# REVERSIBILITY OF STROPHIOLAR PERMEABILITY TO WATER IN SEEDS OF SUBTERRANEAN CLOVER (*TRIFOLIUM SUBTERRANEUM* L.)

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*Summary*

Subterranean clover seeds which were impermeable to water were made permeable by percussion, but the impermeability could be quantitatively restored by covering the strophiolar region with Araldite or Vaseline, indicating that the percussed strophiole was the only site of water conduction.

When percussed seeds were held at relative humidities ranging from 40 to 100%, for 4–12 days before wetting, no change in the proportion of permeable seeds occurred, though the swelling rates of such samples were slightly increased. However, at lower R.H. some seeds reverted to the impermeable condition, the proportion being greater the lower the R.H. This resealing of the strophiole was observed only at R.H. at which the percussed seeds lost water.

When percussed seeds were first held at high R.H. the permeability could not be reversed by subsequent storage at low R.H., nor could the impermeability achieved by initial low R.H. storage be reversed by subsequent storage at high R.H. After initial storage at intermediate R.H., transfer to low R.H. caused some reversion to impermeability.

These results are interpreted in terms of strophiolar structure and their possible practical relevance is indicated.

## I. Introduction

In the papilionate legumes, the micropyle, hilum, and strophiole are specialized regions of the testa concerned with the transfer of water between the seed and environment. Apparently, conduction of water via the micropyle is significant only in some legumes seeds, such as peas and beans (Kyle 1959). The operation of the hilum as a hygroscopic valve permitting passage of water, as vapour, only to the exterior has been elucidated by Hyde (1954).

Less is known about the functioning of the strophiole (the lens, of some authors). It is usually seen as a small swelling of the testa on the raphe, between the hilum and chalaza (Fig. 1*A*). It was pictured by the older anatomists, e.g. Mattirolo and Buscalioni (1892), and detailed descriptions of it were given by Hamly (1932) for *Melilotus alba* Desr., by Zimmermann (1936) for additional species, and by Aitken (1939) for *T. subterraneum* L. Zimmermann reported that a split between the malpighian cells at the centre of the strophiole was always present, while Hamly claimed that the split appeared only after the seeds had been treated in some way, such as percussion.

Percussion increased the proportion of permeable seeds in samples of *M. alba* (Hamly 1932) and of *T. subterraneum* (Aitken 1939; Loftus Hills 1944; Ballard and Grant Lipp 1965). The last authors also showed that, whereas the main effects were

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apparent within 3–4 days, the appearance of permeable seeds still continued, although at a much reduced rate, for as long as 14 days after the percussion treatment. As revealed by osmic acid staining, the strophioles are sites of water entry in such percussed seeds (Hamly 1932; Aitken 1939).

