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RELATIONSHIP BETWEEN LABORATORY
VIGOR TESTS AND FIELD EMERGENCE
OF SOYBEANS (GLYCINE MAX (L.) MERRILL)
IN MICHIGAN

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Donald Floyd Miles, Jr.

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Donald Floyd Miles, Jr.

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ABSTRACT

RELATIONSHIP BETWEEN LABORATORY VIGOR TESTS AND FIELD EMERGENCE OF SOYBEANS (GLYCINE MAX (L.) MERRILL) IN MICHIGAN

By

Donald Floyd Miles, Jr.

A comparison of vigor test and field emergence results of two soybean cultivars (Hodgson and Corsoy) was made in 1979 and 1980. Investigation One utilized high, medium and low quality seed. Seed for Investigation Two came from randomly selected certified seed lots. Warm germination test - four and seven day counts, cold test, accelerated aging test, tetrazolium test, conductivity test (ASA 610), and seed weight were used as laboratory vigor indicators. Three planting dates, each year, were used for field emergence studies.

No single vigor test had the best correlation with field emergence over all planting environments. Combinations of tests in multiple regression equations, for each soil environment, resulted in R^2 values between 0.517 and 0.950. One viability test, plus either the cold test or accelerated aging test, and either the conductivity test or warm germination test - four day count, should give the best indication of field emergence potential on sandy loam soils in southern lower Michigan.

6/16/80

DEDICATION

To Sandy and John, the two most important people in my life. Your sacrifice and patience have made the completion of this graduate degree possible. Your love and support have made it a valuable experience.

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INTRODUCTION

Soybeans have become an important grain crop in Michigan; second only to corn in the number of hectares planted annually. In 1979 396,600 hectares were planted, almost twice the amount planted just ten years earlier. Approximately 23 million kilograms of soybean seed were used to plant that amount of cropland.

While the cost of soybean seed is only about 8% of the total production costs per hectare, the results of planting poor quality seed are costly. Replanting costs of \$40-\$45 hectare along with the increase in labor, soil compaction, and the possible decrease in yield due to delayed planting can drastically reduce the net income on a soybean crop.

Recommendations for early planting and more soybean production in the central and northern counties of Michigan have increased the possibility of poor field emergence resulting from the planting of low quality seed. Producers rely on results of the standard warm germination test, which is printed on the seed tag, to give them the quality information needed to make planting decisions. However, many seed weaknesses are not detected by the warm germination test.

Differences in seed vigor caused by environmental conditions during seed development, harvesting procedures, and storage conditions may be present between seed lots having similar warm germination results. Planting in a pathogen infested seedbed under cold temperatures and/or moisture stress can magnify the expression of these

vigor differences.

Since the development of the cold test in the 1940's, seed scientists have been searching for better ways to measure this complex quality factor called vigor. In recent years many different types of vigor tests have been proposed. Those adopted by the seed industry have been promoted as aides to the farmer for selecting only the highest quality seed lots available and thus maximizing field stand establishment.

The many different factors that affect vigor, the variable conditions under which vigor tests may be run by different seed laboratories, as well as the infinite array of seedbed conditions into which soybean seed is planted, has confounded research efforts to determine which vigor tests best predict field emergence results. This study was initiated with three major objectives: First, to establish the relationship between the results of several vigor tests and field emergence for different seedbed conditions found in Michigan using seed lots with a wide range of vigor potential. Next, to evaluate the use of these vigor tests, singly and in combination, to predict field emergence for seed lots considered to be of acceptable quality by the seed industry. Finally, to use this information to make preliminary recommendations on which vigor tests can be included in a vigor testing program for Michigan soybean producers.

LITERATURE REVIEW

I. Seed and Seedling Vigor

The concept of seed and seedling vigor has for many years been accepted as a seed quality factor by seed scientists. Within the last decade it has also become a vital part of the quality control and marketing programs of many commercial seed companies.

According to Perry (69), one of the earliest recognitions of vigor differences in seed was by Nobbe in 1876, who used the term "energy of germination." However, most of the research on vigor and vigor testing has been done in the last 35 years. In 1950, Franck used the term "vigor" in describing his work with soil germination tests at a meeting of the International Seed Testing Association (ISTA) (69). Seven years later Isley (53) talked to members of the Association of Official Seed Analysts (AOSA) about vigor and vigor testing. Since then a great many papers have been published on these subjects.

The expression of vigor can be described from two different viewpoints. Some researchers speak of seed vigor per se as being an intrinsic property of the seed (96). Perry (69) referred to vigor along with viability, seed health, structural soundness and size as being seed components. Heydecker (49) concluded that a seed cannot be classified as being only good or bad. It has a level of vigor that provides a continuum from poor to good for a population of seeds.

The vigor of harvested seeds in storage has been called storage vigor (48), the vigor of the storage life of the seed (11) and the

non-active vigor state (49). Descriptions of the totality and speed of germination in the absence of environmental influences have included the terms germination vigor (48), germination energy (66), germination capacity (74) and the intensity factor (95). These terms imply the importance of seed viability in describing seed vigor. Delouche (24) concluded that vigor only relates to viable seeds, because a seed that does not germinate has no vigor potential.

The results of the interaction between the seed/seedling and environmental influences such as temperature, moisture, soil crusting and pathogenic microorganisms is the second way vigor can be expressed. Vigorous seeds/seedlings have a greater capacity for germination and emergence when subjected to adverse environmental conditions. These seeds/seedlings are said to have a higher field survival rate (49), a larger environmental range factor (95) or a better stand establishment capacity (28). Once the stand is established, the seedling survival rate (11) and seedling growth can be measured. Thus, seedling vigor (48) on an individual plant basis can have a major effect on the competitive interactions between plants (71) and eventually on yield potential (11, 98).

Although the concept of seed and seedling vigor has been widely accepted, there has not been general agreement on a precise definition of vigor. Many investigators have defined vigor to coincide with their own understanding and experiences. Pollack and Roos (71), Heydecker (49), Perry (69), and Woodstock (96) have presented their own unique definitions of vigor. Two prominent seed testing associations also have different official definitions of vigor. The AOSA has adopted a definition that

states:

"Seed vigor is the sum total of all those properties in seeds which, upon planting, result in rapid and uniform production of healthy seedlings under a wide range of environment including both favorable and stress conditions" (8).

The ISTA definition reads:

"Seed vigor is the sum total of those properties of the seed which determine the potential level of performance and activity of a non-dormant seed or seed lot during germination and seedling emergence" (8).

Each definition is different from all of the others, but all deal with field performance potential. This parameter is the ultimate result of vigor, regardless of whether the vigor expressed is an intrinsic seed property or a result of seed/seedling interaction with the environment.

The AOSA definition of vigor was adopted for the planning and evaluation of this study.

II. Factors Affecting Vigor

The vigor potential of soybean seed increases during seed development until maximum vigor is reached at physiological maturity (6, 25, 90). Throughout the remaining stages of seed life, vigor declines. Several factors are instrumental in determining the rate of decline during seed desiccation, harvest, storage, seed conditioning, planting and emergence. The interactions between these factors also establish the vigor level of a seed at any given point in time.

A. Genetic Factors

Ching (22) realized the important role of genetic factors in determining vigor potential. She observed that if all other factors are

maximized during all phases of seed life, then the genetic components are the greatest limiting factor of maximum seed vigor. Kneebone's (56) review of the genetic influence on seed vigor suggests that efficiency factors like genetic stability and the relative ADP to oxygen ratio have the most effect on seed vigor.

B. Morphological Factors

Seed size is one of the most frequently studied morphological factors in relation to seed vigor. Since large seed is associated with a greater storage capacity for sugars, protein and oils in the cotyledons than smaller seeds, a larger supply of stored metabolites would appear to indicate higher vigor potential. This theory has been supported by several reports (15, 20, 40, 78) of better field performance in terms of emergence and/or yield when large soybean seeds are planted. Contradicting reports have also been made by investigators (5, 51), who have found medium sized seeds to be of higher quality and have rapid emergence capabilities. All of these studies have dealt with the relative seed size within a lot and not with mean seed size between lots. Delouche (24) concluded that normal differences in mean seed size did not have an effect on seed performance. However, Edwards and Hartwig (34) found vigor differences with different seed sizes, as measured by grams per 100 seeds, of near-isogenic soybean lines.

C. Mechanical Damage

According to Pollock and Roos (71) three types of mechanical damage can be found in soybean seed. Visible external damage, which includes large cracks and splits is the most detectable type of damage. Seed lots

having a large amount of this type of damage can easily be diverted from seed channels or possibly upgraded through conditioning. Other kinds of mechanical damage may not be expressed until after seed germination is also possible. This type of damage is usually detected by the appearance of abnormal seedlings showing evidence of fractures in the cotyledons and/or embryonic axis. Microscopic breaks in the seedcoat which allow entry of microorganisms may also be present. This type of damage is virtually undetectable, but the consequence of attack by microorganisms on germination can be great.

There are several ways that seeds may become mechanically damaged. Damage can result from the expansion and contraction of the seed coat, caused by wetting and drying during seed desiccation (67). Repeated wetting and drying may also cause wrinkles to appear in the seedcoat, resulting in cracking of the seedcoat and damage to the embryonic axis. Simon and Raja Harum (76) proposed that wetting and drying may also cause cell cytoplasm leakage. After each rewetting, the lag time for cell membrane reorganization allows cytoplasm to leak out of the cell.

Mechanical damage during harvest is common if threshing machinery is not properly adjusted, especially in seed with low moisture contents. Winjandi and Copeland (93) found that dry bean seed with a moisture content of less than 11% was very susceptible to mechanical damage. Dexter (30) increased the germination of dry bean seed from 39% to 78% by increasing seed moisture at harvest from 11% to 18%. Hartwig (46) reported a decrease in germination and increase in mechanical damage as soybean seed moisture at harvest went below 10%. Moore (64) observed that non-visible damage during threshing contributed to many seed quality problems.

Mechanical damage may also occur during seed conditioning. Bunch (14) discovered more damage to soybean seed with a moisture content of less than 12% during conditioning than seed between 12% and 18% moisture.

Green (45) found large seed to be especially susceptible to mechanical damage, but noted that high quality seed was more tolerant of mechanical abuse than low quality seed. A reduction in field stands results when mechanically damaged seed is planted under adverse soil conditions. Vorst and Mason (89) saw a 25% reduction in emergence when impact damaged seed was compared with undamaged seed under cold soil temperature conditions.

D. Physiological Factors

There are many biochemical and physiological processes that affect seed vigor. Two of these processes that have been studied extensively and have a great impact on seed/seedling field survival are aging and imbibitional chilling response.

1. Aging

Seed deterioration resulting from the aging process occurs during seed desiccation on the plant and while the seed is in storage. Aging begins soon after physiological maturity has been reached and is delayed or hastened by environmental conditions. TeKrony et al. (86) observed that a decline in seed vigor during desiccation was hastened by high temperatures and humidity. Similar observations were reported by Bulat (13) on seed in storage. He found relative humidity (R.H.) to be the most important factor with a R.H. of 55% to 73% causing the greatest decrease in viability and vigor. Egli et al. (37) also reported

deterioration of seed quality when soybean seed was stored at a moisture content of greater than or equal to 13.5% for nine months.

Dehydration from field desiccation causes the mitochondria in seeds to be more susceptible to damage and initiates the degradation processes (2). Abu-Shakra and Ching (3) observed fewer and less efficient mitochondria in aged seeds. Uncoupling of oxidative phosphorylation from respiration may be the major reason for the loss in mitochondrial efficiency. The lower oxidative phosphorylation rate causes slow seedling growth because less ATP is available for protein synthesis. This decrease in the rate of protein and carbohydrate synthesis in aged seeds has been reported by several investigators (1, 2, 22). A lower rate of respiration in aged seeds caused by a decrease in the number of mitochondria has also been observed by several researchers (1, 2, 32, 44, 94). The proposed effect of fewer, less efficient mitochondria on seedling growth was supported by an investigation done by Wahab and Burris (90). They found a linear rate of fresh weight growth for low quality seeds (aged) and an exponential rate of growth for high quality seeds. Dry weight accumulation was also found to be slower for the low quality seeds.

Increased membrane permeability may also contribute to the loss of vigor in aged seeds. An increase in the membrane permeability as seed quality decreases has been reported by many investigators (1, 2, 23, 80, 96, 97). Koostra and Harrington (57) found fewer phosphorous containing polar lipids in artificially aged seeds, which helps to explain the observed increase in permeability. Woodstock (96) observed that an increase in membrane permeability not only allows greater loss

of cell cytoplasm but also increases the possibility of attack by microorganisms, both of which can make a significant contribution to lower seed quality.

2. Imbibitional Chilling Injury

Seed planted in cold, wet soil is exposed to conditions that promote chilling injury during imbibition. Obendorf and Hobbs (50, 68) have found that soybean seeds with a moisture content of less than 12% are most susceptible to chilling injury. They have also shown that temperatures less than or equal to 5°C are needed to induce chilling injury. Littlejohns (58) discovered that the critical temperature for injury to soybeans varied depending upon the cultivar. He classified some cultivars as being intolerant to cold conditions. Some of those cultivars suffered injury at 10°C.

