Evaluating the explosive spore discharge mechanism of *Pilobolus Crystallinus* using mechanical measurements and mathematical modeling

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Abstract

The coprophilous Zygomycete *Pilobolus* uses osmotic turgor pressure as a means of explosive spore discharge, shooting its sporangium up to several meters away from the sporangiophore. This study attempts to clarify the sporangial discharge model by offering the first mechanical measurements of the internal turgor pressure of *Pilobolus*. In order to account for the forces included within the hypothesized shooting mechanism, a mathematical model is used to model the sporangial projectile trajectory. Measurements of subsporangial turgor pressure using a miniature strain gauge were partially successful, showing a pressure of .11 MPa. The mathematical model found that a pressure of .474 MPa would be needed to propel the projectile over the average measured distance, 1.14 m. The difference between the measured and the predicted results is attributed to error within the mechanical measurements. These results emphasize the importance of using innovative and rigorous methods for measuring internal turgor pressure, and encourage further examination of the *Pilobolus* spore discharge apparatus.

Introduction

The unfortunate immobility of the fungi is a limiting factor for their dispersal and distribution. Their sessile state generally leaves two methods of expansion: growth into an adjoining area or dispersal of spores. The latter is often the more feasible option. Fungal spores are lighter and smaller than plant seeds, and consequently encounter increased effects from drag (Vogel 2005). Most fungi do not grow tall enough to effectively send their spores through the sluggish boundary layer of still air surrounding them. Some have adapted to this situation by taking advantage of environmental forces such as wind, water flow, or animal transport (Ingold 1953).

Other fungi have adopted a more active approach, forcibly discharging their spores through the boundary layer. Many ascomycetes explosively shoot meiospore

clusters. As spores mature, water is absorbed by the ascus and insoluble materials are converted to soluble materials, resulting in an increase in internal pressure. When a critical pressure is reached, the spores and cytoplasm are explosively shot away from the ascus (Macdonald, Millward et al. 2002). *Sordaria* shoots its spore clusters over 60 mm, enough to propel it through the surface boundary layer (Ingold and Hadland 1959). Ballistospores of basidiomycete fungi are thrust away from the basidium and into the air flowing along the undersurface of the mushroom cap. This is achieved by the use of a surface-tension catapult which subjects the ballistospore to an acceleration of greater than 25,000 g (Money 1998).

Pilobolus, a fungus assigned to the order Mucorales, is the only member of this order of terrestrial Zygomycetes that makes use of an explosive spore discharge mechanism. All species of this order are coprophilous, occurring on the dung of herbivorous, grazing animals. *Pilobolus* was initially found on horse dung and described by Cohn in 1851 (Buller 1934). The genus is now known to be widely distributed throughout the world, having been isolated from such diverse sources as caribou dung in Alaska and wallaby dung at the southern tip of Australia (Page 1962).

The coenocytic mycelium of the fungus initially grows beneath the surface of the dung substratum. Prior to the formation of the fruiting body, the mycelium begins to form trophocysts which are isolated from the rest of the hyphae through the formation of crosswalls. A solid hypha, called the sporangiophore, emerges from the trophocyst and elongates upward through the medium into the air. Sporangiophores at this stage exhibit marked phototropism, allowing them to grow out of crevices and away from the substratum (McVickar 1942). Fruiting bodies in later stages of development exhibit more

precise phototropism, guaranteeing that the sporangiophore will be oriented toward the sun or open sky (Foster 1977).

After the sporangiophore grows to its full height (2-3 mm), the tip of the sporangiophore swells to form a conspicuously bulbous subsporangial swelling, inside which a considerable osmotic pressure is generated by ion-rich cell sap. On top of the sporangiophore rests a small cap-like structure, the sporangium, which contains between thirty to sixty thousand multinucleate spores (Buller 1934). Immediately prior to discharge, the upper wall of the subsporangial swelling ruptures along a line of weakness just below the sporangium. The wall of the swelling and the stipe contract elastically, propelling the sporangium away from the sporangiophore by a jet of cell sap that shoots into the concave under-surface of the sporangium (Page 1964). The sporangiophore then collapses to the surface.

If at the end of its trajectory the sap-covered projectile lands on a blade of grass, the sticky sporangium adheres to it. When a grazing animal happens to ingest a blade of grass with sporangia, the thousands of spores pass through its digestive tract unharmed and emerge in the dung from the other end. Mycelia grow from these spores, develop into sporangiophores and discharge spores of their own, completing the asexual reproductive cycle.

Although *Pilobolus*, affectionately nicknamed “the shotgun fungus”, is often used as a lab demonstration tool, most of what is known about the fungus is limited to the dramatic phototropic aiming behavior of the spore explosion. The mechanical spore discharge model has hardly been revised, nor has the subsporangial turgor pressure been measured since the work of A.H. Reginald Buller in 1934. This study will attempt to

document the first authentic measurements of subsporangial turgor pressure in *Pilobolus* while using mathematical tools to model spore trajectories. Comparisons of mechanical measurements to mathematical results will be used to test the overall model of spore discharge in *Pilobolus*.

