2. BIOLOGY AND LIFE STRATEGY OF THE ERGOT FUNGI

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2.1. INTRODUCTION

Claviceps species are the causal agents of the ubiquitous ergot disease. About thirty-six different filamentous fungi constitute this genus of phytopathogenic ascomycetes. They parasitize more than 600 monocotyledonous plants of the families *Poaceae, Juncaceae* and *Cyperaceae* (Bové, 1970), including forage grasses and the leading cereals worldwide: wheat, rice, corn, barley, sorghum, oats, rye, millets (Baum *et al.,* 1992). Being epidemic to a greater extent in semi-arid regions than in temperate zones, ergot is of increasing importance in India and Africa, where pearl millet and sorghum are essential crops (Frederickson *et al.,* 1993). Although the fungi cause harvest losses due to replacement of host ovaries with the parasite's resting structures, the ergotcalled sclerotia, the main problem is not a severe loss in seed quantity but arises from complete ruin of grain quality due to the alkaloid content of the sclerotia. Admittedly, ergot alkaloids are secondary metabolites of high pharmacological value and are, therefore, produced worldwide on a large scale, nevertheless, these toxins cause highly dangerous or even deadly ergotism when contaminated grains are fed to animals or are consumed by man. These are the reasons for a continuous interest for ages in ergot fungi and their persistent importance (see chapter 1 in this volume), which will remain valid as long as the main ubiquitous nutritional basis to man and herbivorous livestock is concerned. Worldwide reduction in grain yield and quality causes the permanent necessity for an expensive cleaning of attacked cereals to maintain a minimum of purity standard. A contamination of crops with ergots higher than 0.3% by weight spoils the grain even for feeding (Agrios, 1988). Specific measures for reliable control as well as utilization of positive capacities of ergot fungi closely depend on an overall understanding of host- and pathogen biology.

This article deals with general biology and histopathology of ergot fungi pointing to similarities and differences in the scale of biological forms of *Claviceps* species associated with variable host types and numbers. In contrast to the limited knowledge of fundamental biology in many plantparasite systems (Mims, 1991), numerous investigations, most of them from Mantle's group and mainly based on the few most important ergot species, *C. purpurea* (Fries ex Fries) Tulasne, *C. fusiformis* Loveless, *C. sorghi* Kulkarni, Seshadri and Hegde,

C. africana Frederickson, Mantle & De Milliano, and *C. paspali* Stevens and Hall, add to a considerable body of research on *Claviceps* species reviewed by Taber (1985) and, focused on *C. purpurea,* by Tudzynski *et al.* (1995). This article emphasizes on recent advances in general biology, epidemiology and control as well as histopathology and molecular cytology in the genus *Claviceps*. Although much further work is needed in this field of research on both, the pathogen and the targeted host organ, substantial knowledge and modern methods in fine structural analysis of interaction-specific reactions *in situ* open the opportunity to address unsolved hypotheses in this specific ergot-grass relationship and therewith contribute to general understanding of molecular mechanism in the interaction of hosts and pathogens.

2.2. LIFE-CYCLE

In nature, the parasitic lifes of ergot fungi start with windborne ascospores landing on susceptible hosts in spring. All arising stages of their life-cycle can develop from one single spore, therefore, the ergot fungi are homothallic as shown by Esser and Tudzynski (1978) (see chapter 4 in this volume) for *C. purpurea* (Figure 1). Typically, spores attach and germinate on the pistil surfaces of blooming host florets and initiate a specific pathogenesis pattern with little variation between ergot species (Parbery, 1996). Hyphae invade and colonize the ovary, grow down to the tip of the ovary axis, the rachilla, and establish a specific and persisting host-parasite frontier. The fungi never invade any part further down in the host but proliferate above this site. A sphacelial stroma grows profusely in the ovary, producing masses of anamorphic spores which are exuded into a syrupy fluid (Figure 1). With this honeydew, the conidiospores are transferred to other blooming florets by rainsplash, head-to-head contact or insect vectors. Thereby ergot fungi spread spatially in the field having used the plant gynoecia for their own proliferation.

A few ergot fungi, e.g., *C. africana, C. fusiformis, C. cynodontis* Langdon, *C. paspali* and *C. sorghi,* produce two types of anamorphic spores. They cover a wide range in size and mostly divide into microconidia, measuring about 6 x 2.5 µm, and macroconidia, measuring about 16 x 4 µm. Firstly, the honeydew contains macroconidia, often microconidia as well, which are able to germinate in the honeydew just below the syrup surface. Secondly, conidiophores emerge and differentiate "secondary conidia" outside the liquid in a secondary conidiation cycle. Masses of conidia, mostly microconidia, whiten the surfaces of sticky colourless honey dew droplets one day after their exudation. Since both conidia types can initiate infection, these ergot fungi spread in the field by a second airborne inoculum in addition to the transmittance of macroconidia with the honeydew (Luttrell, 1977; Frederickson *et al.,* 1989, 1993; Parbery, D.G. pers. communication).

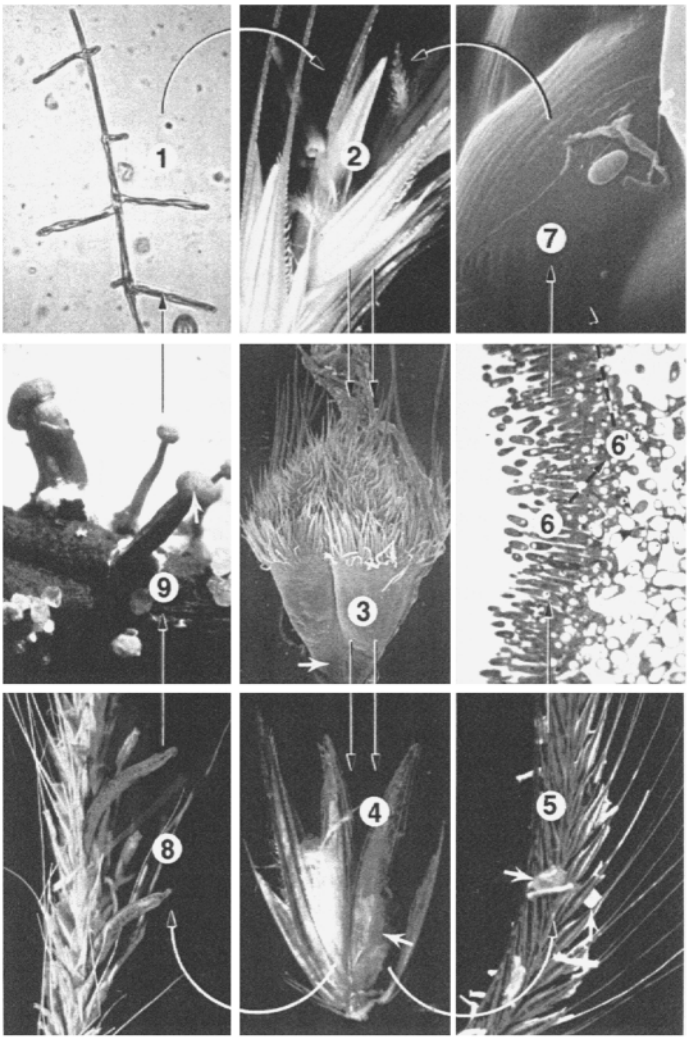

Figure 1 Life-cycle of ergot fungi, shown for *C. purpurea*. The different stages depicted are 1, germinating ascospore; 2, a rye floret at anthesis exposing the stigma between the opened glumes; 3, an infected rye ovary during the endophytic colonization phase with withered stigma and style, long ovary cap hairs and the rachilla (arrow); 4, a biflorescented spikelet of rye after selective inoculation with *C. purpurea* which has formed a sphacelium (arrow) in the infected right floret next to a rye seed developing in the neighbouring uninfected floret; 5, a rye ear with honeydew (arrows) flowing out of infected florets; 6, a sphacelial stroma with phialidic conidiophores producing many anamorphous spores; 6′, pointing to the additional microcycle producing airborne microconidia in some other ergot species; 7, germinating conidiospore on the host ovary cap with subcuticular hyphal growth towards the cellular junction; 8, a mature rye ear with several sclerotia; 9, germinating sclerotium with stromata that differentiate perithecia (arrow) in the head periphery containing asci with ascospores (Figures 1 and 9 courtesy of P.Tudzynski)

Next, honeydew production and conidiation usually cease when the formation of sclerotia starts. Sclerotia mature in about five weeks (Figure 1). Finally, during autumn, instead of a caryopsis, a ripe sclerotium leaves the spike, therewith making ergot a replacement tissue disease (Luttrell, 1980). The hard compact ergot consists of a plectenchymatous whitish medulla consisting of special storage cells and a typically pigmented outer cortex. It serves for sexual reproduction and as a resting structure to survive unfavourable conditions, e.g., in temperate zones for overwintering after having fallen to the ground or having been harvested together with the seed.

