GERMINATION OF Capsicum annuum L. AT
LOW TEMPERATURE

BY

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The cause of slow and nonuniform germination of pepper (Capsicum annuum L.) seeds at low temperature was investigated. There was no evidence of a leachable or extractable germination inhibitor being activated or formed due to low temperature exposure. Auxin and kinetin applications did not effect germination rate. However, gibberellic acid (GA) treatments effectively increased germination rates at 15⁰ and 25⁰C. Gibberellic acid₄₊₇ was more effective than GA₃. Gibberellic acid stimulated germination rate under osmotic stress and the presence of a GA synthesis inhibitor reduced germination rate.

High oxygen (O₂) concentrations stimulated pepper seed germination rates at 25⁰C but inhibited germination rates at 15⁰. Gibberellic acid applications could not overcome the inhibitory effect of 100% O₂ at 15⁰. Seed respiratory activity was greater at 25⁰ in 100% O₂ than in air, but the additional O₂ did not alter respiratory pathways. At 15⁰, total respiration, respira-

tory activities in the cytochrome and alternate pathways were unaltered by additional O_2 .

The endosperm surrounding the embryo appeared to be a mechanical barrier to radicle emergence. The amount of resistance the endosperm imposed on radicle protrusion was measured with the Instron Universal Testing Machine. Endosperm strength decreased as imbibition time increased and the rate of reduction was affected by imbibition temperature. Gibberellic acid treatments and 100% O_2 at 25° increased the rate at which the endosperm strength decreased while 100% O_2 at 15° inhibited the rate at which the endosperm strength decreased.

The endosperm in front of the radicle was visually protruding one day before radicle emergence regardless of imbibition temperature or seed treatment. This protrusion was correlated to a loss of cellular integrity and endosperm thickness in front of the radicle. Gibberellic acid treatments stimulated endosperm breakdown.

Pepper seed cell wall degrading enzyme activity increased with increased imbibition time, with temperature, and GA treatment. Pepper seed enzyme extracts preferentially degraded a galactomannan substrate but did not degrade a carboxymethyl-cellulose substrate indicating that manno-polysaccharides may be components of pepper seed endosperms.

INTRODUCTION

Florida's third most valuable vegetable crop is sweet pepper (Capsicum annuum L.). It accounts for 6.5% of the total vegetable crop value for this state, the leading pepper producing state in the United States. Florida harvested 17,300 acres in 1979 which was approximately one-third of the total United States acreage of peppers. The total value of the pepper crop in the United States in 1979 was 109 million dollars and Florida production accounted for 51 million dollars of that amount.

Germination of pepper seed at low temperatures is very erratic and nonuniform and emergence may be spread out for several weeks when the seeds are sown in cool soils. This problem is not only encountered during direct field seeding in cool soils, but also during seeding for transplants in cold frames and inadequately heated greenhouses during Florida's winter months. This erratic and nonuniform emergence often leads to nonuniform transplants, poor crop stands, and may necessitate crop replanting. Often, reduced yields and delayed harvest (losing early high market prices) can be caused by slow stand establishment.

Emergence data from 80 cultivars of C. annuum suggested that altered germination rates at low temperatures could be controlled genetically (Gerson and Honma, 1978). However, they reported the best mean emergence rate from the 80 cultivars tested at 16°C was 20.6 days. This long period of exposure in a soil environment could lead to pathogenic attack and death of the seed.

The studies contained herein explore the cause or contributing causes of slow and nonuniform germination of C. annuum at low temperature. Investigations for inherent germination inhibitors and applications of growth promoters were made to determine their effects, if any, on pepper seed germination at low temperature. Respiratory control of germination at low temperature and the effects of the seed coat and endosperm on germination were investigated. Additionally, the manner in which the radicle emerges through the endosperm was determined in relation to germination temperature.

CHAPTER I LITERATURE REVIEW

Germination of seeds at low temperature has been correlated with erratic and nonuniform germination, inhibition of germination, abnormal seedling development and embryo death (Gerson and Honma, 1978; Christiansen, 1968; Pollock and Toole, 1966). The response of seeds to germination at low temperatures has been grouped into three general categories: 1) seeds which will germinate at temperatures near freezing 2) seeds which fail to germinate at low temperatures but if moved to a more favorable temperature will germinate normally, and 3) seeds which when imbibed at low temperatures showed injury symptoms when subsequently grown at warmer temperatures. This injury is known as chilling injury (Simon, 1979).

Mustard seed germinated within a day at temperatures between 15 and 25°C (Simon et al., 1976). As the temperature was lowered, seed germination became slower, although mustard seeds were able to germinate at temperatures of 3 to 4°. Thompson (1973) demonstrated that seeds from various populations of 'Grand Rapids' lettuce and Agrostemma githago L., a weed of cereals, could germinate slowly at temperatures near 0°. Mustard, lettuce and Agrostemma githago all germinated at low temperatures without injury to the subsequent seedlings.

Watermelon and tomato seeds do not germinate at constant low temperatures. Sachs (1977) found that no germination of

watermelon seeds occurred at constant temperatures below 15°C while the optimal germination temperature was 30°C. However, when watermelon seeds were imbibed at suboptimal temperatures, as low as 0°C for 10 days, no effect on subsequent germination at optimal temperatures was found. Tomato seed exposed to 8°C for 24 hours at the start of imbibition or just before radicle emergence did not affect the final percent germination (Busnell and Gray, 1976). The cold treatments, however, caused the time to 50% germination to be increased but reduced the time to 95% germination.

In an earlier report, Goodall and Bolas (1942) chilled imbibed tomato seeds at temperatures of 0 to 11°C for periods of 10 to 20 days, then germinated them with untreated seeds at more favorable temperatures. The plants grown from the chilled seeds produced more fruit and had a concentrated yield at the beginning of the cropping season.

Pepper seeds germinate slowly at less than optimal temperatures. Cochran (1935) reported that no peppers germinated after 45 days in a greenhouse with a temperature range between 4 and 10°C. However, with each 6°C increase in temperature range, the speed of germination increased until germination was obtained in five to six days in a greenhouse with a temperature range between 32 and 38°C. Heydecker (1974) reported that when imbibed pepper seeds were held for three weeks at 3, 7 and 10°C, they failed to germinate. But, when they were transferred to 30°C they germinated faster than untreated seeds placed at 30°C from the outset, with no apparent injury.

This was contrary to the work of Harrington and Kihara (1960). They reported chilling injury symptoms on pepper seedlings imbibed at temperatures of 0 to 10⁰ for one-half to four weeks then subsequently germinated at 25⁰. The greatest percentage of chilling injury, 33%, occurred at the 10⁰ imbibitional temperature. Chilling injury symptoms were described as the collapse of cells 1 to 2 mm behind the root tip, necrosis of this area and decaying of the seedlings.

Imbibition and germination at low temperatures may affect subsequent plant growth and development. Highkin and Lang (1966) reported that pea plants obtained from seeds germinated at 3⁰C compared to 27⁰, grew slower, had fewer flowers, pods and seeds and had shorter final heights. Similarly, cottonseed imbibed at low temperatures, produced plants with reduced heights, delayed fruiting, and adversely affected fiber quality (Christiansen and Thomas, 1969). The degree of damage was in direct proportion to the amount of time the seeds were exposed to the low temperature. Imbibition of soybeans at 5⁰ for 12, 24, or 36 hours before planting and germination in a greenhouse at 20 to 30⁰ reduced survival, dry matter accumulation and seedling heights after two weeks compared to soybeans imbibed at 25⁰ (Obendorf and Hobbs, 1970). In a later investigation, Hobbs and Obendorf (1972) reported that surviving plants from soybeans imbibed at 5⁰ for 36 hours were shorter, produced fewer fruits and seeds compared to plants from soybeans imbibed at 25⁰ for 12 hours.

The sensitivity to low temperature during germination appears to occur early during seed imbibition. Pollock and Toole (1966) noted that lima bean seeds and excised embryonic axes had reduced survival rates and seedling size when imbibed at 5 or 15°C then allowed to grow at 25°C. However, if the seeds were initially imbibed at 25°C for two or six hours before low temperature exposure, seed and seedling injury was much less or completely avoided. Exposure of cottonseed to 5°C during imbibition, for as little as 30 minutes, resulted in a reduction in germination rate and an increase in seedling abnormalities (Christiansen, 1968). The sensitivity to low temperatures in cottonseed persisted for the first two to four hours of imbibition. If the seeds were initially imbibed for four hours at 31°C, the optimum temperature for germination, they were less sensitive to subsequent chilling temperatures. Likewise, Bramlage et al. (1978) determined that soybean embryos had reduced axis elongation if imbibed for 30 minutes at 14°C then placed at 28°C for three days. Additionally, injury resulted to soybean embryos which were imbibed at 2°C for as little as five minutes. Bramlage et al. (1978) proposed that chilling injury may occur during the initial hydration of the embryo and the time to injury depends on seed protection structures and the rate of imbibition.

Low Temperature Imbibition and Membrane Reorganization

Chilling injury due to low temperature imbibition of seeds has largely been attributed to the effect water and temperature has on the cellular membranes (Simon, 1974; Bramlage et al., 1978; Leopold, 1980). Using the fluid mosaic model of cellular membranes, Simon (1974) described the effect water content has on membrane architecture. In animal tissues using X-ray diffraction, it was noted that as water content of the membrane fell below 20%, the phospholipids in the cell membrane rearranged from a lamellar phase to a hexagonal phase. The hexagonal state would be more porous, permeable to solutes and would initially allow substances to rapidly diffuse out when cells were rehydrated. Lyons (1973) determined that a similar phase change occurred in plant mitochondrial membranes as temperatures were lowered. The phase change from the liquid-crystalline (lamellar) to the solid-gel (hexagonal) state occurred at 10 to 12°C. This correlated with the temperatures at which injury occurred in chilling sensitive tissues. Ilker et al. (1979) found that ultrastructural chilling symptoms, as examined from electron micrographs, of tomato cotyledons held at 5°C for two to 24 hours, exhibited themselves primarily as a progression of membrane deterioration. Mitochondrial and nuclear membranes became less definite and more disorganized in advance of the plasmalemma which appeared more resistant to chilling.

Lyons et al. (1964) compared several plant species that differed in sensitivity to chilling. Mitochondrial membranes

from chilling sensitive plants exhibited inflexibility when compared to chilling resistant plants. Membrane lipid components also differed between chilling sensitive and resistant species. Mitochondria of chilling resistant species contained a greater content of unsaturated fatty acids than did mitochondria from chilling sensitive species. In seeds, phospholipid composition and synthesis in cell membranes may be critical to low temperature sensitivity (Dogras et al., 1977). During imbibition of a chilling sensitive species, lima bean, more labelled ^{14}C -glycerol was incorporated into phosphatidylethanolamine and phosphatidylglycerol than occurred in broad beans and peas, chilling resistant species. The latter species incorporated a higher proportion of the label into phosphatidylcholine. Broad beans and peas had a greater unsaturated to saturated fatty acid ratio compared to lima beans. The authors speculated, due to the fact that phosphatidylcholine (P.C.) had a lower temperature requirement for phase change than phosphatidylethanolamine (P.E.), that membranes which contained greater amounts of P.C. than P.E. could have lower temperature phase changes and greater chilling resistance.

The ability of germinated pepper seed to withstand low storage temperatures was correlated with the degree of unsaturation in total fatty acids in dry seed (Sosa-Coronel and Motes, 1981). Pregerminated seed from pepper cultivars most sensitive to chilling injury during storage had a lower degree of fatty acid unsaturation in dry seed extracts compared to less sensitive cultivars.

Injury to seeds during low temperature imbibition has been attributed to the inability of cell membrane phospholipids to rearrange from the dry hexagonal state to the hydrated lamellar architecture, because they were gelled in a rigid molecular shape (Simon, 1974). This inability of cellular membranes to reorganize to the semipermeable condition results in the chilling injury demonstrated by the embryo.

Further support for this theory came in work which determined cellular leakage during imbibition. The majority of carbohydrate leakage was restricted to the first 18 hours of imbibition at temperatures of 22 to 30°C in pea seeds (Short and Lacy, 1976). However, at an imbibition temperature of 10°C, significant leakage occurred for 48 hours after the addition of water. Pollock and Toole (1966) noticed that lima bean axes imbibed at 5 or 15°C lost organic material directly and to a greater extent than occurred at 25°C. Bramlage et al. (1978) found that soybean embryos imbibed at 25°C had a short period of rapid leakage that leveled off and became fairly constant. Imbibition of embryos at 10°C or lower prolonged that period of rapid leakage. These authors suggested that the prolonged period of rapid leakage at the low temperatures was evidence that low temperature imbibition interfered with normal membrane reorganization by altering the physical state of the membrane phospholipids.

Additional support to this theory came from evidence that seed leakage can be reduced if seed moisture was increased before low temperature imbibition. A reduced cold sensitivity

of soybean embryos occurred when seed moisture was increased to 35 to 50% by exposure to high humidity before low temperature imbibition (Bramlage et al., 1978). This increased seed moisture was presumed to allow partial membrane reorganization before exposure to the cold stress. Protection against chilling injury has also been achieved by raising seed moisture contents in other cold sensitive species. Chilling resistance has been imparted to lima beans by an increased seed moisture content of 20% (Pollock, 1969), peas by an increase to 30% (Simon and Wiebe, 1975), snap beans by increased moisture contents of 12% or greater (Roos and Manalo, 1976) and corn to 13% or higher (Obendorf et al., 1971).

Contrary to the presumption that seed membranes are in the hexagonal configuration at low moisture contents and before imbibition, McKersie and Stinson (1980) reported that no change in membrane architecture occurred during seed dehydration and reimbibition. Birdsfoot trefoil seeds at moisture contents from 5 to 40% were investigated by low and wide angle X-ray diffraction. No phase change was noted in any sample and all membrane phospholipids were found exclusively in the lamellar state. However, dehydration did appear to induce a subtle reorganization of cellular membranes which altered permeability properties but did not involve a phase change or total loss of selective permeability.

The disorganization of membranes and the increased permeability associated with it during low temperature imbibition can influence other subcellular reactions which can have direct

affects on chilling injury (Lyons, 1973). The phase transition or the inability of membranes to reorganize completely not only increased permeability but also increased the activation energy of membrane-bound enzyme systems. This could lead to a suppressed reaction rate of the mitochondrial krebs cycle and an imbalance with the nonmembrane-bound glycolysis system allowing accumulation of metabolites (ie. pyruvate, acetaldehyde and ethanol). Accumulation of these metabolites can cause injury or death depending on the ability of the species to withstand or metabolize these compounds.

