

THE EFFECT OF A SMALL AXIAL PRESSURE ON THE GROWTH OF THE EPICOTYL OF *VICIA FABA* VAR. *MINOR* BECK

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Summary

Epicotyls of *Vicia faba* var. *minor* Beck, which had been subjected to an axial pressure of 0.5 atm for 48 hr, were 12% shorter than control epicotyls which had supported a negligible load. The state of stress produced in the epicotyl by the axial pressure led to a change in shape which was not accompanied by any change in volume. As the axial pressure of 0.5 atm is regarded as small compared with the point resistance which often has to be overcome by emerging shoots, it is likely that mechanical resistance is an important factor influencing seedling emergence in ordinary soils.

I. INTRODUCTION

Although in nature seeds usually germinate on top of the ground (Haberlandt 1914), the seed of cultivated plants is usually buried within the soil. Haberlandt's observation is interesting because we may suppose that the kinds of emergent organ which have evolved amongst the various species of plants will not necessarily be as well adapted as are roots for penetrating the soil.

Once seeds start to germinate, survival depends on the first seedling organ capable of photosynthesis being exposed to light before metabolic reserves are exhausted. Emergence must therefore occur within a limited time. The shape and size of the emergent shoot varies widely between plant species but, in general, progress through the overlying soil is achieved by elongation of cells formed by division during embryogeny or after germination. When emerging through soil the shoot has to displace particles or cohesive layers from its path, and its movement is opposed by the resistance to deformation shown by the particular soil.

Although there is copious literature on seedling emergence and soil crusting, most of the references deal with the amelioration of extreme structural conditions, or with the relationship between emergence and some empirical parameter which measures the soil's resistance. Little has been learnt of the response of shoots to mechanical stress.

The classical experiments of Pfeffer (1893) provided the first detailed description of the forces which can be exerted by plant tissues. The organs examined included both roots and seedling shoots. In Pfeffer's experiments part of the shoot was held within a gypsum block, and a second but movable block was then cast around the exposed tip or sides of the shoot. With the shoot confined in this way the force exerted by the shoot could be measured by balancing it against a known resistance.

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Pfeffer found that the force increased to a maximum over a period of 36–48 hr and that the maximum force, if distributed over the greatest cross-section within the growing region, corresponded to a pressure of from 5 to 12 atm. Pressures were usually higher in the direction of the longitudinal axis than in the radial direction. Epicotyls of *Vicia faba* L. developed a maximum longitudinal pressure of 5.9 atm, compared with a maximum radial pressure of 5.6 atm. In more detailed experiments with roots, Pfeffer observed that elongation ceased for a number of hours when the root encountered a suitable resistance, and that later it was resumed. The interval was referred to as an "induction period". Pfeffer explained the mechanism for the exertion of force by roots and shoots in terms of the osmotic behaviour of the cells. Measurements of osmotic pressure in the mid-cortex of roots gave values which were similar in magnitude to the maximum longitudinal pressures which the roots could exert.

The effect of mechanical resistance on the growth rate of roots of *Zea mays* (L.) has been examined by Gill and Miller (1956). The roots were grown in a thin bed of glass spheres lying between a rigid base plate and a rubber diaphragm to which pressure could be applied. The resistance to root extension increased with increasing pressure on the diaphragm, but the magnitude of this resistance was not known. When grown in the apparatus in a mixture of oxygen and nitrogen containing 20% oxygen, the roots failed to elongate when the pressure on the diaphragm reached 4.5 atm. Working with roots of *Zea* in a similar apparatus, but with a thin diaphragm bearing directly on the roots, Barley (1962) estimated the resistance per unit cross-section opposed to elongation of the tips. Barley found that root elongation almost ceased at a diaphragm pressure of 4.5 atm, which corresponded to a resistance per unit cross-section, in the direction of elongation, of about 8 atm. This value was in good agreement with the osmotic pressure of cells in the mid-cortex of the tip. In the experiments of Gill and Miller and of Barley, the rate of root elongation decreased with each increment in the diaphragm pressure.