Materials and Methods

Organism and Cultures

Pilobolus Crystallinus cultures were obtained from Carolina Biological Supply Company (Burlington, NC) and grown on rabbit dung agar. The cultures were incubated at room temperature under daylight conditions for one week or until sporangiophores emerged.

Turgor Pressure Measurements

Subsporangial turgor pressure was measured using an apparatus similar to that described in Macdonald et al. (2002). A small steel wire peg was fixed with epoxy at 90° to the end of a miniature strain gauge (type AE-801; SensorOne, Sausalito, CA). Before use, the base of the strain gauge was glued to a micropipette holder for stability and handling purposes. Micropipettes were pulled from glass microcapillaries on a micropipette puller (PUL-100; WPI, Sarasota, FL) and then passed through a Bunsen flame. Smooth, rounded tips with diameters of .05- .2 mm were selected under a

microscope. The appropriate micropipettes were scored with a small hacksaw blade and broken to produce microprobes approximately 1 cm long. The microprobe was carefully fixed on the peg at the end of the strain gauge using forceps (see Figure 1).

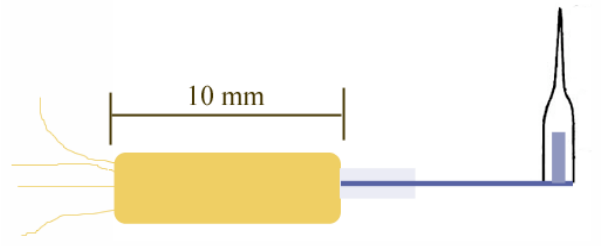


Figure 1. Attachment of microprobe to strain gauge. The beam of the gauge is shown in profile with the metal wire peg glued at a 90° angle, supporting the tip of a micropipette. The header of the strain gauge is secured with epoxy to the head of a micropipette holder. See appendix A for further information.

The strain gauges were used in the half-bridge mode using a Wheatstone bridge (BCM-1; Omega, Waltham, MA) with 5 V input from a highly regulated power supply (PST-4130; Omega, Stamford, CT). Signals were amplified 1000X with a DC amplifier (DAM50; WPI, Sarasota, FL) and filtered through a 30kHz low-pass filter before sampling with a PowerLab system (4sp; ADInstruments, Castle Hill, Australia). Calibration was performed on the strain gauge using milligram and microgram weights, permitting instrument resolution of better than 1 μN (see Figure 2).

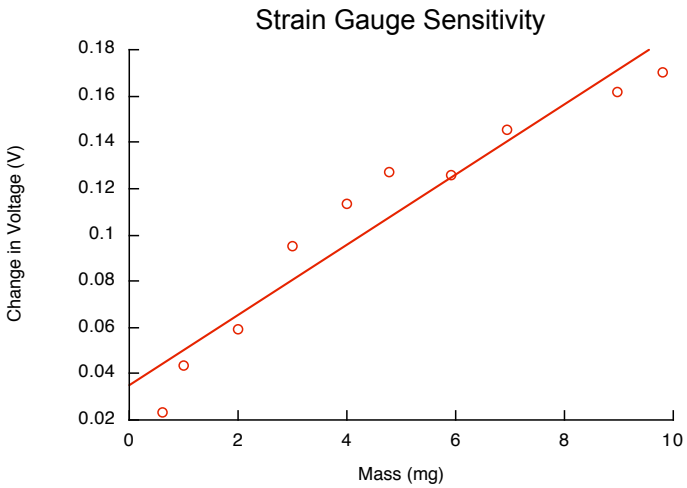


Figure 2. Example of a typical calibration curve obtained using milligram and microgram weights on a miniature strain gauge.

The microprobe was pushed against the sporangiophore at a 90° angle using a microelectrode positioner consisting of a piezoelectric motor (IW-711, Burleigh Instruments, Harpenden, U.K.) and ultra low noise controller (6000 ULN) attached to a micromanipulator (M3301; WPI, Sarasota, FL). The force required to indent the wall of the subsporangial swelling was used as a measure of turgor pressure. This method assumes that the pressure exerted by the internal sap accounts for the shape and stiffness of the sporangiophore and the cell wall offers negligible resistance to compression. This reasoning is justified by the complete collapse of the sporangiophore following sporangium ejection.

The force applied to the extension of the strain gauge was calculated by dividing the recorded force by the area of the tip of the hemispherical microprobe. The force required to slightly indent the cell wall of the subsporangial swelling was used as an estimate of the turgor pressure exerted by the sap within the sporangiophore. Because the

application of force exactly equal to the interior pressure would not cause any visible displacement of the cell wall, these estimates may slightly exceed the actual turgor pressure value. Movement of the sporangiophore away from the microprobe could also result in an overestimate; however this was accounted for by positioning a glass coverslip behind the sporangiophore in order to hold it in a rigid position.

