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CHILLING SENSITIVITY OF PREGERMINATED
PEPPER SEED

presented by

CHRISTINE CAYER IRWIN

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of the requirements for
MASTER'S degree in HORTICULTURE

Hugh G. Price
Major professor

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CHILLING SENSITIVITY OF PREGERMINATED PEPPER SEED

By

Christine Cayer Irwin

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

CHILLING SENSITIVITY OF PREGERMINATED PEPPER SEED

I. Effect of Low Temperature on Emergence

II. Effect of Low Temperature, Potassium, Calcium and EDTA on Solute Leakage

By

Christine Cayer Irwin

Section I

The effect of storage of pregerminated pepper seeds (Capsicum annuum L. cv. Hungarian Wax) at 0°C and 5°C for 2 to 21 days on total percent emergence, time to 50% emergence (T_{50}), and viability was examined. An emergence assay at 25°/20°C showed that only dry seed and seed held at 0°C for 4 days were slower in emerging than fresh pregerminated seed. Assays at 15°/10°C showed that all treatments but 5°C for 4 days resulted in slower seedling emergence. Percent emergence data and tetrazolium chloride assay results supported the T_{50} data.

Section II

Storage of pregerminated pepper seed (Capsicum annuum L. cv. Calwonder) at 5°C for 4 days did not result in increased solute leakage. Exposure to 0°C for the same period, however, resulted in rapid leakage of solutes into distilled water. Calcium chloride in the incubation solution reduced the level of leakage to that of fresh, unchilled seed. Potassium chloride did not reduce leakage, while EDTA increased leakage.

to my mother and father for their support and encouragement

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The paper format which was utilized in this thesis meets the requirements stipulated by the Horticulture Department and the University. The thesis body was separated into two sections. Both Section I and Section II were prepared for the Journal of the American Society for Horticultural Science, and follow the manuscript style of that journal.

LITERATURE REVIEW

LITERATURE REVIEW

Seed germination is often a slow and sporadic process, resulting in non-uniform crop stands. Much effort has been directed towards improving the speed and uniformity of germination with the use of various seed pretreatments. "Hardening" involves cycles of wetting and drying the seed prior to planting. Kanchan and Jayachandra (1974) found that 3 cycles of hardening by soaking in ascorbic acid for 3 hours and drying for 21 hours resulted in quicker germination of pepper seeds. A-As-Saqui and Corleto (1978) improved both the percentage of emergence and the speed of emergence of 4 forage species by soaking the seed for 24 hours in water, then drying at room temperature. Damage to the seed results, however, if it is soaked for too long a time prior to drying. Heydecker and Coolbear (1977) reported no damage to wheat embryos if dried back after 24 hours of imbibition, but drying after 72 hours of imbibition damaged newly synthesized RNA and no recovery occurred.

Polyethylene glycol (PEG) has been used as an osmotic agent, both for simulating water stress and as a 'priming' agent for seeds. Heydecker, Higgins and Gulliver (1974) reported quicker and more uniform seedling emergence when seeds were pretreated, or 'primed', with a PEG solution. Heydecker and Hendy (1975) reported no effect on percent germination (in general), but a large increase in speed of germination with PEG 'priming'. Salter and Darby (1976) found celery seed also benefitted from 'priming'. The concentration of the PEG solution must be high enough to prevent complete germination, but allow sufficient water absorption for initiation of germination processes.

PEG is an inert, large molecule, which does not react with plant material to any appreciable degree, as other osmotic agents such as mannitol.

The germination-promoting properties of potassium nitrate (KNO_3) have resulted in its use in routine germination testing of many crops (Copeland, 1976). Ells (1963) found that treating tomato seed with KNO_3 in combination with K_3PO_4 stimulated germination when seeds were exposed to a 10°C night temperature. More recently, work by Rumpel and Szudyga (1978) has shown a reduced time to germination at 8, 12 and 15°C when tomato seed was presoaked in a PEG solution or a solution of KNO_3 + K_3PO_4.

Larger increases in speed and uniformity of emergence than those obtained with seed pretreatments have been seen with the use of pregerminated seed. Much work in England by Gray (1974), Currah, Gray and Thomas (1974) and Bussell and Gray (1976) has shown that sowing pregerminated seed in a fluid gel can increase the speed of emergence and reduce the spread of emergence of many crops. In the U.S., Taylor (1977) and others have reported similar benefits of pregermination. The advantages of using pregerminated seeds are particularly evident when soil temperatures are suboptimal (in the case of early direct-seeding of warm season vegetables) or supraoptimal (eg. celery transplant production in Florida) for germination of dry seed.

Research into fluid-drilling raised the question of how to handle the pregerminated seed in the event of delayed planting, and how to slow its growth until planting could take place. Low temperature storage has long been used for dry seed, nursery root stock and some fresh produce, since it effectively slows metabolic processes. The applicability and limits of low temperature storage for use with

pregerminated pepper seeds was therefore examined in this research.

Overexposure of plant material of tropical or subtropical origin to low temperature can result in chilling injury. This differs from freezing injury, and occurs in the range of 0 to 15°C. Symptoms of chilling injury manifest themselves in many ways and all plant parts are susceptible. Imbibitional chilling injury can affect not only seed germination, but subsequent growth as well, and can occur very quickly. Pollock and Toole (1966) found that lima beans were most severely injured when chilling occurred during the first 10 minutes of imbibition. Chilling reduced seedling survival and seedling size. Harrington and Kihara (1960) showed with pepper and muskmelon seeds that exposure to 0°C and 5°C during imbibition resulted in collapse and necrosis of tissue behind the radicle. Occurrence of a stelar lesion during imbibitional chilling of a corn strain has been reported by Cohn and Obendorf (1978). Soybeans are also very sensitive to low temperature imbibition. Germination and elongation of embryonic axes were reduced when soybean seeds were imbibed at 5°C (Bramlage, Leopold and Parrish, 1978). Extensive work by Christiansen (1963) with cotton showed that exposure to 5°C resulted in aborted primary roots in 60% of the seedlings examined, with subsequent development of lateral roots. The abnormal root tip failed to stain with tetrazolium chloride. Later studies (1964) showed that early chilling results in a developmental lag, though after some time, development may assume a normal rate. Christiansen also found (1967) there to be two periods of chilling sensitivity in cotton seed - the first during the initial few hours of imbibition, and the second after 18 to 30 hours of imbibition. Along with Thomas, Christiansen (1969) reinforced some

earlier findings and showed that chilling cotton seed during germination reduced plant height, delayed fruiting and reduced fiber quality in a direct relation to the length of exposure to cold. Wiles and Downs (1977) used the term 'nub root' to describe the short, blunt root tip which results from chilling cotton seed, a condition earlier observed by Christiansen (1963).

Chilling can have many complex effects on the metabolism of chilling-sensitive tissue. Lewis (1956) found that protoplasmic streaming ceased after short exposure to about 11°C in Cucurbita pepo and Lycopersicon esculentum (chilling-sensitive), whereas streaming proceeded in chilling-resistant radish (Raphanus sativus) and carrot (Daucus carota) at temperatures as low as 0°C. Chilling-sensitive plants also differ from chilling-resistant plants in their respiratory response to chilling. Lyons and Raison (1970) reported that the Arrhenius plots of the respiration rate of mitochondria isolated from chilling-resistant tissue (cauliflower buds, potato tubers and beet roots) showed a linear decrease over a temperature range of 25°C to 1.5°C. The respiration rates of chilling-sensitive tissues (tomato and cucumber fruit, and sweet potato roots), on the other hand, showed a linear decrease from 25°C to about 9 to 12°C, at which point there was a sharp increase in the slope of the line as temperatures were dropped to 1.5°C. This 'break' in the line of the Arrhenius plot is believed to represent a phase change from fluid to gel in the membrane phospholipids (Kumamoto, Raison and Lyons, 1971). Simon (1974) in a review of the subject states that at the temperature at which the break occurs, the phospholipids can exist in either of two alternative states, whereas at the other temperatures, they can only exist in one state.

The explanation for the differing respiration responses of chilling-sensitive and chilling-resistant plants lies in the degree of unsaturation of the membrane fatty acids. Lyons, Wheaton and Pratt (1964) reported that chilling-resistant plants have more unsaturated fats in the mitochondrial membranes than do chilling-sensitive plants. Therefore, the mitochondrial membranes of chilling-resistant plants have the capacity to swell at low temperatures, whereas those of chilling-sensitive plants do not have such a capacity. Dogras, Dilley and Herner (1977) found that peas and broad beans (both chilling-resistant) imbibed at 10°C incorporated a larger percentage of labelled glycerol into phosphatidylcholine, which is highly unsaturated, than did lima beans (chilling-sensitive). Lima beans incorporated more glycerol into less unsaturated phosphatidylethanolamine and phosphatidylglycerol. The amounts of total phospholipids and the degree of unsaturation has been found to change in some plants in relation to temperature. Gerloff, Richardson and Stahmann (1966) found an increase in both total fatty acids and the degree of unsaturation in alfalfa roots during cold hardening. Grenier and Willemot (1974) reported similar results with alfalfa roots, and noted that cold-hardy cultivars showed greater increases than did less cold-hardy cultivars. DeLaRoche, Andrews and Pomeroy (1972) reported similar increases in the degree of unsaturation in winter wheat seedlings grown at 2°C, but did not find such increases in seedlings grown at 24°C. These changes, however, are gradual, and for the most part limited to tissues which become cold-hardened in preparation for overwintering. The inability of membranes of chilling-sensitive plants to withstand low temperatures results in many metabolic disturbances. Processes catalyzed by

membrane-bound enzymes show an increase in activation energy. Raison, Lyons and Thomson (1971) observed this increased activation energy in the mitochondrial succinate oxidase system of chilling-sensitive plants when exposed to temperatures below 9°C. Raison (1973) later showed this to hold true for other enzyme systems. Shneyour, Raison and Smillie (1973) reported a sharp increase in the activation energy needed for photoreduction of $NADP^+$ when tomato plants were exposed to temperatures below 11°C. In lettuce, a chilling-resistant plant, the activation energy remained constant under the same temperatures. Later work by Raison and Chapman (1976) with Vigna radiata substantiated earlier work with other chilling-sensitive plants. Towers, et. al. (1973) showed that membrane-bound ribosomes are also adversely affected by low temperature, resulting in decreased protein synthesis. Arrhenius plots of the protein synthetic activity of membrane-bound ribosomes displayed characteristic 'breaks', whereas Arrhenius plots for protein synthesis by free ribosomes were linear. As a result of the increased activation energy of many enzymatic reactions, some substances may accumulate within the tissue. Murata (1969), in chilling studies with green bananas, found that the acetaldehyde, α-keto acid and ethanol contents of both peels and fruits increased with the onset of chilling injury. Polyphenols can also accumulate, as shown by Liebermann et. al. (1958). The accumulation of polyphenols is believed to be due, in part, to the destruction of ascorbic acid (ASA) at low temperatures. Miller and Heilman (1952) showed that chilling reduces the ASA content of pineapple fruits. Storage of pepper fruits at 1°C caused a large decrease in the ASA content of the pepper seeds, as observed by Yamauchi, Inaba and Ogata (1978). Simon (1974) indicates that such

'imbalances' of metabolism may be responsible for the appearance of some chilling injury symptoms, such as the appearance of browning with phenol accumulation. In addition, as a result of the disrupted respiratory pathway, large decreases in the ATP content of the tissue may ensue, as seen by Stewart and Guinn (1969) and Wilson (1978).