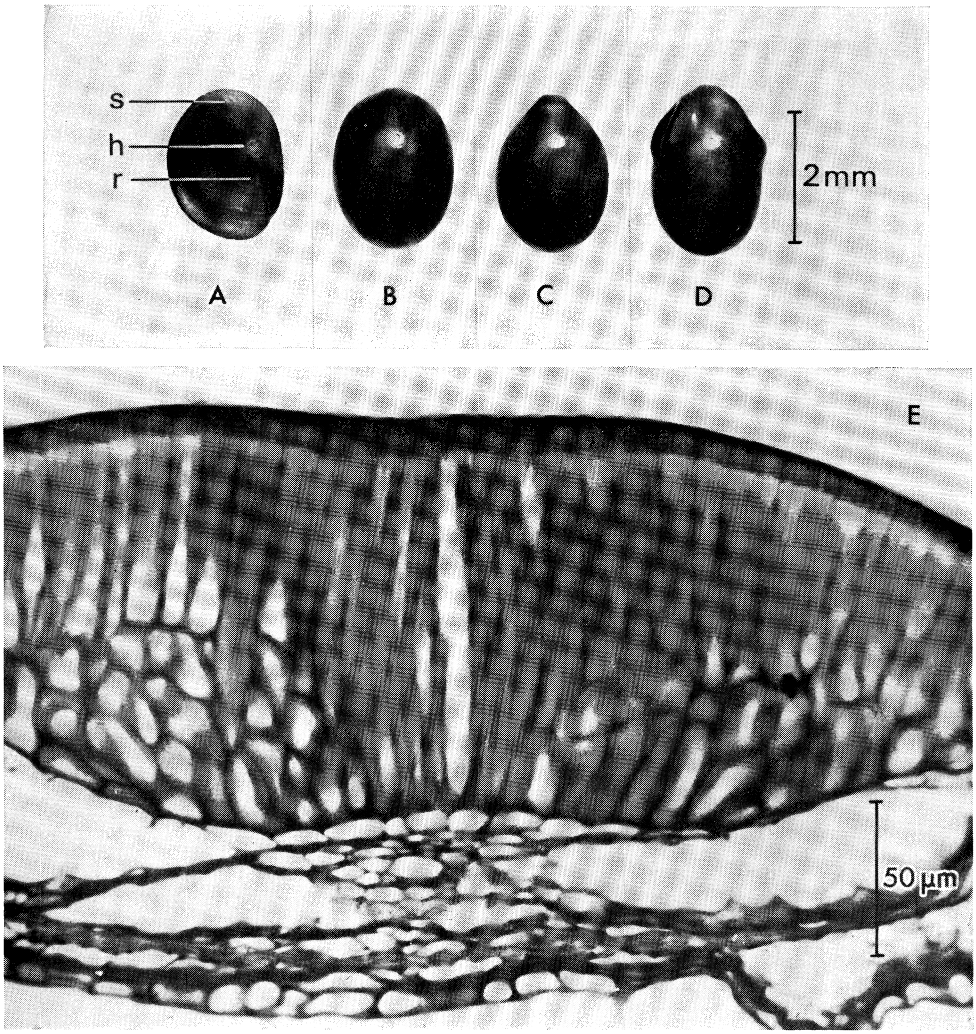


Fig. 1.—Subterranean clover seed (cv. Geraldton). *A*, before wetting, showing location of: *r*, radicle; *h*, hilum; *s*, strophiole. *B*, *C*, *D*, percussed seeds 5, 25, and 95 min respectively after wetting, showing swelling of the strophiolar region and finally wrinkling of the seed coat. *E*, section through the strophiole of a percussed seed showing a split between the malpighian cells extending towards the exterior to the level of the light line.

It is usually taken that impermeability develops in legume seeds only after they have dried to a moisture content of about 14% (Hyde 1954). However, reversions to the permeable condition have also been noted. Behrens (1934) claimed that seeds of

*Vicia villosa* Roth. and *T. pratense* L. could be alternated repeatedly between the impermeable and permeable conditions by storing them for 11–13 days at very low or very high relative humidities respectively. Moreover, he held that the whole of the testa and not any one particular area was concerned. Similarly, in *Lupinus varius* L. (Gladstones 1958; Quinlivan 1968) and in *Ornithopus compressus* L. (Barrett-Lennard and Gladstones 1964) impermeable seeds with moisture contents between 9 and 14% became permeable at high humidities. Below 9% moisture impermeability was irreversible. Nakamura (1962) also noted reversions in other species of legumes.

In this paper evidence will be presented which bears on the question whether strophioles are concerned with changes in the permeability status of legume seeds after they have matured.

## II. Materials and Methods

Seeds of *Trifolium subterraneum* L. cv. Daliak, Dinninup, and Geraldton were used in the experiments. Mature burrs were harvested by hand from material grown in Western Australia in 1966 and 1967 and in both seasons seeds were extracted from them by hand. Seeds were also obtained from the 1967 burrs by gently rubbing them between corrugated rubber boards. This treatment did not greatly increase the proportion of permeable seeds initially present in the Daliak and Geraldton samples, but, for Dinninup, raised it from 2 to 21% ($P < 0 \cdot 01$).

The moisture contents (oven dry basis after heating to 130°C for 1 hr) of the 1967 seeds, on arrival in Canberra were Daliak 2·05%, Dinninup 2·13%, and Geraldton 2·02%. No determinations were made for the 1966 material but it is probable that the water contents were similarly low.

Seeds were percussed as described by Ballard and Grant Lipp (1965), using 100 seeds per container and shaken at 800 oscillations per minute for 1 minute.

When we wished to make the strophiolar region non-conductive of water we covered it with either Vaseline or the water-resistant epoxy resin Araldite, and, in the latter case, the seeds were kept 3–4 days before testing to allow the Araldite to acquire maximum strength and water resistance. As can be seen in Figure 2, both methods were effective and each is advantageous in some respect. Seeds treated with Vaseline can be used immediately, but after the Araldite has set these seeds can be handled more cleanly and easily. Araldite sealing was used for most of the experiments.