Determining the Range of the Projectile

The range of the *Pilobolus* projectile was measured. The floor of a small room was covered with clean sheets of white paper to catch discharged sporangia. A fiber optic illuminator (Intralux 4000-1; Auburn, NY) was used to direct a steady beam of light onto a point in front of the paper sheets. This light was pointed at a 45° angle to the floor. Early in the morning, healthy *Pilobolus* cultures were placed within the beam of light in the otherwise dark room so that the phototrophic sporangiophores would shoot their spores toward the beam of light. The humidity of the room was kept at a constant level by a humidifier (Honeywell HWM255; Morristown, NJ).

In the evening of the same day, the sheets of paper were inspected with a magnifying glass and measurements were made from the point of discharge to determine the horizontal range of all projectiles that were discovered. This procedure was repeated for three days with three different healthy cultures of *Pilobolus*.

Mathematical model

Mathematical modeling techniques were adopted from Fischer et al. (2004). The reasoning behind the modeling exercise was that it should be possible to estimate the pressure contained within the spore discharge system of *Pilobolus* by looking at the range of the projectile. This exercise allowed comparison between the pressure predicted through the back calculations of the model and the actual mechanical measurements, with the intent of understanding the feasibility of the currently accepted mechanism of spore discharge.

The effects of gravity and viscous drag limit the range of the projectile and equations can be used to approximate the x and y position of the projectile as a function of time. The initial velocity of the projectile (spore and sap combined) was calculated using the average horizontal range measured above. Once the initial velocity was determined, it was possible to compute the pressure required to the give the projectile adequate acceleration to reach the measured range (see Appendix B).

When the wall of the sporangiophore breaks transversely just below the sporangia, the elastic wall of the subsporangial swelling and stipe suddenly contracts, violently squirting cell sap out of the open mouth of the subsporangial swelling. The sporangium is thrust from the sporangiophore by this jet, carrying with it a great deal of sap. The mass of the projectile, including cell sap, was taken from Buller (1934). The average volume of the sporangium was estimated using the equation for a sphere, $4/3\pi r^3$. Using Buller's measurements of spore diameter, and assuming that the cell sap accounts for 50% of the volume of the projectile, the volume was estimated at 8.17×10^{-10} m^3 (see Table 1).

Variable in Equation	Significance	Value
V	Volume of projectile	8.17×10^{-10} m^3
m	Mass of projectile	1.1×10^{-8} kg
g	Acceleration due to gravity	9.8 m s^{-2}
η	Viscosity of air	1.8×10^{-5} Nm^{-2} at 20° C

Table 1. Variables and corresponding values used in text and mathematical appendix.

The pressurized sporangiophore exerts a force, F, on the spherical projectile of volume V. Therefore the force acting on the projectile can be determined from the initial velocity using the Work-Energy Theorem. This theorem states that the change in kinetic energy of the projectile equals the integration of the force which is acting on the projectile and the distance over which that force acts. Once this initial force has been found, the internal pressure can be found by dividing the force by the cross-sectional area of the projectile. For this model it was assumed that the projectile falls in a cubic rather than linear path during discharge, reaching zero as the jet of cell sap disperses (see Appendix B). All calculations were performed using Mathematica (version 5.0; Wolfram Research, Champaign, IL).

Results

Turgor Pressure Measurements

Measurements of subsporangial turgor pressure yielded one moderately successful result (Figure 3). The force required to indent the wall of the swelling was approximately equal to 63.7 μN. The contact area between the tip of the microprobe and the wall of the subsporangial swelling was estimated at 628.3 μm^2. Dividing force by contact area of the microprobe produced a mean turgor estimate of .11 μN μm^{-2} (MPa) or approximately 1 atm.

