and lacks ATPase activity (Woods and Gay, 1987). The extrahyphal matrix is more pronounced around the distal end of intracellular hyphae and stains more intensively for polysaccharides (Chong *et al.*, 1981).

Intracellular hyphae commonly do not terminate within host cells. Several light- (Fig. 52) (Colley, 1918; Allen, 1930; Pady, 1935; Gold and Mendgen, 1984b) and electron-microscopic studies (Figs. 53, 54) (Gold *et al.*, 1979; Littlefield and Heath, 1979; Longo and Naldini Longo, 1982; Gray *et al.*, 1983; Gold and Mendgen, 1984b) have confirmed this. Improved methods of location (Zeyen and Bushnell, 1981) should promote future microscopical analyses of intracellular infection structures.

4.4. Host Response

Many investigators have taken a microscopical approach to study the incompatible interaction. The results of these studies suggest several mechanisms for resistance. On *Pinus sylvestris*, a relatively resistant species, basidiospore germination was reduced and delayed compared to more susceptible species (Ragazzi and Dellavalle Fedi, 1982). Natural occlusion of the stomata of *Pinus*

Figures 37–39. Development of *Uromyces appendiculatus* in bean epidermis as seen in whole leaf samples or in cross section. (From Gold and Mendgen, 1984a.) **Figure 37.** A branch of the primary hypha (P) penetrates into an adjacent epidermal cell (E) at arrow. IV, intraepidermal vesicle. (970 $\times$.) **Figure 38.** Primary hypha (P) grows into adjacent epidermal cell (E). Note septum (S) proximal to penetration point and the phase-light collarlike wall apposition (arrow) of the anticlinal wall. (1770 $\times$.) **Figure 39.** Growth of the primary hypha (P) into a palisade parenchyma cell (PC) at arrow. The nucleus (HN) of the PC is closely associated with apex of the intracellular hypha (H). (1560 $\times$.)

Figure 40. Transcellular penetration site of *Gymnosporangium fuscum*. The primary hypha (P) grew from an epidermal cell (E) into a subtending palisade parenchyma cell (PC). Abundant cell wall appositional deposits are associated with the transcellular hypha. Note fungal nucleus (N), narrow penetration site (S), and host nucleus (HN) closely appressed to the intracellular hypha (H). S, septum. (5180 $\times$.) (Courtesy of B. Metzler, unpublished.)

strobis seedlings with wax (Fig. 55) seems to reduce the penetration of *Cronartium ribicola* and the number of needle lesions (Patton and Spear, 1980). However, most other reports on this host–pathogen system suggest that resistance is based on hypersensitive reactions (Kinloch and Littlefield, 1977; Diner and Mott, 1982b). Furthermore, Grill and co-workers (1980) reported that wax occlusions do not hinder penetration by *Chrysomyxa abietis*. They suggested that other physiological factors are responsible for the resistance observed. The thickness of the cuticle has been proposed as a factor preventing direct penetration (Melhus *et al.*, 1920; Melander and Craigie, 1927), although this has not been confirmed in other systems (Reed and Crabill, 1915; Ashworth, 1931; Nusbaum, 1935).

In tissue with hypersensitive lesions, necrosis of host cells (Fig. 57) and host cell wall appositions (Fig. 58) have been observed (Robb *et al.*, 1975; Walkinshaw, 1978; Gray and Amerson, 1983). The hypersensitive response may be triggered by attempted penetration of the

Figure 41. Highly branched, multicellular primary hypha (P) of *Uromyces appendiculatus*. Transcellular penetration sites are shown at arrows. IV, intraepidermal vesicle. (540 $\times$.)

Figure 42. Direct penetration (arrow) by *Cronartium fusiforme* in needle of slash pine seedling. An ovate intraepidermal vesicle (IV) developed in the epidermal cell, prior to forming an intercellular mycelium (I). (1150 $\times$.) (From Miller *et al.*, 1980.)

host epidermis (Fig. 56) or by dead or encased haustoria (Jonsson *et al.*, 1978; Gray and Amerson, 1983).

As most studies have focused on tree rusts, the practical value of the described resistance mechanisms can only be evaluated after long-term field trials (Kuhlmann, 1988).

5. CONCLUSIONS

Recent studies on basidiospores and their germlings have centered around ultrastructural and morphological descriptions of the penetration and infection process, with particular emphasis on the morphology of intracellular infection structures. In contrast to urediniospore research, less emphasis has been given to biochemical and physiological aspects of germination and differentiation of infection structures. Such information is crucial to the understanding of disease initiation and in the development of improved plant disease control methods.

The fact that two haploid, homokaryotic nuclei in basidiospores versus the heterokaryotic condition in urediniospores can effect significantly different penetration and infection patterns on the same host merits further investigation. Apparently, when complementary nuclei are brought together into the same cytoplasm, different parts of the fungal genome are expressed, as evidenced by the distinct penetration and infection process initiated by urediniospores. Future studies should include parallel experiments with basidiospores and urediniospores of the same species for a better understanding of the nuclear control of differentiation processes.

