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Estimates of biomechanical forces in *Magnaporthe grisea*

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ARTICLE INFO

Article history:

Received 20 January 2006

Accepted 27 March 2006

Corresponding Editor:

Nicholas P. Money

Keywords:

Adhesive energy

Elastic modulus

Penetration force

Yield stress

ABSTRACT

The mechanical actions of the fungus *Magnaporthe grisea* raise many intriguing questions concerning the forces involved. These include: (1) the material properties of the appressorial wall; (2) the strength of the adhesive that keeps the appressorium anchored to the rice leaf surface; and (3) the forces involved in the penetration process whereby a peg is driven through the host cell wall. In this paper we give order of magnitude estimates for all three of these quantities. A simple Young-Laplace law type argument is used to show that the appressorial wall elastic modulus is of order 10–100 MPa; and an adaptation of standard adhesion theory indicates a lower bound on the strength of the appressorial adhesive to be of the order 500 J/m². Drawing on ideas from plasticity theory and ballistics, estimates of the penetration force raise interesting questions about experiments performed on the penetration of inert substrates by the fungus.

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Introduction

The mechanisms by which various plant pathogens penetrate their hosts have long been a subject of interest. Given its often damaging impact on the world's rice crop, *Magnaporthe grisea* (commonly referred to as the rice blast fungus) has been the focus of much attention. Detailed descriptions of its life-cycle and the many and complex biological processes involved have been the topic of a number of review articles in recent years [see, for example, Howard & Valent (1996) and Talbot (2003)]. The initial phase begins with the conidium, which lands on the rice leaf surface and strongly attaches to it by means of spore tip mucilage. A germ tube grows out of the conidium, hooks into the surface of the rice leaf, and begins to form an appressorium. The germ tube is eventually shut off from the appressorium leaving the latter as a separate, independent unit. The mechanisms by which the appressorium is initially built, and the interplay with the initial turgor pressure, are apparently not well understood. Our main concern in this paper is with the mechanical actions associated with the appressorium itself and we give a very brief summary of the

key features relevant to the force estimates described in this paper.

- (1) The appressorium is glued to the host surface by means of a very strong adhesive ring around its base. Although it is not clear exactly how far the glue penetrates into the region between the lower appressorial wall and the host surface, it clearly extends no further in than the appressorial pore region (the inner region of contact between the appressorium and host surface) as shown in Fig 1.
- (2) A melanin layer, perhaps 100 nm thick, develops within the appressorium (but not over the appressorial pore). A typical electron micrograph (Fig 1) suggests that the overall appressorial wall thickness is of order 0.1 μm . The permeability properties of the melanin layer are such that significant internal pressures can build up (Howard & Ferrari 1989). Experimental evidence (Money & Howard 1996) indicates that although the appressorium may exhibit (initial) turgor pressures of the order 3–5 atm, there can be significant increases in the pressure after melanization.

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doi:10.1016/j.mycres.2006.03.014

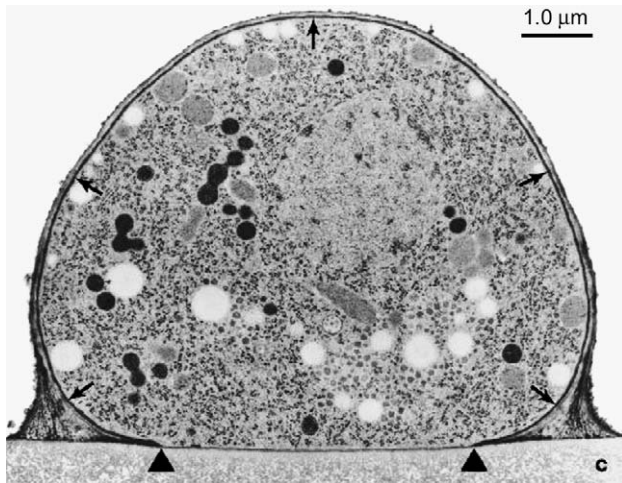


Fig 1 – A typical appressorial structure before peg penetration [reproduced with permission from (Howard & Valent 1996)]. The two arrowheads at the base of the appressorium indicate the range of the appressorial pore. The dark inner circle, indicated by the small arrows, is the melanin layer. The photograph indicates an appressorial radius of approximately 3 μm.