Mitochondrial respiration was suppressed as temperature was reduced below the temperature associated with membrane phase changes in chilling sensitive tissues (Lyons and Raison, 1970). Arrhenius plots of mitochondrial respiration isolated from soybean embryonic axes held at 23 and 10°C differed (Duke et al., 1977). The cold treated axes had an inflection in the Arrhenius plot at 8°C, whereas control axes held at 23°C had an inflection temperature at 12.5°C. Duke et al. additionally determined that the slopes of the plots for axes NADP-isocitrate dehydrogenase were very similar to those for mitochondrial respiration isolated from the control axes. They suggested that this enzyme may limit mitochondrial respiration at low temperatures in soybean tissue.

Other subcellular systems are affected by low temperature inhibition. DNA synthesis in cottonseed was reduced drastically by exposure to 2°C for five hours during a sensitive

period of imbibition between 30 and 36 hours (Clay et al., 1976). The reduced DNA synthesis was correlated with reduced DNA polymerase activity. When a less chilling sensitive genotype of cotton was compared to more sensitive varieties, the resistant type's nuclear DNA polymerase activity was more efficient. Chilling resistance was also correlated with a higher degree of unsaturation in the nuclear membranes of the resistant genotype. Clay et al. (1977) concluded that even though many physiological parameters were probably affected adversely by chilling stress, the capacity to synthesize DNA following chilling was closely associated with seedling performance under unfavorable temperatures.

Protein synthesis, glutamine synthetase, acid phosphatase and fumarase levels decreased in crimson clover seeds when germinated at 10°C compared to 20°C (Ching, 1975). However, no differences were found in soluble protein, protease, α -amylase, ATPase and RNase levels between the two germinating temperatures.

The effect of imbibitional chilling injury on energy metabolism in corn was examined by Cohn and Obendorf (1976). Corn kernels at 5 and 13% moisture were imbibed at 5°C for 12, 24 or 48 hours. Radicle growth was reduced in the low moisture seeds exposed to the 5°C temperature, indicating chilling damage. However seed respiration, ATP content and energy charge of the embryos were the same regardless of initial moisture content. ADP/O ratios and respiratory control ratios of isolated mitochondria were not altered by initial kernel

moisture. The authors concluded that disruption of energy metabolism was not the primary cause of seed moisture-mediated imbibitional chilling injury.

Contrary to previously mentioned work, Simon et al. (1976) found that failure of cucumber seeds to germinate in the cold was not due to imbibition injury, loss of membrane integrity, or failure of mitosis in the cold. Cucumber seed failed to germinate at cold temperatures when first imbibed for 12 hours at 20°C and there was no evidence for loss of membrane integrity in the cold. Mitosis was eliminated as a restriction to low temperature germination when cucumber seeds germinated in the presence of colchicine or after γ -irradiation at 20°C. The authors proposed, after reviewing high Q_{10} figures during low temperature germination, that failure to germinate may result from protein denaturation. Denaturation of proteins may have inhibited low temperature germination by inactivating enzymes or preventing the orderly association of proteins into organelles.

Growth Regulator Activity as a Function of Low Temperature Germination

Growth regulator formation or activation has been indicated to affect the rate, in part, in which tomato seeds could germinate at low temperatures (Abdul-Baki and Stoner, 1978; Maluf and Tigchelaar, 1980). Abdul-Baki and Stoner (1978) reported the presence of a growth promoter and an inhibitor in leachates from tomato seed which were possibly responsible for the ability

of tomato seeds to germinate at low temperatures. Leachate from seeds of PI 341934, a line which germinated well at low temperatures, promoted germination of that line and other tomato cultivars at 10°C . The leachate from 'Red Rock' seeds, a cultivar which germinated poorly at low temperatures, inhibited germination of that and other tomato cultivars at 10° . The growth regulator activity was highest in fresh seed, declined with age and responses were best exhibited at low temperatures. The researchers did not identify the promoting or inhibiting substances nor did they determine the exact role or mode of action these substances had with respect to each other or tomato germination. However, they did find the promotive and inhibitory effects of the leachates were highly specific and restricted to tomato seeds. In support of Abdul-Baki and Stoner's work, Maluf and Tigchelaar (1980) found germination of noncold-germinating lines of tomato could be enhanced by the addition of activated carbon to the germination media. No such enhancement of germination was found when cold-germinating lines of tomato were tested. These researchers determined that inhibition or delay in germination of noncold-germinating lines in tomato was due, in part, to a low temperature activation or formation of an activated carbon adsorbable inhibitor of seed germination.

Low Temperature Germination as it Relates to Genetic
Variability

The ability of seeds to germinate at low temperatures, independent of physiological considerations, appears to be genetically controlled within individual species. Gerson and Honma (1978) in reviewing 105 cultivars from five species of Cap-sicum for emergence response from low soil temperatures found great variability between species and within species. Mean emergence indices of seven pepper species and subspecies at 16°C ranged from 22.9 for C. baccatum v. pendulum to 49.0 for C. baccatum v. microcarpum. Within C. annuum at 16°, mean emergence indices varied from 20.6 to 46.5 for the 80 cultivars tested. From these results the authors suggested the ability to germinate at low temperatures may be heritable in peppers.

Differences in the degree of chilling sensitivity within species, among cultivars, has also been reported for the chilling sensitive crops of cotton (Buxton and Sprenger, 1976), soybean (Hopper et al., 1979), corn (Pinnell, 1949; Cal and Obendorf, 1972 a and b) and carrot (Hegarty, 1973).

The inheritance of low temperature germination ability in tomato has been widely reviewed (Cannon et al., 1973; DeVos et al., 1981; El Sayed and John, 1973; Gatherum et al., 1970; Maluf and Tigchelaar, 1980; Ng and Tigchelaar, 1973). However, the genetic nature and mode of inheritance for low temperature germinability is still unclear and debated. Reported claims from single gene control (Cannon et al., 1973) to polygenic

and primarily additive with dominance for inability to germinate at suboptimal temperatures (Ng and Tigchelaar, 1973) are found within the literature. Cold germinating accessions have been identified in tomato with the ability to reach 50% germination in eight days at 10°C, whereas noncold-germinating accessions may take 22 days or longer to reach 50% germination (Maluf and Tigchelaar, 1980). The emphasis on studying the inheritance of low temperature germination in tomato seed was an attempt to incorporate low temperature sprouting ability into commercially acceptable cultivars to allow early spring direct seeding of tomatoes.

Seed Treatments for Enhancing Low Temperature Germinability

A number of seed treatments have had favorable results on the subsequent performance of seeds under conditions of low temperature stress. Ells (1963) described a seed treatment in which tomato seeds were placed on filter paper moistened with 1% potassium phosphate and 1% potassium nitrate solution for six days. The seeds were dried at room temperature to approximately the original moisture content. This treatment proved beneficial in advancing seedling emergence by up to five days over untreated seed when planted in a greenhouse with a night temperature of 10°C. Ells attributed the effect of this seed treatment not primarily to the salts but to the osmotic pressure maintained by the salt solution. The solution prevented seed germination but permitted enough moisture to enter the seed to "foster enzymatic activities."

Similarly, other researchers increased germination rates of tomato seed at low temperatures by pretreating seeds with potassium salt soaks (Oyer and Koehler, 1966; Wickham and Nicols, 1976; Dimov et al., 1977). The seed treatments increased the speed of germination at low temperatures and increased the uniformity of germination.

Pregermination treatments with potassium salts have had favorable results on other crops. Watermelon (Sachs, 1977), pepper (Sachs et al., 1980), carrot, celery, cucumber, eggplant, muskmelon and Kentucky blue grass (Malnassy, 1971) exhibited increased germination rates at low temperatures when pretreated in aerated potassium salt solutions for several days then redried.

Polyethylene glycol (PEG) has proved favorable as an osmoticum for the pretreatment of seeds for low temperature germination (Heydecker, 1974). Heydecker and associates demonstrated the ability of several species of seeds, including onion, carrot, french bean and geranium, to germinate more rapidly and uniformly after osmotic treatment with PEG (Heydecker et al., 1973; Heydecker, 1974). The benefit gained from osmotic pretreatment before low temperature germination was retained when onion seeds were dried and held in storage for eight weeks. Knypl and Khan (1981) improved the performance of soybean seeds at low temperatures with osmotic pretreatment. The seeds were treated by placing them on filter paper moistened with a PEG-6000 solution (-8.6 to -11.0 bars)

for four to eight days at 15°C in a sealed container. The seeds were then removed from the container, washed and dried. Similar pretreatment of pepper seeds allowed for faster, more uniform germination at 25° in the laboratory and greenhouse, but did not speed germination in the field with cool soil temperatures (Yaklich and Orzolek, 1977).

These hydration-rehydration treatments performed on seeds with salt or PEG solutions have generally been referred to as priming (Malnassy, 1971; Heydecker, 1974). That is, the technique of starting germination processes, bringing them to the brink of cell elongation and radicle emergence then stopping all processes by dehydration. This process apparently advanced the onset of germination by increasing the permeability of the seed coat, and initiating metabolic events which would withstand dehydration, such as increased protease activity (Berrie and Drennan, 1971). Increased protein levels and activation and/or synthesis of a number of enzymes, including acid phosphatases and esterases, have been reported during priming of seeds (Koehler, 1967; Khan et al., 1978). Hanson (1973) noted that oxygen uptake and leucine incorporation in protein was enhanced in aleurone layers, more than in embryos, isolated from primed wheat seed. In addition, amylase activity developed faster in primed seed than in non-primed seed during germination. Sen and Osborne (1974) found that when rye embryos were hydrated for three to six hours then dehydrated, the embryos when rehydrated had a rate of protein synthesis equal to the pretreatment time plus the duration of the second

imbibition time. Additionally, hydration-dehydration treatments enhanced subsequent RNA synthesis and if the hydration time was increased to nine hours before dehydration, DNA synthesis began immediately upon rehydration. Sen and Osborne (1974) concluded that many changes that occurred during the period of hydration pretreatment must be stable to subsequent dehydration.

Priming, however, can be detrimental if during the hydration step embryo growth was apparent or radicle emergence occurred (Berrie and Drennan, 1971). Dehydration usually then resulted in some embryo or radicle damage.

The use of a gibberellic acid (GA) as a pretreatment to seed has proved beneficial in speeding germination of some species at suboptimal temperatures. Nelson and Sharples (1980) reported the rate and total germination of cucumber seeds germinated at 12°C was increased by acetone infusion of fusicoccin, GA_{4/7} or GA₃. Gibberellic acid_{4/7} treatment was less effective than fusicoccin, but more effective than the GA₃. Gibberellic acid_{4/7} treatments to muskmelon seeds increased total germination at 16°C, but did not increase germination rate over untreated seeds. However, no fusicoccin or gibberellic acid treatment was effective in promoting watermelon germination at low temperatures. Cole and Wheeler (1974) noted that chilling injury to cottonseed was reduced at suboptimal temperatures by a preconditioning in water or gibberellic acid. These authors suggested that elevated seed moisture in combination with growth regulator treatments may be a satisfactory treatment for obtaining cotton stands at lower than optimal germination temperatures.

In the study which follows, germination of sweet pepper (Capsicum annuum) seed was investigated to determine the cause or contributing causes of slow germination rate at a low temperature.

CHAPTER II
GROWTH HORMONE INVOLVEMENT IN GERMINATION OF Capsicum
annuum L. AT LOW TEMPERATURE

The control of germination at lower than optimal temperatures may be governed by the presence of growth inhibitors or promoters. Abdul-Baki and Stoner (1978) described the presence of a growth promoter in the leachate from the seed of a tomato accession, PI341984, which germinated well at low temperatures. The leachate promoted germination of seeds at 10°C of the same and other tomato cultivars. Leachates from the seed of the tomato cultivar, Red Rock, which germinated poorly at low temperatures inhibited or slowed germination of tomato seeds. The promotive and inhibitory components of the leachates were not isolated or identified, but they were found to be highly specific and restricted in nature to tomato seeds. The presence of a low temperature germination promoter and inhibitor was further supported by work done by Maluf and Tigchelaar (1980). They found that germination of noncold-germinating lines of tomato was improved by adding activated carbon to the germination media. They felt that inhibited or slowed germination at 10°C by noncold-germinating lines was, in part, due to a low temperature triggered activation or formation of an activated carbon adsorbable inhibitor.

The addition of growth regulators to seeds or to the germination media has proved beneficial in stimulating germination

under adverse temperature conditions. Cytokinins have been used to overcome thermodormancy and the inhibitory effect of abscissic acid on lettuce seed germination (McCoy and Harrington, 1970; Reynolds and Thompson, 1973). Cytokinins have also been suggested as having the permissive role in seed germination because of their ability to oppose the effect of an inhibitor and their inability to trigger seed germination per se (Khan, 1971). The primary role in the control of seed germination can be attributed to gibberellins. The ability of gibberellic acid (GA) to control many germination processes has been well documented (Mayer and Shain, 1974) including the production of α -amylase by de novo synthesis in barley endosperm (Varner, 1964). Gibberellins, GA_3 and GA_{4+7} , have been beneficial in stimulating germination of several species at unfavorable temperatures. Cucumber, cotton, and lilac (Syringa reflexa) have all been stimulated to germinate faster and more uniformly at low temperatures by GA application (Nelson and Sharples, 1980; Cole and Wheeler, 1974; Junttila, 1973).

Auxin, indoleacetic acid (IAA), is an important plant growth hormone which is active in many growth processes. However, its role in seed germination is relatively unknown (Tillberg, 1977). Exogenously applied auxin generally has little or no effect on seed germination. Tillberg (1977) felt that seeds may already contain adequate levels of auxin for germination and thus extra quantities of the hormone would be without effect.

The experiments which follow investigated the effect of growth hormones on Capsicum annuum seed germination at optimal and low temperature (15°C). Determinations were made to find if low temperature stimulated the formation or activation of a germination inhibitor.

Materials and Methods

Seed source. Seeds used in all experiments were from the same seed lot of Capsicum annuum 'Early CalWonder' (Lot number 0031019) obtained untreated from Petoseed Company, Saticoy, California. The seeds were stored in plastic bags at 10°C and 50% relative humidity until used.

