REDUCTION IN SEEDLING VIGOR AND CHANGES IN METABOLISM DURING GERMINATION RELATED TO MECHANICAL ABUSE OF BEAN (PHASEOLUS VULGARIS L.) SEED

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ABSTRACT

REDUCTION IN SEEDLING VIGOR AND CHANGES IN METABOLISM DURING GERMINATION RELATED TO MECHANICAL ABUSE OF BEAN (PHASEOLUS VULGARIS L.) SEED

By

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Seeds of several bean (*Phaseolus vulgaris* L.) varieties were mechanically abused by dropping 9.2 m onto a steel plate. The impaction treatment physically damaged up to 37% of the seeds, depending on their moisture content and variety. These seeds were not used in further studies. Germination and vigor of the remaining seeds planted in the field or greenhouse and in controlled environment chambers was influenced by the germination environment as well as by variety and moisture content during impaction. Emergence was reduced as much as 50%, and up to 62% of the emerging seedlings were morphologically defective. Cotyledon damage, from transverse cracking to complete severence, was the most common abnormality. Severence of the epicotyl and various other breaks, lesions and abnormalities were also prevalent.

Investigation of the growth potential of morphologically normal seedlings indicated a significant decline

in vigor of seedlings grown from damaged seeds. Internode or total seedling elongation was consistently reduced when the germination environment imposed a stress condition. A small delay in flowering and a decrease in yield of some varieties were observed but no reduction in vigor was apparent in several other growth parameters measured. The decline in vigor due to cryptic injury resulting from mechanical abuse was about 1/3 as great as the loss in vigor caused by excision of one cotyledon.

The growth rate of excised embryonic axes was not inhibited by seed abuse, implying that the injury effect is either in the cotyledons or in the transport mechanism between cotyledons and axes. But a microscopic examination revealed no visible injuries or accumulation of wound-response callose in the connective tissues.

Respiration of seeds during the first 30 hours of germination was likewise not influenced by seed damage. However, amylases and succinate-cytochrome c reductase were slightly delayed in reaching peak activity during early seedling growth. These enzymes are believed to be synthesized *de novo* during germination. Cytochrome oxidase, which is apparently present in non-imbibed seeds, was not affected by seed abuse. These facts suggest that the cryptic injury effect may involve protein synthesis.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION

Seed quality encompasses many factors relating to the condition of a seed lot, but in the final practical sense, the most important criterion of seed quality is production potential. Performance potential is dependent on several attributes such as germination, seedling vigor and freedom from disease. Each of these in turn is influenced by a wide range of individual factors.

Most of the factors determining the performance potential of a seed lot can be drastically reduced by maltreatment of the seed before planting. Navy bean seeds are particularly vulnerable to mechanical abuse, especially at low seed moisture contents. It is well known that physical abuse lowers bean seed quality by splitting the seeds; cracking the seed coats, cotyledons, and embryo axes; and decreasing overall germination performance. As early as 1930 Harter (27) also noted that mechanically damaged seeds produce morphologically abnormal seedlings. Since then research has been largely concerned with the gross morphological effects of mechanical abuse and their influence on subsequent performance.

Several reports (10, 38, 47) have suggested that physical abuse of bean seeds may also have more subtle effects on the resulting seedlings: in some cases apparently normal seedlings produced from damaged seeds were less vigorous than seedlings from non-damaged seeds. But these observations included the effects of at least slight morphological defects, such as cracked cotyledons, resulting from the damage treatments.

A drastic delay in maturity was observed when severely damaged navy bean seeds were planted in a field demonstration plot[1] (Figure 1). This striking display of seed injury effects might be largely attributable to abnormal or defective seedlings, but the delayed maturity seemed to be very uniform suggesting that even morphologically normal seedlings were slower to mature.

The objectives of this study were to: (a) establish that seed abuse causes a decline in vigor of even those seedlings showing no physical defects, (b) determine the extent and characterize the expression of this vigor reduction, and (c) investigate possible morphological and physiological bases for the reduced performance. Field trials, greenhouse tests and controlled environment studies were employed to evaluate the performance potential of morphologically normal seedlings from abused seeds and to

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[1] Michigan State University, Agricultural Extension Service, Annual Bean Field Day, 1970.

Figure 1. Delayed maturity resulting from mechanical abuse of Seafarer navy bean seed. Seed for the right three rows was from the same seedlot as for the left rows, but was damaged by repeated tossing of the bagged seed onto a hard floor.

relate this loss in quality to the overall quality reduction resulting from seed damage. Anatomical and physiological techniques were then used to probe for the cryptic physical-biochemical effects of seed damage.

LITERATURE REVIEW

Physical Damage Caused by Seed Abuse

The deleterious effects of mechanical damage on germination have been problems in bean production for many years. The problem assumed serious proportions when bean seed production shifted to the warm dry climate of the West. In 1930 both Harter (27) and Whitney (58) reported cases of severe bean damage and related the injury to mechanical threshing operations. They observed the greater incidence of damage in Western seed and recognized that low humidity during harvest increased susceptibility to injury. Subsequent investigations have substantiated these early claims and described more clearly the types of injury and the factors affecting bean seed damage.

Injuries incurred by bean seeds are often classified into two principle categories (28): (a) external or visible damage and (b) internal injury that becomes evident only after imbibition and germination of the seed. The first group includes seed injuries ranging from slight cracking of seed coats to actual splitting and breaking of the seed. Many types of internal injuries have been observed (5, 8, 53). These range from severe damage of the embryonic axis, which completely inhibits germination; through various types of seedling abnormalities and defects; to abnormalities so slight as to have little practical consequence on seedling performance. Internal injuries not only accompany external damage in bean seed but often occur in seeds showing no evidence of external damage.

Several types of seedling abnormalities resulting from internal injury have been described (5, 8, 53). Two widely recognized types are the 'baldhead' and 'snakehead' seedlings. In baldhead seedlings the entire epicotyl including growing point and primary leaves is missing or damaged so severely it does not develop. Snakeheads retain the growing point but lack both primary leaves. Occasionally the primary root and even part of the hypocotyl are broken off requiring development of adventitious roots for seedling survival. If the break is close to the cotyledons insufficient hypocotylary tissue remains to raise the cotyledons above the soil surface in the normal procedure of epigeal germination. The plumule may then elongate and emerge from the soil in a hypogeal type of germination. Other injury is apparent as numerous types of scars, lesions, and healed-over breaks in the hypocotyls and epicotyls of seedlings.

A very common type of seedling injury is transverse cracking or complete severence of one or both cotyledons.

This condition is mentioned in several reports (8, 10, 37, 57) but is often not given full recognition. According to the official Rules for Testing Seeds (6) bean seedlings lacking one cotyledon or less than half of both cotyledons are not considered abnormal in germination testing.

The amount of injury incurred is directly proportional to the impact load received by the bean (5, 8, 47) and inversely proportional to the seed moisture content at the time of impact (5, 8, 10, 47). Perry (47) also found that seed damage was greater at lower temperatures. Susceptibility to injury from mechanical abuse was greatly influenced by seed size (47) and by the bean variety or strain (7, 10).

Mechanical abuse of seed is not the only factor inducing seed injury and seedling abnormalities. Moore (40) found evidence of natural crushing of embryonic tissues when seeds were exposed to high moisture during ripening or to rapid changes in moisture. Extended storage periods or storage in adverse environments has also been shown to increase bean seedling abnormalities which were indistinguishable from those caused by mechanical abuse (52). And lastly, the commonly observed cotyledon cracking can result from too rapid imbibition as well as from mechanical abuse (37).

Reduced Performance Resulting from Seed Damage

The detrimental effects of seed damage on bean germination and field emergence were reported by Whitney (58) in 1930 and have been observed in many subsequent studies. Asgrow Seed Company (5) and Toole *et al*. (53) noted internal injuries in apparently intact seeds that prevented germination or appreciable growth.

The performance potential of different types of abnormal seedlings has also been studied. Baldhead and snakehead seedlings often develop lateral shoots from axillary buds in the cotyledonary node but the resulting plants are spindly, slower growing, later maturing and much lower yielding than normal plants (5, 18, 26, 42). Waters and Atkins (57) studied the performance of bean seedlings containing various degrees of cotyledon cracks and breaks. They found that loss of cotyledonary tissue resulted in smaller seedlings, fewer pods and lower yields per plant, and that the reductions were proportional to the amount of cotyledon lost. Similar reductions in plant growth and seed yield were observed by McAlister and Krober (36) when they removed cotyledons from soybean seedlings within 2 days after emergence. Removal of the cotyledons later than 4 days after emergence had no influence on plant development. Apparently the critical period for seedling nutrition was during germination and emergence.

Very little literature is available concerning the more cryptic effects of mechanical seed abuse on subsequent seedling vigor and performance. Barriga-Solorio (10) reported a possible vigor reduction in normal seedlings that he attributed to "invisible injury . . . that may have had some effect in the physiological processes of the seed; there may have been some chemical reactions in the seed, or obstructions in the movement of the food reserves in the cotyledons." Similar occurrences of injury-induced reduction in seedling growth were observed for pea (59), peanut (51, 54), and sugar beet (35) seed. However, in all of these studies seedling abnormalities were noted, precluding any deductions as to the vigor of morphologically normal seedlings. No evidence could be found of any attempts to measure the vigor of strictly morphologically normal seedlings produced from mechanically abused seed.

The Concept of Seedling Vigor

As one of the important attributes of seed quality, seedling vigor has been the object of much research and discussion. Extensive reviews of the concepts of vigor were made by Delouche and Caldwell (21) and Isely (28, 29). According to their reviews, two ideas pervade most concepts of vigor: the first concerns performance under unfavorable environmental conditions such as temperature, moisture and pathogens; and the second considers vigor *per se* as reflected in germination and growth rates.

Isely (28) defines vigor as "the sum total of all seed attributes which favor stand establishment under unfavorable field conditions." Grabe (25) disagrees with definitions relating vigor to only unfavorable germination conditions. He feels the concept of seedling vigor should also include effects on yield, storability and performance under favorable conditions. The complexity of the vigor problem was further illustrated in Ching's summary (T. M. Ching, personal communication). She thinks vigor is a product of the genetic complement; maturity factors; harvest, processing and storage manipulations and conditions; and germination environment. The concept of vigor as an intrinsic quality independent of germination conditions seems most amenable to current definitions, but most vigor studies also agree with Perry's (46) conclusion that the expression of vigor depends on the degree of adversity encountered.

Two general categories of vigor tests reflect the two concepts of vigor. Direct vigor tests measure actual performance under adverse conditions while indirect tests purport to indicate the physiological condition of the seed (28). A number of direct and several indirect vigor tests were described and evaluated by Moore (41). Direct tests have the advantages of simultaneously evaluating a number of vigor factors and measuring performance under simulated environmental stresses, but they are difficult to standardize (21, 28). Indirect tests, on the other hand, measure more specifically the physiological condition of the seed and are not influenced by environmental conditions encountered during germination. But these physiological and biochemical tests are usually limited to individual aspects of seed vigor, necessitating the combination of several tests to get an accurate prediction of seed quality.

Physiology of Seedling Vigor

The physiological basis of seedling vigor has not been rigorously defined, but considerable understanding has been gained from studies relating physiological changes to seedling vigor decline.

Respiration rates during the first hours of imbibition and germination often correlate with subsequent seedling vigor. Woodstock and Grabe (60) found the O_2 uptake rates for corn seeds at 6 and 30 hours after planting were highly significantly correlated with seedling lengths at 3, 4 and 5 days after planting. Similar relationships between initial respiration rates and subsequent seedling vigor were reported for wheat (32), crimson clover (16), soybeans (13), peas (59) and lima beans (61). While most of these reports concerned vigor differences due to natural or artificial seed aging, Woodstock and Combs (59) worked specifically with mechanical injury-induced vigor decline in peas. Respiration tests at 6 hours after the start of imbibition readily detected potential vigor reduction in the damaged peas.

