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Imbibition Temperature Sensitivity of Lima Bean Seeds Controlled by Initial Seed Moisture[1, 2]

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Abstract. **Excised embryonic axes and whole seeds of *Phaseolus lunatus* L. were previously shown to be injured if exposed to low (5°–15°) temperature during the initial stages of imbibition. Present data show that this chilling injury during imbibition of liquid water can be prevented if axes are first allowed to absorb water vapor. The increase of initial water content to 20% increases growth even of unchilled axes, and reduces leaching of 264 mμ absorbing compounds. Protection resulting from increased water content is at first independent of the temperature at which water vapor was absorbed. However, longer exposure of high moisture axes to low temperature results in typical chilling injury. The response to initial seed moisture is repeatedly reversible with changes in water content. Because the same response occurs in intact seeds, it may be possible both to protect them against low temperature injury and to increase vigor by increasing seed water-content prior to planting.**

Earlier work with lima beans (15) showed that these seeds are sensitive to chilling injury, even at moderately low temperatures, during the early stages of imbibition. Other reports suggest that a similar response can occur in crimson clover (7), cacao (8), cotton (1), cucumber (18), garden beans (14), and peas (6). Injury has been found to be restricted to specific periods during germination or early growth. The range of plants involved suggests that the phenomenon is of general significance for plant establishment.

There was a major problem of interpreting the original observations (15), and this same problem applies to other published work (1, 2, 10). In the case of an excised embryonic axis placed in water, the outer cells will be hydrated before the cells in the center of the axis. In the case of seeds, water is frequently absorbed only through restricted areas of the seed surface. Thus, until the axes or seeds are fully hydrated, data on water content, which is only a measure of the average of the cell population, cannot be readily interpreted in terms of physiological or biochemical control mechanisms.

To avoid this problem, I have now allowed axes to absorb water vapor instead of liquid water, assuming that the slower rate of water uptake will more nearly match the speed of water distribution within the axis. The results show that temperature sensitivity during subsequent imbibition of liquid water is restricted to axes and intact seeds with a moisture content below 20%. Moreover, even with imbibition at 25°, growth can be significantly increased by raising the initial moisture level above the "air-dried" level.

These data raise interesting theoretical questions about processes occurring at water levels below those considered normal for biological systems. They also provide a practical tool by which seed germination and seedling growth may be raised to the highest and most uniform levels possible.

Materials and Methods

The seeds used were *Phaseolus lunatus* L. cv. Thorogreen, supplied by Ben Fish and Sons, Incorporated, Santa Barbara, California. Most techniques used were those previously described (15). To establish the initial moisture level, axes were equilibrated at 5° or 25° against relative humidities maintained by glycerol solutions (5). Embryonic axes, in groups of 5, were imbibed and grown in 2 ml distilled water in 10 mm × 35 mm plastic Petri dishes. They were imbibed 30 min at either 5° or 25° and changed to fresh water at 25°. They were grown at 25° and at the end of 10 hr the leachate was collected and the axes were placed in fresh water. Growth, as measured by increase in fresh weight, was recorded at 3 hr intervals during the period 10 to 19 hr after the beginning of imbibition.

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[1] Published with the approval of the Director of the Colorado Agricultural Experiment Station as Scientific Series Paper No. 1379.

[2] Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA, and does not imply its approval to the exclusion of other products that may also be suitable.

Results

As axes absorb water vapor at 25°, they become progressively less sensitive to chilling injury (Fig. 1). Surprisingly, even with 25° imbibition, subsequent growth increases until the axes reach about 20 % water (dry wt basis = 21.8 %; fresh wt basis = 17.9 %). This value corresponds to that previously reported (15) for the uptake of liquid water. However, in the present system, uptake of water vapor is so much slower that 28 hr is equivalent to about 10 min in liquid water. As a result, we can consider this value of 20 % to apply to a homogenous cell population.

Immediately after imbibition the axes lose organic materials to the water in which they are growing, in an inverse relationship to their subsequent growth (Fig. 2). In 1 large experiment the relationship of growth 10 to 19 hr after the beginning of imbibition to OD_{264} of leachate from the 0.5 to 10 hr period was described by the equation $y = -64.6x + 48.3$ with a correlation of 0.927. Thus the previously reported (15) relationship between leachate and growth also extends to the effects of axis moisture on growth.