Lower field stands resulting from cold injury are caused by a loss of cytoplasm, especially during the early states of imbibition. Bramlage et al. (12) have shown that exposure to cold temperatures during imbibition increases the time needed for membrane reorganization. Therefore, more exudates are lost than if imbibition occurred under warmer conditions. An increase in the leakage of sugars and amino acids (50, 68) as well as proteases and phosphatases (12) resulting from chilling injury, attracts soil-borne fungi and bacteria. These two results of chilling injury help to explain poor field emergence results when seed is planted in cold soil.

E. Pathological Factors

The complex interactions between the seed and microorganisms can reduce viability and vigor. De Tempe and Limonard (29) discussed

the antagonistic and stimulatory effects between seeds, pathogens and saprophytes. The effect of microorganisms on seed quality varies depending upon the type of organism and environmental conditions.

Diaporthe phaseolorum var. sojae and Phomopsis sp. can infect the seed anytime during seed development but infection is enhanced by high temperatures and humidity and when harvest is delayed (77). The fungus invades the seedcoat and may spread to the cotyledons and embryos in heavily infected seed. Large infections of these organisms in a seed usually prevent seed germination. Smaller infections will affect germination the most when soil temperatures are between 30°C and 35°C. When diseased seeds germinate they provide inoculum for dissemination to surrounding noninfected plants. Kmetz et al. (55) discovered that as the percentage of Phomopsis sp. infection increased, the amount of seed decay increased and the percent emergence decreased.

Pseudomonas glycinea can also infect soybean seed prior to harvest. A study by White et al. (92) shows a reduction in field emergence when seed infected by P. glycinea is planted in soil with temperatures between 20°C and 30°C.

During seed storage many fungi of the genera Aspergillus, Alternaria, Penicillium and Rhizopus can cause deterioration of high moisture soybean seed (77). High temperatures and humidity aid the invasion and spread of these fungi in seed. To avoid infection, seed should be stored at less than 13% moisture, 50% R. H. and 10°C. Seed that is heavily infected with storage fungi often fails to germinate under near ideal conditions. Less infected seeds usually produce stunted seedlings which have decayed cotyledons and reduced plumule growth.

Wallen and Seaman (91) proposed that the germination and emergence phases of seed life were critical for disease development since many fungi and bacteria spread during these stages. Matthews (59) found that exudates from cotyledons enhance the spread of these microorganisms. Exudates also predispose the seed to infection by soil-borne pathogens of the genera Pithium and Fusarium. These fungi can cause reductions in field emergence.

III. Vigor Testing

Interest in vigor testing began when seed scientists and technologists realized that there were components of seed quality that could not be detected by the warm germination test. In the last decade, as seedsmen became aware of vigor testing and customers became more dissatisfied when poor field stands occurred, the demand for the use of vigor testing as a quality control tool and marketing aid has increased tremendously.

Vigor testing can benefit both the seedsman and the farmer (61). With the help of a vigor testing program, seedsmen can monitor seed quality and improve marketing practices. Farmers who plant only good quality seed, as determined by vigor tests, can benefit from earlier planting dates, lower seeding rates, and more uniform field stands. Unfortunately many farmers and seedsmen believe in the "seed testing myth" (84). It is:

"That one laboratory test will measure all of a seed lot's physiological traits and potential and apply to a wide range of field conditions."

A vigor test needs to meet several criteria before it can be used as a routine test of seed quality. McDonald (61) and Heydecker (48)

have outlined the attributes that an "ideal" vigor test should possess. It must be sound in principle, objective, inexpensive, rapid, related to field emergence and involve uncomplicated procedures yielding reproducible results. Many reports (19, 54, 84, 85, 95) have shown that no single test can satisfy all of these requirements. Since different vigor tests measure different aspects of seed quality and because seed is planted in a wide range of soil conditions, a combination of several vigor tests would seem most likely to meet the expected criteria. The results of these tests reported either separately or in an index form would give the most information about the quality of a seed lot and its potential field performance.

One of the biggest problems with vigor testing is that different procedures may be used by different seed laboratories for the same test. Isley (53) and Delouche (26) have encouraged all persons using vigor tests to work towards standardization. Until standard procedures are established, the primary value of vigor testing will be as an "in-house" quality control tool. Schoorel (75) has stated that vigor tests presently can be used only as "local" indicators of a seed lot's planting value. The Vigor Test Committee of the Association of Official Seed Analysts has attempted to attain standardization for some popular vigor tests by publishing a progress report on a seed vigor testing handbook (8).

Vigor tests are classified in numerous ways. A popular way of viewing vigor tests is to classify them as direct or indirect indicators of seed vigor (28, 48, 52). Direct tests are variations of the warm germination test which are used to indicate seed quality. Many also

incorporate some type of stress simulation. The cold test, warm germination test-four day count and accelerated aging test could be classified as direct tests. Direct tests can indicate how a population of seeds will perform under specified conditions, while indirect tests utilize the seed as the only biological variable. For example, the tetrazolium vigor test (66) places emphasis on the seed's attributes and not the environment. The conductivity test could also be classified as an indirect test. These tests allow for quality interpretation on an individual seed basis. A valid vigor testing program should use a good balance of both direct and indirect tests.

A 1976 survey (43) of 102 seed laboratories in the United States revealed that 68% were already using vigor tests on a regular basis, or expected to be routinely testing for vigor by 1980. The three most commonly used tests were the cold test, the accelerated aging test, and the tetrazolium vigor test. A similar survey among members of the International Seed Testing Association (11) showed that the conductivity test, cold test, and tetrazolium vigor test were used most often by its members.

The factors that determine which vigor tests will be included in a seed laboratory's vigor testing program are the availability of equipment, the expertise and abilities of laboratory personnel, and advice from "experts." Biases based on previous experiences with vigor tests and their relationship to field performance also influence these decisions.

The standard germination test has correlated well with field emergence in many studies (16, 17, 36, 39, 79, 85). These studies involved planting seed in favorable seedbed conditions or used artificially

damaged or deteriorated seed having a warm germination of below 85%. The cold test, which was developed to test the effectiveness of fungicide seed treatment (73), has correlated well with emergence when seed is sown in adverse soil conditions (9, 54, 63, 82, 83). Speed of germination, or the four day count of the warm germination test has also shown promise in evaluating seed vigor (19, 31, 70, 85). It is used extensively in European countries as an "in-house" check of seed quality (43). However, differences in hypocotyl elongation rates between cultivars (47) and the lesser sensitivity of this test to detect quality changes than other vigor tests (37) has caused concern about its use as a vigor test. The accelerated aging test has been shown to detect differences in seed vigor (9, 27, 62, 63, 83, 84, 85), especially if the seed has deteriorated or is subjected to adverse planting conditions. It does not do as well when seed is planted in a favorable seedbed (79), and modifications in procedure are needed to account for differences in initial seed moisture (62, 81). Recent reports from investigators in the United States (31, 63, 83) have indicated that the conductivity test may also correlate well with seed vigor. The tetrazolium test can be used to detect quality differences if seeds are categorized from high to low vigor based on the color and soundness of seed tissues (13, 65, 98). The extensive training needed for accurate interpretation of tetrazolium test results is the major disadvantage of this test.

The cold test and/or the accelerated aging tests have been recommended for inclusion into vigor testing programs by several seed scientists (24, 54, 84). The tetrazolium vigor test (24) and the warm germination-four day count (84, 85) have also been mentioned as candidates

for vigor testing programs. TeKrony (85) has proposed using a vigor indexing system, combining the results of several vigor tests with the warm germination test results. Yaklich (98) prefers the use of coefficients of multiple determination (R^2), involving the multiple regression of several tests, to evaluate the vigor of seed lots.

IV. Use of Fungicide Seed Treatment

Evidence for the use of fungicide seed treatments on soybean seed has not been conclusive. Fungicide costs and the unsuitability of treated soybeans for the commercial grain market have caused many seedsmen to sell their seed untreated. However, it has been shown that in certain cases seed treatment can be very beneficial.

Several reports (10, 33, 48, 87) have shown that use of a fungicide seed treatment can improve the field emergence capabilities of poor and medium vigor lots of soybean seed. The extent of this improvement is influenced greatly by the soil environment. TeKrony et al. (87) and Edje and Burris (33) suggest the use of a fungicide seed treatment when seed has a warm germination below 85%. Seed treatment should also be considered on early planted seed that may encounter cold soil temperatures.

V. The Relationship Between Vigor and Yield

Vigor is important in determining the ultimate yield of soybeans on an individual plant basis. Since vigorous seeds/seedlings have a definite competitive advantage (42, 71), the resulting plants should produce more branches, pods, seeds and other components of yield. Less vigorous seeds produce seedlings which have a slower rate of growth (20,

21). These plants then tend to be lower yielding or barren (20).

Rajanna and de la Cruz (72) have documented this relationship between the early plant growth ratio and yield of individual plants.

Although farmers who use high vigor seed expect greater yields, they do not always obtain them under actual field conditions. Seed vigor, as it is used by many in the seed trade, usually refers to the quality of a population of seeds. Athow and Caldwell (10) discovered that the spacing between plants within the row could vary from 2.5 cm to 10.2 cm without affecting yield. Byrd (21) found no difference in yield between low quality and high quality seed lots when plant populations were equalized to 23 plants/meter. Egli and TeKrony (35) concluded that there was no relationship between initial vigor level and yield when they compared the yield results of natural field stands of high, medium and low vigor seed planted at rates of 16 viable seeds/meter and 33 viable seeds/meter. Edje and Burris (33) summarized the sentiments of many seed scientists when they concluded that if a satisfactory field stand is established, yield is not affected by seed/seedling vigor. Therefore, vigor tests are best used to identify and eliminate seed lots that will not produce acceptable field stands over a wide range of soil environmental conditions.

MATERIALS AND METHODS

Two investigations were conducted in both 1979 and 1980. In the first study, high quality seed lots of two soybean cultivars were selected. Samples of each lot were subjected to impact damage and artificial aging to obtain low and medium quality levels in addition to the undamaged control. Randomly selected seed lots of the same two cultivars were collected from lots eligible for certification in Michigan for use in the second investigation. Laboratory studies were done in both investigations using eight different tests to determine seed quality. Field studies were also conducted to determine the ability of the seed to emerge under various environmental conditions.

I. 1979 Studies

Investigation One consisted of two high quality seed lots of the cultivars Hodgson and Corsoy that had been produced in 1978. These cultivars are well adapted to Michigan and of maturity groups I and II respectively. They also represent the most popular Michigan Certified seed cultivars produced for their respective maturity groups. Each seed lot was uniformly mixed and subdivided into four 2 kg samples. One sample was kept untouched as a high vigor control lot. Another sample was dried to 10% seed moisture and subjected to mechanical impact damage by using a pulley-driven propeller to project the seed against a 1.5 cm thick board. Two additional samples were artificially aged, by exposing them to a temperature of 40°C at a seed moisture of 16% for

one week and two weeks respectively. After treatment, each of the four samples represented a different vigor level.

Each 2 kg sample was divided into two 1 kg subsamples. One subsample was treated with a slurry fungicide seed treatment of N (trichloromethyl) thio-4-cyclohexene 1, 2-dicarboximide (Captain 80W) at a rate of 35.4 grams of formulation per 27.2 kg of seed. The other subsample was left untreated. The 32 subsamples (eight from each original high quality lot) were adjusted to a seed moisture of 13% and then stored at 10°C and 50% relative humidity until laboratory tests and field studies were completed.

For Investigation Two, five lots each of the cultivars Hodgson and Corsoy were randomly selected from seed lots eligible for certification in 1978. A 2 kg sample was collected from each lot. All samples were between 12% and 14% moisture when collected. Each sample was divided into two 1 kg subsamples. As in the first study, one subsample was treated with Captan 80W fungicide seed treatment and the other was left untreated. All twenty subsamples were stored at 10°C and 50% relative humidity until completion of the laboratory tests and field studies.

A. Laboratory Tests

Laboratory tests for Investigation One were done in a completely randomized design with a 2x2x2x4 factorial arrangement of treatments. The 32 treatment combinations consisted of two cultivars, two fungicide seed treatments (one with Captan 80W and one without fungicide), two lots per variety and four vigor levels per lot. The completely randomized

design for Investigation Two had a 2x2x5 factorial arrangement of treatments. The 20 treatment combinations were from two cultivars, five lots per cultivar and two fungicide treatments.

The procedures for each laboratory test were identical for all experimental units in both investigations, unless otherwise specified. Standard analysis of variance methods were used to analyze the laboratory test data.

1. Standard Warm Germination Test - Seven Day Count (WG-7)

Warm germination tests were done at the Michigan Crop Improvement Association laboratory in a Warren-Sherer walk-in germination chamber. Four 100 seed replicates were placed on moist Kimpac germination media (100 seeds per tray). The seeds were germinated at 25°C and 90% relative humidity. Seedlings were classified as normal, abnormal, and dead after seven days in the germinator. The percentage of normal seedlings was recorded for this test. Classification of normal seedlings was based on the criteria listed on pages 113-114 in the AOSA "Rules for Testing Seeds" (7).

