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Germination and Seedling Vigor of Snap Beans
(Phaseolus vulgaris L. cv. Slenderette)

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Jack R. Staig

Laboratory and greenhouse studies were undertaken to elucidate the relationship between chilling and soaking stresses to imbibing snap bean seed. Studies in which slow imbibitional chilling of medium moisture seed at 5 °C was followed by soaking gave no indication that injury due to soaking was enhanced by prior chilling. Slow imbibition at either 5 °C or 20 °C reduced the susceptibility to soaking injury, with the time required to reach minimum susceptibility to soaking injury being inversely related to temperature. Seed moisture content at the point of minimum susceptibility was the same for both temperatures of slow imbibition: about 60%. The duration of slow imbibition and the seed moisture content that were necessary to minimize soaking injury were inversely related to initial seed moisture. Rapid rate of water uptake was less injurious when seed moisture was

higher. No evidence was found to indicate that soaking of snap bean seed prior to 5 °C chilling resulted in increased injury due to chilling. The simultaneous soaking and chilling of high (17.6%), medium (9.5%), and low moisture (6.8%) seed resulted in an injury pattern which tended to parallel water uptake which was faster at 20 °C than at 5 °C, and faster with higher initial seed moisture contents. The exception to this was high moisture seed which possibly showed a slower rate of injury at 20 °C soaking than at 5 °C. No consistent relationship was found between amount of soaking injury and free water accumulations. There was no definite evidence of chilling injury in any of the experiments.

The Effects of Soaking and Low Temperatures on
Germination and Seedling Vigor of Snap Beans
(Phaseolus vulgaris L. cv. Slenderette)

by

Timothy W. Walls

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Dean of Graduate School

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Typed by Ilene Anderton for

Timothy W. Walls

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THE EFFECTS OF SOAKING AND LOW TEMPERATURES
ON GERMINATION AND SEEDLING VIGOR OF SNAP BEANS
(Phaseolus vulgaris L. cv. Slenderette)

I. INTRODUCTION

Snap beans (Phaseolus vulgaris L.) are a major commercial crop in the Willamette Valley of Western Oregon. They are, in addition, grown extensively by home gardeners. While conditions in the valley during June and July can be nearly ideal for seed germination and seedling growth, plantings made during the cold and wet conditions of late April and early May are subject to significant reductions in stand, as well as decreased vigor and yield of surviving plants.

There are two important aspects to the problem. One of these is injury due to soaking which is defined simply as "the complete immersion of seeds in water" (31), but which would also apply in a less strict sense to seeds imbibed in media containing a high percentage of water. Seeds of some cultivars of Phaseolus vulgaris are damaged by only brief periods of soaking. The second is "chilling injury", a term given to damage caused by exposure to low temperatures above freezing. In this case, it is specifically the negative effects upon seeds germinated in a cold medium.

When one considers the hypothesized causes and remedies for soaking and chilling injury, there are many similarities:

- 1) Both soaking and chilling injury have been reported in the literature to be decreased by slowing the rate of water uptake (51, 53, 54, 55, 72, 78, 79).
- 2) Both have been decreased by raising seed moisture slowly prior to imbibition (8, 11, 23, 32, 34, 40, 45, 48, 51, 52, 53, 59, 78).
- 3) Injury to both is accompanied by exudation of electrolytes, suggesting the involvement of membrane dysfunction (32, 41, 42, 44, 51, 54, 63, 64, 67).
- 4) The injurious effects of each stress are reportedly intensified by the simultaneous administration of the other (23, 33, 34, 44, 56).

Research has tended to consider chilling and soaking separately. Yet, the high-moisture conditions under which much of the work on chilling of imbibing seeds has been done suggest that soaking actually may have contributed to injury attributed solely to chilling.

The objective of this research is to elucidate the interaction between chilling and soaking. What would be

the effect, for example, of sequencing the soaking and chilling stresses? Can exposure to one prior to the other enhance the injury from the second? Injury due to soaking is thought to be related to free water in the seed (48). Could chilling have an effect on free water so that soaking injury was intensified? Does free water from soaking appear to intensify the amount of injury from chilling? Initial seed moisture plays a role in resistance to chilling injury (8, 11, 32, 34, 40, 46, 52, 53, 59). What is its role in soaking injury? How do patterns of total and free water vary during soaking and chilling with initial seed moisture?

The experiments in Chapter IV are directed toward answering these questions and in doing so, help us arrive at a better understanding of the relationship between chilling and soaking. It is hoped that this increased understanding will in turn suggest methods to improve germination and seedling vigor of early plantings.

II. REVIEW OF THE LITERATURE

Soaking Injury

Symptoms

The seeds of a number of cultivars of snap beans (Phaseolus vulgaris L.) can be damaged by soaking in water for short periods of time. As soaking time for a given cultivar increases, one observes progressive decreases in germination rate, an even greater reduction in the number of mature plants (since many of those seeds from which radicles emerge do not survive), a decrease in the average height and weight of the above-ground portions of the plants and an increase in the time required to reach maturity (33). In addition, plants from soaked seed have leaves which are thinner and cells which are more tightly packed with fewer open spaces. The ratios of the thicknesses of phloem to xylem and of carbohydrates to nitrogen-containing compounds are higher for plants grown from soaked seed (2). Harrison (29), using the tetrazolium test with corn and pea seed, found that the tissues most likely to be injured during soaking were the meristematic and surrounding areas of the root and shoot.

Powell and Matthews (55) found an association between slower growth of seedlings from damaged embryos and a slower rate of food reserve transfer from the cotyledons to the growing axis. They observed a large decline in the respiration rate of the cotyledons, and attributed it to the death--also observed by tetrazolium staining--of large numbers of cells in the cotyledons.

Kidd and West (33) noted that the immediate effects of soaking on bean seed might appear to be invigorating, while at the same time resulting in decreased subsequent growth and increased mortality. In their study almost all of the seeds soaked for 24 hours germinated with radicles which at first appeared to be larger and stronger than those of unsoaked beans. By the 12th day, however, about half had died and the rest showed only about one-sixth of the growth of the non-soaked controls.

Soaking increases the exudation of ions such as potassium, amino acids, sugars and other compounds, especially from poor vigor or damaged seed. This exudate is thought to encourage the growth of, and attack by, pathogens such as Fusarium and Pythium and greatly increase seedling mortality (41, 42, 44, 51, 63, 67).

Transverse cracking of the cotyledons of the seeds often result from soaking. This is attributed to the "...physical stresses imposed by differential hydration of the outer and inner tissues of cotyledons..."(32). It is most likely to occur to seeds of low moisture content during rapid hydration (19, 32, 43, 53). It has also been reported by various investigators to be either independent of temperature (19, 53) or-- despite slower rate of water uptake-- more serious at low temperatures (43). It is a mechanical injury that seriously reduces seedling growth by impairing nutrient flow from the cotyledons to the axes.

Factors Influencing the Amount of Soaking Injury

There are a number of factors which can influence the degree of injury sustained during soaking:

1) Temperature: Several authors (23, 33, 34, 44, 56) have shown that soaking at low temperatures greatly reduced the germinative capacity of snap bean seed, although Kidd and West (33) noted--in contrast to other researchers--that the dry weight per surviving plant was not markedly reduced. Harrison (29) found more injury to the meristematic regions of the root and shoot in pea seed soaked at 1^o C than in those soaked at 20^o C.

(2) Vigor: Woodstock and Tao (78) found that soybean embryonic axes which had been artificially aged were susceptible to injury by rapid water uptake during early imbibition, whereas unaged axes were not. Woodstock and Taylorson (79) found both high and low vigor soybean seeds to be susceptible to soaking injury. However, they also found that low vigor seeds had additional injury after 1 hour of imbibition which high vigor seeds did not show, and that increases in acetaldehyde and ethanol were more pronounced in low than in high vigor seeds.

3) Seed moisture at the onset of soaking: A number of researchers (23, 48, 51, 78) have determined that by raising seed moisture slowly to some minimum level prior to soaking, it was possible to reduce the severity of the injury.

4) Rapidity of water uptake: Slowing rate of water uptake by imbibing seeds in solutions of polyethylene glycol (Carbowax) has resulted in decreased injury to peas (51, 55) and soybeans (78, 79).

5) Depth of soaking solution: Mullet and Considine (44) noted radicle growth of snap bean seeds was curtailed and ⁺K leaching increased by increasing the depth of the soaking solution.

6) Number of seeds soaked: Eyster (23) noted that increasing the number of seeds in a given volume of water increases the percentage of seeds injured by soaking.

7) Sterility of seeds and soaking media: Soaking injury has been shown to increase when seeds and/or soaking solution are not sterilized beforehand (23, 72).

Causes of Soaking Injury

Many hypotheses have been advanced to explain soaking injury. Kidd and West (34) felt that the injury was due to "(1)...disorganized metabolism resulting from deficiency in oxygen supply and accumulation of carbon dioxide, (2)...leaching out of essential soluble food reserves, or , (3)...a combination of (1) and (2)." Tilford, Abel, and Hibbard (72) attributed the largest portion of the injury caused by soaking of bean seeds to bacterial action and, to a lesser degree, to the absence of oxygen and the accumulation of carbon dioxide. Bailey (2), however, showed that even under sterile conditions soaking is a major cause of injury. Eyster (23) hypothesized that injury resulted from an insufficient supply of oxygen that caused an alteration in the permeability of cytoplasmic membranes which, in turn,

brought about a loss of essential cell constituents. Barton (4), however, showed that oxygen gas bubbled through the soaking water seriously aggravated the injury and increased water uptake, while carbon dioxide bubbled through almost completely alleviated it and also reduced water uptake. Nitrogen gas produced much less deleterious effect than oxygen, although the germinative capacity of the soaked seeds was much reduced below the level of those in the carbon dioxide treatment. Several authors (5, 23, 48) showed that the addition of hydrogen peroxide to the soak water was effective in preventing injury, while at the same time reducing the amount of water taken up by the seeds.

Focusing on the substances leached out of seeds during soaking, Barton and McNab (5) were able to isolate sucrose, raffinose, amino acids, and IAA. However, neither the addition of IAA nor amino acids into the soak water had any effect in preventing the injury. Wheeler (74) found a gibberellin and a betaine in the soak water of beans. The deleterious effect of soaking seeds was not alleviated, however, by treating seeds or seedlings with either gibberellic acid or glycine betaine. Mullett and Considine (44) in their study of potassium release and

reabsorption by soaked seeds, found a correlation between lack of radicle growth and failure to reabsorb K^+ ion lost to the soaking solution.

The experiments of Orphanos and Heydecker (48) indicated that the ill effects of soaking were caused by the seed's taking up more water than the cotyledons were ultimately able to absorb, resulting in free water in the intercotyledonary cavity. This occurs, they felt, because as cotyledons swell they maintain their original concave shape, thus causing the volume of the cavity between them to steadily increase, thereby pulling in more water. Some water is absorbed by the cotyledons, but any that is not is trapped in the seed because the cotyledons swell excessively during long periods of soaking, are kept tightly pressed together by the seed coat, and form a water seal. (The micropyle is not an exit.) The penetration of the seed coat by the radicle which might give the interior access to oxygen is prevented by the continuing anaerobic condition.

Orphanos and Heydecker found that treatments which eliminated or decreased the amount of free water in the intercotyledonary cavity largely prevented the damage done by soaking. These treatments included:

- 1) removing the seed coat before or after soaking;
- 2) drying of the soaked seed;
- 3) draining the seed after soaking by cutting off its end portion; and
- 4) treatment of the seed with hydrogen peroxide before, during, or after soaking.

This last treatment worked, they felt, through the enzymatic breakdown of hydrogen peroxide to yield more oxygen to the interior of the seed, and drive out the free water.

Several investigators (23, 48, 51, 78) have noted that seeds which are first imbibed slowly at favorable temperatures lose a great deal of their susceptibility to soaking. Because of this fact Orphanos and Heydecker (48) postulated that the injury is caused by an oxygen deficiency in the interior of the seed during the critical early stages of germination. In testing this hypothesis, they found that when air was bubbled continuously through the soaking water the seeds suffered even greater injury. They also allowed seeds to imbibe--without soaking them--for 24 hours in a nitrogen atmosphere. All seeds germinated when transferred to favorable conditions. Their conclusion was

...that temporary anaerobic conditions do not by themselves harm imbibed Phaseolus seeds but that damage is caused specifically when the access of oxygen to the interior of the embryo at the rate required for germination is prevented whilst aerobic conditions exist at the surface of the seed (48).

Roos and Pollock (59) in their study of soaking in lima bean axes found that submergence during the first 10 minutes of imbibition conditioned the axes to reduced growth. They considered three alternatives to explain why this initial period of water uptake is critical:

- 1) There is an initial oxygen dependent reaction;
- 2) There is mechanical injury due to rapid water uptake; and
- 3) There is a blockage of intercellular air spaces of the seed axis by water absorbed during the initial imbibition period, thereby slowing oxygen diffusion into the cells.