- (3) Experiments have been performed in which the fungus is grown on inert substrates, and studies performed by Howard *et al.* (1991) with Mylar[®] sheets [which are composed of poly-(ethylene terephthalate) fibre] suggest that pressures as high as 80 atm (8 MPa) can be achieved. Based on optical methods, Bechinger *et al.* (1999) reported an invasive force of approximately 17 mN (5.35 MPa) exerted by the appressoria of *Colletotrichum graminicola*.
- (4) After a period ranging from tens of hours to a few days, during which time turgor pressure builds up, a penetration peg is driven through the surface of the rice leaf. Although some enzymatic degradation of the host surface may be involved, the fact that peg penetration can occur on inert surfaces such as Mylar strongly suggests that mechanical force plays a significant role in this process, especially as the indications are that harder Mylar surfaces require higher pressures for penetration to occur (Howard *et al.* 1991). Typical peg diameters are of the order of 0.7 mm and, depending on the host surface, penetration can be of the order of 3 mm; and on some surfaces much further [see Koga (1994) for a variety of peg penetration micrographs].

There are many intriguing mechanical features associated with the high turgor pressures that can develop in the appressorium. These include the observations that: (1) there does not appear to be any significant change in the size of the appressorium after large increases in turgor pressure; (2) the adhesive is strong enough to keep the appressorium firmly anchored to the rice leaf (or other host surface) at all times; and (3) higher pressures are apparently required for the penetration of harder surfaces.

Using simple dimensional analysis and scaling arguments, and the basic physical principles governing the elasticity of thin shells, adhesion theory, the hardness of materials,

plasticity theory, and ballistics, we give some order of magnitude estimates of the material properties and forces involved in the appressorial processes. These estimates are based on standard arguments commonly used in the physical sciences. This approach can be illustrated by considering the properties of an elastic material. For a homogeneous and isotropic elastic material the classical stress–strain relations for (linear) elasticity are such that the shear stress, σ_{xy} , is related to the corresponding strain, u_{xy} , according to (Landau & Lifshitz 1986)

$$\sigma_{xy} = \frac{E}{1 + \sigma} u_{xy},$$

where E is the elastic modulus of the material, and σ is the Poisson ratio. The strain and the Poisson ratio are dimensionless quantities of order one. Indeed, in linear elasticity theory the strains are typically assumed to be much smaller than one. For classical materials $0 \leq \sigma \leq 1/2$ with σ being closer to $1/2$ for rubber-like materials. As the only dimensional quantity on the right-hand side of the above formula is the physical parameter E , and given that the strains and Poisson ratio are order one quantities, the overall scale of the stress is thus set by the size of E and is relatively insensitive to the precise value of σ . (In the case of a Poisson ratio close to $1/2$ the principal stresses, such as σ_{xx} may become large, but to within an order of magnitude the scale of the stresses is still set by the scale of E .) Thus we state, when estimating orders of magnitude, that the stresses ‘scale like’ E (and hence the notation used below of $\sigma \sim E$). The same argument still holds for a nonlinear elastic material: although the stresses may now be complicated nonlinear functions of the deformation gradient (the strain) the overall scale of the stresses is set by the order of magnitude of the (nonlinear) elastic moduli.

Material properties of the appressorial wall

In a recent study (Tongen *et al.* 2006) we undertook a theoretical and numerical study of the basic appressorial architecture using elastic shell theory. Our studies indicated that typical appressorial structures under moderate turgor pressures were well modelled by a neo-Hookean material (essentially a ‘linear’ elastic material). However, under higher pressures the shape could only be maintained by a material able to exhibit considerable strain stiffening, i.e. a nonlinear elastic response. To set the scales in our model an estimation of the elastic and bending moduli of the appressorial wall is required. An order of magnitude estimate can be obtained from the Young-Laplace law for a spherical membrane under pressure; namely the relationship between the pressure difference ΔP across the membrane wall, the sphere radius R , and the membrane stresses, which we express in the form

$$\Delta P = \frac{h(\sigma_{\theta\theta} + \sigma_{\phi\phi})}{R}, \quad (1)$$

where h is the membrane thickness and $\sigma_{\theta\theta}$, $\sigma_{\phi\phi}$ are, respectively, the meridional (longitudinal) and azimuthal (hoop) stresses¹. Because of the spherical symmetry $\sigma_{\theta\theta} = \sigma_{\phi\phi}$.