2. Standard Warm Germination Test - Four Day Count (WG-4)

Counts for this test were made on the same trays used in the seven day warm germination test. The percent normal seedlings with a hypocotyl length of 4 cm or greater were recorded after four days in the germination chamber.

3. Cold Soil Germination Test (CG)

Four replicates of 50 seeds each were placed in plastic boxes (50 seeds per box) measuring 30 cm x 16.5 cm x 8 cm on 2.5 cm of a sieved soil mixture containing 1/3 peat, 1/3 sand and 1/3 unsterilized field

soil. Another 2.5 cm of the soil mixture was placed over the seed. The water holding capacity of this soil mixture was 50% of its dry weight. The soil was premixed with enough water to equal 60% of its water holding capacity (800 ml per 4.5 kg of soil). Plastic lids were placed on the boxes to maintain proper moisture conditions. The boxes were placed in a 10°C cold chamber for seven days and then transferred to a 25°C growth chamber for seven days. The percent of normal seedlings that had emerged from the soil after seven days was recorded.

4. Accelerated Aging Test (AA)

Four 100-seed replicates were placed in accelerated aging chambers (200 seeds per chamber). The chambers consisted of a plastic box 11 cm x 11 cm x 3.5 cm, that had a tight fitting cover and a brass wire screen basket to elevate the seed 2 cm above the bottom of the box (62). The wire mesh basket, containing one layer of 200 seeds, was placed in the box over 50 ml of distilled water. The covered chambers were placed in an incubator at 41°C for 72 hours. Then, the seeds were removed and germinated on Kimpac using the same procedures as for the standard warm germination test. The number of normal seedlings was recorded after seven days.

5. Tetrazolium Test (TZ)

Two 100-seed replicates from the seed lots without fungicide treatment were used for this test. Moisture imbibition was accomplished by placing the seeds in folded brown germination paper, that had been saturated with distilled water, for 14 hours at 25°C. Then the seeds were immersed in a 0.5% solution of 2,3,5-triphenyl tetrazolium chloride

salts for three hours at 25°C. A longitudinal bisection of each seed was made through the embryo and between the cotyledons with a sharp razor blade after the staining period. The exposed embryo (radicle, hypocotyl, and epicotyl) and cotyledonary surfaces were evaluated for sound, weak, dead, and fractured tissues. Tissue soundness was based upon tissue integrity and the intensity, amount and location of staining as described in the AOSA "Tetrazolium Testing Handbook" (88) on pages 24-25. Based upon tissue soundness, seeds were classified as viable and non-viable. The percent of viable seeds was recorded.

6. Tetrazolium Vigox Index (TZV)

Viable seeds from the tetrazolium test were further categorized into high, medium, and low vigor seeds. High vigor seeds exhibited only slight damage to the lower portion of the cotyledons and had a light pink staining of the sound tissue. A more intense and extensive staining of the cotyledons was typical in medium vigor seeds. Some medium vigor seeds also showed slight damage to the surface of the embryo. Low vigor seeds were characterized by deep red staining of the cotyledons with a low frequency of cotyledons containing small areas of dead (white) tissue. Some low vigor seeds also showed damage to the lower one-fourth of the radicle. The number of seeds in the high, medium, and low vigor classes were multiplied by index factors of six, four and two respectively. These indexes were summed to obtain a cumulative index of vigor.

7. Conductivity Test (ASA 95 and ASA 60)

Conductivity measurements were made by using the Automatic Seed Analyzer (Model 610) manufactured by Agro Sciences Incorporated, Ann Arbor, Michigan. All procedures for testing were done as described in the

instruction manual (4).

Four 100-seed replicates of seed lots that were not treated with the fungicide were used for this test. One seed was placed in each cell of a tray containing 100 cells. The dimensions of each cell were 2 cm x 2 cm x 1.5 cm. All cells were filled to an equal volume with distilled water. The seeds soaked in the water for 18 hours at 25°C. A cover plate containing 100 pairs of electrodes (one pair per cell) was placed into the trays.

The ASA 610 measured the number of microamperes passing between the two electrodes in each cell. A printer recorded the microampere reading for each cell and an electronic microprocessor compiled and displayed the number of seeds below a selected microampere partition (e.g. 95 microamperes). Seeds with microampere values less than the partition value were considered to be of better quality than those equal to or above the partition value. Partition values for estimating the warm germination percentage and selecting vigor levels were selected using the root mean square equation, $m = \left[\frac{\sum (P-A)^2}{N} \right]^{1/2}$, where P equals predicted germination of a lot by the ASA 610, A equals actual germination of the lot and N equals the number of samples tested. Preliminary studies using 50 soybean seed lots showed that the minimum values for the equation were obtained at 95 microamperes for predicting warm germination results and 60 microamperes to predict emergence from cold soil. These minimum values represented the greatest prediction accuracy and therefore were used in this study.

8. Seed Weight (SD. WT.)

Four replications of seeds were weighed to determine the average weight per 100 seeds of each seed lot for both investigations. All seed was sized to between 4.75 mm and 8.0 mm with a Clipper fanning mill before weighing.

B. Field Studies

Field plots were planted at the Michigan State University Experiment Station's Soil Science Farm at East Lansing, Michigan. All plots were planted with two modified John Deere Maxi-merge planting units mounted on a tool bar that was adapted for three-point hitch attachment to a tractor. A ground driven belt metering mechanism was mounted on top of the planting units. The seeds were planted at a rate of 23 seeds/m of row and at a planting depth of approximately 3.8 cm. Plot length was five meters. Weed control consisted of preplant incorporation of chloramben (2.2 kg/ha) mixed with trifluralin (0.8 kg/ha) and hand weeding. Fertilizer (6-24-24) was applied with a four-row John Deere planter at a rate of 213 kg/ha prior to planting the seed. The planting dates of 8 May, 16 May and 31 May represented early (stress), the earliest recommended, and normal planting environments for the two cultivars.

Four-row plots were arranged in a completely randomized design with two replications per experimental unit for Investigation One. A 2x2x2x4 factorial arrangement of the 32 treatment combinations included two cultivars, two lots per variety, four vigor levels per lot, and two fungicide treatments for each of the three planting dates. A spring plowed Capac loam (Aeric Ochraqualfs, fine-loamy, mixed, mesic) with

zero percent slope was used for this investigation. Secondary tillage included the use of a disc harrow and a field cultivator just prior to the planting of each planting date.

A 2x2x5 factorial arrangement of 20 treatment combinations in a completely randomized design with two replications was planted at the three planting dates for Investigation Two. The factors included two cultivars, five lots per cultivar and two fungicide treatments. Four-row plots were planted in a Riddles sandy loam (Typic Hapludalf, fine-loamy, mixed, mesic) with two percent slope. The plots were spring plowed, then disc harrowed and field cultivated just prior to planting each planting date.

Final field emergence counts were made after all seedlings had emerged. Most seedlings were at the V2 stage of growth as described by Fehr and Caviness (38). The number of days after planting to final emergence was 29 days for the first planting date, 28 days for the second planting date, and 24 days for the third planting date.

Soil temperatures were recorded at a soil depth of 3.8 cm, from the first planting date until the last emergence count of the third planting date, with a Weksler seven day mechanical temperature recorder.

Field emergence was analyzed using standard one way analysis of variance methods. Correlation coefficients were calculated for associations between all laboratory tests and between laboratory test and field emergence results. Multiple regression equations were used to establish the relationships between laboratory test results and field emergence for each planting date.

II. 1980 Studies

Seed material for Investigation One consisted of two high quality lots each of the cultivars Corsoy and Hodgson. Each lot was thoroughly blended for homogeneity and separated into three 2 kg samples. One of the samples was retained as a high vigor control lot. A second sample was impacted as in the 1979 study. The last sample was artificially aged for three days at 41⁰C and near 100% humidity in an aging chamber. Two subsamples weighing 1 kg each were made from each 2 kg sample. One of these subsamples was treated with a fungicide seed treatment, Captan 30-DD, applied as a slurry at the rate of 240 ml of diluted formulation (one to three dilution with water) to 45.3 kg of seed. All 24 samples were readjusted to a seed moisture of 13% and stored at 10⁰C and 50% relative humidity until all laboratory and field testing was completed.

The seed material for Investigation Two consisted of three randomly selected lots of the cultivars Hodgson and Corsoy from fields eligible for seed certification in 1979. A thoroughly blended 2 kg sample was drawn from each lot and divided into two 1 kg subsamples. One subsample was treated with Captan 30-DD, the same as samples in Investigation One, and one was left untreated. All samples had a seed moisture content of between 12% and 14%. The samples were stored at 10⁰C and 50% relative humidity until completion of the laboratory and field tests.

A. Laboratory Tests

A 3x2x2 factorial arrangement of treatments in a randomized complete block design was used for all laboratory tests in Investigation One. The 12 treatment combinations included two cultivars, three

lots per cultivar and two fungicide treatments (one with Captan and one without fungicide). Investigation Two was designed as a 3x2x2x2 factorially arranged randomized complete block experiment. The 24 treatment combinations consisted of two cultivars, two lots per cultivar, three vigor levels per lot and two fungicide treatments. Four replications of each test were done for both investigations unless it is otherwise specified in the procedures.

All laboratory procedures were the same as those used in 1979 with a few minor modifications: The standard germination tests (four and seven day counts) and the accelerated aging germinations were done in a Stults germinator at 25°C. The cold soil test samples were exposed to the 25°C conditions in a Precision incubator. Four 100-seed replicates of seed not treated with Captan were used for the tetrazolium and tetrazolium vigor tests. Four 100-seed replicates of both fungicide treated and untreated seeds were used for the conductivity test. Statistical analysis of laboratory data was by analysis of variance procedures.

B. Field Studies

Field plots were again planted at the Michigan State University Soils Farm in East Lansing, Michigan. Seedbed preparation, type of planter, planting depth, seeding rate, and rate and method of fertilizer application were the same as that used in 1979. Plot length was changed to 2.5 m to attain more uniform planting of the seeds while maintaining the same seeding rate. A postemergence application of bentazon (0.8 kg/ha) was made in addition to the 1979 rates of chloramben and trifluralin

applied preplant incorporated.

Two cultivars, two lots per cultivar, three vigor levels per lot and two fungicide treatments were the components of a 2x2x2x3 factorially arranged randomized complete block design for Investigation One. Four replications of the 24 treatment combinations were planted for each of three planting dates. Two-row plots were planted in a Metea loamy sand (Arenic Hapludalf, loamy, mixed, mesic) with two percent slope.

A 2x2x3 factorial arrangement of treatments in a randomized complete block design was used for Investigation Two. The 12 treatment combinations included two cultivars, three lots per cultivar for each planting date, and two fungicide treatments. Four-row plots were planted in an Owosso sandy loam (Typic Hapludalf, fine-loamy, mixed, mesic) with two percent slope.

Planting dates for both investigations were May 2, May 23 and June 13 which corresponded to very early, normal, and "more optimal" planting environments. Soil temperatures were again recorded at a 3.8 cm soil depth. Final field emergence counts were made at the same vegetative growth stage used in 1979. The time from planting to final emergence was 35 days for the first planting date, 27 days for the second planting date, and 27 days for the third planting date.

Statistical analyses of field emergence data and the relationships between laboratory tests and field emergence were similar to these used in 1979.

RESULTS AND DISCUSSION

I. Laboratory Tests

A. Means and Standard Errors

The difference between means of WG-7 and TZ was 1.4% for Investigation One - 1979 (Table 1). A difference of 4.7% was found between means of WG-7 and ASA 95 for the same investigation. Similar differences occurred in 1980 with the largest being 7.7% between WG-7 and ASA 95. Mean differences between WG-7 and these two tests were also small for Investigation Two in both 1979 and 1980 (Table 2). The smallest difference was 0.1% between WG-7 and ASA 95 in 1979 and largest was 2.6% between WG-7 and TZ in 1980. These results support the use of TZ and ASA 95 to estimate seed viability.

The wide range within results of these three tests showed the large variation in seed quality for Investigation One in both 1979 and 1980. This is also reflected by the large standard errors of these tests. Although viability tests usually do not detect vigor differences, they are useful in determining some differences when such large variation in seed quality exists.

Examination of the means, standard errors, and ranges for WG-7, CG, AA, TZV, and ASA 60 indicates that lots included in Investigation One - 1979 were of lower quality than those in 1980. This is largely a result of the inclusion of lots artificially aged for two weeks in the 1979 investigation.

Table 1. Mean, standard error, and range of laboratory test results averaged over all factors, Investigation One, 1979 and 1980.