The first possibility was dismissed after an experiment similar to that of Orphanos and Heydecker: lima bean axes which were imbibed in a nitrogen atmosphere for the first hour of water uptake showed no reductions over controls in subsequent growth. Mechanical damage to membranes by increased rate of water uptake during soaking could not, they felt, account for reduced growth because even though injury due to soaking was significantly greater than that

resulting from imbibition on filter paper, the rates of water uptake as well as the amounts of substances leached out by the two methods of imbibition were not greatly different during the critical first hours of water uptake. Therefore, according to Roos and Pollock, the third explanation--blockage of intercellular air spaces by water absorbed during the initial imbibition period--is the most logical. This fits with the observed

...small, yet statistically significant, increase in weight of axes when imbibed submerged vs. on filter paper (which) might be an actual measurement of the intercellular air spaces which become filled with water during the submerged treatment (59).

Perry and Harrison (51) working with whole pea seeds (in contrast to the isolated lima bean axes of Roos and Pollock) reasoned that oxygen deficiency would not be a cause of injury, because increased injury was observed in seeds soaked at low temperatures when oxygen needs would be decreased due to slower respiration rates. They did however, in contrast to Roos and Pollock, see large differences in the total water uptake and rates of exudation between blotter-imbibed seeds--which were not injured--and soaked seeds, which were injured. Consequently, they tied soaking injury to rapid rate of water uptake and the deleterious effects it has on the

coordination in the dry seed of the sub-cellular events necessary to resume the active state.

Powell and Matthews (55), observed--through the use of tetrazolium staining--dead tissue on the abaxial surface of cotyledons following very short periods (2 minutes) of rapid water uptake by dry pea embryos (i.e. without seed coats). They felt that this supported the conclusion of Larson (36) that increased leakage and reduced seedling growth after soaking of seeds without seed coats were due to damage as a result of the disruption of cell membranes caused by rapid inflow of water. The fact that the tissue was actually killed did not, they said, tend to support the conclusion of Simon and Raja Harun (69) that the initial flood of exudate from a soaked seed was the result of lower membrane integrity at lower seed moisture, and the later decreased leakage the result of membrane conformational changes which permit less exudation. Powell and Matthews concede, however, that the rise and decline of seed exudate upon hydration may result from, first, cell damage to the outer layers of the tissues, and second--because inner layers would take up water more slowly than outer layers, and consequently remain alive--a rehydration, reformation and consequent decrease in permeability.

The demonstration by Woodstock and Tao (78) that artificially aged soybean axes which were subjected to rapid water uptake during early imbibition leaked nearly 6 times more electrolytes than high vigor unaged axes given the same treatment--despite the fact that rates of water uptake were identical--shows that rapidity of water uptake can interact with membrane "health", which perhaps includes its ability to reform upon hydration and prevent electrolyte loss. When imbibition of low vigor axes was slowed with PEG, leaching of low vigor seeds was greatly reduced.

Support for membrane dysfunction as a result of soaking is found in other work. Mullet and Considine (44) as mentioned above, found a correlation between lack of radicle growth and failure to reabsorb K^+ lost to the soaking solution when they compared a high performance line of snap beans--with better laboratory, greenhouse, and field emergence, less leaching in water, and greater mean plant height 14 days after sowing--to a low performance line. Re-uptake of K^+ occurred only for the high performance line under partial immersion. This is also the only treatment which showed radicle growth. The other treatments--the high performance line totally

immersed, and the low performance line at both partial and total immersion--showed no net K^+ re-uptake and no radicle growth. It is believed that K^+ uptake is mediated by a membrane-bound ATPase. Failure to reabsorb K^+ could be due to interference with membrane function through the accumulation of toxic byproducts of anaerobic respiration, or perhaps also, as Mullett and Considine suggest, to the failure of glycolysis to produce adequate ATP to fuel the uptake process.

There are a number of writers who feel that at least part of the long term effects of soaking are due to the accumulation of the toxic by-products of anaerobic respiration. Sherwin and Simon (65) working with Phaseolus vulgaris L. noted a greater accumulation of lactic acid prior to radicle emergence in seeds germinated under wet conditions (moist sand), than in those germinated under normal conditions. They suggested that the lactate may be one factor contributing to the injury from soaking.

Crawford (18), however, felt that tolerance to lactate accumulation might be a way to avoid soaking injury. He found that soaking tolerant species such as rice and lettuce apparently have the ability to prolong the period over which lactate can serve as an endproduct

of glycolysis, thereby avoiding the accumulation of ethanol--which poisons by (1) disrupting lipid arrangements in, and possibly removing phospholipids from, the cell membrane; and (2) possibly through the alteration of the configurations of proteins associated with membranes. The resultant decreased membrane integrity manifests itself as loss of germinability and vigor (56). Avoidance of ethanol production is accomplished by low anaerobic respiration rates, and the avoidance of the Pasteur effect.

Woodstock and Taylorson (79) in their comparison of high (HV) and low vigor (LV) seeds noted that

- 1) in HV seeds, injury does not increase with periods of submergence longer than 1 hour, but in LV seeds the longer the soaking period, the greater the injury,
- 2) in HV seeds, the increase in the respiratory quotient--which signals a shift toward fermentative pathways and the production of acetaldehyde and ethanol--is transient, while in LV seeds it persists longer,
- 3) in HV seeds, levels of ethanol even after 8 hours of submergence are less than they are in LV seeds at 2 hours, and

- 4) imbibition in 30% PEG prevents injury to both LV and HV seeds.

Their conclusion is that since O_2 concentration in 30% PEG is less than that in water, the initial injury, at least, is not caused by anaerobic conditions, but by rapid water uptake. For LV seeds the injury due to rapid water uptake is apparently followed by anoxic conditions which elevate respiratory quotient, increase levels of ethanol and increase the injury.

Chilling Injury During Germination

Symptoms

The seeds of many species are sensitive to low temperatures during germination. This sensitivity is expressed as reduced germination and emergence of seedlings, and as decreased vigor and yield of the surviving mature plants (7, 9, 13, 14, 15, 16, 27, 32, 46, 54, 60, 62, 77). With cotton, for example, chilling during germination reduced plant height, delayed fruiting, and reduced fiber quality in a direct relation to the length of exposure to cold (16). In studies with snap beans, axes weights of seedlings at emergence from cool (12.5 C) seedbeds were much reduced compared to axes

weights of seedlings emerging from seedbeds held at 20 C (27), and early sowing dates are thought to produce a lasting negative effect on seedling growth rate (26). Imbibition of soybean seed at 5 C has been shown to cause reduction in survival, dry matter accumulation, and height of seedlings (46), as well as reductions in fruit, seeds and stover (32). Lima beans (52, 54, 77) react similarly to low temperatures during germination.

A number of studies have examined the specific location of the injury to the germinating seedling. Harrison (29), using the Tetrazolium test with cold imbibed corn and pea seed, found that the tissues most likely to be injured were the meristematic and surrounding areas of the root and shoot. He also observed that spirally twisted radicles were more common in seeds soaked at 1 C, than those soaked at 20 C. Christiansen (12) found two types of injury caused by chilling of germinating cotton seed. The first was radicle tip abortion, and the second was root cortex disintegration. The type of injury depended upon the time during germination that low temperatures were administered. Woodstock and Pollock (77) observed a decreased and abnormal elongation of the lima bean radicle. Elongation

of the hypocotyl was not affected. Injuries of these types to the radicle lead to reduced water uptake, slow recovery, and reduced resistance to disease.

Cotyledonary damage during low temperature imbibition may also contribute to decreased seedling growth rate. This has been shown with soybeans (32, 46) and snap beans (19, 43, 53). Some studies have shown no temperature dependence (19, 32, 53) while others have shown it to increase at low temperatures (43).

A number of researchers have observed increased exudation from germinating seeds as a result of chilling (32, 51, 54, 64, 67). Bramlage, et al (8) working with soybeans found that the amount of exudation, measured as OD 280, was negatively correlated with germination at low temperatures among near-isogenic strains, but was not closely correlated when different cultivars were compared; that is, there was considerable variation in leakage due to chilling among cultivars with similar growth and germination rates. That these substances encourage the growth of and attack by pathogens such as Fusarium, Pythium, and Rhizoctonia is well established (24, 42, 63, 64, 66, 67). In addition to increasing the amount of exudate, low temperatures may reduce the rate of consumption of this exudate by soil bacteria, thereby

increasing the size of the zone into which the exudates diffuse (66). This can enhance the injury. Nevertheless, leachate stimulation of soil-borne pathogens is not the sole cause of reduced survival of low-temperature germinated seeds. This is shown in experiments in which seeds were germinated at low temperatures in either sterilized or unsterilized soil. There were significant reductions in stands even in the sterilized soil where damage due to exudate stimulation of pathogens would be minimal (71).

Factors Influencing the Amount of Chilling Injury

There are a number of factors which can influence the degree of injury sustained by the low-temperature germinated seed:

(1) Seed vigor. The less vigorous the seed due to, for example, aging, poor storage conditions, or rough handling during harvest the more susceptible, generally, the seed will be to imbibitional chilling injury (53, 54).

(2) Seed moisture. Several species, among them lima beans (52), soybeans (8, 32, 34, 46), snap beans (40, 53), pea (60), fababeans (60), and chick peas (11) show reduced chilling injury when seed moisture during the

initial period of low temperature imbibition is at or above some critical level. Conversely, injury is greater as seed moisture is reduced. Increasing seed moisture by slow imbibition in warm temperatures, before exposure to chilling, is therefore one method of reducing injury (8, 34, 54). Pollock (52), in his experiments with lima bean seed axes, has shown that this effect of seed moisture level on temperature sensitivity was repeatedly reversible.

(3) Stage of imbibition. In addition to the beginning of imbibition, seeds may be especially sensitive to low temperatures at other times during germination. Christiansen (14) found that, in cotton, there was a period between 18 to 30 hours after the start of imbibition where "chilling hypersensitivity" was observed. This period coincides with the period which begins with rapid radicle elongation and ends with initiation of rapid hypocotyl elongation.

(4) Bleaching of seed coat. Bleached (white) lima bean seeds are more susceptible to injury by low temperatures during germination than are unbleached (green) seeds (54). Heydecker (30) suggests that the pigmentation of colored seeds confers an antibiotic property which is lacking in the bleached seeds, so that

they are less susceptible to attack by microorganisms in the soil.

(5) Rate of imbibition. The slower the rate of water uptake, the less likely is chilling injury. Pollock and Toole (54) note that hard seed coats of lima bean seeds could prevent the uptake of water and enable the seed to escape chilling injury. They felt that this hardseededness is itself temperature dependent; that is, it is most operative at low temperature. Tully, et al. (73) in a study of soybeans and peas, suggested that chilling injury is due more to the rapid uptake of cold water than it is to the uptake of cold water per se. Peas which imbibed slowly at 0^o C were not injured, whereas soybean seeds which imbibed relatively rapidly were injured. Removal of the seed coats of the peas accelerated imbibition, and also made them more susceptible to chilling injury. Slowing soybean imbibition with PEG 6000, on the other hand, decreased chilling injury. Tetrazolium staining showed that the outer layers of seeds which had imbibed rapidly were injured, whereas in seeds in which imbibition was slowed, no dead layer appeared. Perry and Harrison (51) were also able to lessen chilling injury to peas by slowing imbibition with Carbowax (PEG). Pollock (52) found that

the protection from subsequent rapid low temperature imbibitions conferred on lima bean axes by gradually increasing moisture content through vapor equilibration was at first independent of the temperature at which the water vapor was absorbed. Longer exposure of high moisture axes to low temperatures, however, resulted in injury. Closely related to the above is the observation by Pollock, et al. (53) that chilling injury to snap bean is increased in fine sand with high moisture and low oxygen. Roos and Pollock (59), in their study of soaking injury in lima beans noted that chilling injury in limas "has always been coupled with initial submergence stress."

The implication, however, that rate of water uptake rather than low temperature is the major cause of injury is disputed by Chen, et al. (11) who found that even though chickpeas imbibed by a rapid method at 2^o C, and those imbibed by another method at 20^o C had similar rates of water uptake, the seeds imbibed at 20^o C showed about three times the amount of germination as those at 2^o C.

(6) Conditions during maturation of seed. Bramlage, et al (9), in a comparison of chilling sensitivity of 15 soybean cultivars found that embryos from early maturing varieties were more susceptible to injury during imbibition than embryos from late maturing varieties.

They hypothesized that lower temperatures during the developmental period can harden seed against chilling injury.

(7) Inhibitors and promoters. Abdul-Baki and Stoner (1), in work with tomato found that the leachate from a chilling-sensitive tomato cultivar could inhibit the germination of seeds of some other tomato cultivars, whereas the leachate from a more cold-tolerant variety could shorten the time necessary for seeds of some other cultivars to germinate.