¹ Here we recall that stress is defined as force per unit area and tension as force per unit length. Thus, for a spherical bubble, equation (1) can be written in the familiar form $\Delta P = 2T/R$ where T is the surface tension.

To a first approximation these stresses scale as $\sigma \sim E$, where E is the membrane's elastic modulus. The experimental data indicate a typical appressorial radius of order 3 mm and a possible range for the turgor pressure of $\Delta P \sim 1\text{--}8$ MPa. Using a wall thickness estimate of $h \sim 0.1$ μm , the Young's modulus E is thus estimated to be of order of magnitude

$E \sim 10\text{--}100$ MPa.

This estimate of E is consistent with that of many other biological materials shown in the elastic moduli atlases of Ashby *et al.* (1995). We also note that this estimate is based on a linear constitutive relationship, so if the radius of the appressorium stays approximately constant under an increase in turgor pressure, the corresponding estimate of the elastic modulus increases proportionately. Thus, if the turgor pressure increases five-fold and the appressorial radius is, to a first approximation, unchanged, the elastic modulus (which is really the local slope of the stress/strain plot) of the wall could be considered to have increased five-fold. In this sense the appressorial wall could be considered a 'smart material'; namely adjusting its properties in response to a change in its local environment.

Elastic shell theory (Landau & Lifshitz 1986) also tells us that the bending modulus for a thin sheet is given (in the framework of linear elasticity theory) by the relation $B = E h^3 / (12(1 - \sigma^2))$, where σ is the Poisson ratio. Although not currently known for the appressorial wall, we assume $\sigma = 1/2$, i.e. an incompressible material. This leads to the estimate

$B \sim 10\text{--}100 \times 10^{-13}$ Nm

Clearly bending effects will only be significant if the cell wall exhibits regions of high curvature. These types of estimates for E and B have been used by Boudaoud (2003) to analyse morphological scaling laws for a large class of microorganisms, and used to distinguish families that are tension dominated (where E is the significant parameter) and bending dominated (where B is the significant parameter).

Adhesive forces

Without very strong adhesion to the host surface, the high turgor pressures that develop in the appressorium would cause it to break contact with that surface (essentially deforming into a sphere with a point of vanishing contact). To date, little progress has been made in analysing the structure of the adhesive or its action. Here, using elementary ideas from adhesion theory, we attempt to give a simple estimate of adhesive strength necessary to keep the appressorium attached to the host surface.

The basic picture we employ is one in which the internal appressorial pressure provides an effective 'peeling force' that tries to lift the base of the appressorial shell off the substrate surface. Standard theory [see, for example, Kendall (1975)] for thin-film peeling gives

$$\gamma = \frac{F^2}{2b^2hE} + \frac{F}{b}(1 - \cos\psi), \quad (2)$$

where γ is the adhesive energy (of the film/substrate interface), F the applied force, b the film width, h the film thickness, E the film's elastic modulus, and ψ the peeling angle (see Fig 2). This equation represents the critical balance (per unit area) between

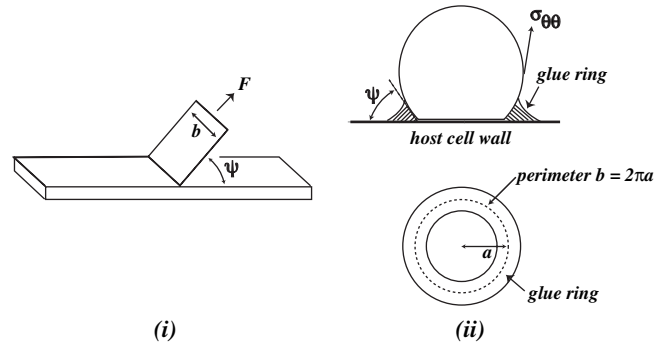


Fig 2 – (i) Schematic representation of peeling model embodied in eqn (2). (ii) For the appressorium the meridional stress $\sigma_{\theta\theta}$ plays the role of the peeling force. The glue film 'width' is estimated as the mean glue ring perimeter, $b = 2\pi a$, leading to $(F/b) \sim \sigma\pi a^2/2\pi a = \sigma a/2$.