Lab Test	1979			1980		
	\bar{x}	SE	Range ⁺	\bar{x}	SE	Range
WG-7 [‡]	74.0	25.3	9 - 98	82.8	15.8	50 - 99
WG-4	69.4	26.1	8 - 97	27.1	13.3	2 - 99
CG	55.7	33.6	0 - 98	66.9	21.1	20 - 99
AA	42.6	38.9	0 - 98	74.0	21.7	4 - 99
TZ	74.4	21.4	13 - 97	84.6	16.1	47 - 99
TZV [§]	292.5	121.3	32 -536	462.0	99.5	234 -586
ASA 60	38.3	24.8	0 - 85	50.3	25.2	13 - 98
ASA 95	78.7	18.7	18 - 98	90.5	8.2	68 - 99
SD. WT. [#]	16.5	0.6	15.5-17.7	17.3	1.8	15.0-21.6

⁺The range of values are for individual replications.

[‡]Values for WG-7, CG, TZ, ASA 60, and ASA 95 expressed as %.

[§]Values for TZV expressed as the total frequencies of vigor classes.

[#]Seed weight values expressed as grams/100 seeds.

Table 2. Mean, standard error, and range of laboratory test results averaged over all factors, Investigation Two, 1979 and 1980.

Lab Test	1979			1980		
	\bar{x}	SE	Range ⁺	\bar{x}	SE	Range
WG-7 [†]	93.1	3.2	84 - 98	94.2	3.0	84 - 99
WG-4	67.6	18.1	35 - 95	39.3	13.0	16 - 74
CG	59.4	28.6	0 - 98	83.5	8.6	60 - 98
AA	78.7	11.5	49 - 98	77.5	18.0	31 - 98
TZ	92.9	3.2	86 - 97	96.8	2.3	92 - 99
TZV [§]	424.8	35.0	352 -490	530.2	23.9	460 -574
ASA 60	56.9	26.7	14 - 93	31.9	16.7	8 - 64
ASA 95	93.2	7.7	78 -100	92.8	4.3	81 - 99
SD. WT. [#]	18.5	1.6	16.6-21.7	16.8	1.1	14.9-18.3

⁺The range of values are for individual replications.

[†]Values for WG-7, WG-4, CG, TZ, ASA 60, and ASA 95 expressed as %.

[§]Values for TZV expressed as the total frequencies of vigor classes.

[#]Seed weight values expressed as grams/100 seeds.

All seed lots selected for Investigation Two were of acceptable market quality. Acceptable market quality is defined as seed with viability of at least 80% (16). The results of the three viability tests in Tables 1 and 2 show that higher quality seed was used for Investigation Two than Investigation One, both years.

The mean and standard error of the CG results indicate that higher quality seed was used in 1980 than in 1979 for Investigation Two. This is supported by the TZV results. Results of WG-4 and ASA 60, however, show 1979 lots to be of higher quality. Lower WG-4 results in 1980 may have resulted from a diurnal fluctuation of the germination temperature. ASA 60 results could have been influenced by soak temperature, which was constant in an incubator set at 25°C in 1980 and at approximately room temperature of 23°C in 1979. Differences in seed size may also have been a factor. The seed weight per 100 seeds shows the seed size of lots used in 1980 was smaller than in 1979. Smaller seed has a larger surface to weight ratio which can cause a greater electrolyte diffusion rate thus erroneously indicating lower seed quality. The discrepancy in conclusions based on these test results emphasizes the need for standardization of vigor test procedures.

B. Linear Correlation Coefficients

To establish the relationship among laboratory tests, linear correlation coefficients were calculated. Many significant correlations with $r \geq 0.500$ were found among results for Investigation One and Two, both years (Tables 3, 4, 5 and 6), except for Investigation Two in 1980, where only six such correlations exist.

Table 3. Linear correlation coefficients between laboratory test results for 1979, Investigation One.

<u>Lab Tests</u>	<u>SD.WT.</u>	<u>ASA 95</u>	<u>ASA 60</u>	<u>TZV</u>	<u>TZ</u>	<u>AA</u>	<u>CG</u>	<u>WG-4</u>
WG-7	-.098	.946**	.854**	.891**	.926**	.743**	.814**	.989**
WG-4	-.151	.927**	.863**	.880**	.896**	.757**	.837**	
CG	-.116	.756**	.803**	.804**	.773**	.801**		
AA	-.016	.678**	.894**	.804**	.732**			
TZ	-.072	.942**	.804**	.967**				
TZV	-.119	.878**	.848**					
ASA 60	-.073	.794**						
ASA 95	-.114							

**Significance at the 0.01 level of probability.

Table 4. Linear correlation coefficients between laboratory test results for 1979, Investigation Two.

Lab Tests	<u>SD. WT.</u>	<u>ASA 95</u>	<u>ASA 60</u>	<u>TZV</u>	<u>TZ</u>	<u>AA</u>	<u>CG</u>	<u>WG-4</u>
WG-7	-.639**	.883**	.637**	.813**	.890**	.731**	.259	.638**
WG-4	-.531*	.723**	.844**	.743**	.613**	.722**	.483*	
CG	-.075	.393	.709**	.269	.289	.651**		
AA	-.534*	.808**	.882**	.670**	.681**			
TZ	-.674**	.913**	.609**	.880**				
TZV	-.711**	.791**	.605**					
ASA 60	-.576**	.755**						
ASA 95	-.649**							

*,** Significance at the 0.05 and 0.01 levels of probability respectively.

Table 5. Linear correlation coefficients between laboratory test results for 1980, Investigation One.

<u>Lab Tests</u>	<u>SD. WT.</u>	<u>ASA 95</u>	<u>ASA 60</u>	<u>TZV</u>	<u>TZ</u>	<u>AA</u>	<u>CG</u>	<u>WG-4</u>
WG-7	-.348	.429*	.462*	.935**	.926**	.804**	.732**	.748**
WG-4	-.456*	.610**	.574**	.620**	.566**	.814**	.742**	
CG	-.354	.615**	.700**	.653**	.613**	.772**		
AA	-.519**	.594**	.584**	.622**	.586**			
TZ	-.230	.206	.302	.985**				
TZV	-.326	.316	.400					
ASA 60	-.617**	.809**						
ASA 95	-.577**							

*,** Significance at the 0.05 and 0.01 levels of probability respectively.

Table 6. Linear correlation coefficients between laboratory test results for 1980, Investigation Two.

<u>Lab Tests</u>	<u>SD. WT.</u>	<u>ASA 95</u>	<u>ASA 60</u>	<u>TZV</u>	<u>TZ</u>	<u>AA</u>	<u>CG</u>	<u>WG-4</u>
WG-7	-.425	.600*	-.389	.291	.613*	.497	.706*	.067
WG-4	-.089	-.303	-.244	-.220	-.112	.593*	.141	
CG	-.337	.156	-.290	.433	.532	.731**		
AA	-.301	-.003	-.423	.134	.184			
TZ	-.240	.700*	-.006	.503				
TZV	-.276	.440	.051					
ASA 60	-.568	-.250						
ASA 95	-.523							

* ** Significance at the 0.05 and 0.01 levels of probability respectively.

The WG-7 results correlated best with TZ and ASA 95. The highest significant correlation coefficient between WG-7 and TZ was 0.926 for Investigation One in both 1979 and 1980. A highly significant correlation between ASA 95 and WG-7 of $r = 0.946$ was found for Investigation One - 1979. The correlation between these two tests for Investigation One in 1980 of $r = .0429$ was lower than expected. The highest correlations between TZ and ASA 95 of $r = 0.492$ for Investigation One and $r = 0.913$ for Investigation Two were found in 1979. Correlations for these two tests were not as high in 1980.

The high correlation coefficients among the WG-7, TZ and ASA 95 support the use of these tests as indicators of viability. The TZ has long been used as a viability test and because of the short time for completion (24 hours), it has been used to predict warm germination test results (98). The conductivity test has not had extensive use as a viability test because it is normally run on a bulk sample of seeds. However, the partitioning of seeds into individual cells, as in the ASA method, makes it possible to compare the conductivity of individual seeds. These correlation coefficients suggest that under the proper conditions the ASA 95 may also be used as an indicator of viability. Advantages of the ASA 95 include speed of testing (less than 24 hours) and elimination of the interpretation variability inherent with the TZ.

WG-4, CG, AA and TZV had highly significant correlations with WG-7 for Investigation One in both 1979 and 1980. Each test was also significantly correlated with WG-7 results for Investigation Two in either 1979 or 1980. The good correlation of these tests with WG-7

in Investigation One for both years shows that the spread in seed lot quality was large enough for viability tests to detect some of the differences in vigor.

WG-4 was significantly correlated with AA in both investigations, both years. Significant correlation coefficients also existed between WG-4 and TZ, TZV, ASA 60, and ASA 95 in three of the four investigations. Only Investigation Two in 1980 did not show good correlation between WG-4 and those four tests. Investigation One in both 1979 and 1980 showed significant correlations between the CG and WG-4.

These data illustrate the good correlation that was expected between WG-4 and other vigor tests like AA, TZV, ASA 60 and CG. However, since WG-4 is a modification of WG-7, it is not surprising to see it correlate well with viability tests.

The CG and AA had highly significant correlation coefficients for both investigations, both years. Good correlation between these tests was expected since both are a direct type vigor tests. Another test that correlated well with CG was the ASA 60. In Investigation One - 1979 and 1980 and Investigation Two - 1979 highly significant r values between 0.700 and 0.803 were found. The 60 microampere partition used in the conductivity test was selected based on cold test information from previously tested soybean seed lots.

The AA was also significantly correlated with WG-4 in both investigations, both years. The TZ, TZV, ASA 60, ASA 95, and WG-7 correlated well with AA in three of the four investigations. Of these five tests, ASA 60 was best correlated with AA in 1979. Since electrolyte leakage can result from seed deterioration, AA would be expected to

correlate well with ASA 60.

The TZ had significant correlation coefficients not only with WG-7 and ASA 95 but also with the TZV in Investigation One, both years and Investigation Two in 1979. Since the TZV is a modification of TZ using seed tissue soundness as an index of vigor, it is not surprising to find a good correlation between these two tests.

C. Fungicide Seed Treatment Effects

Seed treatment caused a highly significant ($p = .01$) increase in the CG percentage for both investigations, both years (Figures 1 and 2). This relationship was the same for all seed quality levels. In the cold test, the fungicide seed treatment protects the seed from soil-borne fungal pathogens. Several species of Pithium can attack soybean seed during the critical cold incubation period. During this time exudates predispose untreated seed to pathogen attack (59). Decay of the cotyledons and embryonic axis by fungi can severely decrease germination and emergence. Low vigor seeds, which exude more sugars and amino acids (97) than seed of higher quality, are especially susceptible to attack. In both 1979 and 1980, the lower quality seed lots used in Investigation One had a greater response to treatment with Captan than the higher vigor lots of Investigation Two.

AA results were significantly increased ($p = .01$) by seed treatment in three of the four investigations. The increase for Investigation Two - 1979 was less significant ($p = .10$). These results concur with those of Delouche and Baskin (27). The high temperature and humidity during the AA incubation period were ideal for infestation by storage

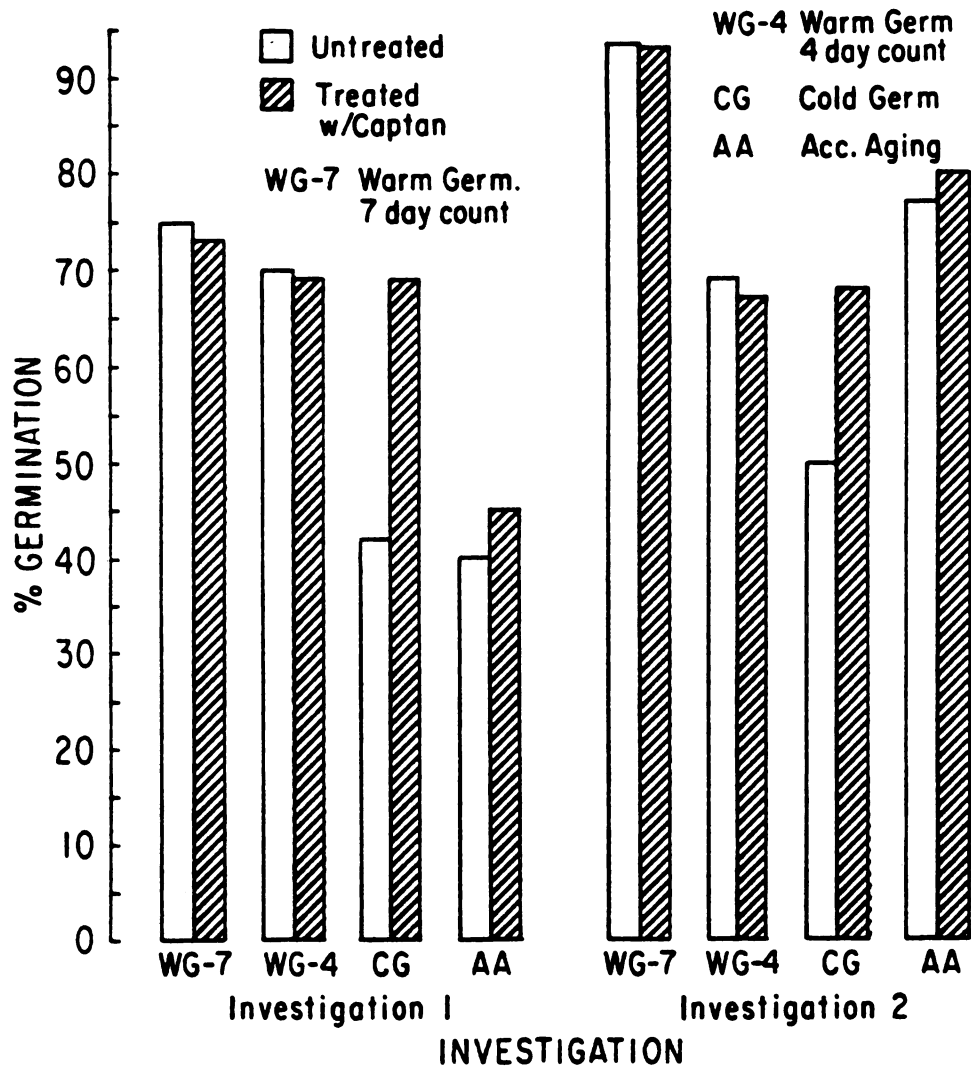


Figure 1. Influence of fungicide seed treatment on germination results of several laboratory tests, averaged over cultivar, lot and quality level for 1979 investigations.