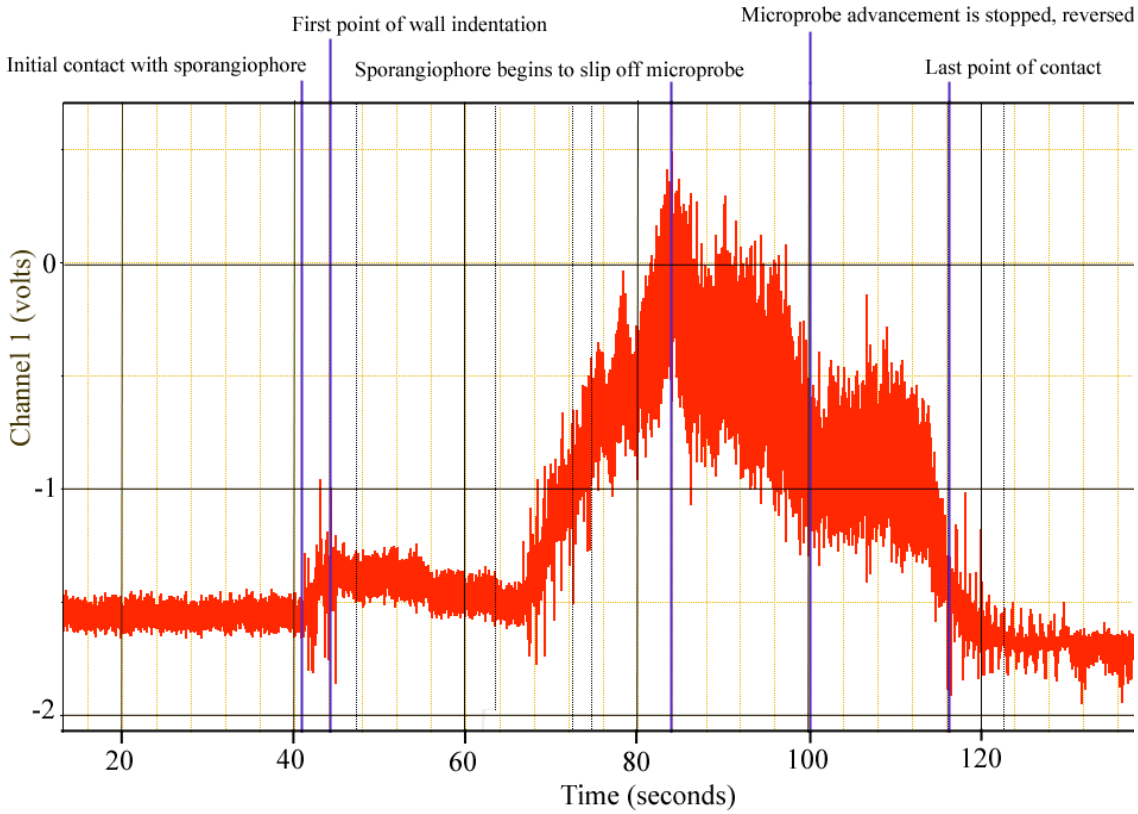


Figure 3. A Voltage trace vs. time obtained from the change in resistance over a miniature strain gauge induced by the indentation of the wall of the subsporangial swelling of the fungus *Pilobolus*.

Projectile Range

Thirty-four discharged sporangia were counted. The average range for the projectiles was 1.14 m (Figure 4).

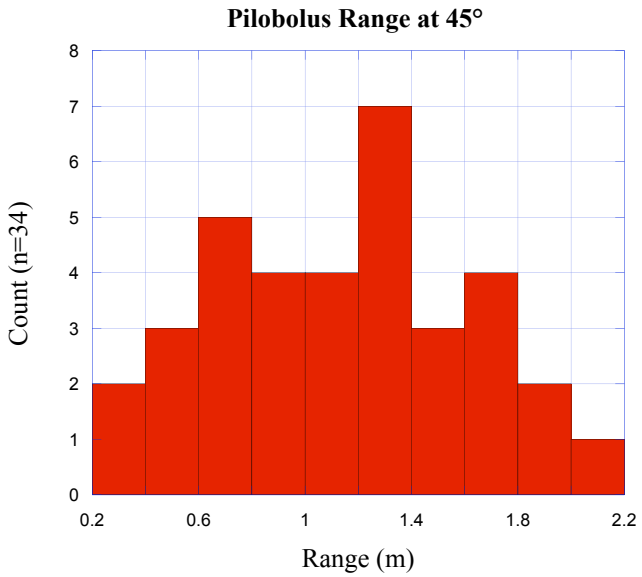


Figure 4. Measured range of *Pilobolus* sporangia projectiles discharged at 45° to the horizontal (mean=1.14, s.d.=.49).

Mathematical Model

The results of the mathematical model showed that for sporangiophores oriented at a 45° angle, an initial velocity of 42.35 m s^{-1} would propel the projectile over a range of 1.14 m. This initial speed would require an internal pressure of .474 MPa, equal to approximately 4.8 atm.

Discussion

Mechanical Measurements of Turgor Pressure

The mechanical measurements of turgor pressure are distinctly different from the predictions of Buller (1934), who used plasmolytic experiments to estimate an osmotic pressure of 5.5 atmospheres for the internal cell sap of *Pilobolus*. Due to the sloppiness and imprecision of these measurements, the mechanical results probably do not accurately reflect the actual conditions that exist within the sporangiophore of *Pilobolus*. The point at which the wall of the subsporangial swelling was indented was subject to the inaccuracy of my personal judgment, further obscured by the difficulty of observing a tiny microprobe advancing at a rate of μm/sec. with a large black sporangium partially blocking my view of the interaction. As can be seen from the trace (Figure 3), the microprobe was also slipping off the side of the swelling and the forces measured by the strain gauge may have been affected by the direction in which load was being applied to the fungus. Variation between *Pilobolus* individuals could also contribute to the surprisingly low forces measured, and encourages the use of a larger sample size that would more accurately represent the mechanical characteristics of the *Pilobolus* sporangiophore.

The complications encountered during the mechanical investigation of *Pilobolus* bring into question the methods used in this paper and outlined in Fischer et al. (2004). Although with sufficient practice it may be possible to consistently probe the wall of the sporangiophore, it does not seem systematically rigorous to visually resolve when the

wall experiences indentation. Mechanical behavior will depend upon cell wall elasticity and stiffness as well as the internal turgor pressure, creating a very specific threshold that might be difficult to detect while simultaneously operating a step-motor and looking through a microscope. It is difficult to accept that reliable measurements of turgor pressure can be obtained based on a correlation between visual identification of wall indentation and internal pressure. Perhaps the use of digital video recording would improve the accuracy with which these measurements were taken.