Vigor reductions may also be accompanied by changes in activities of specific enzymes. Several enzymes found to decline in activity with seed deterioration and loss of vigor were catalase (23), amylase (3), glutamic acid decarboxylase (24), cytochrome c oxidase (16), acid phosphatase (16) and dehydrogenases (39).

Other changes associated with vigor losses due to seed aging are loss of selective permeability of cellular membranes (17), decreased membrane phospholipids (34), incapacitated glucose utilization during early germination (1), and increased hydrolytic activity resulting in increased free fatty acids (9), amino acids and inorganic phosphate (17). Phosphorylative efficiency appears to be related to seedling growth rate and may be influenced by deteriorative effects on the structural integrity of mitochondria (2).

In her work with crimson clover seed Ching (16) observed correlations between vigor decline and the capacities for ATP, DNA, RNA and protein synthesis. She noted, however, that these criteria as well as respiration rates and enzyme activities were not always closely proportional to seedling growth measurements,

> simply because . . . growth requires the cooperative effort of all biochemical systems in the right places at the proper times. A precise coordination of quantity and quality is essential for optimum growth. Whatever system we measure would only show a partial picture of the whole.

After discussing the complex interaction of biochemical processes in different tissues during germination and early seedling growth she suggested that "The weakest link in this chain of events often limits seed vigor. . . ."

In commenting on possible relationships between mechanical abuse and seedling vigor decline, Ching (T. M. Ching, personal communication) suggested that although isolated molecules would probably not be damaged, membrane associated enzymes and cellular organelles might be damaged by severe abuse. The resulting decline in functional efficiency may reduce the supply of energy and impair synthetic ability. App, Bulis, and McCarthy (4) lend support to this postulation. They found that severe fracturing of rice embryos prevented dissociation of ribosomes into functional subunits required for protein synthesis.

MATERIALS AND METHODS

Seed Material

Bean (Phaseolus vulgaris L.) seed stocks used in these studies were from the three commercial varieties: 'Seaway' and 'Seafarer' white navy beans, and 'Charlevoix' red kidney beans. Seed samples for the initial field and greenhouse experiments were from high quality mechanically harvested and processed certified seed lots grown in 1968 and obtained from the Michigan Foundation Seed Association. All subsequent experiments utilized carefully handharvested and handprocessed Seafarer seed grown in 1969.

Seed lots were handpicked to remove all discolored and non-uniform seeds. The seed material was stored in controlled humidity containers at about 55% relative humidity at 5 or 20 to 25 C.

All seed lots were screened with official, slotted, grain grading screens to improve uniformity in seed size. Seeds passing through 4.8 mm-wide slots and seeds too large to pass through 5.4 mm slots were discarded. For the experiments using handharvested Seafarer, the seed was further divided into three size classes: 4.8 to 5.0 mm, 5.0 to 5.2 mm and 5.2 to 5.4 mm. Only one size class was used per experiment or per replication in order to minimize variability due to variations in seed size.

Mechanical Abuse Treatment

One half of each seed lot was given a mechanical impaction treatment by dropping the seeds in a slow stream 9.2 m onto a steel plate inclined about 45°. Severity of the mechanical abuse was controlled by conditioning the bean seed lots to predetermined moisture levels.

Three damage levels were obtained for the initial studies by abusing seeds at about 8, 11 and 13.5% moisture contents (wet basis). The respective moisture levels were achieved by storing the seed lots four weeks over saturated salt solutions of magnesium chloride, magnesium nitrate and sodium nitrate. At room temperature these solutions produced equilibrium relative humidities of about 30, 50 and 65%, respectively. All subsequent experiments used the handharvested Seafarer seed, one half of which were mechanically abused by dropping at 11 ± 0.5% moisture content.

The impacted seed (hereafter referred to as damaged seed) were carefully examined and all broken seeds and seeds with cracked seedcoats were discarded. Only physically sound, intact seeds showing no external evidence of damage were used for these studies.

Field Performance Trial

Samples of the three varieties, Seaway, Seafarer and Charlevoix, damaged at the three previously mentioned moisture levels, and corresponding moisture level non-damaged (control) samples were planted in a randomized block field trial, June 17, 1969, on the bean research farm west of Saginaw, Michigan. Three varieties, three seed moisture levels and two treatments (control and damaged) resulted in 18 treatment combinations which were replicated in 6 blocks. Plots consisted of 100 seeds planted in each of two 25 foot rows for each treatment combination.

The seed samples were treated prior to planting by shaking each sample in a cup with several drops of a 'Dalsan' ('Captan' and 'Dieldrin') slurry. At planting time insecticide and fertilizer were banded beside the seed according to commercial practices.

Total seedling emergence was recorded for all plots and the seedlings of several replications classified into the following categories: (a) completely normal, (b) weak or low vigor, (c) lacking primary leaf, (d) lacking cotyledon, (e) baldhead, (f) baldhead lacking cotyledon, and (g) dead after emergence.

Since the seed damage treatment reduced emergence and field stand, control plots in four blocks were hand thinned to approximately the same plant density as the corresponding damaged seed plot. This eliminated differences in field performance resulting from unequal plant densities between control and damaged seed plots.

At maturity the plots were harvested and threshed, and seed yield determined.

Greenhouse Performance Trials

Several greenhouse studies were conducted to obtain more detailed seedling vigor data on an individual plant basis and to eliminate the performance reduction contributed by abnormal seedlings. Seed lots used for these studies were the same as used previously in the field trial except that only Seaway navy and Charlevoix kidney bean varieties were used. One non-damaged lot from each variety provided a control with which to compare the three lots of different injury levels. Again, only seeds with no external evidence of injury were used.

Thirty-six seeds from each control and damaged lot of both variety were planted 3 cm deep and 2.5 cm apart in rows in framed greenhouse benches filled with 15 cm of a sterilized peat-soil mixture. A completely randomized design was used with three replications.

The date of emergence for each seedling was recorded and each seedling was classified as normal or as one of the several abnormal categories previously mentioned (p. 13). After emergence was complete, all abnormal seedlings were removed and each row was thinned to nine plants. Therefore, in damaged as well as control plots, only normal appearing seedlings showing no morphological abnormalities or injuries were used for subsequent measurements.

At the time of thinning all morphologically normal seedlings removed were dried for 3 days at 85 C and their dry weights determined.

Thirteen days after planting, the internode between the cotyledonary and primary leaf nodes was measured on five plants from each row. Seventeen days after planting, the internode between primary leaf and first trifoliate leaf nodes on the same five plants was measured.

The date of first flowering was recorded for each plant. At maturity the seedpods were collected from each row, counted and shelled. The number of seeds per pod, number of seeds per plant, weight per seed and total seed weight were determined.

Additional seedling vigor comparisons were obtained from seedling growth tests in greenhouse flats. Fifty seeds each of the control and medium damage level (dropped at 11% moisture content) of both previously mentioned Charlevoix and Seaway varieties were planted 2.5 cm deep in a sand-peat-soil mixture in each of two flats. Emergence and primary leaf expansion were counted daily. At nine days, when emergence was complete, the control sections were thinned to the same plant density as the damaged seed sections. After 14 days all morphologically normal seedlings from control and damaged treatments were harvested, measured for length and placed in individual coin envelopes for drying. The seedlings were dried about 2 days at 90 C in a forced air oven and weighed.

Standard Germination Test

For this and all subsequent studies only the hand-harvested and handprocessed Seafarer seed and only the medium damage level were used. An official laboratory germination test was conducted on control and damaged samples by the Michigan Crop Improvement Association. Two hundred seeds from each treatment were germinated on Kimpak and blotter paper for 8 days at 25 C according to standard procedures of the Association of Official Seed Analysts (6). Lengths of morphologically normal seedlings were determined at 6 days after planting.

Germination in Vermiculite

A test for individual seed performance under optimal environmental conditions was conducted in the growth chamber. Seventy control seeds and 105 damaged seeds were planted, individually, 3 cm deep in vermiculite in 206 ml paper cups. These were incubated in a growth chamber at alternating conditions of 30 C for 8 hours with light and 20 C for 16 hours without light.

Emergence, primary leaf opening and first trifoliate leaf opening for each seedling were recorded to the nearest 1/2 day. All seedlings showing any type of physical abnormality were then removed and the normal appearing seedlings for both control and damage treatments were grouped to provide similar plant density environments. After 15 days seedling lengths and fresh weights were determined.

Germination in Sand

Another common germination technique was used to evaluate mechanical injury effects on seedling vigor. Fifteen seeds of control and damaged Seafarer lots were planted 2.5 cm deep in sand in each of four 19 x 27 cm plastic germination trays. About 400 ml of distilled water were added, after which the trays were incubated at 21.5 ± 0.5 C.

The dates of emergence and primary leaf opening were recorded for each seedling. Lengths of morphologically normal seedlings were determined after incubation for 8 days.

Stress Tests for Seedling Vigor

Cold Temperature

An early spring outdoor germination and seedling growth study was conducted to determine the effect of stress conditions in accentuating vigor differences between control and damaged seeds. Thirty seeds each of control and damaged samples were planted 2.5 cm deep in a soil-sand mixture in each of 4 greenhouse flats. The flats were then placed outdoors for the duration of the study except for four nights when the temperature was expected to drop below 5 C. The experiment was conducted about one month prior to normal planting time for commercial beans. Soil temperature was often considerably below the commonly accepted 18 C minimum for bean seed planting. Emergence date for each seedling was recorded. Twenty-one days after planting, the internode between the cotyledonary and primary leaf nodes was measured. Thirty-five days after planting, all morphologically normal seedlings from the damage treatments and 10 normal seedlings from each control replication were harvested and measured for length. Seedling dry weights were determined after the roots were removed and the seedlings were dried 4 days at 70 C in individual coin envelopes.

Cold Temperature and Lack of One Cotyledon

Another cold temperature study was initiated to further investigate the extent of vigor reduction in normal appearing seedlings resulting from mechanical abuse and to compare this vigor loss to that resulting from lack of one cotyledon. Loss or breakage of cotyledons was one of the most common seedling abnormalities observed to result from mechanical damage.

About 75 control and 150 damaged seeds were planted, individually, 3 cm deep in a soil-sand mixture in 206 ml cups. A growth chamber at 18 C and with a 12 hour photoperiod was used for incubation. After emergence seedlings were selected or treated to achieve the following four groups of plants: (a) Control, normal seedlings--perfect seedlings from non-dropped seeds, (b) Control, minus one cotyledon--seedlings as in Treatment #a except that one cotyledon was excised with a razor blade after emergence, (c) Damaged, normal seedling--morphologically normal seedlings from damaged seed retaining both cotyledons and having no physical defects, (d) Damaged, minus one cotyledon--seedlings from damaged seed showing no physical defects except that one cotyledon was missing due to the impaction treatment. Any other abnormal seedlings were discarded. All seedlings selected for the above treatment categories were completely randomized within the growth chamber.

The first and second internodes above the cotyledonary node were measured 17 days after planting. After 24 days the seedlings were harvested and their total length measured. Seedling dry weights were determined after drying for three days at 80 C.

Growth Rate of Excised Embryonic Axes

Embryonic axes were excised from a number of control and damaged seeds by splitting the cotyledons of air-dry seeds apart and cutting the axes off the cotyledons to which they remained attached. The axes were imbibed between wet blotter papers for 3 hours prior to measurement of respiratory activity as described on page 25. After measurement of respiration for 2 hours the axes were immediately planted on vertical wet filter paper. The axes were planted in rows 8 cm from the bottom of 16 x 20 cm filter paper sheets and covered with 'Saran Wrap' to hold them to the paper. The filter papers were then suspended from glass rods in a covered plastic container so that 1 cm of the paper was submerged in distilled water in the bottom of the container. The entire container was placed in a germinator at 25 C in the dark.