Germination procedure and data. Seeds were germinated at 15 and 25°C on moistened Whatman No.1 and No.3 filter papers in 5.5 or 9 cm Petri dishes. Germination data for all of the experiments were taken at 24 hour intervals and all treatments were replicated 4 times with 25 seeds per replicate. Seeds with visible radicles were counted as germinated. Data taken for each treatment included total percent germination and mean number of days to germination. The mean number of days to germination (MDG) was calculated by the formula adapted from an emergence index used by Gerson and Honma (1978):

$$MDG = \frac{\sum(\text{days to germination}) (\text{number of seeds germinated})}{\text{total number of seeds germinated}}$$

Imbibition rate of pepper seeds. Seeds were imbibed at 15 and 25°C in distilled water. After the appropriate treatment time, the seeds were removed, blotted dry, then weighed.

The total number of seeds which germinated were counted, the seeds were dried at 70° for 48 hours, and dry weights were recorded.

Determination of inhibitor activity after low temperature imbibition. To determine if there was a water soluble leachable low temperature germination inhibitor present in pepper seeds, 5 g of air dry seed were leached in 40 ml of cold distilled water, on a reciprocating shaker for 0, 2, 4, or 8 days at $4^{\circ} \pm 0.5^{\circ}\text{C}$. The water was changed midway during each leaching period and saved. Moist, fully imbibed, and dry seeds were also subjected to the 4° temperature as controls. At the end of each leaching period, seeds were taken for germination tests.

Samples were prepared by combining the leachate from each of the time periods, freezing at -20°C and then concentrating the leachate by freeze-drying. The freeze-dried residue was taken up in 10 ml of distilled water then filtered through a double thickness of Miracloth. Pepper seeds were germinated in 1 ml of the filtered, concentrated sample, representing leachate from 0.5 g of seed. The osmotic potential of the sample was determined by a vapor pressure osmometer (Wescor Inc. Logan, Utah) and suitable osmotic comparison treatments were prepared using a polyethylene glycol (PEG-6000) (Michel and Kaufmann, 1973) or a 0.2M sodium phosphate buffer (ph 6.5) (Ross, 1974) diluted to the appropriate osmotic potential.

Seeds leached for 0, 2, 4 or 8 days, representing approximately 4.5 g of air dry seed, were homogenized in distilled water by a Sorvall Omni-mixer for 5 minutes. The homogenate

was filtered and washed through a double thickness of Miracloth, centrifuged for 10 minutes at 3600 X g, and concentrated in a freeze dryer. The residue was taken up in 9 ml of distilled water and 1 ml was used for germination tests.

Organically extractable inhibitor activity after low temperature imbibition. Ethanol and acetone extractions were performed on 5 g of either a) air dry pepper seed b) seeds imbibed 2 days at 25⁰C, or c) seeds imbibed 4 days at 15⁰. The seeds were homogenized in 10 ml of absolute ethanol or redistilled acetone in a Virtis homogenizer for 5 minutes then filtered through a double thickness of Miracloth. Seeds were treated in Petri dishes containing 1 ml of the filtrate and 4 ml of distilled water or the appropriate organic solvent. The Petri dishes were sealed and placed in a constant temperature chamber (25⁰) for 2 hours. Immediately after treatment, the solutions were decanted and the seeds were vacuum dried in a desiccator for 2 hours. After drying, germination tests were performed.

Application of growth hormones to pepper seeds germinated at low temperatures. IAA and gibberellin A₃ or A₄₊₇ were applied in concentrations from 0 to 1000 ppm to pepper seeds to determine if they would stimulate germination at low temperature. Kinetin was similarly tested at concentrations of 0 to 100 ppm. The IAA, GA₃ and kinetin were obtained from Sigma Chemical Company, St. Louis, Missouri, and GA₄₊₇ was obtained from Imperial Chemical Industries, Plant Protection Limited, Surrey, England. The IAA was dissolved in 2 ml of

acetone whereas the GA's and kinetin were dissolved in 10% KOH. The solutions were corrected to pH 6.5 with 10% HCl and used to moisten the filter papers for germination tests.

Redistilled acetone was also used to apply GA₄₊₇ at 100 ppm to pepper seeds. Seeds were soaked in 5 ml of acetone or acetone containing GA₄₊₇, 100 ppm, for 0, 2, 4, 8, 12, or 24 hours at 25°C. After the prescribed durations, the solutions were decanted and the seeds were dried in a vacuum desiccator for 2 hours. Germination tests were then performed.

Effect of osmotic potential on pepper seed germination.

Pepper seeds were germinated in PEG-6000 solutions from -1 to -8 bars with and without GA₄₊₇, 100 ppm. The osmotic potentials of the aqueous solutions of PEG-6000 were determined from Michel and Kaufmann's (1973) paper.

Pepper seed germination with a GA synthesis inhibitor.

At 25°C, 1.5 ml of GA₄₊₇ at 0, 10, 100 and 1000 ppm in combination with 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine carboxylate, (AMO-1618), a GA synthesis inhibitor, at 0, 100 and 1000 ppm was used as a germination medium for pepper seeds.

Results

Total germination of pepper seeds does not differ at 15 and 25°C (Figure 2-1). However, germination at 15°C becomes less uniform and the MDG is 2 times that at 25°C (Figure 2-2).

Figure 2-1. Germination of pepper seeds at 15 and 25°C.
zMean separation within columns by Duncan's
multiple range test, 5% level.

Figure 2-1

Figure 2-2. Germination of pepper seeds at 15 and 25°C.
Mean separation within columns by Duncan's
multiple range test, 5% level.

Figure 2-2

Water uptake at 15 and 25°C was similar and increased with imbibition time until 12 hours (Figure 2-3). After 12 hours, the moisture content of pepper seeds remained constant until germination exceeded 50%, then the moisture content increased.

Leaching pepper seeds in cold distilled water for 2, 4 or 8 days had no effect on the mean number of days to germination at 25°C when compared to unleached seed (Table 2-1). Seeds which were kept moist for 8 days germinated slower than unleached seed. A similar treatment for 4 days germinated slower than 4 and 8 day unleached seeds. There was no difference noted in the total germination of leached, moist or unleached seeds when germinated at 25°C (Table 2-2).

Pepper seeds leached in cold distilled water for 8 days and germinated at 15°C, germinated at the same rate as the unleached and moist seeds after the same treatment time (Table 2-3). Leaching pepper seeds for 2 or 4 days speeded the germination rate of these seeds when compared to unleached or moist seeds after the same treatment times. However, pepper seeds leached 2 days, germinated at a faster rate than any other treatment. Total germination of pepper seeds was unaffected by leaching when germinated at 15°C (Table 2-4).

Seeds germinated slower in the leachate extract than the untreated control at 25°C (Table 2-5). Germination in the -2 bar sodium phosphate buffer was also slower than the untreated control. There was no difference in the total germination with any treatment at 25°C. When germinated at 15°C, untreated seeds

Figure 2-3. Water uptake of pepper seeds during imbibition
at 15 and 25°C
*Indicates greater than 50% of radicles have emerged .

Figure 2-3

Table 2-1. The effect of leaching seed at 40°C for various times on the mean number of days to germination (MDG) when germinated at 25°C

	Days Treated			
	0	2	4	8
	(MDG)			
Leached Seed	--	6.4 c	7.1 abc	7.2 abc
Moist Seed	--	6.6 c	7.8 ab	8.2 a
Unleached Seed	6.7 bc ^Z	6.7 bc	6.5 c	6.6 c

^ZMean separation by Duncan's multiple range test, 5% level.

Table 2-2. The effect of leaching seed at 40°C for various times on the total germination when germinated at 25°C

	Days Treated			
	0	2	4	8
	(%)			
Leached Seed	--	79 a	73 a	75 a
Moist Seed	--	83 a	75 a	70 a
Unleached Seed	76 a ^Z	74 a	81 a	75 a

^ZMean separation by Duncan's multiple range test, 5% level.

Table 2-3. The effect of leaching seed at 40C for various times on the mean number of days to germination (MDG) when germinated at 150C

	Days Treated			
	0	2	4	8
	(MDG)			
Leached Seed	--	8.0 c	9.4 b	9.2 b
Moist Seed	--	9.2 b	10.7 a	10.2 ab
Unleached Seed	10.1 ab ^Z	10.6 a	11.0 a	10.1 ab

^ZMean separation by Duncan's multiple range test, 5% level.

Table 2-4. The effect of leaching seed at 40C for various times on the total germination when germinated at 150C

	Days Treated			
	0	2	4	8
	(%)			
Leached Seed	--	76 a	74 a	78 a
Moist Seed	--	80 a	67 a	75 a
Unleached Seed	72 a ^Z	68 a	78 a	77 a

^ZMean separation by Duncan's multiple range test, 5% level.

Table 2-5. The effect of leachate, from 0.5 g of seed, on the germination of pepper seed at 25 and 15°C

Treatments	25 ^o		15 ^o	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Untreated control	4.6 b ^z	78 a	8.5 d	79 ab
2 day leachate	5.3 a	80 a	10.8 b	82 ab
4 day leachate	5.4 a	80 a	10.1 bc	86 ab
8 day leachate	5.6 a	85 a	10.6 b	86 ab
-1 bar PEG-6000	4.6 b	82 a	8.8 d	91 a
-2 bar PEG-6000	4.4 b	84 a	9.7 c	84 ab
-2 bar sodium phosphate buffer	5.4 a	79 a	11.6 a	77 b

^zMean separation within columns by Duncan's multiple range test, 5% level.

and seeds soaked in -1 bar PEG-6000 germinated more rapidly than all other treatments. Seeds soaked in -2 bar sodium phosphate buffer germinated slower than all other treatments. Total germination in all treatments was similar to the untreated control at 15⁰.

Filtrate from ground seeds which had been previously leached slowed the rate of germination compared with the untreated control at 25⁰C (Table 2-6). Total germination of treated seeds at 25⁰ was similar to the untreated control. After 2 or more days of leaching, the extract of ground leached seeds reduced germination rate at 15⁰ compared to the untreated control. When these leached seed treatments were compared to the -3 bar PEG or -2 bar sodium phosphate treatments, germination rate was unaffected. Total germination was again unaffected by germinating the seeds in a ground seed extract compared to the untreated control.

Soaking pepper seeds in 20% ethanol extract had no affect on germination at 25⁰C (Table 2-7). Adding ethanol seed extracts to seeds germinated at 15⁰ slowed germination rates compared to the untreated control. The ethanol extracts did not affect total germination at 15⁰.

When seeds were soaked in similar extracts containing absolute ethanol, total germination at 25 or 15⁰C was unaffected (Table 2-8). However, ethanol extracts slowed germination rate over both controls at 25⁰. All ethanol treatments increased the MDG of pepper seeds germinated when compared to the untreated control at 15⁰.

Similar 20% acetone extracts had no affect on germination rate at 25⁰C or total germination at 25 or 15⁰ (Table 2-9).

Table 2-6. The effect of an aqueous solution from ground leached seed, 0.5 g/treatment, on pepper seed germinated at 25 and 15°C

Treatments	25 ^o		15 ^o	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Untreated control	4.8 b ^z	79 ab	9.3 c	84 ab
0 day leached seed	7.2 a	77 ab	10.9 abc	77 b
2 day leached seed	6.6 a	71 b	12.2 a	80 ab
4 day leached seed	7.5 a	77 ab	11.2 ab	78 ab
8 day leached seed	7.2 a	82 ab	11.2 ab	77 b
-2 bar PEG-6000	4.4 b	84 a	9.7 bc	84 ab
-3 bar PEG-6000	4.5 b	85 a	12.3 a	88 a
-2 bar Sodium phosphate buffer	5.4 b	79 ab	11.6 a	77 b

^zMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-7. Germination of pepper seeds at 25 and 15°C when treated with 1 ml of ethanol seed extract diluted with 4 ml of distilled water

Treatments	25 ⁰		15 ⁰	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Untreated control	4.8 a ^Z	84 a	10.4 c	78 a
Dry seed-extract	5.9 a	82 a	13.1 b	81 a
4 day imbibed seed at 15 ⁰ -extract	5.3 a	87 a	15.9 a	87 a

^ZMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-8. Germination of pepper seeds at 25 and 15°C when treated with 1 ml of ethanol seed extract diluted with 4 ml of ethanol

Treatments	25 ⁰		15 ⁰	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Untreated control	4.8 b ^Z	84 a	10.4 b	78 a
Ethanol soak control	6.4 b	88 a	14.1 a	84 a
Dry seed-extract	7.6 a	85 a	14.3 a	76 a
2 day imbibed seed at 25 ⁰ -extract	8.6 a	80 a	14.8 a	73 a
4 day imbibed seed at 15 ⁰ -extract	8.6 a	80 a	13.8 a	81 a

^ZMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-9. Germination of pepper seeds at 25 and 15°C when treated with 1 ml of acetone seed extract diluted with 4 ml of distilled water

Treatments	25 ⁰		15 ⁰	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Untreated control	4.8 a ^Z	84 a	10.4 b	78 a
Dry seed-extract	5.1 a	77 a	12.3 a	86 a
4 day imbibed seed at 15 ⁰ -extract	4.6 a	86 a	11.9 a	74 a

^ZMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-10. Germination of pepper seeds at 25 and 15°C when treated with 1 ml of acetone seed extract diluted with 4 ml of acetone

Treatments	25 ⁰		15 ⁰	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Untreated control	4.8 b ^Z	84 a	10.4 b	78 a
Acetone soak control	10.9 a	79 a	17.7 a	78 a
Dry seed-extract	13.8 a	75 a	17.3 a	78 a
4 day imbibed seed at 15 ⁰ -extract	10.8 a	76 a	16.4 a	84 a

^ZMean separation within columns by Duncan's multiple range test, 5% level.

Germination rate at 15⁰ was slower in the acetone treatments when compared to the untreated control.

Extracts in 100% acetone slowed the rate of germination when compared to the untreated control at both 25 and 15⁰C (Table 2-10). The treatments did not alter total germination percentage at either temperature.

IAA concentrations from 0 to 500 ppm did not effect the speed of germination at 25⁰C (Table 2-11). At 1000 ppm, IAA reduced the germination rate at both 25 and 15⁰. Concentrations of IAA from 0 to 250 ppm had no effect on germination rate at 15⁰; however, higher concentrations slowed germination. IAA treatments had no effect on the total germination of pepper seeds at 25 or 15⁰.

Kinetin, 1 to 100 ppm, did not alter germination rate or total germination of pepper seeds germinated at 25⁰C (Table 2-12). At 15⁰, 0 ppm treated seeds germinated faster than the 50 or 100 ppm kinetin treatments. Total germination was unaffected by kinetin at 15⁰.