Pilobolus Range and the Mathematical Model

The observed range for the *Pilobolus* projectiles at 45° was comparable to that of past studies (Grove 1884; Buller 1934) and was used for the mathematically modeling of sporangial trajectory. The results of the mathematical model indicated that a pressure of .474 MPa would be sufficient to propel a *Pilobolus* projectile launched at an angle of 45° approximately 1.14 meters, with an initial velocity of 42.35 m s^{-1}. This initial velocity is nearly triple that reported by Buller (1934). Buller's measurement, in which he used two rotating discs of paper, may be significantly lower since it was measuring the velocity slightly removed from the actual point of discharge. This could make a large difference for a projectile that experiences such a high acceleration and pays a massive drag tax early in its trajectory.

The prediction generated by this model should be considered cautiously due to a variety of possible sources of error. For example, a simplified drag equation was used to model projectile trajectory. This equation directly reflected Stokes' law despite recent

revisions to methods of evaluating drag-related phenomena in the field of biomechanics. The resistance of drag is the defining ballistic factor for projectiles at such low Reynolds numbers (Vogel 2003). This mathematical oversimplification was necessary because application of a more complex drag equation resulted in pressures ten times greater than those predicted using the current model. The model used in this paper, and advocated by Fischer et al. (2004), was used because it offered reasonable values for subsporangial turgor pressure.

Several other factors that question the validity of this model stem from the mystery surrounding the mechanics of *Pilobolus* spore discharge. It is not clear how the ballistics of the projectile affect the importance of drag; for example, the shape that the sap-covered spore assumes in flight. It has been suggested that the sap may provide a streamlined tail, reducing drag on the projectile (Vogel 2005). Secondly, there are few clues to help understand how pressure decreases at the point of explosion. The rate at which the pressure is applied could seriously change the efficiency of the mechanism. Add to that the further complication of estimating applied force from a jet of cell sap and it is apparent that the exchange of energy between the two bodies cannot be simplified to a neat Newtonian equation. Taking into account these general sources of error and the fact that the model did not factor in friction between the projectile, the cell sap, and the sporangiophore, it is reasonable to conclude that the internal turgor pressure of *Pilobolus* lies in between .3 and .6 MPa.

Conclusion

The observations made in this study, both qualitative and quantitative, support Buller's model of sporangial discharge. More importantly, these results indicate that the methods outlined in Fischer et al. (2004) and employed in this paper should be seriously reconsidered before they are applied to the investigation of other systems that manage internal pressures. An alternative of superficially determining an internal pressure would be to measure a quantifiable deformation resulting from an applied force. Still, this technique would not eliminate the difficulties introduced by the mechanical substance of the surrounding cell wall. Perhaps the only way to avoid the confounding influence of a cell wall of an unknown elasticity and stiffness is to use internal measurements, such as the oil-filled pressure probes that have been used extensively to measure turgor and water relations of plant cells (Steudle 1993). This might be effective if the device could be manipulated to form a tight seal and prevent the sporangiophore from deflating. Future studies of *Pilobolus* and other spore launching fungi should make use of innovative methods to study the pressure-driven discharge mechanism in an attempt to elucidate the finer details of these remarkable systems.

Appendix A: Materials and Methods

1. The Power Supply

Connect an AC line to terminals 6 and 7. Terminals 2 and 4 (B+, B-) should be connected to the power terminals of the Wheatstone bridge (B+ & EX+, B- & EX-). The

sense terminals(1, 3) must also be connected to their corresponding excitation leads. This can happen either at the sensor or directly on the power source. The easiest solution is to install jumpers between 1 & 2 terminals, 3 & 4 terminals. The sense leads must be connected for the power supply to create an output voltage. The output voltage can be adjusted from 4 to 15 V of DC power. This voltage can be adjusted using the OUTPUT ADJUST pot. It should generally be kept around 5 or 6 V. The maximum recommended output current is 150 mA.

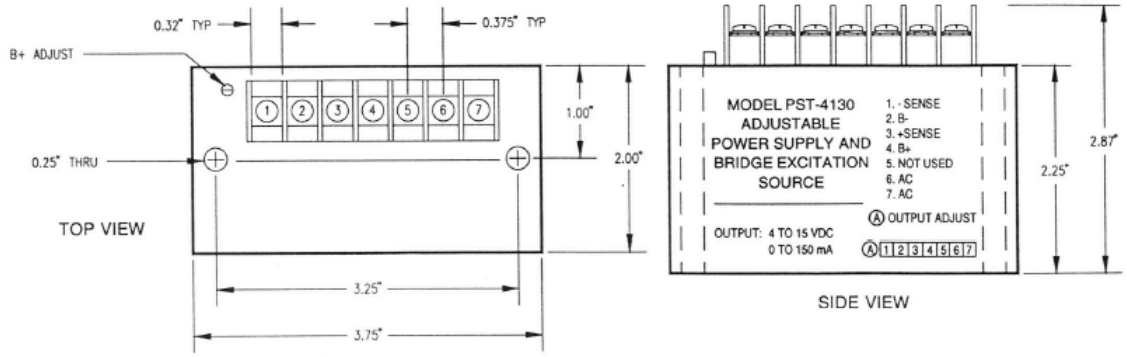


Figure 1. Section and schematic of the power supply (Omega Engineering 2005).