Another serious consequence of the phospholipid phase change incurred at chilling temperatures is increased membrane permeability. This can be determined by measuring leaked solutes, either spectrophotometrically or with the use of a conductivity bridge. Increased solute leakage has been shown to be a symptom of chilling injury in seeds, leaf and fruit tissue. Christiansen, Carns and Slyter (1970) reported large steady increases in solute leakage from radicles of cotton seeds exposed to 5°C. Guinn (1971) found cotton cotyledons to also suffer increased solute leakage when exposed to 5°C for 3 hours of more. Chilling greatly increased the amounts of reducing sugars, ninhydrin-positive material, and ionic material which subsequently leaked from the cotyledons. Powell (1969) measured the resistance of the solution surrounding chilled and unchilled cotton leaf discs, and found increased conductivity after chilling. Bramlage, Leopold and Parrish (1978) found that dry soybeans, placed in water, leak solutes profusely at first, but leakage quickly subsides to a much slower rate as the membranes reorganize. In chilled cotyledons, however, the decline in leakage takes considerably longer to occur. Minchin and Simon (1973) found increased leakage of water and electrolytes from cucumber leaf discs, resulting in wilting. Some later work by Tanczos (1977) with cucumber leaf discs showed no increase in conductivity with 3 days or less at 2°C, but longer exposure

resulted in increased conductivity. Phaseolus vulgaris leaves also suffer increased solute leakage with chilling, as observed by Wright (1974). Lewis and Workman, in some early work (1964) showed that exposure of mature green tomato fruits to 0°C for 4 weeks caused a 3-fold increase in cell membrane permeability, but had no effect on the permeability of cabbage leaves. Recently, Tatsumi and Murata (1978) found that Arrhenius plots of ion leakage from cucumber and pepper fruits showed a dramatic increase in leakage around 7 to 10°C, indicated by a change in slope, but a straight line response from potato tubers was observed. Some other recent work (1978) by Fukushima and Yamazaki revealed that phospholipid phase changes are not the only factors involved in increased membrane permeability with chilling. They found in cucumbers, bananas and sweet potato roots that chilling results in an increase in hot water insoluble pectin and a decrease in hot water soluble pectin, contributing to increased cell wall rigidity.

The significance of solute leakage was recognized as early as 1928, when Hibbard and Miller reported that leakage and percent seed germination showed a negative correlation. Matthews and Bradnock (1967, 1968) showed a significant negative correlation between solute leakage and field emergence of peas and French beans, and believe that a measurement of seed exudation is a better indicator of seed vigor than standard laboratory germination tests.

If chilling injury has not been too severe, it can be reversed by subsequent exposure to warmer temperatures. Work by Brand, Kirchanski and Ramirez-Mitchell (1979) has recently shown with a blue-green alga, Anacystis nidulans, that cells grown at 25°C, then exposed to 0°C for 30 minutes underwent a phase separation which was totally reversed by

re-exposing the cells to 25°C. However, growth at 39°C prior to exposure to 0°C resulted in considerably more morphological alterations than the 25°C growth temperature, and subsequent exposure to 39°C resulted in only partial reversal of the phase separation. Ibanez (1963), working with cacao seed, showed that if seeds were exposed to 4°C for 10 minutes or less, cold inhibition of germination could be reversed by exposure to 37°C, but longer exposure to 4°C resulted in irreversible injury. Creencia and Bramlage (1971) observed warm temperature reversal of chilling injury incurred during short term exposure to 0.3°C in corn seedlings. A decrease in solute leakage from cucumber leaf discs exposed to 2°C for 4 days or less was noted by Tanczos (1977) when the discs were transferred to 25°C. Five days or more, however, did not allow for recovery.

Cold hardening refers to the gradual process which plants undergo in preparation for winter. Wheaton and Morris (1967) succeeded in applying the same principles to tomato plants and reduced their chilling sensitivity by exposing the plants to 12.5°C, slightly above the chilling range for tomatoes, for 48 hours. Higher temperatures provided less protection than did 12.5°C.

Various treatments have been used, both to prevent chilling injury and to reduce the symptoms, with varying degrees of success. Pollock and Toole (1966) found they could reduce imbibitional chilling injury in lima beans by imbibing them for a brief period at 25°C, prior to continued imbibition at 5°C. Miller and Corns (1957) reported increased low temperature resistance in sugar beet seedlings treated with Dalapon or trichloroacetic acid. Ilker, et. al. (1976) found ethanolamine lended some increased low temperature resistance to tomato seedlings,

perhaps by lowering the temperature of the phospholipid phase transition. Bartowski, Katterman and Buxton (1978) were able to increase the germination of some cotton cultivars at 14°C by applying exogenous fatty acids, but failed to increase the germination of all cultivars tested. Another approach has been taken by Christiansen and Ashworth (1978), who observed a reduction in chilling injury of cotton seedlings by placing plants in plastic bags to elevate humidity or by the use of antitranspirants. Since water uptake by roots of cotton and other tropical species is considerably restricted at temperatures below 15°C, reducing transpiration reduces foliar necrosis and other injury symptoms associated with water loss. Spraying cucumber seedlings with abscisic acid (ABA) was shown by Rikin and Richmond (1976) to reduce chilling injury. They found in other work (1976) that subjecting the seedlings to water stress, which resulted in an elevated ABA content, had the same effect as an ABA spray application. The role of ABA in reducing water loss was suggested as the mechanism of ABA-induced chilling resistance.

Calcium has long been known to be an integral part of plant cell walls, and Marinos (1962) showed in some submicroscopic studies of calcium deficient barley, that lack of calcium results in membrane disorganization. With this information in mind, several researchers have utilized Ca to reduce or prevent leakage resulting from membrane damage. Van Steveninck (1965) found he could reverse EDTA-induced leakage from beet root tissue with equivalent amounts of $CaCl_2$. He observed reduced leakage to some degree with several other divalent cations, but Ca was most effective. Sucrose leakage from corn scutellum slices was reduced with as low as .001M Ca by Garrard and Humphreys

(1967). Using artificial membranes, Gary-Bobo (1970) showed that $CaCl_2$ decreases pore size, further evidence of calcium's role in controlling membrane permeability. Christiansen, Carns and Slyter (1970) found that Ca was effective in reducing chill-induced membrane leakage from cotton radicles. They suggested that part of calcium's function in membrane stabilization may be in influencing cell surface charge. Poovaiah and Leopold (1976) reduced leakage from beet root tissue and Rumex obtusifolius leaf discs with $CaCl_2$ solutions. Most recently, Poovaiah (1979) has been successful in preventing ethephon-induced membrane leakage from beet root tissue with $CaCl_2$. Other divalent cations were somewhat less effective, and monovalent cations were not effective.

Chilling injury is a time-temperature response, ie. the lower the temperature or the longer the period of exposure, the greater the injury. Christiansen (1968) points this out in some of his work with imbibing cotton seed. Chilling incurred in the field may be cumulative with chilling incurred in storage, as may occur with produce going into post-harvest storage. The reverse situation could be the case as well, with transplants stored prior to field planting. With pregerminated seed, this is also a consideration if it is to be stored prior to planting. Other stresses may accentuate injury caused by low temperature exposure. Soil compaction has been shown to adversely affect emergence and growth of many crops, including calabrese (Hegarty and Royle, 1976), barley (Wilson and Robardo, 1977) and pepper (Fawusi, 1978), and combined with chilling, could produce more injury than either stress alone.

The need for accurate assessment of the viability and vigor of

seeds has resulted in the development of several types of tests. The biochemical test most widely used today, according to Copeland (1976) is the tetrazolium chloride (TTC) test for seed vigor and viability. In contact with actively respiring tissue, the tetrazolium salt forms a red, water insoluble substance, formazan. Nonviable tissue does not stain. Kittock and Law (1968) found with wheat seeds that vigor, TTC reduction and rate of respiration all were positively correlated. However, Lobanov (1967) warns that chemical seed viability determinations of spring crops are only approximations and cannot be used for determining sowing rates. Heydecker (1973) contends this may be because such tests as the TTC test reflect only a measurement of seed condition and not the interaction between seed and environment that occurs in the field. Germination tests are also routinely used for assessing seed viability. Standard germination tests are carried out under optimum conditions, and results are often not as closely correlated with field performance as are results of the cold test (Johnson and Wax, 1978), or other stress-imposing tests. The cold test involves germinating the seeds in cool, moist soil, then moving them to warmer conditions. This test more accurately reflects the seed/ environment interaction which Heydecker (1973) discusses than either the standard germination test or the TTC test.

In light of all this, the research for this thesis was undertaken with the following objectives:

1. assessment of the performance of pregerminated pepper seed vs. dry seed under various conditions.
2. determination of the chilling sensitivity of pregerminated pepper seed, and the limits of cold tolerance.

3. application of such knowledge to development of a cold storage technique for pregerminated pepper seed.
4. application of such knowledge to a better understanding of what happens with early sowings of pregerminated pepper seed.
5. definition of some of the symptoms of chilling injury in pregerminated pepper seed.
6. examination of the effectiveness of some preventative and remedial measures for reducing chilling injury.
7. assessment of the value of the TTC test and modifications of other vigor tests for use with pregerminated pepper seed.

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SECTION I

EFFECT OF LOW TEMPERATURE ON EMERGENCE

Chilling Sensitivity of Pregerminated Pepper Seed

I. Effect of Low Temperature on Emergence.

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Additional index words: Capsicum annuum, tetrazolium chloride assay, cold storage

Abstract. The effect of storage of pregerminated pepper seeds (Capsicum annuum L. cv. Hungarian Wax) at 0°C and 5°C for 2 to 21 days on total percent emergence, time to 50% emergence (T_{50}), and viability was examined. An emergence assay at 25°/20°C showed that only dry seed and seed held at 0°C for 4 days were slower in emerging than fresh pregerminated seed. Assays at 15°/10°C showed that all treatments but 5°C for 4 days resulted in slower seedling emergence. Percent emergence data and tetrazolium chloride assay results supported the T_{50} data.

Fluid-drilling is a direct-seeding technique utilizing seed pregerminated under controlled conditions. The seed is dispersed in a fluid gel just prior to planting. The gel acts as a carrier for the seed and minimizes the amount of mechanical damage during the planting process. The advantages of this technique over conventional direct-seeding techniques include quicker and more uniform seedling emergence (4,11), especially when temperatures are suboptimal for germination of dry seed.

To develop a means of holding pregerminated seed in the event of delayed planting, low temperature storage was examined. For successful storage of pregerminated seed, the temperature must be low enough to temporarily halt radicle growth, but not so low as to cause chilling

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injury. Elongated radicles are more subject to damage during planting. A further objective was to study the effect of low soil temperature on stored and unstored pregerminated pepper seed.