the adhesive energy, the elastic work done by the applied force deforming the film elastically [the first term on the right-hand side of (2)], and the peeling work [the second term on the right-hand side of (2)]. Thus in controlled experiments, say in which F and ψ are measured, this equation can be used to determine γ . This well-established model shows us that at the level of dimensional analysis, the peeling work done (per unit area) scales as σa where σ is the applied stress (which here is taken to be the meridional stress $\sigma_{\theta\theta}$) and a is the length scale of the adhered region; and that the corresponding elastic work done scales as $\sigma^2 a^2 / 2 h E$. We also note that in the above model (2) there is direct adhesive contact between the film and the substrate, whereas here it is mediated by a (nonuniform) layer of glue that provides the adhesion. Again using the fact that σ scales as $\sigma \sim E$ we take $\sigma \sim E \sim 50$ MPa, the contact length scale to be $a \sim 1.5$ μm and an appressorial wall thickness (the elastic film thickness in this model) of $h \sim 0.1$ μm . With these estimates we find that

$$\frac{\sigma^2 a^2}{2hE} \sim 500 \text{ N/m}, \quad \text{and} \quad \sigma a \sim 75 \text{ N/m},$$

indicating that for these parameter choices the elastic work is significantly bigger than that due to peeling. Overall, we can now make the order of magnitude estimate

$$\gamma \sim 500 \text{ J/m}^2.$$

It is important to note that this estimate represents very much of a lower bound on the strength of the appressorial adhesive and, given the great stubbornness with which the appressorium stays attached to the host surface, γ could easily be significantly higher. The current estimates of γ are comparable with the adhesive energy of various adhesive tapes (Gent & Kaang 1986), but orders of magnitude less than epoxy glues. Thus although our estimate indicates that the appressorial adhesive is quite strong, it does not appear to be in the 'super-glue' category.

Penetration forces

Perhaps the most intriguing of the appressorial processes is peg penetration. At the level of mechanics this process is

probably best understood within the framework of plasticity theory (Hill 1950) and an understanding of the hardness of materials (Tabor 1951). We first recall the basic ideas of hardness testing in which a small indenting tool (with a spherical tip in the case of the Brinell test, and a pyramidal tip in the case of the Vicker test) is pressed, under a known load, W , into the test surface. The ‘hardness’ of the material is defined as the ratio of the load to the projected area (onto the test surface plane) of the indentation. When the indentation is permanent its formation is a result of a plastic yielding of the material, and in principle, these tests can be used to determine the yield stress, Y , of the material. As plastic deformations are primarily the result of shear, and the load in the hardness test is applied normally, the relationship between the loading and shear stresses has to be determined. This can be achieved through the theory of slip-lines (Hill 1950, Tabor 1951) and the net result, depending on the indenter geometry and choice of plastic yielding criterion, is that typically $W \sim 3Y$. Thus for the Mylar sheets used by Howard *et al.* (1991) the authors reported hardnesses ranging from 140–250 MPa, indicating yield stresses ranging from 50–80 MPa. In the discussion that follows it should be emphasized that the concepts of plasticity theory and the associated notions of yield stress, slip-lines, etc. have been primarily developed for ductile materials like metals. In the case of biological materials, where the structure is very different from the regular crystal lattice structure of metals, many of the equivalent plasticity concepts have yet to be fully understood.

For deep penetration into ductile materials, models based on plasticity theory (Bishop *et al.* 1945) indicate that loads of four to five times the yield stress are required. However, in the case of deep penetration of some plastics that are subject to fracture the formation of a micro-fracture zone around the penetrator can reduce the required load by a factor of two or three (Wright *et al.* 1992). In the case of penetration of rubber-like materials (including mammalian skin) a more appropriate modelling may be in terms of fracture initiated by the penetrator, and a nonlinear elastic response of the material to accommodate the penetrator after fracture has been initiated (Stevenson & Abmalek 1994). However, in these models the ‘wound’ closes up elastically after the penetrator is removed. As the penetration peg leaves a permanent hole this last scenario does not appear to be relevant to our discussion.

The mechanical work done in penetrating a target surface is considered to be the key quantity in ballistic studies (Taylor 1948). Accordingly, our challenge is to estimate the work done in the peg penetration process (Fig 3). Although reminiscent of the ballistics problem, the slow time scale over which the penetration occurs means that kinetic and dissipative effects (Thomson 1955) can be neglected; although we note that even in the ballistics context these do not appear to be dominant effects (Woodward 1978a). The work done per unit volume in a mechanical deformation is

$$\int \sigma_{ij} d\epsilon_{ij},$$

where σ_{ij} , ϵ_{ij} are the stress and strain tensors respectively. Although a determination of the strain profile during the penetration process is a nontrivial problem, and involves different considerations depending on whether the penetrated sheet is