2. The Wheatstone Bridge

A Wheatstone bridge consists of four resistive arms across which a voltage is applied. The BCM-1 strain gauge completion module can be used for either quarter-bridge or half-bridge measurements. In the case of the SensorOne AE-801 strain gauge, the half-bridge mode is used. The half-bridge configuration yields an output that is linear and about double the output of the quarter-bridge.

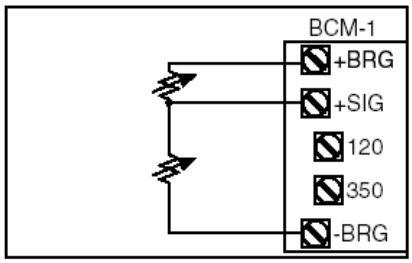


Figure 2. Schematic of Wheatstone bridge in half-bridge mode (Omega Engineering 2005).

3. The Strain Gauge

The two active resistors on the left in Figure 2 are provided by the AE-801 strain gauge. The AE-801 is extremely delicate and the beam should not be touched. The maximum force applied to the gauge should be less than 12 g. Fixing the wire peg to the strain gauge is the most difficult step in this procedure. While the gauge is still in its glass case, solder wires onto the four leads of the strain gauge. Then clamp these wires onto a micromanipulator stand and remove the glass case. Score the glass with a hacksaw and crack off the end to produce a hollow cylinder. Insert this cylinder over the strain gauge so that only the very tip of the beam protrudes. Then reclamp the gauge to the stand using the glass cylinder. Set the beam up exactly parallel to the floor under a dissecting microscope.

Take a small piece of Plexiglas, approximately 1 cm by 1 cm, and drill a hole through the center that is slightly larger then the 1 mm wire. Cut a small piece of wire for the peg, about 3 mm long, and file down the tips to be flat. Position the Plexiglas under the microscope so that the tiny hole rests exactly above the end of the strain gauge beam. This hole will act as a brace for the peg while the epoxy dries, so it must keep the peg

exactly perpendicular to the beam. Mix up some epoxy and apply a tiny drop to the tip of the beam. The epoxy should only touch the outer quartile of the beam as it may interfere with the resistors closer to the header. Making sure that they are exactly perpendicular, insert the wire peg through the hole in the Plexiglas until it rests upon the beam. Let dry for at least 12 hours.

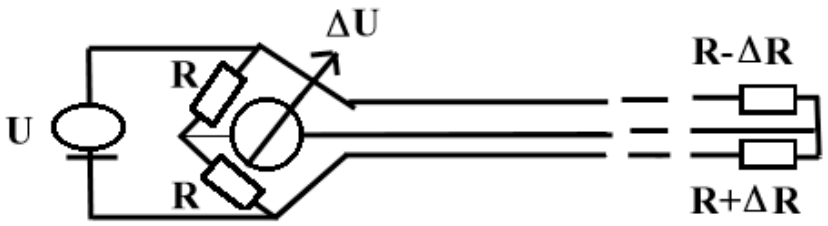


Figure 3. Schematic of Wheatstone bridge circuit.

In Figure 3, the right side of the diagram represents the strain gauge and the left represents the Wheatstone bridge. Of the two resistors in the strain gauge, R+ΔR is in tension and R-ΔR is in compression. The unbalance of the bridge due to a deflection d is given by the equation

$$\Delta U = U\frac{\Delta R}{2R} = 0.75 \cdot U \cdot \lambda \frac{\ell - 0.5}{\ell} \cdot \frac{h}{\ell^2} \cdot d$$

where λ is the gauge factor of the resistors. It is the change in voltage, U, that will indicate the force applied to the beam. Other variables are displayed in Figure 5.

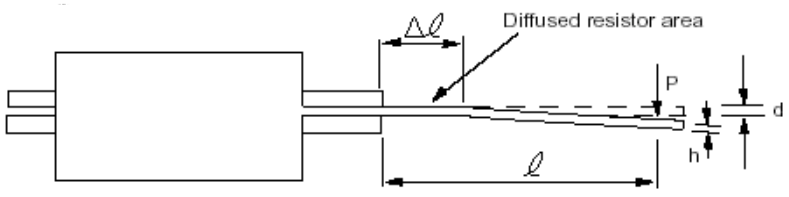


Figure 5. Diagram of AE-801 strain gauge (SensorOne 2005).

4. The DC Amplifier

Before it is recorded, the signal U must be amplified using a DC amplifier. The V_{out} terminals on the bridge should be wired into the amplifier as shown in Figure X. The two ground wires can be connected to the EX- terminal on the bridge. The amplifier (WPI DAM 50) should be in DC mode, set to A-B Input, and at 1000X DC gain. The output on the amp should be connected directly to the CH1 terminal on the PowerLab.