Materials and Methods

Pepper seeds, Capsicum annuum L. cv. Hungarian Wax, were germinated for 5 days in aerated glass water columns at room temperature. Radicles after 5 days were 2 to 4 mm in length. Germinated seeds were removed from the columns and placed in cold storage wrapped in 2 thicknesses of moist cheesecloth 15 x 15 cm and loosely wrapped in polyethylene bags. Storage temperatures of 0°C and 5°C were used since preliminary experiments had shown that 10°C did not stop radicle elongation. Based on preliminary experimentation, seeds were stored at 0°C for 2 and 4 days, and 5°C for 4, 8, 12 and 21 days. Dry seed and fresh pregerminated seed (unstored) were used as controls. Four replicates of 10 seeds/treatment were used for the tetrazolium chloride (TTC) assay for seed viability. Replicates were placed in glass test tubes, and covered with 4 ml of a 0.5% 2,3,5-triphenyl tetrazolium chloride solution. The tetrazolium salt had been dissolved in a .01 M phosphate buffer solution of pH 7.0. Reduction of the tetrazolium salt by actively respiring tissue results in the formation of water-insoluble formazan, which is red. Test tubes were kept at room temperature in darkness for 24 hours. The TTC solution was decanted, the seeds rinsed with distilled water, and each replicate was then covered with 4 ml of 2-methoxyethanol for extraction of the formazan. After 4 hours, each sample extract was measured for absorbance at 480 nm in a Bausch & Lomb Spectronic 20. Tetrazolium chloride reduction has been shown to be

positively correlated with vigor (9).

For the emergence assay, 8 replicates of 25 seeds/treatment were planted at a 1.5 cm depth in flats containing moistened vermiculite. Treatments were arranged in a completely randomized design. Four replicates were placed in a Percival environmental growth chamber with a 25°C 14 hour day and 20°C 10 hour night, and the other 4 in an identical growth chamber with a 15°C 14 hour day and 10°C 10 hour night. Flats were subirrigated as needed, and daily emergence counts were taken. The experiment was terminated 41 days after planting, and time to 50% emergence, an index of seedling vigor (10), and percent emergence were calculated for each treatment. Data were analyzed using analysis of variance and LSD was used for mean separation.

Results

The tetrazolium assay showed a significant reduction in formazan absorbance compared with fresh pregerminated seed after 2 days storage at 0°C, and an even greater reduction with 4 days at 0°C (Fig. 1). Examination of the seeds prior to color extraction showed that the tips of the radicles had been injured by the 0°C treatments, indicated by the lack of formazan in the tips. Though the assay showed no significant reduction in viability with storage at 5°C, formazan absorbance and days at 5°C showed a significant negative correlation (Fig. 2).

Emergence assay results obtained in the 25°/20°C growth chamber indicated that only dry seed, and pregerminated seed stored at 0°C for 4 days is slower in emergence than fresh seed, as seen by the higher T_{50} values (Fig. 3). Storage of seeds for 21 days at 5°C did not increase the T_{50} emergence. In the 15°/10°C assay, however, all

Fig. 1. Absorbance (480 nm) of 2-methoxyethanol extracts from tetrazolium chloride treated fresh pregerminated pepper seeds (unstored) and seeds held at 0°C and 5°C for 2 to 21 days. ($LSD_{.05}$ = 0.25)

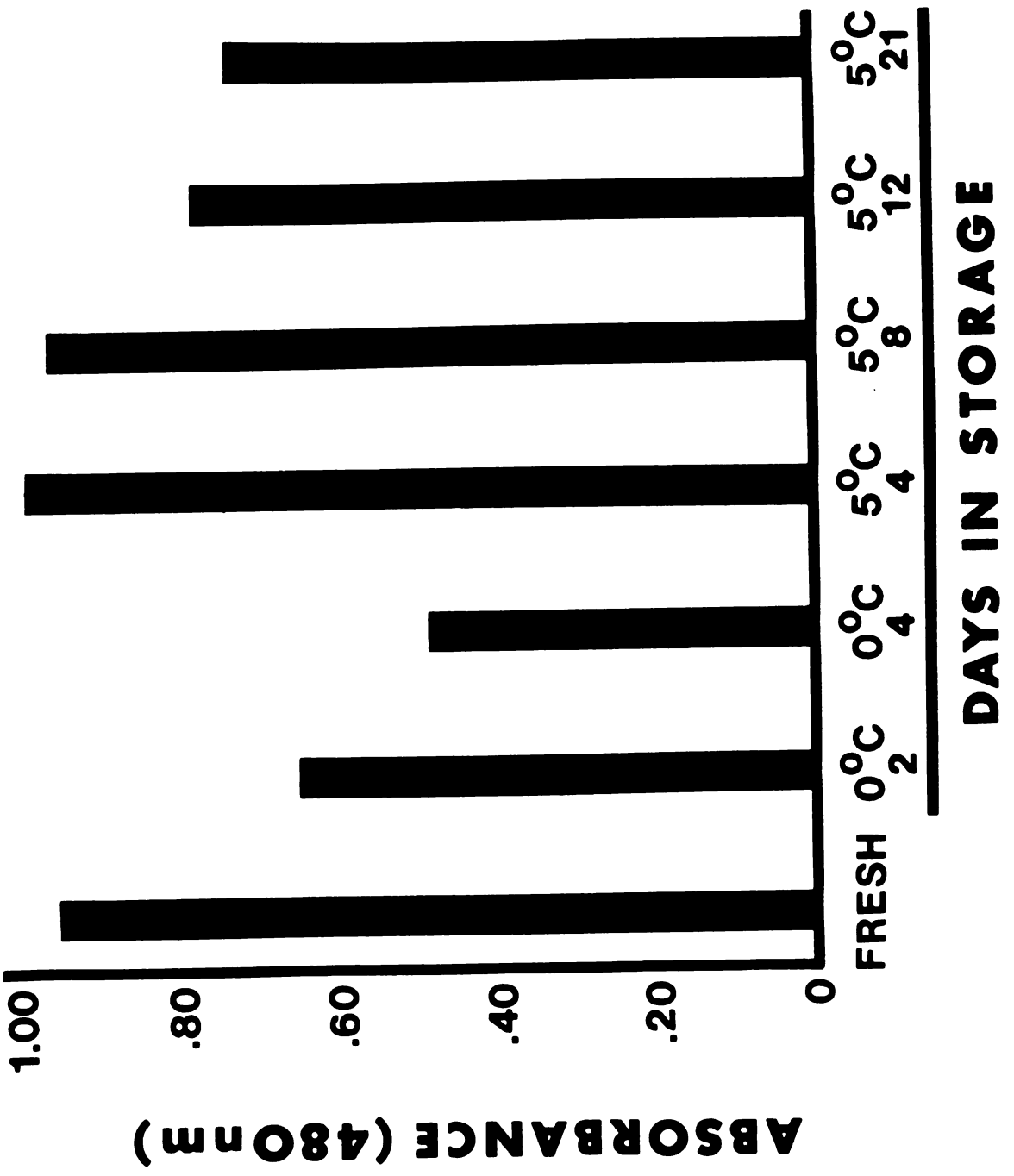
ABSORBANCE (480nm)
1.00
.80
.60
.40
.20
0
FRESH
0°C 2
0°C 4
5°C 4
5°C 8
5°C 12
5°C 21
DAYS IN STORAGE

Fig. 1

Fig. 2. The relationship between days at 5°C and absorbance (480 nm) of 2-methoxyethanol extracts from tetrazolium chloride treated pregerminated pepper seeds.

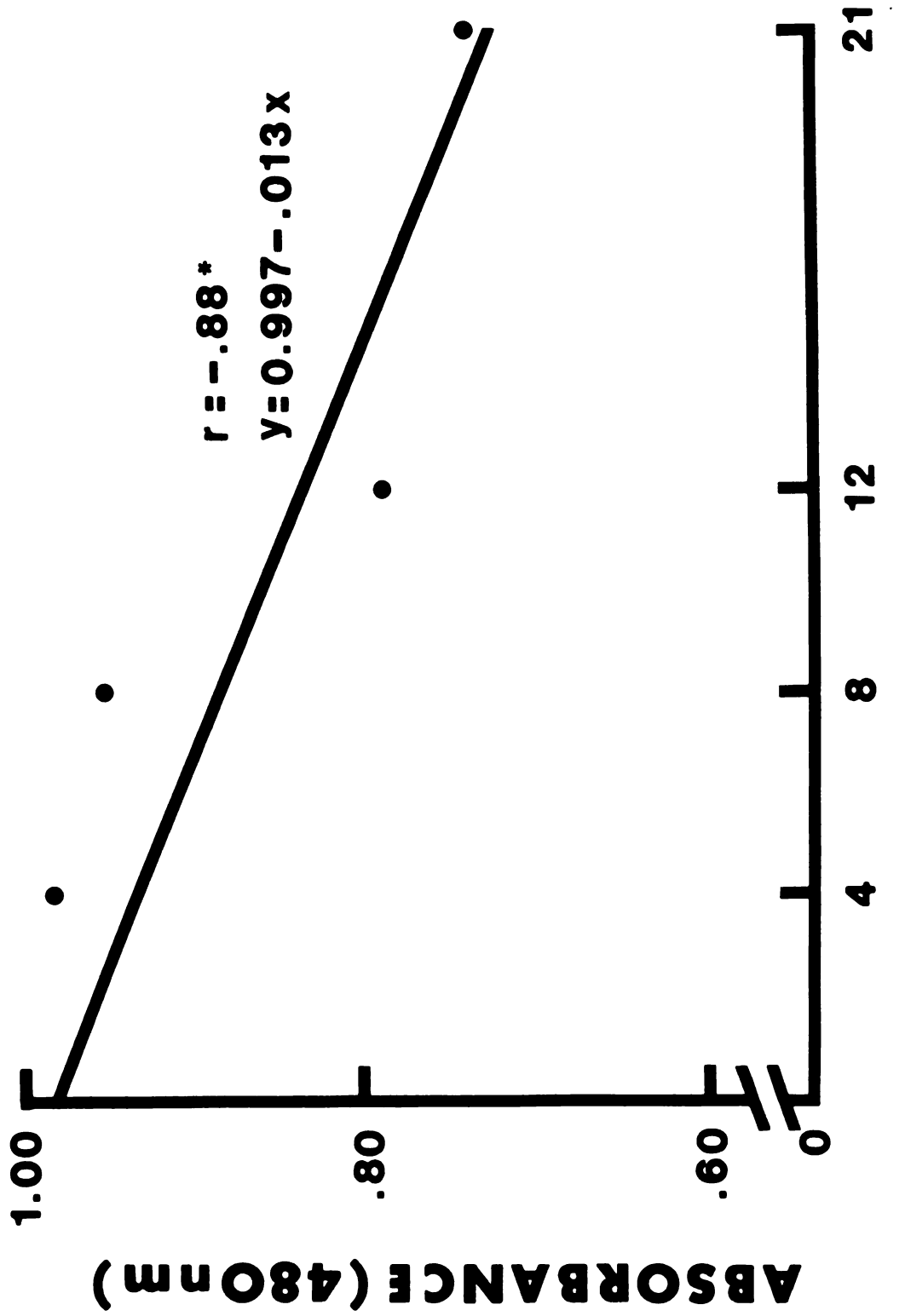

Fig. 2

Fig. 3. Effect of days at 0°C (A) and 5°C (B) on time to 50% emergence (T_{50}). Emergence assays were conducted at 25°/20°C and 15°/10°C. Dry seed was significantly slower in emerging than fresh (unstored) pregerminated seed under both assay temperatures. ($LSD_{.05}$ = 1.89)