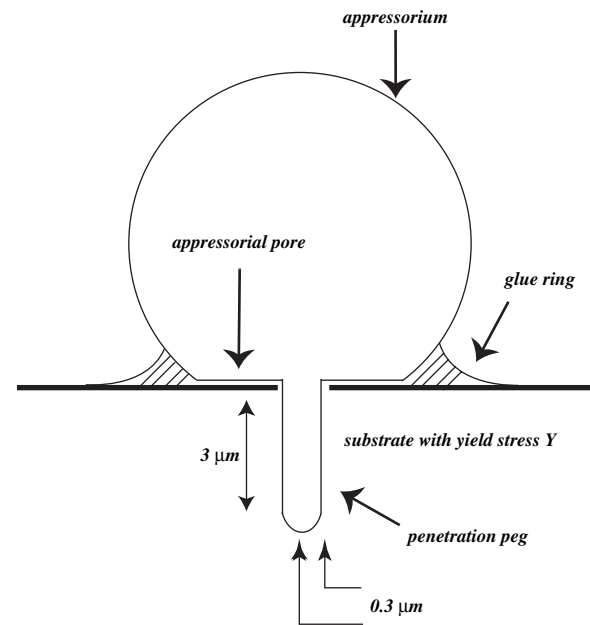


Fig 3 – Schematic representation of penetration process.

thick or thin relative to the penetrator radius (Taylor 1948, Woodward 1978b), a simple estimate of the total work done, W_d , during penetration can be made by assuming that the host substrate yields plastically, i.e. $\sigma_{ij} \sim Y$, and hence to a first approximation,

$$W_d \sim YV,$$

where V is the volume of substrate displaced in the course of the penetration (which we will estimate as the peg volume). In fact, this estimate is quite close to the more precise one calculated for thick armour penetration (Woodward 1978a). Thus for $Y \sim 80$ MPa, and penetration peg dimensions of radius $\sim 0.3 \mu\text{m}$ and length $\sim 3 \mu\text{m}$, and hence a volume $V \sim 1 \mu\text{m}^3$, we estimate

$$W_{\text{penetration}} \sim 8 \times 10^{-11} \text{ J}$$

It should be born in mind that this estimate is linear in the yield stress. Thus, to a first approximation, to make the same sized penetration in a sheet of hardened steel with a yield stress of $Y \sim 1200$ MPa (Woodward 1978a) would involve work of order $W_{\text{penetration}} \sim 1.2 \times 10^{-9}$ J.

Returning to the experimental work of Howard *et al.* (1991) a somewhat puzzling point needs to be observed. Indentation requires normal loads of (at least) the order of the hardness. In the case of the Mylar sheets used, their hardness was reported to be 140–250 MPa, yet the apparent turgor pressures (which provide an estimate of the normal load driving the penetration peg) achieved in these experiments is of order 8 MPa. Various explanations of this apparent mismatch of forces include an overestimate of the Mylar hardness, an underestimate of the turgor pressure, or the involvement of some other agent able to degrade the substrate and facilitate the penetration. This conundrum has also been noted by Money (1999). A revisiting of the Mylar hardness measurements would be a helpful first step in resolving this issue. We also note, as discussed earlier,

that many of the ideas associated with plasticity and penetration mechanics are only properly understood for ductile materials and the application of these ideas to biological and other materials (such as Mylar) requires further justification. The apparent mismatch between the substrate hardness and the turgor pressure couples to another puzzle: namely that in any indentation process it is assumed that the indenter is harder than the material it is penetrating. One imagines that the penetration peg produced by the fungus is in the form of a pressurized, cylindrically shaped, biological membrane. However, the presence of high pressure within the peg is, by itself, not enough to ensure sufficient 'hardness' of the peg itself. If the material properties of the peg wall are such that it has a lower yield stress than the substrate (and one could imagine this to be the case in the experiments on Mylar), then as it is pushed against a harder substrate the membrane itself can undergo plastic yielding, possibly to the point of rupture. Whether the peg membrane undergoes stress-induced rigidification during penetration is an intriguing question.

Acknowledgements

This work is supported by the NSF grant DMS-0307427. The authors thank Nicholas P. Money for a number of valuable conversations, and also thank Anthony Tongen, Arezki Boudaoud and Axel Buguin for helpful discussions at the beginning of this project.

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