II. METHODS

(a) *Plant Material*

Vicia faba var. *minor* Beck was chosen as the test plant for a number of reasons: it had already been used in Pfeffer's classical experiments, its seed was smaller and more convenient to handle than the seed of the large *V. faba*, the apical hook provided a convenient point at which to apply a load, and, as its germination is epigeal, the plant could be conveniently held by the cotyledons during early growth of the shoot. The seed was graded for weight, and seeds which appeared to be sound and weighed between 0.30 and 0.35 g were chosen.

(b) *Apparatus*

The apparatus shown in Figure 1 consisted of a piston of known weight (D) which could be elevated within a glass cylinder by an elongating shoot. The cotyledons at the base of the shoot were held stationary in a holder (E). Ten piston-cylinder units were evenly spaced around the circumference of a circular rack of 22 cm diameter, which was housed within a box constructed of "Perspex". Each cylinder was fitted to a flange (C) which rested on the lid of the box, and which

could be centred and fixed so that the cylinder was located over the plant shoot in a position suitable for loading with the piston. The load could be varied by adding lead shot to the cup (A). The lid of the box was provided with an inlet (H) for gases, and a magnetic stirring device (G) for mixing gas within the container. A gap was left between the shaft of the piston and the cylinder to permit gas to circulate around the seedling and flow freely out of the container. The air supplied to the apparatus was first passed through activated charcoal, and was then humidified by bubbling through water at 30°C and cooling in water at 20°C. A flow rate of 300 cm³/hr of air supplied about 200 times the amount of oxygen being consumed by the seedlings. The seedlings were normally grown in darkness, but filtered light ($\lambda = 0.48\text{--}0.62\ \mu$) from a fluorescent tube was used for short periods to illuminate the working area or to make observations. Vermiculite (F) was retained within the boundary of the rack and, when supplied with nutrient solution, provided a medium for the growth of roots of the seedlings.

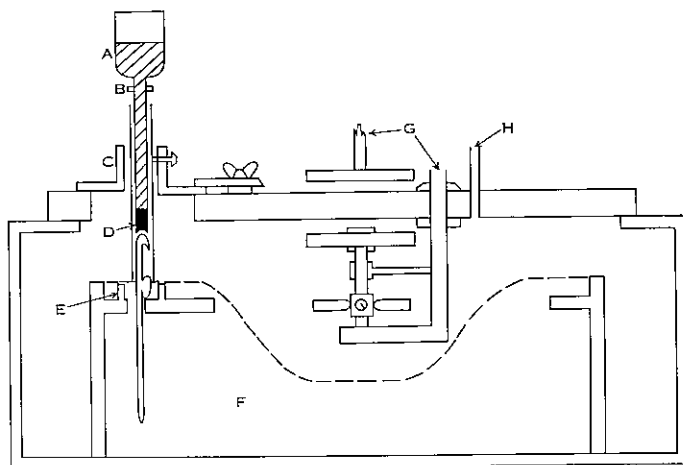


Fig. 1.—Construction of the apparatus used to load and aerate the epicotyls. A, container for lead shot; B, arrestment; C, movable flange; D, piston (end coated with paraffin wax); E, seed holder; F, vermiculite; G, magnetic stirrer; H, air inlet.

(c) *Measurement of Epicotyl Elongation*

Twenty-five seeds were planted in a loose pack of vermiculite wetted with tap water (water content 325%), and allowed to germinate at a temperature of 20°C. After 42 hr the 10 most uniform seeds, with radicles just showing, were transferred onto a bed of vermiculite in the apparatus. The 600-g bed of vermiculite had been wetted with 2 l. of a complete nutrient solution. Additional vermiculite was packed around each seed until it was level with the top of the seed. After being transferred to the apparatus the seedlings were allowed to grow in darkness for 70 hr, by which time the epicotyls were 2–3 cm long. After the length of each epicotyl had been measured (l_0), the cylinders were placed over the shoots and allowed to rest on the vermiculite. Next the pistons were gently lowered onto the apical hooks and the