5. The Powerlab

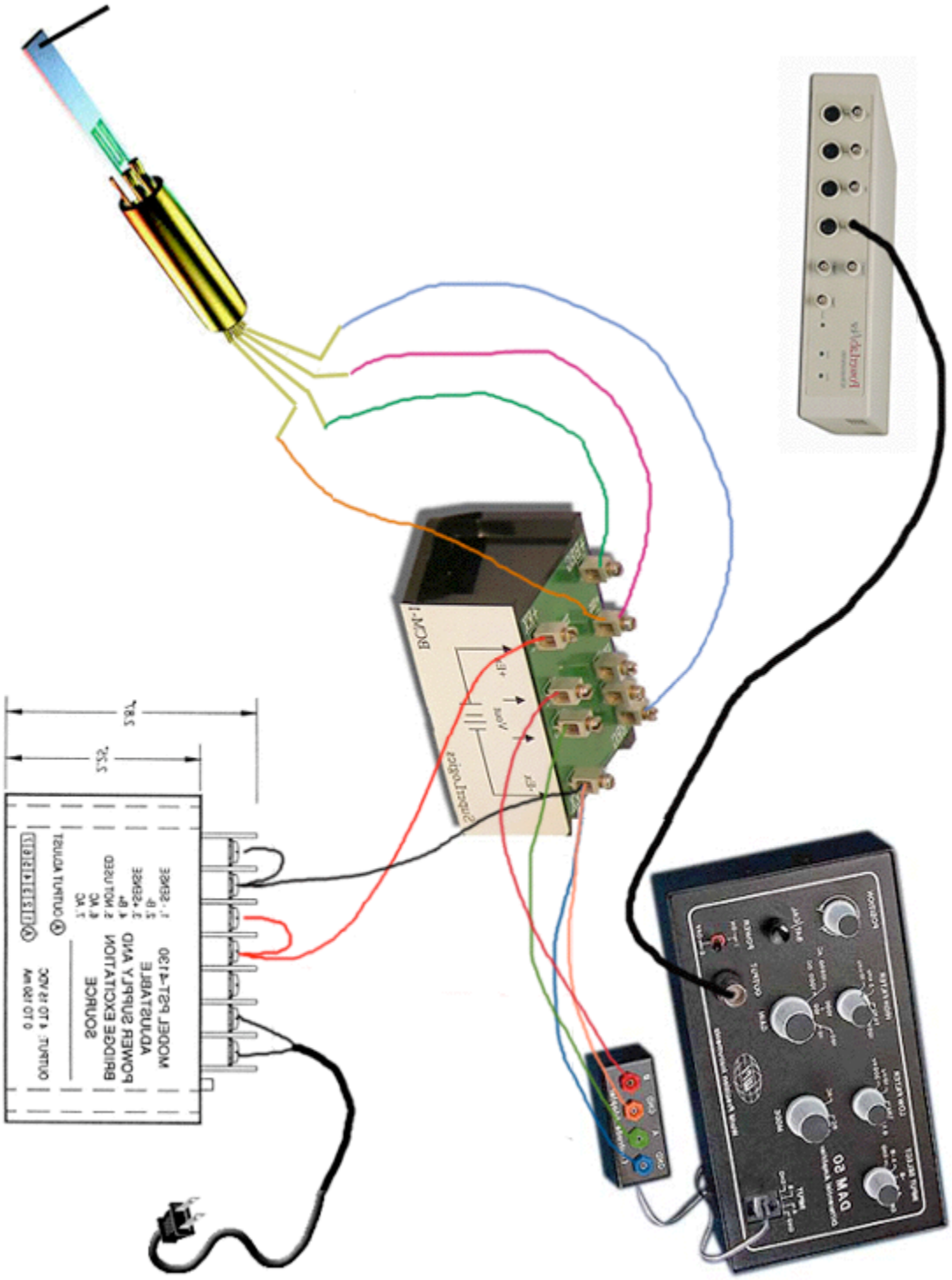
The program Chart can be used to record traces off the bridge. Isolate Channel 1 and set the range to between 1-5 volts (depending on what sort of forces will be measured). With the amplifier on and the power supply plugged in, press the start button to record.

6. Calibrating the Strain Gauge

The easiest way to calibrate the gauge is to make a series of small weights using a very accurate scale and apply them to the peg. I used 1-10 mg weights, measured to .0001 mg, made out of paper with a hole in the center so that they could easily be slipped over the peg and then removed with forceps. While recording with the Powerlab apply each weight to the beam, wait for the trace to level off then remove the weight. Take the average voltages using the Data Pad function and plot the differences to obtain a calibration curve for a variety of weights.

6. Reducing Noise

Wrapping the wires that connect the bridge to the amp and the strain gauge significantly reduces noise. Grounding the aluminum foil helps even further. Putting the device in a Faraday cage with the power supply outside may help. Air currents and changes in light and temperature can also affect noise levels. It is advisable to use consistent light conditions and to recalibrate the gauge before each use.