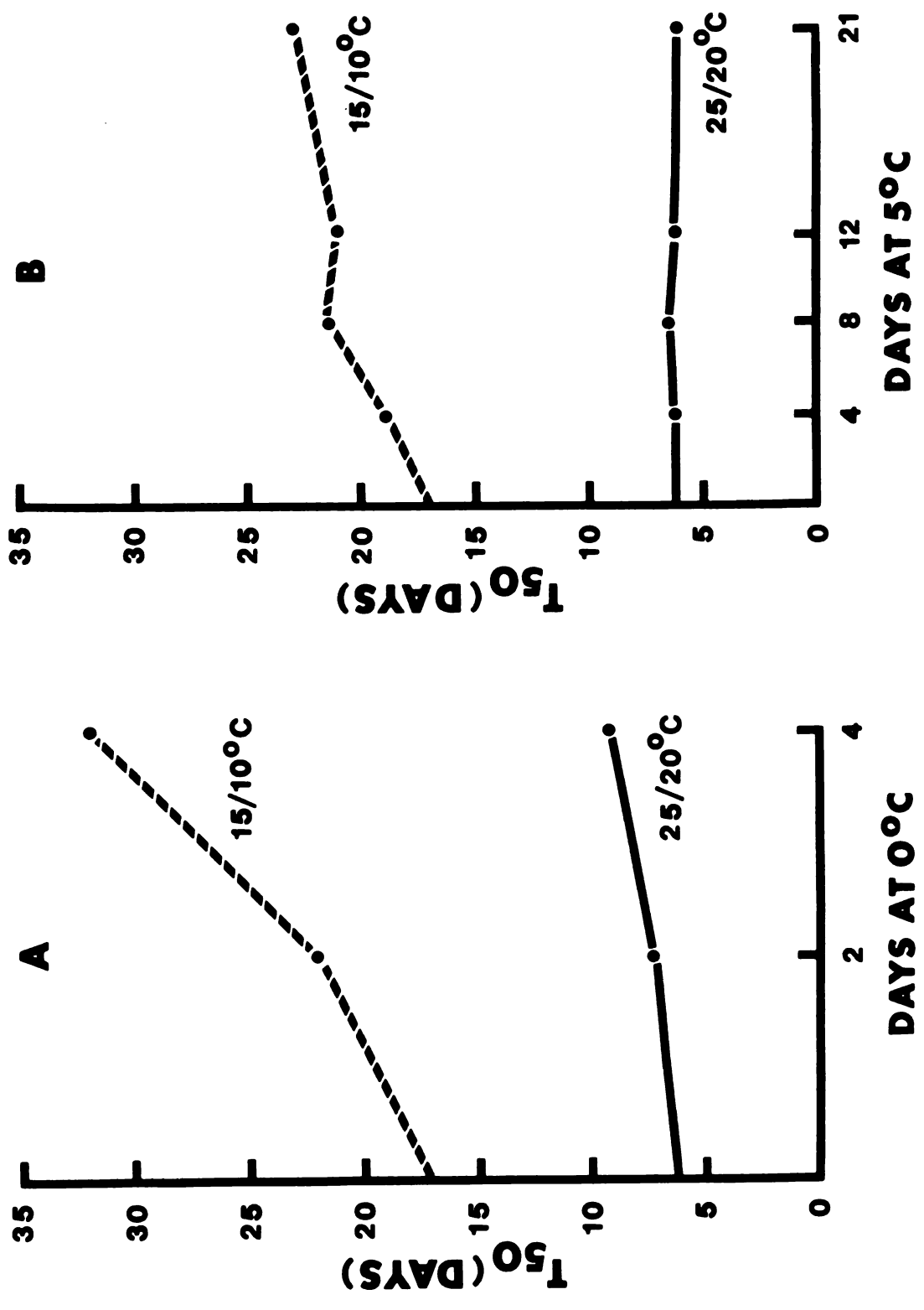
A
B
T_{50} (DAYS)
35
30
25
20
15
10
5
0
15/10°C
25/20°C
2
4
DAYS AT 0°C
4
8
12
21
DAYS AT 5°C

Fig. 3

treatments except 5°C for 4 days had higher T_{50} values than fresh seed. Seeds stored at 0°C emerged much slower than seeds held for any duration at 5°C. At the lower assay temperature, dry seed had a T_{50} of 33.5 days compared with 17.0 days for fresh pregerminated seed.

Under warm growing conditions, only dry seed and seed stored at 0°C for 4 days had a significantly lower percentage of emerged seedlings than the fresh treatment (Fig. 4). Assay under 15°/10°C resulted in a reduction in percent emergence, compared with emergence in the 25°/20°C chamber, with all storage treatments. Fresh pregerminated seed and dry seed did not exhibit a reduced stand under the low temperature regime. When compared with the percentage of emerged seedlings from fresh pregerminated seed, all treatments except 5°C for 4 days and dry caused a reduction in percent emergence.

Discussion

Injury to the root tip, evidenced by non-staining in the tetrazolium assay, has been reported by others (1,2,5,13) to be a result of low temperature exposure. If completely killed back, early proliferation of adventitious roots occurs, and there is no tap root.

Assay of seedling emergence under near optimum conditions may not accurately reflect the condition of the seed. This was evidenced by the large differences between results of the high and low temperature assays. It may be that a slight amount of low temperature injury incurred during storage is reversed once the chilled seed is moved to nearer optimum conditions. Reversal of chilling injury has been shown with corn seedlings and cucumber leaf discs, but reversal does not occur if injury has been too severe (3,12). If this is happening with

Fig. 4. Effect of days at 0°C and 5°C on percent emergence. Emergence assays were conducted at 25°/20°C and 15°/10°C. ($LSD_{.05}$ = 13.88)

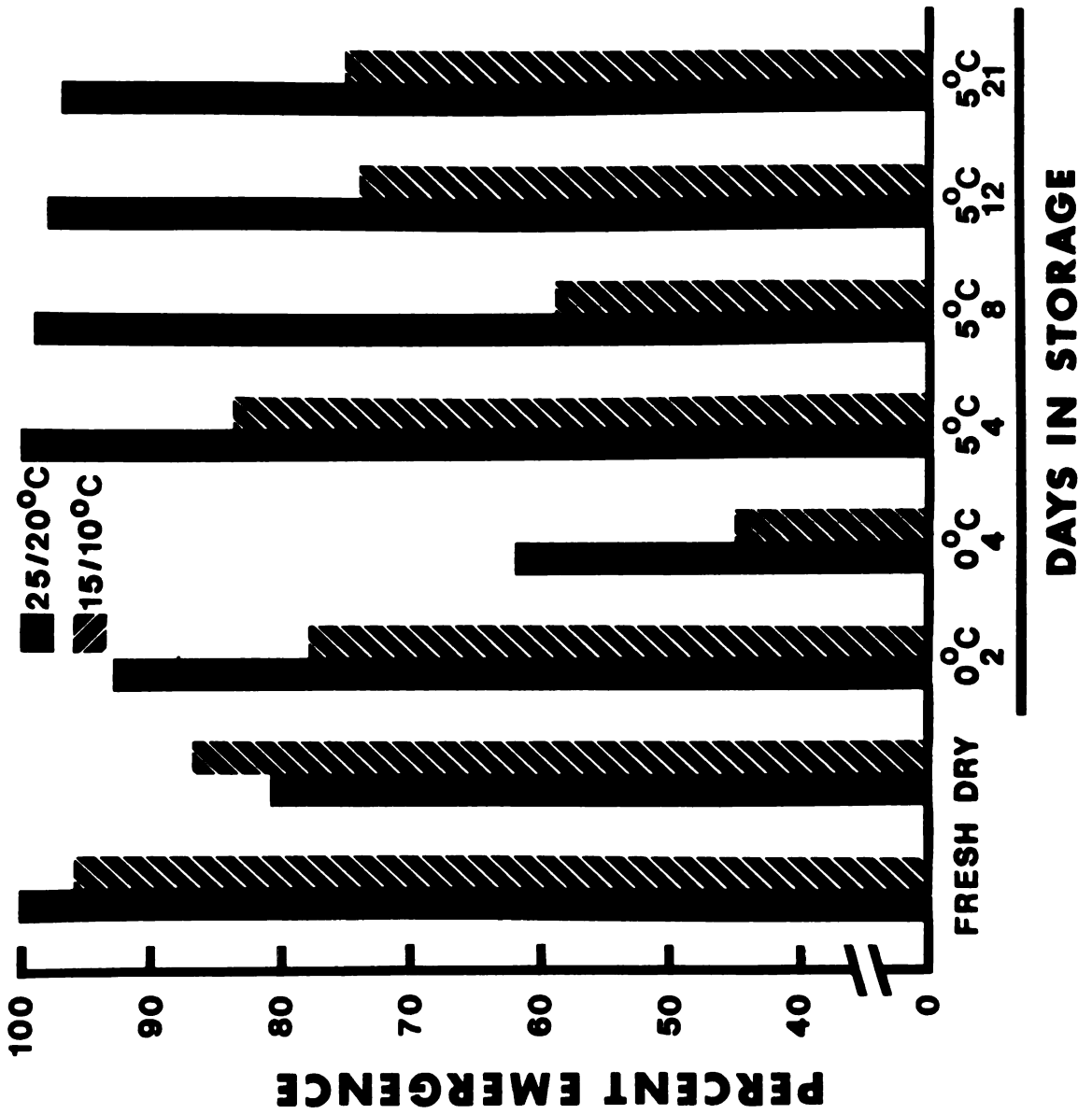
PERCENT EMERGENCE
100
90
80
70
60
50
40
0
25/20°C
15/10°C
FRESH DRY
0°C 2
0°C 4
5°C 4
5°C 8
5°C 12
5°C 21
DAYS IN STORAGE

Fig. 4

the pregerminated pepper seeds, 0°C for 4 days may cause too much injury for reversal to occur. Imposing the additional stress of low temperatures after planting accentuates any weakened state incurred during storage. Treatments which did not result in slower emergence or a reduced stand when assayed at 25°/20°C, did in fact, when assayed at 15°/10°C, except for 4 days at 5°C, which appears to be safe for storage of pregerminated pepper seeds. Longer periods of time at 5°C, and 2 days or more at 0°C apparently weakened the seed so that the additional low temperature exposure in the 15°/10°C growth chamber caused injury symptoms resulting in slower emergence and/or a reduced stand. Seed analysts have found similar results with germination tests - when carried out under optimum conditions, results often show a poor correlation with field performance (6,8).