The time relationships of growth and leaching show that the growth response follows the cell damage which results in leaching. However, the data do not prove that the reduction in growth is the direct result of cell damage because the leachate may stimulate growth of injurious microorganisms. The system used is not sterile, and Schulz and Bateman (17) have shown that seeds injured by imbibition temperature are subject to subsequent attack by microorganisms.

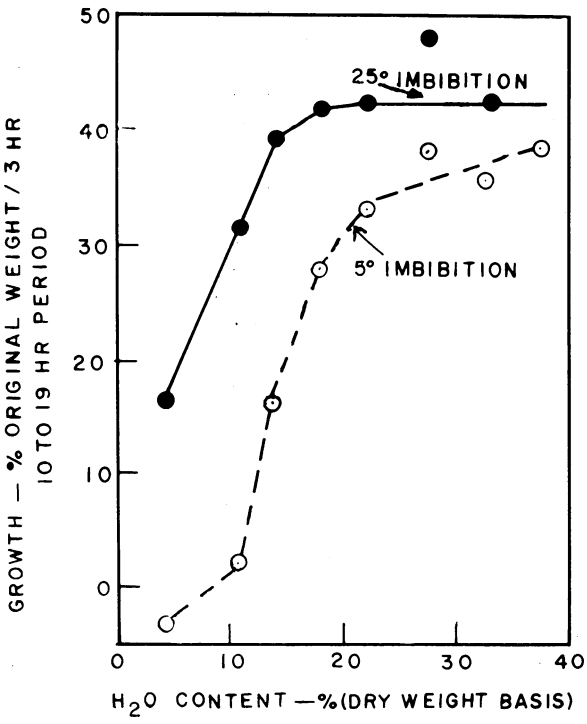

FIG. 1. Growth of embryonic axes as a function of axis moisture level at the time of imbibition of liquid water. Axis moisture levels obtained by equilibrating 28 hr at 25° against relative humidities controlled by glycerol solutions.

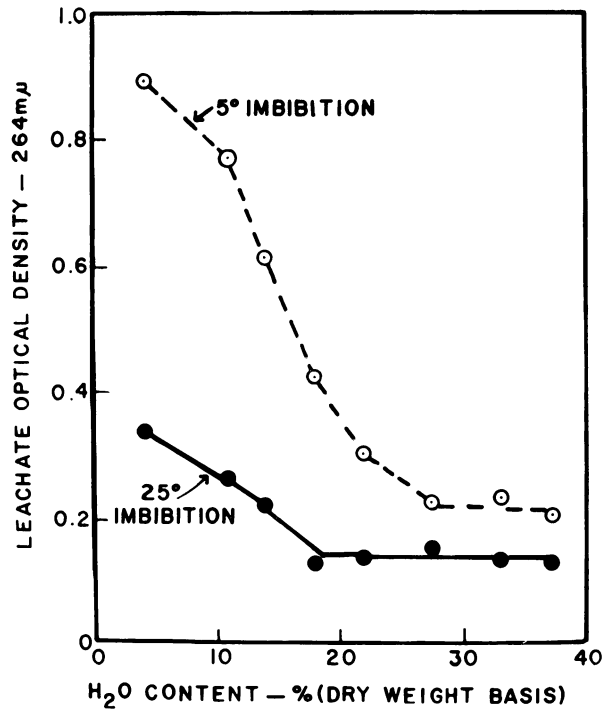

FIG. 2. Leaching from embryonic axes during the period 0.5 to 10 hr following the beginning of imbibition. Growth of these same axes in the 10 to 19 hr period is shown in Fig. 1.

The initial effect of water vapor uptake is independent of the temperature at which vapor uptake occurs (Fig. 3). As the axes are allowed to equilibrate at 5° to a constant moisture level of 40 %, the protective effect of water content is quickly established (Fig. 4). However, this protection then slowly disappears, accompanied by a parallel decrease in growth after 25° imbibition. This disappearance of protection at 5° is not typical of storage injury resulting from high moisture, because it occurs at a low temperature but not at 25°. Therefore, this decrease appears to be the result of typical chilling injury. Other experiments failed to show a relationship between this injury and moisture content (above 20 %). Experiments in which axes were imbibed at low temperatures and then dried and returned to 25° failed to show any recovery from injury.