Causes of Chilling Injury

Studies of soybean (8, 37, 49) have found that the embryos, with seed coats removed, leak solutes profusely during the first minutes of imbibition. Chilling during these first few minutes markedly stimulates the leakage, but chilling begun after as little as one minute of water uptake results in very little leakage increase. Moreover, a great reduction (60%) in respiration was observed for chilling administered during the first minutes of imbibition, and little or no reduction for chilling begun at 5 to 15 minutes after the start of imbibition (37).

It has been proposed that the initial, profuse leakage of solutes is a critical period of membrane reorganization during which the membranes change from a

porous to a non-porous condition (8, 49, 67). A chilling stress applied during the period before reorganization is complete can result in abnormal reorganization, and a consequent prolonged period of leakage as well as decreased germination and axis elongation. Humidifying soybean embryos to 35-50% moisture before chilling

...probably allows at least partial reorganization of membranes before imbibition, thereby lessening the destructive effects of membrane phase changes during imbibition at a chilling temperature (8).

Some workers, however, (36, 55) feel that the evidence points more to cellular rupture than to membrane reorganization as the cause of leakage of solutes during seed imbibition. In either case,

...a disorganized membrane or a fragile easily ruptured membrane, the rate of imbibition appears to be critical. In the case of the former, too-rapid imbibition would not allow for an orderly reconstitution of the bilayer membrane prior to the rehydration and mobilization of other cellular components, while for the latter, the degree of membrane rupture would be proportional to the rate of water uptake (76).

Chilling sensitivity has been found to be related to membrane lipid composition. Resistance to chilling injury, in fact, has been correlated with a higher ratio of unsaturated to saturated fatty acids (50). In addition, in certain plants injury from low temperature has been associated with marked increases below a critical

temperature in the energy of activation (E_a) of a number of membrane-bound respiratory enzymes (40). These increases in E_a are thought to be the result of configurational changes in these enzymes which occur because of physical phase transitions taking place in the lipids of the membranes. The phase change has been detected by electron spin resonance, and the temperature at which it takes place is in most cases the same as that below which the activation energies of the membrane-bound enzymes increase, and the plants fail to grow (58). The observed higher ratio of unsaturated to saturated membrane fatty acids of chilling resistant plants could result in a more flexible membrane, as well as a significant lowering of the temperature at which the membrane phase change occurs (39). The polar group of the phospholipid can also affect solidification temperature (20).

Lyons (39) has suggested that in chilling-sensitive plants, the lowering of the temperature results in the solidification of the membrane lipids at the critical temperature. This solidification would be expected to be accompanied by a contraction that causes cracks or channels and the consequent increased permeability observed during chilling. The activity of membrane-bound enzyme systems would be suppressed while that of

nonmembrane-bound enzyme systems would not. The result would be an imbalance, with metabolites such as pyruvate, acetaldehyde, and ethanol being produced at normal rates, but not being used rapidly enough to prevent toxic accumulations. These compounds do actually accumulate very early in chilling. Lyons also states that

...a greatly reduced energy supply accompanying the suppressed mitochondrial respiration, along with the possibility of altered activity of the membrane-bound ATP-ase system, would greatly upset the normal energy balance of the cell (39).

The question of whether or not membrane composition, phase transition, and energy anomalies play the same role in chilling injury for germinating seeds as they may in whole plants has not been settled. Priestly and Leopold (57), for example, in a comparison of soybean seeds (chilling sensitive) and pea (chilling insensitive) found few differences between the phospholipids, fatty acids, and free sterols of the two species. Neither did they find significant differences in permeability to KCl, glucose or glycerol of liposomes reformed from the membranes lipids of the two species. Likewise, Stewart and Bewley (70) found no differences in the composition of the phospholipid fatty acids from the axes of the chilling-intolerant cultivar of soybean, Fiskeby, and the chilling-tolerant soybean cultivar, Biloxi. Wolk and

Herner (75), however, compared the degrees of unsaturation in the phospholipid fractions of the seeds of the snap bean cultivars 'Kinghorn Wax' and 'Tendercrop'. 'Kinghorn Wax', which is more resistant to imbibitional chilling injury, also showed the greatest amount of phospholipid unsaturation.

The observed amelioration of injury from chilling by raising seed moisture levels does not appear to be connected either to an increase in unsaturation of phospholipids (75) or to the type of energy anomalies predicted by membrane phase-change hypotheses. For example, Cohn and Obendorf (17) compared energy metabolism in chilled low and high moisture corn seed. They observed reduced radicle growth only in the low moisture seeds, but saw no differences in the oxygen uptake or ATP levels between the two seed moisture levels. Their conclusion is that a

...disruption of energy metabolism is not a primary cause of kernel moisture-mediated imbibition chilling injury...

as would be expected if suppression of mitochondrial respiration were involved in the injury. In short, it is

...clear that chilling injury during germination presents unique problems not encountered elsewhere. Most appear to be related to the extremely dehydrated condition of the seed prior to imbibition... It

appears that this injury may be due in part to 1) structural changes of membranes due to dehydration, 2) physical state changes related to membrane composition, or 3) disruption of membranes as a result of too-rapid rehydration. All of these factors could cause membrane disruption and thus solute leakage, disruption of metabolism, abnormal

metabolism with the development of toxins, and the mixture of substrates and enzymes normally sequestered from one another (76).

Similarities Between Soaking and Chilling Injury

There are a number of similarities between chilling and soaking injury. In both, germination and growth are reduced. Both stresses tend to increase exudation of essential cell constituents from the seed, and consequently--in non-sterile field conditions--result in increased jeopardy through pathogen attack to the germinating seeds. In both chilling and soaking, increased rapidity of water uptake results in greater injury. In both, the injury is most severe at the beginning of seed imbibition, and, by raising the initial seed moisture through some method of slow imbibition, a certain degree of immunity to both soaking and chilling can be conferred. It has been hypothesized that both soaking and chilling injury may involve lack of oxygen during the critical early stages of imbibition. In neither case, however, has slow imbibition in a nitrogen

environment proven to be a method of duplicating injury (8, 35, 48). And, because chilling injury seems to be most readily obtainable under conditions which might also cause soaking injury, it has been suggested that much of the injury previously attributed to chilling may in fact be the result of rapid imbibition (55).

It appears then that more work needs to be done to elucidate the effects of these two stresses.

III. METHODS AND MATERIALS

General

Experiments were conducted in controlled temperature rooms, laboratory and greenhouse at Oregon State University, Corvallis, Oregon, during 1979 and 1980. The seeds used were bush-type snap beans Phaseolus vulgaris, L. cv. Slenderette, with a germination rating of 85%, supplied by the Musser Seed Co., Inc. of Twin Falls, Idaho. They were not treated with fungicide or insecticide.

Germination and Growth

Germination and growing of seed were carried out in plastic flats in the greenhouse. Each treatment was planted in 2 rows, side by side with 4 treatments per flat. Depending upon sample size there were 10-13 seeds per row. The flats (45 cm x 36 cm x 6 cm) were filled with No. 2 (2 to 3 mm) grade vermiculite to within about 1-1/2 to 2 cm of the top, watered thoroughly, and allowed to equilibrate to greenhouse temperature (daytime 22 C to 27 C; nighttime 17 C to 20 C). Seeds were laid in rows on top of the vermiculite, covered with more vermiculite, to

the top of the flat and rewatered with water equilibrated to greenhouse temperatures. Subsequent waterings were direct from the tap. No fertilizers or insecticides were applied.

Slow Imbibition of Seed

Seeds were imbibed by placing them on 1 sheet (11 cm x 14.5 cm) regular weight Anchor germination papers (Anchor Paper, St. Paul, MN) which had first been dipped in water equilibrated to the desired temperature and loosely wrung out. The papers were folded in half, rolled up, secured loosely with a rubber band, placed in plastic boxes sealed to retard water loss to the air, and returned to a room at the desired temperature to be stored for specified lengths of time.

Scarification of Seed

Seeds were scarified by chipping off a small portion of the seed coat on the abaxial side of one of the seed's cotyledons with a razor blade. Care was taken to avoid injury to the cotyledon itself.

Soaking of Seed

Seeds were soaked in one of two ways depending on the experiment: (1) by placing them in 50 ml of distilled water in 250 ml Ehrlenmyer flasks, or (2) by placing them in 50 ml of distilled water in 75 ml sample jars. Depths before soaking were 2 cm in the flasks and 4 cm in the jars.

Choice of method of soaking was largely a matter of convenience, but was, in all cases, kept uniform within experiments. Soaking temperature was 17 - 18^o C unless specified otherwise.

Adjustment of Seed Moisture Levels

"Medium" moisture seed was taken directly out of the seed bag, and was in the range 9 to 12% moisture (dry weight basis) with the average being about 10%. To establish initial "low" and "high" moisture levels, seeds were placed on wire mesh screens suspended over solutions of varying percentages of glycerol and water in sealed plastic buckets (10). The table below details desired seed moisture levels, approximate necessary relative humidities needed, and the percent glycerol by volume used to maintain each relative humidity (25, 38).

Desired % Seed Moisture f.w.b.	% Seed Moisture d.w.b.	Approx. Nec. R.H.	% Glycerol by Volume
3 - 4%	3 - 4%	10%	98%
15 - 18%	18 - 22%	75%	52%

f.w.b. = fresh weight basis
d.w.b. = dry weight basis

A number (10-15) of seed samples at the desired moisture levels were usually taken out of the equilibration buckets at one time, so that the buckets did not have to be opened prior to each treatment. Storage of these seed samples for short periods in small plastic vials (previously tested and verified as suitable) was employed as a means of insuring constancy of seed moisture prior to treatment.

Determination of Seed Moisture Levels

Seed moisture level (dry weight basis) was calculated in the following manner:

$$\frac{\text{weight before drying} - \text{weight after drying}}{\text{weight after drying}} \times 100$$

Seeds were dried at 100°C for 24 hours.

The percent free water was determined by patting dry and weighing the whole seeds; then splitting open, patting dry, and reweighing; and finally, drying them in the drying oven and reweighing. Percent moisture for the whole and split seeds was then calculated by the above method and the difference taken to give percent free water on a dry weight basis.

Determination of Total Seedling Dry Weight

When germination was completed, and primary leaves were fully expanded--about 14 days after planting--the seedlings were harvested by cutting the stems at soil level. Cotyledons were removed. Only "normal" seedlings--those with an intact growing point, at least 1 complete primary leaf, and not markedly stunted by, for example, incomplete hypocotyl elongation--were included in the sample. The harvested seedlings were then dried for a minimum of 48 hours in a tunnel dryer held at a constant ^o60 C before being weighed.

IV. EXPERIMENTS, RESULTS AND DISCUSSION

Experiment 1: The Effect of Temperature and Duration of Slow Imbibition of Snap Bean Seed on Susceptibility to Injury From Subsequent SoakingExperiment and Results

The objective of this experiment was to explore whether low temperatures predispose bean seeds to injury from soaking. Vigorous seeds of 11.9% moisture were imbibed slowly on paper towels at either 5 °C or 20 °C for various lengths of time. They were then planted in flats of coarse vermiculite in the greenhouse, or soaked for 2 hours at room temperature (17 ° - 18 °C) and then planted. Each experimental unit consisted of a lot of 25 seeds. Each treatment was replicated 4 times. The start times of the treatments were staggered so that all would be planted at the same time. The resultant seedlings were harvested about 14 days after planting and total seedling dry weight was determined.