At 25⁰C, seeds germinated in GA₃ had faster germination rates than the 0 ppm treatment (Table 2-13). At 15⁰, only the three highest concentrations of GA₃ (100, 500 and 1000 ppm) significantly increased the germination rate when compared to the 0 ppm treatment. The GA₃ applications had no effect on the total germination of pepper seeds at 25 or 15⁰.

All GA₄₊₇ applications at 25 or 15⁰C stimulated germination rates when compared to the 0 ppm (Table 2-14). Applications of GA₄₊₇ did not affect the total germination.

Table 2-11. The effect of indole acetic acid (IAA) concentration on germination of pepper seeds at 15 and 25°C

IAA Concentration	25 ^o		15 ^o	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
(ppm)	(MDG)	(%)	(MDG)	(%)
0	4.0 bc ^Z	82 a	8.8 c	85 a
10	4.2 bc	86 a	8.7 c	86 a
25	4.1 bc	83 a	8.6 c	82 a
50	4.0 bc	85 a	8.6 c	83 a
100	4.0 bc	87 a	8.6 c	83 a
250	4.2 bc	77 a	8.7 c	82 a
500	4.4 b	85 a	9.3 b	87 a
1000	5.3 a	78 a	9.7 a	81 a
Acetone control	3.8 c	84 a	9.3 b	85 a

^ZMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-12. The effect of kinetin (Kn) concentration on germination of pepper seeds at 15 and 25°C

Kn Concentration	25 ^o		15 ^o	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
(ppm)	(MDG)	(%)	(MDG)	(%)
0	3.8 a ^z	77 a	7.8 b	86 a
1	4.0 a	86 a	8.1 ab	78 a
5	4.2 a	85 a	8.3 ab	80 a
10	4.0 a	84 a	8.2 ab	76 a
25	3.9 a	87 a	8.1 ab	85 a
50	4.2 a	86 a	8.4 a	74 a
100	3.8 a	79 a	8.6 a	82 a

^zMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-13. The effect of gibberellic acid₃ (GA₃) concentration on germination of pepper seeds at 15 and 25°C

GA ₃ Concentration ^z	25 ^o		15 ^o	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
(ppm)	(MDG)	(%)	(MDG)	(%)
0	4.7 a ^y	79 a	12.9 a	81 a
10	4.5 b	91 a	12.2 ab	79 a
50	4.3 bc	83 a	12.3 ab	80 a
100	4.4 bc	78 a	11.6 b	84 a
500	4.2 c	84 a	11.7 b	76 a
1000	4.4 bc	85 a	11.7 b	86 a

^zGibberellic acid was in 0.05 M Sodium phosphate buffer.

^yMean separation within columns by Duncan's multiple range test, 5% level.

Table 2-14. The effect of gibberellic acid₄₊₇ (GA₄₊₇) concentration on germination of pepper seeds at 15 and 25°C

GA ₄₊₇ Concentration ^z	25 ^o		15 ^o	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
(ppm)	(MDG)	(%)	(MDG)	(%)
0	4.7 a ^y	79 a	12.9 a	81 a
10	4.2 b	82 a	11.5 b	79 a
50	3.9 bc	78 a	10.7 c	79 a
100	3.8 c	75 a	10.8 c	86 a
500	3.7 c	80 a	10.7 c	82 a
1000	3.7 c	82 a	10.9 c	85 a

^zGibberellic acid was in 0.05 M Sodium phosphate buffer.

^yMean separation within columns by Duncan's multiple range test, 5% level.

All GA₄₊₇ applications in acetone generally increased the germination rate of pepper seeds compared to the untreated control or acetone treated seeds at 25⁰C (Table 2-15). Seeds soaked in acetone for 2 or 24 hours and GA₄₊₇ in acetone for 8 or 24 hours, reduced total germination at 25⁰ (Table 2-16). All GA₄₊₇ applications in acetone increased the germination rate compared to acetone treatments or untreated control at 15⁰ (Table 2-17). Acetone treatments with or without GA₄₊₇ had no affect on the total germination compared to the untreated control (Table 2-18).

Increasing the osmotic concentrations of the germination medium over -4 bars greatly reduced germination rate at 25⁰C (Table 2-19). Gibberellic acid₄₊₇ applications increased germination rate at 25⁰ regardless of osmotic concentration. Total germination was reduced at -8 bars when compared to the untreated control. Similar results were observed with GA₄₊₇ application at 15⁰ when the osmotic concentration of the germination medium was varied (Table 2-20). The -4 bar treatment germinated more rapidly than the -6 bar treatment and -8 bar treatment did not germinate. Gibberellic acid₄₊₇ applications reduced the time to germination at the -4 and -6 bar osmotic concentrations. Total germination was not affected by osmoticum, except that no seeds germinated at the -8 bar treatments.

The application of AMO-1618 at 100 or 1000 ppm in the absence of GA₄₊₇ reduced the rate of germination when compared to the untreated control (Table 2-21). Application of GA₄₊₇ overcame the inhibition of AMO-1618 on germination rate. There was no

Table 2-15. The effect of acetone or GA₄₊₇, 100 ppm, applied in acetone on the germination rate of pepper seeds at 25°C

Treatments	Hours Treated					
	0	2	4	8	12	24
	(MDG)					
Untreated control	3.9 abc ^z	--	--	--	--	--
Acetone	4.1 a	3.9 abc	4.0 ab	3.7 c	3.8 bc	3.5 d
GA ₄₊₇ , 100 ppm	3.4 d	3.1 ef	3.3 de	3.0 f	3.0 f	3.0 f

^zMean separation by Duncan's multiple range test, 5% level.

Table 2-16. The effect of acetone or GA₄₊₇, 100 ppm, applied in acetone on the total germination of pepper seeds at 25°C

Treatments	Hours Treated					
	0	2	4	8	12	24

^zMean separation by Duncan's multiple range test, 5% level.

Table 2-17. The effect of acetone or GA₄₊₇, 100 ppm, applied in acetone on the germination rate of pepper seeds at 15°C

Treatments	Hours Treated					
	0	2	4	8	12	24
	(MDG)					
Untreated control	8.4 a ^z	--	--	--	--	--
Acetone	8.4 a	8.3 a	8.8 a	8.4 a	8.6 a	8.5 a
GA ₄₊₇ , 100 ppm	7.5 b	7.1 c	7.4 bc	7.0 c	7.1 c	7.2 bc

(MDG)

^zMean separation by Duncan's multiple range test, 5% level.

Table 2-18. The effect of acetone or GA₄₊₇, 100 ppm, applied in acetone on the total germination of pepper seeds at 15°C

Treatments	Hours Treated					
	0	2	4	8	12	24
	(%)					
Untreated control	84.7 ab ^z	--	--	--	--	--
Acetone	83.0 ab	85.0 ab	77.0 ab	80.5 ab	85.0 ab	89.0 a
GA ₄₊₇ , 100 ppm	80.5 ab	84.5 ab	86.0 ab	80.0 ab	85.0 ab	75.0 b

(%)

^zMean separation by Duncan's multiple range test, 5% level.

Table 2-19. The effect of GA₄₊₇, 100 ppm, on pepper seed germination in osmotic solutions at 25°C

Treatment	Germ. Rate		Total Germ.	
	+GA	-GA	+GA	-GA
	(MDG)		(%)	
Untreated control	3.8 h ^z	6.0 efg	81 ab	80 ab
-1 bar PEG	4.6 gh	6.3 def	89 a	80 ab
-2 bar PEG	4.8 gh	7.0 de	85 ab	78 ab
-4 bar PEG	4.5 fgh	7.8 d	86 ab	86 ab
-6 bar PEG	7.1 de	10.7 c	80 ab	75 b
-8 bar PEG	13.1 b	17.8 a	41 c	6 d

^zMean separation within main observations by Duncan's multiple range test, 5% level.

Table 2-20. The effect of GA₄₊₇, 100 ppm, on pepper seed germination in osmotic solutions at 15°C

Treatment	Germ. Rate		Total Germ.	
	+GA	-GA	+GA	-GA
	(MDG)		(%)	
Untreated control	7.6 f ^z	9.6 def	84 a	82 a
-1 bar PEG	7.4 f	9.7 def	85 a	76 a
-2 bar PEG	8.6 ef	10.3 de	79 a	82 a
-4 bar PEG	11.3 d	13.9 c	86 a	83 a
-6 bar PEG	22.3 b	26.6 a	83 a	84 a
-8 bar PEG	-- ^y	--	0	0

^zMean separation within main observations by Duncan's multiple range test, 5% level.

^yNo seeds germinated and this data was not included in the statistical analysis.

Table 2-21. The effect of AMO-1618 on the germination rate of pepper seeds at 25°C when GA₄₊₇ is applied

GA ₄₊₇ Concentration (ppm)	AMO-1618 Concentration (ppm)		
	0	100	1000
	(MDG)		
0	5.6 b ^Z	6.7 a	6.7 a
10	4.4 cd	4.5 cd	4.8 c
100	3.8 d	4.2 cd	4.2 cd
1000	4.0 d	4.0 d	4.2 cd

^ZMean separation by Duncan's multiple range test, 5% level.

Table 2-22. The effect of AMO-1618 application on the total germination of pepper seeds at 25°C when GA₄₊₇ is applied

GA ₄₊₇ Concentration (ppm)	AMO-1618 Concentration (ppm)		
	0	100	1000
	(%)		
0	84.0 a ^Z	87.0 a	85.0 a
10	82.5 a	77.0 a	83.0 a
100	83.0 a	85.0 a	84.5 a
1000	80.0 a	80.0 a	85.5 a

^ZMean separation by Duncan's multiple range test, 5% level.

effect of varying the concentration of GA on this enhancement. AMO-1618 did not affect the total germination (Table 2-22).

Discussion

Pepper seed germination under optimal conditions was uniform with most radicle emergence occurring after 4 days of imbibition. However, at 15°C, germination became less uniform with radicle emergence occurring after 7, 8 and 9 days of imbibition. Similarly, Gerson and Honma (1978) screened 80 cultivars of Capsicum annuum for emergence rates at low temperatures. The average emergence indices for all C. annuum cultivars increased from 17.8 at 18°C to 26.5 at 16°C and further increased to 41.5 at 13°C.

Water uptake at 15 and 25°C was similar and did not differ until 50% or more of the seeds germinated. Sachs et al. (1980) also found little difference in the rate of water uptake in pepper seeds imbibed at 15 and 25°C. Water content increased appreciably only after germination. The authors reported that any differences in water uptake due to temperature did not lead to a delay in germination.

There does not appear to be any evidence for a leachable inhibitor to low temperature germination of pepper seed as was found for tomato seed (Abdul-Baki and Stoner, 1978; Maluf and Tigchelaar, 1980). No increase in the rate of germination was found at 25°C when seeds were leached at 4°C for up to 8 days. However, the germination rate generally increased at 15°C when seeds were leached compared with the unleached seeds.

This may provide evidence for a leachable low temperature inhibitor, but leaching only for 2 days proved effective in speeding germination rate when compared to unleached seeds. Increasing leaching times to 4 or 8 days was not beneficial in speeding germination. Heydecker (1974) reported that pepper seeds imbibed at 3, 7 and 10⁰ failed to germinate but when transferred to 30⁰ germinated faster than seeds placed at 30⁰ from the outset. In similar experiments, Sachs et al. (1980) observed that pepper seeds imbibed at 30⁰ for 2 days enhanced germination rates at 15⁰ when the seeds were not re-dried after treatment. The benefit of the 2 day leaching time on pepper seed germination rates may be similar to what Heydecker (1974) and Sachs et al. (1980) found.

When seed leachate was applied back to unleached seed, it slowed germination at 25 and 15⁰C. The PEG-6000 treatments, osmotic concentrations equal to the leachates, did not account for the reduction in the speed of germination, but a -2 bar sodium phosphate buffer treatment reduced the speed of germination to the same rate or greater than the leachates. Heydecker (1974) found similar results with pepper seed extracts. The delay in germination rate imposed by pepper extracts could not be fully explained by the osmotic potential of the extract, duplicated by PEG-6000. He felt that one of the components of the extract imposed a delay in germination chemically. However, in this study, germination rate was not reduced further by increasing leaching time. Furthermore, germination temperature had no effect on these trends. Thus, there does not appear to

be a leachable inhibitor which was formed or activated during low temperature exposure, but merely a salt-osmotic effect on germination rate due to the addition of the leachate itself.

Pepper seeds were extracted in distilled water, ethanol or acetone to determine if there was a non-leachable germination inhibitor formed or activated during low temperature imbibition. Aqueous extracts of leached pepper seeds delayed germination regardless of leaching time. The delay in germination at 15⁰ was similar to the osmotic treatments. No "build-up" or activation of germination inhibitors at low temperatures was observed in aqueously extracted pepper seeds. Ethanol extracts inhibited germination rates at 15⁰ when diluted in water and at 25⁰ in ethanol. Acetone extracts did not affect germination rate at 15 or 25⁰ regardless of how they were applied. Singh and Khattra (1977) found levels of an abscissic acid-like compound, equivalent to 2 ppm, present in 80% methanol extractions of Capsicum annum seeds. If an abscissic acid-like compound was present in the ethanol extracts of the present experiments, it did not appear to be formed or activated in response to low temperature.

Auxin or kinetin applications had no effect on increasing the germination rate of pepper seeds. However, GA₃ and GA₄₊₇ did effectively increase the germination rate at both 15 and 25⁰C. Gibberellic acid₄₊₇ stimulated the germination rate to a greater extent than GA₃. Gibberellic acid stimulated germination in cucumber (Nelson and Sharples, 1980), cotton (Cole and Wheeler, 1974), and Syringa reflexa (Junttila, 1973) at

suboptimal temperatures. Gibberellic acid₄₊₇ was more effective than GA₃ in stimulating germination in cucumber (Nelson and Sharples, 1980) and Labiatae species (Thompson, 1969). Gibberellic acid applications were effective in increasing germination rates whether the seeds were in continuous contact with the growth regulator or whether it was applied with an organic solvent before imbibition was initiated.

