

Contractile Characteristics of *Mimosa pudica* L.

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ABSTRACT

The force-velocity characteristics of the primary pulvinus of *Mimosa pudica* have been determined using a new polytonic measurement technique. The contractile characteristics were determined from a modified form of Hill's equation (Hill, A. V. 1938. Proc. Roy. Soc. London B126: 136-195) describing the physiological contractile behavior of animal muscle. The values of the resulting Hill's constants were found to be remarkably similar to those of intact animal muscle and reconstituted contractile collagen.

Over the years, the workers in the field of animal physiology have developed instruments for recording tracings of muscular contractions and relaxations. These instruments (generally called "myographs") in the past have been used mainly to study only two basic types of muscle testing. In the first type, a force-time curve is generated while the muscle length is held constant. These are called "isometric" tests. In the second type, the force-time or force-velocity curve is generated while the load on the muscle is held constant. These are called "isotonic" tests. Recently (2) a new myograph has been developed which allows a variable force to be exerted on the muscle during the test. This type of test has been named "polytonic," and it is thought to be superior to both the isometric and isotonic apparatus because of its ease of operation and because it allows the muscle to undergo a more natural motion.

One of the results of many years of study of the physiology of animal muscle by A. V. Hill was his now well known empirical isotonic force-velocity correlation (6):

$$v = \frac{b(p_0 - p)}{p + a} \quad (1)$$

where v is the velocity of contraction, p is the force of contraction and p_0 is the isometric force. The Hill constants a and b represent the internal resistance of the muscle and a measure of the absolute rate of energy liberation of the muscle respectively. They have been found to be nearly constant for all animal muscle tissue, but do vary with temperature. The mechanism of muscle contraction, though not directly observable since it is a molecular phenomenon, is thought to be fairly well understood, with only two or three major theories in vogue. Whatever the detailed molecular mechanism may be, it seems certain to be different from the seismonastic reaction in *Mimosa pudica* and other "sensitive" plants. The purpose of the work presented here is to investigate the applicability of Hill's equation to the seismonastic movements of the primary pulvinus of *Mimosa pudica*.

Although Hill's equation was developed from data taken with an isotonic myograph, he felt that equation 1 was characteristic of the contractile system and therefore should be generally independent of the type of loading used in generating the data (6, p. 184). Balmer and Soto (2) have shown that a slightly modified form of equation 1 will correlate the polytonic contractile characteristics of reconstituted cross linked collagen fibers extracted from bovine tendons.

POLYTONIC THEORY

A simple polytonic myograph can be built using a flexible cantilever beam with strain gages attached to the constrained end of the beam. In cantilever beam theory, the load is uniquely related to the deflection of the free end of the beam. A force p applied to the free end of the beam of uniform cross-section will cause a deflection of the free end given by:

$$\delta = \frac{p}{K} \quad (2)$$

where K is the beam constant given by:

$$K = \frac{3EI}{L^3} \quad (3)$$

where E is the elastic modulus of the material of the beam, I is its moment of inertia, and L is the length of the beam from the point of support to the point where δ is measured.

Now, the velocity of the free end of the beam is given by:

$$v = \frac{d\delta}{dt} \quad (4)$$

Substituting equations 2 and 4 into equation 1 and integrating gives:

$$(a + p_0) \ln \left(1 - \frac{p}{p_0} \right) + p = -bKt. \quad (5)$$

Equation 5 predicts that p approaches p_0 as t becomes infinite. This only occurs in the isometric case, that is, with very rigid cantilever beams. However, if p_0 in equation 5 is replaced by p_∞ —the actual force observed as t becomes large—then equation 5 can be written as:

$$\left(\frac{a}{p_\infty} + 1 \right) \ln \left(1 - \frac{p}{p_\infty} \right) + \frac{p}{p_\infty} = -\left(\frac{b}{p_\infty} \right) Kt. \quad (6)$$

Equation 6 has been shown to correlate polytonic contractile data accurately (2).