In temperate zones, sclerotia germinate in spring after a period of low temperature, which favours germination of *C. paspali* sclerotia (Luttrell, 1977). For *C. purpurea,* a temperature of 0°C for at least 25 days would be optimal for germination (Kirchhoff, 1929). Possibly, low temperatures are needed to activate enzymes for lipid mobilization in sclerotia (Cooke and Mitchell, 1967). Optimally, at about 20°C, germination can occur above or just beneath of the soil surface (Kirchhoff, 1929). Germination results in one to sixty clavicipitaceous stromata, formed of mushroom-like stalks with spherical capitula (Figure 1). Both, stalk and capitula have species-specific pigmentation (Frederickson *et al.,* 1991) and are 0.5 to 3.8 cm long, growing positively phototrophic (Hadley, 1968) to reach the air. Except for *C. paspali* (Taber, 1985), female ascogonia and male antheridia develop in the periphery of the capitula and fuse to form dikaryotic ascogenous hyphae. The hyphae surrounding the fertilized ascogonia build flask-shaped perithecia within which karyogamy and meiosis occur, producing asci with thin, needle-like, hyaline, nonseptate ascospores. *Claviceps* ascospores appear to be comparable in different species (Frederickson *et al.,* 1991) and measure 40 to 176×0.4 to 1.2 µm. Under suitable moist conditions, eight ascospores are forcibly ejected through the apical pores of asci, which emerge through the ostiole of perithecia in *C. paspali* (Luttrell, 1977). About four weeks after sclerotia germination, these ascospores represent the airborne primary inoculum and give rise to new infection foci.

2.3. HOST RANGE AND SPECIFICITY

2.3.1. Host Range

As mentioned above, ergot species are common on cereals, cultivated forage grasses and many wild grasses, in addition, ergot infects sedges and a few rushes (Taber, 1985). Although *Claviceps* infects over 600 host species worldwide, most of the thirty-six different *Claviceps* species have a monogeneric host range, a few are tribe-specific (Loveless, 1971), and some are species-specific (Parbery, 1996). Some ergot fungi are widely endemic, e.g., *C. africana,* living solely in eastern and south-eastern Africa, or *C. sorghi,* confined to India (Frederickson *et al.,* 1994). In conclusion, endemism is not due to spatial host restrictions, since both species share the same host type.

C. purpurea, however, parasitizes mainly rye, wheat and barley as well as numerous forage- and roadside grasses (Campbell, 1957; Loveless, 1971). All in all, *C. purpurea* attacks about 400 species of grasses throughout the world (Taber, 1985). Such wide host range is unique in the genus, and rises the question whether physiological races have been evolved. Although substantially investigated, the existence of *formae specialis* has not been verified thus far (Stäger, 1922; Campbell, 1957; Loveless, 1971; Darlington *et al.,* 1977; Frauenstein, 1977; Taber, 1985; Tudzynski *et at.,* 1995). Nevertheless, a high variation in random amplified polymorphic DNA (RAPD) pattern with considerable strain-specificity among 29 field isolated of *C. purpurea* indicated an unusual high degree of genetic diversity in this ergot species; host specificity is indicated to some extend, since most strains from specific host plants group together in preliminary tree analysis (Tudzynski and Tudzynski, 1996; Jungehülsing and Tudzynski, 1997; see chapter 4 in this volume). With few exceptions and regardless of locations, most strains of *C. purpurea* isolated from one host can pass over onto another and vice versa (Campbell, 1957), in particular individuals from ryegrass can infect rye (Mantle, 1967). More aggressive strains can replace other ergot strains having already settled in the ovary (Swan and Mantle, 1991). This multidirectional infection is not without consequences for epidemiology and control (see below).

Notably, all natural ergot-plant associations share two conspicuous host features, which obviously reflects special adaptations of the antagonist (see below): (a) all hosts are anemophilous monocotyledons and (b) the fungal objectives are host gynoecia solely.

2.3.2. Organ Specificity

In all *Claviceps* species studied thus far, infection is confined to host ovaries (Parbery, 1996). In general, organ specificity is poorly understood (Schäfer, 1994). In ergot, in particular, this phenomenon is a matter of speculations, because the biological function of the targeted host organ intended for sexual reproduction and its distinctive adaptations thereupon offer some additional concepts, however, are not conceived at their molecular function itself.

Although the inoculum reaches most likely every host surface area, in nature, successful ergot infection is strictly specific to florets. Florets can prevent infection completely by denying access of inoculum to their pistils due to tightly closed bracts (see Chapter Ergot Virulence and Host Susceptibility). However, one can artificially induce sclerotia development by wounding and inoculating young tissue, e.g., on stalks (Stoll and Brack, 1944), on the shoot apex of rye seedlings (Lewis, 1956), or on nodes and internodes of rye (Garay, 1956). Jointly, these observations point to unique features of pistil surfaces which appear to be indispensable for the establishment of infection. Additionally, imitation of specific pollen-stigma interaction has been suggested. Therefore, molecular cytological investigations of the pistil surface and the interaction-specific reactions are

necessary (see Chapter Histo- and cytopathology of infection). Mechanisms might be analogous to pollen adhesion and penetration processes that have been examined in grasses (Heslop-Harrison and Heslop-Harrison, 1980, 1981; Heslop-Harrison *et al.,* 1984, 1985). There are some striking similarities between the process of fungal colonization and natural fertilization, for the plant's ovary mainly serves as a host for the male gametophyte in order to favour and to guide pollen tube tip-growth. Furthermore, both invaders interfere with each other when landing on their objective at the same time, in particular, simultaneous pollination favours fungal penetration, e.g., in *C. purpurea* (Williams and Colotelo, 1975), however, pollen tubes grow much quicker reaching the ovule in wheat in about 30 min (You and Jensen, 1985). Admittedly, the ovary appears to be dispensable for fungal development subsequent to primary infection, because sclerotia are formed even after artificial inoculation of florets, the ovaries of which have been removed previously (Cherewick, 1953) or after advanced kernel growth (Mantle, 1972). Nevertheless, mimicry of pollen tube growth might also occur in the ovary colonization process.

A second reason for organ specificity might be the exceptional molecular architecture of the monocotyledonous cell wall (Carpita and Gibeaut, 1993) certainly with some additional unique cell wall modifications in different pistil tissues (Tenberge *et at.,* 1996a, b; see below), e.g., pistil epidermis with stigma hairs, ovary mesophyll, transmitting tissue and integuments.

Host floret biology is fundamentally important in every respect. Since ergot hosts are anemophilous, floret morphology and the time course of anthesis determines pollination. Likewise, access of fungal spores, especially of airborne primary inoculum, is subject to floret biology. In most grasses, single florets gape for several hours, then close tightly, sometimes leaving the stigmas exposed between the glumes. After pollination, stigmas wither directly in pearl millet, quickly in rye or remain turgid over a period of two month, even after infection, in sorghum. Regarding complete ears, florets open, starting near the top of the ear and progressing in a series basipetally over several days, as in sorghum (Frederickson and Mantle, 1988) or rye. Hence, cereal fields are in bloom for about two weeks.

2.4. HISTO- AND CYTOPATHOLOGY OF INFECTION

Different ergot species exhibit little variation in overall pathogenesis and their cytopathology of infection, which has recently been reviewed with emphasis on *C. purpurea* (Tudzynski *et al.,* 1995), is basically identical (Luttrell, 1980; Parbery, 1996). In the few species investigated at the microscopical level, so far, starting with the first detailed description of infection by Tulasne (1853), there exists both, some peculiar findings and some conflicting data still open to question.

2.4.1. Host Infection

Infection Site and Route

Infection of a single host plant is naturally induced by spores landing on the pistil of open florets for which less than ten conidia are sufficient (Puranik and Mathre, 1971). The precise site of spore germination and the resulting infection route, i.e., either via stigma or style with help of the pollen tube path in contrast to ovary wall or ovary base, has been controversially discussed (Engelke, 1902; Kirchhoff, 1929; Campbell, 1958; Luttrell, 1980; Shaw and Mantle, 1980a; Tudzynski *et al.,* 1995). Conflicts arose because the penetration site was deduced from hyphal locations, i.e., in the transmitting tissue after inoculation, but was not shown in micrographs itself. Rye stigmas were shown to be penetrated by *C. purpurea* (Luttrell, 1980), however, the use of squash mounts is a questionable approach, since ergot fungi will grow into almost every young tissue after its epidermis has been wounded. Thin sectioning appears to be not feasible, but, employing scanning microscopy, spore adhesion, spore germination and host cuticle penetration could be documented to occur on either part of the pistil surface (Figures 1 and 2) (Tenberge, 1994; Tenberge and Tudzynski, 1995). Therefore, germination of *C. purpurea* is not restricted to the stigma; it does obviously not depend on stigmatic fluid. Likewise, regarding other *Claviceps* species, each one of the penetration site has been reported, in particular the ovary wall, and in some cases visualized (Luttrell, 1977; Thakur and Williams, 1980; Willingale and Mantle, 1987a; Frederickson and Mantle, 1988), but never has proof been presented for a distinct epidermal region of the pistil to be resistant to ergot penetration. In conclusion, ergot fungi most likely are able to penetrate the pistil epidermis anywhere.