Seeds were subjected to a range of relative humidities by holding them in perforated containers above sulphuric acid solutions of appropriate concentration (Wilson 1921) in sealed vessels at 20°C.

In all experiments the proportion of permeable seeds was determined by placing seeds on moist filter paper in petri dishes maintained at 20°C, and counting and removing swollen seeds, usually daily, for 14 days. Results are expressed as means of four replicates each of 100 seeds. Statistical analyses were carried out on values of percentage swollen seed after angular transformation.

Strophioles were prepared for microscopic examination by embedding pieces of testa containing them in 8–10% gelatine containing 2% (v/v) dimethyl sulphoxide. The gelatine block was frozen and 6–10 $\mu$m sections were cut at −24°C in a cryostat. The sections were stained with 0·02% toluidine blue in 0·01M acetate buffer at pH 4.

## III. Results

### (a) *Percussion Effects*

The proportion of permeable seeds in these carefully handled samples was small, usually about 5%, and it increased very little either during dry storage in the laboratory for 2–3 years, or under moist conditions during a 14-day test period.

Percussion, however, greatly increased the proportion of permeable seeds [Figs. 2(*a*) and 2(*b*)]. Most seeds were swollen after 4 days of the test, though a few more became swollen during the next 10 days. This agrees with the pattern observed by Ballard and Grant Lipp (1965) using seed of subterranean clover (Commonwealth Plant Introduction No. 19465), though their final value differed from those of Figure 2. Still different final levels can be noted in other data of the present paper. It seems that the capacity to be rendered permeable by percussion is a sample characteristic, depending partly on the properties of the cultivar and partly on conditions of growth and maturation—e.g. values for swollen seeds at 14 days for Geraldton 1966 and 1967 were 71 and 47% respectively.

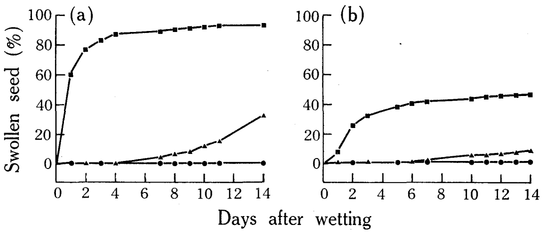


Fig. 2.—Time course of swelling of subterranean clover seeds after percussion, with and without strophioles covered with a water-impermeable material. (*a*) cv. Daliak; (*b*) cv. Geraldton. ● Unpercussed (control). ■ Percussed only. ▲ Percussed and strophioles covered with: Araldite (*a*), Vaseline (*b*).

When the strophioles of the percussed seeds were covered with either Vaseline or Araldite before wetting, the level of permeability was reduced to that of the unpercussed state and remained at this level for several days. We attribute the gradual slight increase in permeability after day 5 either to loss of water resistance in the covering material or to loss of adherence to the testa, allowing slow seepage of water.

A similar result has been recorded for all the cultivars in both seasons. This quantitative restoration of impermeability to the unpercussed level, achieved by covering the strophioles, permits the conclusion that the latter are the only sites of permeability in percussed seeds.

It is unnecessary to use dyes to make apparent the dominant role of the strophiole in the early entry of water into percussed seeds. Under low magnification, seeds about to swell become distinguished by a slight enlargement of the strophiole and some lightening of tone in the immediate vicinity due to pigment leaching. Soon a small blister or protuberance appears as both the testa and, to a smaller extent, the cotyledon tissues swell locally. The subsequent seed enlargement and coat wrinkling and stretching spreads from this focal point (Figs. 1*B*, 1*C*, 1*D*).

## (*b*) *Storage Effects*

If seeds are not placed in appropriate conditions for imbibing water immediately after percussion, the result obtained on doing so depends on the relative humidity at which they have been held meanwhile. As seen in Figure 3, when held at 99% R.H.

the final proportion of swollen seeds does not differ from that in the unstored control, though the rate of swelling is somewhat increased, but following storage at 0% R.H.