The tetrazolium assay for seed viability provided additional evidence for the observed reduction in viability occurring with storage at 0°C for 2 and 4 days. Comparison of the TTC results with emergence assay results showed that it is more sensitive to viability differences than an emergence assay carried out under near optimum temperature conditions (25°/20°C). However, it did not reveal treatment differences which were obvious from the low temperature emergence assay. These discrepancies may result because the TTC test reflects seed condition, not the interaction between seed condition and environment (7), as does the emergence assay.

Pregerminated pepper seeds should not be stored at 0°C, since that temperature results in a reduction in viability and vigor. Depending on the temperature at the time of planting, 5°C may be a safe storage temperature for 4 to 21 days. The safe period for storage is shorter the lower the soil temperature at the time of planting.

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SECTION II

EFFECT OF LOW TEMPERATURE, POTASSIUM, CALCIUM AND EDTA ON SOLUTE LEAKAGE

Chilling Sensitivity of Pregerminated Pepper Seed

II. Effect of Low Temperature, Potassium, Calcium and EDTA on Solute Leakage

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Additional index words: Capsicum annuum, fluid-drilling, membrane integrity, cold storage

Abstract. Storage of pregerminated pepper seed (Capsicum annuum L. cv. Calwonder) at 5°C for 4 days did not result in increased solute leakage. Exposure to 0°C for the same period, however, resulted in rapid leakage of solutes into distilled water. Calcium chloride in the incubation solution reduced the level of leakage to that of fresh, unchilled seed. Potassium chloride did not reduce leakage, while EDTA increased leakage.

Exposure of plants of tropical or subtropical origin to temperatures in the range of 0°C to 15°C often results in chilling injury. Among other things, this injury is characterized by a disruption of membrane integrity, resulting in leakage of solutes from the chilled tissue. Membranes of chilling-resistant plants have been shown to be more flexible than membranes of chilling-sensitive plants due to the higher degree of unsaturation of their membrane phospholipids (11). Upon exposure to low temperature, phospholipids in chilling-sensitive plants undergo a 'phase change' from fluid to gel, the membrane cracks, and solutes leak profusely (9). Increased solute leakage has been observed from seeds (1,3), leaf tissue (6,13,16,17,21) and fruit (10,18) upon exposure to low temperatures. Divalent cations,

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especially calcium, have been shown effective in reducing or inhibiting leakage induced by chilling (3). Monovalent cations, such as potassium, have been ineffective in reducing ethephon-induced membrane leakage. Ethylenediaminetetracetate (EDTA) has been used to induce membrane leakage, and equal amounts of calcium were found to reverse leakage (20).

Pregerminated seeds are utilized in fluid-drilling, a seeding technique which results in faster and more uniform seedling emergence (5,19). Recent work has shown low temperature storage of the pregerminated seed to be useful in the event of delayed planting (8), however, with definite limits. The objectives of this research were to assess the effects of low temperature exposure on membrane integrity, using solute leakage as an indicator.

Materials and Methods

Capsicum annuum L. cv. Calwonder pepper seeds were germinated for 5 days in aerated water columns at room temperature. Seeds were removed from the columns and stored at 0°C or 5°C for 4 days in moist cheesecloth. Fresh (unstored) pregerminated seed was used as a control. Five replicates of 50 seeds per treatment were placed in 50 ml Erlenmeyer flasks containing 15 ml of distilled water. Flasks were incubated in a 25°C water bath under constant agitation. A sample from each flask was measured for absorbance at 262 nm in a Gilford UV spectrophotometer after 3.5, 6.5 and 9.5 hours. To assess the effect of calcium, potassium and EDTA on leakage, pregerminated seeds held at 0°C for 4 days were incubated in distilled water, .05 M $CaCl_2$, .05 M KCl or .005 M EDTA for 3 hours. The solutions were then decanted and replaced with distilled water. Absorbance at 262 nm was measured 3 and 7.5 hours

later. The area under the curve of the absorbance values from the initial time to the final time for each replicate was calculated. Values were analyzed using standard analysis of variance techniques and LSD was used for mean separation.

Results

There was no difference in solute leakage between seeds stored at 5°C for 4 days and fresh (unstored) pregerminated seeds. Levels of leakage from these seeds remained fairly constant throughout the sampling period. Leakage from seeds held at 0°C for 4 days, however, was not only initially higher than the other treatments, but increased in a linear fashion over the same sampling period (Fig. 1).

Incubation of chilled seeds in .05 M $CaCl_2$ reduced the level of leakage to that of fresh unchilled seeds. Leakage was not reduced by .05 M KCl. Incubation in .005 M EDTA significantly increased solute leakage from chilled seeds (Table 1).

Table 1. Absorbance of incubating solutions after treatment of chilled pregerminated pepper seed with water, $CaCl_2$, KCl and EDTA. Fresh pregerminated seed are included as a control. Delta absorbance is a comparison of all treatments to the chilled seeds incubated in water. Absorbance values at 3 and 7.5 hours were analyzed for statistical significance.

	3 hours		7.5 hours	
Treatment	A262 nm	Δ A	A262 nm	Δ A
Chilled/H_2O	.082		.125	
Chilled/$CaCl_2$	.075	-.007	.092	-.033
Chilled/KCl	.099	+.017	.143	+.018
Chilled/EDTA	.123	+.041	.186	+.061
Fresh	.058	-.024	.083	-.042
$LSD_{.05}$	.023		.019	

Fig. 1. Solute leakage, measured as absorbance at 262 nm, from pregerminated pepper seeds stored at 0°C or 5°C for 4 days, or not stored. Measurements were taken over a 9.5 hour period. The fresh (unstored) and 5°C treatments are not significantly different.

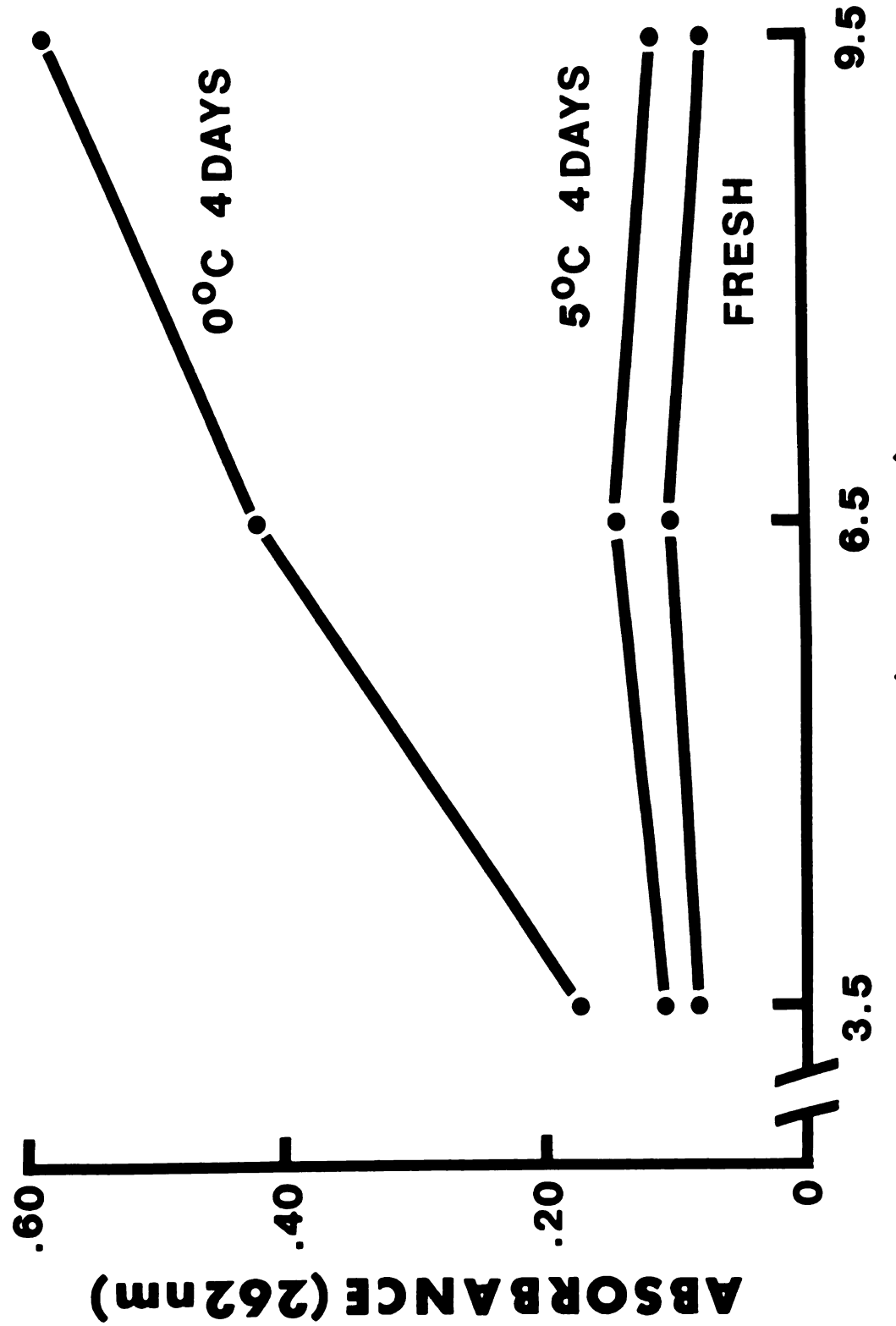
ABSORBANCE (262nm)
.60
.40
.20
0
3.5
6.5
9.5
TIME(HOURS)
0°C 4DAYS
5°C 4DAYS
FRESH

Fig. 1

Discussion

The chilling sensitivity of pepper seeds and fruit has already been established. Imbibing pepper seeds at 0°C or 5°C results in collapse and necrosis of tissue behind the root cap (7). Arrhenius plots of solute leakage from pepper fruits have shown a dramatic increase in slope in the range of 7 to 10°C, implying that a membrane disruption has occurred in that temperature range (18). Pregerminated pepper seed, however, did not exhibit increased solute leakage when exposed to 5°C for 4 days. Other researchers have shown imbibition to be a particularly chilling-sensitive period (1,2,14). Hydration of dry seed at low temperatures results in improper reorganization of membranes, leading to solute leakage and other disfunctions. By pregerminating the seed before exposure to low temperature, the problem of membrane reorganization is avoided. The membrane phospholipids, however, due to their degree of saturation, are still susceptible to low temperature. Four days exposure to 0°C is sufficient to induce a membrane phase change, resulting in increased solute leakage.

The effectiveness of calcium in reducing solute leakage has been attributed to its ability to decrease pore size (4) and to restabilize cell surface charge (3). Other divalent cations, and several monovalent cations have not been as effective in reducing leakage (15,20). This was substantiated by the ineffectiveness of KCl in reducing leakage from chilled, pregerminated pepper seeds. EDTA has been used to induce betacyanin leakage from beet root tissue (20). It was found that the compound removed 69 to 76% of the total calcium in the beet tissue, and that subsequent treatment with $CaCl_2$ reversed leakage. Incubation of chilled pregerminated pepper seeds in .005 M EDTA resulted in the

greatest amount of leakage.

Levels of solute leakage have been negatively correlated with emergence of peas and French beans, and measurement of seed leakage has been suggested as a standard test for seed vigor (12). The results of this experiment correlate with other work on pregerminated pepper seed in which 5°C for 4 days did not reduce seedling emergence, but 0°C for 4 days did (8).