Gibberellic acid₄₊₇ treatments also had the effect of increasing germination rate under osmotic stress at 15 and 25°C. Conditions of high osmoticum have been associated with inhibition of cellular expansion while not affecting cell division (Haber and Luippold, 1960). Gibberellic acid treatments have also been correlated with increasing the water potential of embryos (Junttila, 1973). This suggests that GA may promote germination rate in pepper by increasing the water potential (rate of radicle elongation) of the embryo or by stimulating processes other than cell division. This was supported by the fact that GA₄₊₇ increased germination rates of pepper seeds at high (35 and 40°C) and low (10 and 12°C) temperatures (Appendix). High temperatures inhibit germination in lettuce but not cell division (Haber and Luippold, 1960). At low temperatures, cell division was delayed in lettuce until after radicle emergence occurred.

AMO-1618, an inhibitor to GA synthesis, delayed germination of pepper seeds at 100 and 1000 ppm. However, the delay in germination was overcome with GA applications. Harvey and Oaks (1974) found AMO-1618 at 200 µM concentrations inhibited protease and amylase production in excised corn endosperms.

Additions of GA_3 did not overcome this inhibition. Moore (1967) found that 1 μg of GA_3 could overcome the inhibitory effect that 10 μg of AMO-1618 had on hypocotyl elongation of cucumber seedlings. It appears from this study that low levels of GA may be synthesized preceding germination of pepper seeds. Gibberellic acid synthesis may not be a prerequisite of germination but in effect could be necessary to allow germination to proceed at a rapid pace. Added GA stimulated germination at 15 and 25°C by possibly triggering or enhancing some germination process, such as the formation or activation of enzymes as seen in monocots or by increasing embryo growth potential allowing germination to proceed at a more rapid rate.

Summary

Germination of pepper seeds was reduced by 1/2 and became less uniform as the temperature was decreased from 25 to 15°C. Water uptake at 15 and 25°C was similar and increased appreciably only after radicle emergence occurred.

There was no evidence for a leachable or extractable germination inhibitor being activated or formed due to low temperature exposure. Leachates and aqueous extracts of pepper seeds did slow germination but the effect appeared to be a salt-osmotic effect due to the addition of the leachates or extracts to the germination medium.

Auxin and kinetin applications had no effect on increasing germination rates of pepper seeds at 15 or 25°C. However, GA_3

and GA₄₊₇ effectively increased germination rates at both temperatures. Gibberellic acid₄₊₇ was more effective than GA₃. Gibberellic acid applications stimulated germination whether the seeds were in continuous contact with the growth regulator or whether it was applied with an organic solvent and redried before imbibition was initiated.

AM0-1618 applications inhibited pepper seed germination rate indicating GA synthesis may be necessary for rapid germination but is not a prerequisite for germination. Gibberellic acid applications were effective in increasing germination rates at 15 and 25°C when seeds were under osmotic stress, a condition associated with inhibition of cellular expansion but not cellular division.

CHAPTER III
RESPIRATORY CONTROL OF Capsicum annuum L. GERMINATION AT
LOW TEMPERATURE

The availability of oxygen for germination is critical for most species. With the exception of certain water plants, most seeds will not germinate in its absence (Come and Tissaoui, 1973). A 2% oxygen environment inhibited germination of all dicots tested except Cucumis sativa, Celosia plumosa and Celosia cristata (Heichel and Day, 1972). However, these three species exhibited severely suppressed growth in the low oxygen environment. Embryos may receive low oxygen concentrations when seeds are first imbibed (Come and Tissaoui, 1973). The only oxygen that can pass to the embryo must be dissolved in water that is covering the structures of the seed during imbibition.

Under optimum conditions, increased germination of several species has been achieved by increasing the oxygen concentration above normal (Edwards, 1973). Sachs et al. (1981) found that clay-coated pepper seeds germinated faster when placed in 100% oxygen. They felt the clay coating limited oxygen supply to the embryo and thus inhibited germination. In addition, the authors felt that water was also a barrier to oxygen diffusion in clay-coated pepper seeds leading to a reduction in germination rate.

The supply of oxygen may regulate respiratory pathways during seed germination (Roberts, 1973). Roberts (1973) found

that in dormant seeds, any treatment that increased alternate respiration tended to decrease or alleviate dormancy. Such treatments included the removal of covering structures, increased oxygen pressure, or application of respiratory inhibitors to the cytochrome mediated-cyanide sensitive pathway. Higher oxygen pressure may have stimulated alternate respiration by reducing the competition between the high affinity cytochrome oxidase and the lower affinity oxidase of the alternate respiratory pathway. Yentur and Leopold (1976) concluded that the alternate respiratory pathway, was needed during the early stages of soybean germination. In addition, they found that the early stages of soybean germination required a normal tension of oxygen but later when alternate respiration was lower, this was no longer required. Burgullio and Nicolas (1977) reported that the alternate pathway of respiration was not the major respiratory pathway early in germination of chick peas, but reached its maximum between 72 and 96 hours. Oxygen stimulated the appearance of the alternate respiratory pathway and the stimulation was dependent on cytoplasmic protein synthesis.

In the following study, the effect of oxygen on germination of Capsicum annuum seeds was investigated to determine the ability of oxygen to control cyanide sensitive and resistant respiratory pathways at 15 and 25⁰C. Gibberellic acid has been shown to stimulate germination of pepper seeds at 15 and 25⁰ (Chapter II). The effect of GA on respiratory activity at 15 and 25⁰ was determined to find out if it correlated with increased germination rate. Effectiveness of GA and O₂ on stimulating germination rate were compared.

Materials and Methods

Effect of O_2 on germination. Twenty-five pepper seeds were placed on a moistened No.3 Whatman filter paper in an open 5.5 cm Petri dish. The Petri dishes were placed into 450 ml glass jars and sealed. The lid to each jar had 2 rubber septums. Jars receiving the same gas treatments were connected via needles and rubber tubing. The jars were placed into constant temperature chambers, 15 or 25°C, and attached to gas streams containing the desired atmospheric concentrations of O_2 and N_2 . The gas streams were humidified by bubbling them through distilled water at the desired germination temperature. Oxygen mixtures of 10, 21, 40, 60 and 100 percent, were achieved by mixing 100% O_2 and 100% N_2 in a 1000 ml erlenmeyer flask, after passing the gases through distilled water. Flow rates for individual gases were monitored by the use of flowmeters (Airco Industrial Gases, Scientific Products, McGaw Park, Illinois) and the gas mixture before and after passing through the sealed jars, was monitored with a Beckman oxygen analyzer (Beckman Instrument Inc., Fullerton, California).

Effect of O_2 and GA combinations on germination. Twenty-five pepper seeds were placed on a No.3 Whatman filter paper in an open 5.5 cm Petri dish. Three milliliters of distilled water or GA_{4+7} at 100 or 1000 ppm was used to moisten the filter paper. The Petri dishes were placed into 450 ml glass jars, sealed and treated with 21 or 100% O_2 gas streams at 15 and 25°C as described above.

Germination data for all the experiments in this section were taken at 24 hour intervals and all treatments were replicated 4 times. Seeds with visible radicles were counted as germinated. Data taken for each treatment included total percent germination and mean number of days to germination (MDG) as described in Chapter II.

Respiratory measurements. One hundred pepper seeds per treatment were imbibed or germinated in the manner described above in 2l or 100% O_2 at 15 or 25°C for various time durations. The percent germination was recorded and all the seeds were quickly transferred to 15 ml Warburg respirometer flasks. One milliliter of distilled water was added to each flask with or without the respiratory inhibitors, KCN (Fisher Scientific Company, Fair Lawn, New Jersey) at 10 mM or salicylhydroxamic acid (SHAM) (Sigma Chemical Company, St. Louis, Missouri) at 1 or 10 mM. The center wells contained 0.4 ml of 10% KOH (weight/volume) and a filter paper wick. The 10% KOH was used to absorb the CO_2 during the O_2 uptake determinations. The KOH may have absorbed some CN from the respirometer flask environment (Purvis, 1980) but the concentrations of CN in the reaction medium remained sufficient to maximally inhibit O_2 uptake. This was apparent from the observation that any concentration of CN above 5 mM inhibited O_2 uptake to the same extent at both 15 and 25°C. The flasks were mounted onto a Gilson differential respirometer (Gilson Medical Electronics, Middleton, Wisconsin), submerged into the water bath and equilibrated at the appropriate treatment

temperature, 15 or 25⁰. The flasks for the high O₂ treatments were flushed through a sidearm on the respirometer flask with 100% O₂ as previously described (Umbreit et al., 1959). The flasks were equilibrated for one hour before commencing O₂ uptake measurements. Oxygen uptake was measured three times for 15 or 30 minutes with a 15 minute equilibration between readings for each treatment. Upon the termination of each experiment, the seeds were removed from the flasks, rinsed, dried at 70⁰ and weighed. The results were expressed on a dry weight basis. Each treatment was replicated 4 times.

Determination of total respiratory activity in germinating pepper seeds treated with GA₄₊₇ at 100 ppm was performed as described above, but without the use of any respiratory inhibitors. One hundred pepper seeds were imbibed or germinated in 9 cm Petri dishes in a constant temperature chamber at 15 or 25⁰C. Whatman No.1 and No.3 filter papers were placed in the bottom of the Petri dish and moistened with 0.05 M pH 6.5 sodium phosphate buffer or 100 ppm GA₄₊₇ in buffer solution. Seeds were removed at the appropriate time intervals from the Petri dishes, total percent germination was determined, and the seeds were placed into the Warburg respirometer flasks. One milliliter of the treatment solution was added to each flask. Oxygen determinations were taken as described above. Treatments were replicated 4 times.

Results

Oxygen concentrations above 21% increased the rate of pepper seed germination at 25°C (Table 3-1). A 10% O₂ concentration reduced the speed of germination when compared to the 21% level. No apparent trends in altering total germination were observed by increasing O₂ concentrations above 21% O₂, although there was a significant increase at 60% O₂. Reducing the O₂ concentrations to 10% at 25°C led to a reduction in total germination. At 15°C, the O₂ concentrations above or below 21% generally reduced the rate of germination. Total germination was unaffected by O₂ treatments at 15°C.

At 25°C, GA₄₊₇ application or 100% O₂ increased germination rate above 21% O₂ alone; however, combining both materials had no further effect on germination rate (Figure 3-1). Total germination was unaffected by these treatments at 25°C. All treatments at 15°C exhibited different germination rates (Figure 3-2). Oxygen concentrations of 100% delayed germination, while GA₄₊₇ and 21% O₂ increased the germination rate above 21% O₂ alone. When GA₄₊₇ was added to seeds treated in 100% O₂, it increased the germination rate but did not totally overcome the inhibitory effect of 100% O₂. No treatment affected total germination of pepper seeds at 15°C. Increasing the GA₄₊₇ concentrations to 1000 ppm did not decrease the inhibition effect of high O₂ on the germination rate of pepper seeds at 15°C (Table 3-2).

Total respiratory activity was greater at 25°C when pepper seeds were germinated in 100% O₂ compared to 21% O₂ (Figure 3-3).

Table 3-1. The effect of O_2 concentration on germination of pepper seed at 25 and 15°C

Oxygen concentration	25°		15°	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
(%)	(MDG)	(%)	(MDG)	(%)
10	12.0 a ^z	70.0 c	12.3 a	80.0 a
21 (air)	4.2 b	80.3 b	8.0 d	82.7 a
40	3.4 c	86.0 ab	9.0 c	85.0 a
60	3.2 c	88.0 a	8.6 cd	85.0 a
100	3.4 c	80.0 b	11.2 b	81.3 a

^zMean separation within columns by Duncan's multiple range test, 5% level.

Table 3-2. The effect of high O_2 concentration and various GA_{4+7} levels on germination of pepper seeds at 15°C

GA_{4+7} Concentration	Oxygen concentrations (%)			
	Germ. Rate		Total Germ.	
	21	100	21	100
(ppm)	(MDG)		(%)	
0	9.4 c ^z	14.6 a	80 a	73 a
100	8.5 d	12.7 b	82 a	85 a
1000	8.3 d	12.2 b	75 a	77 a

^zMean separation with main observations by Duncan's multiple range test, 5% level.

Figure 3-1. The effect of GA₄₊₇, 100 ppm, and O₂ concentration on the germination of pepper seeds at 25°C.
zMean separation within columns by Duncan's multiple range test, 5% level.

Figure 3-1

Figure 3-2. The effect of GA₄₊₇, 100 ppm, and O₂ concentration on germination of pepper seeds at 15°C
Mean separation within columns by Duncan's multiple range test, 5% level

Figure 3-3. Respiration (Cyanide sensitive and resistant components) of pepper seeds germinated in 21 or 100% O₂ environments at 25°C

Cyanide sensitive respiration was more active than cyanide resistant respiration (indicated by the hatched areas in Figure 3-3) throughout germination. The alternate respiratory pathway generally accounted for 5 to 8% of the total respiration.

At 15°C, respiratory activity was similar at the 21% and 100% O₂ (Figure 3-4). However, total respiration was higher in 21% O₂ at 6 and 12 days. Cyanide resistant respiration was low and no differences were observed between O₂ treatments throughout germination.

Respiratory activity was similar during the first two days of germination at 25°C between GA₄₊₇ treated and untreated seed (Figure 3-5). However, after day 4, GA₄₊₇ treated seeds had higher respiratory activity than untreated seeds. At 15°, respiration was similar until day 6 when respiration rates were higher in GA treated seeds. At both temperatures, GA treated seeds had higher respiration rates than untreated seeds after radicle emergence occurred.

Discussion

Oxygen concentrations above that found in air stimulated pepper seed germination rate at 25°C. When seeds imbibe water, O₂ becomes less soluble and less available to the embryo as germination temperatures increase (Come and Tissaoui, 1973). At 25°, diffusion of O₂ to the pepper embryo may be limited by solubility or covering structures. Increasing the O₂ concentration, 40% or greater, may allow the embryo to proceed with metabolic processes near optimal rates. This is supported

Figure 3-4. Respiration (cyanide sensitive and resistant components) of pepper seeds germinated in 21 or 100% O₂ environments at 15°C

Figure 3-5. Respiration of pepper seeds germinated at 15 and 25°C with or without GA₄₊₇, 100 ppm

by the increased total respiratory activity in 100% O_2 and that GA_{4+7} did not further stimulate germination rate in 100% O_2 . Ohmura and Howell (1960) found that when corn, soybean and barley tissues were soaked in water, a marked decrease in O_2 uptake occurred at 29°. This was overcome by substituting 100% O_2 for air. They felt that O_2 solubility and diffusion through the tissues was reduced by the water.