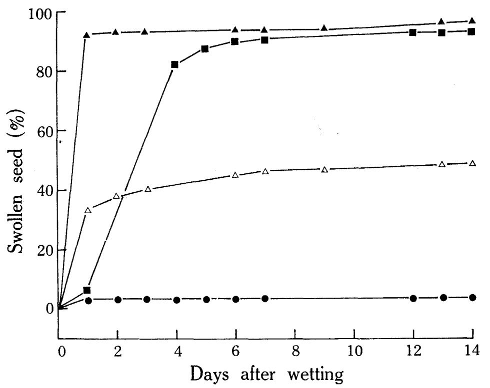


Fig. 3.—Effect of storage prior to wetting on subsequent course of swelling of Dinninup seeds.
● Unpercussed (control).
■ Percussed, no storage.
▲ Percussed, stored 12 days at 99% R.H.
△ Percussed, stored 12 days at 0% R.H.

the final proportion of swollen seed is much reduced. Results over the whole relative humidity range show that this restoration of impermeability, or resealing of strophioles, becomes apparent following storage at R.H. < 20% (column 2, Table 1).

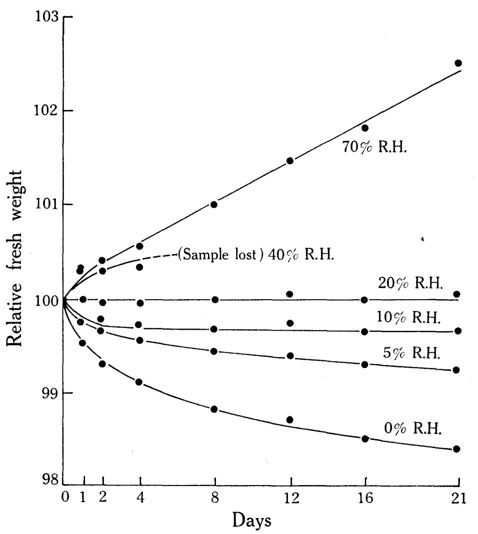


Fig. 4.—Relative weight changes of Dinninup seeds during storage at several relative humidities.

In the above experiment, other samples of percussed seeds, placed in each of the relative humidities, were weighed at intervals (Fig. 4). Above 20% R.H. the seeds gained weight, below 20% R.H. they lost weight, and there was virtually no change at 20% R.H. Their moisture content was thus in equilibrium with 20% R.H., and only when drying was possible was resealing observed.

The results of similar storage experiments with different cultivars from both seasons are also presented in Table 1. It seems general that at low relative humidities there is some reversion to impermeability, the amount depending on cultivar and being greater the lower the relative humidity during storage.

TABLE 1

EFFECT OF RELATIVE HUMIDITY DURING STORAGE OF PERCUSSED SEEDS ON THEIR SUBSEQUENT SWELLING

Values are percentage swollen seed 14 days after wetting. Within columns, values without asterisks do not differ significantly from values for percussed seeds which were tested immediately whilst those with one and two asterisks differ significantly at $P<0\cdot05$ and $P<0\cdot01$ respectively. n.t., not tested

| Storage R.H. (%) | Dinninup 1966,† Stored | | Geraldton 1966,† Stored 12 Days | Geraldton 1966,‡ Stored | | Daliak 1967,‡ Stored 20 Days |
|---|---|---|---|---|---|---|
| | 12 Days | 22 Days | | 12 Days | 20 Days | |
| 99 | 94 | n.t. | n.t. | n.t. | n.t. | n.t. |
| 90 | n.t. | n.t. | 70 | 38 | 38 | 96 |
| 85 | 93 | n.t. | n.t. | n.t. | n.t. | n.t. |
| 70 | 91 | 97 | 63 | 41 | 31 | 95 |
| 40 | 95 | 94 | 58* | 30 | 37 | 94 |
| 20 | 89 | 92* | 62 | 33 | 28 | n.t. |
| 10 | 81** | 87** | n.t. | 33 | 29 | n.t. |
| 5 | 77** | 71** | 31** | 16** | 13** | 44** |
| 0 | 49** | 52** | 12** | 6** | 2** | 15** |
| Controls: | | | | | | |
| Seeds not percussed, not stored | 4 | 0 | 6 | 5 | | 6 |
| Seeds percussed and tested immediately | 93 | 96 | 67 | 35 | | 97 |

† Seeds hand separated. ‡ Seeds rubbed on board.

Results given in Table 1 suggest that the effects of storage are completed in 12 days. Those given in the following tabulation, which shows the effect of duration of storage at 0% R.H. of percussed Dinninup seeds on their subsequent swelling as measured 14 days after wetting, indicate that significant effects are found after only 1 day, and that little further change occurs after about 4 days:

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Duration of storage (days): | 0 | 0·33 | 1 | 2 | 4 | 8 | 12 | 16 |
| Swollen seed (%) | 93 | 88 | 79 | 75 | 53 | 49 | 42 | 48 |
| Transformed values* | 74·5 | 70·2 | 62·9 | 60·1 | 46·9 | 44·6 | 40·2 | 44·0 |

* Least significant differences: $P<0\cdot05$, 6·6; $P<0\cdot01$, 8·9.

