was still activated, demonstrating that estrogen acts extracellularly. Estradiol can still activate Maxi K^+ channels reconstituted into artificial membranes (provided both α and β subunits are present), indicating that no intracellular signaling is required and that the Maxi K^+ channel is a receptor for estrogen. To corroborate this deduction, Valverde and colleagues performed binding studies. Oocytes expressing Maxi K^+ channels composed of α and β subunits, but not of α subunits alone, bound greater amounts of ^{3}H-estradiol. Human embryonic kidney cells expressing the α/β channels show greater fluorescence after exposure to estradiol tagged with a fluorescent label compared with cells expressing channels containing only the α subunit. These experiments indicate that the direct binding of estradiol to an external site on Maxi K^+ channels—which is available only when the β subunit is present—increases channel activity.

New methods for treating cardiovascular disease are continually being sought. There is accumulating evidence that postmenopausal estrogen-replacement therapy decreases the risk of major coronary heart disease (*1*). However, the benefit of estrogen treatment decreases with long-term hormone use because of the increased risk of breast cancer (*11*). Consequently, tissue-specific, estrogen-like drugs that preserve the beneficial effects of estrogen on the cardiovascular system without having deleterious effects on other organs are needed. The finding by Valverde *et al.* that estrogen directly activates vascular smooth muscle cell Maxi K^+ channels may pave the way for the rational design of new drugs for the prevention of cardiovascular disease.

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PERSPECTIVES: PLANT BIOPHYSICS

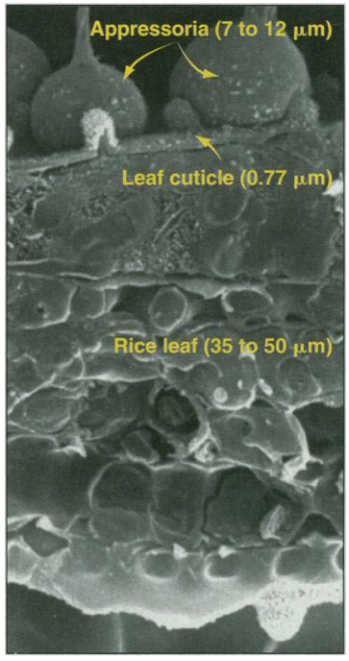
Forcible Entry

Nicholas J. Talbot

When a fungal pathogen lands on a plant leaf, the most obvious obstacle it faces is how to gain entry to the underlying tissue. Unlike bacteria, which have to circumvent the problem by locating stomata (pores in the plant epidermis), wounds, or other natural openings, many fungal species can rupture the cuticle (the tough outer layer of a plant) directly (*1*). How they do so remains controversial (*2*). In the case of some fungi, enzymatic action is clearly visible at the point of infection, suggesting that the plant cuticle is dissolved ahead of the infecting pathogen (*1*). In other species, specialized infection structures called appressoria are formed that can generate high pressures, indicating a mechanical infection process (*3*). On page 1896 of this issue, Bechinger *et al.* (*4*) report that enormous invasive forces are applied by appressoria of a fungal pathogen, directly demonstrating for the first time that appressoria can exert sufficient pressure to enable mechanical infection of plants by fungi. By allowing appressoria to form on an optical waveguide, the forces exerted by the fungal penetration pegs could be visualized and quantified.

Many of the most severe and economically important plant diseases are caused by fungi, and the initial infection processes have been studied extensively to develop effective disease control strategies (*1*). Fungi have evolved many methods for entering plants, including mechanisms for locating stomata and the ability to rapidly colonize wound sites (*3*, *5*). But many fungal species simply penetrate plant cuticles directly, either as threadlike fungal cells called hyphae or, more frequently, by growing specialized appressoria (see the figure) (*3*). Appressoria are swollen, dome-shaped, or cylindrical cells that differentiate from the end of fungal germ tubes and during maturation can further differentiate to produce a thick, rigid cell wall (*3*). Appressoria allow tight adhesion to the plant surface, followed by rupture of the cuticle with a narrow hypha called a penetration peg. During this process, the fungus changes its axis of growth and reestablishes polarized growth as the penetration peg extends into the plant.

The infection process by appressoria can involve enzymatic action, and the external matrix around appressoria often contains cutinase, cellulases, and other nonspecific esterases to help soften the cuticle, thereby aiding adhesion and penetration (*1*, *3*). However, experimental proof of an absolute requirement for enzymatic activity has remained elusive (*2*), and it has been apparent ever since the pioneering work of Miyoshi (*6*) and others that some fungi can physically break their way through plant cuticles.



Breaking in. A spore from the rice blast fungus *Magnaporthe grisea* has germinated on the surface of a rice leaf and formed a dome-shaped appressorium. The appressorium has to breach the thin but tough rice leaf cuticle to invade the leaf and cause disease.

Fungi such as *Colletotrichum* and *Magnaporthe* species produce appressoria with tough melanin-pigmented cell walls (*3*, *7*). Howard *et al.* previously showed that appressoria of *Magnaporthe grisea*, the causal agent of rice blast disease, generate very high internal pressure (turgor) (*8*). A cell collapse assay was used to predict the appressorial turgor of *M. grisea* by calculating the concentration of polyethylene glycol required to collapse an appressorium. Howard *et al.* showed that *M. grisea* appressoria generate pressures of between 6 and 8 megapascals—the equivalent of 30 to 40 times the pressure of an average car tire—an astounding pressure for a cell to generate. Appressoria were also shown to be able to puncture artificial plastic membranes. In the same series of experiments, ap-

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pressorium turgor was found to be essential for plant infection (*8*). When appressoria were incubated in solutions of increasing concentration, their ability to penetrate epidermal cell layers decreased with increasing external solute concentration. The high pressure of appressoria from these fungi is due in part to the melanin layer that forms an inner cell wall layer, especially in mature appressoria. Mutants of *Magnaporthe* and *Colletotrichum* species that fail to synthesize melanin are nonpathogenic and do not accumulate turgor (*7*). These studies strongly implicated turgor generation in plant infection, but direct evidence that appressoria produce enough force to puncture plant cuticles has been lacking until now.