At 15°C, high O_2 concentrations inhibited the rate of pepper seed germination. Gibberellic acid $_{4+7}$, 100 ppm, applications could partially relieve the inhibitory effect that high O_2 concentrations had on germination rate. Increasing GA_{4+7} concentration to 1000 ppm in high O_2 did not further increase germination rate. At 15°, respiration rates were similar in 21 and 100% O_2 . Apparently, at 15° pepper seed respiration cannot utilize higher O_2 concentrations. Siegel and Gerschman (1959) found that excised bean embryos which received pure O_2 at 25° exhibited reductions in growth and enzymatic activity. Wheat root growth was inhibited by exposure to pure O_2 for longer than 2 days (Eliasson, 1958). The effect of pure O_2 on root growth was an inhibition of cellular elongation. Albaum et al. (1942) found that when oat seeds were soaked in oxygenated water, subsequent growth was inhibited when compared to aerated controls. Enzyme assays on the extracts of the oat seedlings indicated little or no change in cytochrome oxidase due to oxygenation, but, catalase and dehydrogenase activity was lower. The authors suggested that high O_2 levels may interfere with proteolytic breakdown

of the endosperm, preventing nitrogen transport from the endosperm to embryo, development of enzyme activity and growth. Thus, in the present study, at 15° lack of O₂ utilization may be related to altered metabolic processes at the low temperature. It should be noted that total germination was unaffected by increasing O₂ concentrations at 15°.

At 25°, cyanide sensitive and resistant respiration were at higher levels with 100% O₂. However on a percentage basis, total respiratory activity was the same as in air. At 15°, respiratory activity was similar, regardless of the concentration of the O₂ in the environment. In any event, cyanide resistant respiration regardless of O₂ treatment made up a small percentage of the total respiratory activity at both 15 and 25°.

Yentur and Leopold (1976) found that respiration in soybean seeds was dominated by cyanide resistant respiration during the first 4 to 5 hours of germination. After this time, the cytochrome pathway gradually took over and after 9 hours nearly all respiration was cyanide sensitive. Burguillo and Nicolas (1977) reported that in chick pea, respiration was dominated by the cytochrome pathway for the first 12 hours of germination shifting to more cyanide resistant respiration, reaching maximal amounts at 72 to 96 hours. They also found that high O₂ concentrations could initiate the appearance of the alternate respiratory pathway. Pepper seeds did not exhibit an increase in respiration in the alternate pathway in this study. These differences could be due to variations among

species and the time periods that data were recorded. However, McCaig and Hill (1977) found that 100% O_2 did not stimulate the appearance of the alternate pathway in wheat mitochondria isolated from etiolated coleoptiles. In fact, germination in 100% O_2 seemed to have little effect on either the cytochrome or cyanide resistant pathways.

The addition of GA to pepper seeds stimulated germination and respiration rates. However, respiration rates of GA treated seeds did not increase over untreated seeds until radicle emergence had already taken place. Therefore, GA does not appear to stimulate respiratory controlled metabolic activities prior to radicle emergence.

Summary

Higher than normal O_2 concentrations stimulated germination of pepper seeds at 25°C and inhibited germination rates at 15°. Low O_2 concentration, 10%, slowed germination rates at both temperatures. Gibberellic acid₄₊₇ increased germination rate at 15 and 25° in air. At 25° in 100% O_2 , GA did not further increase the germination rate. Gibberellic acid treatments did not overcome the inhibition 100% O_2 treatments had on the germination rate at 15°.

Total respiratory activity of pepper seeds germinated at 25°C was higher in 100% O_2 treated seeds compared to air treated seeds. However, high O_2 treatments did not affect the proportion of respiration which was in the cyanide sensitive and resistant pathways. Cyanide resistant respiration

comprised only a small percentage of total respiratory activity. At 15⁰, total respiration and the cyanide sensitive and resistant components were similar regardless of O₂ treatment.

The addition of GA to pepper seeds increased respiratory activity only after radicle emergence occurred at 15 and 25⁰C.

CHAPTER IV
MECHANICAL RESISTANCE OF THE SEED COAT AND ENDOSPERM
IN THE CONTROL OF *Capsicum annuum* L. GERMINATION
AT LOW TEMPERATURE

Seed coats and surrounding structures may have a profound influence on the ability of a seed to germinate (Mayer and Shain, 1974). These structures have been attributed to interfering with water uptake, gas exchange and diffusion of endogenous inhibitors. In addition, seed coats and surrounding endosperms may offer mechanical restriction to embryo growth (Mayer and Shain, 1974; Ikuma and Thimann, 1963).

In seeds that do not have hard seed coats or require scarification for water uptake, the endosperm in endospermic dicotyledonous seeds, may mechanically restrict embryo expansion preventing radicle emergence (Pavlista and Haber, 1970). Junttila (1973) described the mechanism of low temperature dormancy in *Syringa* species. Low temperature dormancy (9 to 15°) was imposed by an endosperm embedding the radicle and could be alleviated by removal of endosperm from around the radicle. The endosperm did not appear to contain significant quantities of germination inhibitors, and his results indicated that dormancy was mainly due to mechanical restriction of radicle emergence through the endosperm.

In lettuce, a 2-celled layer of endosperm, characterized by thick cell walls and dense cytoplasm, encases the lettuce

embryo (Jones, 1974). The ability of this endosperm to control or to mechanically restrain lettuce radicle emergence has been greatly debated. Ikuma and Thimann (1963) proposed that red light relieved endosperm restriction on the embryo in photosensitive lettuce varieties. They proposed that the red light triggered cellulolytic enzyme production in the embryo weakening the endosperm layer. Nabors and Lang (1971) also felt the lettuce endosperm restricted radicle emergence; however, this could be overcome by increasing the growth rate of the embryo. Red light could induce greater growth rate in the lettuce embryo enabling it to grow through the endosperm. Pavlista and Haber (1970) proposed that mechanical force of the growing embryo pushing against the endosperm and chemical, enzymatic, weakening of the endosperm were necessary for lettuce seed germination. This was suggested from their observation that when chemical weakening of the endosperm was inhibited, the embryos grew and buckled in the endosperm encasing but did not germinate.

Jones (1974) in microscopic and electron microscope investigations determined that the cell walls of lettuce endosperm were increasingly degraded with longer imbibition times and at the time of germination the walls were extensively broken down. In a study using the Instron Universal Testing Machine to test puncture forces of endosperm in lettuce seed, Tao and Khan (1979) determined that endosperm strength did not appear to be directly related to radicle protrusion. Halmer et al. (1976) reported that an enzymatic degradation of a mannose containing

polysaccharide, a major component of lettuce endosperm cell walls, was stimulated by red light or gibberellin. The appearance of this process did not correspond with radicle emergence and enzymatic activity increased markedly only after radicle emergence.

Pepper embryos are totally surrounded by endosperm materials making up the bulk of food reserves for the embryo and young seedling (Cochran, 1938). The experiments which follow investigate the possible mechanical control that the seed coat and endosperm have on germination of Capsicum annuum at optimum and suboptimal temperatures.

Materials and Methods

Decoated seeds. Air dry pepper seeds were decoated by inserting a scalpel or probe into the seed cavity and peeling back the seed coat material until a coatless seed was obtained. Twenty-five decoated or raw (untreated) pepper seeds were placed on a moistened No.1 and No.3 Whatman filter paper in a 9 cm Petri dish. The Petri dishes were placed in 15 or 25°C constant temperature chambers for germination.

Scarified endosperm test. Decoated air dry seeds were scarified by removing 0.5 mm of endosperm material directly in front of the radicle. Scarified seed and decoated control seeds were then germinated as described above.

Germination data for both experiments were taken at 24 hour intervals and all treatments were replicated 4 times.

Seeds with visible radicles were counted as germinated. Data taken for each treatment included total percent germination and mean number of days to germination (MDG) as described in Chapter II.

Endosperm strength measurements. The Instron Universal Testing Machine (Instron Engineering Corporation, Canton, Massachusetts) was used to determine resistance to radicle emergence. Seeds were previously treated with distilled water, 100 ppm GA_{4+7} or 100% O_2 when imbibed at 15 and 25°C. Fifty pepper seeds per treatment time were imbibed in 5 ml of distilled water or GA_{4+7} , on Whatman No.1 and No.3 filter paper in 9 cm Petri dishes at 15 or 25°C. Seeds treated with 100% O_2 were gassed in a continuous flow system at 15 and 25°C as described in Chapter III. After the appropriate pretreatment time, the seeds were immediately subjected to testing with the Instron. Each of the seeds was prepared immediately before measurement on the Instron. The seeds were decoated and approximately 1/4 of the seed, the area of radicle emergence, was excised. The radicle was teased out of the surrounding endosperm material with a microprobe, leaving a clean undamaged radicle cavity in the endosperm. The endosperm section was placed onto a No. 78 drill blank, 0.4 mm in diameter, attached to a basal load cell (Figure 4-1). The crosshead had a bolt placed in it with a 0.8 mm diameter counter hole drilled into it. The load cell was set to 100 g (0.98 newton) full scale load. Cross-head and chart speeds were 1.3 and 25.4 cm/minute respectively. Each treatment consisted of 5 seeds and all treatments were replicated 5 times.

Figure 4-1. The endosperm section placed onto the drill blank ready for puncture force measurement with the Instron Universal Testing Machine. The drill blank is held by a drill chuck mounted on a platform attached to the basal load cell. The crosshead of the Instron has a bolt placed in it with a counter hole drilled into it.

Figure 4-1

Results

Decoated seeds germinated more rapidly than control seeds at 25°C (Figure 4-2). There was no difference in total germination between the two treatments. At 15°C, germination rate was unaffected by decoating (Figure 4-3). Again, decoating did not affect the total germination of pepper seeds at 15°C.

When decoated pepper seeds were scarified by removing 0.5 mm of endosperm directly in front of the radicle, germination rates increased dramatically at both 15 and 25°C (Table 4-1). The scarification treatment did not affect the total germination of pepper seeds at 15 or 25°C.

Puncture force of the endosperm directly in front of the radicle decreased with increasing imbibition time at both 15 and 25°C (Figure 4-4). The endosperm resistance decreased more rapidly when imbibed at 25°C than at 15°C. Puncture force decreased to 0.3 to 0.4 newtons at both 15 and 25°C before the majority of radicle protrusion occurred.

Gibberellic acid treatment at 25°C reduced the endosperm resistance and increased the germination rate compared to untreated seeds (Figure 4-5). Gibberellic acid decreased endosperm resistance rapidly after 24 hours of treatment whereas in untreated seeds, endosperm resistance did not decrease appreciably until 48 hours. At 15°C, GA treatments had a similar effect on endosperm weakening and germination rate as at 25°C, except that the effects were slower (Figure 4-6).

Figure 4-2. The effect of seed coat removal on
pepper seed germination at 25°C
Mean separation within columns by
Duncan's multiple range test, 5% level.

Figure 4-3. The effect of seed coat removal on
pepper seed germination at 15°C
Mean separation within columns by
Duncan's multiple range test, 5% level.

Table 4-1. The effect of removing 0.5 mm of endosperm directly in front of the radicle on decoated pepper seed germination at 15 and 25°C

Treatment	25°		15°	
	Germ. Rate	Total Germ.	Germ. Rate	Total Germ.
	(MDG)	(%)	(MDG)	(%)
Decoated control	3.1 a ^z	72 a	9.1 a	94 a
Scarified Seed	1.7 b	78 a	3.4 b	90 a

^zMean separation between columns by Duncan's multiple range test, 5% level.

Figure 4-4

Mechanical resistance of the endosperm directly in front of the radicle and germination of pepper seeds as affected by germination temperature (15 and 25°C)

Figure 4-4

Figure 4-5. Mechanical resistance of the endosperm directly in front of the radicle and germination of pepper seeds as affected by GA₄₊₇, 100 ppm, at 25°C

Figure 4-5

Figure 4-6. Mechanical resistance of the endosperm directly in front of the radicle and germination of pepper seeds as affected by GA₄₊₇, 100 ppm, at 15°C

Figure 4-6

Gibberellic acid treatments decreased endosperm strength after 1 day; however, endosperm strength reductions occurred after 2 days of imbibition in untreated seeds at 15⁰.

When seeds were exposed to high O₂ concentrations at 25⁰C, endosperm puncture force decreased and germination rate increased compared to the air control (Figure 4-7). Decreases in the puncture force of the endosperm did not become apparent in high O₂ or air treated seeds until the second day of treatment. Conversely, high O₂ treatments at 15⁰ slowed the rate at which endosperm strength decreased (Figure 4-8). Germination in 100% O₂ was slower than in air. No significant reduction in endosperm puncture force was evident until after the second day in the control seeds or the fourth day in high O₂ treated seeds. Endosperm strength in 100% O₂ treated seeds was higher at all times compared with the corresponding air treatments.

Discussion

Decoated pepper seeds germinated at a faster rate than coated seeds at 25⁰C. The removal of the seed coat at 25⁰ may remove a water barrier to O₂ movement or gas exchange allowing germination to proceed at a faster rate as was the case in elevated O₂ environments (Chapter III). The removal of the seed coat at 15⁰ did not increase germination rate possibly because there already was a sufficient O₂ supply to the embryo for maximal metabolic and germination activities at this temperature. Junttila (1973) found similar results with Syringa species. The seed coat and endosperm inhibited O₂ uptake at high temperatures but not at low temperatures.

Figure 4-7.

Mechanical resistance of the endosperm directly in front of the radicle and germination of pepper seeds as affected by 100% O₂ at 25°C

Figure 4-7

Figure 4-8. Mechanical resistance of the endosperm directly in front of the radicle and germination of pepper seeds as affected by 100% O_2 at 15°C

Figure 4-8

There does not appear to be any mechanical restriction to germination imposed by the seed coat per se. If there was, seed coat removal would be expected to increase germination rates at 15 and 25°C.

Scarification of the endosperm material directly in front of the radicle dramatically reduced the time to germination at 15 and 25°C. Removal of 0.5 mm of endosperm material led to germination. Removal of more than 0.5 mm caused the embryos to be "pushed" from the endosperm by imbibitional pressure and damage to the radicle was usually evident. This points to a mechanical resistance imposed by the endosperm; but, the effect of the mechanical restriction was greater at the lower temperature. Junttila (1973) improved germination in Syringa reflexa at low temperatures by total removal of the endosperm around the radicle.

No inhibitor was found at low temperatures (Chapter II). Water uptake by pepper seeds was similar at 15 and 25°C (Chapter II) and O₂ levels found in air did not affect germination rate at 15°C but may be a factor at 25°C in slowing germination (Chapter III). However, mechanical resistance of the endosperm does appear to be directly affected by germination temperatures as measured by the Instron Universal Testing Machine. The amount of mechanical resistance imposed by the endosperm on the radicle in pepper seeds is dependent on imbibition time, germination temperature and treatment.