Axes lengths from tip of root to base of primary leaf were measured at 2.5 and 5 days after initiation of imbibition.

Examination for Callose Tissue

Three-, five- and eight-day-old normal appearing seedlings were examined for evidence of callose tissue in the hypocotyl-cotyledon juncture area. Several seedlings from control and damaged treatments from previously described studies were harvested, stored in a killing-fixing solution, embedded in paraffin, sectioned and mounted, stained, and examined with an ultraviolet-lighted microscope.

The 5-day-old seedlings were fixed and stored in an ethanol:water:formalin (60:40:4) solution then dehydrated and embedded in paraffin according to Sass (49). The 3- and 8-day-old seedlings were fixed and stored in FAA (50% ethanol:5% glacial acetic acid:10% formalin:35% water) before dehydrating and embedding according to the method of Knobloch (33). The embedded specimens were sectioned (20 or 25 μ thick) using a rotary microtome, and the sections were mounted on glass slides with a potassium dichromate-gum arabic solution according to Harrison (C. M. Harrison, personal communication). The mounted sections were then deparaffinized and hydrated in a serial coplin-jar procedure similar to the method outlined by Knobloch (33). After staining for 10 minutes in a 0.01% aniline blue solution in 0.067 M potassium phosphate buffer (pH 9.5) the cover slips were mounted with a hygroscopic solution of equal volumes of potassium acetate (crystals), methanol and water according to Eschrich and Currier (22). Cover slips were ringed with melted paraffin and the slides stored temporarily in a refrigerator to minimize drying.

The prepared slides were examined under a microscope using a UV light source and filter with peak transmittance at about 366 nm. UV light at this wavelength is quite specific for callose in aniline blue-stained material. Photomicrographs were taken with a mounted Kodak camera using Ektachrome X film.

Respiration Determination

Respiration (0_2 uptake) of whole seeds, excised cotyledons and excised embryonic axes was measured periodically during the first 20 to 30 hours of germination. Five whole seeds, or the equivalent excised cotyledons or axes of five seeds, were used per respiration flask. Six replicate samples were used for each determination for control and damaged seed lots. Sample volumes needed for calculating flask constants were computed from the initial sample dry weight using previously established regression equations for imbibed volumes and dry weights.

Whole seeds were first surface sterilized by submerging 2 min in a 1.0% hypochloride solution ('Clorox') and washed for 20 sec in flowing distilled water. The whole seed, cotyledon or embryonic axis samples were placed on wetted Kimpak between wet germination blotter paper and placed in a germinator at about 27 C. After imbibition for 2.5 hours the samples were placed in respirometer flasks containing 2.5 ml of water in the main compartment, and 0.2 ml of 10% KOH and folded filter paper wick in the center well. The flasks were then attached to mannometers and placed in the 25 C water bath of a Warburg apparatus. Three flasks in each run contained water only to serve as thermobarometers for monitoring changes in water bath temperature and atmospheric pressure. The flasks were allowed to equilibrate for 20 to 30 minutes at about 100 oscillations per minute before measurement of oxygen uptake was begun.

During measuring periods the mannometers were read every 30 min. The first measurement period was for 2 hours beginning 3 hours after start of imbibition. The stopcocks were then opened and the system left to oscillate until oxygen uptake was again measured during the 1 hour period beginning 10 hours after planting. After the 10-hour measurement the embryo axes samples were again left in the system and a final series of measurements made for the period of 20 to 22 hours after planting. The excised cotyledons respired quite rapidly so that by the end of 20 hours the KOH solution had become saturated with carbon dioxide. After replenishing the KOH and adding a new filter wick, oxygen uptake was again measured for 1 hour starting 22 hours after planting. Respiration of whole seeds at 22 hours after planting was determined from additional seed samples which had been surface sterilized and imbibed for 21.5 hours on Kimpak between blotters. After measuring oxygen uptake for 2 hours the system was left to incubate and a final 1-hour measurement taken at 28 hours after planting.

All mannometric readings were corrected for changes in the thermobarometers and converted to μl oxygen uptake according to the method of Umbreit, Burris and Stauffer (55).

Enzyme Assays

The activity levels of three enzymes in bean seedling cotyledons were determined daily for the first 8 to 14 days of germination and seedling growth. From 12 to 16 control and 50 to 70 damaged seeds were planted daily 2.5 cm deep in washed and graded no. 7 Wausau quartz sand in plastic trays. Approximately 400 ml of tap water were added and the trays were incubated at 17 to 18 C in the dark. The excess damaged seeds were planted in order to obtain sufficient morphologically normal seedlings since 70 to 90% of the seedlings from damaged seeds were abnormal in some way.

On the day for an enzyme assay, seedling samples from the daily plantings were harvested and kept cool in small bottles containing water until they were to be used. Only morphologically normal seedlings showing no physical defects or evidence of mechanical injury were used.

Cytochrome Oxidase

The histological distribution and activity of cytochrome oxidase during germination and early seedling growth were determined similar to the procedure used by Castelfranco, Lott and Sabar (14). Both cotyledons of one seedling were used for each determination. Determinations were replicated 9 to 14 times for control and damaged seed lots for each seedling age from one through six days and five times for eight-day old seedlings.

The cotyledons were sectioned at right angles to their long axis into 125 μ sections with a rotary microtome. Ten sections were taken from the center portion of each cotyledon to make a 20-section composite sample for each seedling. The sections were incubated with occasional stirring 1.5 hours at room temperature in a para-amino diphenylamine (PADA) solution prepared as follows: 96 mg of PADA were dissolved in 4 ml of ethanol and diluted to 400 ml with 0.2 M tris buffer (pH. 7.5). Because of the tendency to photodecompose the PADA solution was prepared just prior to use. Tissue incubation was done in black tape-wrapped vials and subsequent procedures were performed in subdued light.

After incubation the cotyledon sections were rinsed in 0.2 M tris buffer and stored temporarily in a descicator in the dark.

The color produced during incubation with PADA was extracted into lipid solvents and measured spectrophotometrically. Dried samples were placed in small 'Thomas' homogenizers and crushed with the pestle after freezing with a small amount of liquid nitrogen. The crushed samples were then homogenized 1.5 min in 1.5 ml of a chloroform:methanol (1:1) solution. One ml of distilled water and 2.5 ml of n-hexane were added to the homogenate in small test tubes. The tubes were shaken vigorously 30 sec and centrifuged about 2 min in a clinical centrifuge to separate the phases. The upper chloroform:hexane phase containing the color was transferred to black tape-wrapped vials and the absorbance at 485 nm determined soon after extraction using a Beckman model DU spectrophotometer. Activity of cytochrome oxidase was expressed as absorbance at 485 nm per 1.5 hours per twenty 125 μ-sections.

Succinate-Cytochrome c Reductase

Succinate-cytochrome c reductase activity was determined in the debris-free homogenates of cotyledons of germinating bean seeds. Ten cotyledons from five seedlings for each determination were homogenized for 5 min in 10 ml of 0.1 M potassium phosphate buffer (pH 7.4) with sand in a mortar and pestle. Filtering through 4 layers of tissue, and centrifugation for 8 min at 5,000 x *g* cleared the homogenate of cellular debris. All manipulations were performed at 3 to 5 C.

The procedure of Ragland and Hackett (49) was used to assay for succinate-cytochrome c reductase activity. The following reagents were prepared for the assay media:

0.2 M potassium phosphate buffer (pH 7.4), 0.6 M sodium succinate containing potassium cyanide to a concentration of 0.03 M, and 1.5 x 10^{-4} M cytochrome c (Sigma, Type III). These reagents, distilled water and the prepared homogenate as an enzyme extract were pipetted into a 1 cm cuvette in the following quantities producing final reaction concentrations as shown in parentheses: 1.4 ml phosphate buffer (0.1 M), 0.1 ml succinate:KCN solution (20 mM;1mM), 0.4 ml distilled water, 1.0 ml cytochrome c solution (5 x 10^{-5} M), and 0.1 ml enzyme extract (0.33%). The buffer, succinate and distilled water were mixed in the proper proportions prior to use and pipetted at once into the cuvette. The contents were mixed thoroughly by inverting in the cuvette about seven times immediately after adding the enzyme extract. The rate of reduction of cytochrome c at 22 C was determined by following the increase in absorbance at 550 nm using a blank containing all of the above ingredients except the enzyme extract. Absorbance changes with time were recorded with a Beckman model DK-2A recording spectrophotometer or determined by taking periodical 30 sec readings in a Beckman model DU spectrophotometer.

Only the linear sections of the rate curves were used for computing enzyme activities. Activity was expressed as increase in absorbance at 550 nm per min per 10 cotyledons.

Amylases

Amylase activity was also determined in homogenates of germinating cotyledons. Ten cotyledons from five seedlings per sample were homogenized with 10 ml of cold 1.0 mM acetate buffer (pH 4.8) for 1 min in a Virtis blender. After filtering through four layers of cheesecloth the homogenate was centrifuged at 27,000 x *g* for 30 min at 4 C.

Amylase activity of the supernatant fluid was determined according to the technique described by Bernfeld (11) and modified by Penner (D. P. Penner, personal communication). To four test tubes for each sample were added 0.8 ml of 0.06 M potassium phosphate buffer (pH 4.5), 0.2 ml of cotyledon homogenate, and 1.0 ml of 0.067% soluble starch in 0.06 M phosphate buffer. Immediately after adding the starch to two of the test tubes the reaction was stopped by addition of 2 ml of a 0.4 N sodium hydroxide solution containing 1% 3,5-dinitrosalicylic acid and 30% potassium sodium tartrate. The other two tubes were incubated 10 min with shaking in a 25 C water bath before stopping the reaction as above. All tubes were then heated 5 min in a boiling water bath and cooled. Light absorbance at 540 nm compared to a blank without enzyme was determined with a Beckman DU spectrophotometer. Mean values for the non-incubated tubes were subtracted from means of the incubated tubes for each sample assayed and amylase activity expressed as absorbance at 540 nm per 10 min per 10 cotyledons.

The assay for amylase activity was replicated at least twice for all seedling ages from 1 through 14 days after planting. A third replication was run for several seedling ages.

RESULTS AND DISCUSSION

Effects of Mechanical Abuse on Seed Breakage

Dropping bean seed 9.2 m onto a metal plate caused extensive seed and seedcoat breaking (Table 1). The extent of this damage was largely determined by seed moisture contents at the time of impactation: the severity of damage increased directly with the decline in seed moisture.

Seed size or class of bean also influenced susceptibility to damage. The larger more oblong Charlevoix kidney seeds were much more susceptible to seed coat cracking than were the small spherical navy beans. The kidney and navy beans were about equally susceptible to actual seed breaking. The two navy varieties also differed in the type of injury incurred. Seafarer seemed to be slightly more susceptible to seed coat cracking but more resistant to splitting than Seaway.

These data agree closely with the findings of previous studies. Extensive work by Asgrow Seed Company (5), Barriga-Solorio (10), Perry (47) and others has shown the relationship between mechanical damage and seed moisture content, bean varieties, and seed size. Moisture content is clearly the major factor influencing the extent

Table 1. Percentage of seed breakage caused by dropping three varieties of bean seed 9.2 m at three seed moisture levels.