The data presented in Figure 1 show that for both slow imbibition temperatures susceptibility to soaking injury was progressively reduced as duration of slow imbibition prior to soaking increased. Seeds imbibed slowly at 5 °C, however, took approximately 24 hours to

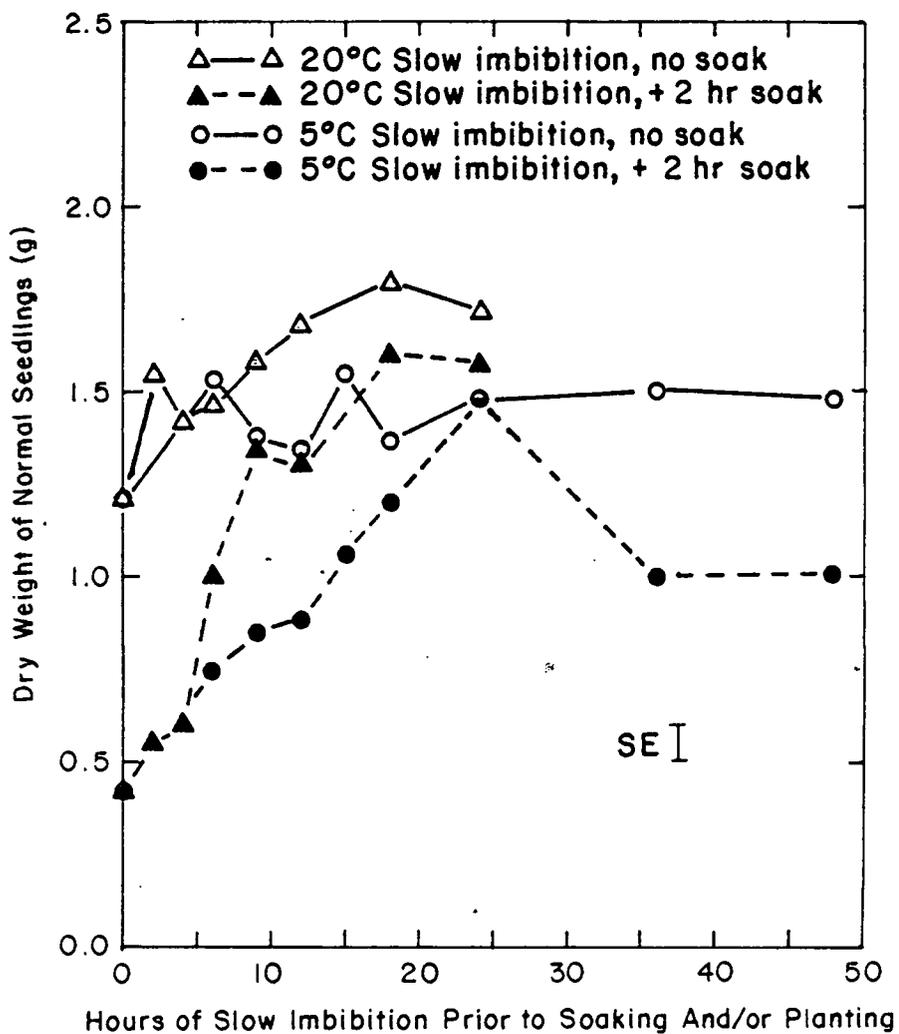


Figure 1. The effect of slow imbibition temperature and duration and 2 hours of soaking of snap bean seed on dry weight of normal seedlings.

reach minimum susceptibility to soaking--the point at which 2 hours of soaking reduced the dry weight of the resultant seedlings the least amount--while those imbibed slowly at 20 °C required only 9 hours to reach this point. There was no time when 2 hours of soaking did not have at least a small effect on diminishing dry weight regardless of duration of previous slow imbibition.

The data for 5 °C slow imbibition show that after this point of minimum susceptibility, there was another period of increased susceptibility. To determine whether the apparent increases in susceptibility were actual, the experiment was run again for 5 °C only, with more times after 18 hours being explored. This second run at 5 °C (data not shown) did not show any increased susceptibility to soaking for slow imbibition times greater than 24 hours. They did, however, agree fairly well with data from the first part of the experiment with regard to the duration of slow imbibition at 5 °C required to reach minimum susceptibility to soaking; that is, at about 24 hours.

Total seedling dry weight from seeds which were imbibed slowly for 8 or more hours at 20 °C and not soaked tended to be larger than those imbibed slowly at 5 °C and not soaked (Figure 1). This is a reflection of a

slightly greater number of normal seedlings at 20 C than at 5 C for most slow imbibition times (data not shown). The 5 C and 20 C lines do not show increasing divergence with greater chilling duration as would be expected if chilling were the cause of reduction in dry weight. Though the seed moisture was fairly high, the answer may lie in hard seededness, which Pollock (53) feels would tend to exaggerate damage caused by 5 C imbibition. The effect of the "slow-starting" seeds would appear as a decrease in the number of seedlings emerging and in the size of the surviving seedlings.

Moisture uptake during slow imbibition at 5 C and 20 C was measured (Figure 2) to determine the relation between moisture content of seed and susceptibility to soaking injury. When the points of minimum susceptibility to soaking (9 hours at 20 C, and 24 hours at 5 C) are compared with moisture uptake for the same durations, it is apparent that minimum susceptibility is reached at about 60% seed moisture for both of the slow imbibition temperatures.

Discussion

The results of the experiment do not support the hypothesis that chilling predisposes a seed to injury from

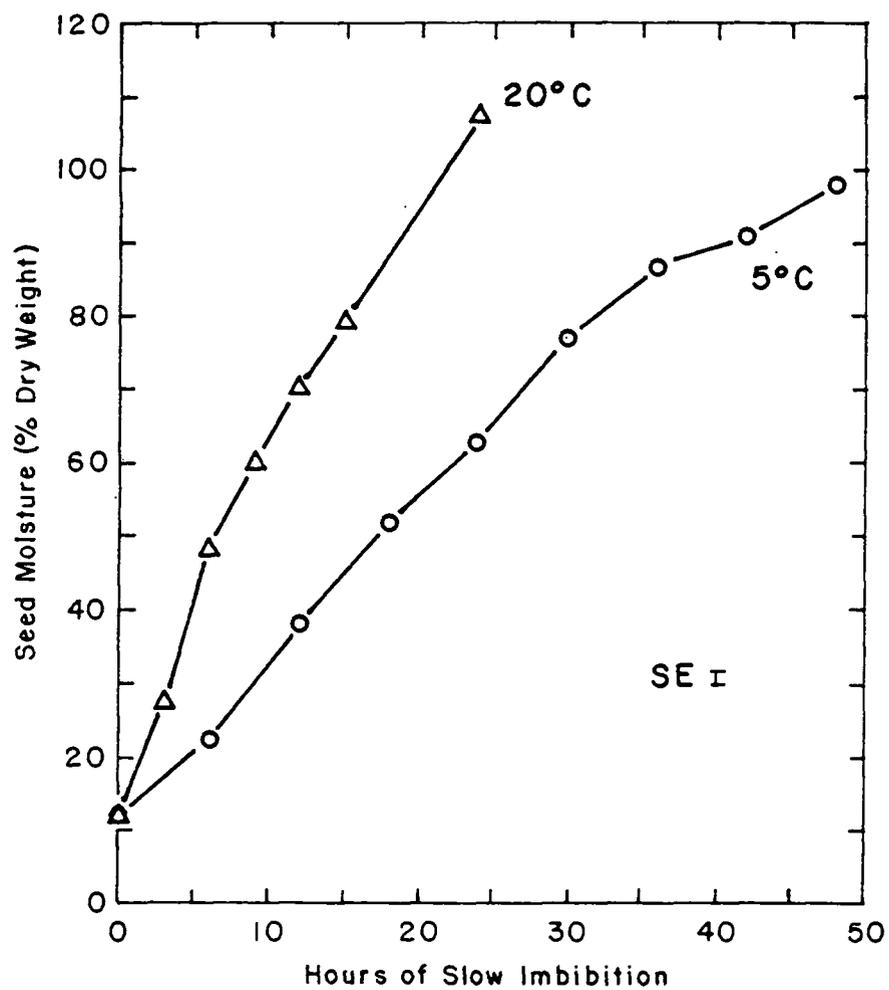


Figure 2. The moisture uptake during slow imbibition of snap bean seed at 5°C and 20°C.

soaking. What they do show, however, is that lower temperatures of imbibition can lengthen the period over which the seed is susceptible to soaking stress, thereby making it less likely to germinate into a healthy viable seedling. Early plantings into fairly dry, but still cool soils would result in seed remaining susceptible to soaking injury from later rains for a longer period of time than they would in warmer soils.

Moreover, seed lots for commercial plantings would probably be of lower moisture content than the 11.9% seed used in these experiments. There would be more hard seed in these lots, with water uptake restricted even further, and susceptibility to soaking injury prolonged even more.

Other investigators have noted that bean seeds lose much of their susceptibility to soaking after a period of slow imbibition. Eyster (23) placed bean seeds in moist cloth at moderate temperature until they were observed to absorb water and swell. He then soaked them in water at 10 °C for as long as 2 weeks, noting that they were still able to germinate if the radicle had not yet begun to protrude from the seed. Orphanos and Heydecker (48) found that bean seeds imbibed slowly (aerobically) to about 37% moisture (dry weight basis) and then soaked for 24 hours were all capable of germinating. They suggested that if

oxygen requirements to the interior of the seed can be fulfilled during the earliest part of imbibition, subsequent oxygen deprivation through soaking may not result in injury. They further suggest that this point might coincide with the end of the first stage of hydration noted by Opik and Simon (47) in their study of the relationship of water uptake and respiration rates of P. vulgaris cotyledons.

It was surprising to see that the 5^o C treatment did not itself result in greater injury to the seeds. It may be that injury would have resulted had the seeds been planted into a non-sterile media or had seeds of lower vigor and moisture content been used (53). A faster rate of water uptake during the imbibition prior to soaking might also have resulted in chilling injury (11, 23, 51, 53). This rate was purposely slowed, however, to avoid soaking the seeds.

Experiment 2: The Effect of Initial Seed Moisture Level and Duration of Slow Imbibition of Snap Bean Seed on Injury From Subsequent Soaking

Experiment and Results

The objective of this experiment was to determine how initial seed moisture level and duration of slow imbibition affects sensitivity to subsequent soaking. Seeds of each of 3 initial moisture levels--low, 7.0%; medium, 9.2%; and high, 19.4%--were imbibed in moist germination papers at 20 C for various lengths of time. The seeds were then planted directly in flats in the greenhouse or soaked for 2 hours before being planted. Experimental units consisted of 35 seeds. Twenty-five of these were ultimately planted. Moisture uptake data for the various slow imbibition times and for slow imbibition + 2 hours of soaking were taken on the remaining 10 seeds. The treatments were replicated 4 times. Because of the large number of treatments, starting, planting, and harvesting times, as well as moisture uptake measurements were staggered by replication.

When seeds of the three initial moisture levels were imbibed slowly and not soaked before planting, the total dry weights of normal seedlings were generally similar,

although they tended to be slightly lower for low moisture seed (Figure 3a).

Soaking the seeds for 2 hours reduced total dry weight of normal seedlings (Figure 3b). The reduction was greatest for seeds of medium moisture, and about equal for seeds of high and low initial seed moisture. Imbibing seeds slowly at 20 °C prior to soaking generally reduced the injury sustained by soaking. The longer the duration of slow imbibition prior to soaking the less the injury sustained until it was essentially minimized

The duration of slow imbibition that is necessary to minimize soaking injury depends on the initial seed moisture level (Figure 3b). For high moisture seed, injury was minimized at 6-8 hours, for medium moisture at about 12 hours, and for low moisture at over 24 hours. These results suggest that the planting of higher moisture seed would reduce the likelihood of soaking injury by minimizing the duration of sensitivity.

The rate of water absorption during soaking, and to a lesser degree during slow imbibition was dependent on the initial moisture level of the seed (Figure 4). Medium and high moisture seeds absorbed water at the same rate during slow imbibition. This is indicated by the similarity in the slopes of their lines in Figure 4b. Low moisture seed

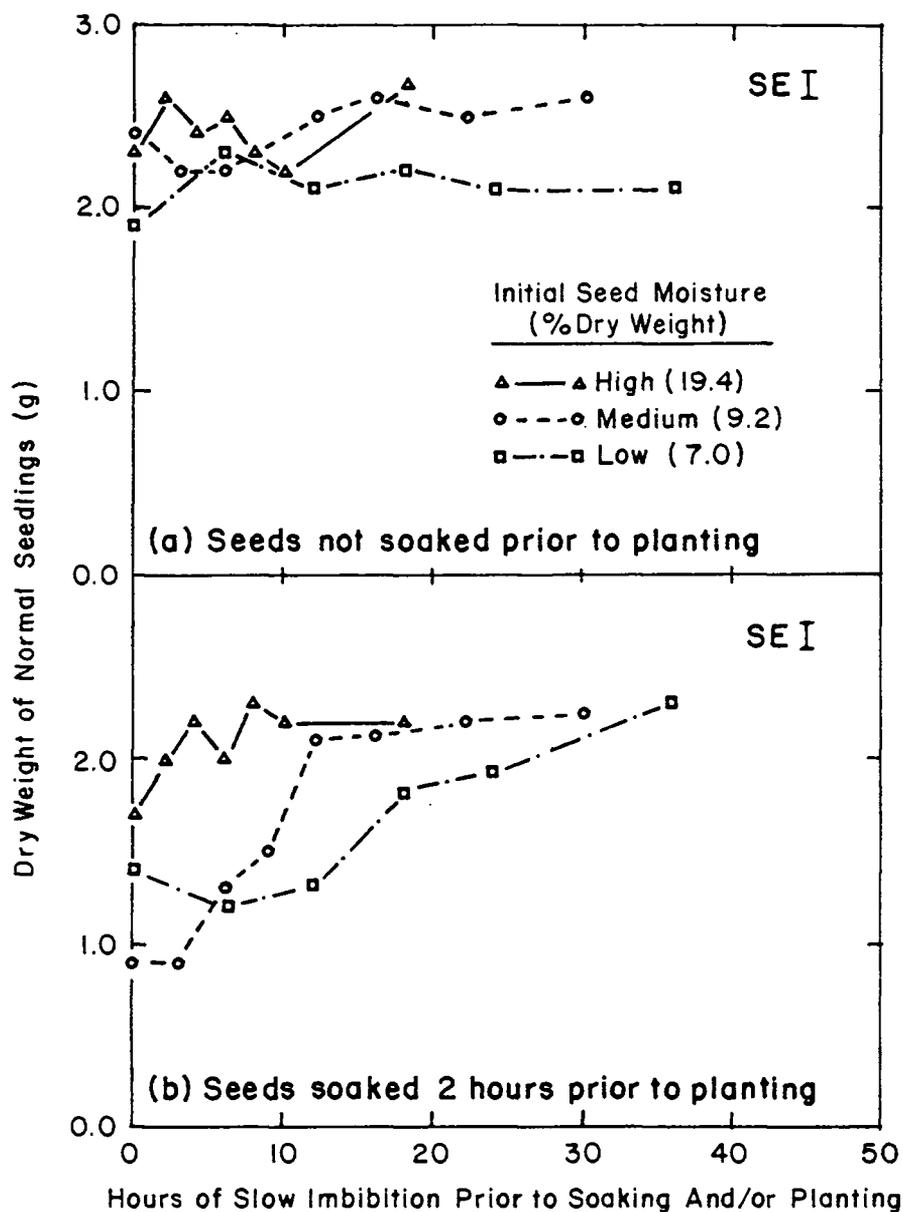


Figure 3. The effect of initial seed moisture, duration of slow imbibition prior to soaking and 2 hours of soaking of snap bean seed on dry weight of normal seedlings.