Figure 43. Direct penetration from appressorium (Ap) of *Cronartium fusiforme* in hypocotyl of loblolly pine. The vacuolated intraepidermal vesicle (IV) and primary hypha (P) are bordered by the invaginated plasmalemma (IP) and a prominent extrahyphal matrix (EhM). The IP is fused with noninvaginated plasmalemma (arrow) where P exits the epidermal cell to become an intercellular hypha (IH). Note septate (S) neck region of the vesicle. (7160 $\times$.) (From Gray *et al.*, 1983.)

Figure 44. Intercellular hyphae (IH) of *Chrysomyxa abietis* in spruce needle. Note clearly visible septa (S). (1000 $\times$.) (From Grill *et al.*, 1980; Courtesy of Center for Electron Microscopy, University of Graz, Austria.)

Figures 45–48. Inter- (IH) and intracellular hyphae (H) of *Uromyces appendiculatus* in bean leaf tissue. (From Gold and Mendgen, 1984b.) **Figure 45.** IH in cross section showing extracellular matrix (EM) along its periphery and at points of contact with host cell. L, lipid body; PC, parenchyma cell. (21,260 $\times$.) **Figure 46.** Terminal, fingerlike H. Note septum (S) prior to penetration site of parenchyma cell (PC). (1180 $\times$.) **Figure 47.** IH with two H that formed as side branches. Note septa (S) near penetration sites (980 $\times$.) **Figure 48.** Median longitudinal section through a penetration site into a parenchyma cell (PC). The invaginated plasma membrane and extrahyphal matrix are only clearly visible surrounding adjacent cross-sectioned H (arrows). Patches of glycogenlike particles (arrowheads) are seen in the IH. (6210 $\times$.)

Figure 49. Intracellular hypha (H) of *Cronartium fusiforme* in loblolly pine cell with associated extrahyphal matrix (EhM) and invaginated host plasmalemma (IP). A well-developed collar (C) containing membranous components surrounds the neck region of the H. The EhM appears similar to the extracellular matrix (EM) surrounding the intercellular hyphae (IH). N, nucleus; S, septum. (9960 $\times$.) (From Gray *et al.*, 1982.)

Figure 50. Intracellular hypha (H) of *Peridermium pini* in a pine parenchyma cell (PC). Note the collarlike wall apposition (C) and the septum (S). The intercellular hypha (IH) is only slightly constricted at the penetration site. N, nucleus; EhM, extrahyphal matrix. (10,000 $\times$.) (From Walles, 1974.)

Figure 51. Two intracellular hyphae (H) of *Puccinia recondita* in epidermal cell of *Thalictrum speciosissimum*. Note the branched, hyphalike morphology of the H. (1900 $\times$.) (From Gold *et al.*, 1979.)

Figures 52–54. Transcellular penetration sites. Figure 52. Intracellular hypha (H) of *Uromyces appendiculatus* between two parenchyma cells (PC). (1590 $\times$.) (From Gold and Mendgen, 1984b.) Figure 53. Intracellular hypha (H) of *Gymnosporangium fuscum* in pear parenchyma cells (PC) with septum (S) prior to penetration site. N, nucleus. (8000 $\times$.) (Courtesy of B. Metzler, unpublished.) Figure 54. Coiled intracellular hypha (H) of *Puccinia malvacearum* in parenchyma cell of hollyhock. Note the branched, hyphalike nature of the H and exit from the host cell (arrow) to become an intercellular hypha (IH). (5400 $\times$.) (From R. E. Gold and L. J. Littlefield, unpublished.)

Figure 55. Basidiospore germlings (Bs) of *Cronartium ribicola* on a leaf of a resistant pine cultivar. The germ tubes grew randomly across the wax-covered epidermis without attempting to penetrate the wax-occluded stomatal antechamber (arrow). (500 $\times$.) (Courtesy of R. F. Patton, unpublished.)

Figure 56. Attempted penetration by basidiospore germling of *Uromyces vignae* on the nonhost *Vicia faba*. The germling was removed during processing for light microscopy. The incompatible host-parasite interaction resulted in necrosis of the epidermal cell; note rippled appearance of the epidermis and darker-stained anticlinal walls (arrows). The necrotic intraepidermal vesicle (IV) and primary hypha (P) are visible. (500 $\times$.) (Courtesy of S. Freytag, unpublished.)

Figure 57. Longitudinal section through a disorganized intracellular hypha (H) of *Cronartium ribicola* in an axenic-cultured pine cell. The H is surrounded by host vacuoles (HV) that contain densely staining tannin deposits and the intercellular space is filled with cellular debris (arrow). IH, intercellular hypha; N, nucleus; S, septum. (4320 $\times$.) (From Robb *et al.*, 1975.)

Figure 58. An aborted intracellular hypha (H) of *Cronartium fusiforme* in a cortical cell (CC) of a resistant loblolly pine seed line. A large papillar wall apposition (Pa) formed in response to attempted penetration. Note the heterogeneous appearance of the Pa and abundance of electron-dense membranous material (arrows) in the apposition and in the adjacent host cytoplasm. The intercellular hypha (IH) is also necrotic. (12,000 $\times$.) (From Gray and Amerson, 1983.)

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Note added in proof: Since this chapter was written, experiments with basidiospores of *M. pinitorqua* on shoots of *P. sylvestris* have provided information on the effect of the epicuticular wax structure on pre-penetration development of germlings (Desprez-Loustau and Le Menn, 1989). Also, two reports on host response to basidiospore infections have appeared (Heath, 1989; Hopkins and Reid, 1988).

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