The effect of axis moisture on imbibition temperature sensitivity is, however, repeatedly reversible

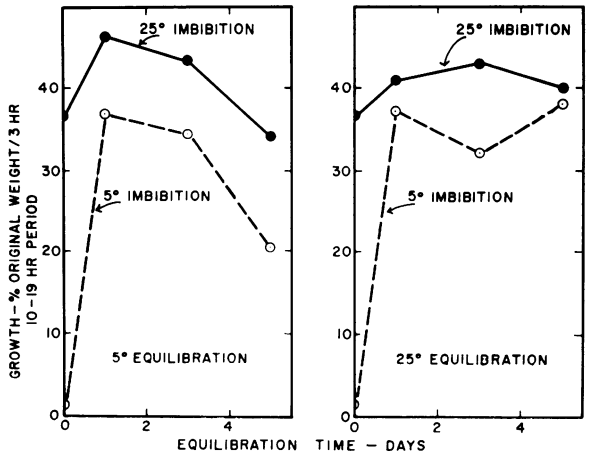

FIG. 3. Comparison of protective effect of water vapor absorbed at 5° with water vapor absorbed at 25°. Axes equilibrated against relative humidities to give moisture contents of 27 to 45 % (dry wt basis) from the first day of the equilibration, in contrast to 8.8 % at the beginning of the experiment.

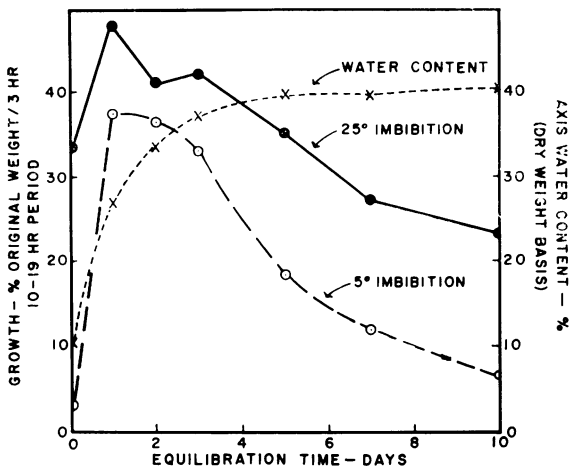

FIG. 4. Time course of protection and temperature injury in axes equilibrated at 5° against a relative humidity controlled by 20 % glycerol.

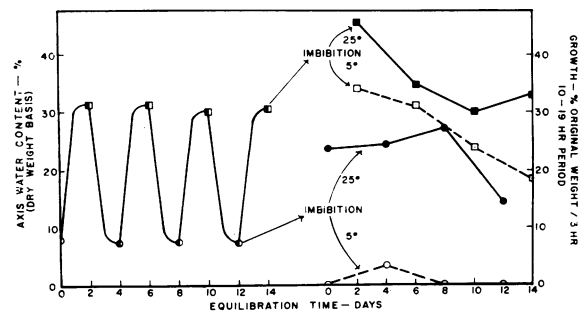

FIG. 5. Change in imbibition temperature sensitivity and growth following repeated changes in axis hygroscopic moisture level at 25°.

(Fig. 5). Thus, whatever the mechanism of the response, it depends only on the moisture level at the time of imbibition, not on an irreversible reaction (2). However, axes subjected to repeated periods of high moisture show decreased growth following both 5° and 25° imbibition. This decrease may be storage damage resulting from the length of time the axes were exposed to a high moisture level. The mechanism which causes axis moisture sensitivity may then be the same mechanism as that destroyed by unfavorable seed storage conditions.

The effect of initial seed moisture also applies to whole seeds, both in terms of size of seedlings (Fig.