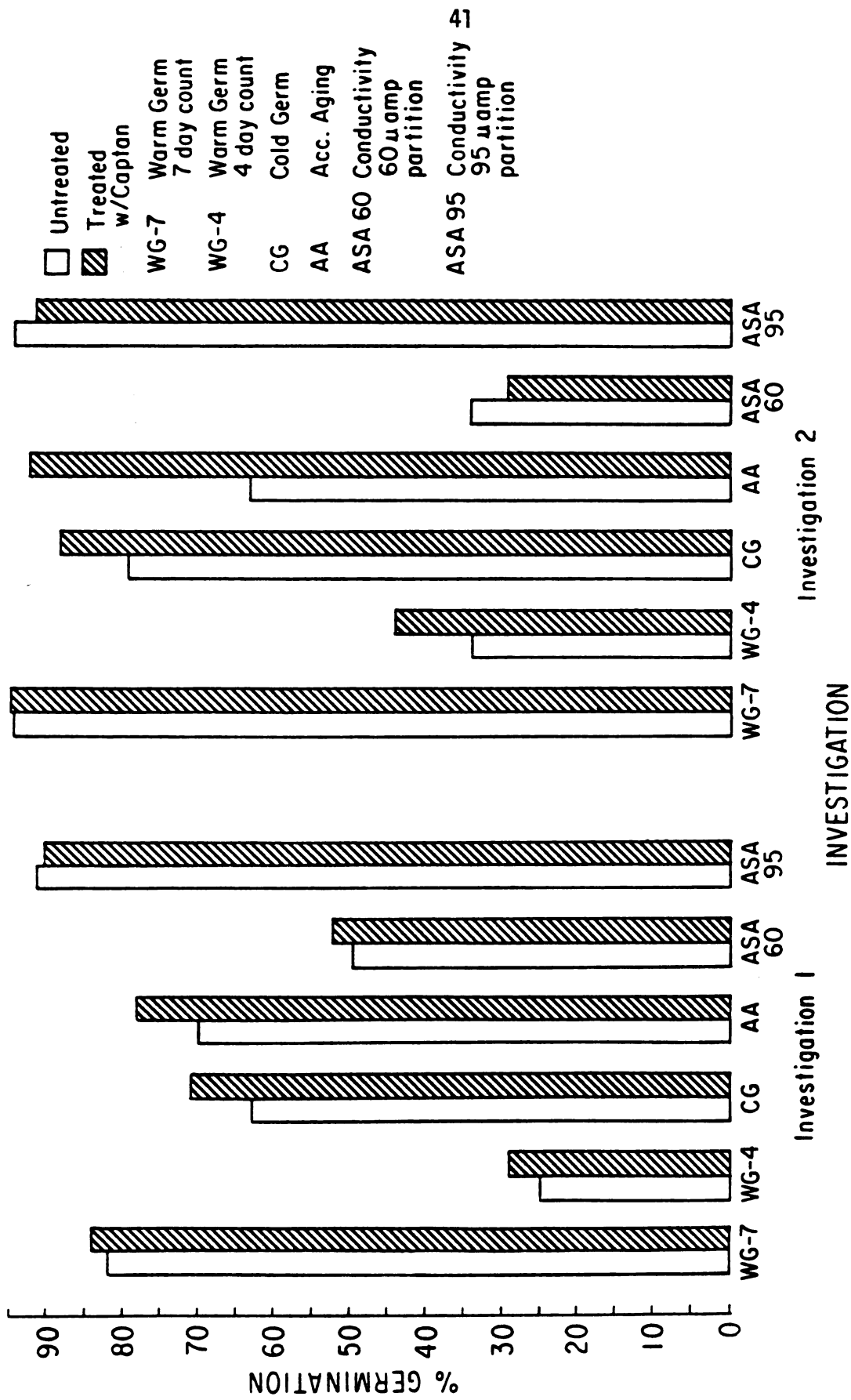


Figure 2. Influence of fungicide seed treatment on germination results of several laboratory tests, averaged over cultivar, lot and quality level for 1980 investigations.

fungi like Aspergillus flavus. Conditions favorable for fungi growth caused cotyledon and embryo decay during the germination phase. This decay decreased the percentage of normal seedlings produced.

Both investigations in 1980 showed a significant increase ($p = .05$) of WG-4 results with seed treatment. Seed treatment did not improve WG-4 results in 1979. For Investigation One this may be due to the different method of artificial aging used in 1980. The higher relative humidity produced may have greatly increased infestation by storage fungi. A significant cultivar by fungicide interaction ($p = .01$) indicates that the Corsoy lots used in 1980 may be the reason for significance in Investigation Two. Delayed harvest probably increased infestation of fungi in the fields of this cultivar. The WG-7 results were not affected by seed treatment in these investigations. Therefore fungal decay could not have affected the embryonic axis. Damage to the cotyledons would reduce the amount of food reserves available for seedling growth. This may account for a decrease in the speed of germination (WG-4 results).

The effect of seed treatment on the conductivity tests (ASA 95 and ASA 60) was evaluated in 1980. No significant difference was obtained for either test in both investigations. These results show that Captan treated service samples may be interpreted the same as untreated samples by a seed testing laboratory using this method of conductivity. Different fungicides, insecticides and bacteriacides would have to be tested before similar conclusions could be reached about their effect on the conductivity results.

II. Field Emergence

A. Influence of Soil Environment on Field Emergence

Field emergence results from these studies should only be related to other fields of similar soil types with similar temperature, moisture, and pathogen conditions (16). The soil types used in this study for both 1979 and 1980 were the same as about 10% of the tillable cropland in southern lower Michigan. The soil moisture conditions at all three planting dates, both years, were adequate for germination and growth. Pathogen populations were assumed to be similar to those found in other loam and sandy loam soils having corn as the preceeding crop.

Soil temperature, an important variable in these studies, was characterized using a method for determining heat units related to germination and seedling growth. A growing degree days equation of
$$\left[\frac{1}{2}(\text{maximum soil temperature (}^{\circ}\text{C)} + \text{minimum soil temperature}) - 10^{\circ}\text{C} \right] (1.8)$$
 was used to determine daily heat units (41). Littlejohns (58) and Hatfield and Egli (47) suggested 10°C as the minimum temperature for soybean germination and growth. A conversion factor of 1.8 was included to convert the number of heat units into Fahrenheit degree terms, which are usually used for calculating growing degree days. By summing heat units, a single number can be obtained to characterize soil temperature conditions during soybean germination and hypocotyl elongation to the VE stage of growth. Adequate moisture availability for germination and growth was assumed in using this method.

The number of accumulated heat units at five and thirty days after planting are presented in Table 7. Soil temperature conditions for the first five days after planting are the most critical since

chilling imbibition injury and infestation by soil-borne pathogens can occur during that time (12, 77). At thirty days after planting, almost all seedlings had emerged for all planting dates, both years. Incorporation of the soil moisture factor into this method of soil temperature characterization could make it a more useful tool in field emergence studies.

Table 7. Total heat units, characterizing the soil environment at five and thirty days after planting, 1979 and 1980.⁺

1979			1980		
Planting Date	5 Days	30 Days	Planting Date	5 Days	30 Days
8 May	62	260	2 May	54	416
16 May	46	334	23 May	86	391
31 May	60	396	13 June	72	645

⁺Heat units - $\left[\frac{1}{2}(\text{max. daily temp. (}^{\circ}\text{C)} + \text{min. daily temp.}) - 10^{\circ}\text{C} \right] (1.8)$.

Comparison of the field emergence means (Tables 8 and 9) with heat units (Table 7) shows that the heat units accumulated in the first five days had more influence on final emergence than the heat units accumulated at thirty days. This was true for all planting dates in 1980 and the 16 May and 31 May planting dates in 1979. Soil crusting caused poorer emergence for the 8 May, 1979 planting date than would be expected by analysis of the heat units accumulated at five days. The best planting conditions and field emergence results for 1979 were for the 31 May planting date.

A period of cold weather from 15 June to 20 June, 1980 caused

Table 8. Mean, standard error and range of field emergence (%) as influenced by planting date, averaged over all factors, Investigations One and Two, 1979.

Planting Date	Investigation 1			Investigation 2		
	\bar{x}	SE	Range ⁺	\bar{x}	SE	Range
8 May	32.0	21.3	0 - 73	45.4	15.2	20 - 73
16 May	36.8	19.7	0 - 65	62.2	15.8	31 - 85
31 May	45.5	29.5	1 - 85	67.0	12.4	43 - 84

⁺The range values are for individual plots.

Table 9. Mean, standard error and range of field emergence (%) as influenced by planting date, averaged over all factors, Investigations One and Two, 1980.

Planting Date	Investigation 1			Investigation 2		
	\bar{x}	SE	Range ⁺	\bar{x}	SE	Range
2 May	33.0	16.1	2 - 74	42.0	12.9	12 - 74
23 May	61.4	16.7	16 - 88	77.5	8.9	54 - 90
13 June	39.8	14.8	7 - 73	60.4	11.3	29 - 79

⁺The range values are for individual plots.

field emergence for the 13 June planting to be lower than expected. The best soil temperatures occurred after 23 May, 1980. Field emergence was the most rapid and uniform for that planting date. The coldest soil conditions came after the 2 May, 1980 planting. Examination of the data in Table 9 shows the decrease in field emergence due to cold temperature stress at that planting date.

B. Influence of Fungicide Seed Treatment on Field Emergence

Fungicide seed treatment significantly increased ($p = .05$) field emergence in both investigations for all 1979 planting dates (Figure 3). Since the soil temperatures after each planting date imposed at least a slight cold stress on the seed, these results are not surprising. The increase in emergence was greatest for Investigation Two after the 16 May planting date and after the 8 May planting date for Investigation One. These results confirm other reports on the effectiveness of seed treatment to improve field emergence, especially with lower seeding rates (10, 87). The results for Investigation Two conflict with findings of Edje and Burris (33) which showed improvement of field emergence only when lots had an 84% or lower warm germination.

In 1980 the only significant increase in field emergence occurred for Investigation Two after the 2 May planting date (Figure 4). The non-significant differences for the 23 May and 13 June planting dates were expected since soil conditions after these dates were more favorable for emergence. The greater accumulated heat units for these two dates when compared to 2 May, 1980, allowed the untreated seed to emerge faster with less chance of attack by soil-borne fungi.