Following penetration in the outer epidermal wall of the pistil, the hyphae keep on growing towards the rachilla (Figure 2). They grow either down the style in the transmitting tissue following the pollen tube path outside the ovule and leaving this way at the micropylar region in direction of the rachilla or, after lateral entrance into the ovary, in the carpel mesophyll to the ovary basis (Luttrell, 1980; Shaw and Mantle, 1980a; Tudzynski *et al.,* 1995). The ovary wall gets completely colonized after about 2 days post inoculation (dpi) in *C. paspali,* 4 dpi in *C. fusiformis* or 8 dpi in *C. sorghi* depending on temperature, geographical location (Willingale and Mantle, 1987a) and species. However, the ovule first remains uninvaded (Kirchhoff, 1929) due to the integuments which appear to form a temporary barrier to the fungus (Campbell, 1958; Willingale and Mantle, 1987a). Ergot of dallisgrass is much quicker and colonizes the ovule already after 2 dpi (Luttrell, 1977). Sorghum ovules are additionally invaded through the chalazal region (Frederickson and Mantle, 1988). The same occurs in rye. Integuments typically collapse during development of caryopses. However, possibly due to chitinases or chitinbinding lectins found in rye seeds (Raihkel *et al.,* 1993; Yamagami and Funatsu, 1996), growing kernels remain noncolonized after late infection.

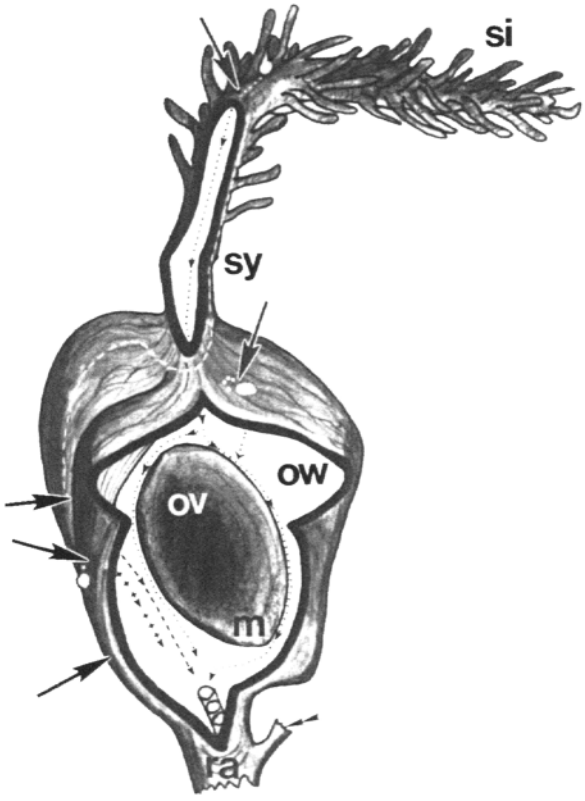

Figure 2 Illustration of different conidia germination places and penetration sites (long arrows) with resultant infection routes of *Claviceps purpurea* in a cereal pistil……, spore germination and infection via stigma or style or ovary cap partly corresponding to the pollen tube path; +++, spore germination and infection at the base of the ovary wall;——, spore germination on the stigma and infection at the base of the ovary wall suggested by Kirchhoff (1929). Double-arrow, indicating filament base; m, micropylar region; ov, ovule; ow, ovary wall; ra, rachilla; si, stigma; sy, style

Fungal cells colonize the entire ovary wall but in the ovarian axis the hyphae of all ergot species stop to spread in the plant tissue. No hyphae emerge beyond the rachilla tip. Thus, a narrow frontier between the fungal stroma and the noncolonized host tissue develops, which is finished approximately six days after infection with *C. purpurea* and persists throughout the remaining life-span (Luttrell, 1980; Shaw and Mantle, 1980a; Tudzynski *et al.,* 1995). The completion of this frontier coincides with the exudation of honeydew, 4 to 10 dpi depending on the species, and indicates the begin of the sphacelial phase (see below). Honeydew presents the first macroscopic evidence for infection in

most host grasses but not in pearl millet, where browning, withering and constriction of stigmas and stylodia is obvious already after 36 h post inoculation (hpi) (Willingale and Mantle, 1987a). Stoppage of fungal growth in the rachilla of *C. purpurea* has been shown to be most likely caused by host phenolics that accumulate during infection at this site (Mower and Hancock, 1975; Shaw and Mantle, 1980a; Hambrock, 1996) and might inhibit fungal pectin-degrading enzymes (Mendgen *et al.,* 1996).

The route which is usually used, however, depends on floral biology and spore vehicles. Short floret gaping followed by stigma exposure between tightly closed glumes causes the pollen tube path to be of importance in nature, such is valid for *C. paspali* in dallisgrass (Luttrell, 1977) and *C. fusiformis* in pearl millet with very large feathery stigmas (Thakur and Williams, 1980; Willingale and Mantle, 1985). Additional routes in rye, however, are obvious from successful infection with *C. purpurea* after previous ovary colonization with the bunt fungus *Tilletia caries* (Willingale and Mantle, 1987b) or after fertilization with advanced kernel development (Mantle, 1972), since in both cases the pollen tube path is blocked. This shows that, regardless of the infection route, the growth of ergot fungi is strongly directed at the vascular tissue supply of the ovary, which itself represents the entrance but is dispensable (Luttrell, 1980; Willingale and Mantle, 1987b) although incidentally consumed for overall nutrition.

Spore Adhesion, Infection Structures and the Infection Process

The attachment process of ergot spores is not investigated precisely. Since the attaching force of ungerminated spores of *C. fusiformis* increases during the first 8h following inoculation (Willingale and Mantle, 1987a), one may infer adhesive strategies of spores to the plant surface. But in *C. sorghi* conidia appear to become detached from the surface during germination and penetration (Frederickson and Mantle, 1988). On stigma or style, the stigmatic fluid might offer hydrophilic conditions or, in case of honeydew-mediated transmittance, the syrupy fluid may support adhesion to the host surface. After attachment, conidia germination starts with the formation of one to several germ-tubes, supported by dew periods, and is accomplished very quickly in some species, e.g., in *C. paspali* within 4 hpi (Luttrell, 1977), in *C. fusiformis* within 12–16 hpi (Willingale and Mantle, 1987a), in *C. sorghi* within 16–48 hpi (Frederickson and Mantle, 1988).

In the most important ergot fungus, *C. purpurea,* early infection events are not described and growth modes of ergot fungi are not analyzed functionally. To study the penetration and colonization mechanisms of ergot fungi, early events of the infection process of *C. purpurea* were first documented and then cytochemically analyzed in detail in our laboratory. Spores attached everywhere on the pistil epidermis. Its outer epidermal wall comprises a faint cuticular membrane, measuring about 15 nm in thickness, and forms a continuous outer

barrier of the host ovary. Germination results in one or two germ-tubes, e.g., on the ovary cap (Figure 1). Sometimes a limited external mycelium is formed. Next, the faint plant cuticle of the outer epidermal wall is directly penetrated. An indirect entry via natural openings is unimportant because the pistil is free of stomata and natural wounding was never observed. Infection hyphae originate either directly from the germ-tube or from the external mycelium (Figure 1). Since no changes in hyphal shape were apparent, it appears that *C. purpurea* penetrates without specialized infection structures (K.B.Tenberge, unpubl.). At suitable sites, infection hyphae pass through the outer epidermal cell layer growing intercellularly into the anticlinal epidermal walls. However, hyphae may as well pass through the outer epidermal cell wall away from cellular junctions (Shaw and Mantle, 1980a; Tudzynski *et al.,* 1995).

So far investigated, all ergot fungi penetrate directly into the anticlinal walls between epidermal host cells, sometimes after a period of subcuticular growth similar to that of *C. purpurea* (Figure 1), e.g., *C. sorghi* (Frederickson and Mantle, 1988). However, the hyphae of some species develop special morphological structures prior to penetration. *C. gigantea* produces an appressorium (Osada Kawasoe, 1986). In *C. fusiformis,* an external mycelium is formed and bulbous infection structures arise at the tips of several germtubes of a single macrospore (Willingale and Mantle, 1987a). Whether these structures fulfil appressorial function is not described so far.

Mechanisms of Adhesion and Penetration

The mechanism of cuticle penetration still needs to be elucidated, although, in most ergot fungi, the direct push of a infection hypha into the epidermal wall obviously matches the more simple type of penetration as classified by Mims (1991). Infection structures of phytopathogenic fungi are specialized hyphae adapted for the invasion of the host and show considerable variations in penetration strategy (Mendgen and Deising, 1993; Mendgen *et al.,* 1996). The bulbous structures of *C. fusiformis* suggest the utilization of turgor pressure, the one used for penetration is mediated by functional appressoria and has been shown to be obligatory in the rice blast pathogen (Howard and Valent, 1996). Turgor pressure itself is essential for fungal tip growth (Wessels, 1994), however, it is an open question whether it is sufficient for penetration. According to Mendgen *et al.* (1996), directly penetrating fungi that do not form appressoria clearly need cell wall-degrading enzymes for penetration. During penetration of grass stigma cuticles, tip growth of pollen tubes is mediated by cutin-degrading enzymes (Baum *et al.,* 1992; Heslop-Harrison and Heslop-Harrison, 1981), however, in case of ergot fungi, the secretions of such enzymes have not been demonstrated so far.