Neither equation 5 nor 6 will satisfy relaxation data accurately because in relaxation, at $t = 0$, $p = p_\infty$ (or p_0). But, in both equations the logarithmic term becomes infinite as p approaches p_∞ (or p_0). If Hill's equation is again modified by setting

$$p = p_\infty - p_r \quad (7)$$

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where p_r is the actual applied force during relaxation, then equation 6 becomes

$$\left(\frac{a}{p_\infty} + 1\right) \ln\left(\frac{p_r}{p_\infty}\right) - \frac{p_r}{p_\infty} + 1 = -\left(\frac{b}{p_\infty}\right)Kt \quad (8)$$

which is seen to satisfy to initial condition at $t = 0$.

Consequently, the resulting modified Hill's equation to be applied to polytonic contractions is given by substituting p_∞ for p_0 in equation 1 yielding:

$$v = \frac{b(p_\infty - p)}{p + a} \text{ (contraction only)} \quad (9)$$

and the corresponding modified equation for polytonic relaxation is given by substituting equation 7 into equation 9 yielding:

$$v = \frac{bp_r}{p_\infty + a - p_r} \text{ (relaxation only).} \quad (10)$$

Finally, the polytonic technique has the additional advantage that the work done during a contraction process, starting, for instance, at $p = 0$, is given by:

$$\text{work} = \int_0^p p d\delta = \int_0^p K\delta d\delta = \frac{p^2}{2K} \quad (11)$$

and the maximum work done during a polytonic contraction, then, is given by:

$$\text{maximum work} = \frac{p_\infty^2}{2K}. \quad (12)$$

Balmer and Soto (1) have used the polytonic technique to investigate the contractile characteristics of reconstituted collagen

fibers extracted from bovine tendons. The fibers were extruded into a 0.135-cm by 0.004-cm tape and cross-linked by dipping into a 1.22% formaldehyde solution and air-dried. The collagen tape would contract when brought into contact with various salt solutions and would relax again when washed with fresh H₂O. In this instance, too, it is unlikely that the molecular contractile mechanism is exactly identical to that of *in vivo* animal muscle, yet the resulting contractile characteristics were remarkably similar (2).

MATERIALS AND METHODS

A mature, healthy *Mimosa pudica* about 30 cm tall was used for these tests. No special nutrients were used in its development, and it was grown on a window sill. Young upper branches were used in the experiments. Figure 1 illustrates the experimental apparatus used. A special polytonic cantilever beam was fabricated from 2024-T351 aluminum (elastic modulus = 7.24×10^{11} dyne/cm²), 1 cm wide, 0.066 cm thick, and 15.24 cm long. Four standard wire strain gages were epoxied 13.3 cm from one end of the beam (two on the top and two on the bottom of the beam), and were connected in such a way as to produce a maximum signal for a given deflection (3). The end of the beam near the strain gages was securely clamped onto a ring stand so that its height could be easily adjusted. The four strain gages formed a bridge (3) which was then connected to a Sanborn strain gage amplifier and strip chart recorder. The resulting sensitivity of the system was ± 15 dynes.

A small hole was put in the free end of the beam and a wire hook was passed through it and supported in such a manner that it was allowed to turn freely in any direction. The ring stand holding the cantilever beam assembly was set on a commercial lab jack for fine height adjustments. The system was adjusted so that the hook end of the beam passed between the pinnae and