Appendix B. Mathematical Modeling

The forces of drag and gravity act against the projectile during flight :

$$F_{drag} = -6\pi\eta r v \text{ and } F_{grav} = -mgy$$

where
m = mass of projectile
g = acceleration due to gravity
y = a unit vector in the y − direction
r = radius of the projectile
η = the viscosity of air
Gravity and drag act against the projectile in the y direction :

$$\sum F_y = ma_y = -mgy - 6\pi\eta r v_y$$

Drag resists movement in the x direction :

$$\sum F_x = ma_x = -6\pi\eta r v_x$$

The derivatives of these equations yield expressions for acceleration.

$$a_x = -\frac{6\pi\eta r v_x}{m} \text{ and } a_y = -g - \frac{6\pi\eta r v_y}{m}$$

Solving the acceleration (in the x direction) as a differential equation without any initial conditions
yields :

$$x[t] = -\frac{e^{-\frac{6\pi r t\eta}{m}} m C[1]}{6\pi r\eta} + C[2]$$

where C[1] and C[2] are the constants of integration.

One can manually solve for C[1] and C[2] using the initial conditions of the model (xi = 0, vi Cos[θ] = x'[0]) to find that ...

$$C[2] = xi + \frac{m\, vi\, Cos[\theta]}{6\pi r\eta} \text{ and } C[1] = vi\, Cos[\theta]$$

Substituting these constants back into the definition for x[t] yields the equation for x as a function of time ...

$$\mathbf{x[t] = xi + \frac{m\, vi\, Cos[\theta]}{6\pi r\eta} - \frac{e^{-\frac{6\pi r t\eta}{m}} m\, vi\, Cos[\theta]}{6\pi r\eta}}$$

This procedure is now repeated to find an equation describing movement in the y − direction.

Solving the acceleration (in the y − direction) as a differential equation without any initial conditions yields ...

$$y[t] = -\frac{g m t}{6\pi r\eta} - \frac{e^{-\frac{6\pi r t\eta}{m}} m C[1]}{6\pi r\eta} + C[2]$$

where C[1] and C[2] are the constants of integration.

One can manually solve for C[1] and C[2] using the initial conditions of the model (yi = 0, v_i Sin[θ] = y'[0]) to find that ...

$$C[2] = -\frac{-g m^2 - 36\pi^2 r^2 yi\eta^2 - 6 m\pi r vi\eta Sin[\theta]}{36\pi^2 r^2\eta^2} \text{ and } C[1] = \frac{g m}{6\pi r\eta} + vi\, Sin[\theta]$$

Substituting these constants back into the definition for y[t] yields the equation for y as a function of time ...

$$y[t] = -\frac{g\,m\,t}{6\,\pi\,r\,\eta} - \frac{e^{-\frac{6\,\pi\,r\,t\,\eta}{m}}\,m\left(\frac{g\,m}{6\,\pi\,r\,\eta} + vi\,Sin[\theta]\right)}{6\,\pi\,r\,\eta} - \frac{-g\,m^2 - 36\,\pi^2\,r^2\,yi\,\eta^2 - 6\,m\,\pi\,r\,vi\,\eta\,Sin[\theta]}{36\,\pi^2\,r^2\,\eta^2}$$

To find the horizontal range of the projectile,
solve for time as the y – position returns to 0, or y[t] = 0.

$$t = \frac{vi}{\sqrt{2}\,g} + \frac{6\,yi\,\pi\,\alpha\,\eta}{g\,m} + \frac{1}{6\,\pi\,\alpha\,\eta}\left(m\left(1 + \text{Productlog}\left[e^{-1-\frac{36\,\pi^2\,r^2\,vi\,\eta^2}{g\,m^2}-\frac{3\sqrt{2}\,vi\,\pi\,\alpha\,\eta}{g\,m}}\,vi\left(-1-\frac{3\sqrt{2}\,\pi\,\alpha\,\eta}{g\,m}\right)\right]\right)\right)$$

The horizontal range can be found by evaluating x[t] at the time defined by this equation. Due to problems encountered in Mathematica,
this should be done by manually solving the equations without initial conditions then solving for the constants of integration by specifying the initial conditions later.

Once the horizontal range has been determined,
the equation for x[t] can be inverted to solve for vi

The Work – Energy Theorem can be used to solve for pressure (P) from this initial velocity ...

$$\frac{m\,vi^2}{2\,V} = P$$

where V is the volume of the projectile.

This expression can be used to determine the lower limit of internal pressure, assuming that the pressure within the sporangiophore is constant. However, it is more likely that the pressure exerted by the jet of cell sap decreases as the projectile is discharged. This would require a greater initial pressure to propel the projectile the same distance. Consider, for example,
if the pressure were to decrease uniformly from P to 0. This would be described by the equation ...

$$\frac{m\,vi^2}{V} = P$$

Taking into account the extreme elasticity of the wall that causes the sudden contraction, it is likely that P decreases even faster than this linear case. For modeling Pilobolus, the force is described as decreasing in a cubic fashion as the projectile is launched, resulting in the
equation :

$$\frac{2\,m\,vi^2}{V} = P$$

This is the equation that was used to calculate pressure from the initial velocity defined above. For a detailed explanation see Fischer et al. 2004.

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