Recently, genes coding for hydrophobin-type proteins have been isolated from a *Claviceps* sp. (Arntz and Tudzynski, 1997) and from *C. purpurea* (V.Garre and P.Tudzynski, pers. communication, see chapter 4 in this volume), in addition,

hydrophobin-type proteins have been identified and purified from corresponding axenic cultures (O.de Vries, S.Moore, C.Arntz, J.G.H.Wessels and P.Tudzynski, pers. communication). Hydrophobins can act as adhesive to the host cuticle (Wessels, 1994) and have been shown to play a crucial role in formation, adhesion and infection court preparation of phytopathogenic fungi (Beckerman and Ebbole, 1996; Talbot *et at.,* 1996). Therefore, the présence of such proteins suggests similar functions in ergot. In addition, one can speculate that they might mediate the intimate contact and abundant wrapping of superficial ergot hyphae with the host cuticle demonstrated by TEM (Tudzynski *et al.,* 1995; K.B.Tenberge, unpubl.) and therewith might cause a mechanical disruption of the thin cuticle itself. Cytological expression analysis of the hydrophobin gene with *in situ* hybridization technique showed that transcripts were located in external and penetrating hyphae as well as in *C. purpurea* conidiophores (Tenberge *et al.,* 1998). This localization indicates another function of hydrophobins that are thought to be essential for the formation of aerial hyphae and fruit bodies (Wessels, 1994) to be met in *C. purpurea.*

2.4.2. Fungal Mechanisms for Host Colonization

Ectotrophic Growth in the Host Ovary with Limited Endotrophism

After penetration, ergot fungi live inside the ovary, i.e., endophytically, during the colonization phase. Subcuticular hyphae, in fact, are located within the outer epidermal cell wall and then the fungi usually grow between epidermal cells into the host apoplast. Before tapping the vascular traces, fungal growth during the colonization phase has been reported to be exclusively intercellular, i.e., ectotrophic, in all ergot fungi investigated (Luttrell, 1977, 1980; Shaw and Mantle, 1980a; Willingale and Mantle, 1987a; Frederickson and Mantle, 1988). However, a limited intracellular growth has been documented electron microscopically in *C. purpurea* (Tenberge and Tudzynski, 1994; Tudzynski *et al.,* 1995). Therefore, at least the mycelium of this ergot species is ectotrophic but with limited endotrophism (Figure 3). The vegetative hyphae exhibit ultrastructural features typical for ascomycetous fungi and well preserved during processing for TEM. The thin fungal cell wall and the host cell wall build up an intimate zone of contact while both, host and pathogen, appear to be healthy (Tudzynski *et al.,* 1995). This is particularly valid for intracellular hyphae and the penetrated host cells and points to haustorial function. For this purpose, the endotrophic mycelium is well positioned in the chalazal region that serves for the host ovule nutrition. The interface of the intracellular hyphae, which are completely encapsulated by the host plasma membrane (Figure 3B), has developed special adaptations (Tenberge *et al.,* 1996a; Müller *et al.,* 1997), however, nutrient uptake into hyphal cells is not yet investigated.

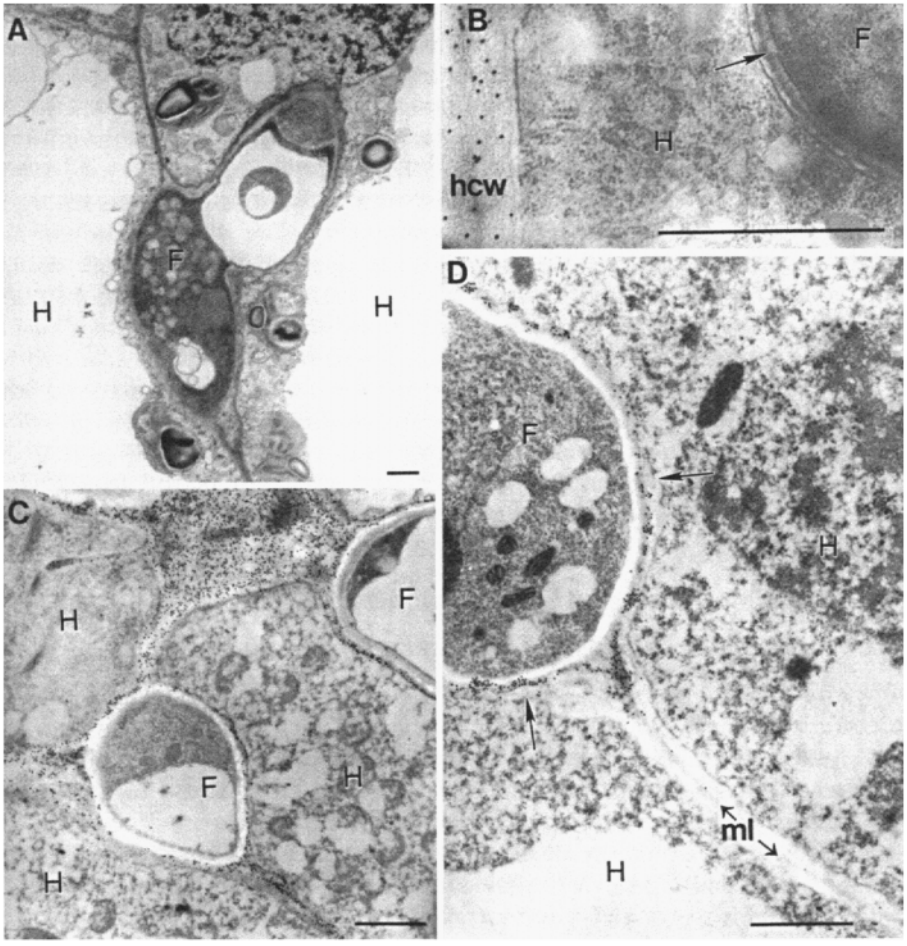

Figure 3 TEM micrographs of rye ovaries during the colonization phase with *C. purpurea* showing inter- and intracellular growth in host mesophyll cells. **A,** An intercellularly growing hyphal cell (F) actively penetrating a living host mesophyll cell (H). **B,** Enzyme-gold localization of *ß*-1,4-glucan with a cellulase-gold sol showing gold label in the host cell wall (hcw) distant from the fungus (F) while no label is visible immediately at the interface of the intracellular hypha (arrow). C and D, Immunogold localization of homogalacturonan epitopes in pectin with a monoclonal antibody JIM5 specific for non-methyl-esterified polygalacturonic acid visualized with gold-linked secondary antibody. Distant from intercellular hyphae (F) in the ovary mesophyll (H), low JIM 5 label was restricted to the middle lamella (ml) (**D**)**;** however, JIM 5 label was high throughout host walls after demethylation with sodium carbonate (**C**). At the interface of hyphae, high JIM 5 label was present above the entire host wall (arrows) and above the altered middle lamella zone (**D**). **A, B,** Glutaraldehyde fixation, osmication, epoxy resin embedding. **C, D,** Formaldehyde-glutaraldehyde fixation, no osmication, LR White embedding. Scale bars=1 μm

Mechanisms of Fungal Growth in the Host Tissue

The chemical composition of the infection court is a matter of speculation if assuming that the cell wall type of grass leaves (Carpita and Gibeaut, 1993) is also valid for the ovary neglecting the ovary's specific function. We currently are analysing the molecular architecture of the host-parasite association with emphasis on interaction specific reactions, e.g., polymer alterations and protein secretion, at the electron microscopical level. This molecular cytological study is intensely co-ordinated with a molecular genetical approach (see chapter 4 in this volume) in order to elucidate fungal mechanisms utilized for infection court preparation, penetration and further ecto- and endotrophic host colonization, because functional studies are very limited on ergot pathogenicity.