Variety and severity of abuse	Seed moisture at dropping (%)	Physical effects of mechanical abuse (%)		
		Broken seed	Cracked seedcoats	Total external damage
Seafarer				
Light	13.7	0.4	1.3	1.7
Medium	11.1	1.2	9.6	10.8
Severe	8.4	4.3	17.3	21.6
Seaway				
Light	13.6	0.6	0.0	0.6
Medium	10.9	2.5	4.9	7.4
Severe	8.2	5.8	14.9	20.7
Charlevoix				
Light	12.7	0.2	3.8	4.0
Medium	10.0	1.1	13.3	14.4
Severe	7.7	5.5	31.7	37.2

of physical damage caused by a given impact load. Seed variety, size and shape are also important factors determining injury susceptibility and injury type but their effects are less pronounced and less well defined.

Effects of Mechanical Abuse on Seedling Emergence and Abnormalities

External physical damage was not the only result of mechanical abuse. Even more significant was the internal injury incurred by the seeds resulting in markedly reduced germination and increased seedling abnormalities. Field emergence was reduced by as much as 50% for kidney beans receiving the severe (low moisture) treatment (Table 2). Mechanical abuse at both the medium (11%) and low (8%) moisture levels reduced emergence performance for all varieties of beans tested. These and all subsequently reported results are from seed samples from which all seeds showing external damage had been removed.

It is apparent that emergence performance was considerably affected by environmental conditions. Temperature, moisture or soil conditions in the field trial, greenhouse flats and cold test appear to have imposed more stresses preventing germination or emergence of the more seriously injured seeds. Under more optimal conditions of the greenhouse bench and germination in vermiculite these seeds were able to germinate and produce seedlings.

Table 2. Effects of mechanical abuse of bean seed on seedling emergence.

Germination media and variety	Emergence at various injury levels (% of control)		
	Light	Medium	Severe
Vermiculite[1]			
Seafarer	--[2]	88.6	--
Field			
Seafarer	110.5	82.6**	59.8**
Seaway	100.6	77.6**	68.9**
Charlevoix	93.7	71.7**	49.9**
Greenhouse Bench			
Seaway	100.9	99.1	90.3*
Charlevoix	100.9	90.6**	80.3**
Greenhouse Flat[1]			
Seaway	--	83.7	--
Charlevoix	--	85.6	--
Cold Soil[1]			
Seafarer	--	84.8	--

[1] Statistical evaluation was not feasible for these results.

[2] Not determined.

*Reduction in emergence was statistically significant at the 5% level.

**Reduction in emergence was statistically significant at the 1% level.

Typical patterns of emergence for seeds given three levels of abuse are shown in Figure 2. Severe mechanical abuse not only lowers total emergence but also reduces the rate of emergence. A relative index of the emergence rate can be calculated by summing the quotients: percentage of seedlings emerging each day divided by the number of days elapsed since planting. Emergence indices thus computed and expressed as percentage of the control index were 102.4, 95.3 and 91.1% respectively for the light, medium and severe damage treatments of Seaway seed in the greenhouse bench study. Almost identical reductions in emergence rates were observed for the Charlevoix kidney bean seeds.

The most striking effect of mechanical injury in bean seed is not evident in the gross seedling emergence counts. Even though most of the damaged seeds may germinate a large proportion of the resulting seedlings have lower productive potential because of physical abnormalities. Table 3 summarizes the types and extent of seedling abnormalities resulting from mechanical abuse.

The most common type of seedling injury was cracking or breaking of the cotyledons. Many seedlings emerged with one or both cotyledons completely missing. In other cases the cotyledons were attached but were of little value to the growing seedling because of substantial cracks near the point of attachment. Callus tissue and

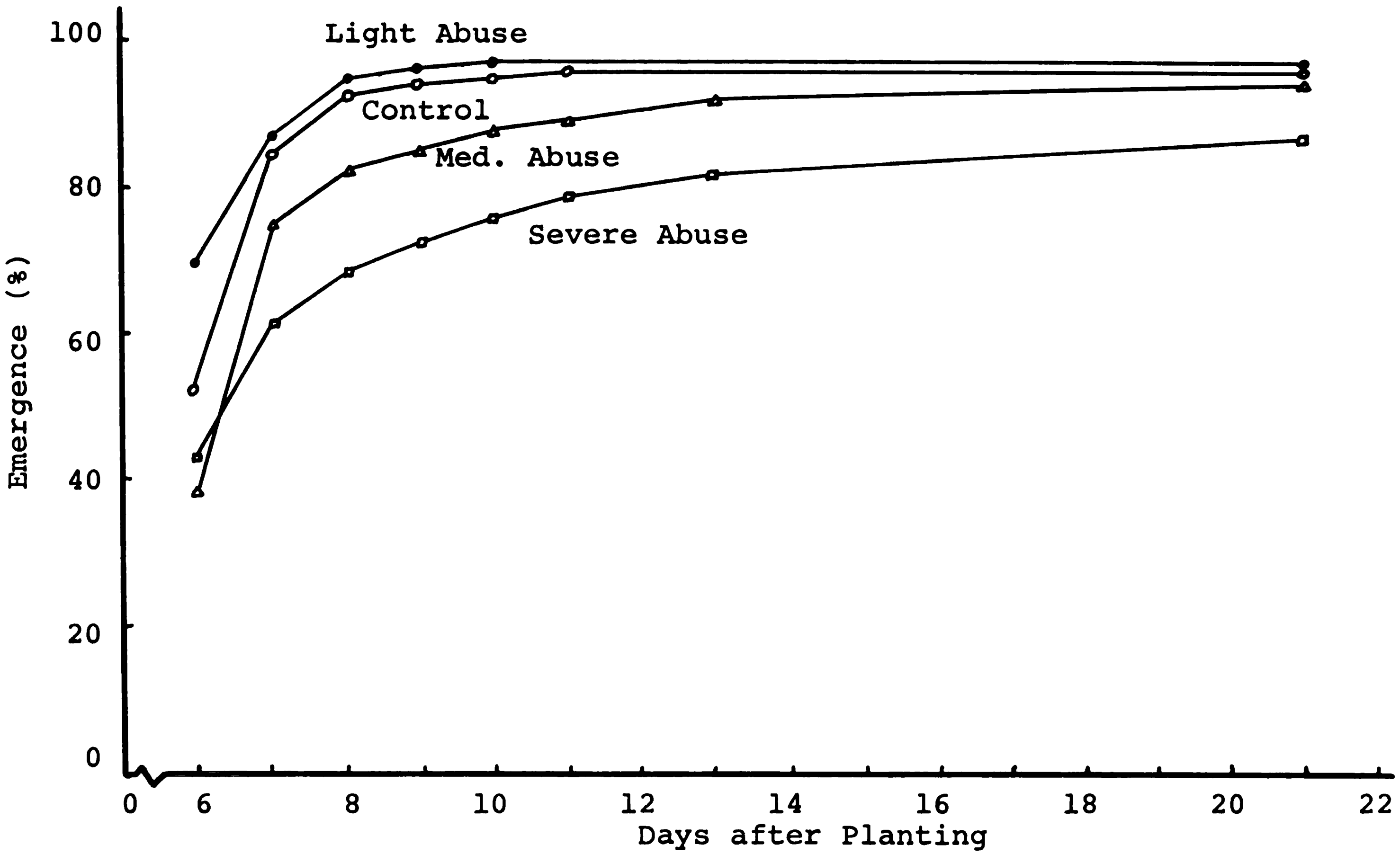

Figure 2. Emergence of Seaway navy bean seedlings as influenced by mechanical seed abuse. Both morphologically normal and abnormal seedlings were counted.

Table 3. Classification and frequency of seedling abnormalities resulting from mechanical abuse of bean seeds.

Experiment, variety and damage level	Seedling abnormalities[1]						
	Baldheads	Broken or missing cotyledons	Missing primary leaf	Hypogeal emergence	Misc. injury	Total abn.	Weak seedlings
I. Field Trial							
Seafarer							
Light	1.1	--[2]	3.0	--	--	--	0.4
Medium	13.9	--	1.3	--	--	--	1.9
Severe	12.1	--	3.8	--	--	--	6.7
Seaway							
Light	5.7	--	2.8	--	--	--	0.6
Medium	14.4	--	7.2	--	--	--	3.7
Severe	13.5	--	3.2	--	--	--	9.3
Charlevoix							
Light	6.4	--	1.5	--	--	--	0.6
Medium	23.8	--	6.4	--	--	--	3.5
Severe	24.0	--	4.7	--	--	--	3.3
II. Greenhouse Bench							
Seaway							
Light	0.0	1.8	0.9	0.0	0.0	2.7	0.0
Medium	18.3	13.6	0.0	2.9	0.9	35.7	0.0
Severe	16.0	21.6	1.5	5.3	0.0	44.4	5.7

Table 3. Continued.

Experiment, variety and damage level	Seedling abnormalities[1]						
	Baldheads	Broken or missing cotyledons	Missing primary leaf	Hypogeal emergence	Misc. injury	Total abn.	Weak seedlings
<u>Charlevoix</u>							
Light	6.5	7.4	0.9	0.0	0.0	14.8	2.8
Medium	16.0	23.8	1.0	4.1	1.0	45.9	7.8
Severe	26.8	37.7	1.1	9.3	2.4	77.3	0.0
III. Germination in Vermiculite							
<u>Seafarer</u>							
Medium	6.4	48.4	1.1	1.1	5.0	62.0	1.1
IV. Cold Test							
<u>Seafarer</u>							
Medium	3.0	42.4	0.0	0.0	7.1	52.5	--

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[1] Values reported are percentages of emerged seedlings and not percentages of total seeds planted.

[2] Not determined.

adventitious roots were often found to develop from the injured areas. These cotyledons did not diminish in size during germination, indicating that the food reserves were not being utilized by the growing axis. Many cotyledons which appeared to be intact at the point of attachment were still poor food sources because of transverse cracks which prevented metabolite transport from that portion of the cotyledon distal to the crack. Borthwick (12) as early as 1932 noted that detachment of the cotyledons was one of the most common symptoms of thresher injuries.

Baldhead seedlings were the second most frequent type of abnormality. Plumules of these seedlings were severed or damaged such that they never developed beyond the small, white, folded-leaf stage found in imbibed seeds. Other types of abnormalities were loss of one primary leaf; hypogeal rather than epigeal germination; and various splits, breaks or abnormalities of the hypocotyl. The types of seedling abnormalities observed in these studies were very similar to those reported and described by Toole, *et al*. (53).

The extent of seedling abnormalities caused by mechanical abuse of the seed ranged from about 3 to 77% of the emerged seedlings depending on the variety and severity of abuse. Charlevoix kidney beans were again the most seriously injured in comparison to the two navy bean varieties.

Many of the abnormal seedlings were capable of developing into plants but they were usually less vigorous and slower growing. Seedlings missing only one cotyledon or possessing cracked cotyledons are usually able to develop into plants visibly indistinguishable from completely normal plants. However, under sub-optimal growing conditions often encountered in field situations the reduced supply of nutrients from the cotyledons may delay seedling development and render the seedling less competitive with both neighboring seedlings and weed species. Evidence and further discussion of the cotyledon effect on seedling vigor will be presented in a later section (p. 59).

Even baldhead seedlings survived and developed axillary shoots from lateral buds in the cotyledon axes. These plants, however, were so late in developing that they behaved essentially as competitors against rather than contributors to crop production. These observations were similar to those made by Asgrow Seed Company (5).

Effects of Mechanical Abuse on Field Performance

Seed yields from plots planted with mechanically damaged seed revealed a reduction in productivity resulting from seed injury. Table 4 shows the relative seed yields from the four blocks of the field trial in which plant densities of the control plots had been adjusted to approximate the density of treated plots. The medium and severe injury levels consistently reduced yield 11 to 17%.

Table 4. The influence of mechanically damaged seed on subsequent crop yield.

Variety	Bean yield from seed given three levels of mechanical abuse (% of control)		
	Light	Medium	Severe
Seafarer	94.5	85.3*	87.9
Seaway	110.4	86.5*	89.2
Charlevoix	98.3	86.9	86.6

*Reduction in seed yield was statistically significant at the 5% level.