The precise times found would probably be different at other storage relative humidities.

### (c) *Stability of Storage Effects*

Whether these changes in strophiolar opening caused by exposure to certain relative humidities were permanent, or could be modified by later exposure to different humidities, was investigated by holding percussed seeds for 12 days at several humidities, withdrawing samples for test, and transferring the remainder to other humidities.

The results of one such experiment are presented in Figure 5. At both ends of the humidity scale changes in strophiolar opening were no longer possible—those strophioles opened by percussion and exposed to 100% R.H. remained open and could not be resealed by exposure to 0% R.H., nor could those opened by percussion and resealed by exposure to <20% R.H. be reopened by exposure to 90% R.H. However, at intermediate humidities changes occurred. Some of the strophioles opened by percussion, and which remained open during exposure to 40–90% R.H., were resealed by later exposure to 0% R.H., the amount of resealing being greater the lower was the initial storage relative humidity.

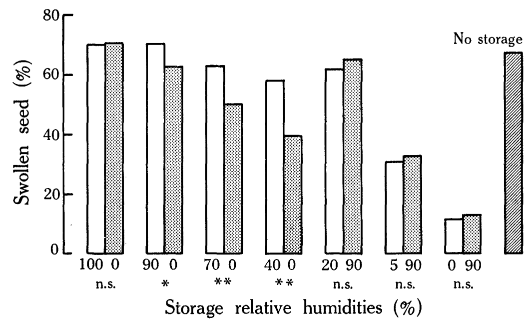


Fig. 5.—Effect of alternation of humidity during storage on strophiolar opening in percussed Geraldton seeds, as indicated by percentage of swollen seed 14 days after wetting. Open columns show values after storage for 12 days at indicated relative humidity, stippled columns after a further 12 days at different relative humidities. Significance of difference between pairs: n.s. = not significantly different; * $P<0\cdot05$; ** $P<0\cdot01$.

In an attempt to determine why percussed, and thus permeable, seeds which had been stored at high R.H. could not be made impermeable again, groups of percussed seeds were held for 12 days at a range of humidities. The strophioles of half of each group were then covered with Vaseline and half remained uncovered. Both lots were immediately wetted and the results are recorded in Table 2. At 70% R.H. and lower, blocking the strophioles restored the impermeability to the unpercussed level [as in Figs. 2(*a*) and 2(*b*)] but at 90% R.H. most of the seeds with covered strophioles remained permeable, indicating that they were then permeable at sites other than the strophiole.

## (*d*) *Strophiolar Structure*

The main feature seen in sections of percussed strophioles is the fissure extending through the malpighian cells of the median region. In some cases this is almost of the nature of a tear, carrying through to the exterior. It is difficult to determine whether such cases arise as a form of relatively severe damage during percussion itself or through tearing during sectioning. Probably both causes operate. In other cases, as in Figure 1*E*, the slit extends only to, or just beyond the light line and cannot be seen to reach the exterior. However, such strophioles would be conductive, since in subterranean clover (Aitken 1939) and other papilionate species (Kühn 1925; Hamly 1932) the cuticle and subcuticular layer (matrix) have been found to be permeable to water.

In our sections it is also apparent that the long, narrow malpighian cells of the central region have definite caps, as described by Hamly (1932), while the adjacent cells have more flattened ends.

TABLE 2

EFFECT, ON THE SWELLING OF PERCUSSED SEEDS, OF COVERING THEIR STROPHIOLES AFTER STORAGE AT VARIOUS RELATIVE HUMIDITIES

Geraldton seed stored 12 days. Strophioles covered with Vaseline. Values given are percentage swollen seed 7 days after wetting. Unpercussed controls = 8%; percussed unstored controls = 64%

| Condition of Strophiole | Storage R.H. (%) | | | | |
|---|---|---|---|---|---|
| | 90 | 70 | 40 | 20 | 5 |
| 1: not covered | 71 | 65 | 65 | 56 | 39 |
| 2: covered | 54 | 12 | 13 | 12 | 5 |
| Significances: | | | | | |
| Between line 1 and percussed unstored controls | n.s. | n.s. | n.s. | n.s. | * |
| Between line 2 and unpercussed control | ** | n.s. | n.s. | n.s. | n.s. |
| Between lines 1 and 2 within columns | * | ** | ** | ** | * |

* $P < 0 \cdot 05$. ** $P < 0 \cdot 01$. n.s., not significantly different.