Increased solute leakage results from exposure to 0°C, reinforcing the fact that peppers are chilling-sensitive. $CaCl_2$ was effective in reducing leakage from chilled seeds, but KCl was ineffective. EDTA induced the highest amount of leakage from chilled seeds.

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APPENDICES

APPENDIX A1

CHILLING SENSITIVITY OF VARIOUS RADICLE LENGTHS OF PREGERMINATED PEPPER SEEDS

Chilling Sensitivity of Various Radicle Lengths of Pregerminated Pepper Seeds

Several researchers have shown the chilling sensitivity of seed from tropical or subtropical origin. Exposure of pepper seed to 0°C or 5°C during imbibition results in collapse and necrosis of tissue behind the root cap (Harrington and Kihara, 1960). Christiansen (1963) observed that low temperature can result in abnormal root tips which fail to stain with tetrazolium chloride, or in 'nub root', a term Wiles and Downs (1977) used to describe the short, blunt root tip which results from chilling some seeds.

Observation of pregerminated pepper seeds exposed to 0°C revealed what appeared to be differences in chilling sensitivity among radicles of various lengths. The objectives of this experiment were to determine whether different radicle lengths do vary in their chilling sensitivity and the effect of chilling on their emergence.

Materials and Methods

Capsicum annuum L. cv. Staddon's Select pepper seeds were germinated for 4, 5, 6 or 7 days in aerated water columns at room temperature. The average length of 10 radicles was 0 mm (radicles yet to emerge), 3.20 mm, 4.25 mm and 8.45 mm, respectively. Seeds were then stored at 0°C for 2 or 4 days in moist cheesecloth bags.

The emergence assay utilized 8 replicates of 25 seeds per treatment planted at a 1.5 cm depth in horticultural grade vermiculite in a completely randomized design. Four replicates were placed in a Percival

environmental growth chamber with a 14 hour 25°C day and a 10 hour 20°C night. The other 4 were placed in a similar growth chamber with a 14 hour 15°C day and a 10 hour 10°C night. Flats were watered as necessary, and daily emergence counts were taken. At the culmination of the experiment, time to 50% emergence (T_{50}), an indicator of seedling vigor (Orchard, 1977), and percent emergence were calculated for each treatment. Data were analyzed using standard analysis of variance techniques and LSD was used for mean separation.

Results

After 2 days of exposure to 0°C, number of days of germination and time to 50% emergence were negatively correlated (Figure 1A). The longer the radicle, the more quickly the seedlings emerged. However, 4 days at 0°C had the opposite effect on the speed of emergence, ie. at 25°/20°C, the longer the radicle, the greater the T_{50}, or the slower the emergence (Figure 1B). The T_{50} data from the 15°/10°C growth chamber was not highly correlated with radicle length.

Two days exposure to 0°C did not produce any significant differences in percent emergence among treatments, except for the 51% stand seen with 7 days of germination in the 15°/10°C chamber (Table 1). This was

Table 1. Percent emergence of Staddon's Select pepper seeds germinated for 4 to 7 days, exposed to 0°C for 2 days, then grown at 25°/20°C or 15°/10°C. ($LSD_{.05}$ = 12.91)

No. of days germinated	25°/20°C	15°/10°C
4	93.0	87.0
5	88.0	84.0
6	86.0	93.0
7	89.0	51.0

Figure 1. Effect of exposing pepper seeds germinated for 4 to 7 days to 0°C for 2 days (A) and 4 days (B) on time to 50% emergence. (A) represents the average T_{50} values from the 25°/20°C and 15°/10°C growth chambers. (B) represents T_{50} data from the 25°/20°C chamber only.

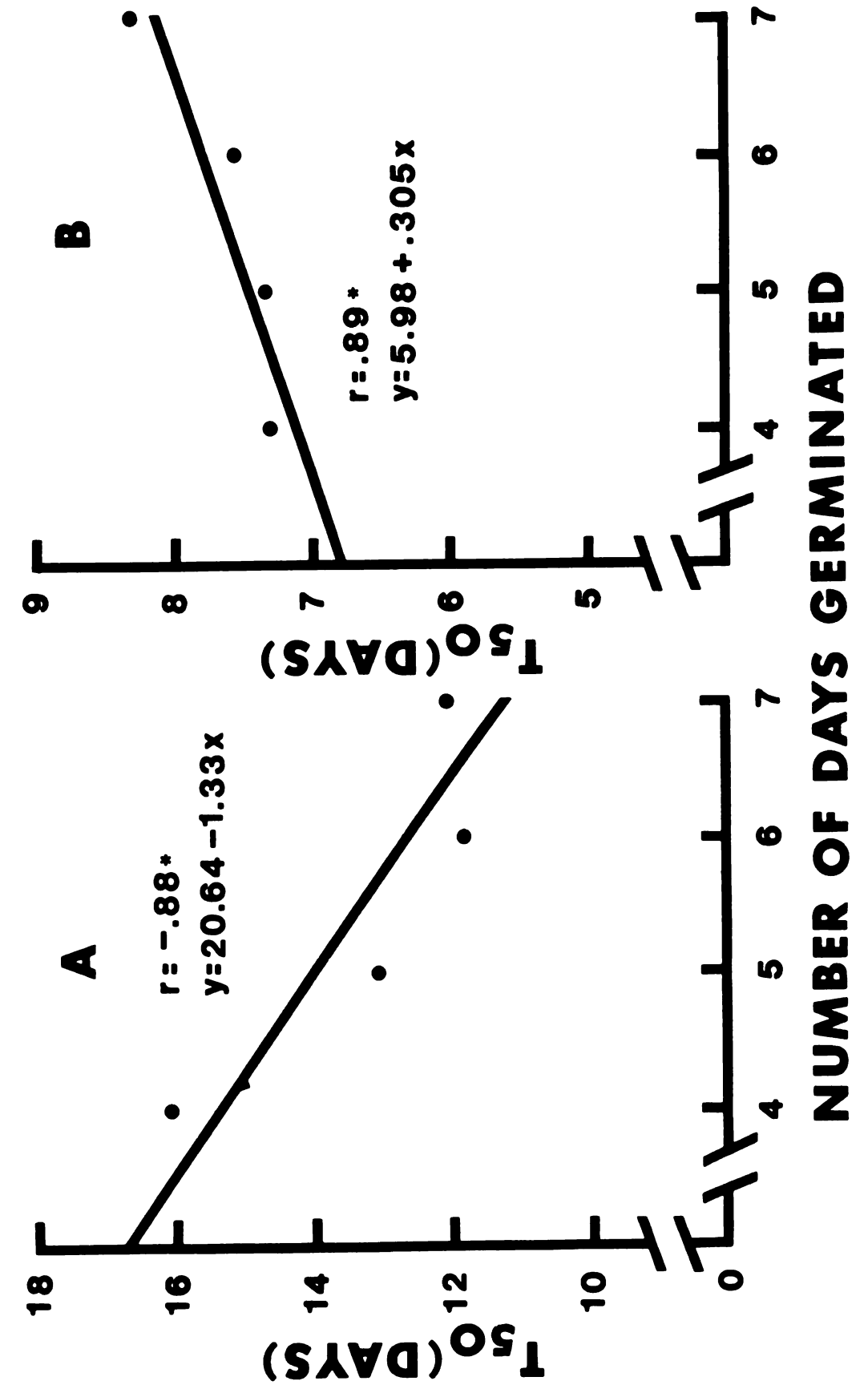
A
r= -.88*
y=20.64 -1.33x
T_{50} (DAYS)
18
16
14
12
10
0
4
5
6
7
NUMBER OF DAYS GERMINATED
B
r=.89*
y=5.98 +.305x
T_{50} (DAYS)
9
8
7
6
5
4
5
6
7

Figure 1

Figure 2. Effect of exposing pepper seeds germinated for 4 to 7 days to 0°C for 4 days on percent emergence. Values represented are the average of values from the 25°/20°C growth chamber and the 15°/10°C growth chamber.

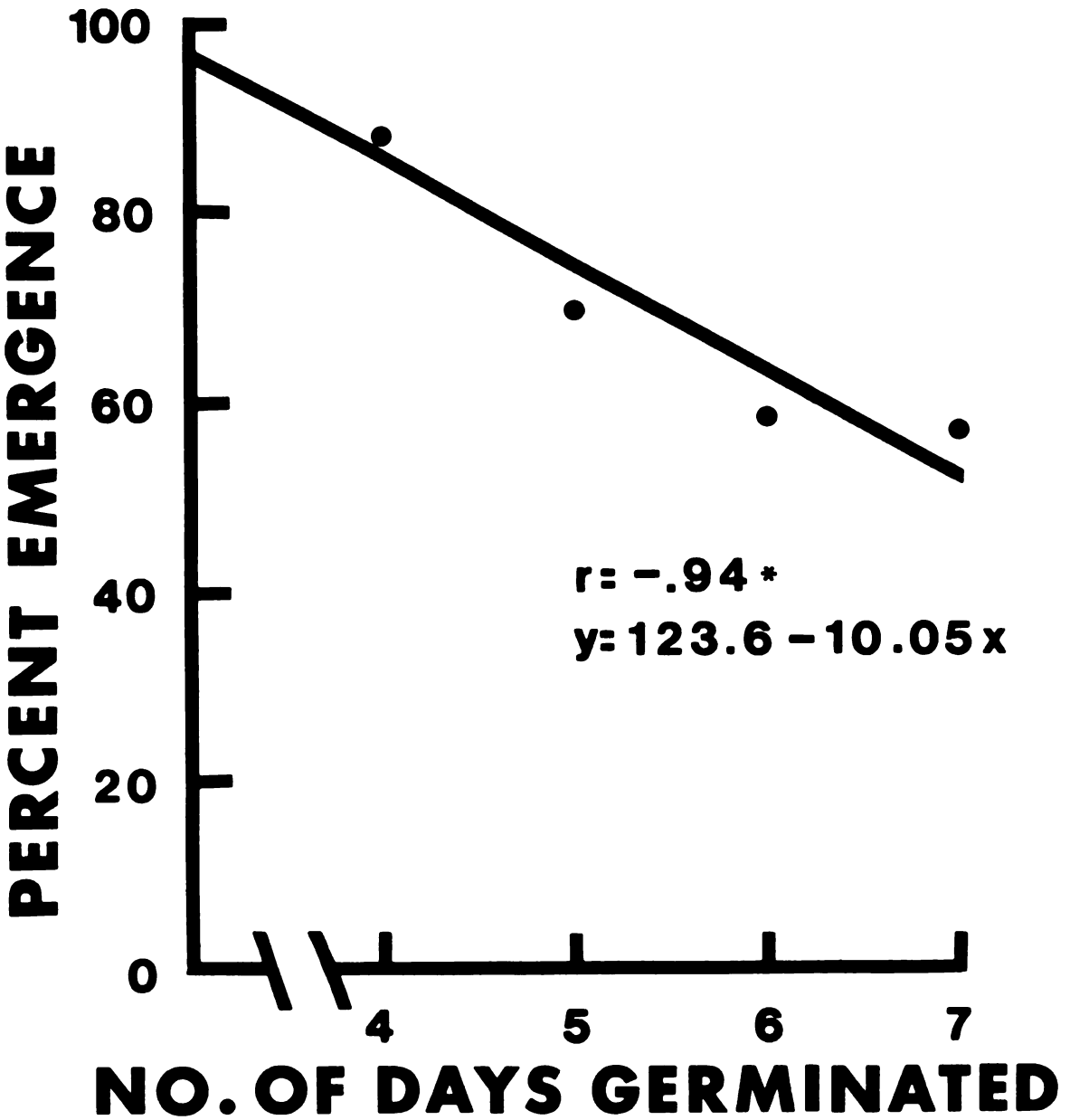
100
80
60
40
20
0
PERCENT EMERGENCE
r= -.94*
y= 123.6 - 10.05x
4
5
6
7
NO. OF DAYS GERMINATED

Figure 2

considerably less than the percentage of any other treatment. An additional 2 days at 0°C, for a total of 4 days, however, resulted in number of days of germination and percent emergence having a high negative correlation (Figure 2) in both growth chambers.