Thinning the control plots to plants-per-row densities similar to complementary damaged-seed plots eliminated the influence of emergence differences on yield. All seedlings whether normal or abnormal were retained in the field performance trial. The yield reductions observed may, therefore, largely reflect the influence of abnormal seedlings. However, many of the seedlings which were physically malformed because of seed abuse recovered and developed into mature plants. For the first few weeks after emergence some of the severely damaged-seed plots were visibly distinguishable from control plots because of their slower development and generally lower vigor. By flowering time visible differences were not apparent. The delayed maturity effect of seed injury was more dramatically evident in the demonstration plot shown earlier (Figure 1). All abnormal seedlings were retained in that comparison as well and likely account for much of the obvious delay in maturity.

These observations agree with those of Mezynski (38) who observed similar results from mechanically abused Michelite and Sanilac varieties of navy bean. Impaction at lower moisture levels consistently reduced subsequent yields even though no visibly injured seeds were planted.

Effects of Mechanical Abuse on Vigor of Morphologically Normal Seedlings

The types, extent and consequences of gross mechanical injury (Tables 1-4) have established that several currently important bean varieties are as seriously damaged by mechanical abuse as were other varieties in previous studies.

A main objective of this research, however, was to examine some more subtle effects of mechanical injury, particularly to investigate and try to substantiate suggestions from several sources that mechanical injury reduces vigor even in seedlings showing no morphological defects.

To determine seedling vigor, several parameters of early seedling growth and development were investigated. All abnormal seedlings in the several growth studies were either eliminated or disregarded; only perfect appearing, completely normal seedlings from control and damaged seed stocks were considered. These seedlings, subsequently referred to as morphologically normal seedlings, showed no physical defects resulting from the damage treatment. Both cotyledons were firmly attached, were not transversely cracked and were uniformly utilized by the growing seedling.

Growing conditions for the vigor determinations varied from the optimal 20 to 30 C growth chamber germination in vermiculite to cold outdoor germination and growth in wet soil.

Emergence Time and Rate

Table 5 summarizes the seedling emergence results from several studies. There was essentially no difference between the mean times required for emergence of morphologically normal seedlings from control or mechanically abused seeds. The rates of emergence as indicated by the emergence index also did not differ significantly.

The striking effect of sub-optimal germination conditions is readily observed by comparing the cold test results to results of the other two experiments. The germination in vermiculite at 20 to 30 C alternating

Table 5. Time and rate of emergence of morphologically normal and total seedlings from damaged or non-damaged seeds.

	Ave. no. of days to emergence and emergence index[1]		
		Damaged seed	
Experiment	Control normal seedlings	All seedlings	Normal seedlings
Germination in vermiculite	4.9 (20.5)	5.0 (20.3)	4.8 (20.9)
Germination in sand	4.3 (23.5)	4.5 (22.7)	4.6 (22.1)
Cold test	14.6 (7.1)	15.5* (6.7)	14.2 (7.2)

[1] Values in parentheses are emergence indexes.

*Average emergence time was significantly (5% level) longer than for normal seedlings from both control and damaged seedlots.

temperature and the germination in sand at 22 C apparently provided favorable conditions for germination and emergence. The early spring outdoor germination in cold soil delayed emergence about 10 days and reduced the emergence index by about 65%. Also, in the cold test total seedling emergence from damaged seed was significantly slower (about 1 day) than emergence of morphologically normal seedlings from both control or damaged seed. This germination delay was not apparent in the other two tests. Stress conditions of the cold test appeared to accentuate vigor differences that were not observable under more optimal germination regimes. When only normal seedlings were compared, little or no difference in emergence performance was evident even in the cold test. Although the mechanical damage treatments were shown to significantly reduce total emergence (Table 2), the seed injury incurred apparently did not affect the speed of germination or emergence of those seedlings sustaining no physical defects.

Primary and Trifoliate Leaf Opening

The time lapse from seed planting to primary and first trifoliate leaf unfolding for morphologically normal seedlings was determined in several of the experimental studies. The results (Table 6) show little or no effect of mechanical injury on this growth parameter.

Table 6. Time required for primary and first trifoliate leaf opening of morphologically normal seedlings from damaged or non-damaged seeds.

Experiment and variety	Ave. no. of days to leaf opening			
	Primary leaves		First trifoliate leaf	
	Control	Damaged	Control	Damaged
Germination in vermiculite				
Seafarer	5.8	5.6	13.8	13.8
Charlevoix	7.4	7.6	13.5	13.5
Germination in sand				
Seafarer	5.8	5.8	--[1]	--
Greenhouse flats				
Seaway	7.7	7.9	--	--
Charlevoix	8.0	8.1	--	--

[1] Not observed.

It was interesting to note the difference in seedling development between Seafarer navy and Charlevoix kidney varieties in the germination experiment in vermiculite. Although Charlevoix primary leaves opened almost two days later than Seafarer, initiation and opening of the first trifoliate leaf was about the same for both varieties.

Internode and Total Seedling Lengths

Seedling length measurements and rates of seedling elongation are often used as indicators of seedling vigor. In these studies internode and total hypocotyl-epicotyl lengths were used to compare vigor of normal seedlings produced from abused and non-abused seeds. Figure 3 illustrates the marked length reduction observed in one experiment. The results from several experiments are shown in Table 7. Mechanical abuse uniformly reduced elongation of morphologically normal bean seedlings when growing conditions were somewhat less than optimal. Seed abuse reduced internode length as much as 45% in the Seaway seedlings in the greenhouse bench experiment, and total seedling length as much as 24.5% in the standard germination test of Seafarer. Differential response can be observed between varieties, between experimental conditions and in interactions between these factors.

Figure 3. Reduction in elongation of morphologically normal seedlings produced from mechanically abused seed. Control and damaged Charlevoix red kidney bean seeds were grown in greenhouse flats.

Table 7. Effects of seed abuse on internode or total length of subsequent morphologically normal seedlings.

Experiment, variety and severity of abuse[1]	Internode lengths[2]			Total hypocotyl-epicotyl length[2]
	Cotyledon to primary leaf	Primary to first trifoliate leaf	Cotyledon to trifoliate leaf	
<u>Germination in vermiculite</u>				
Seafarer	--[3]	--	--	107.7
Charlevoix	--	--	--	104.9
<u>Germination in sand</u>				
Seafarer	--	--	--	98.8
<u>Standard germination test</u>				
Seafarer	--	--	--	75.5**
<u>Greenhouse flats</u>				
Seaway	--	--	--	93.0**
Charlevoix	--	--	--	92.3**

Table 7. Continued.

Experiment, variety and severity of abuse[1]	Internode lengths[2]			Total hypocotyl-epicotyl length[2]
	Cotyledon to primary leaf	Primary to first trifoliate leaf	Cotyledon to trifoliate leaf	
<u>Greenhouse bench</u>				
Seaway: Light	98.3	84.6	91.4	--
Medium	87.3	55.1**	71.4**	--
Severe	91.1	65.6**	78.5*	--
Charlevoix: Light	131.1	116.0	126.2	--
Medium	101.6	93.3	98.9	--
Severe	90.4	90.7	87.8	--
<u>Cold tests</u>				
Seafarer: Outdoor	89.8	--	--	94.3**
Growth cham.	92.1*	93.3	92.5*	97.3

[1] Unless otherwise indicated, severity of abuse was the medium level.

[2] Values shown are expressed as percentage of control.

[3] Not determined.

*Length reduction was statistically significant at the 5% level.

**Length reduction was statistically significant at the 1% level.

A reduced growth potential was not evident in the favorable environments provided in the vermiculite and sand germination tests, again illustrating the necessity of an environmental stress for detecting small vigor differences. Results from the standard germination test would at first appear to disagree with this hypothesis. Moisture, light and temperature should have provided nearly optimum conditions for germination and early seedling growth. However, morphologically normal 6-day-old seedlings from damaged seed averaged 24.5% shorter than seedlings from non-damaged seed. Sufficient stress to elicit this vigor response may have resulted from the customary procedure of placing a wet blotter over the seeds for the first few days of germination. The blotters were removed after 2 to 3 days but by this time most seeds had germinated and the seedlings had lifted the blotter 3 to 5 cm.

Varieties appeared to interact with environmental conditions in expressing seedling vigor differences. Seedling lengths of both Seaway and Charlevoix varieties were significantly reduced by mechanical abuse when grown in soil in greenhouse flats. But in the greenhouse bench study Charlevoix did not illustrate a significant vigor reduction due to injury as did Seaway. These measurements, however, were of internode lengths rather than total seedling length as in the vigor test in greenhouse flats, so a direct comparison of results may not be valid.

The significance of these seedling growth measurements is twofold. First, they demonstrate a true loss of growth potential even in those seedlings which are not visibly damaged by mechanical abuse. Secondly, the expression of vigor differences is greatly influenced by environmental conditions.

Seedling Weight

Weight accumulation is another growth parameter used to measure comparative seedling vigor. Seedling fresh or dry weights were determined for several of the experiments (Table 8). No significant reductions in weight accumulation were observed for normal seedlings produced from damaged seeds.

Previous work on beans (10) and peas (59) had shown damaged-induced weight reductions. However, neither of these studies attempted to eliminate those seedlings which lacked cotyledons or which were injured such that the cotyledons were not readily used as a nutrient source. Weight differences observed in those studies was likely influenced to a large degree by these types of seed injury.

Dry weights of 12-day-old seedlings thinned from the greenhouse bench study were also determined but were not reported in Table 8 because some of the seedlings included in the weight determinations lacked a cotyledon. Mean seedling weights for Seaway were reduced about 44, 45, and 60% by light, medium and severe levels of mechanical abuse.

Table 8. Fresh or dry weights of morphologically normal bean seedlings produced from abused or non-abused seed.

Experiment and variety	Mean seedling weight		
		Damaged	
	Control (mg)	(mg)	(% of control)
Germination in vermiculite (fresh wt. at 15 days)			
Seafarer	1379.6	1379.0	100.0
Greenhouse flats (dry wt. at 14 days)			
Seaway	132.4	130.7	98.7
Charlevoix	271.9	247.0	90.8
Cold tests (dry wt. at 30 days)			
Seafarer			
Outdoor flats	225.3	236.1	104.8
Growth chamber	478.3	436.5	91.3

Date of Flowering

To obtain a better picture of plant performance, the plants in the greenhouse bench study were grown to maturity. A record of the date of first blossom opening for each plant showed that the medium and severe levels of seed abuse significantly delayed Seaway flowering by 1 day (Table 9). No delay in flowering was observed for Charlevoix plants produced from damaged seeds. Mechanical abuse of seed again appears to have a differential influence on performance of different bean varieties.

A striking injury-induced delay in maturity was previously shown in Figure 1. Delayed maturity in that case could likely be contributed to the influence of abnormal seedlings, but the visual impact was outstanding.

Seed Yield

Seed yield and several components of yield were also determined in the greenhouse bench study (Table 10). Pod set and seed yield were poor for all treatments, including the control, in this experiment. High temperature and intense sunlight during anthesis may have been responsible. Consequently, variation between plants was higher than usual for some of the variables measured, resulting in lowered statistical precision.

Nevertheless, Seaway and Charlevoix varieties appeared to respond differently to the seed abuse treatment.

Table 9. Time required for initial blossoming of morphologically normal bean plants produced from non-damaged seed and seed given three levels of mechanical abuse.

Variety and severity of abuse	Ave. no. of days to first blossoming		Damaged expressed as % of control
	Control	Damaged	
<u>Seaway</u>	38.0		
Light		38.4	100.9
Medium		39.0*	102.6
Severe		38.7*	101.8
<u>Charlevoix</u>	37.1		
Light		36.7	98.9
Medium		37.1	100.1
Severe		36.8	99.2

*The increased time required for first flower opening was statistically significant at the 5% level.