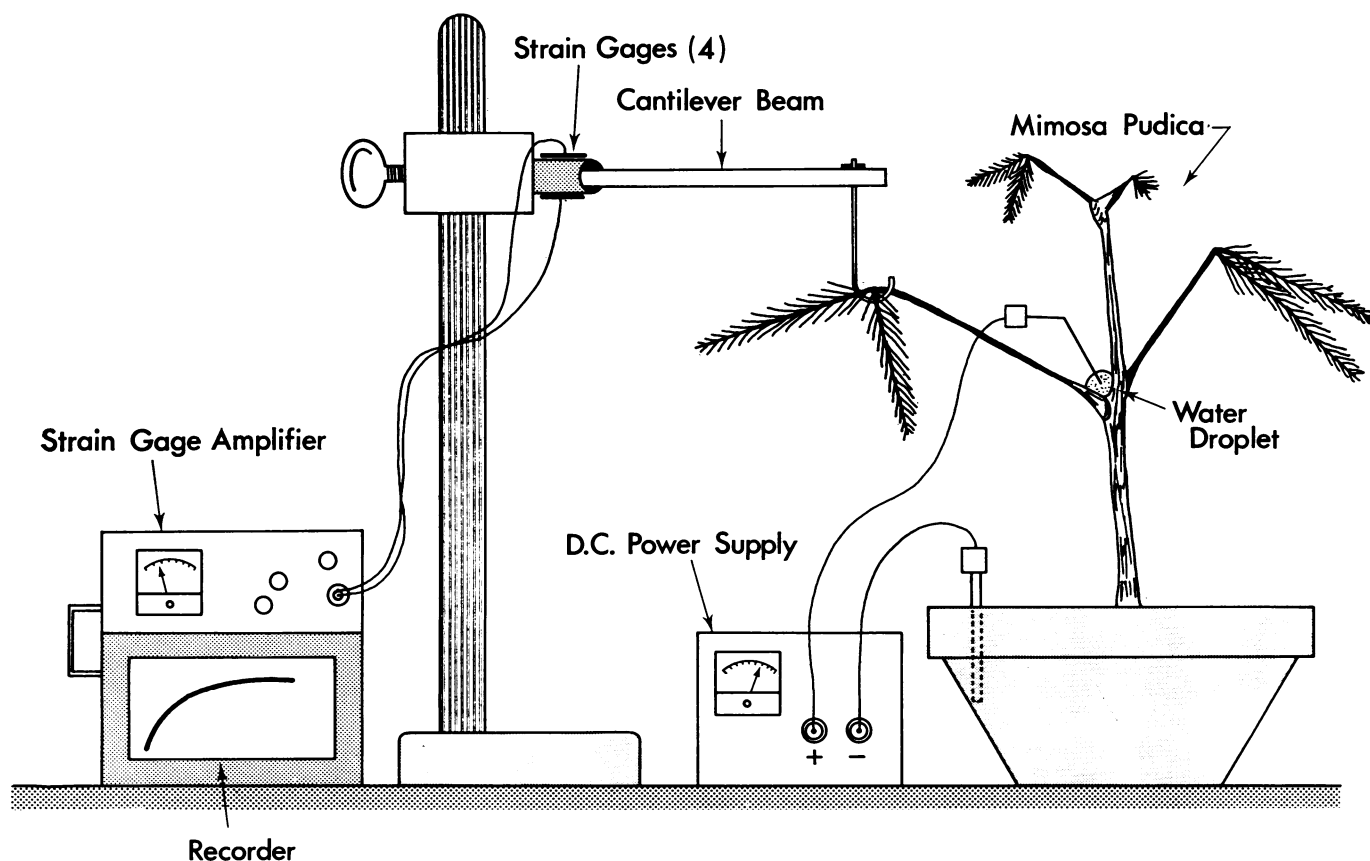


FIG. 1. Schematic diagram illustrating experimental apparatus used in generating force-time data on *Mimosa pudica*.