Discussion

Early exposure to low temperature can adversely affect subsequent performance of pregerminated pepper seeds. Similar effects have been seen by Pollack and Toole (1966) with lima beans and with cotton seed by Christiansen (1964), and Christiansen and Thomas (1969) after exposure to low temperature during imbibition. Storing pregerminated pepper seeds for 4 days at 0°C resulted in slower emergence and a reduced stand. Exposure to 0°C for 2 days did not appear to cause injury. It has been noted in other work by this researcher that severe chilling compounded with low growing temperatures can have such an adverse effect on the emergence of pepper seedlings that the effect of other variables is masked. This might explain the poor correlation of T_{50} values for seeds held at 0°C for 4 days with radicle length at 15°/10°C.

The greater the number of days pepper seeds have been germinated, the longer the radicle. When uninjured, the more advanced seeds with the longer radicles are the first ones up. The longer the radicle, however, the greater the amount of tissue susceptible to chilling injury, thus the negative correlation seen between radicle length and speed and percent of emergence after 4 days at 0°C. In view of this data, it appears that a short radicle is desirable, not only to minimize damage during planting, but to minimize the adverse effects of chilling during storage or in the field.

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APPENDIX A2

THE EMERGENCE OF CHILLED AND UNCHILLED PREGERMINATED SEED AND DRY PEPPER SEED IN FIELD SOIL AND GREENHOUSE SOIL MIX

The Emergence of Chilled and Unchilled Pregerminated Seed and Dry Pepper Seed in Field Soil and Greenhouse Soil Mix

Poor seedling emergence can be a problem in field planting situations, and is often a result of soil compaction, crusting, chilling injury or various combinations of these. The objectives of this experiment were to assess the effects of soil type and chilling and the interaction of the two on the speed of emergence and percent emergence of pregerminated and dry pepper seed.

Materials and Methods

Capsicum annuum L. cv. Hungarian Wax pepper seeds were germinated for 5 days in aerated water columns at room temperature. Pregerminated seeds were removed from the columns and stored at 0°C for 2 days, wrapped in moist cheesecloth. Fresh (unstored) pregerminated seed and dry seed were used for controls. Three replicates of 25 seeds per treatment were planted at a 1.5 cm depth in a standard greenhouse potting mix of peat and sand, and 3 replicates were planted at the same depth in Miami silt loam from the Horticultural Research Center, East Lansing. Flats were placed in a Scherer environmental growth chamber with a 14 hour 15°C day and 10 hour 12°C night, and watered as necessary.

Daily emergence counts were taken, and at the termination of the experiment, time to 50% emergence, an indicator of seedling vigor (Orchard, 1977) and percent emergence were calculated for each treatment.

Data were analyzed using standard analysis of variance techniques and LSD was used for mean separation.

Results

The time to 50% emergence (T_{50}) of fresh pregerminated seed was lower than that of chilled or dry seed from both field and greenhouse soil (Table 1). With all treatments, field soil reduced the speed of

Table 1. The effect of chilling and soil type on the time to 50% emergence (T_{50}) of pepper seedlings. ($LSD_{.05}$ = 1.78)

	T_{50} (mean of 3 reps)	
Treatment	Greenhouse mix	Field soil
Fresh	10.75 days	17.74 days
0°C for 2 days	17.38	19.67
Dry	16.65	21.44

emergence when compared with emergence from the greenhouse soil. The field soil had become compacted soon after initiation of the experiment, while the greenhouse mix remained friable. Field soil increased the T_{50} by as much as 7 days.

The effect of soil type on percent emergence is quite clear. Although there was no significant difference in percent emergence of chilled and unchilled pregerminated seed and dry seed when grown in greenhouse soil, large differences can be seen with field soil (Figure 1). Fresh pregerminated seed suffered the least reduction in percent emergence. Field soil reduced the dry seed stand from 84 to 37%, and the chilled pregerminated seed stand from 67 to 4%.

Figure 1. The effect of chilling and soil type on the percent emergence of pepper seedlings. ($LSD_{.05}$ = 24.71)

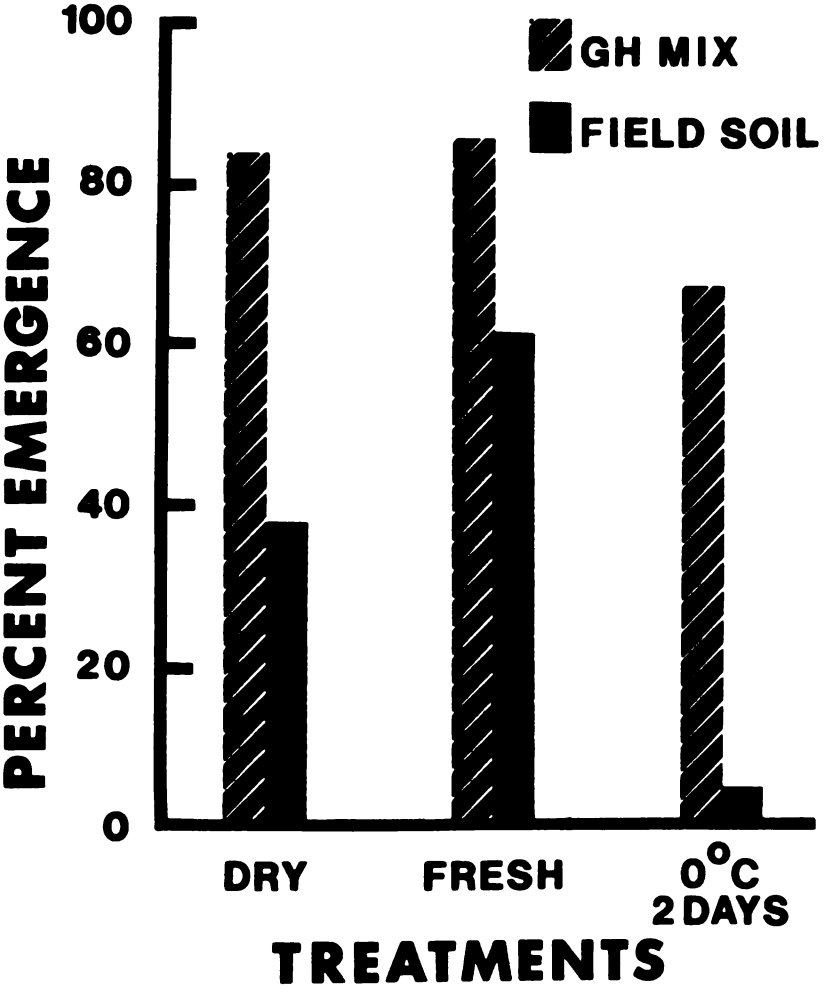
100
80
60
40
20
0
PERCENT EMERGENCE
GH MIX
FIELD SOIL
DRY
FRESH
0°C
2 DAYS
TREATMENTS

Figure 1

Discussion

Based on the results of this experiment, it appears that pregerminated seed is better able to emerge through field soil than dry seed, provided it has not been chilled. Storage of pregerminated seed at 0°C for 2 days, in combination with planting in field soil, resulted in a larger reduction in percent emergence than any other combination of conditions. The adverse effect of 2 days storage at 0°C on emergence has been seen in other work with pregerminated pepper seed, especially when raised at the suboptimal temperatures of 15°/12°C (Irwin and Price, 1979). Imposing an additional stress in the form of heavy field soil accentuates the weakened condition of the chilled seed, resulting in the poor stand. Pepper seedling emergence has been observed to be adversely affected by soil compaction (Fawusi, 1978). Fritz (1965) recognized the correlation between vigor and the ability of seedlings to emerge through a layer of paper and sand, and standardized such a test for use with small grains. The emergence force exerted by seedlings of small-seeded legumes has been shown by Williams (1956) to be a direct function of seedling vigor. Therefore, the reduction in vigor resulting from storage at 0°C was reflected in the reduced ability of the pregerminated pepper seed to penetrate the compacted field soil. When unchilled, however, pregerminated pepper seed emerges considerably faster and produces a better stand in field soil than does dry seed.

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APPENDIX A3

DETERMINATION OF THE OPTIMUM TETRAZOLIUM CHLORIDE (TTC) CONCENTRATION AND DURATION OF THE TTC SOAK FOR TETRAZOLIUM TESTING OF PREGERMINATED PEPPER SEED

Determination of the Optimum Tetrazolium Chloride (TTC) Concentration and Duration of TTC Soak for Tetrazolium Testing of Pregerminated Pepper Seed

Tetrazolium testing has been used in the U.S. since shortly after WWII, and is now a routine test for determining seed viability of many crops (Grabe, 1970). The test is a non-specific indicator of dehydrogenase activity. In contact with actively respiring tissue, the water soluble tetrazolium salt is reduced to a water insoluble red substance, formazan. Non-respiring tissue, indicative of non-viability, does not stain. Tetrazolium testing normally is performed on non-germinated seed, which is then examined individually, and termed germinable or non-germinable, depending on the location and extent of unstained tissue. For pregerminated seed, however, a modification of the technique used by Kittock and Law (1968) with germinating wheat was made. The objective of this experiment was to determine the optimum concentration of the tetrazolium chloride solution, and the shortest length of time the pregerminated seeds could be soaked in the solution to get good staining results.

Materials and Methods

Capsicum annuum L. cv. Hungarian Wax seeds were germinated for 5 days in aerated glass water columns at room temperature. Pregerminated seeds were removed from the columns and placed in .1%, .5% or 1% solutions of 2,3,5-triphenyl tetrazolium chloride in .01 M phosphate buffer of pH 7.0. Intact seeds were soaked in the TTC solutions for

6, 12, 18 or 24 hours. Four replicates of 10 seeds per treatment were placed in test tubes, covered with 4 ml of solution, and maintained in darkness in a randomized block design. At the end of the soaking period, the TTC solution was decanted and discarded, seeds were rinsed with distilled water, and 4 ml of 2-methoxyethanol were added to each tube for extraction of the formazan. After 4 hours, the 2-methoxyethanol was decanted, and each sample measured for absorbance at 480 nm in a Bausch and Lomb Spectronic 20. Data were analyzed using standard analysis of variance techniques and LSD was used for mean separation.