Table 10. Effects of mechanical seed abuse on yield and yield components of morphologically normal bean plants grown in the greenhouse.[1]

Variety and severity of abuse	Pods per plant	Seeds per pod	Weight per seed	Total seed yield
Seaway				
Light	101.5	103.0	95.1	99.5
Medium	109.5	99.3	94.4*	102.4
Severe	107.3	99.3	97.8	104.0
Charlevoix				
Light	93.5	101.9	99.2	94.5
Medium	88.9	90.7	99.4	80.1*
Severe	79.9*	97.4	98.6	76.7*

[1] Values shown are expressed as percentage of control.

*The reduction in yield or yield component was statistically significant at the 5% level.

Mechanical injury consistently reduced pod set on Charlevoix but not on Seaway plants. Neither variety showed consistent reductions in number of seeds per pod or in seed size. Charlevoix yield was significantly reduced by the two more severe seed damage treatments while Seaway yield was not affected.

Since seed weight and number of seeds per pod were not significantly affected, the yield reduction obtained from damaging Charlevoix seed appears to be attributable to the reduction in number of pods formed.

Comparative Vigor Reduction from Cryptic Injury and Loss of One Cotyledon

Cotyledon cracking, breaking and actual severence was shown to be the most common type of seedling abnormality resulting from mechanical abuse of bean seed (Table 3). Preliminary observations supported the speculation that actual or functional loss of cotyledonary food supply would effectively reduce growth potential of those seedlings. A detailed investigation of vigor reduction resulting from loss of one cotyledon due to seed abuse or by hand excision substantiated the hypothesis (Table 11). Loss of one cotyledon highly significantly reduced seedling growth in every parameter measured in this performance test.

Table 11. A comparison of seedling vigor reduction resulting from cryptic injury incurred during seed abuse and vigor reduction due to loss of one cotyledon.

	Internode lengths[1]				
Treatment	Cotyledon to primary leaf	Primary to first trifoliate leaf	Cotyledon to trifoliate leaf	Total seedling length[1]	Seedling dry weight[1]
Non-damaged					
(One cotyledon excised)	78.3**	72.3**	76.4**	91.1*	77.8**
Damaged					
(Normal seedlings)	92.1*	93.3	92.5*	97.2	91.3
(Lacking one cotyledon)	77.5**	63.3**	73.4**	80.1**	59.9**

[1] Values shown are expressed as percent of control.

*Vigor reduction was statistically significant at the 5% level.

**Vigor reduction was statistically significant at the 1% level.

In agreement with data of the other vigor tests (Tables 7, 9 and 10), an injury-induced decline in vigor was also observed. While only internode length reduction was statistically significant, the morphologically normal seedlings produced from damaged Seafarer seed showed reduced growth in every parameter. This reduction in growth, however, was only about 1/3 as much as that caused by lack of one cotyledon.

It is clear from these results that loss of a cotyledon greatly reduced seedling growth potential. Similar cotyledonary influence on growth potential was previously shown for garden beans (57) and for soybeans (36).

When the two detrimental factors occurred in the same seedlings (damaged, lacking one cotyledon) the combined effect on seedling length and dry weight is more than additive. This observation may be explained by the findings of McAlister and Krober (36) that removal of one soybean cotyledon later than four days after emergence had no influence on plant development. Evidently the critical period for seedling nutrition is during early germination and emergence. Excision of a cotyledon from control seedlings after emergence did not elicit the total vigor reduction observed in damaged seedlings which had lost their cotyledons before germination commenced.

Effects of Mechanical Abuse on Growth of Excised Embryonic Axes

After establishing that mechanical seed abuse did in fact reduce the growth potential of even morphologically normal seedlings, additional studies were initiated to investigate possible sites and mechanisms for the vigor reduction phenomenon. The growth of excised embryonic axes from Seafarer was measured to determine if the injury-induced vigor loss was located within the axis itself. No reduction in growth was observed for embryonic axes from damaged seeds. In fact, damaged axes were about 13 and 15% longer than control axes at 2.5 and 5 days after planting. These differences were both statistically significant.

There is no apparent explanation for the more rapid growth of excised axes from damaged seeds. Respiration (rate of 0_2 uptake) of these axes had been measured prior to planting them on filter paper. Damaged axes tended to use 0_2 slightly more rapidly than control axes but the differences were not statistically significant. Both respiration rates and axes length measurements were quite uniform for the six replications, decreasing the probability of random variation as an explanation.

Nevertheless, it is apparent that the cryptic injury did not reduce growth potential of the embryonic axis itself. This suggests that the injury is located either in the cotyledon or in the transport system between axis and cotyledon.

Effects of Mechanical Abuse on Development of Callose Tissue in the Vascular Traces

The search for callose accumulations in the connective tissues between cotyledons and axes showed no apparent differences between normal seedlings from control or damaged seeds. Figure 4 illustrates the representative callose development in 3-, 5- and 8-day-old seedlings. No callose was observed in 3-day-old seedlings. By 5 days considerable callose had developed in sieve tube cells of the vascular tissues between the hypocotyl and cotyledon but no increase due to mechanical abuse was observed. Stainable callose was found both in pits of the lateral cell walls and on the sieve plates.

Currier (19) and Currier and Strugger (20) reported that wounding of tissues by sectioning or other methods stimulated rapid synthesis of stainable callose. Currier (19) states that, "while the first appearance of callose as a result of wound stimulation was confined to the pits, the substance later developed in some instances to cover practically the entire wall surface." In some cases callous plugs were formed in sieve tubes and laticifers as a response to wounding.

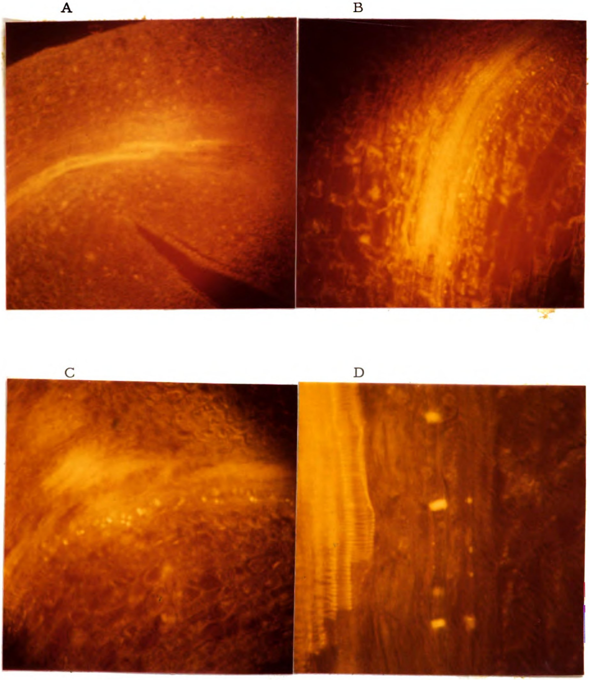

Figure 4. Stainable callose (brightly fluorescing spots) in vascular tissue between cotyledons and embryonic axes of morphologically normal bean seedlings. (A) Typical 3-day-old control or damaged seedling. (B) Five-day-old seedling from control seed. (C) Five-day-old seedling from damaged seed. (D) Enlargement showing callose in ends and sidewalls of sieve tube elements.

The absence of wound stimulated callose synthesis in damaged bean seedlings does not disprove the hypothesis that tissue wounding may be at least partially responsible for the observed decline in vigor. A disruption in the vascular linkage between axis and cotyledon could impair metabolic transport and still not be severe enough or of the required nature to stimulate callose synthesis. On the other hand, the absence of a wound response and the microscopic examination itself did indicate that no extensive internal injury was present.

Effects of Mechanical Abuse on Respiration During Early Germination

The rate of 0_2 uptake during the first 20 to 30 hours of germination for whole seeds and embryo components is shown in Figure 5. It is apparent from these results that mechanical abuse of Seafarer bean seeds did not reduce the rate of 0_2 uptake by intact seeds or by excised embryonic axes or cotyledons. In fact, respiration of the damaged samples was slightly higher for many of the measurements. Preliminary trials with other bean varieties also indicated no influence of mechanical damage on respiration during early germination.

Combined respiration of excised axes and cotyledons is much higher than for intact seeds probably because of the restrictive effects of seed coats on intact seeds.

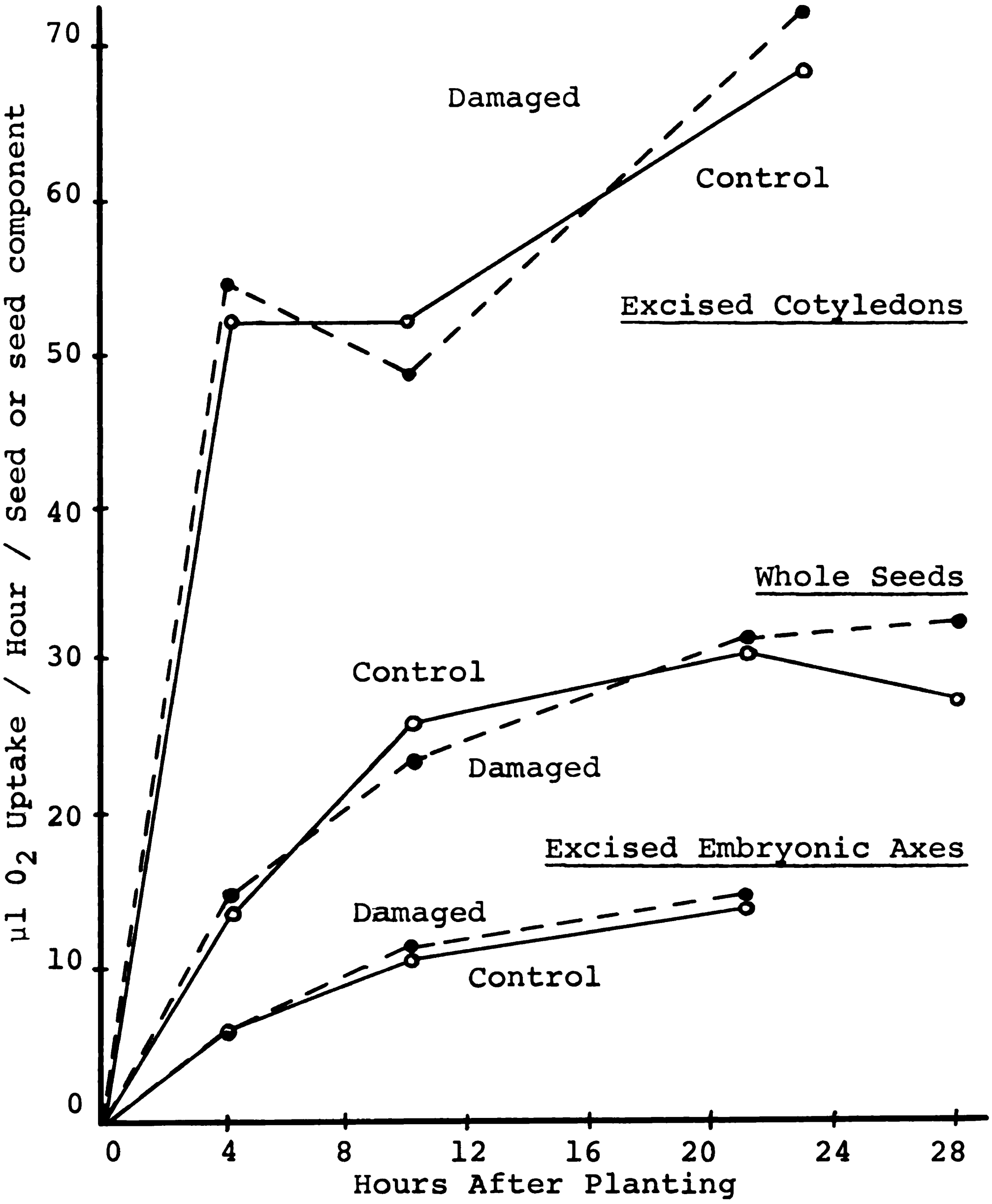

Figure 5. Respiration during imbibition of whole seeds, excised embryonic axes, and excised cotyledons from control and damaged navy bean seed lots.