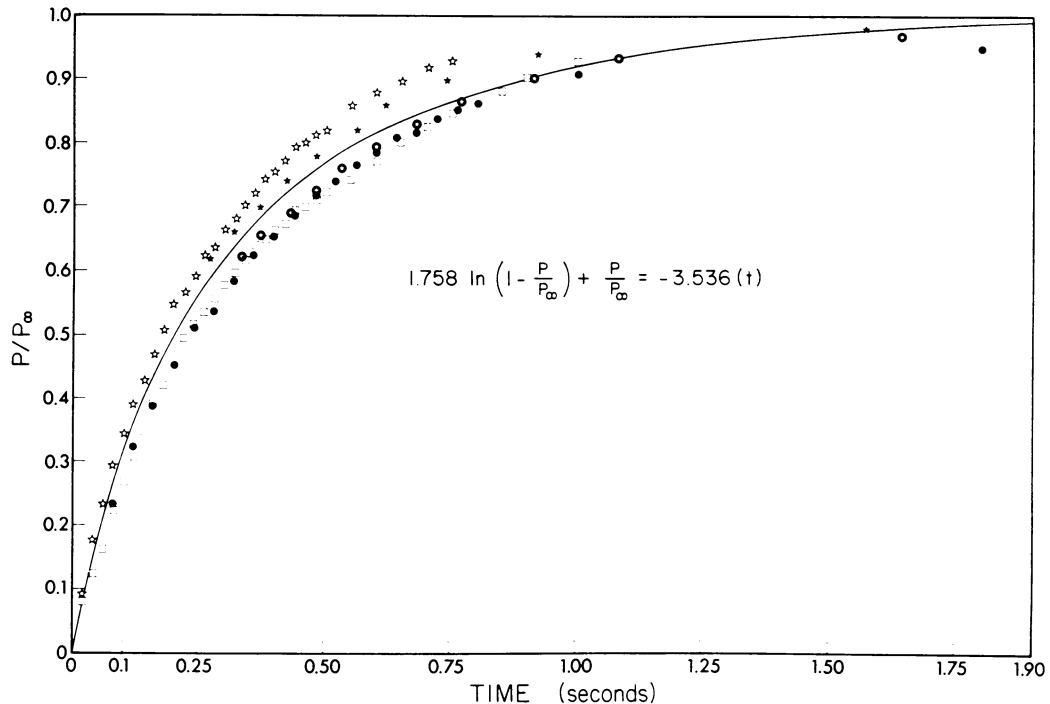


FIG. 2. Normalized stimulation force versus time for five experimental runs on *Mimosa pudica*. The solid line is equation 6 with $a/p_\infty = 0.758$ and $b/p_\infty = 2.75 \times 10^{-4}$ cm/dyne·sec. The polytonic beam constant is 1.286×10^4 dyne/cm.

held (but did not puncture) the primary petiole at the secondary pulvinus. This adjustment usually stimulated the plant slightly, so sufficient time was always allowed for it to recover (the recovery could be seen on the strip chart recorder). The primary pulvinus was stimulated electronically using a dc power supply with one probe inserted deeply into the soil at the base of the plant, and the other probe connected to a fine wire which was inserted into a drop of tap water which was carefully placed at the stipule of the primary pulvinus and the stem (Fig. 1). In this way, no electrical connections were made directly to the plant.² The stimulation voltage was only briefly applied and varied between 200 and 400 v dc, which was far above the approximately 25 v threshold voltage necessary to cause movement. No effect of probe polarity or actual voltage was detected. Generally, only the stimulated primary pulvinus and its associated tertiary pulvina reacted to the voltage.

The relaxation phenomenon following the stimulation was recorded simply by allowing the electronic system to continue to run until the branch had returned to its original position before stimulation.

RESULTS

Figure 2 presents the results on fitting five sets of force-time data to equation 6. The solid line represents the best fit of all five data sets taken together. The resulting Hill's dynamical constants for the solid line are:

$$(a/p_\infty)_{\text{avg}} = 0.758; (b/p_\infty)_{\text{avg}} = 2.75 \times 10^{-4} \text{ cm/dyne} \cdot \text{sec.}$$

Occasionally the hook at the end of the beam would not hold the

² In fact, if a metal hook is used on the beam, the electrical connections can be made to the beam (or ring stand) and to the soil probe. Also, if a very high voltage transformer is used (for instance, 10,000 v, but very low current), then only one probe is necessary and the plant can be made to react selectively by bringing the high voltage probe near the plant and allowing an arc to jump from the probe to the plant.

secondary pulvinus sufficiently tightly at the beginning of the stimulation and some slippage would occur. This was detectable as erratic behavior on the chart recorder and could be accounted for by compensating the time scale to the point where the hook finally seated.

Typical stimulation and relaxation curves illustrating the use of both equations 6 and 8 are shown in Figure 3. In this instance, for the stimulation $(a/p_\infty) = 1.26$ and $(b/p_\infty) = 3.87 \times 10^{-4}$ cm/dyne·sec, and for the relaxation $(a/p_\infty) = 22.9$ and $(b/p_\infty) = 2.18 \times 10^{-6}$ cm/dyne·sec.

For the data presented here, the maximum force (p_∞) was typically 980 dynes and $K = 1.28 \times 10^4$ dyne/cm. Thus equation 12 gives the maximum work done by the primary pulvinus during a typical contraction as 37.3 dyne·cm.