Bechinger *et al.* (*4*) measure the force exerted by penetration pegs from *Colletotrichum graminicola*, a fungus producing similar melanized appressoria to *M. grisea* and the causal agent of anthracnose disease of corn (*9*). The technical difficulty of accomplishing this feat is difficult to overstate. Appressoria from *C. graminicola* are 7 to 9 μm in diameter, and the penetration peg breaching the plant cuticle is only 2 μm in diameter. The invasive force produced by the penetration peg was measured by growing appressoria on an optical waveguide composed of a polydimethylsiloxane membrane sandwiched between two thin films of aluminum. Light was focused on the underside of the waveguide through a prism and the reflected light detected by a charge-coupled device linked to an image processor and computer. The invasive force exerted by *C. graminicola* could be detected because the fungus deformed the waveguide as the penetration peg pushed against it. The resulting alteration in the thickness of the optical waveguide was detected by a change in the intensity of the reflected light. Penetration of the optical waveguide by appressorial penetration hyphae typically produced an indentation of 10 nm. The force necessary to produce this indentation was calibrated by comparison with the action of a glass capillary applying exactly defined forces against the waveguide. Bechinger *et al.* show that *C. graminicola* appressoria can exert an invasive force of 16.8 ± 3.2 micronewtons, a considerable force capable of breaching most plant cuticles. This force is consistent with the generation of enormous cellular turgor inferred by Howard *et al.* for *M. grisea* (*8*). The force exerted by *C. graminicola* increases quite rapidly (within 2 to 3 hours) after maturation of the appressorium and continues to be exerted for several hours thereafter, indicating that the force is not a sudden release of turgor but rather a sustained application of pressure by the penetration peg.

How such large forces are generated by appressoria of these fungal pathogens remains unknown, but two things are apparent. First, appressoria must adhere very tightly to the leaf surface, because if the force of adhesion did not exceed the force of penetration, the cell would simply lift away from the leaf. Second, appressoria of *C. graminicola* must accumulate large amounts of solute. Turgor pressure in appressoria appears to be the result of an influx of water into the cells, which develop within dew drops on the leaf surface. In *M. grisea*, the solute providing the osmotic potential for turgor generation is glycerol, which accumulates to concentrations in excess of 3 M (*10*). The glycerol is held in place, at least in part, by the thick melanin wall layer that prevents leaking of the small lipophilic molecule from the cell (*10*, *11*). The forces exerted by appressoria of *C. graminicola* suggest a turgor pressure of over 5 megapascals. This means that if a single solute accumulates in *C. graminicola*, it must do so to very high concentrations (as in *M. grisea*). Alternatively, the fungus would need to use a combination of solutes to produce the necessary turgor.

How the turgor is translated to physical force and applied at the penetration peg remains unknown (*11*). Localized cell wall dissolution and cytoskeletal reorientation play a role in the process (*7*, *12*), but the genetic and biochemical control of appressorium function is largely unstudied. The optical waveguide technique developed by Bechinger *et al.* (*4*) provides a wonderful opportunity to select mutants that are unable to produce functional appressoria and to identify and characterize the genetic components of this fascinating process.

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PERSPECTIVES: BIOCHEMISTRY

How Serpins Are Shaping Up

Robin W. Carrell

Survival of the fittest is a rule that applies just as much to protein families as it does to organisms. The serpins are a protein family of highly adaptable serine protease inhibitors that have outmaneuvered their many relatives to become the predominant protease inhibitors in human plasma. Their success can be attributed to their ability to trap and irreversibly bind to their target proteases (see the figure) (*1*). Cleavage of the serpin's reactive loop by the protease triggers entrapment and inactivates the serpin. Furthermore, the conformational changes in the serpin that accompany this mousetrap-like action are now known to act as signals for a range of physiological responses (*2*). A striking example of this is reported by O'Reilly *et al.* (*3*) on page 1926 of this issue. They show that small conformational changes in the serpin antithrombin III can profoundly alter its activity—from that of an inhibitor of thrombin and other clotting proteases in plasma to an agent that blocks blood vessel formation (angiogenesis) and induces tumor regression.

Antithrombin differs from other serpins in that it is able to bind (through a cation site on its A and D helices) to heparin-like molecules attached to the cells that line blood vessels. Upon binding to these molecules antithrombin becomes immobilized and undergoes a series of small conformational changes resulting in exposure of the carboxyl-terminal loop (which contains the active site) and a dramatic increase in its affinity for thrombin and other coagulation proteases. Proteases that bind to the heparin-bound antithrombin cleave the exposed loop reversing the conformational changes at the heparin-binding site, with a consequent release of the protease-antithrombin complex into the circulation (see figure, panel A) (*4*). Exactly the same loss of heparin affinity occurs whenever antithrombin undergoes a transition from its active five-stranded structure to an inactive six-stranded form (*5*). This takes place not only when the serpin binds to its protease but also after cleavage of the loop by incidental proteolysis or as a result of a slow spontaneous conversion of antithrombin to its latent form present in trace amounts in

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