## IV. DISCUSSION

The central finding of the above results is that the permeability induced by percussion and opening of strophioles is not absolute, but can be reversed by manipulation of relative humidity. This type of reversal differs from that previously recorded (Esdorn 1930; Behrens 1934; Gladstones 1958; Barrett-Lennard and Gladstones 1964; Quinlivan 1968), where the permeability of the whole testa, or random sites on it, is concerned, and which occurs only in the range of 9–14% seed water content. In our case the reversibility is concerned only with the strophiole and it occurs at seed moisture contents as low as 2%. We do not know whether there is an upper seed moisture content at which strophiolar reversibility is no longer possible.

The only previous relevant report is that of Hamly (1932) who found no change in the permeability of percussed seeds of *Melilotus alba* Desr. after storing "for weeks in a dry place". But this scarcely conflicts with our findings, since the relative humidity may not have been low enough in relation to seed moisture content to permit drying.

We suggest that the restoration of impermeability by exposure to low relative humidity may be caused by the shrinkage that is likely to occur on drying. The slits of percussed strophioles do not all extend to the exterior (Fig. 1*E*), and the re-apposition of surfaces over only a part of the length of the slit would probably be sufficient to restore impermeability. The details of the resealing–relative humidity table can be interpreted if percussion, with normal biological variation, produced a range of strophiolar disturbance. The strophioles least-opened would probably be easiest to reseal, and more resealing could be effected with greater shrinkage as a result of greater drying (lower humidity).

Other features of our results (Fig. 5) can be explained on a similar basis:

(1) The inability to restore permeability by high after low humidity: The low humidity seals the strophioles through which water vapour can no longer enter, and the subsequent high humidity induces hilar closure. Judging from the results with *Lupinus* (Quinlivan 1968), at 2% moisture content, the seeds are outside the range of conditional hardness so water entry through the testa is impossible and the seeds remain unswollen.

(2) The inability to restore impermeability by low after very high humidity: Seeds can absorb water vapour through the opened strophioles and so swell, apparently sufficiently, to make minute cracks in the testa (Table 2). If the testa were completely breached it would not be repairable. Even superficial cracks in the testa generally would probably not be repairable, because there the middle lamellae of the malpighian cells are not arranged in a definite plane, as at the strophiole (Hamly 1932). In any case this amount of swelling is likely to open the strophioles beyond their repairable limits.

(3) The partial resealing by low after intermediate humidities, more seeds being resealed the lower the initial humidity: This cannot be due to partial drying in the first phase becoming completed in the second, since, at these intermediate humidities, not drying but water absorption occurs (Fig. 4). It seems that the resultant swelling which is insufficient to split the testa [as in (2) above] is nevertheless sufficient to enlarge the strophiolar openings so that many of them cannot be repaired. However, more can be resealed from the lower humidities where swelling and strophiolar enlargement would be less.

### *Practical Considerations*

Since the elegant descriptions of Hyde (1954) the part played by the hilum in regulating water loss from developing seeds has been recognized. After the testa becomes impermeable, the hilum functions as a one-way hygroscopic valve. It opens when the relative humidity of the atmosphere round it is lower than that in equilibrium with the moisture content of the seed, permitting water loss and seed maturation, and closes in the reverse situation.

The results presented here indicate that the strophiole, in addition to being a predetermined site of weakness in the testa through which imbibition can ultimately occur, can also exhibit movements in response to changes in relative humidity, but in the opposite sense to those of the hilum. These movements, however, are more restricted than those of the hilum which can be repeatedly opened and closed, whereas the strophiole is closed only by dry conditions. If it is either open or shut, moist conditions are without effect on the opening.

There is yet no evidence that this strophiolar response has adaptive significance, but it could possibly affect the properties of commercial seed samples. If the latter, after processing, ever contained some seeds which had been rendered permeable at the strophiole only, storage under low humidity during the summer months may result in some strophioles being resealed, with a consequent increase in impermeability. Such lots may not then meet required standards, though at the time of processing it appeared they would do so.

## V. Acknowledgments

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