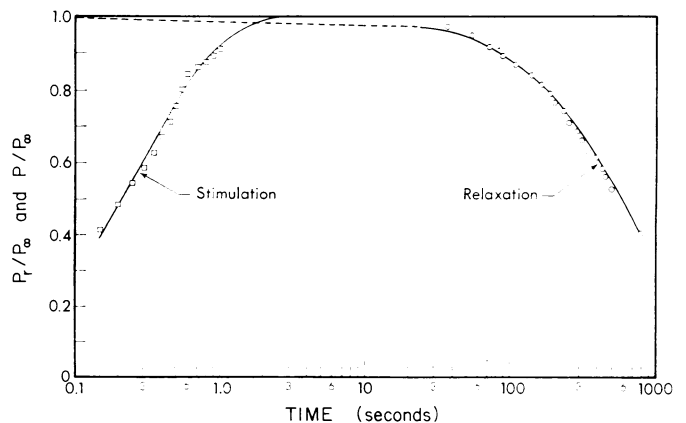


FIG. 3. Normalized typical stimulation and subsequent relaxation forces versus time for *Mimosa pudica*. The solid lines are equations 6 and 8 with $a/p_\infty = 1.26$ and $b/p_\infty = 3.87 \times 10^{-4}$ cm/dyne·sec for the stimulation and $a/p_\infty = 22.9$ and $b/p_\infty = 2.18 \times 10^{-6}$ cm/dyne·sec for the relaxation.

DISCUSSION

For almost a century now, investigators have alluded to the possibility of some commonality in the motions of animal muscles and turgor movements in plants. Gardiner (5) remarked that the tissue of the primary pulvinus of *Mimosa pudica* reacted to electrical stimulation in a manner almost identical to that of some animal muscles. Darwin (4) noted that "it is impossible not to be struck with the resemblance between the foregoing movements of plants and many of the actions performed unconsciously by the lower animals." Weintraub (9) suggests that the loss of turgidity in the cells of motor tissue of *Mimosa pudica* is due to the contraction of small vacuoles, and that this contraction is caused by a contractile protein substance in the cell protoplasm or cell wall. Sibaoka (7) has shown that elongated parenchyma cells in the phloem and protoxylem of the primary petiole of *Mimosa pudica* behave electrically similar to animal nerve and muscle cells. He demonstrated that the interior of these excitable cells is about 160 mv below their exterior and that during stimulation this potential difference decreases. Toriyama and Jaffe (8) after many years of research on *Mimosa pudica* have concluded that the central vacuole of the motor cell contains a contractile protein which undergoes a conformational change during seismonastic movement. These conformational changes are thought to change the permeability characteristics of the tonoplast and plasmalemma which in turn allows a rapid potassium ion efflux from the cell. This results in water loss (probably of hydration) from the cell with consequent loss in turgor.

The values of a/p_0 in equation 1 or a/p_∞ in equation 9 have been found generally to lie in the range from zero to approximately unity for a large variety of animal muscles and reconstituted contractile collagen. The constancy of this ratio must reflect somehow upon the molecular mechanism of contraction. It is remarkable that the average value of a/p_∞ reported here for the stimulation of the primary pulvinus of *Mimosa pudica* also lies in this range. This macroscopic observation adds to the evidence of commonality in the plant-animal contractile movement mechanism.

The fact that the value of this parameter is nearly the same for

in vivo animal muscles, reconstituted cross-linked contractile collagen, and the *Mimosa pudica* cannot be viewed as mere coincidence. At the molecular level there cannot be a multitude of mechanisms, and whatever occurs must be governed by the physical laws of molecular mechanics. Therefore, the concept of "some" commonality at this level is quite acceptable.

The average value of b/p_∞ for the stimulation of the primary pulvinus is generally greater than that of reconstituted collagen (typically 10^{-6} cm/dyne-sec), but it is comparable to that of intact animal muscles (typically 10^{-5} cm/dyne-sec or greater). For the same values of a/p_∞ and total chemical energy input, the larger the value of b/p_∞ , the more efficient is the mechanochemical energy conversion process. Thus, these results would tend to indicate that the seismonastic stimulation of the primary pulvinus of *Mimosa pudica* may be more efficient than typical animal muscle movements.

The values of a/p_∞ and b/p_∞ for the relaxation process following stimulation follow the same behavior as with reconstituted collagen (1). The value of a is generally larger in relaxation than in stimulation, and the value of b is generally smaller in relaxation than in stimulation. This mainly reflects the fact that the relaxation process is slower than the stimulation process.

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