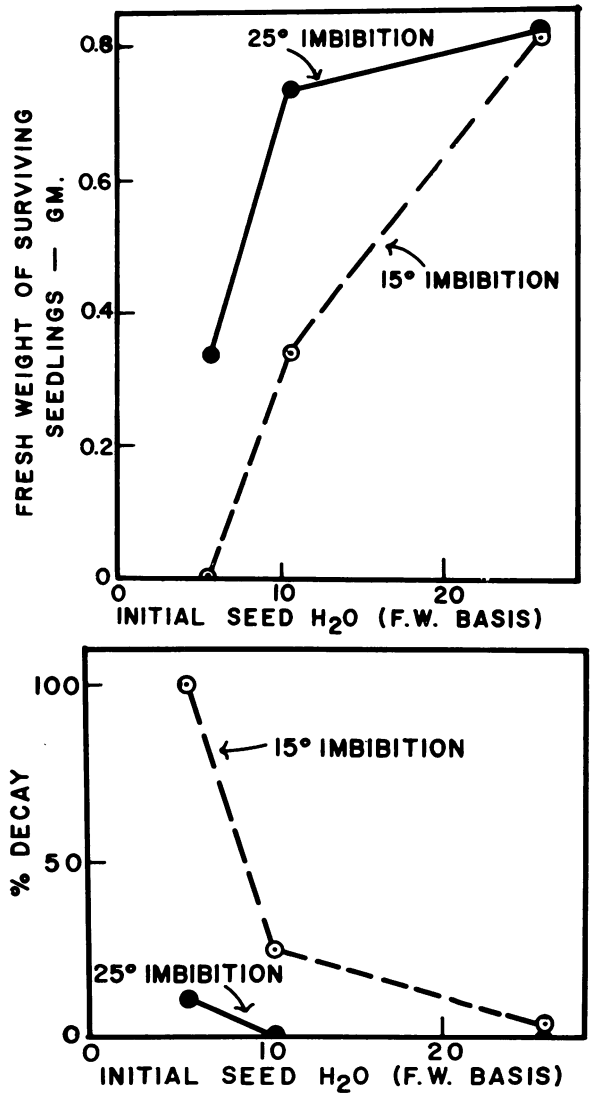

FIG. 6, 7. Effect of initial seed moisture on imbibition temperature sensitivity of scarified lima bean seeds as measured by growth of surviving seedlings and decay of seeds. Moisture levels established by equilibrating at 25° against dry $CaCl_2$ (1 week) or water (1 and 2 weeks). Imbibed 24 hr in sand at 15° or 25°; germinated 9 days at 25° on a 16-hr day in individual rolled towels.

6) and number of surviving seedlings (Fig. 7). Thus, in agreement with previous results (15), physiological studies with excised axes have direct practical applicability to whole seeds.

Discussion

Our knowledge of cell metabolism is based almost entirely on studies of hydrous systems isolated from fully hydrated cells. However, the imbibition temperature-sensitive stage is controlled by physical conditions or metabolic events in cells normally considered "dry", *i.e.*, cells lacking water as a solvent phase. This is not the only phenomenon known to occur in seeds at low moisture levels, since afterripening of dormant seeds in dry storage is well known (12).

In the earlier paper (15) it was suggested that temperature sensitivity might be a physical phenomenon, perhaps operative on a membrane system such as that of mitochondria. However, Christianson (2) has questioned the probability of a purely physical mechanism, preferring some type of metabolic control and concluding from his data: "An irreversible event that is blocked or disrupted by chilling apparently occurs during early seed hydration." This statement represents a major change from the classical idea of imbibition as a purely physical phenomenon (12). The rationale is very strong for this statement. A body of evidence is accumulating (11) to show that the change from seed development to seed germination involves a major change in metabolism from synthesis of reserve materials during seed development to the degradation and utilization of these reserves during germination. In both lima (9) and garden beans (13), electron microscopy during seed maturation shows the disappearance of polysomes with simultaneous appearance of monosomes, suggesting control at the translation level. In barley, gibberellic acid from the embryo appears to act at the transcription level in causing the synthesis of α-amylase (4). In early imbibition of wheat, ribosomes appear to be unable to synthesize protein because of a lack of mRNA (10).

However, it is obvious that some enzymes formed during seed development must persist, or be activated, to provide the metabolic machinery essential for the initiation of germination (11). At this time we cannot be sure of the relative importance of "old" and "new" enzyme systems in the initial control of germination. It is therefore vital to recognize that all previous data (1, 2, 10, 15) were obtained by using material in which only part of the cells were fully hydrated. Thus these concepts, originally developed from experiments using fully hydrated cells, could be applied to the hydrated cells of the seed. However, the present data show that response occurs in cells near the limit of existence of solvent water. If the control is metabolic, then the elements of the metabolic system must be placed in close physical proximity during maturation of the seed, and the system must be freely reversible. Therefore, the physical organization of the system must be critical to the initiation of germination. However, it is equally possible that the effect of hydration is to influence the structure of some macromolecular component of the system which, as a result of its initial level of hydration, has a secondary influence on metabolic events.

While these data raise interesting theoretical questions about the mechanisms controlling germination, they may also provide a practical method for increasing germination and vigor of seeds. I have presented data to show that these results are applicable to intact lima bean seeds. It has also been shown that initial seed moisture can have a major influence on the germination of such diverse crops as garden beans (16) and cotton (3).

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