Although only very low pectin content is expected in grass cell walls according to Carpita and Gibeaut (1993), host cell wall loosening during subcuticular and intercellular growth indicated that actions of pectolytic enzymes, which Shaw and Mantle (1980a) have proved to be active in culture, in honeydew and in parasitic tissue extracts, play a role in parasitism. Then, the simultaneous présence of both pectin types, non-methyl-esterified and methyl-esterified galacturonan, in the cell walls along the usual infection path in healthy carpels has been documented, using the two monoclonal antibodies JIM 5 and JIM 7 (Tenberge *et al.,* 1996a, b). With the same experimental design applied to parasitic culture of ergot on rye, a local molecular pectin modification as well as degradation have been demonstrated for the host cell wall and the middle lamella zone at the interface of subcuticularly and intercellularly growing hyphae *in situ* (Figure 3D). Chemical demethylation and immunogold labelling indicated a high total content of galacturonan that, in late infection phases, was completely absent, emphasizing its use for nutrition together with other plant polysaccharides. The observed host wall alterations provide evidence for the secretion and activity of extracellular pectinolytic enzymes *in planta*. From on-section saponification studies, the local reactions specific for interaction were concluded to comprise an enzymatic demethylation mediated by pectin-methylesterases, converting the pectin into the appropriate substrate of endo-polygalacturonases for final degradation (Figure 3, C and D). While the analysis of the pectin-methylesterase is a matter of future research, two genes, putatively encoding two endo-polygalacturonases, have already been isolated from *C. purpurea* and were shown to be expressed during infection of rye (Tenberge *et al.,* 1996a). This strongly indicates the fungal origin of the pectinolytic activities found in infected ovaries by Shaw and Mantle (1980a).

The cellular junctions were shown to consist of high amount of unesterified pectin. Therefore, polygalacturonase activity seems to be a well fitting means to enable an entry into the middle lamella from the intercellular spaces which is not continuous along the infection route towards the rachilla. The outer cellular junctions in epidermal cells were rich in homo-polygalacturonan, too, clearly representing the right conditions for an entry into the anticlinal epidermal walls,

which were usually selected for the primary penetration of the host dermal tissue as concluded from carefully directed hyphal branching above those sites (Tudzynski *et al.,* 1995). During primary penetration, degradation of host pectin could be detected (Tenberge *et al.,* 1996a, b), however, elicitation of host defence reactions causing incompatible interactions has not been observed. Since pollen tubes secrete pectic enzymes during tip-growth (Derksen, 1996), too, one can speculate that modifications of host pectin by fungal pectinases does not betray the pathogen présence.

The ergot fungus actively penetrates plant cell walls to produce an endotrophic mycelium during the primary penetration of the epidermis, the colonization of the mesophyll (Figure 3A) and later for the tapping of xylem vessels. At the interface of intracellular hypha, the host cell wall is obviously lacking, as seen in TEM (Tudzynski *et al.,* 1995). Since grasses have developed a special cell wall type containing low amounts of pectins and considerably high amounts of xylans in addition to the major polysaccharide portion of cellulose (Carpita and Gibeaut, 1993), xylanases and cellulases as well as pectinases are expected to be necessary for breaking down the major cell wall components during infection. At the host-pathogen interfaces of intracellular hyphae (Figure 3B), and additionally of intercellular hyphae, a lack of ß-1,4-glucan in host cell walls has been found with the use of a specific enzyme-gold probe, pointing to the enzymatic action of cellulases in ergot infection (Tenberge and Tudzynski, 1994). Correspondingly, a putative cellobiohydrolase gene (*cel*1) has recently been isolated from *C. purpurea* and was found to be induced during the first days of infection of rye (Müller *et al.,* 1997; see chapter 4 in this volume). Therefore, this cellobiohydrolase may be involved in the penetration and degradation of host cell walls by depolymerising plant ß-1,4-glucan as is characteristic of true cellulolytic fungi.

ß-1,4-Xylan, i.e., the substrate of the fungal ß-1,4-xylanase used in the enzyme-gold technique, has been localized in rye ovary cell walls throughout the infection route (Giesbert *et al.,* 1998), confirming that this major cell wall component in grass leaves is in fact a structural compound in ovary cell walls. The ß-1,4-xylan is expected to represent only the backbone of the typical grass heteropolysaccharid, glucuronoarabinoxylan (GAX). Arabinofuranosyl epitopes, one of the possible side chains in GAX, were localized in ovary cell walls (Giesbert *et al.,* 1998). Giesbert and Tudzynski (1996) isolated a xylanase gene (*xyl*1), cloned another putative xylanase gene (*xyl*2) and proved their expression during parasitic culture of *C. purpurea* of rye (Giesbert *et al.,* 1998; see chapter 4 in this volume). Using three different heterologous antibodies in tissue printing experiments, the secretion of ergot xylanases in axenic culture and during infection of rye has been localized *in situ* (Giesbert *et al.,* 1998). Currently, the assumed xylan alteration during infection is being investigated.

While in necrotrophs the release of cell wall-degrading enzymes results in tissue maceration and host cell death immediately ahead of invading hyphae

(Parbery, 1996), in the biotrophic *C. purpurea,* secretion of these enzymes causes no such drastic effects but only limited damage to the host during colonization. To restrict the enzymatic action to an adequate but limited area, the fungus might control the physico-chemical properties of the interface (Tenberge *et al.,* 1996a).

In conclusion, these different cell wall-degrading enzymes appear to be essential for the preparation of the infection court and the establishment of infection. The cell wall material is thought to be important for nutrition during colonization of the ovary supported by their complete use and also because cell wall extracts of ears stimulated growth in culture (Garay, 1956). In order to evaluate the importance of enzymes for ergot pathogenicity, deficient mutants have been created by targeted gene replacement (Giesbert *et al.,* 1998; see chapter 4 in this volume) which are currently being investigated microscopically.

A novel finding in fungal phytopathology and particularly in ergot was the detection of a fungal catalase secreted in axenic culture of *C. purpurea* and most likely during infection of rye (Tenberge and Tudzynski, 1995; Garre *et al.,* 1998a, b). Catalase activity has been measured in axenic culture of *C. purpurea* (Tenberge and Tudzynski, 1995). Isoelectric focusing together with diaminobenzidine (DAB)-mediated activity staining showed the présence of a specific catalase in parasitic culture of *C. purpurea* as well as in infected ovaries and in honeydew and that it is likely induced during infection (Garre *et al.,* 1998b). Electron dense deposits have been found *in situ* in multivesicular bodies as well as in cell walls of hyphae from axenic culture (Figure 4) and during infection of rye if using ultrastructural enzyme-activity staining with DAB but not after inhibition with aminotriazole, indicating catalase activity (Garre *et al.,* 1998b). Putative catalase proteins have been immunogold localized in axenic (Figure 4) and parasitic culture using different heterologous antibodies (Tenberge and Tudzynski, 1995; Garre *et al.,* 1998b). It has been shown therewith that the detected antigen is secreted via fungal multivesicular bodies into the fungal cell wall diffusing further into the adjacent host apoplast at the host-pathogen interface exclusively. Moreover, one catalase gene comprising a putative signal sequence has been isolated from *C. purpurea* and shown to be expressed during infection of rye (Garre *et al.,* 1998a; see chapter 4 in this volume) providing further evidence for the fungal origin of the detected catalase and the possibility of a functional analysis by gene replacement experiments. Since infection-induced H_2O_2 production has been shown to occur outside host cells (Mehdy, 1994), we suggest a multiple function of fungal catalase in pathogenesis due to its hydrogen peroxide decomposing activity: (a) Cytotoxic effects to fungal cells are prevented, (b) Mechanical barrier formation during host defense reaction, i.e., H_2O_2-mediated cross-linkage of cell wall components during oxidative burst or lignification, are suppressed, (c) In particular, in grass cell walls, a phenolic cross-linkage of polysaccharides is supposed to occur while cell walls expand

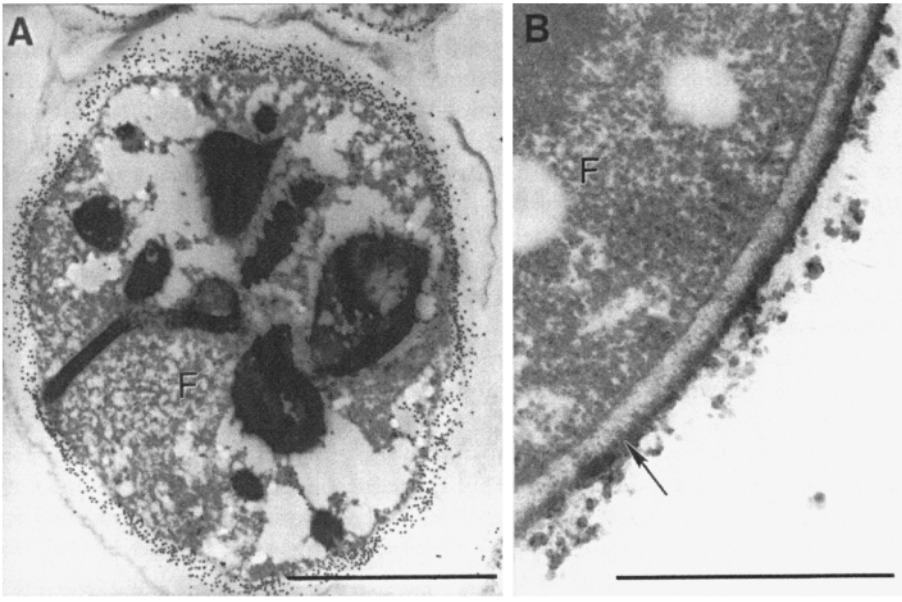

Figure 4 TEM micrographs of *C. purpurea* showing hyphal cells from axenic culture. **A,** Immunogold localization with a polyclonal anti-catalase antibody, raised against native sunflower catalase (Tenberge and Eising, 1995), and protein A-gold showing gold label for catalase-like proteins in the fungal cell wall. Preimmune controls lack gold particles (not shown). **B,** Activity staining for catalase with diaminobenzidine showing moderate electron dense deposits throughout the fungal wall and strong depositions at the wall periphery (arrow). Controls with aminotriazole for catalase inhibition lack electron dense deposition (not shown). Scale bars=1 μm

during their ontogeny causing high cell wall rigidity (Carpita and Gibeaut, 1993). By suppression of those reactions, ergot fungi might maintain a convenient habitat for colonization. In the rachilla, infection induced cross-linkage of phenolics (see above) forestalls fungal colonization and, therefore, might efficiently control fungal growth.