A hypothesis was proposed by Pavlista and Haber (1970) that germination of lettuce seed requires a) a mechanical force of a growing embryo pushing against the endosperm and b) chemical or enzymatic weakening of the endosperm. The studies presented here, do not explain how pepper seed germination proceeds but indicate that endosperm strength has to be reduced by embryo growth and/or reduced by enzymatic weakening before germination will occur. The ability of GA_{4+7} to reduce endosperm resistance at 15 and 25°C offers little evidence to the mechanics of pepper seed germination. However, stimulation of cell division by GA does not appear to be the cause of increased germination rates (Chapter II). Junttila (1973) stated that conditions which favor germination of Syringa embryos, high temperature, chilling and GA_3 , favored enhanced radicle elongation. Gibberellic acid $_{4+7}$ was able to stimulate pepper germination rates in osmotic solutions which have been associated with inhibition of cellular expansion (Chapter II). Gibberellic acid can stimulate enzymatic activity in monocot seeds (Varner and Chandra, 1964; Mayer and Shain, 1974) and in lettuce seeds after radicle emergence (Halmer et al., 1976). Thus, the possibility exists that GA may regulate pepper seed germination by controlling radicle elongation and/or enzymatic degradation of endosperm materials.

Without further study, O_2 control of endosperm weakening is hard to elucidate. At 25°C, pepper seeds which germinated in high O_2 concentrations, had a higher respiratory rate thus higher metabolism than seeds germinated in air (Chapter III).

At 15⁰, higher O₂ concentrations did not increase respiration rate. At this temperature, endosperm strength decreased at a slower rate than occurred in air. In excised bean embryos cultured in 100% O₂, reductions in growth and enzymatic activity were found (Siegel and Gerschman, 1959). Eliasson (1958) reported that pure O₂ inhibited wheat root growth due to inhibition of cell elongation. Albaum et al. (1942) suggested that subsequent growth of oxygenated oat seeds was inhibited due to high O₂ interference with proteolytic breakdown of the endosperm, preventing nitrogen transport from the endosperm to embryo, development of enzyme activity and growth. Yet, high O₂ did not affect cytochrome oxidase. This may indicate that the reduced endosperm weakening in pepper seeds germinated in 100% O₂ at 15⁰ may be due to the inability of embryos to utilize the greater O₂ levels at 15⁰ for respiration, than at 25⁰. These higher endogenous levels of O₂ may then be adversely affecting radicle cell elongation or enzymatic breakdown of pepper endosperm, thus slowing germination.

Summary

Decoated pepper seeds germinated faster at 25⁰C, but not at 15⁰ when compared to coated seeds. The seed coat did not appear to impose a mechanical restriction on pepper seed germination. Scarification of the endosperm material directly in front of the radicle dramatically reduced the time to germination at both 15 and 25⁰.

The amount of mechanical resistance imposed by the endosperm on radicle emergence before germination was measured using the Instron Universal Testing Machine. Endosperm strength reduced as imbibition time increased. Imbibition at 25°C caused a faster reduction in the puncture force when compared to seeds imbibed at 15°C. The reduction in puncture force corresponded with the ability of pepper seeds to germinate. The majority of radicle emergence occurred at 15 and 25°C after the puncture force was reduced to 0.3 to 0.4 newtons.

Applications of GA₄₊₇, 100 ppm, stimulated earlier germination at 15 and 25°C and correspondingly increased the rate in which the puncture force of the endosperm decreased. High O₂ concentrations at 25°C had similar effects on germination and endosperm strength as GA₄₊₇. However, at 15°C high O₂ concentrations inhibited germination rate and the rate at which endosperm strength decreased.

CHAPTER V
GIBBERELIC ACID STIMULATION OF EARLY ENDOSPERM
DEGRADATION IN Capsicum annuum L.

The endosperm, in endospermic dicotyledonous seeds, may mechanically restrict radicle emergence (Pavlista and Halmer, 1970). In lettuce, a two-celled layer of endosperm, characterized by thick cell walls and dense cytoplasm, encases the lettuce embryo (Jones, 1974). The means by which the lettuce radicle penetrates the endosperm for germination, and the role of endosperm in lettuce seed germination has been greatly debated. Ikuma and Thimann (1963) felt that the main effect of light on photosensitive lettuce seeds was exerted primarily on the mechanical properties of the endosperm. They proposed that red light initiated the production of an enzyme enabling the radicle to penetrate through the endosperm. However, no detection of an enzyme was found in radicle but the cotyledons did produce such enzymes. Nabors and Lang (1971) proposed that lettuce endosperm restricted radicle emergence. This restriction could be overcome by increasing the growth rate of the embryo. Red light was capable of increasing the growth rate of lettuce embryos, possibly enabling it to grow through the endosperm.

Pavlista and Haber (1970) determined that increased growth of the embryo and enzymatic weakening of the endosperm

were needed for lettuce germination. In support of this theory, they observed that when weakening of the endosperm was prevented the embryo grew and buckled inside of the endosperm but did not germinate normally. Jones (1974) in microscopic and electron microscopic investigations determined that the cell walls of the lettuce endosperm degraded with increased imbibition time and were extensively broken down at the time of germination. He found that digestion of the endosperm walls proceeded from the plasmalemma outward in each cell, thus suggesting the enzymes may be produced by the endosperm cells themselves. Tao and Khan (1979) using the Instron Universal Testing Machine to test endosperm strength during germination concluded that endosperm strength was not directly related to radicle protrusion. This was partially supported by Halmer et al. (1976) where they found enzymatic degradation of a mannose containing polysaccharide, the major component of lettuce endosperm cell walls, increased markedly only after radicle emergence.

Pepper seed endosperm totally surrounds the pepper embryo and mechanically restricts radicle emergence (Chapter IV). A reduction in endosperm thickness greatly reduced the time to germination at 25 or 15°C. Endosperm strength decreased as imbibition time increased and the rate of decrease was affected by imbibition temperature and GA₄₊₇ application. The means by which the radicle penetrates this endosperm is not known. It is not apparent if the enzymatic and/or increased growth of the embryo are needed for germination. However,

increased embryo growth before radicle emergence has been indicated in Chapters II and III as part of the germination process. In the experiments which follow, visual observations were made to clarify the manner in which the pepper radicle penetrates the restrictive endosperm layer during germination.

Materials and Methods

Scanning electron microscope observations of pepper seed.

Longitudinal sections (parallel to the plane of the embryo) of pepper seeds imbibed one day at 25°C or whole seeds imbibed four days at 25°C were investigated. The longitudinal sections were prepared by halving the imbibed seeds with a single edge razor blade. The moist seed sections and whole seeds were then mounted on aluminium stubs with tube coat (G.C. Electronics Co., Rockford, Illinois) and viewed immediately in a Hitachi scanning electron microscope, model S-450, using an accelerating voltage of 20 kV.

Radicle penetration during germination. Pepper seeds were imbibed or germinated at 15 or 25°C in constant temperature chambers for various lengths of time on a moistened No.1 and No.3 Whatman filter paper in a 9 cm Petri dish. The filter papers were moistened with either 5 ml of distilled water or 5 ml of 100 ppm GA₄₊₇. After the appropriate treatment time (0, 1, 2, 3 and 4 days at 25°C or 1, 2, 4, 6, 7 and 8 days at 15°C) the seeds were removed from the chambers, decoated then examined and photographed with a Zeiss Stereomicroscope SR (Zeiss, Oberkochen, West Germany).

Endosperm degradation during germination. Pepper seeds were prepared for sectioning and viewing of the endosperm directly in front of the radicle. The seeds were imbibed in distilled water or GA₄₊₇, 100 ppm, solutions as described above at 15 or 25°C. After the appropriate treatment time (same times as above) the seeds were removed from the chambers, decoated and dissected. The area directly around the radicle tip was removed and placed immediately into Karnosky's chemical fixative, a cacodylate buffered glutaraldehyde-formaldehyde (3% each) fixative (Dawes, 1971). The endosperm-radicle pieces were then fixed for 3 hours under aspiration, washed for 3 hours in cacodylate buffer under aspiration, then post-fixed in 2% osmium tetroxide in distilled water for 12 hours. The osmium tetroxide was changed and the pieces were placed under aspiration for 2 hours, then rinsed in distilled water for 2 hours under aspiration. The endosperm-radicle pieces were then dehydrated in a graded ethanol series over 24 hours with a final period of 2 hours in propylene oxide. Each step received at least 2 hours of aspiration. The dehydrated tissues were then infiltrated in a graded ethanol-Spurr's epon resin mixture (Polysciences, Inc., Warrington, Pennsylvania) under aspiration with all steps receiving a 3 hour treatment. The endosperm-radicle sections were then embedded in fresh Spurr's resin in plastic boats and polymerized overnight in a 70°C oven. All fixation, dehydration and infiltration steps were performed under a hood at room temperature, $\approx 22^{\circ}$.

One micron sections were cut from the plastic-embedded tissues with a Sorvall Ultra Microtome, MT 5000 (Dupont Company

Instrument Products, Biomedical Division, Newtown, Connecticut) using glass knives (LKB Knife Maker, Type 7801 B, LKB Instruments, Inc., Rockville, Maryland). Care was taken to section in the center of the radicle and endosperm to supply median sections for observation. The sections were then examined and photographed with phase-contrast optics at 100 X and 400 X using a Nikon Optiphot light microscope (Nikon, Japan).

Results

The pepper embryo is totally surrounded by endosperm material which is fairly uniform in nature except directly in front of the radicle where the endosperm cells become smaller and more tightly packed (Figure 5-1A). The radicle emerged through the endosperm material and the micropylar cavity on the fourth day of imbibition at 25°C (Figure 5-1B).

The pepper endosperm directly in front of the radicle became visibly enlarged after 3 days of imbibition in distilled water at 25°C (Figure 5-2A). When pepper seeds were imbibed in GA₄₊₇ solutions at 25°C, enlargement or protrusion of the endosperm directly in front of the radicle occurred after 2 days and radicle emergence occurred on the third day (Figure 5-2B). After 4 days of imbibition, both untreated and GA₄₊₇ treated seeds had radicles protruding through the endosperm (Figure 5-2C). Radicle elongation was greater and root hairs were apparent on the GA treated seeds.

At 15°C, pepper seeds imbibed in distilled water appeared similar in their appearance through the sixth day (Figure 5-3A).

Figure 5-1. Scanning electron micrographs of pepper seed
A) Longitudinal section of 1 day imbibed seed at
25°C, 32X B) Germinated seed, imbibed 4 days at
25°C, 86X

Figure 5-1

Figure 5-2. Radicle penetration through decoated pepper seeds imbibed at 25°C 8X A and B- top left, dry seed; top right, imbibed 1 day; bottom left, imbibed 2 days; bottom right, imbibed 3 days. A) Imbibed in distilled water B) Imbibed in GA₄₊₇, 100 ppm C) Imbibed 4 days in: left, distilled water; right, GA₄₊₇, 100 ppm

Figure 5-2

Figure 5-3. Radicle penetration through decoated pepper seeds imbibed at 15°C 8X A and B)- top left, dry seed; top center, imbibed 1 day; top right, imbibed 2 days; bottom left, imbibed 4 days; bottom center, imbibed 6 days; bottom right, imbibed 7 days.

A) Imbibed in distilled water B) Imbibed in GA₄₊₇, 100 ppm C) Imbibed 8 days in: left, distilled water; right, GA₄₊₇, 100 ppm

Figure 5-3

On the seventh day, the endosperm material in front of the radicle was markedly protruding. Gibberellic acid treated seeds at 15⁰ also exhibited the same protrusion of endosperm (Figure 5-3B). However, the change in the physical appearance of the endosperm occurred on the sixth day and radicle emergence on the seventh day. After 8 days at 15⁰, both untreated and GA₄₊₇ treated seeds had radicles emerged (Figure 5-3C). Greater radicle growth and root hairs were apparent on GA treated seeds.

Pepper seeds imbibed 1 day at 25⁰C with or without GA₄₊₇ were similar in appearance (Figure 5-4). The endosperm in both untreated and GA treated sections were 7 to 9 cells thick with no loss of cellular integrity or compression noted.

Endosperm sections imbibed 2 days at 25⁰C in distilled water were not different when compared to 1 day sections (Figure 5-4C and 5-5A). However, endosperm sections from 3 or 4 day untreated imbibed seeds had marked reductions in endosperm thickness (Figures 5-5B and 5-5C). Cellular integrity adjacent to the radicle was lost and compression of cells in front of the radicle was evident. The endosperm on the side of the radicle after 4 days of imbibition did not exhibit any of the characteristics demonstrated by the endosperm in front of the radicle after the same imbibition time (Figure 5-5D).

Endosperm of GA treated pepper seeds imbibed at 25⁰C for 2 or 3 days had a reduced thickness, loss of cellular integrity and compressed cells in front of the radicle (Figures 5-6A and 5-6B).

Figure 5-4. Light microscope viewed median sections of pepper seed endosperm directly in front of the radicle. Seeds were imbibed 1 day at 25°C. A) Imbibed in distilled water; X100 B) Imbibed in GA₄₊₇, 100 ppm, X100 C) Imbibed in distilled water, X400 D) Imbibed in GA₄₊₇, 100 ppm, X400

Figure 5-4

Figure 5-5. Light microscope viewed median sections of pepper seed endosperm directly in front of the radicle, X400. Seeds were imbibed in distilled water at 25°C.
A) 2 days B) 3 days C) 4 days
D) Endosperm on the side of the radicle, imbibed 4 days

Figure 5-5

Endosperm sections from untreated pepper seeds imbibed at 15°C did not exhibit any change in endosperm characteristics until after the fifth day of imbibition (Figure 5-7). On the sixth day of imbibition, some loss of cellular contents was noted but no change in the thickness of endosperm occurred (Figure 5-7C). After 7 days, untreated endosperm sections were reduced in thickness, cellular integrity was lost, and compression of cells in front of the radicle was evident (Figure 5-7D).

Endosperm sections from GA treated seeds at 15°C did not exhibit any change until after 4 days of imbibition (Figure 5-8). On the fifth day of imbibition, some loss of cellular contents was noticed but endosperm thickness was not changed (Figure 5-8B). Six days of imbibition in GA solutions resulted in reduced endosperm thickness, lost cell integrity and compressed cells (Figures 5-8C and 5-8D).