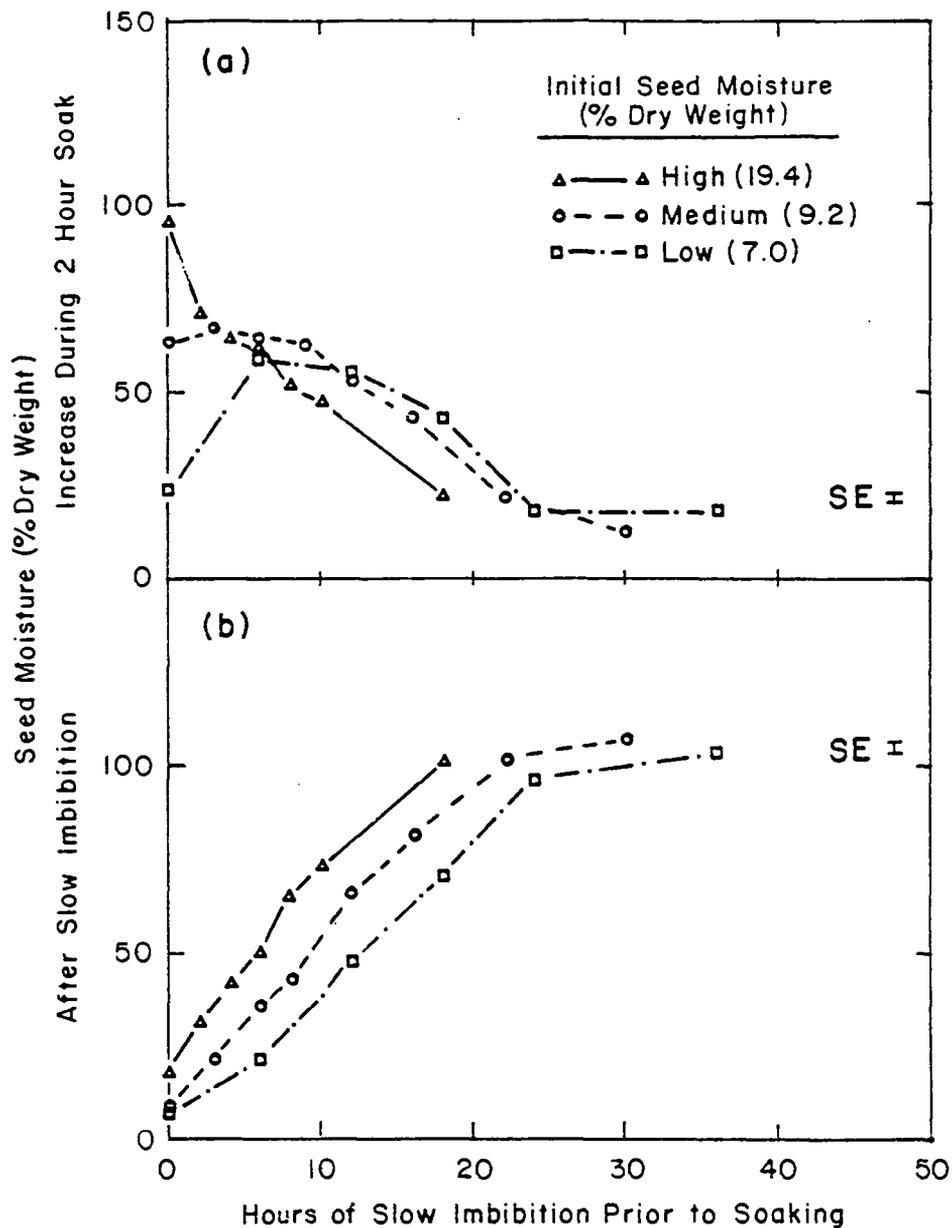


Figure 4. The moisture uptake of three initial moisture levels of snap bean seed after slow imbibition and 2 hours of soaking.

appeared to have a slightly slower rate of water absorption during the first 6 hours of slow imbibition, probably due to some hardseededness, and afterward a rate comparable to that of the high and medium moisture seeds.

When soaked for 2 hours without prior slow imbibition (Figure 4a), high moisture seed absorbed the most water (95.7%) and low moisture seed absorbed the least (24.6%), probably again due to hardseededness.

High moisture seeds that were imbibed slowly prior to soaking absorbed less water during the soaking. As the duration of slow imbibition increased, the amount of water absorbed during soaking steadily decreased. The medium and low moisture seeds absorbed the most water during soaking after prior slow preimbibition of about 3 and 6 hours, respectively. With longer durations of slow-imbibition the amount of water absorbed during soaking decreased.

Comparison of Figures 3 and 4 suggests that the degree of soaking injury was not consistently related to the amount of water absorbed during soaking. Within a given initial seed moisture level, the greatest injury from a 2 hour soaking did occur when the greatest amount of water was absorbed: prior to slow imbibition of the high moisture seed, after 0 to 3 hours of slow imbibition

of the medium moisture seed, and after 6 hours for the low moisture seed. When the different initial seed moisture levels were compared, however, this relationship did not hold. The high moisture seed with no prior slow imbibition absorbed more water during soaking than the medium moisture seed did, over 95% compared to about 64%, but the high moisture seed sustained far less injury. The results show that the rapid rate of water uptake associated with soaking is less injurious when initial seed moisture is higher.

The percent seed moisture at which injury from the 2 hour soaking was minimized can be estimated from Figure 5 in which moisture content of seed at the onset of soaking is plotted against seedling dry weight after soaking. The graph shows that as initial seed moisture level declines, the seeds must be imbibed to a higher moisture before susceptibility to soaking injury is minimized, i.e. total dry weight of seedlings reaches a plateau. For high moisture seeds, this minimal susceptibility was attained when seed moisture content reached about 40 to 60% by slow imbibition. It was attained at about 60% moisture for medium moisture seed and at over 100% for low moisture seeds. The moisture content at which medium moisture seed

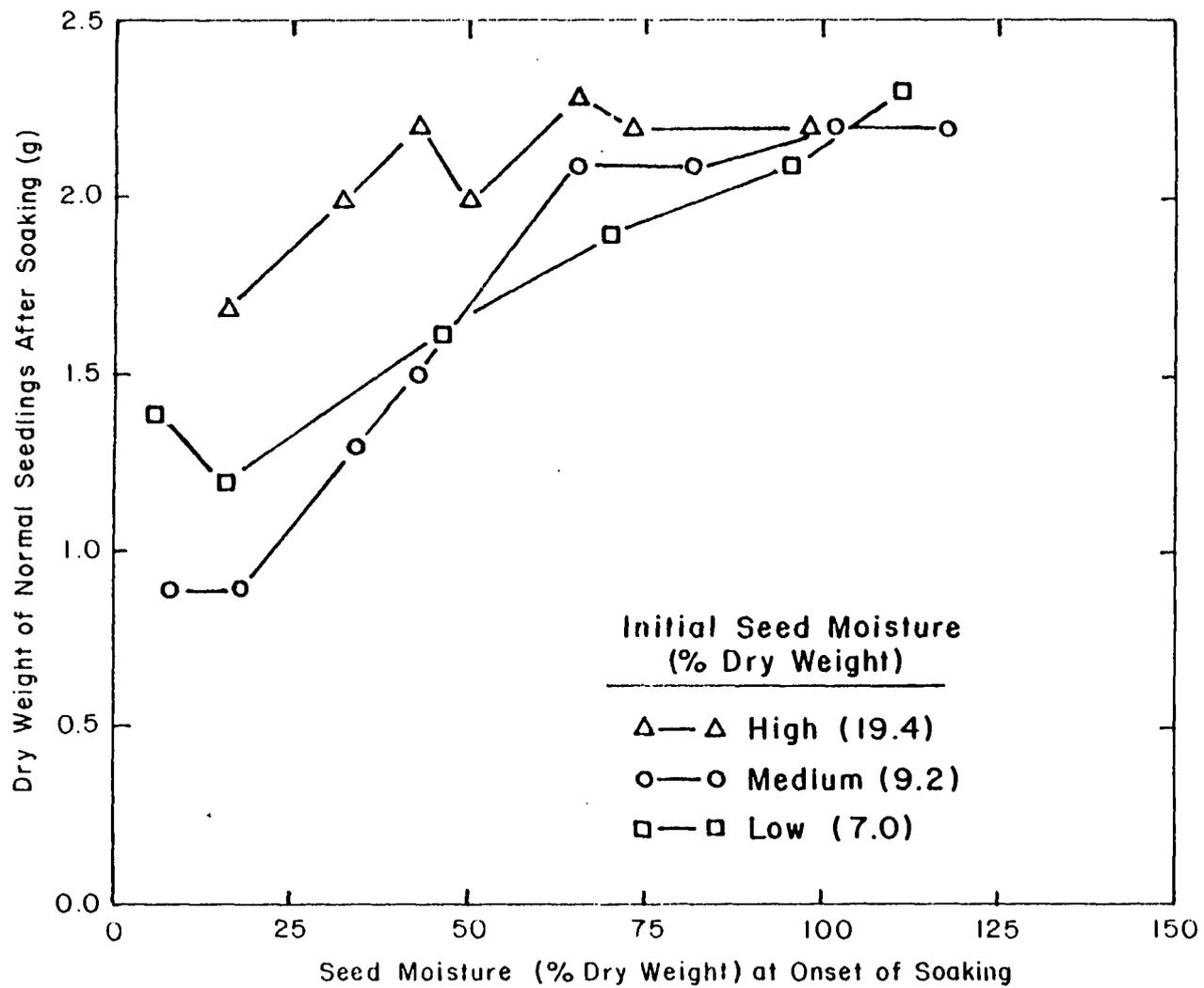


Figure 5. The relationship of seed moisture at the onset of soaking to dry weight of normal seedlings after soaking for three initial moisture levels of snap bean seed.

reached minimum susceptibility to soaking is consistent with the results of Experiment 1.

Discussion

The greater resistance of high moisture seed to transverse cotyledonary cracking is one factor which could contribute to its greater tolerance to soaking and rapid water uptake. Hobbs and Obendorf (32), for example, noted that the

...cracking of cotyledons was probably caused by the physical stresses imposed by differential hydration of the outer and inner tissues of cotyledons of low moisture seeds during imbibition.

They observed a brittleness of tissues of low moisture seeds as opposed to a flexible rubberlike consistency of high moisture seed. They felt that this greater flexibility accounted for the physical protection from cracking of cotyledons in high-moisture seeds.

Unequal hydration may also account for the observation that seed moisture content at which soaking injury is minimized varies with initial seed moisture. As a seed absorbs water the outer tissues in closest contact with water would be expected to have higher moisture content than those farther away. If some minimum moisture content is required by all tissues in the seed in order to

minimize soaking injury, then the lower the initial seed moisture, the greater must be the average moisture content of the seed to reach least susceptibility. The more rapid the imbibition, the greater would be the difference between the moisture contents of the outer and inner tissues, and consequently the higher the average moisture content required to minimize injury from soaking. If this is the case, the point of minimum susceptibility to soaking injury would probably have been the same if the method of slow imbibition used in this experiment had been vapor equilibration rather than moist paper towels.