Direct Tapping of the Vascular Nutrition Supply

Plant synthates, primarily intended for the developing seed, are the main nutrition source to the fungus (Mower and Hancock, 1975) which obviously is exploited at about 5 dpi depending on the ergot species. To use this natural sink, several enzymes are secreted such as the cell-wall bound inducible fructosyltransferase (invertase) (Bassett *et al.*, 1972; Taber, 1985; Tudzynski *et al.*, 1995) and the fungal foot is developed structurally for attaching and absorbing (Luttrell, 1980). While intense exudation of honeydew is reported to occur without penetration

of vascular cells, e.g., in *Pennisetum americanum* infected by *C. fusiformis* (Willingale and Mantle, 1987a), tapping of the vascular traces in rye by intracellular hyphae of *C. purpurea* has been documented (Luttrell, 1980; Tudzynski *et al.,* 1995).

The cytological basis of assimilate flow is an ectotrophic mycelium with limited endotrophism in the host phloem, which appears to be unchanged in vitality. In sharp contrast to uninfected ovaries, however, common phloem callose was not found in infected ovaries at all or was distinctly reduced as outlined by Tudzynski *et al.* (1995). This unblocking of sieve elements may be the reason for increased flow of assimilates to the infected floret causing limited growth of neighbouring seeds. The current opinion of the mechanisms is that ergot fungi enzymatically degrade the phloem callose by secreting ß-1,3-glucanases, which have been purified from axenic cultures of *C. purpurea* (Dickerson and Pollard, 1982; Brockmann *et al.,* 1992). Using immunofluorescence, Dickerson and Pollard (1982) localized ß-1,3-glucanase in the sphacelium but not before the tenth day after inoculation. Recently, the callase has been immunogold localized throughout the colonization phase. Detection of antigens in the fungal secretion pathway proved the fungal origin of the ß-1,3-glucanase activity found in infected ovaries and honeydew (Tenberge *et al.,* 1995). Furthermore, immunogold electron microscopy documented that the secreted enzyme is diffusing into the host apoplast up to the host periplasmic area, which is the site of host callose deposition. Cross-reactivity of the antiserum produced by Dickerson and Pollard (1982) with callase from *C. fusiformis* (Willingale and Mantle, 1987b) indicates that this enzymatic action might be used in other ergot fungi, too. In addition, it is likely used to suppress callose deposition during potential defence reaction of the host, because only limited aniline blue fluorescence for callose has been detected in the ovary wall during its colonization with *C. purpurea* (Hambrock *et al.,* 1992).

2.4.3. Sphacelial Stromata for Secondary Propagation

The sphacelial stroma is evident between 4 dpi, e.g., in *C. fusiformis,* and 6 dpi depending on ergot species and sporulation ceases approximately 11 dpi (Luttrell, 1980). The sphacelial plectenchyma is formed intercalarly (Tulsane, 1853) and accumulates lipids later in this phase. These filamentous hyphae are of the type normally found in axenic culture. At the base of the ovary, proliferation of the fungal cell starts (Kirchhoff, 1929); hyphae accumulate beneath the host cortical layers and break through the epidermis towards the ovarian outer surface (Luttrell, 1980). Finally, fungal cells cause ovary replacement, which is not necessary but a consequence of acropetal development (Willingale and Mantle, 1987a). Phialidic conidiophores emerge from the sphacelial stroma possibly favoured by ergot hydrophobins (see above). Numerous oblong conidia are produced (Figure 1). These conidia do not germinate in the honeydew, which is excreted simultaneously, due to high osmotic pressure (Kirchhoff, 1929; Taber,

1985). Some isolates of *C. purpurea* do not produce normal exudates of honeydew, only a few spores were detected later. However, these strains produced normal sclerotia (Mantle, 1967) indicating that abundant formation of honeydew is important for secondary infection but appears not to be a necessary prerequisite for sclerotial formation. In some species, macroconidia are produced that are able to germinate in honeydew and subsequently conidiophores emerge for the production of microspores (see above).

2.4.4. Ergot Sclerotia

After the secondary conidiation has ceased, the sclerotia start growing. Within about three, e.g., *C. paspali* (Luttrell, 1977), up to five weeks post inoculation, e.g., *C. purpurea* (Kirchhoff, 1929), the maturity of sclerotia is achieved. In general, morphology and anatomy of sclerotia is species-specific. They measure 2–50 mm in length and a few millimetres in diameter. In *C. purpurea,* they are oblong, in *C. fusiformis* spherical with large variation at different location (Thakur *et al.,* 1984; Chahal *et al.,* 1985). Sclerotia clearly grow epiphytically on top of the ovary stalk and they mostly emerge out of the florets. Since the sclerotia are no longer enclosed between the glumes, energy must be provided to protect them from desiccation, UV radiation and mycoparasitism (Parbery, 1996). The outer rinds get naturally pigmented resulting into different sclerotia colours, such as dark-purple in *C. purpurea* (Luttrell, 1980), red-brownish in *C. sorghi* and *C. africana* (Frederickson *et al.,* 1991), dark-brown in *C. fusiformis* (Thakur *et al.,* 1984), except those of *C. paspali,* which are white-to-brown (Luttrell, 1977).

The differentiation mode of the sclerotium varies between *Claviceps* species. In *C. purpurea,* first at several places within the sphacelium, sphacelial hyphae differentiate into sclerotial hyphae, later at the sclerotial base, sclerotial hyphae are formed directly (Shaw and Mantle, 1980b). The sclerotial plectenchyma develops intercalarly above the stromatic fungal foot by a generative zone as previously did the sphacelium (Campbell, 1958). *C. sorghi* forms an proximal plectenchyma below an extended sphacelial stroma, within which elongation is evident from a thin red core, representing cortical tissue enclosing some of the first differentiating sclerotial medulla (Frederickson *et al.,* 1991). Remnants of sphacelial stroma with conidia as well as the ovary cap may persist on top of the growing sclerotia, but no internal conidiogenous locules were found. Hence, in these species, the sclerotial hyphae are newly formed. *C. africana,* on the other hand, largely differentiates the sclerotium be transformation of the spherical sphacelium with little change in total size (Frederickson *et al.,* 1991).

Purple pigmentation is the first sign of sclerotial development in *C. purpurea* about 12 dpi (Shaw and Mantle, 1980b), but the trigger for the change from sphacelial into sclerotial growth is unknown (Parbery, 1996). It has been speculated that nutrition is a major factor, supported by the transition effect of certain amino acids found in axenic culture (Mantle and Nisbet, 1976). Changes

in cytology of the cells coincide with increasing levels of lipids which are predominantly triglycerides with the fatty acid ricinoleate (Corbett *et al.,* 1974). This increase in total lipid content from 10% to 30% of the dry weight is the first metabolic indicator of the morphogenesis of sclerotial cells (Bassett *et al.,* 1972). The youngest fungal cells of the sclerotial stroma are longitudinally organized, distinct, frequently septate hyphae forming the prosenchymatous region at the proximal end of the sclerotium. Lipid content is evident in these storage cells, thus they are packed with osmiophilic globules in contrast to the differentiating hyphae of the generative zone in the lower ergot region. In the medulla, distal from the prosenchymatous region, fungal cells form a region of a compact plectenchyma which is build out of bulbous storage cells interspersed with narrower hyphae (Shaw and Mantle, 1980b). The absorbing hyphae, which connect the sclerotium to the ovary stalk and form the stable host-parasite frontier, however, are neither of the sclerotial type nor typically sphacelial cells. They exhibit no parallel orientation, lack lipids and contain large vacuoles (Shaw and Mantle, 1980b). A special function of the fungal foot is also suggested by xylanase activity which has been localized in the sclerotial phase at this host-parasite frontier exclusively, using tissue printing experiments (Giesbert *et al.,* 1998).

Sclerotia are the only ergot structure containing alkaloids (Ramstad and Gjerstad, 1955) and the pigmentation of the sclerotial cortex might protect these light sensitive alkaloids (Taber, 1985). In axenic culture, differentiation of the sphacelial-like hyphae into sclerotial-like cells occurs (Kirchhoff, 1929). They accumulate up to 40% of triglyceride/ricinoleate, but cells show no pigmentation and, as in the parasitic state, do not always produce alkaloids (Bassett *et al.,* 1972).