Discussion

The endosperm of pepper seeds was demonstrated to mechanically restrict radicle emergence (Chapter IV). The amount of mechanical force needed to puncture the endosperm decreased with increased imbibition time and the rate was controlled by imbibition temperature and seed treatment. However, the means by which the radicle penetrated the endosperm previously was not fully understood.

Little change occurred to the endosperm material in front of the radicle until 1 day before radicle emergence at either 15 or 25°C, with or without GA treatment. At that time, the

Figure 5-6. Light microscope viewed median sections of pepper seed endosperm directly in front of the radicle, X400. Seeds were imbibed in GA₄₊₇, 100 ppm, at 25°C
A) 2 days B) 3 days

Figure 5-6

Figure 5-7. Light microscope viewed median sections of pepper seed endosperm directly in front of the radicle, X400. Seeds were imbibed in distilled water at 15°C.
A) 4 days B) 5 days C) 6 days D) 7 days

Figure 5-7

Figure 5-8. Light microscope viewed median sections of pepper seed endosperm directly in front of the radicle, X400. Seeds were imbibed in GA₄₊₇, 100 ppm, at 15°C.
A) 4 days B) 5 days C and D) 6 days

Figure 5-8

cellular mass in front of the radicle appeared to be compressed and it decreased in thickness. This led to a protrusion of the outer layers of the endosperm. Gibberellic acid stimulated this process by 1 day regardless of temperature. Low temperature delayed this process by 4 days. The breakdown of the endosperm material appeared to be very localized in front of the radicle. Similarly, Pavlista and Valdovinos (1978) examined the surface of lettuce endosperm with a scanning electron microscope for visual indications of germination preceding radicle emergence. Cracks and pits in the walls and surface of endosperm cells occurred and increased with time to 12 hours after imbibition. The percentage of lettuce seeds with these openings corresponded to the amount of seeds which later germinated.

In pepper seeds, the visual indications of germination occurred 1 day before radicle emergence. However, the amount of puncture force required to penetrate the endosperm decreased well before visual reductions in the endosperm thickness occurred (Chapter IV). This indicated that the endosperm cells in front of the radicle were weakened before visual degradation was apparent. Jones (1974) found cell wall degradation in lettuce increased with increasing imbibition time and digestion of the endosperm cell walls proceeded from the plasma-lemma of each cell outward. This indicated that the cells were responsible for their own cellulolytic enzyme production and breakdown. However, the outer endosperm cell walls next to

the integument never degraded and digestion always proceeded in a direction away from these walls. In pepper seeds, the direction of cellular degradation appeared to occur from the radicle outward with no degradation of the exterior cell walls of the endosperm.

Increased embryo growth potential is apparently necessary and an integral part of pepper seed germination. This is supported by the fact that conditions which favor increased embryo growth potential favored increased germination rates: GA treatments (Chapter II) and high oxygen treatments at 25°C (Chapters III and IV). It has been found in lilac species, that conditions which favor increased embryo growth favored germination (Junttila, 1973). However, this does not preclude endosperm enzymatic activity as a necessary and regulatory component of pepper seed germination. Gibberellic acid treatments are known to stimulate enzymatic activity in monocot seeds (Mayer and Shain, 1974) and in lettuce after radicle emergence (Halmer et al., 1976). Enzymatic activity could be stimulated in pepper seeds in advance or in response to increased embryo growth. Thus, enzymatic activity could weaken the endosperm and cause the decrease in endosperm puncture force in front of the radicle well in advance of actual visual cell degradation and compression.

Summary

The external appearance of the endosperm of pepper seeds did not change until 1 day before radicle emergence. The day before radicle emergence, the endosperm in front of the radicle enlarged and protruded outward. Gibberellic acid applications decreased the time to the appearance of the protruding endosperm by 1 day at 15 and 25°C. Reducing the imbibition temperature from 25 to 15°C delayed this occurrence by 4 days.

The protrusion of the endosperm correlated with breakdown and loss of endosperm cellular integrity and a reduction in endosperm thickness directly in front of the radicle as observed in prepared endosperm sections. Gibberellic acid treatment stimulated the breakdown of the endosperm by 1 day at 15 and 25°C. Imbibition at 15°C delayed the degradation of the endosperm by 4 days. The degradation of endosperm cells was localized previous to radicle emergence and occurred only in front of the radicle.

CHAPTER VI
THE APPEARANCE OF CELL WALL DEGRADING ENZYMES
DURING GERMINATION OF Capsicum annuum L.

The presence of either a low temperature germination inhibitor, restricted water uptake or restricted gas exchange was not responsible for the slow germination rate of pepper seeds at moderately low temperatures. No low temperature germination inhibitors were found in pepper seeds (Chapter II). Imbibition rates were similar at 15 and 25°C (Chapter II) and at 15% oxygen enrichment did not promote increased germination rates (Chapter III). However, mechanical resistance of the endosperm did directly affect the germination rate of pepper seeds (Chapter IV). The amount of mechanical resistance imposed by the endosperm on the radicle was dependent on imbibition time, imbibition temperature, and seed treatment. Gibberellic acid₄₊₇ was effective in stimulating the germination rate of pepper seeds at 15 and 25°C (Chapter II) and also the rate in which the endosperm strength in front of the radicle decreased (Chapter IV).

The means by which the radicle moves through the endosperm has not been totally understood. However, increased embryo growth potential is apparently necessary for radicle emergence to occur (Chapter II). Endosperm resistance decreased in front of the radicle well in advance of visual endosperm degradation, indicating a weakening of endosperm material before endosperm

cellular compression by the radicle (Chapters IV and V).

In this section, enzymatic activity on pepper seed cell wall preparations was determined to investigate the possibility of increased enzymatic activity prior to radicle emergence. Untreated and GA₄₊₇ treated seeds were imbibed at 15 and 25°C for various time intervals and extracted and assayed for cell wall enzymatic activity.

Materials and Methods

Pepper seed cell wall preparations. Pepper seeds (100 g) were boiled in distilled water for two hours to kill the seed and seed enzymes. The boiled seeds were homogenized in distilled water for five minutes using a Waring blender at maximum speed. The homogenate was filtered through premoistened Miracloth and washed with 1 l of distilled water. The remaining material was rehomogenized in 50% (volume/volume) redistilled acetone for five minutes at maximum speed. The homogenate was refiltered through acetone moistened Miracloth and washed with 500 ml of redistilled acetone. The residue (cell walls) were transferred to a Buchner funnel fitted with a No.1 Whatman filter paper and the liquid was removed by applying aspiration. The dry cell walls were stored under vacuum in a desiccator until used.

Enzyme extraction. Fifty pepper seeds (air dry - 6% moisture) were used per extraction treatment and their air dry weight was recorded before imbibition. The seeds were imbibed in 5 ml of distilled water or GA₄₊₇, 100 ppm, in 9 cm Petri dishes

containing Whatman No.1 and No.3 filter papers. After the appropriate treatment times (0,1,2,3,4 and 5 days at 25°C; 0,2,4,6 and 8 days at 15°C) percent germination was recorded, the seeds were blotted briefly, and extracted in 20 ml of ice-cold phosphate/citrate buffer (pH 7.0; 16.6 ml 0.2 M Na₂HPO₄ and 3.4 ml 0.1 M citric acid) (Halmer et al., 1976) by homogenizing for 5 minutes with a Sorvall Omni-mixer. The homogenate was centrifuged at 17,000 X g for 10 minutes at 5°C. The volume of the supernatant was determined and recorded before storage at -20°C. Each treatment was replicated four times.

Enzyme assay. Five milliliter samples of the crude extracts were dialyzed against the assay buffer (pH 5.0; 0.02 M phosphate/0.01 M citrate) (Halmer et al., 1976) for 24 hours at 4°C. The dialyzed extract was centrifuged at 17,000 X g for 10 minutes at 5°C and the volume of the supernatant recorded. One milliliter of dialyzed enzyme solution was boiled for 15 to 20 minutes in a boiling water bath to kill any active enzymes as a control for the assay. One milliliter samples of the dialyzed enzyme solutions or boiled enzyme control were added to 15.0 to 18.0 mg (dry weight) of the prepared pepper seed cell walls or galactomannan substrate (Sigma Chemical Company, St. Louis, Missouri) in small test tubes. The enzyme solutions contained 0.02% sodium azide to suppress microbial growth (Huber, 1980). The tubes were capped and incubated at 30°C for 24 hours. After incubation, the samples were filtered through glass fiber filters (Whatman Gf/C) and the volume of the filtrate was brought up to 4 ml. The filtrate was assayed for total sugars using the phenol-sulfuric assay (Hodge and Hofreitor, 1962). The data

were expressed as μg glucose-equivalents/mg cell wall or substrate (dry weight)/g seed extracted (dry weight). Carboxymethylcellulose was used as a substrate for a viscometric assay with pepper seed extracts (Halmer et al., 1976).

Results

The quantity of total sugars (expressed as glucose-equivalents) liberated were greater at each time tested when enzyme extracts were prepared from GA_{4+7} treated seeds compared to untreated seeds imbibed at 25°C (Figure 6-1). The capacity of the extracts to degrade isolated cell walls increased with increasing imbibition time and large amounts were found after the 5 day imbibition time.

Enzyme preparations from GA_{4+7} treated seeds imbibed at 15°C demonstrated a greater capacity to degrade pepper seed cell walls at 2, 4 and 8 days but not at 6 days when compared to enzyme preparations from untreated seeds (Figure 6-2). Increased liberation of total sugars from cell walls was evident with enzyme preparations derived from GA and untreated seeds prior to the onset of radicle emergence. However, the greatest degradation of cell wall materials occurred after radicle emergence.

Enzyme solutions prepared from GA treated seeds liberated total sugars from the galactomannan substrate to a greater extent than did enzyme solutions prepared from untreated seed (Figure 6-3). The greatest amount of substrate degradation in both treatments occurred employing enzyme derived from seeds one day after greater than 50% of the radicles emerged.

Figure 6-1.

Pepper seed cell wall degrading activity from enzyme preparations extracted from GA₄₊₇, 100 ppm, treated and untreated pepper seeds after various imbibition times at 25°C.
Data expressed as μg glucose-equivalents/mg cell wall (dry weight)/g seed extracted (dry weight).

Figure 6-1

Figure 6-2. Pepper seed cell wall degradation activity from enzyme preparations extracted from GA₄₊₇, 100 ppm, treated and untreated pepper seeds after various imbibition times at 15°C

Data expressed as μg glucose-equivalents/mg cell wall (dry weight)/g seed extracted (dry weight).

Figure 6-2

Figure 6-3. Glactomannan degrading activity from enzyme preparations extracted from GA₄₊₇, 100 ppm, treated and untreated pepper seeds after various imbibition times at 25°C. Data expressed as µg glucose-equivalents/mg substrate (dry weight)/g seed extracted (dry weight).

Figure 6-3

Enzyme solutions prepared from GA treated and untreated seeds exhibited little or no activity when used in a viscometric assay with carboxymethylcellulose (data not presented).

Discussion

Cell wall degrading activity was present and increased well before radicle emergence occurred in pepper seeds. Gibberellic acid treatments stimulated this activity. However, the greatest enzymatic activity was found after radicle emergence. Additionally, pepper seed enzyme preparations degraded a galactomannan and appeared to prefer this substrate over pepper seed cell walls. In a viscometric assay employing carboxymethylcellulose as substrate, little or no activity was found even with enzyme extracts prepared from 5 day imbibed seed at 25°C (data not presented).

Halmer et al. (1976) did not find increased enzyme activity during the period prior to radicle emergence in lettuce. However, similar to the observations made here, they did find marked increases in enzyme activity after radicle emergence. The enzyme assay employed by Halmer et al. (1976) exclusively determined mannanase activity despite reports from other researchers who found or speculated that a cellulase type activity occurred prior to radicle emergence in lettuce seeds (Pavlista and Valdovinos, 1975; Ikuma and Thimann, 1963). Halmer et al. (1976) reasoned that due to the composition of lettuce endosperm cell walls, mostly mannose containing polysaccharides, cellulases would not likely

lead to major cell wall breakdown. However, Bewley and Halmer (1980) stated that mannanase although the most important polysaccharidase in terms of lettuce cell wall composition, was only one polysaccharidase involved in endosperm cell wall degradation. Activity of other enzymes along with low levels of mannanase activity might be sufficient to weaken endosperm cell walls preceding radicle emergence.

Halmer et al. (1976) documented that many seed endosperms are rich in manno-polysaccharides and speculated that this compound may be a widespread feature of endosperm containing seeds. The fact that pepper enzyme extracts reacted with the galactomannan substrate may allow speculation that mannose-containing polysaccharides are a component of pepper seed endosperm. However, further investigation would be necessary to ascertain the composition of pepper endosperm.

The increased enzymatic activity prior to radicle emergence observed with pepper seeds may cause the reduction in endosperm strength (Chapter IV) which occurred before any visual degradation of the endosperm was observed (Chapter V). Reduced enzyme activity at 15⁰C may account for the slower germination rate and slower rate at which endosperm strength is decreased. Gibberellic acid stimulation of germination rates at 15 and 25⁰ corresponded with increased enzyme activity at both temperatures. Thus, germination of pepper seeds at 15 and 25⁰ may require enzymes that weaken the endosperm in front of the radicle and increased growth of the embryo (Chapter II) to push through the endosperm. Low temperatures appear to affect both enzymatic

activity and the ability of the pepper embryo to elongate, thus slowing the germination rate.

Summary

Pepper seed cell wall degrading enzyme activity increased with increasing imbibition times. Gibberellic acid₄₊₇ treatments and optimal germination temperatures stimulated this enzyme activity. However, the greatest amount of enzyme activity occurred after radicle emergence.

Pepper seed enzyme preparations degraded a galactomannan substrate, indicating that manno-polysaccharides may be a component of pepper seed endosperms. Carboxymethylcellulase activity was not found in the extracts.

APPENDIX
GERMINATION RATE OF PEPPER SEEDS AT VARIOUS TEMPERATURES

Germination rate of pepper seed was determined at various constant temperatures with or without GA₄₊₇, 100 ppm. The germination rate increased as the temperature increased to 30°C. Higher temperatures than 30°C inhibited germination rates. Gibberellic acid₄₊₇ stimulated germination rate of pepper seeds at all temperatures tested and allowed germination of pepper seeds at 40°C, although total germination was reduced compared to the other treatments (data not presented).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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