The role of membrane hydration must also be considered in attempting to explain the greater resistance of seed with high initial moisture to soaking. Simon (67) has suggested that the membranes of dry seeds are leaky, allowing passive diffusion of low molecular weight cellular substances through pores or channels in the membrane phospholipids. Upon rehydration the "normal" phospholipid structure reforms, the membranes reorganize, and selective permeability is reestablished. While in the rigid dehydrated hexagonal configuration the membrane is particularly vulnerable to stress. Chilling temperatures are thought to result in "abnormal organization of membranes... and extensive internal disruption"(8) while

rapid water uptake from soaking can result in membrane rupture, copious exudation of cell constituents, and cell death (21, 55). Studies, for example, with isolated axes from soybean seeds whose vigor was lowered by accelerated aging (78) show the most severe injury at the onset of rapid water uptake: up to 6 times the release of electrolytes, and less than half the seedling axis length of high vigor controls. When initial water uptake of the low-vigor axes was slowed sufficiently by, for example, imbibition on blotters containing polyethylene glycol or if seed moisture was raised by vapor equilibration, injury from subsequent rapid water uptake was reduced or eliminated. Enough time is apparently being allowed for at least partial membrane reorganization, at which time rapid water uptake is not nearly as detrimental. Transverse cotyledonary cracking would not be a factor here, because isolated embryonic axes were used.

Experiment 3: The Effect of Soaking
of Snap Bean Seed Injury
From Subsequent Chilling Treatments

Experiment and Results

The objective of this experiment was to determine whether soaking stress predisposes the seed to greater injury from low temperatures. Medium moisture seeds (10.7%) were imbibed at 20 °C on imbibition papers for either 0, 6, or 12 hours before being soaked for either 0, 45, 90, or 180 minutes at 17 to 18 °C. The seeds were then planted in flats of vermiculite and held at either 5 °C or 20 °C for 24 hours. Afterwards the flats were transferred to the greenhouse where, in order to terminate the post-soak temperature treatments, and make growing conditions thereafter more uniform, they were rewatered with water equilibrated to house temperature. There were 3 replications and 20 seeds to each treatment. Starting times of the experiments, planting and harvesting--which began about 14 days after planting--were staggered by replication because of the large number of treatments.

The purpose of the range of pre-soak slow imbibition treatments and duration of soaking was to induce differing initial levels of soaking stress. Table 1 shows that the interaction between these two factors was highly

Table 1. Analysis of variance of the effect of soaking of snap bean seed on injury sustained by subsequent chilling.

Main Effects		
Temperature After Soak	(T)	***
Hours of Slow Imbibition	(I)	***
Prior to Soaking		
Minutes of Soak	(S)	***

2-Way Interactions		
T x I		NS
T x S		NS
I x S		***

3-Way Interactions		
T x I x S		NS

NS = Not Significant

* = <0.05

** = <.01

*** = <.005

significant. This is illustrated graphically in Figure 6. As the duration of slow imbibition prior to soaking increases from 0 to 12 hours, soaking is less damaging. In fact, the results are consistent with those of Experiment 1, in which susceptibility to soaking is also minimized between 6 and 12 hours. For 6 hours of imbibition most of the injury is caused by the first 45 minutes of soaking, with additional duration of soaking causing little if any more injury. At 12 hours of prior slow imbibition, soaking has no effect on seedling dry weight.

Table 1 shows a significant effect of the temperatures after soaking on the seedling dry weight. Total weight of the seedlings was slightly lower for the 5 °C post-soak treatment. Part of this effect may be due to the younger physiological age of those seeds treated at 5 °C after soaking. Part, however, is due to the fact that the 5 °C treatments averaged 13.2 normal seedlings, while those for 20 °C treatments averaged 13.7 (data not shown). This reduction in the number of normal seedlings and its consequent effect in reducing total seedling dry weight may be the result of chilling injury.

Whatever chilling injury there may be, however, is apparently not affected by a previous soaking stress.

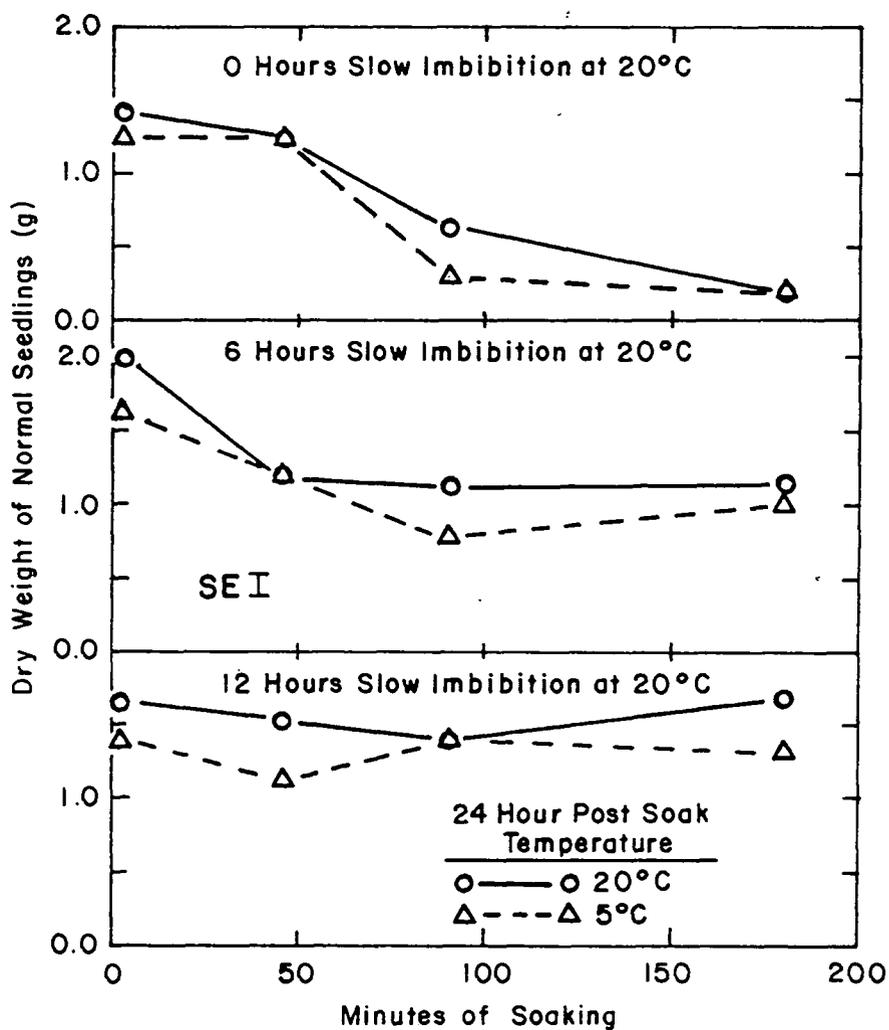


Figure 6. The effect of slow imbibition at 20°C prior to soaking of snap bean seed, soaking duration, and post-soak temperature on dry weight of normal seedlings

Table 1 shows that the response to temperature after soaking was unaffected by the duration of slow imbibition prior to soaking or the duration of the soaking treatment. Figure 6 does not show the increasing divergence of the 5 °C and 20 °C lines with increasing soaking durations which one would expect if greater soaking injury caused more injury from subsequent low temperatures.

In order to study the relationship between soaking injury and the amount of free water in the seed, moisture uptake data were collected during slow imbibition and soaking.

The initial rates of total water absorption during soaking were roughly the same for the 3 durations of slow imbibition (Figure 7). After 45 minutes of soaking, however, the rates of uptake began to decline for seeds that had been imbibed slowly for 6 and 12 hours, while the rate for 0 hours increased before beginning to decline at 90 minutes. Free water for each of the slow imbibition durations followed the same patterns as total water uptake.

Although seeds with no prior imbibition had absorbed the least total water for any given duration of soaking, these seeds contained the most free water. Twelve hours of imbibition prior to soaking resulted in the greatest

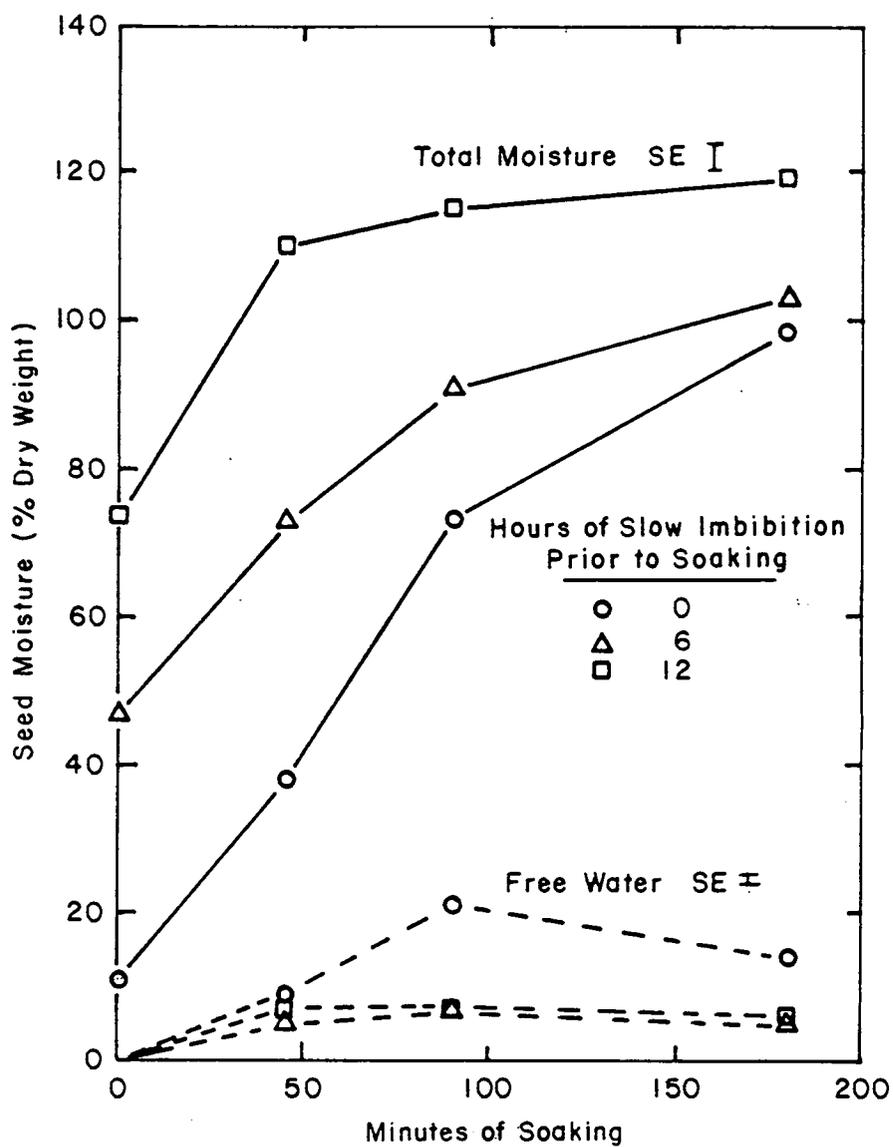


Figure 7. The effect of slow imbibition prior to soaking and duration of soaking of snap bean seed on free and total seed moisture.

amounts of total water for any given duration of soaking, but the amount of free water in the seeds was only slightly greater than that of seeds imbibed slowly for 6 hours before soaking.

The most injury was sustained by the seeds with the greatest amount of free water after soaking--those with no prior imbibition. However, this relationship between injury and the amount of free water did not hold across all three slow imbibition times. Six hour imbibed seeds contained less free water than those imbibed for 12 hours, yet soaking caused moderate injury to the 6 hour seeds, and very little to the 12 hour seeds.

Seeds imbibed for 12 hours before soaking were at about 73% moisture before soaking and were essentially immune to damage from up to 3 hours of soaking. This is fairly consistent with data from previous experiments which indicate that medium moisture seeds (9-12%) imbibed gradually in imbibition papers at 20 C reach immunity to 2 hours of soaking when they have attained around 60% seed moisture.

Discussion

The results of the first part of this experiment--in which soaking was shown to have no effect on the reaction

to a subsequent chilling treatment--are similar to those reported by Harrison (29). In that work, corn and pea seeds were soaked in water for 24 hours before being chilled between blotters. No effect on the germination of either species was seen. It might be in both these instances that the effect of the temperature was overwhelmed; that the injury caused by soaking the seeds is so serious, either through mechanical disruption of membranes, or the accumulation of the toxic by-products of oxygen starvation, that the effects of subsequent low temperature are relatively minor in comparison, not to say, increased by the soaking stress.

There is evidence that chilling stress must be administered simultaneously with relatively rapid water uptake (51, 72). The results of this experiment, along with those of Experiment 1--in which chilling during slow imbibition prior to soaking resulted in increased resistance to soaking--reinforce this conclusion. One apparently cannot "weaken" the seed with one stress and increase its susceptibility to the other.