2.5. ERGOT VIRULENCE AND HOST SUSCEPTIBILITY

Different *Claviceps* species cause ergot disease in a scale of crops, e.g., wheat, rye, sorghum and millet with various levels of virulence. To date, knowledge about resistance in the hosts is fragmentary. Resistance is reported only in a few cases, such as spring and durum wheat (Platford and Bernier, 1970). In the experiments with four different *C. purpurea* strains, however, obscure ovary necrosis occurred only in some of the florets, others produced sclerotia in these hosts. Necrotic ovary response also has been found after double inoculation with two different *C. purpurea* strains (Swan and Mantle, 1991). In sorghum, some ergot resistant lines had been identified (Musabyimana *et al.,* 1995). In pearl millet, *Pennisetum glaucum,* a female nuclear genetic factor contributes to high resistance to *C. fusiformis* (Rai and Thakur, 1995). So far, only limited evidence for a gene-determined somatic resistance has been discovered in diverse hosts. In pearl millet, some lines are resistant to ergot, other genotypes expressed

considerably reduced susceptibility to *C. fusiformis* (Willingale *et al.,* 1986). Resistance in these lines, however, is not based on a specific gene-for-gene interaction but on host floret biology. Distinctive ecological and morphological adaptations to pollination determine gaping and therewith affect susceptibility to ergot drastically. Firstly, pollination induces closing of florets and withering of stigma and style. Hence, competition arises between inoculation and pollination, although, admittedly, pollen landing aside of spores on the pistil surface can stimulate conidia germination in *C. purpurea* (Williams and Colotelo, 1975). Since settlement of inoculum on the pistil surface can initiate infection exclusively, the period of exposure determines the infection success. Susceptibility is highest at anthesis and declines rapidly afterwards in sorghum (Frederickson and Mantle, 1988); but in rye, wheat or barley infection with *C. purpurea* remains possible at a lower rate even after fertilization (Kirchhoff, 1929; Campbell and Tyner, 1959; Puranik and Mathre, 1971). Therefore, floret biology is important for epidemiology and control (see below). The smaller the time window of gaping is, the lower the susceptibility of the host is, e.g., in sorghum (Musabyimana *et al.,* 1995). While host male-sterility or protogyny enhances gaping duration and consequently susceptibility (Willingale and Mantle, 1985), cleistogamy is most effective in avoiding ergot, as has been shown for sorghum (Frederickson *et al.,* 1994).

In addition to gaping control, pearl millet (Willingale and Mantle, 1985) and maize (Heslop-Harrison *et al.,* 1985) evolved a pollination-induced constriction of host stylodia. Constriction occurs about 6 h after pollination, rapidly after passage of compatible pollen tubes and after 4 to 5 days of ageing of unpollinated pistils. This controlled mechanical barrier, based on unique pistil anatomy, effectively directs fertilization and produces passive resistance to *C. fusiformis* in some lines of *Pennisetum americanum*. Passive resistance is efficiently accomplished by early pollination and immediate stigmatic constriction response, which is quicker than ergot establishment and, therefore, always blocks infection (Willingale *et al.,* 1986; Willingale and Mantle, 1987a). In contrast, the susceptibility of wheat and rye to *C. purpurea* persists at least a few days after fertilization and withering of the stigmas (Willingale *et al.,* 1986).

The limited reports on resistance could point to special adaptations of the ergot fungi to the monocotyledonous host ovaries. Assuming that the ergot fungi indeed mimic pollen tube growth with the genius of using components of the specific signal exchange of the pollen-stigma interaction, they possibly grow unrecognized in the ovary and completely avoid host defense reaction of any type. This would suggest that the ergot fungi have coevolved, as assumed for anthracnose fungi's use of their host ripening hormone (Flaishman and Kolattukudy, 1994). One can speculate that successful defense reactions producing host resistance might interfere with the basic function of the ovary and, therefore, do not evolve, since no progenies arise.

2.6. TROPHISM AND ECOLOGY

The more precisely fungal-plant associations are studied, the less clear do the original distinctions between biotrophs, necrotrophs and saprophytes become, as profoundly outlined in an outstanding review by Parbery (1996). Since *C. purpurea,* in contrast to some biotrophic rusts, is able to survive for a short time outside living host tissue while growing saprophytically in axenic culture, *C. purpurea* and other *Claviceps* spp. were distinctively classified as belonging to the hemibiotrophs. Since this is conflicting with widely accepted terminology, according to which hemibiotrophs live part of their lifes in living tissue and part in tissue that they have subsequently killed (Luttrell, 1974), the trophism type of *Claviceps* needs to be reconsidered. In addition, other fungal pathogens, such as *Cladosporium fulvum,* grow easily in axenic culture, although belonging to the biotrophs (van den Ackerveken and de Wit, 1995).

Ergot fungi never kill host cells in advance of colonizing with the intention to draw nutrition from the killed cells. Hence, they are clearly no necrotrophs but are biotrophs according to Münch (1929) which colonize and draw their nutrients from living host tissue (see Chapter Histo- and cytopathology of infection). Admittedly, throughout the ergot-infected pistil, host cells die not in advance but only subsequent to fungal exploitation of living tissue and possibly some due to induced senescence. In addition, due to the unique pathogenesis pattern, the ergot fungi proliferate intercalarly in the ovary basis that inevitably results into an separation of the host ovary cap. Nevertheless, the separated and colonized tissue stays alive for a while, possibly with nutritional support from the sugary honeydew. Furthermore, already fertilized ovules can develop on top of sphacelia into mature seeds of normal function (Mantle, 1972). Thus, ergot-grass interactions are classified as belonging to a pathosystem free of necrosis in fully susceptible hosts; cell death is not intended but inevitably induced after a while, similar to early host senescence by other true biotrophs (Parbery, 1996). The onset of fruit ripening and host senescence does not trigger necrotrophic vegetative growth of *Claviceps* species, which would be typical for necrotrophs and hemibiotrophs, but coincides with sclerotia formation for sexual reproduction and survival, hence matching another feature of holobiotrophic fungi (Parbery, 1996). In conclusion, ergots are true holobiotrophs which are, following Luttrell's (1974) terminology, ecologically obligate parasites and in nature obtain nutrients only from living host tissue (Mims, 1991) while managing to maintain host cell viability for extended periods, and serve as sink for plant metabolites (Mendgen and Deising, 1993).

Claviceps species appear to be most efficient biotrophs. They don't establish themselves in living tissue in order to wait for host death to use the remainings. They don't establish pathogenic sinks in organs actually intended for synthate export. Instead, *Claviceps* species likely take advantage of the most common source-sink system for synthate in a host by directly tapping the host's nutrition supply network and exploiting the plant resources in a working sink (see

Chapter Direct tapping of the vascular nutrition supply). However, the fungus likely utilizes principles to maintain the phloem synthate flow. In addition, the parasite might enhance the sink, which is supported by the suppression of seed development in noninfected florets of the same ear. Likewise, the inverse correlation of sclerotia size and number indicates a competition of sinks in one ear.

While being true holobiotrophic parasites, *Claviceps* species are symbiotrophic at the same time. In exchange for a habitat and nutrition, they protect their hosts and thereby themselves against grazing animals. The introduction of *C. paspali* in Italy caused its host, *Paspalum distichum,* to become a widespread weed. In contrast to earlier days the grass was avoided by animal, which might have recognized ergot infected grasses by its typical smell, reminding the animal of negative experience. This is due to the toxic ergot alkaloids which, although representing dispensable secondary metabolites in the pathogenesis, benefit the fungal's survival by supporting its host's survival (Parbery, 1996).

In this respect and regarding the infection path, there exists great resemblance between *Claviceps* species and some relatives in the *Clavicipitaceae,* e.g., *Acremonium* and some other balansoid genera, which are grass endophytes using toxic alkaloids for defense against herbivory, too (Taber, 1985; Cheeke, 1995). Both fungal groups infect florets and get access to the host ovary probably via the stigma. While ergot fungi sometime enter and later destroy the ovule incidentally, some balansoids, e.g., *Acremonium lolii,* immediately get into the ovule but do not interfere with seed development to ensure systemic inoculation (Parbery, 1996). Since they never leave the plant, i.e., they are living totally endophytic, they are systemic symptomless endophytes. However, some of these symbiotrophs are able to grow and conidiate in axenic culture. Between the two ecological forms, pathogenic ergot fungi on the one hand and symptomless protective commensals on the other, there exist intermediates among the balansoids with great similarities in each direction. Like *Claviceps* species, they first grow endophytic and symptomless in the host, but in order to multiply, they produce damaging epiphytic stromata (White *et al.,* 1992). This is accompanied by suppression of anthesis which is easily tolerated by the host but this phytopathogenic potential characterizes these symbiotrophs to be holobiotrophic. The trophism type varies with the host in some species (White *et al.,* 1992). In summary, there is a great body of evidence for the fact that biotrophic pathogenesis offers several ecological advantages, conserved in obviously similar pathogenesis pattern across different taxa. However, it is controversial whether biotrophism is the origin (Parbery, 1996) or a secondary, younger development derived from saprophytism (Luttrell, 1974; Mendgen *et al.,* 1996) during the evolution of trophism types.