height between the top of each cylinder and a reference point on its piston (B on Fig. 1) was measured. A load of 35 g wt. was imposed on the treated epicotyls compared with a load of 5 g wt. on the controls. The loaded seedlings were grown for a further 48 hr in darkness, when the height of the pistons was again measured. Finally the apparatus was dismantled, and each epicotyl was severed at a distance l_0 from its base. The length of epicotyl produced in 48 hr was then measured by straightening the severed portion against a scale. Additional measurements made at harvest were the length of the apical hook between the uppermost part of the epicotyl and the apex of the hook, and the diameter of the epicotyl 2 cm below its uppermost part. The harvested portions were weighed fresh, and also after being dried for 48 hr at 80°C. Epicotyls which showed any brown lesions or splits, or which were bent other than in a simple curve, were rejected from the experiment. The roots of the seedlings were also examined for lesions, but very few were found.

TABLE 1
EFFECT OF A SMALL AXIAL PRESSURE ON THE GROWTH OF EPICOTYLS OF VICIA
FABA VAR. MINOR BECK

Measurement	Load, 5 g wt. $n^* = 63$	Load, 35 g wt. $n^* = 53$	S.D. of Difference between Means
112-160 hr from germination			
Length increment (cm)	5.61	4.94	0.18
Fresh weight increment (mg)	320	318	12
Dry weight increment (mg)	24	25	0.7
160 hr from germination			
Diameter of epicotyl 2 cm below uppermost part of shoot (cm)	0.275	0.292	0.004
Length of plumular hook (cm)	1.03	1.08	0.02

* Number of seedlings.

(d) Construction of Height-Time Curves

Additional data were obtained to try to detect any lag or induction period which may have occurred after loading. The technique was similar to that described above, with the following exceptions: 10 epicotyls were loaded with 5 g wt. at the 2-3 cm stage. After 12 hr the load on alternate seedlings was increased to 35 g wt. Vector displacement (height) of the pistons was measured at intervals before and after loading.

(e) Measurement of the Rate of Deformation

The rate of bending under load was examined further with the constant-moment apparatus of Lockhart (1959), as the apparatus used in the main experiment was not sufficiently sensitive to measure the initial sequence of deformations produced by the application of the load. Sections of epicotyls (2 cm long) were excised a few millimetres below the apical hook. The basal end was secured, and the apical

end was loaded with a glass tube of 3 g wt. The time course of deformation under a constant bending moment of 4.5 g wt. cm was then recorded.

III. RESULTS

Data from the main 48-hr experiment are summarized in Table 1. The loaded epicotyls were shorter and wider than the controls. However, the fresh and dry weight increments were almost identical for the two groups of epicotyls, as were the volume increments computed from the length-increment and diameter data given in Table 1 (controls 0.333 cm³, loaded group 0.331 cm³).

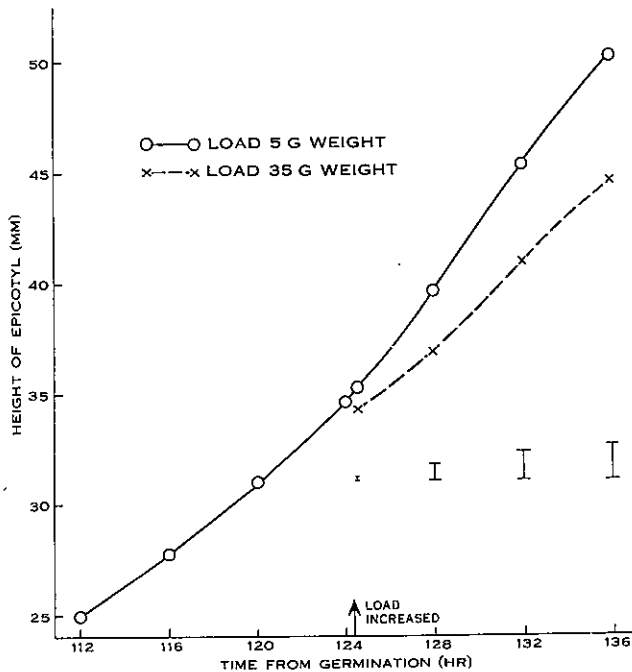


Fig. 2.—Height-time curves before and after the application of an additional load. Lengths of vertical lines indicate standard deviation of difference between means.