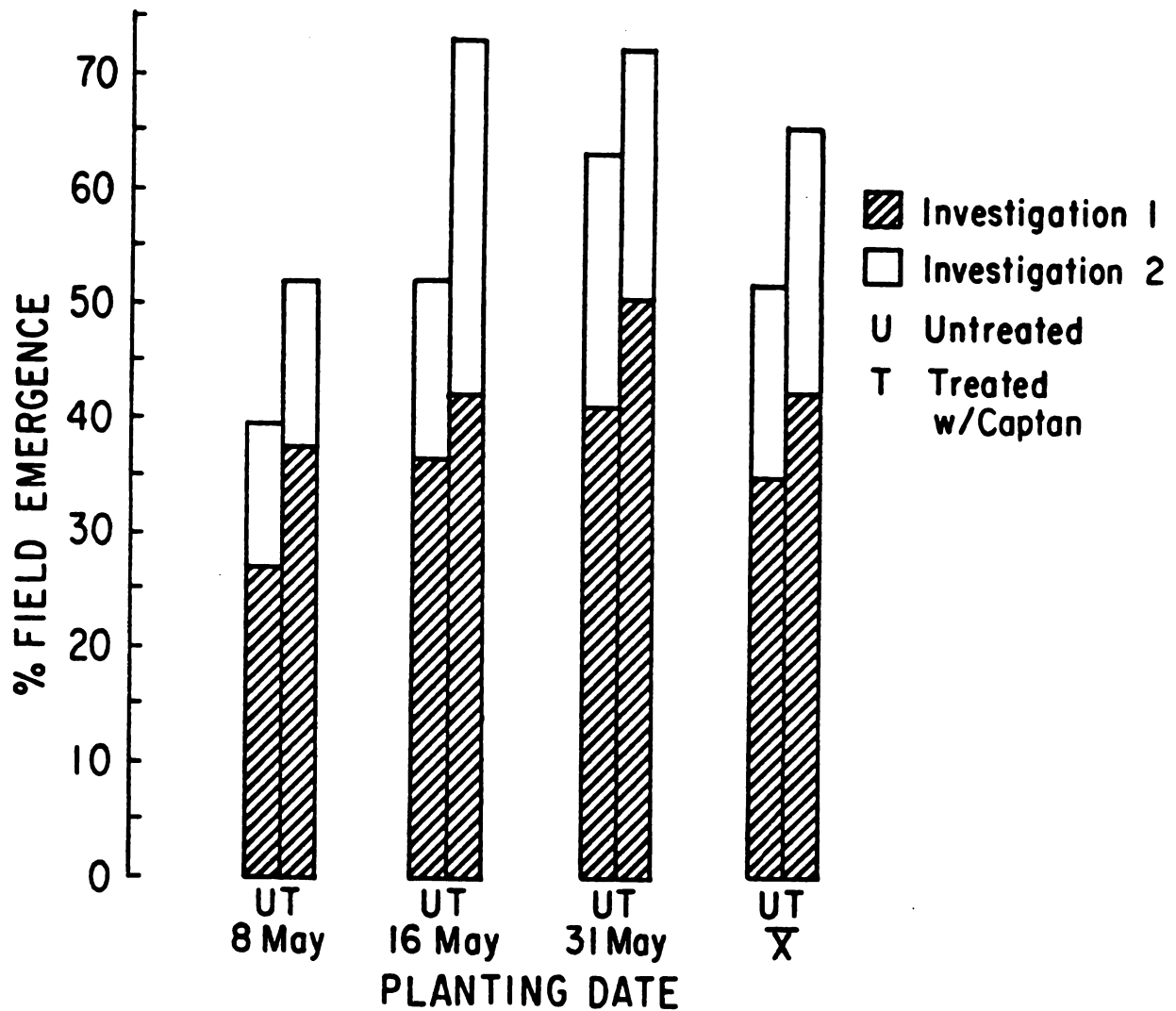


Figure 3. Influence of fungicide seed treatment and planting date on field emergence, averaged over cultivar, lot, and quality level for 1979 investigations.

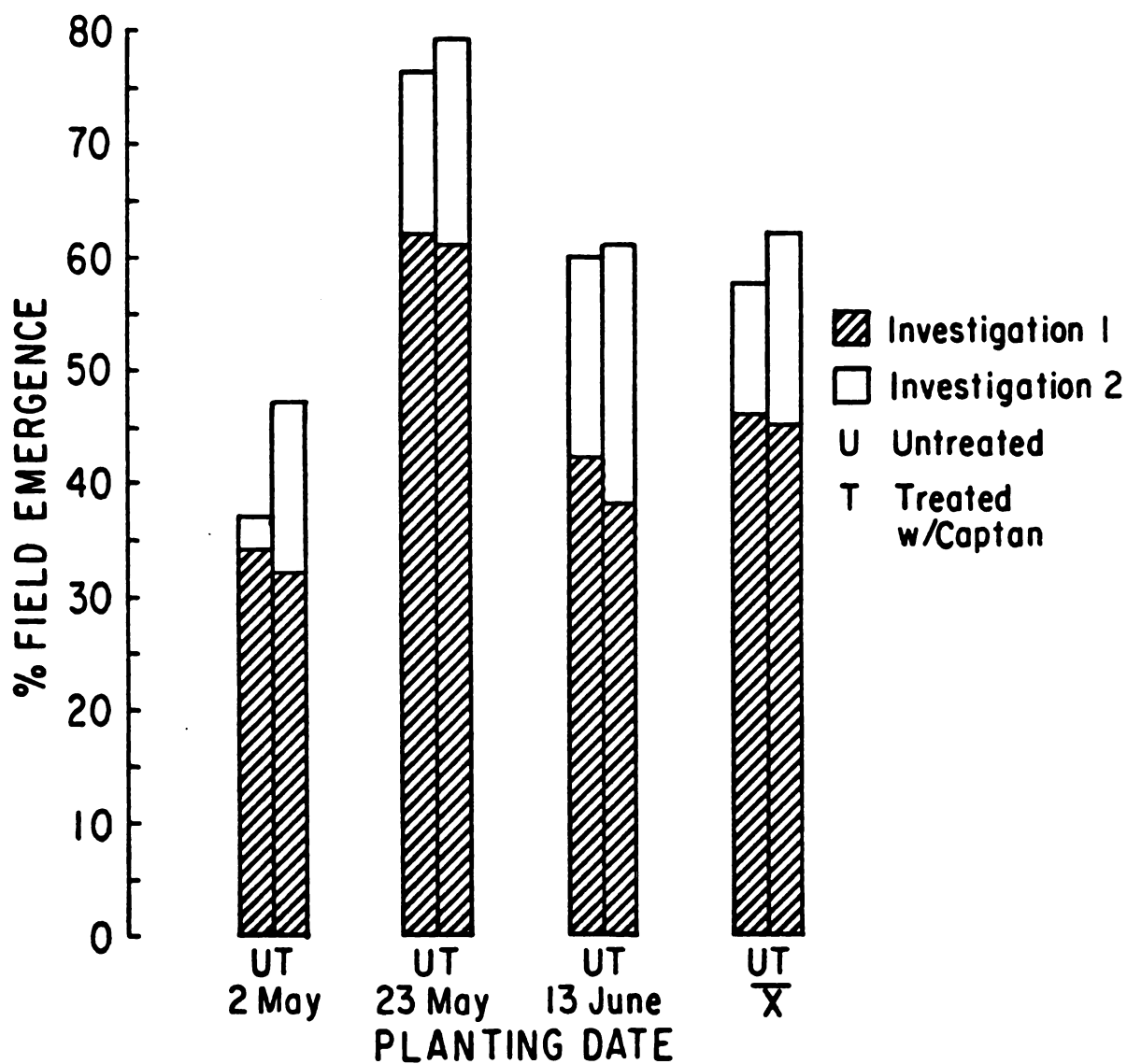


Figure 4. Influence of fungicide seed treatment and planting date on field emergence, averaged over cultivar, lot and quality level for 1980 investigations.

The only surprising result in 1980 was the decrease in emergence of treated seed for Investigation One after the 2 May planting date. This unexpected result was caused by a significant decrease in the field emergence of artificially aged seed lots. Contrary to expected results, seed treatment in this case had a negative effect on emergence.

III. Relationships Between Laboratory Test and Field Emergence Results

A. Linear Correlation Coefficients

Linear correlation coefficients were computed between all laboratory tests and field emergence for each planting date. There were many significant correlation coefficients higher than 0.500 for Investigation One in both 1979 and 1980 (Tables 10 and 11). The correlations for this investigation were much higher than those for Investigation Two, both years (Tables 12 and 13). This is consistent with reports by Burris (16) who observed that when seed lots with WG-7 results below 80% were included in vigor test studies, the correlations between laboratory tests and field emergence were much higher than if only seed of acceptable market quality was used.

All vigor tests had higher correlations with field emergence than WG-7 for all three planting dates in Investigation One - 1979. CG, AA, and ASA 60 had the highest correlations of $r \geq 0.827$. WG-7 had slightly higher correlations than the other two viability tests, TZ and ASA 95.

The WG-7 had higher correlations than CG, WG-4, AA and ASA 60 for Investigation One at the two 1980 planting dates with the most

Table 10. Linear correlation coefficients between individual laboratory tests and % field emergence as influenced by planting date for 1979, Investigation One.

Planting Date	Laboratory Tests						
	WG-7	WG-4	CG	AA	TZ	TZV	SD. WT.
8 May	.786**	.791**	.874**	.902**	.746**	.796**	-.145
16 May	.808**	.881**	.883**	.827**	.869**	.904**	-.139
31 May	.869**	.892**	.928**	.899**	.807**	.847**	-.146

** Significance at the 0.01 level of probability.

Table 11. Linear correlation coefficients between individual laboratory tests and % field emergence as influenced by planting date for 1980, Investigation One.

Planting Date	Laboratory Tests						
	WG-7	WG-4	CG	AA	TZ	TZV	SD. WT.
2 May	.677**	.478**	.700**	.422*	.719**	.682**	.005
23 May	.819**	.630**	.768**	.591**	.837**	.825**	-.120
13 June	.824**	.582**	.730**	.632**	.812**	.820*	-.470*

** Significance at the 0.05 and 0.01 levels of probability respectively.

Table 12. Linear correlation coefficients between individual laboratory tests and % field emergence as influenced by planting date for 1979, Investigation Two.

Planting Date	Laboratory Tests								
	<u>WG-7</u>	<u>WG-4</u>	<u>CG</u>	<u>AA</u>	<u>TZ</u>	<u>TZV</u>	<u>ASA 60</u>	<u>ASA 95</u>	<u>SD. WT.</u>
8 May	.393	.397	.665**	.636**	.417	.498*	.571**	.421	-.400
16 May	.347	.414	.625**	.560 *	.510*	.433	.578**	.560*	-.401
31 May	.513*	.633**	.785**	.767**	.492*	.520*	.792**	.541*	-.473*

*,** Significance at the 0.05 and 0.01 levels of probability respectively.

Table 13. Linear correlation coefficients between individual laboratory tests and % field emergence as influenced by planting date for 1980, Investigation Two.

Planting Date	Laboratory Tests								
	<u>WG-7</u>	<u>WG-4</u>	<u>CG</u>	<u>AA</u>	<u>TZ</u>	<u>TZV</u>	<u>ASA 60</u>	<u>ASA 95</u>	<u>SD. WT.</u>
2 May	-.147	.024	.235	.375	-.045	-.195	.026	-.420	.545
23 May	.106	.479	.168	.393	.216	-.067	.215	-.186	.616*
13 June	.368	.574	.301	.420	.387	.038	-.153	.039	.306

* Significance at the 0.05 level of probability.

favorable soil temperature conditions; 23 May and 13 June. These results agree with other reports (36, 79) that under favorable soil conditions WG-7 correlates better with field emergence than these vigor tests. CG had slightly higher correlations than WG-7 under the cold stress conditions of the 2 May planting date.

The highest correlation coefficients for Investigation Two - 1979 were at the 31 May planting date. This planting date also had the greatest number of significant correlations. These results agree with the findings of Johnson and Wax (54) that as soil conditions become more favorable a greater number of laboratory tests will correlate well with field emergence. All three planting dates, for this investigation, had five vigor tests (CG, AA, WG-4, ASA 60, and TZV) with higher correlations than WG-7.

Correlations at all three planting dates for Investigation Two - 1980 were low. This could be due to the small sample size of an investigation having smaller vigor differences among lots and generally higher quality lots than those used in 1979. Seed weight (SD. WT.) had the best correlation with field emergence of any test at the most favorable and unfavorable planting dates; 23 May and 2 May respectively. These results show some support for evidence that smaller seeds tend to be of lower quality (5, 20). Burris (18) concluded that larger seeds produce larger embryos and thus should perform better under favorable conditions.

Comparisons of the correlation coefficients from all investigations show CG had the highest or second highest correlation with field emergence for the first planting date in each investigation, except

Investigation Two - 1980. Since CG measures emergence under artificially induced cold soil conditions it would be expected to correlate well with the stress field situations encountered at the first planting date both years. CG also had one of the four highest correlations for the second and third planting dates of the same three investigations. These results agree with other reports (9, 54, 63, 82, 83) that the cold test correlates well with field emergence especially under cold stress conditions.

For the first planting date, AA had the highest correlation in Investigation One - 1979 and the second highest correlation in Investigation Two, both years. It had the third highest correlation in Investigation Two, both years and was significantly correlated in Investigation One, both years for the second planting date. AA also had the third highest correlation for the third planting date in Investigation Two-1979 and the second highest correlation in Investigation One - 1979 and Investigation Two - 1980. These results agree with the reports of three years of results from the AOSA Vigor Test Referee Program (9, 63, 83) that show the AA as having very high correlations with field emergence.

Correlations of the ASA 60 results for the first planting date were the third highest for both investigations in 1979. It also had highly significant correlations in both of these investigations and a significant correlation in Investigation One - 1980 for the second and third planting dates. Matthews and Bradnock (60) found a good correlation between conductivity and field emergence in french bean. High correlation coefficients have also been reported for the classic

conductivity test in the AOSA Vigor Committee reports (9, 63, 83).