Both imbibition and gaseous exchange rates are greatly influenced by the seed coat. Respiration of excised tissues might also be stimulated by a wound response mechanism.

Another artifact of the experimental method is evident in the apparent decline in O_2 uptake rate by cotyledons after the initial 4-hour measurement. The rapid respiration of the cotyledons apparently produced sufficient CO_2 to saturate the KOH solution. The 10-hour O_2 uptake measurement was therefore depressed due to the counteracting increase in CO_2 pressure. A decline in O_2 concentration should not have been responsible for the depression because the system was left open to the atmosphere between measurement periods. Before the 23-hour measurement of respiration the flasks were opened to aerate, and the KOH and filter paper wicks were replaced in the center wells.

An explanation for the lack of correlation between bean seed respiration and subsequent seedling vigor is not readily apparent. Several previous studies have shown positive correlations between respiration rates during the first hours of imbibition and subsequent seedling vigor (13, 32, 59, 61). While most of these reports concerned vigor differences due to natural or artificial seed aging, Woodstock and Combs (59) worked specifically with mechanical damage-induced vigor decline in peas. Respiration tests at 6 hours after the start of imbibition readily detected potential vigor reduction in the peas.

Respiration in seeds involves a wide range of metabolic processes that may be differentially influenced by a number of factors. In their study relating water content to respiration rate of bean seed, Opik and Simon (45) suggested that "a differential activation of enzymes in early germination is quite possible." Mechanical damage often breaks or weakens the bean seed coat causing an increase in imbibition. The resulting subtle changes in metabolism may influence subsequent seedling development without immediate effects on overall respiration.

Opik (43) observed in a later study that mitochondrial activity and structure develop at different rates in different cotyledonary tissues, but this differentiation in development begins only after the first 24 hours of germination. Consequently, respiration during this first 24 hours would probably not reflect possible future malfunction in the mitochondrial respiratory system. Opik (44) also noted that the vascular cells in bean cotyledons completed differentiation only after germination began. Injuries affecting these tissues or their development may have little influence on early respiration but could profoundly reduce subsequent translocation of metabolites to the growing embryo.

Whatever the reason may be, it is apparent that the respiration test is not a good indication of vigor losses in mechanically damaged navy bean seeds. It should be pointed out that the material used for these determinations could not be selected to eliminate those seeds which were destined to produce abnormal seedlings. Abnormal seedlings cannot be detected until at least the 3- or 4-day stage of normal germination. If mechanical injury were effective in reducing the respiration rate one would have expected an exceptionally large decline due to the presence of the potentially abnormal seedlings.

Another similarly discrepant response by bean seed has been observed. The estimation of glutamic acid decarboxylase activity (GADA) in seeds has recently been shown to correlate highly with seed viability and vigor for many crop species. James (30), however, found that correlations of GADA and viability for several bean varieties were very inconsistent and generally low.

Effects of Mechanical Abuse on Enzyme Activity in Bean Seedling Cotyledons

A more detailed picture of metabolism during early seedling growth was obtained by determining the developmental patterns of activity of three enzymes in the seedling cotyledons. Figures 6, 7 and 8 show the activities of cytochrome oxidase, succinate-cytochrome c reductase and amylases, respectively, in cotyledons of normal seedlings produced from control and damaged seeds.

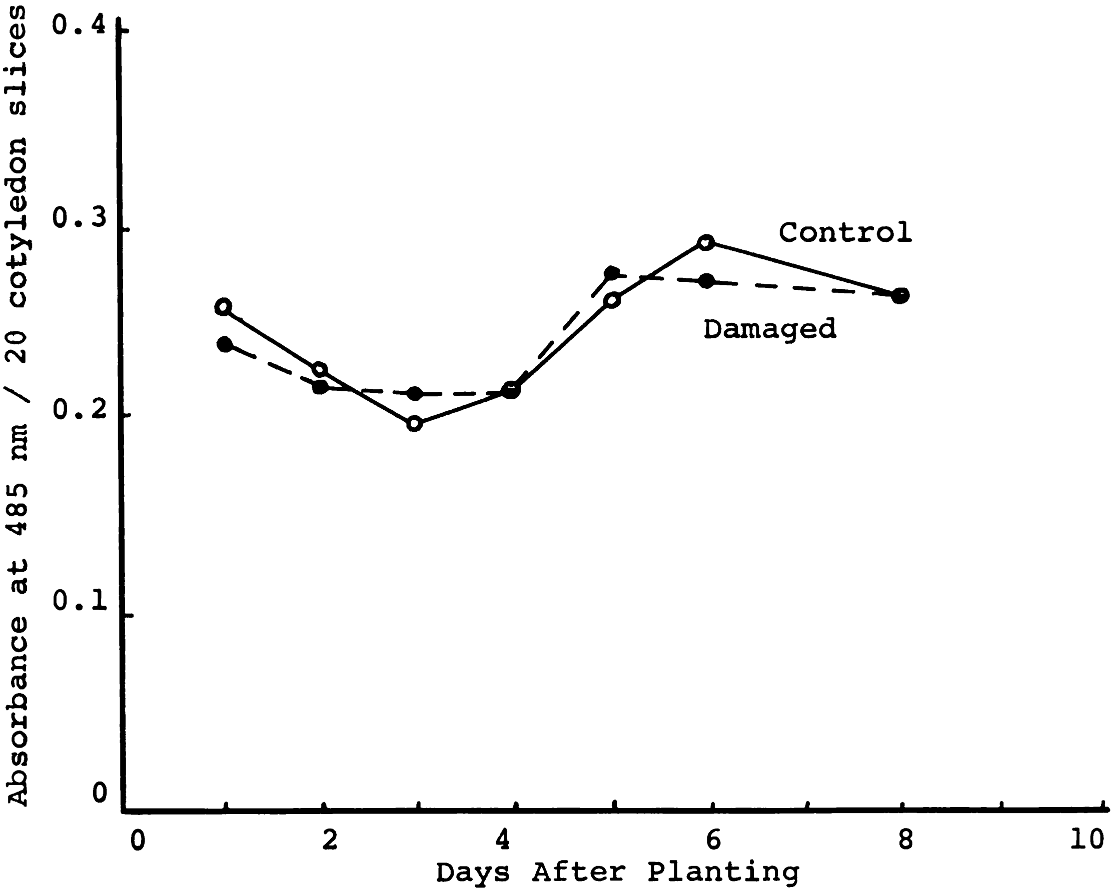

Figure 6. Cytochrome oxidase activity in cotyledons of normal appearing seedlings from control and damaged navy bean seeds.

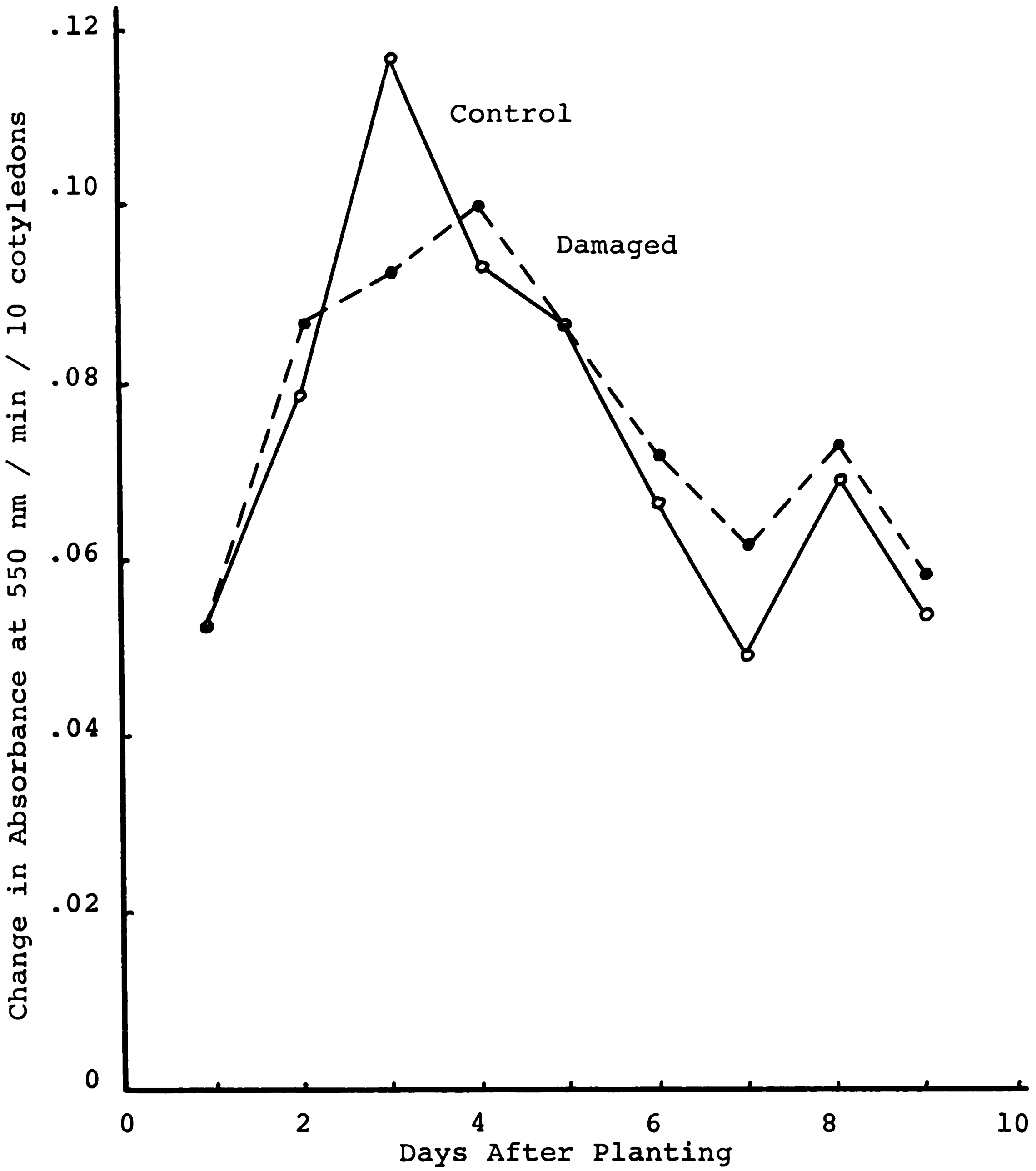

Figure 7. Succinate-cytochrome c reductase activity in cotyledons of normal appearing seedlings from control and damaged navy bean seeds.