The percentage of epicotyls rejected because of damage during loading or because of the development of longitudinal splits in the tissue during growth was 4% for the controls and 15% for the loaded group. The mean displacement of the piston by the rejected epicotyls was only 12 mm, showing that the rejected epicotyls constituted a distinct, damaged group. The height of the epicotyls at the time of loading did not differ significantly between the retained and rejected groups of epicotyls, and there is no evidence to suggest that the faster growing epicotyls were disproportionately represented in the rejected group. The accidental damage apparently occurred at the time of loading.

Bending due to the loads of 5 and 35 g wt. can be compared by finding the difference between the increase in length of the epicotyl and the vertical displacement

of the piston. For the controls (5 g wt.) the magnitude of the displacement was 1.2 mm less than the increase in length, compared with a difference of 3.3 mm for the loaded epicotyls (35 g wt.). The maximum angle of deflection from the vertical was about 8°. It was observed that loads greater than 35 g wt. often bent the epicotyls to a considerable extent.

The height-time curves for treated and control shoots are shown in Figure 2. There was a small (<1 mm) decrease in height in the first half hour after loading, but subsequently showed a continuous increase with time. The mean velocity of the apex during the period 0.5–12.0 hr after loading was 1.29 and 0.89 mm per hour for the control and treated groups of epicotyls. Although this difference was significant ($P < 0.05$), it should be noted that, unlike the data on lengths given in Table 1, the data on height and velocity are influenced by the small additional degree of bending shown by the loaded epicotyls.

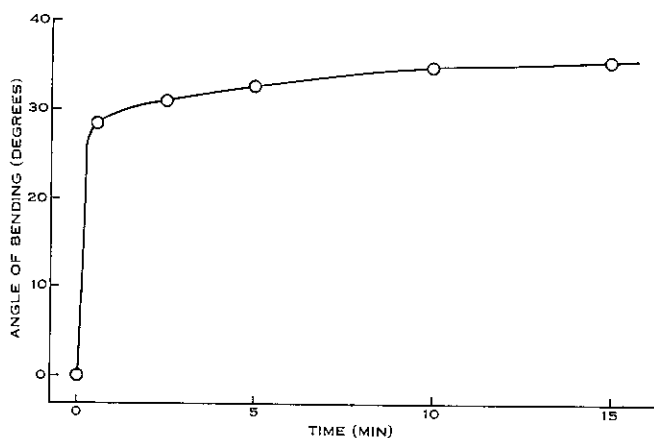


Fig. 3.—Epicotyl bending as a function of time from the application of a small, constant moment.

The time course of the deformation under a constant bending moment is shown in Figure 3. Sections of both control and loaded epicotyls grown in the main experiment underwent rapid initial bending followed by relatively slow bending which continued for up to 30 min at a continuously decreasing rate.

IV. DISCUSSION

(a) *Nature of the Response*

The data in Table 1 comprise the only quantitative evidence known to the authors which shows that an axial pressure can reduce the rate of elongation of seedling shoots. Pfeffer's (1893) apparatus was designed to measure maximum forces developed by tissue in essentially static systems rather than the dynamic effects of an axial pressure on the rate of growth. Moreover, although the pressure on the apex could be specified in Pfeffer's work, aeration could not be adequately controlled or specified. In the experiment of Gill and Miller (1956) aeration was

well controlled but the load could not be specified. In the present experiment, adequate provision was made for aeration, and bending was minimized by restricting the magnitude of the load.