With regard to moisture uptake, there are--as discussed in Experiment 2--several reasons why higher moisture seed would be more tolerant to the effects of rapid water uptake. The first would be resistance to

transverse cotyledonary cracking, which might in turn be one reason there is so much less free water as a result of soaking in seeds which are imbibed slowly for 6 or 12 hours before soaking than there is in seeds with no prior imbibition. If low moisture cotyledons are cracked by the "physical stresses imposed by differential hydration of outer and inner tissues" (32), it seems logical that they would not be able to absorb water as rapidly, and a greater percent of the total water absorbed would be free within the seed.

The amount of free water in the seed, however, cannot be the only factor which determines injury. The 12-hour slow imbibition treatments absorbed as much or more water than the 6 hour treatments after soaking, yet showed no significant decreases in seedling dry weight as a result of soaking. The 6-hour treatments, on the other hand, showed injury after as little as 45 minutes of soaking. It is possible that some necessary requirement--possibly "the adequate oxygen supply for a relatively short time during the earliest stages of imbibition" (48) had been met by the seeds imbibed beforehand for 12 hours, and/or that the membranes were "conditioned" beyond susceptibility to rapid water uptake, as discussed in Experiment 2.

Orphanos and Heydecker (48) proposed that soaking injury occurs when there is more intercotyledonary water than the embryo could ultimately absorb. Based on a water uptake curve, "saturation deficit" is the amount of water which a seed can still absorb. The point at which free water in the cavity is greater than this amount is the duration of soaking which will result in injury. Our own data are at variance with this. For example, seed which is imbibed slowly for 6 hours and soaked for 45 minutes is injured by the treatment. This seed would ultimately absorb over 100% moisture, yet at the point of injury the total water in the seed was only slightly over 70%. This difference may be the result of cultivar, or it may be that seed imbibed slowly before soaking behaves differently in this regard than does seed at 10-11% moisture at the time of soaking.

Experiment 4: The Effect of Soaking Temperatures,
Initial Seed Moisture, and Scarification
On Injury Sustained by Snap Bean Seeds

Experiment and Results

This experiment tested the effect of soaking temperature, initial seed moisture, and scarification on injury sustained by snap bean seeds soaked in water. Treatments consisted of soaking seeds of high (17.6%), medium (9.5%) or low (6.8%) moisture as well as seeds which had been scarified and equilibrated to low moisture (7.9%) at either 5 °C or 20 °C for various lengths of time. The intention of the scarification was to see the effects of soaking on low moisture seed without the complication of hardseededness. For some reason, however, this seed equilibrated to only 7.9%, so that a direct comparison to the low moisture seed could not be made.

After soaking, the seeds were planted in flats of vermiculite in a greenhouse, and rewatered with water equilibrated to greenhouse temperature. There were three replications with 20 seeds per treatment. Starting, planting, and harvesting--which began about 14 days after planting--were staggered by replication because of the large number of treatments.

Mean total dry weights for all normal seedlings are shown in Figure 8. The results illustrate the relatively short soaking times necessary to produce injury in 'Slenderette' snap beans. The results did not agree, however, with earlier reports showing that low temperatures during soaking increased injury (23, 34, 44, 51, 74). Medium and low moisture, and scarified seeds, were generally injured less when soaked for a given period at 5 C than when soaked at 20 C. For high moisture seed, however, it appears that for longer soaking times at least--soaking at 5 C may be more injurious than at 20 C.

As seed moisture of unscarified seeds decreased, the difference in rate of injury between 5 C and 20 C soak increased. This is shown by the increasing divergence of the 20 C and 5 C lines in Figure 8. In the case of scarified low moisture seed, on the other hand, the difference in rate of injury between 5 C and 20 C soak was much less than it was for the unscarified low and medium moisture seeds.

The low moisture seed was less sensitive to injury from either 5 C or 20 C soaking than was the medium moisture seed. This is indicated by the generally lower seedling dry weight for the medium moisture seed after a given duration of soaking. Soaking at 5 C for short

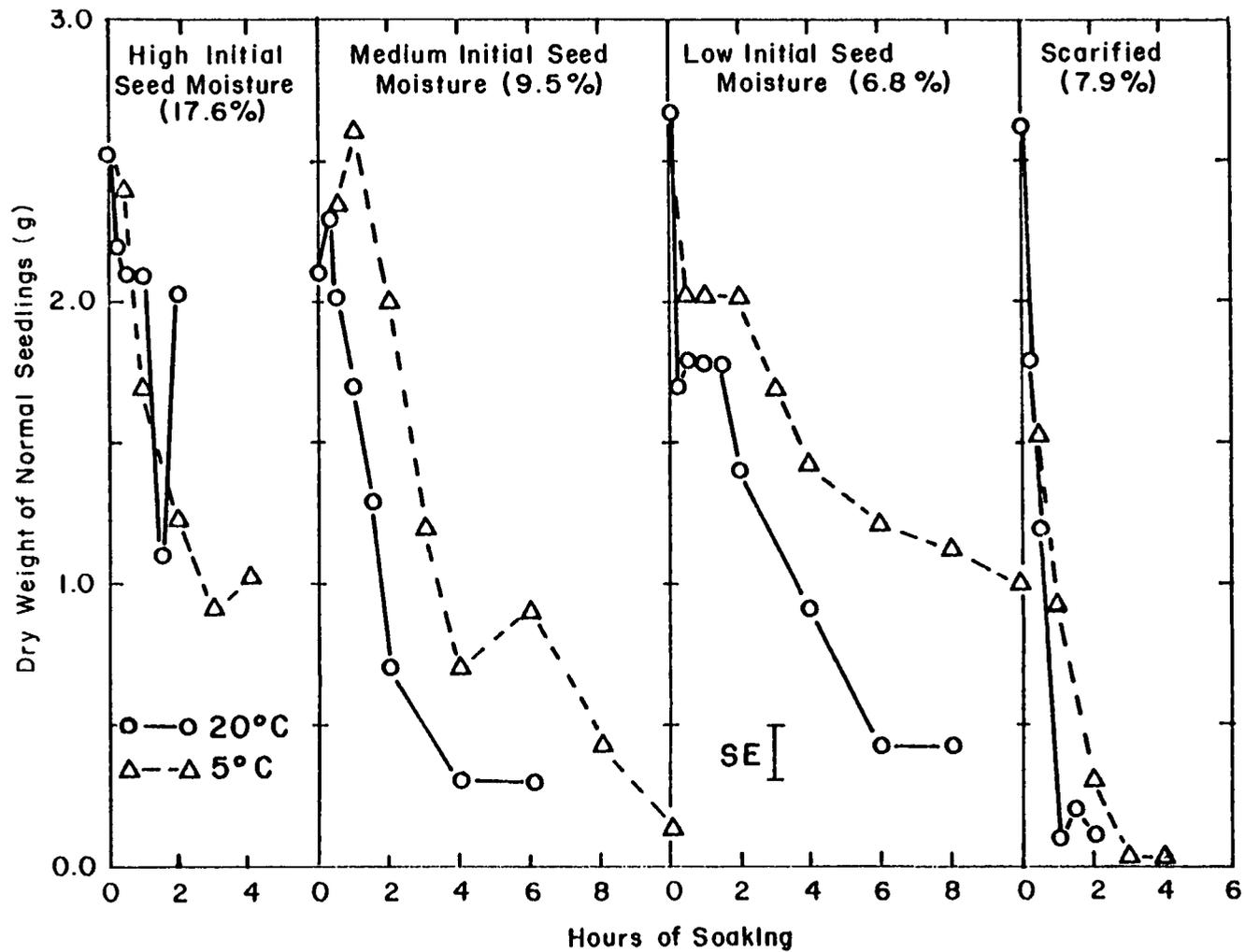


Figure 8. The effect of temperature and duration of soaking of snap bean seed on dry weight of normal seedlings for scarified seed and seed of 3 initial moisture levels.

periods of time, 2 to 3 hours, was more injurious to high moisture seed than to medium or low; however, the high moisture seed tended to be less sensitive to soaking at 20 C. Scarified low moisture seed was extremely sensitive to soaking. One hour at 20 C and 3 hours at 5 C resulted in nearly complete lack of germination. This suggests that the seed coat plays an important role in sensitivity to soaking injury.

In order to relate seed water to soaking injury, moisture uptake data--both total and free--were taken in a separate experiment. Though the seed lot used was the same as that in the planting experiment, the initial moisture contents of the seed were slightly different. High moisture seed were at 18.7%, medium at 9.1%, low at 6.6%, and scarified low moisture seed at 7.9%. Three replications of 10 seeds per treatment were used. The results are presented in Figures 9 and 10.

Total water uptake (Figure 9) was, as expected, more rapid for each of the four lots of seed at 20 C than at 5 C.

Water uptake for unscarified seed at each of the temperatures was most rapid for high moisture seed, next most rapid for medium seed, and least rapid for low moisture seed. Rate of uptake for scarified seed fell

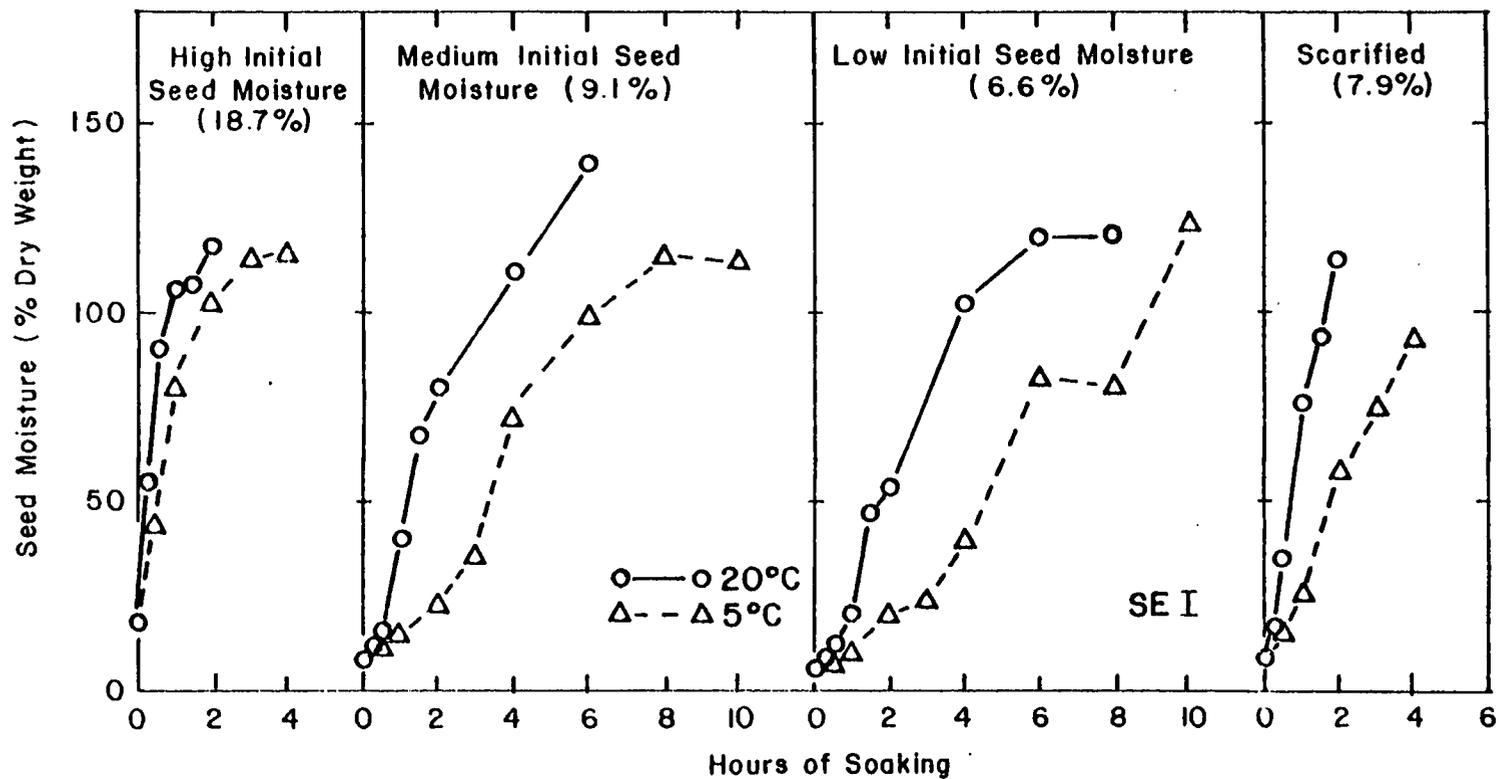


Figure 9. The effect of temperature and duration of soaking of snap bean seed on seed moisture for scarified seed and seed of 3 initial moisture levels.