The strategies for the parasite's survival covers several highly specialized ecological adaptations interconnected with the biology of the host, (a) Sclerotia

dormancy, germination and ejection of airborne primary inoculum are perfectly located and timed, in particular in case of monogeneric host specificity, corresponding to the brief receptive phase of blooming florets of the annual anemophilous host plant, (b) The fungi are clearly able to penetrate the outer epidermal wall of the host pistil solely, (c) The fungi might mimic the pollen tube growth, including the mechanisms and mutual recognition, (d) Fungal growth mechanisms are likely adapted to the specialized molecular architecture of monocotyledonous cell walls. Colonization is endophytic, mainly ectotrophic with limited endotrophic hyphae and very cautious, hence nearly symptomless. (e) Ergot fungi directly utilize an already working synthate sink. This exclusive niche might compensate the limitations caused by the narrow time window open for infection, (f) Quickly after infection, sphacelial stromata produce anamorphic conidia for secondary infection to ensure the expansion of the fungal population in the field within the small flowering period of the host. In nature, the infectious inoculum consists of several spores from various strains, resulting in sclerotia of heterogeneous composition. Admittedly, *C. purpurea* is homothallic, however, the mixed inoculum might facilitate the hybridization of strains (Swan and Mantle, 1991) and have some evolutive advantages, e.g., adaptation to host range, (g) Alkaloid content creates a mutualistic interaction assuring the survival of the host together with the parasite.

Although the strategies are not fully perceived on the molecular functional level, it is clear that they bring the ergot fungi in full use of three fundamental advantages of parasitism given by Parbery (1996). (a) There is little competition in the host ovary. In addition, *C. purpurea,* for example, can settle on wheat and barley already infected with smuts like *Ustilago tritici* or *U. nuda* (Cherewick, 1953). (b) The habitat is more than large enough with sufficient nutrition to multiply, to spread and to produce a succeeding generation within six weeks, (c) The preservation of exclusiveness and supply of renewable substrate is guaranteed. Ergot fungi are seldom that highly virulent strains causing a complete suppression of host reproduction; some florets remain noninfected and fertile in every ear. Even if epidemic in the field, the fungi only mix sclerotia in the seed. The susceptibility of hosts evolved to an degree that maintains a certain level of infection with ergot. Symbiotrophism is likely the reason for tolerance and is antagonistic to evolution of resistant species. In conclusion, ergot disease appears not to be a severe problem to the plant but to animal and mostly to man in the need for clean crops.

2.7. EPIDEMIOLOGY AND CONTROL

2.7.1. Occurrence and Spread of Ergot Disease

As already mentioned, ergot disease is common throughout the world. Even single species, e.g., *C. purpurea,* are ubiquitous and several forms may occur in

one locality (Swan and Mantle, 1991), producing a mixed inoculum. However, there are recent reports on immigration of individual ergot species into areas not occupied, so far, and, occasionally, ergot epidemics flame up for some reasons. Alternate hosts for *C. purpurea* proliferated on field borders or due to shut-down of arable land; small sclerotia which can not be shifted out during harvest remained in the seed. Increase in ergot severity or natural epidemics were correlated with reduced male-fertility or malesterility in sorghum (Frederickson *et al.,* 1993), in wheat after limited pollen supply (Mantle and Swan, 1995) and in pearl millet (Rai and Thakur, 1995). However, the highly susceptible male-sterile plants were used for hybrid breeding on a large scale. Highly protogynous hosts, e.g., pearl millet, are particularly susceptible to ergot by exposing the stigmas for two to five days before pollination.

In 1991, *C. paspali* was newly recognized in France after ergotism on cattle (Raynal, 1996). In 1995, *C. africana* was discovered on sorghum in Brazil for the first time, causing a widespread and economically important epidemic in commercial forage and hybrid seed production fields (Vasconcellos, 1996), and in 1996, *C. africana* was detected on sorghum in Australia for the first time (Ryley *et al.,* 1996). Ergot is severely epidemic on sorghum in Eastern Africa, i.e., Ethiopia-Swaziland, and in southern India (Frederickson and Mantle, 1988). In the USA, ergot is one of the most serious diseases in Kentucky bluegrass (*Poa pratensis* L.) (Johnston *et al.,* 1996) with up to 504 sclerotia per gram seed, which is 47% ergot by weight in one strain. Between 1991 and 1994, seed replacement amounts up to 0.44% at year's average, making 9% seed loss during cleaning to meet purity standards (Alderman *et al.,* 1996). In 1994, *C. purpurea* was epidemic in eastern Germany with average 10.4 sclerotia per rye ear and up to 10% seed loss during cleaning (Amelung, 1995).

In spite of its large host range, epidemics are in most cases spatially restricted with *Claviceps* species, they don't even threaten nearby cereal crops (Frederickson *et al.,* 1989, 1993). Host floral biology (see above) largely determines epidemics, nevertheless, this indicates rather unfavourable climatic conditions or limited inoculum transmission only at rather low distances. Though maximum ascospore release is perfectly timed with respect to host anthesis (Mantle and Shaw, 1976) and this primary inoculum is wind distributed, it induces only the primary infection focus in the field but is not supposed to contribute much to the disease spread (Mantle, 1988). Spatial distribution of ergot disease is achieved by a secondary inoculum and is thought to be transmitted together with honeydew by rain-splash, insect vectors, physical head-to-head contact between ears and dripping down onto florets inserted below in the same ear (Tulasne, 1853; Engelke, 1902; Kirchhoff, 1929; Swan and Mantle, 1991). However, conidia transmittance, in particular, by insect vehicles is not investigated in detail (Luttrell, 1977; Mantle, 1988) but a matter of current research (von Tiedemann, pers. communication). On the contrary, recent aerobiological investigations into the spread of ergot in semi-arid zones showed that it are not the more infectious macroconidia but the microconidia produced outside of honeydew

during microcycle are effective windborne propagules, which provide the principle epidemiological agent in the transmittance of *C. africana* in sorghum fields (Frederickson *et al.,* 1989, 1993). Higher humidities and lower temperature favour conidiation and drying-off periods support spore distribution by air (Marshall, 1960; Frederickson *et al.,* 1993).

2.7.2. Control Measures

Although the ergot attack is a minor problem or even beneficial to the host, it is a great disadvantage in agriculture and, for certain reasons as discussed above, ergot disease gained strength in recent years. To enhance quantity and, more important, quality of grain and to minimize cleaning expenditure, control of ergot is needed, which has mainly been based on sanitary and cultural procedures (Kirchhoff, 1929) since many years. Protective fungicides have been ineffective or technically infeasible in controlling ergot (Puranik and Mathre, 1971; Thakur and Williams, 1980), because appropriate amounts must reach the ovary surfaces directly before spores landing. Unfortunately, the same is true for the fungicide benomyl, therewith the more feasible application of one systemic fungicide failed, so far. Recently, triazole fungicides have been identified as highly efficient for the control in experimental trials and in seed production fields (Vasconcellos, 1996). Hyperparasites growing on sclerotia of *Claviceps* species, e.g., *Fusarium heterosporum, F. roseum* and *F. acuminatum* are not yet developed for biological control (Chelkowski and Kwasna, 1986; Ali *et al.,* 1996).

Actually executed control measures are the removal of sclerotia from seeds before sowing combined with postharvest deep plowing and crop-rotation (Agrios, 1988). Usually, ergots do not live longer than one year (Taber, 1985). Normally, they do not survive if buried deep in the ground and, 1–1.5 inch below the soil surface, they do not produce functional stromata (Mantle and Shaw, 1976). Sometimes, however, even one and the same ergot can produce functional perithecia in a second and a third season, although their germination rate is much reduced (Kirchhoff, 1929). Cutting of intermediate hosts at field borders, particularly ryegrass or blackgrass, before flowering is recommended (Mantle, 1967; Mantle and Shaw, 1976). In USA, field burning is traditionally used but the control effect is controversial, because sclerotia viability is only markedly reduced above soil by temperatures higher than 100°C (Johnston *et al.,* 1996). Since host floral biology widely determines passive resistance and susceptibility, floral features antagonistic to ergot, e.g., pollination-induced host stigmatic constriction, has been exploited in the breeding of ergot-resistant strains for commercial use (Willingale *et al.,* 1986). Additionally, provision of pollen donor lines in the protogynous pearl millet efficiently reduces ergot attack (Thakur *et al.,* 1983). Inoculation with an ergot strain, which itself turned out to be relatively incompetent in sclerotia formation, could reduce the rate of successful infection of other strains which were already in place (Swan and Mantle, 1991).

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