The load on the epicotyls may be specified as the axial force applied per unit cross-section. An increase in the diameter of the loaded epicotyls (see Table 1) had the effect of reducing the applied pressure by 12% below its original value. Few of the epicotyls bent sufficiently to touch the cylinder wall. If, for those epicotyls which touched the wall, we let

W = applied load,

F_1 = the component of the load acting in the axial direction on the epicotyl above the contact with the cylinder wall,

F_2 = the axial force acting in the upwards sense below the contact,

θ = the angle of deflection of the epicotyl measured clockwise from the vertical direction,

ν = the coefficient of friction (epiderm/glass),

then

$$F_1/W = \cos \theta.$$

If it is assumed that the epicotyls were bent symmetrically around the contact, then, considering the equilibrium of forces at the contact,

$$F_2/W = \cos \theta - \nu \sin \theta.$$

The maximum angle of θ was 8° , and ν is unlikely to exceed 0.3 (cf. Barley 1962). On substituting these values, $F_1/W = 0.99$ and $F_2/W = 0.95$. Thus we may conclude that neither change in shape nor "vectoring out" of the applied load was likely to have had much effect on the magnitude of the axial pressure.

The reduction in elongation caused by loading may most readily be interpreted as the result of a change in shape of the cells elongating within the epicotyl. The stress due to the load was superimposed on, and interacted with, the stresses existing within the tissue. Although the original state of stress was not known, it is probable that most planes through the epicotyl sustained additional shearing stresses after application of the load. These stresses are thought to have caused irreversible plastic deformation of the cell wall together with viscous flow of the protoplast. In the root tip of *V. faba* shearing stresses acting a few millimetres behind the apical meristem can cause displacements of the young cells leading to a change in cell number across the diameter of the radicle (Hottes 1929); however, it is not known whether this occurred in the epicotyls.

No lag or induction period occurred after applying the load which could be distinguished from the lag due to the initial deformation of the epicotyl. The data obtained with Lockhart's apparatus (see Fig. 3) suggests that most of the initial deformation would have occurred within 15 min from the time of application of the load, and this observation is consistent with the shape of the height-time curves shown in Figure 2. The way in which the rate of bending of the epicotyl sections changed with time under a constant moment agreed well with the behaviour of *Avena* coleoptile sections observed by Tagawa and Bonner (1957). For the *Avena*

coleoptile the rapid and slow phases of the bending largely corresponded with the elastic and plastic parts of the deformation. The uniformity of the fresh weight data, and the shape of the height-time curves, suggest that if any induction period occurred (in Pfeffer's sense of the term), its duration would not have exceeded 1-2 hr.

As no intercalary meristem is present in the epicotyl of *V. faba*, the reduction in elongation is unlikely to have involved a change in cell number. Thus, the absence of any difference in epicotyl volume between loaded and control groups probably implies that there was no difference in cell volume. This appears to be inconsistent with a hypothesis of Barley (1962), which interprets the effects of a compressive stress on tissues in terms of the dynamics of enlargement of an elastic-walled cell osmometer. However, a critical test of this hypothesis requires control of the ambient rather than the axial pressure.

As only a single perturbation occurred at the time of loading, and as pistons were placed on both groups of epicotyls, any haptotropic response to the perturbation is unlikely to have produced the observed differences in growth. The sensitivity of the epicotyls to mechanical damage by knocking with the pistons is itself an interesting phenomenon which warrants further study.

(b) Ecological Implications

The need to restrict the experiment to small loads in order to prevent bending suggests that bending may delay emergence in field soils whenever lateral support is inadequate. Little data are available on the resistance likely to be encountered by shoots during emergence. The most appropriate data are those of Morton and Buchele (1960), who forced small penetrometers upwards through a 3 in. layer of soil and measured the point resistance. For a 2.7 mm diameter point in moist sandy loam at low bulk density (water content 16%; bulk density 1.1 g/cm³), the maximum resistance per unit cross section encountered in traversing the layer was 4 atm. After compaction of the surface of the layer the resistance rose to 12 atm. These values may be compared with the axial load of 0.5 atm used in the present experiments. The significant and appreciable (12%) reduction in the rate of elongation caused by this small load leads us to expect that mechanical resistance may have a considerable influence on seedling emergence in ordinary soils. The effects of mechanical stress on seedling emergence cannot, however, be adequately predicted until more is known about the effects of various stress distributions on elongation.

V. ACKNOWLEDGMENT

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