Results

Use of a .1% tetrazolium chloride solution did not result in sufficient coloration for routine use with pregerminated pepper seed (Table 1). Although a 24 hour soak in a .1% solution did produce an acceptable absorbance of 0.70, this concentration would not be high enough were chilled seeds soaked in it, since chilling reduces the amount of stainable tissue. A .5% solution, however, produced

Table 1. The effect of TTC concentration and number of hours seeds were soaked in the TTC solution on absorbance (480 nm) of formazan in 2-methoxyethanol extracts. ($LSD_{.01}$ = 0.27).

	TTC concentration (%)		
Hours in solution	.1%	.5%	1%
6	0.00	0.26	0.44
12	0.16	0.78	1.08
18	0.30	0.93	1.20
24	0.70	1.28	1.38

satisfactory staining of the pregerminated seed. Results of the .5% concentration and 1% concentration were not significantly different at

the 1% level except when the seeds were soaked for 12 hours. Differences between absorbance values obtained with .1% solution and .5% solution were, in general, considerably larger than between the .5% and the 1% solutions. Soaking seeds for 12 hours or more in TTC produced acceptable results. Six hours in TTC were not long enough for detectable coloration to occur at all concentrations. The interaction between percent concentration and length of soaking period was significant.

Discussion

The results indicate that the reduction of TTC is a time/concentration response. The longer the seeds are soaked, or the higher the concentration of the TTC solution, the greater the formazan development. Although the recommended time in solution (Grabe, 1970) for any crop on which TTC testing is routinely used does not exceed 6 to 8 hours (some of the grasses), it is obvious that this is not a sufficient length of time for use with pregerminated seeds. Recommended preparation for TTC testing usually involves dissecting, piercing or removing the seed coat to facilitate quicker staining. None of these were done with the pregerminated seed, however, and the presence of the seed coat may slow the staining reaction. Sato (1962) working with embryos of Phaseolus vulgaris found that the rate of TTC reduction and the number of embryos in a tube of TTC solution were directly proportional when up to 12 embryos per tube were used. Above 12 embryos, the linear relationship no longer existed. It may be that routine testing involves larger numbers of seeds than what were used in this experiment (10 seeds/tube), therefore a shorter soaking period

is necessary. Other factors enter into the rate of TTC reduction - eg. temperature (Grabe, 1970) and pH (Sato, 1962), however, recommendations regarding these two were followed in this work.

Based on the obtained results, use of a .5% solution or greater for at least 12 hours is necessary for detectable and measurable levels of formazan production.

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APPENDIX A4

THE EFFECT OF 'PRIMING' WITH POLYETHYLENE GLYCOL ON GERMINATION OF PEPPER SEEDS

The Effect of 'Priming' with Polyethylene Glycol on Germination of Pepper Seeds

Soaking seeds in a concentrated solution of polyethylene glycol (PEG), termed 'priming', prior to planting has been shown by several researchers to be beneficial to germination. Heydecker, Higgins and Gulliver (1974) reported quicker and more uniform seedling emergence when seeds were 'primed' with a PEG solution. Heydecker and Hendy (1975) found that percent germination was usually unaffected by PEG pretreatment. Celery is a crop characterized by non-uniform germination, but Salter and Darby (1976) were able to greatly improve the uniformity by 'priming' the seed. The PEG solution allows enough imbibition to initiate germination processes, but not enough for radicle emergence to occur. The objective of this experiment was to determine the effect of PEG 'priming' on the germination and emergence of 4 cultivars of pepper seeds.

Materials and Methods

Four replicates of 50 seeds of Capsicum annuum L. cvs. Atlas, Sonnette, Shepard and Yolo Wonder L were placed in 9.0 cm Petri dishes containing 2 pieces of Whatman #2 filter paper. The filter paper was moistened with 6 ml of a solution of Carbowax[1] (polyethylene glycol) 6000 with an osmotic potential of -11.5 bars. Seeds were 'primed' for

[1]Union Carbide Corporation

4, 8, or 12 days, and dry seed was used as a control. During the 'priming', the seeds were maintained at room temperature. At the end of the 'priming' period, 25 seeds from each replicate were planted in a completely randomized design at a 1.5 cm depth in horticultural grade vermiculite. Flats were placed under Gro-Lux lights and watered as necessary. Daily emergence counts were taken until the number of emerged seedlings had not changed for 3 days. Percent emergence and time to 50% emergence, an indicator of seedling vigor (Orchard, 1977), were calculated for each treatment within each cultivar. Data were analyzed using standard analysis of variance techniques and LSD was used for mean separation.

Results

PEG pretreatment reduced the T_{50} emergence for all 4 pepper cultivars (Figure 1). The decrease was linear until 8 days of soaking, with no additional decrease at 12 days. Percent emergence exhibited very little change with any of the 'priming' treatments (Table 1). There was no significant interaction between cultivars and the PEG treatments.

Table 1. Effect of number of days in a PEG 6000 solution with an osmotic potential of -11.5 bars on percent emergence of 4 cultivars of pepper seeds. ($LSD_{.01}$ = 12.12)

	Number of days in PEG solution			
Cultivar	0 (dry)	4	8	12
Atlas	77	74	76	70
Sonnette	91	95	96	97
Shepard	84	89	89	91
Yolo Wonder L	86	78	85	83

Figure 1. Effect of number of days in a PEG 6000 solution of -11.5 bars on the time to 50% (T_{50}) emergence of 4 cultivars of pepper seeds. ($LSD_{.01}$ = 1.09)

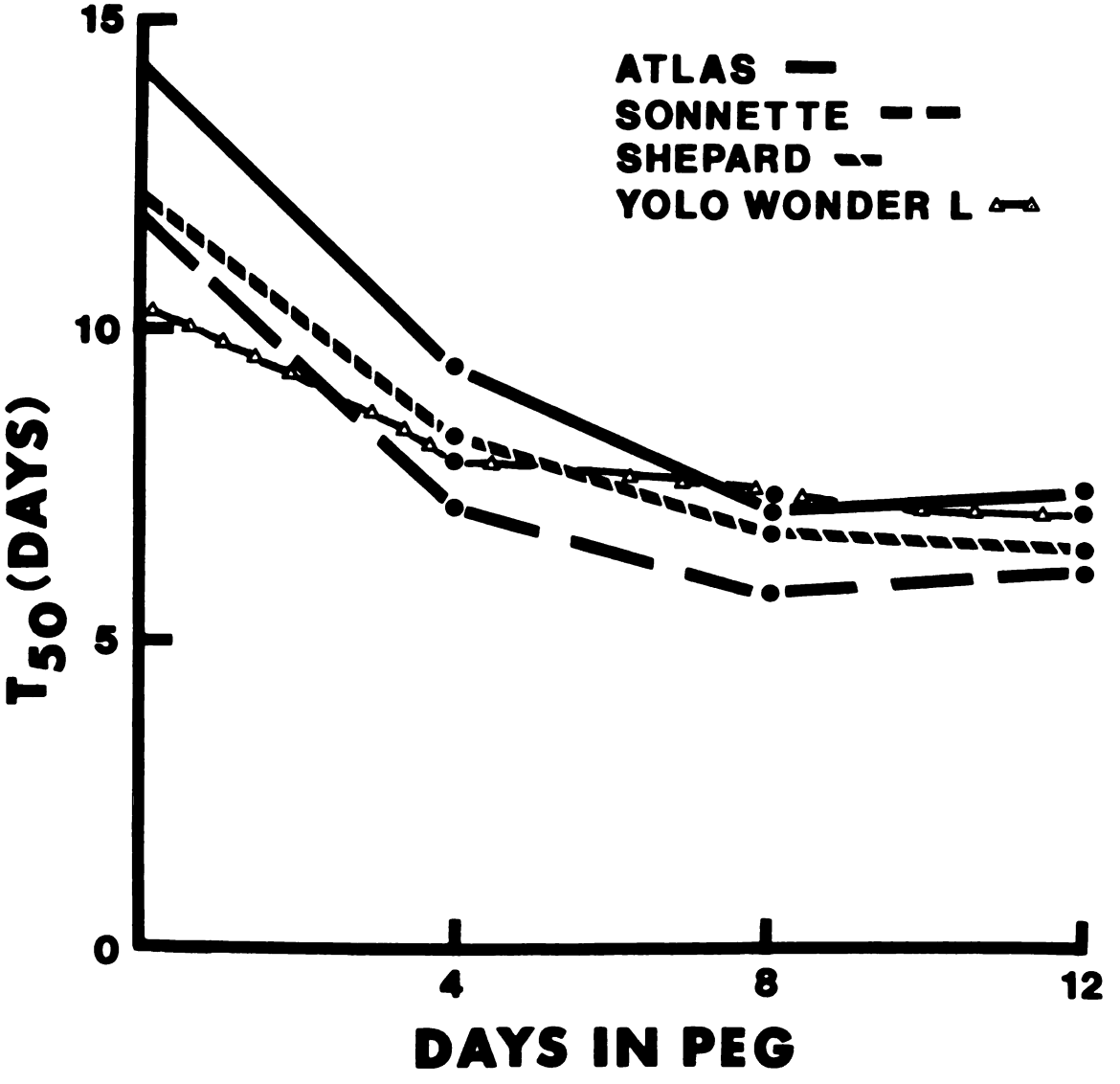
15
10
5
0
T_{50} (DAYS)
ATLAS
SONNETTE
SHEPARD
YOLO WONDER L
4
8
12
DAYS IN PEG

Figure 1

Discussion

The results of this experiment substantiate the benefit of PEG 'priming' proclaimed by Heydecker and coworkers (1974,1975) and Salter and Darby (1976). In all 4 cultivars tested, the T_{50} emergence was reduced by nearly 50% with an 8 day soak in a PEG 6000 solution. There appears to be no added advantage to an additional 4 days of 'priming', however, as T_{50} values differed very little from those obtained with an 8 day soak. Salter and Darby (1976), working with celery seed, found germination to be very rapid after a 14 day treatment with a PEG solution of -12.5 bars. The results of shorter treatment periods were not discussed. Heydecker, Higgins and Gulliver (1974) also recommend 'priming' periods of 2 to 3 weeks for onion, carrot, beet and celery seed. Though the osmotic potential of the PEG solutions used by these researchers was in the range of -11.5 bars (-10 to -12.5), the temperature used for 'priming' by Salter and Darby and Heydecker et. al. was different. Both groups worked with 15°C, while the 'priming' of pepper seeds was carried out at room temperature (20-25°C). The higher temperature could be responsible for those processes initiated within the seed proceeding at a faster rate than at 15°C. This could result in the maximum benefit to be obtained from 'priming' showing up in 8 days at 25°C versus 14 or 21 days at 15°C. Another factor could be simply the difference between pepper seeds and seeds of onion, beet, carrot and celery.

'Priming' with a PEG solution of -11.5 bars is beneficial to germination of pepper seeds. An 8 day soak was most successful in reducing the number of days to 50% emergence in all 4 cultivars tested, but 'priming' had no effect on percent emergence of any cultivar.

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