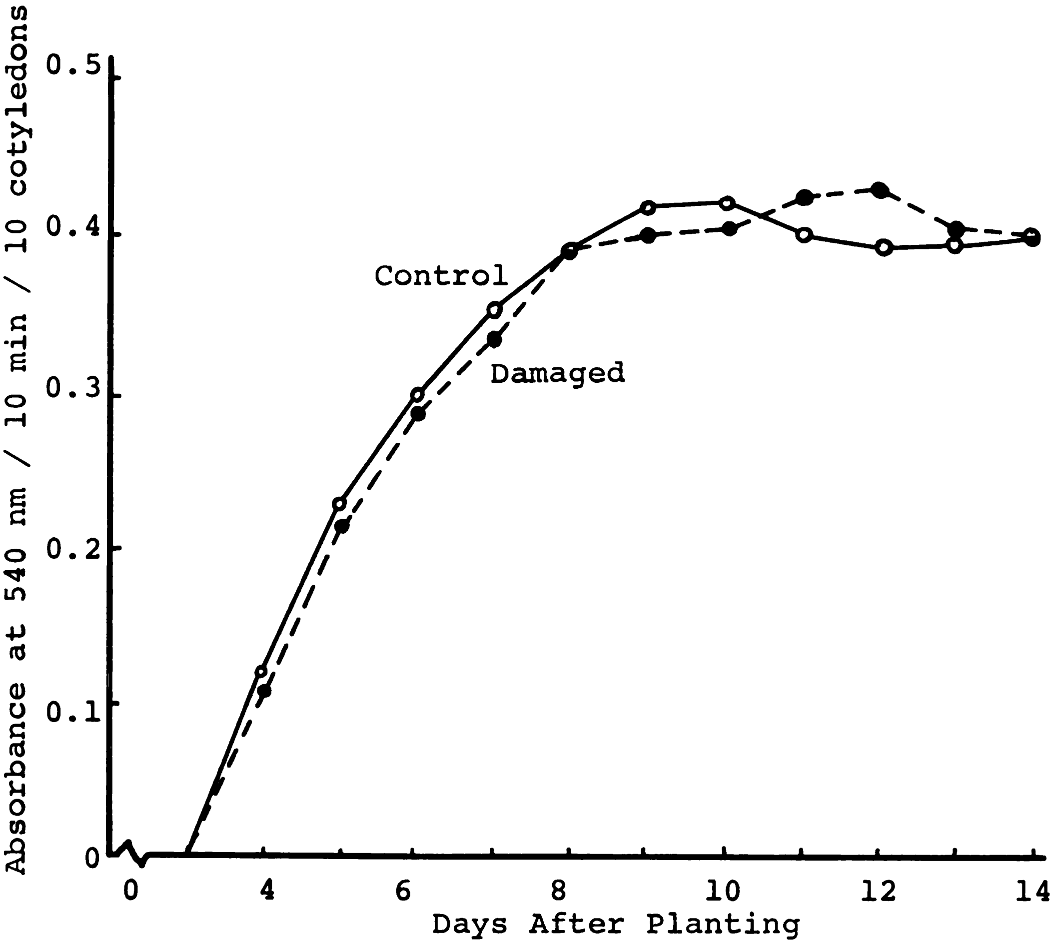

Figure 8. Amylase activity in cotyledons of normal appearing seedlings from control and damaged navy bean seeds.

Cytochrome oxidase activity showed relatively little change during germination while activity of the other two enzymes markedly increased during early seedling growth. Cytochrome oxidase was quite active on the first day of imbibition and was probably present in active form in the dry seed. Imbibition was the only requirement for full activation. The assay technique used for cytochrome oxidase in this study could not be applied to dry seeds. Cherry (15) used a different assay for cytochrome oxidase activity in peanut cotyledons and found an almost identical pattern of activation as shown in Figure 6. The time-scale for peanut was about double that for beans, but the initial high activity, followed by a slight decline and final increase were the same. Cytochrome oxidase was present in active form in dry peanut cotyledons.

There were no differences in the development of cytochrome oxidase activity in normal seedlings from control or damaged seeds.

Activity of the succinate-cytochrome c reductase system (Figure 7) was very different from that of cytochrome oxidase. Activity increased from a probable zero in dry seeds to a definite peak at 3 or 4 days after planting, followed by a decline and secondary small increase at 8 days. This pattern is also identical to that established for peanut cotyledons including the small incline late in cotyledonary life (15). Activity of this enzyme in peanut was again extended over a longer time span due to slower development of the peanut seedling. No reductase activity was found in peanut cotyledons before 2 days of germination, which supports extrapolation of the reductase curve to zero for dry bean seeds.

Mechanical seed abuse appears to have influenced development of succinate-cytochrome c reductase activity in morphologically normal seedlings. While the initial increase in activity was similar for both control and damaged seeds, the subsequent activity for damaged seedlings was slightly lower and delayed one day.

No amylase activity was found in the bean cotyledons until 4 days after planting (Figure 8). Activity increased steadily thereafter, reached a peak after 9 days of seedling growth, and then declined slightly. Development of amylase activity was again very similar for both control and damaged seedlings during early growth. However, while activity in control seeds rose steadily and peaked at 9-1/2 days, activity in damaged seedlings plateaued between 8 and 10 days, then increased to its maximum level at about 11-1/2 days. The difference between control and damaged seedlings was slight in terms of total activity but was consistent for the several replications.

Relating the enzyme activities to the morphological stages of germination may help to put them in proper perspective. When cytochrome c reductase activity reached its maximum at 3 to 4 days the radicles had protruded from the seed coat and elongated to 1 to 2 cm. At 8 days when the cytochrome enzymes were declining in activity and amylase activity was still increasing the seedlings had emerged from the sand media and were 8 to 9 cm tall. The primary leaves were just beginning to protrude from between the cotyledons. Soon after amylase activity reached its maximum rate at 10 to 12 days the cotyledons began to shrivel and senesce.

It is clear from these data that enzymes involved in metabolism during seed germination differ considerably in their patterns of activity, illustrating the complexity of these processes. Even the two cytochrome enzymes which are both mitochondrial enzymes and components of the same metabolic pathway differ markedly. Cytochrome c reductase shows peak activity at the same stage of germination that cytochrome oxidase activity is at its lowest level. Amylase activity was still zero at this stage. Young and Varner (62) found similar enzyme relations in the cotyledons of germinating pea seeds. Both phosphatases and proteases were activated several days earlier than the amylases. Cherry (15) concluded from his study of changes in several enzymes of the oxidative phosphorylation system during peanut germination, "that certain enzymes, even though involved in a metabolic pathway, may not be affected the same way as other enzymes of the same pathway."

Examination of the data in relation to mechanical damage effects shows that seed abuse may be responsible for metabolic changes during subsequent germination. Two of the three enzymes studied exhibited delays in activation resulting from seed damage. Although these delays are not of large magnitude, they may be indicative of a more widespread metabolic alteration. The damage effects in these two enzymes alone are almost certainly not responsible for the reduced vigor observed in normal seedlings produced from mishandled seeds. However, if other metabolic enzymes are similarly affected, the combined effect on metabolism could conceivably reduce seedling growth. The extremely complex interactions between the many processes of metabolism have been well established. And since most processes occur in a step-wise sequence, a delay in activation or reduction in rate of one small step may influence the entire chain of events (16). If several steps are even slightly inhibited the cumulative effect may be multiplicative rather than additive.

It is interesting to note that the enzymes affected by seed damage were those which were not present in active form in resting seeds but appeared only after initiation of germination. Cytochrome oxidase which was evidently active in dry seeds was not influenced by seed abuse.

Young and Varner (62) presented strong evidence for de novo synthesis of amylase in pea cotyledons during germination. And Cherry (15) found that the pattern of enzyme activation, one of which was succinate-cytochrome c reductase, closely resembled the level of RNA during germination. These facts may indicate that the physiological effect of mechanical injury in seed is related to the mechanism of protein synthesis rather than to enzyme activity itself.

Possible injury effects influencing protein synthesis can only be speculated upon. Ching reported (16) that phosphorylative efficiency is related to seedling growth rate. Injury-induced delays in activation of mitochondrial enzymes as was observed for cytochrome c reductase will affect phosphorylative efficiency. Impairment of this ATP producing process could have profound effects on subsequent enzyme synthesis, metabolism and seedling growth. While Ching (T. M. Ching, personal communication) did not feel that isolated molecules were likely to be damaged she did suggest that membranes and membrane associated enzymes might be affected by severe seed abuse. She felt that damaged cells or cellular organelles "would result in reduced functional efficiency which may cause lowered energy supply (ATP content) and synthetic ability." According to these hypotheses the delayed activation of the membrane-bound cytochrome c reductase enzyme may have been related to mitochondrial damage which may subsequently have influenced amylase synthesis and other metabolic processes.

Another possibility is that seed abuse may in some way damage the transport system between the embryonic axes and cotyledons. Besides impairing translocation of metabolites from cotyledon to axis, this type of injury could also influence enzyme activation or synthesis within the cotyledon. Many investigations have shown that metabolism in storage tissues during germination is controlled by stimuli from the embryonic axes. While most work in this area has been with barley, other seeds including legumes have been investigated. Some factor or factors from the axis tissue of germinating pea seeds have been found to control development of amylase and phosphatase activities (63) and other metabolic processes in the cotyledons (56). Preliminary work, not reported in this dissertation, suggested a similar stimulatory mechanism in bean germination. Activation of succinate-cytochrome c reductase was 1 to 3 days slower in excised cotyledons. An injury sustained by the vascular system would impede translocation of the stimulating factor(s) to the cotyledon thereby delaying resumption of metabolism and production of the nutrients required for embryo growth.

Although these studies have not established the true physiological basis for mechanical injury-induced decline in seedling vigor, the data do suggest several possibilities. Final elucidation of the physiological or morphological defects in normal appearing seedlings awaits further study.

SUMMARY AND CONCLUSIONS

Seed quality reduction and related metabolic changes caused by mechanical abuse of bean seed were investigated. Seeds of several bean varieties were preconditioned to specific moisture contents and damaged by dropping 9.2 m onto a steel plate. After determining the extent of physical damage caused by the impaction treatment, all seeds showing external damage were discarded. Performance of the sound seeds was compared to performance of non-damaged seeds in field trials, greenhouse tests and controlled environment studies. Cryptic effects of seed damage were sought by anatomical and physiological techniques.

The impaction treatment caused extensive physical damage; cracking or breaking from 0.5 to 37.2% of the seeds and seed coats. The extent of damage depended on seed moisture content at the time of impaction and on seed variety. Performance of the remaining sound seeds was dependent on these two factors and on environmental conditions of the various tests. Mechanical abuse reduced seedling emergence from 0 to 50%. Of the emerged seedlings 3 to 62% were morphologically abnormal. The most common type of defect was cotyledonary damage, ranging from transverse cracking to complete severence of one or both cotyledons. Severence of the epicotyl was also common. The defective seedlings were probably largely responsible for reduced yields observed in the field trials.

The growth potential of morphologically normal seedlings was investigated to determine possible cryptic effects of seed abuse. Measurement of internode or total seedling elongation indicated a significant reduction in vigor of normal seedlings produced from damaged seeds. A slight delay in flowering of one variety and decreased yield of another variety were observed, but the vigor reduction was not apparent in several other growth parameters examined. Injury-induced loss of vigor in normal seedlings was about 1/3 as great as the vigor decline caused by excision of one cotyledon.

The growth rate of excised embryonic axes was not reduced by seed abuse, suggesting that the cryptic injury is located in the cotyledon or in the transport mechanism between cotyledon and embryo axis. However, a search for wound-response callose tissue in the connective tissue between cotyledons and embryonic axes revealed no apparent differences between normal seedlings from control or damaged seeds. No evidence of microscopic damage to the vascular system could be found.

Respiration during the first 30 hours of imbibition and germination was likewise not influenced by seed damage.

But two of three enzyme assayed were slightly delayed in reaching peak activity during early seedling growth; amylase and succinate-cytochrome c reductase were affected but cytochrome oxidase was not. Both enzymes which were affected are thought to be synthesized *de novo* during germination, while cytochrome oxidase is apparently present in an active form in non-imbibed seeds. These facts may imply that the cryptic injury effect involves protein synthesis.

These data have established that mechanical seed abuse reduces the performance potential of those seedlings showing no visible morphological defects. Although the physiological basis for this effect has not been determined, damage to some metabolic mechanism(s) in the cotyledons are implicated.

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