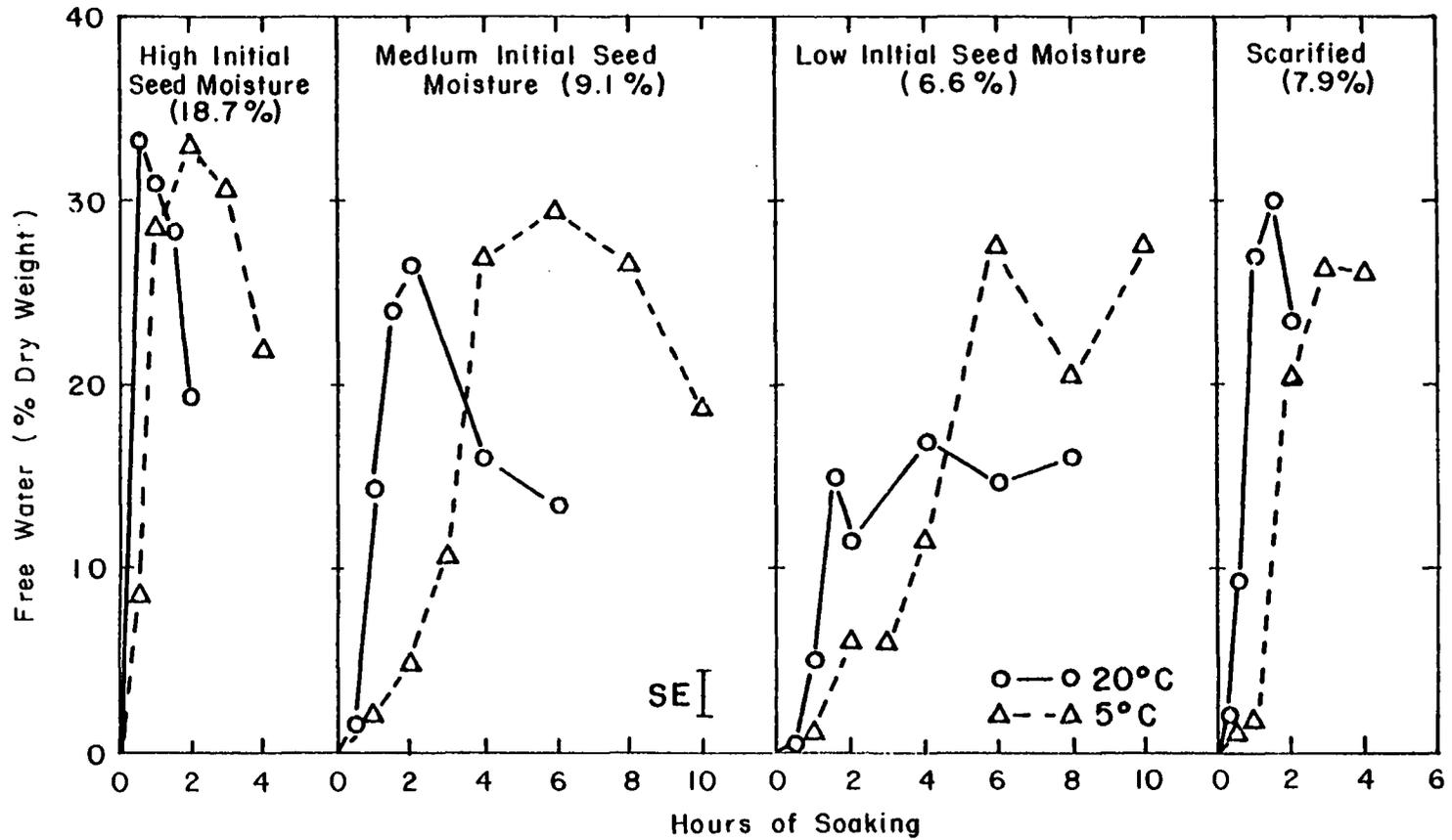


Figure 10. The effect of temperature and duration of soaking of snap bean seed on seed free water for scarified seed and seed of 3 initial moisture levels.

between high and medium moisture. For non-scarified seed, there was a tendency for the difference between rates at 5 °C and 20 °C to be greater as seed moisture decreased. Once again, scarified seed fell between high and medium moisture seed.

Some regularity is found in the relationship between rate of total water uptake, and rate of soaking injury, but the relationship is not consistent across all four seed groups. For medium, low, and scarified seed, injury patterns parallel moisture uptake: the more rapid the rate of water uptake, the greater the rate of injury. But this relationship does not hold for high moisture seed, which had the highest rate of water uptake at 20 °C, but which tended to have less injury for equal soaking durations than medium, low or scarified seeds at 20 °C. High moisture seed also--like the other seed groups--had a higher rate of water uptake at 20 °C than at 5 °C but, unlike the others, had somewhat slower rate of injury at 20 °C than at 5 °C. Therefore, the rate of water uptake does not by itself determine injury.

The basic pattern of percent free water peak and decline in the seed (Figure 10) roughly follows the patterns of total water uptake. It peaks and declines more rapidly at 20 °C than at 5 °C, (except for low moisture

seed) and it peaks and declines more rapidly with high seed moisture--excepting scarified seed, which has a free water pattern similar to that of high moisture seed. Furthermore, as with total water, the difference in patterns of free water uptake between 5 °C and 20 °C increased as seed moisture level is lower. A comparison of the maximum amount of free water reached at 5 °C versus that reached at 20 °C shows little difference for high or medium moisture, or scarified seed. For low moisture seed, on the other hand, the free water levels reached higher values when seed was soaked at 5 °C. Comparing initial seed moistures, no clear difference was found in maximum amount of free water reached with a 5 °C soak; it tended, however, to reach higher levels with the 20 °C soak when seed moisture was higher.

No consistent relationship was apparent between degree of soaking injury and either the rapidity at which free water accumulates, or the maximum level of free water reached. While the peak and decline of percent free water tended to be faster at 20 °C than at 5 °C, the rate of injury tended to be less at 20 °C for high moisture seed, and greater at 20 °C for medium, low, and scarified seed. High moisture seed soaked at 20 °C reached a greater maximum percent free water than medium moisture seed at

20 °C and had a slower rate of injury, while medium moisture seed soaked at 20 °C reached a greater maximum than low moisture seed at 20 °C, yet had a faster rate of injury. For medium and high moisture seed, respectively, the free water maximums for 5 °C and 20 °C soaks are, as noted, about equal, yet high moisture seed shows the fastest rate of injury from a 5 °C soak, while medium shows the fastest from a 20 °C soak. For low moisture seed there is a lower maximum free water at 20 °C, yet it shows an injury pattern similar to that of medium moisture seed; i.e. with greater rate of injury at 20 °C than at 5 °C.

Discussion

The expectation in this experiment was that snap bean seed soaked at low temperatures would show greater injury than those soaked at room temperature. Kidd and West (33), for example, found that while dry weight per snap bean plant was not markedly affected by soaking of seeds at low temperatures (10 °C), the number of seeds germinating was greatly reduced, so that the total seedling dry weight was considerably less than that after room temperature soaking. Eyster (23) likewise found greater reductions in germination of snap beans by soaking

at 10^o C than at 25^o C. Perry and Harrison (51) in an experiment with peas, found that at

...lower temperatures more seeds were killed, more electrolytes were exuded, and less water was absorbed than at 20^oC. The detrimental effect of soaking at low temperatures was more pronounced in the low-vigor lot B than in the high-vigor lot A.

Powell and Matthews (55) found that pea seeds soaked at low temperatures (7^o C) were injured more than at 20^o C despite the fact that the rate of imbibition was considerably reduced at low temperatures.

In view of these findings, it is puzzling that the results of our experiments with chilling and soaking showed greater injury--with the possible exception of high moisture seed--at 20^o C than at 5^o C. The difference may perhaps be the result of a much slower rate of water uptake at 5^o C for 'Slenderette' than for other varieties, which could nullify the effect of the low temperatures (51, 72). This may account for the results of preliminary studies with 'Slenderette' and other cultivars adapted to Northwest conditions which showed them to be very difficult to damage by chilling and extremely easy to injure with excess moisture. It is possible that if other cultivars had been chosen, the results would have been more consistent with the work of other researchers. It is possible, also, that had a higher temperature--say

10 C--been chosen for chilling, rates of water uptake would have been greater, and a greater rate of injury would have been observed than at 20 C.

Transverse cotyledonary cracking (TVC) would be increased by soaking and would contribute to decreased seedling vigor. However, it does not appear that TVC alone would result in the patterns of damage seen here. TVC is most severe at low seed moistures (43, 53), while the trend here is toward a faster rate of injury--if one excepts high moisture seed soaked at 20 C--as seed moisture level increases. TVC is also reported by various investigators to be either not dependent on temperature (19, 53) or more serious at lower temperatures (43). The results here, however, show the rate of injury to be less severe at 5 C than at 20 C except for high moisture seed.

Injury from low temperature imbibition is reported to be more serious when initial seed moisture is low (8, 11, 32, 34, 40, 46, 52, 53, 59). Our results, however, show that the lower the seed moisture, the slower was the rate of water uptake at 5 C, and consequently the lower the rate of soaking injury. The effect of low seed moisture on germinability and vigor of seedlings is complicated, however, by hard-seededness, which tends to increase as seed moisture level and temperature decrease. As

temperature declines, there is a progressive resistance to water uptake. Data by Pollock (53) indicate that hardseededness would tend to exaggerate damage caused by 5 C imbibition: the effect of the "slow-starting" seeds appeared as a decrease in the number of seedlings emerging and in the size of the surviving seedlings. That is, hardseededness would tend to influence the results in the same direction as those expected for this experiment, and not in the direction which actually occurred--a greater rate of injury at 20 C. Eyster (23), however, states that hardseededness will result in the seeds remaining hard and unswollen at the lower temperature, with no loss of viability. If seeds are scarified, he states, low temperature soaking will produce greater injury than soaking at room temperature. Our data do not confirm this: the scarified seed soaked at 20 C show a greater rate of injury than those soaked at 5 C.

The finding here that high moisture seed is more resistant to soaking at 20 C than medium or low moisture seed is consistent with the results of Experiment 2. As mentioned in the discussion of that experiment, less damage from soaking may be due to enhanced resistance to transverse cotyledonary cracking, as well as to the "conditioning" of seed membranes. The fact that high

moisture seed apparently has a greater rate of injury at 5°C than at 20°C may be the result of its rate of water uptake at 5°C being sufficient for chilling to produce injury (51, 72), whereas for medium and low moisture and scarified seeds it is not. This may also explain why, rather than decreasing as seed moisture increases, the rate of injury for 5°C soaking of unscarified seed increases: the conditioning effect of high seed moisture is overcome by the opposite tendency for more rapid water uptake as initial seed moisture increases and its consequent greater susceptibility to injury from chilling.

V. SUMMARY

1. There was no definite evidence of chilling injury in any of the experiments.
2. There was no evidence that slow low temperature imbibition of snap bean seed prior to soaking increased injury from soaking.
3. Slow imbibition of seed--regardless of temperature--prior to soaking generally reduced the injury sustained by soaking.
4. The longer the duration of slow imbibition of seed prior to soaking the less the injury sustained until it was essentially minimized.
5. The duration of slow imbibition of seed that was necessary to minimize soaking injury depended on temperature. For medium moisture seed it took approximately 24 hours at 5 °C and 9 hours at 20 °C to reach immunity. This corresponded at both temperatures to about 60% seed moisture.
6. The duration of slow imbibition of seed prior to soaking that is necessary to minimize soaking injury depends on the initial seed moisture level. For high moisture seed injury was minimized at 6-8 hours, for medium moisture at about 12 hours, and for low

moisture at over 24 hours. This corresponded to 40-60% for high moisture seed, about 60% for medium moisture, and over 100% for low moisture seed.

7. Within a given initial seed moisture level, the greatest injury from 2 hours of soaking occurred when the greatest amount of water was absorbed; however, a comparison of the three different initial seed moisture levels shows that the rapid rate of water uptake from 2 hours of soaking is less injurious when initial seed moisture is higher.
8. There is no evidence that soaking of seed prior to chilling results in increased injury due to chilling.
9. The relationships between duration of slow imbibition of seed prior to soaking and amounts of free water and injury sustained are not consistent. Seeds with no prior imbibition absorb the least amount of total water during soaking, yet contain the greatest amount of free water and sustain the greatest injury. However, seeds imbibed slowly for 6 hours had similar amounts of free water after all durations of soaking to those imbibed for 12 hours before soaking, yet were injured more by soaking.
10. The simultaneous soaking and chilling of high, medium and low moisture seed as well as scarified seed

resulted in an injury pattern which tended to parallel water uptake. Water uptake was faster at 20 °C than at 5 °C and faster with higher initial moisture content. The rate of injury tended to be greater at 20 °C than at 5 °C, and greater as initial seed moisture content increased. The exceptions to this are high moisture seed which possibly shows a slower rate of injury at 20 °C soaking than at 5 °C, and scarified seed which was injured at both temperatures of soaking more severely than its rate of water uptake would have indicated.

11. No consistent relationship was apparent between rate of soaking injury to the seed and either the rapidity of accumulation or the maximum level of free water in the seed reached.

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