TZV had significant correlations in Investigation One in both 1979 and 1980 for all three planting dates. It had the highest correlation of all tests in Investigation One - 1979 for the second planting date. Significant TZV correlations were also found for the first and third planting dates in Investigation Two - 1979. Moore (65, 66) has proposed that the TZV classification system is very useful in determining the vigor level of seed especially when it is mechanically damaged or deteriorated due to aging. The results from Investigation One support this proposal. Good correlation between TZV classes and field emergence has also been reported by Yacklich and Kulik (98). The TZ viability correlation coefficients were usually lower than those for TZV in the 1979 investigations.

While correlation coefficients for WG-4 were highly significant in Investigation One - 1979 and 1980, they were usually lower than those of CG, AA, and TZV. WG-4 correlations were better than correlations for WG-7 for all planting dates in both investigations in 1979. Tekrony and Egli (85) found WG-4 to have higher correlations with field emergence than AA but lower than WG-7.

Significant correlations of $r \geq 0.677$ were found between WG-7 and field emergence for all planting dates in Investigation One in both 1979 and 1980. Similar results were found by Burris (17) when seed lots of below acceptable market quality were used. As soil conditions became more favorable, the WG-7 correlations for Investigation One were higher. The only significant WG-7 correlation with emergence in Investigation Two was at the 31 May, 1979 planting date.

No single laboratory test had the highest linear correlation with field emergence for all planting dates over all investigations. TeKrony (84) stated the feeling of many seed scientists, that a single laboratory test cannot correlate well with field emergence over the whole range of possible planting conditions. Although these data confirm this hypothesis, they also show some definite trends for correlations of the different laboratory tests.

CG had good correlations over all planting dates for three investigations, and along with AA, had higher correlations than most other tests at the planting dates with the greatest cold temperature stress. ASA 60 also had good correlation in 1979, with the best correlations at the planting dates with the most favorable soil conditions. TZV results had a strong association with field emergence results when aged and mechanically injured lots were tested. The other tests in this study (WG-7, WG-4, TZ, ASA 95) had their best correlations under the more favorable planting conditions and when large vigor differences were being measured.

B. Multiple Regression Analysis

Laboratory and field emergence results were analyzed using multiple stepwise regression techniques with a forward selection strategy. The results of Investigation One - 1979 (Table 14) show very good coefficients of multiple determination (R^2) of between 0.877 and 0.950. The amount of the total variability explained by the regression equation increased as planting conditions became more favorable to a high of 95% at the 31 May planting date. CG was a significant variable in the

Table 14. Independent variables significantly contributing to a step-wise multiple regression equation of laboratory tests and % field emergence (dependent variable) and the coefficients of multiple determination for these equations, as influenced by planting date for 1979, Investigation One.

<u>Planting Date</u>	<u>Independent Variables</u>	<u>R²</u>
8 May	CG + AA	.877
16 May	CG + TZV	.883
31 May	WG-4 + CG + AA	.950

Table 15. Independent variables significantly contributing to a step-wise multiple regression equation of laboratory tests and % field emergence (dependent variable) and the coefficients of multiple determination for these equations, as influenced by planting date for 1979, Investigation Two.

<u>Planting Date</u>	<u>Independent Variable</u>	<u>R²</u>
8 May	CG + SD. WT.	.565
16 May	CG + SD. WT.	.517
31 May	CG + SD. WT.	.788

regression equations for all three planting dates. The contribution of WG-4 to the regression of the third planting date, while significant, was only 2%.

Two independent variables, CG and SD. WT., were a part of all three regression equations selected for Investigation Two - 1979 (Table 15). The R^2 values ranged from 0.517 for the second planting date to 0.788 for the third planting date. Although SD. WT. did not have significant linear correlations with field emergence, in the presence of CG, it made a significant contribution to the regression equations for this investigation.

The R^2 values for Investigation One - 1980 (Table 16) of between 0.765 and 0.848 were very good but not as high as for the same investigation in 1979. CG again appeared in the regression equation at all three planting dates. TZ, AA, and SD. WT. all appeared in two of the three equations for this investigation.

The coefficients of multiple determination for Investigation Two - 1980 (Table 17) were high for all three planting dates. Although none of the linear correlation coefficients were very high for this investigation, when combined, the regression of several of the independent variables accounted for 80-95% of the variability. WG-4 and SD. WT. appeared in all three equations.

The inclusion of SD. WT. in many of these stepwise regression equations is contrary to expected results. Delouche (24) concluded that while the small and large extremes in seed size within a lot may differ in vigor, the differences in mean seed weight between lots usually had little effect on seed performance. The ranges and linear correlation

Table 16. Independent variables significantly contributing to a step-wise multiple regression equation of laboratory tests and % field emergence (dependent variable) and the coefficients of multiple determination for these equations, as influenced by planting date for 1980, Investigation One.

<u>Planting Date</u>	<u>Independent Variables</u>	<u>R²</u>
2 May	CG + AA + TZ + TZV	.765
23 May	CG + TZ + SD. WT.	.833
13 June	WG-7 + CG + AA + ASA 60 + SD. WT.	.848

Table 17. Independent variables significantly contributing to a step-wise multiple regression equation of laboratory tests and % field emergence (dependent variable) and the coefficients of multiple determination for these equations, as influenced by planting date for 1980, Investigation Two.

<u>Planting Date</u>	<u>Independent Variables</u>	<u>R²</u>
2 May	WG-7 + WG-4 + AA + SD. WT.	.801
23 May	WG-4 + AA + ASA 95 + SD. WT.	.950
13 June	WG-4 + TZ + ASA 60 + SD. WT.	.891

coefficients, for these investigations where SD. WT. significantly contributed to the stepwise regression equation, shows that lots with mean seed weights of 16.5-18 g/100 seeds had the best field emergence. Seed that was smaller or larger than that had lower field emergence percentages for these investigations. These data indicate that seed size can be an important variable in predicting field emergence through multiple regression, when large and small seeded lots are included in the investigations.

Multiple regression equations, using variables frequently appearing in Tables 14-17, were computed to find the best equation for similar planting environments (Table 18). The resulting R^2 values were usually less than those of the stepwise multiple regression equations. In most cases at least one variable was not significantly contributing to the equation for at least one of the investigations.

Since CG and SD. WT. appeared in most of the stepwise multiple regressions, equations with only those two variables were computed for all planting dates of both investigations, both years. Table 19 shows that the R^2 values were no better, and usually less, than those for the stepwise multiple regression equations. SD. WT. did not significantly contribute to the regression of Investigation One - 1979 because of the narrow range of SD. WT. values. The contribution of CG for Investigation Two - 1980 was non-significant for all three planting dates.

The use of a combination of tests to predict field emergence results has been suggested by other investigators (36, 98). Yaklich (98) used the best R^2 values from all possible multiple regressive equations to evaluate tests that had similarly measured vigor. By using a number

Table 18. Independent variables and coefficients of multiple determination (R^2) of multiple regression equations for Investigations One and Two under similar planting conditions.

<u>Planting Conditions</u>	<u>Independent Variables</u>	<u>R^2</u>	
		<u>Investigation 1</u>	<u>Investigation 2</u>
8 May, 1979 (most severe)	CG + AA + SD. WT.	.882	.568
16 May, 1979 2 May, 1980	CG + TZV + SD. WT.	.883 .676	.518 .518
31 May, 1979 13 June, 1980	WG-4 + CG + AA + SD. WT.	.952 .797	.585 .600
25 May, 1980 (most favorable)	CG + TZ + SD. WT.	.833	.581

60

Table 19. Coefficients of multiple determination (R^2) for regression equations having CG and SD. WT. as the independent variables predicting % field emergence for both investigations, both years.

<u>Planting Date</u>	<u>1979</u>		<u>1980</u>	
	<u>Investigation 1</u>	<u>Investigation 2</u>	<u>Planting Date</u>	<u>Investigation 1</u> <u>Investigation 2</u>
8 May	.764	.565	2 May	.563 .495
16 May	.780	.517	23 May	.617 .593
31 May	.861	.788	13 June	.581 .278

of laboratory tests that measure several different aspects of vigor, test combinations having high R^2 values have been found, that will predict field emergence results under the seed bed conditions of this investigation.

SUMMARY AND CONCLUSIONS

Comparisons of vigor tests and field emergence results were made in 1979 and 1980. Investigation One utilized high, medium and low quality seed. The medium and low quality lots were fabricated from the high quality lots by subjecting them to artificial aging and mechanical damage. Seed lots for Investigation Two were randomly selected from lots of certified seed. One-half of each lot, for both investigations, was treated with a slurry mixture of Captan fungicide seed treatment.

The laboratory tests used to evaluate seed vigor were the standard warm germination test - four and seven day counts, cold soil germination test, accelerated aging test, tetrazolium test, tetrazolium vigor index, conductivity tests (ASA 60 and ASA 95), and seed weight per 100 seeds. Field emergence data were collected at one East Lansing, Michigan location for each investigation, both years.

I. Laboratory Tests

A strong relationship between WG-7, TZ, and ASA 95 was found both years, indicating that all three are good tests for viability. Tests that are direct indicators of vigor, like CG, AA, and WG-4, correlated well with each other. The good correlation of these tests with WG-7 for Investigation One, both years indicates a wide range of seed quality in the lots used for that investigation. There was a good association between AA and CG results for both investigations, both years.

Fungicide seed treatment improved CG results by protecting the seed from soil-borne pathogens. The spread of storage fungi was

limited by seed treatment in the accelerated aging test. Fungicide treatment did not affect conductivity results (ASA 60 and 95).

Additional research is needed to standardize procedures for WG-4 and the conductivity tests.

II. Field Emergence

Seed from both investigations was planted at three planting dates, to expose it to three different planting environments, both years. Heat units were used to characterize the soil environment. Total heat units accumulated in the first five days after planting had the greatest effect on final emergence. The largest improvement in field stands due to fungicide seed treatment was under adverse planting conditions.

III. Relationship of Laboratory Tests with Field Emergence

WG-4, CG, AA, and ASA 60 were better correlated with field emergence than WG-7 for Investigation One, 1979. However, WG-7 had better correlations with emergence under favorable soil conditions for this investigation in 1980. Correlation of these four vigor tests with final field stands was better than WG-7 for Investigation Two, 1979. All correlations for Investigation Two - 1980 were low.

CG results were best correlated with field emergence under adverse soil temperature conditions but also did well as soil temperatures improved. The correlation of emergence and AA results was also good for most soil environments. The conductivity test had a good association with field stands for both investigations in 1979 and for Investigation One - 1980. The relationship between emergence and TZV was best for Investigation

One, both years. Correlations between final emergence and WG-4 were better than WG-7 for both investigations in 1979.

Multiple stepwise regression equations were calculated for each planting date of both investigations, both years. Each equation had a different set of independent variables (laboratory tests) in it. For all equations, the regression of these variables on field emergence accounted for over 50% of the variability. CG and SD. WT. appeared in most of these equations. When these two variables were regressed on field emergence for all planting dates of both investigations, both years, the resulting R^2 values were usually lower, and in some cases, one of the variables did not significantly contribute to the regression. Similar results were obtained when one multiple regression equation was calculated for each set of planting dates with similar soil temperature.

IV. Conclusions

The following conclusions can be drawn from this study:

1. No single test will best predict field emergence under all planting conditions in Michigan, since the possible number of different planting environments is almost infinite.

2. Since each vigor test measures a different attribute of vigor, each will correlate best with field emergence when the seed is planted in particular soil environments. Tests like CG and AA are best correlated with emergence when seed is planted under adverse conditions, whereas the viability tests and the other vigor tests in this study are

better vigor indicators when deteriorated or mechanically damaged seed is used or when seed is sown in soil with favorable temperatures.

3. Mean seed weight may be helpful in determining seed vigor, especially when it is evaluated in the presence of the other vigor tests.

4. The use of one viability test plus either CG or AA and either the conductivity vigor test (ASA 60) or WG-4 should give the best indication of potential field emergence under most field conditions found in southern lower Michigan on sandy loam soils. The tetrazolium vigor test index is best used in special cases when mechanical damage or deterioration are suspected causes of low vigor.

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APPENDIX

Table A1. Analyses of variance of the cold soil germination test and the accelerated aging test results for Investigation One, 1979 as influenced by fungicide seed treatment (F), cultivar (C), lot (L), and vigor level (V).

<u>Source</u>	<u>df</u>	<u>Cold Soil Test</u>		<u>Accelerated Aging</u>	
		<u>Mean Square</u>	<u>F</u>	<u>Mean Square</u>	<u>F</u>
F	1	11718.06	718.35***	425.39	23.99***
C	1	1387.56	85.06***	4505.77	254.07***
FC	1	169.00	10.36**	185.64	10.47**
L(C)	2	4675.16	286.60***	4562.64	257.28***
FL(C)	2	54.53	3.34*	339.89	19.17***
V(LC)	12	3630.93	222.59***	6495.37	366.26***
FV(LC)	12	367.91	22.55***	158.74	8.95***
Error	32	16.31		17.72	

*, **, *** Significance at 0.05, 0.01, and 0.001 probability levels respectively.

Table A2. Analyses of variance of the cold soil germination test and the accelerated aging test results for Investigation Two, 1979 as influenced by fungicide seed treatment (F), cultivar (C), and lot (L).

<u>Source</u>	<u>df</u>	<u>Cold Soil Test</u>		<u>Accelerated Aging</u>	
		<u>Mean Square</u>	<u>F</u>	<u>Mean Square</u>	<u>F</u>
F	1	3204.10	28.51***	70.23	3.03
C	1	8410.00	74.82***	1600.23	69.05***
FC	1	360.00	3.20	46.23	1.99
L(C)	8	1987.58	17.68***	343.73	14.83***
FL(C)	8	236.05	2.10	29.85	1.29
Error	20	112.40		23.18	

*** Significance at the 0.001 level of significance.

Table A3. Analyses of variance of the warm germination test-four day count, cold soil germination test, and accelerated aging test results for Investigation One, 1980 as influenced by replication (R), fungicide seed treatment (F), cultivar (C), lot (L), and vigor level (V).

Source	df	Warm Germ-4 day		Cold Soil Test		Accelerated Aging	
		Mean Square	F	Mean Square	F	Mean Square	F
R	3	201.23	2.51	98.51	2.08	35.26	2.18
F	1	341.26	4.25*	1625.26	34.29***	1625.26	100.67***
C	1	168.01	2.09	2784.26	58.74***	0.26	0.02
FC	1	3.76	0.05	380.01	8.01**	894.26	55.39***
L(C)	2	1767.84	22.03***	7990.89	168.60***	9238.01	572.21***
FL(C)	2	25.43	0.32	608.80	12.84***	711.93	44.10***
V(LC)	8	712.95	8.89***	1859.97	39.24***	2210.41	136.91***
FV(LC)	8	123.66	1.54	232.47	4.90***	442.53	27.41***
Error	69	80.23		47.39		16.14	

*, **, ***, Significance at the 0.05, 0.01 and 0.001 levels of probability respectively.

Table A4. Analyses of variance of the warm germination test-four day count, cold soil germination test, and accelerated aging test results for Investigation Two 1980 as influenced by replication (R), fungicide seed treatment (F), cultivar (C), and lot (L).

Source	df	Warm Germ-4 day			Cold Soil Test			Accelerated Aging		
		Mean Square	F		Mean Square	F		Mean Square	F	
R	3	129.39	1.98		32.22	0.95		31.24	1.13	
F	1	1220.08	18.66***		936.33	27.60***		9890.02	357.46***	
C	1	520.08	7.96**		3.00	0.09		1441.02	52.08***	
FC	1	588.00	8.99**		133.33	3.93		391.02	14.13***	
L(C)	4	275.33	4.21**		189.50	5.59**		365.58	13.21***	
FL(C)	4	489.91	7.49***		113.33	3.34*		270.21	9.77***	
Error	33	65.37			33.92			27.67		

*, **, *** Significance at the 0.05, 0.01 and 0.001 levels of probability respectively.

Table A5. Analyses of variance of final field emergence (%) for the three planting dates of Investigation One, 1979 as influenced by fungicide seed treatment (F), cultivar (C), lot (L), and vigor level (V).

<u>Source</u>	<u>df</u>	<u>8 May</u>			<u>16 May</u>			<u>31 May</u>		
		<u>Mean Square</u>	<u>F</u>		<u>Mean Square</u>	<u>F</u>		<u>Mean Square</u>	<u>F</u>	
F	1	1681.00	16.51***		462.25	4.29*		1350.56	23.31***	
C	1	1640.25	16.11***		841.00	7.81**		2997.56	51.74***	
FC	1	110.25	1.08		105.06	0.98		0.00	0.00	
L(C)	2	2443.63	23.99***		2934.03	27.25***		4349.28	75.07***	77
FL(C)	2	316.63	3.11		3.66	0.03		115.66	2.00	
V(LC)	12	1405.29	13.80***		1484.41	13.78***		3260.28	56.27***	
FV(LC)	12	119.96	1.18		213.86	1.99		137.22	2.36*	
Error	32	101.84			107.69			57.94		

*, **, *** Significance at the 0.05, 0.01 and 0.001 levels of probability respectively.

Table A6. Analyses of variance of final field emergence (%) for the three planting dates of Investigation Two, 1979 as influenced by fungicide seed treatment (F), cultivar (C), and lot (L).

Source	df	8 May			16 May			31 May		
		Mean Square	F		Mean Square	F		Mean Square	F	
F	1	1512.90	6.56*		4223.03	110.91***		756.90	10.30**	
C	1	4665.60	20.21***		1575.03	41.37***		3168.40	43.14***	
FC	1	36.10	0.16		819.03	21.51***		108.90	1.48	
L(C)	8	177.75	0.77		328.03	8.62***		142.44	1.94	
FL(C)	8	147.38	0.64		16.40	0.43		75.21	1.02	
Error	20	230.80			38.08			73.45		

*, **, *** Significance at the 0.05, 0.01, and 0.001 levels of probability respectively.

Table A7. Analyses of variance of final field emergence (%) for the three planting dates of Investigation One, 1980 as influenced by replication (R), fungicide seed treatment (F), cultivar (C), lot (L) and vigor level (V).

Source	df	2 May			23 May			13 June		
		Mean Square	F		Mean Square	F		Mean Square	F	
R	3	202.68	1.07		334.92	2.99*		103.23	1.19	
F	1	75.26	0.40		22.04	0.20		333.76	3.83	
C	1	981.76	5.20*		1066.67	9.52**		0.51	0.01	
FC	1	90.09	0.48		425.04	3.79		5.51	0.06	
L(C)	2	452.48	2.39		1674.67	14.95***		2453.01	28.16***	
FL(C)	2	251.68	1.33		185.04	1.65		390.05	4.48*	
V(LC)	8	784.21	4.15***		1422.04	12.70***		910.42	10.45***	
FV(LC)	8	266.27	1.41		158.79	14.18		152.81	1.75	
Error	69	188.98			112.01			87.11		

*, **, *** Significance at the 0.05, 0.01, and 0.001 levels of probability respectively.

Table A8. Analyses of variance of final field emergence (%) for the three dates of planting of Investigation Two, 1980 as influenced by replication (R), fungicide seed treatment (F), cultivar (C), and lot (L).

<u>Source</u>	<u>df</u>	<u>2 May</u>		<u>23 May</u>		<u>13 June</u>	
		<u>Mean Square</u>	<u>F</u>	<u>Mean Square</u>	<u>F</u>	<u>Mean Square</u>	<u>F</u>
R	3	156.31	1.30	59.86	0.90	184.08	2.06
F	1	1240.33	10.29**	80.08	1.21	25.52	0.29
C	1	176.33	1.46	602.08	9.07**	1054.69	11.82**
FC	1	4.08	0.03	2.08	0.03	13.02	0.15
L(C)	4	391.08	3.25*	114.90	1.73	131.85	1.48
FL(C)	4	106.96	0.89	56.15	0.85	221.52	2.48
Error	33	120.49		66.36		89.23	

* ** Significance at the 0.05 and 0.01 probability levels respectively.

Table A9. Daily maximum and minimum soil temperatures ($^{\circ}\text{C}$) and precipitation (mm) at the Michigan State University experimental farm, East Lansing, Mi., 1979-80.

<u>Date</u>	<u>1979</u>			<u>1980</u>		
	<u>Max.</u>	<u>Min.</u>	<u>pptn.</u>	<u>Max.</u>	<u>Min.</u>	<u>pptn.</u>
April (month)	-	-	73.7	-	-	70.1
1 May	11.1	0.0	0.5	20.6	9.4	
2	15.6	4.4		22.2	10.0	
3	11.1	8.9	15.2	23.3	8.9	
4	8.9	2.2		24.4	13.3	
5	11.1	1.1		24.4	13.3	
6	17.8	5.6		22.2	10.0	
7	15.6	8.9		13.3	6.7	
8	22.2	10.0		12.2	4.4	
9	24.4	14.4		14.4	5.6	
10	25.6	15.6		15.6	10.0	
11	21.1	15.6	4.6	20.0	11.1	2.3
12	10.0	8.9	15.0	21.1	12.2	T
13	15.6	5.6	6.6	21.1	7.8	9.4
14	17.8	11.1	1.3	16.7	7.8	13.5
15	17.8	10.0	1.0	17.8	6.7	T
16	20.0	6.7		18.9	10.6	0.8
17	18.9	7.8		14.4	12.8	
18	22.2	10.0		15.6	11.1	31.2
19	21.1	11.1		22.8	10.0	0.3
20	23.3	10.0	1.0	23.3	12.2	
21	17.8	10.0	2.0	26.7	12.8	
22	21.1	7.8		29.4	14.4	
23	14.4	10.0		26.1	16.7	
24	10.0	7.8	0.5	25.6	16.7	
25	10.0	6.7	0.3	26.7	14.4	
26	12.2	7.8	0.3	23.3	12.2	
27	15.6	7.8	0.5	21.1	12.8	
28	13.3	8.9	4.8	20.0	13.9	
29	20.0	7.2		19.4	16.1	
30	14.4	8.9		20.2	16.7	1.5
31	20.0	10.0	0.3	23.3	12.2	14.2
1 June	20.0	14.4		16.7	14.4	
2	24.4	11.1		23.3	17.2	8.9
3	25.6	12.2		23.9	11.7	17.8
4	26.7	12.2		22.2	10.0	
5	23.3	15.6		22.2	16.1	
6	25.6	13.3		26.7	17.8	12.4
7	21.1	15.6		23.3	11.1	T
8	28.8	17.8	1.3	18.3	11.1	23.9
9	30.0	20.0	3.6	16.7	5.6	2.0
10	27.8	21.1		18.9	6.7	0.3

Table A9 (Continued)

<u>Date</u>	<u>1979</u>			<u>1980</u>		
	<u>Max.</u>	<u>Min.</u>	<u>pptn.</u>	<u>Max.</u>	<u>Min.</u>	<u>pptn.</u>
11 June	23.3	12.2	8.9	23.3	10.0	
12	23.3	11.1	3.8	28.3	14.4	
13	26.1	10.6		28.9	17.8	
14	27.8	12.2		23.3	15.6	9.4
15	30.0	16.7		17.8	8.0	1.5
16	32.8	17.8		22.2	9.4	8.1
17	27.8	18.9		24.4	12.2	
18	24.4	15.6		25.0	14.4	
19	27.8	12.2		17.8	10.0	2.3
20	24.4	15.6		24.4	11.7	9.9
21	25.6	15.6	14.2	25.6	13.3	
22	26.7	15.6		29.4	15.6	
23	21.1	13.3		30.6	17.8	
24	25.6	11.1		31.1	18.9	
25	27.8	10.0		34.4	21.1	
26	30.0	13.3		34.4	18.9	
27	31.1	15.6		33.3	18.3	
28	31.1	16.7	3.3	34.4	18.3	T
29	23.3	18.9	1.8	33.3	16.7	
30	15.6	14.4	71.1	26.7	14.4	
1 July	16.7	15.6	17.3	27.8	17.8	
2	21.1	15.6	0.5	32.8	15.6	0.5
3	22.2	13.3		35.0	19.4	
4	22.8	13.9	2.3	26.7	20.0	
5	21.7	11.1		28.9	17.8	10.4
6	25.0	11.1		33.3	15.6	4.8
7	26.7	12.2		30.6	23.3	T
8	25.6	14.4		36.7	18.9	T
9	25.6	16.7		28.9	20.0	
10	27.8	15.6	T	36.7	20.6	

Table A10. Regression coefficients and standard error of the estimate of the stepwise multiple regression equations for three planting dates, both years, both investigations.

	<u>Intercept</u>	<u>WG-7</u>	<u>WG-4</u>	<u>CG</u>	<u>AA</u>	<u>TZ</u>	<u>TZV</u>	<u>ASA 60</u>	<u>ASA 95</u>	<u>SD.WT.</u>	<u>SE of Estimate</u>
<u>1979</u>											
<u>8 May</u>											
Investigation 1	4.269			0.267	0.305					-3.300	7.70
Investigation 2	86.018			0.346							10.57
<u>16 May</u>											
Investigation 1	-4.531			0.273			0.099				7.44
Investigation 2	106.502			0.338						-3.477	11.61
<u>31 May</u>											
Investigation 1	-7.380		0.315	0.349	0.278					-3.188	6.91
Investigation 2	106.259			0.333							6.02
<u>1980</u>											
<u>2 May</u>											
Investigation 1	-15.883			0.422	-0.197	1.367	-0.175			5.347	5.80
Investigation 2	56.149	-1.360	-0.410		0.522						4.92
<u>23 May</u>											
Investigation 1	-32.419			0.340	0.134	0.543			0.741	1.485	6.48
Investigation 2	-100.792		0.235							5.349	1.56
<u>13 June</u>											
Investigation 1	48.704	0.627		0.414	-0.310			-0.186		-3.257	5.59
Investigation 2	-308.791		0.401			2.848		-0.235		5.090	3.05