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SEVIM CINKI for the M. S. in Farm Crops
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Title THE EFFECTS OF MECHANICAL DAMAGE, INSECT INFESTATION, AND STORAGE CONDITIONS ON SEED VIABILITY OF WHEAT (TRITICUM VULGARE)

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The effects of mechanical damage, insect infestation and storage conditions on seed viability of spring wheat were studied by three known tests, laboratory germination, greenhouse growth, and tetrazolium staining, so that the relationship of these tests could be discerned to facilitate seed testing work.

Mechanical damage was induced by threshing, insect infestation was accomplished by inoculation of granary weevils, and storage conditions were provided by simulated climatic zones around the world, i. e. tropical (hot-wet), arctic (cold-wet), desert (hot-dry) and temperate.

Seed moisture content reached an equilibrium under tropical conditions with three weeks to 18 percent and desert conditions within three months to six percent. Under temperate conditions, seed gained moisture gradually to 12 percent at the end of six months,

while under arctic conditions, seed took up moisture rapidly in the first month and then only gradually to 19 percent in six months. Seed moisture was not greatly affected by mechanical damage and insect infestation.

Seed viability was reduced rapidly under tropical conditions, gradually under arctic conditions, and slightly under temperate and desert conditions for six months. Mechanical damage and insect infestation caused a substantial decrease under all the conditions studied. Both mechanical damage and insect infestation increased abnormal seedlings and internal injuries, and decreased seedling vigor.

Results from laboratory germination and greenhouse growth tests were comparable for all the treatments. The results of tetrazolium and germination tests in the laboratory or greenhouse were highly correlated. Regression equations for predicting germination percentages from results of tetrazolium tests were calculated for each storage condition and treatment.

THE EFFECTS OF MECHANICAL DAMAGE, INSECT INFESTATION, AND STORAGE CONDITIONS ON SEED VIABILITY OF WHEAT (TRITICUM VULGARE)

by

SEVIM CINKI

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Redacted for Privacy

Professor of Farm Crops

In Charge of Major

Redacted for Privacy

Head of Department of Farm Crops

Redacted for Privacy

Dean of Graduate School

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INTRODUCTION

Maintaining seed viability after harvest until the time of planting is an important problem in crop production. Better stands are obtained from carefully handled and stored seed. During processing and storage, seed viability may be affected by inherited characteristics of the species, seed maturity, moisture content and chemical composition of the seed, processing methods, contamination of seed by mold and insects, or storage period and conditions. In most cases when seed with an unknown life history comes to a seed laboratory, it is impossible to determine the causes for seedling abnormalities and low seed germinability. Information is needed regarding the factors affecting seed germinability and vigor. The aim of this research was to determine the influences of various treatments during the processing and storage of wheat on subsequent germination capacity, vigor and the tetrazolium (TZ) test results.

Visible mechanical damage to seeds can easily be avoided by the proper adjustment of the machines in processing, but evidence of internal injuries from mishandling are not easily discernible. A considerable reduction in seed viability and an increase in abnormal seedlings may result from these internal injuries. Seeds which are injured and weakened are very susceptible to diseases and insect attacks. Under adverse storage conditions, mechanically damaged and infested grain loses its planting value completely in a few weeks, but the loss from insect attacks would be negligible if special care were taken. The effects of storage conditions, i. e. temperature and moisture, on seed viability are clearly understood. However, the interactions of mechanical damage, insect infestation, and storage condition are unknown.

Generally the only method used in the measurement of seed viability is the standard germination test which takes at least six days, depending upon the kind of seed used. Since germination tests may not always correlate with the stand establishment, investigators have been looking for another more accurate and rapid test for the measurement of seed viability and vigor. At present the TZ test is the most promising viability test. It was first developed by Lakon in Germany and has been used unofficially by many seed laboratories in the United States. The underlying principle of this test is the reduction of colorless triphenyl tetrazolium chloride, TZ, to red formazan by the activity of respiratory enzymes present in living tissue. The TZ test is suggested as a good viability and vigor test because it reveals the internal condition of the seed. Furthermore, with this test dormant seed and hard seed can be examined. These hard and dormant seeds will not germinate in standard laboratory tests without special treatment to break dormancy of the seed.

In this study, wheat with a known life history and high germination capacity was subjected to simulated mechanical and insect damage and stored under four conditions for six months. These conditions were: hot-wet with a high temperature (90° F) and high relative humidity (90 percent), hot-dry with high temperature (90° F) and low humidity (40 percent RH), standard condition (70° F and 66 percent RH), cold-wet (35° F and 85 percent RH). Seed viability was measured by standard germination test, greenhouse "growth test," and the TZ test. The results, obtained from these three tests were correlated to compare the TZ test with the germination and growth test.

REVIEW OF LITERATURE

The literature related to seed germinability, seed vigor, and seed testing methods is voluminous. This is understandable since seed quality is an important factor in crop production and seed trade. An exhaustive review of literature would be impossible, thus only pertinent ones will be included in the following sections.

Factors Affecting Seed Germinability and Vigor

Inherited characteristics of the species, environmental conditions under which the seeds are grown, harvesting and processing methods, and storage conditions are the major influential factors affecting seed germinability and vigor. The inherited characteristics determine the life span and growth potential of the seed, whereas the other factors usually modify these potentials to various degrees.

Species. Hutchinson (26) stated that under ideal conditions the ability of seed to maintain its germinability depends entirely upon the species. Stickle and Wasson (64) found a varietal effect on emergence and seedling vigor of birdsfoot trefoil. In the experiments with buried seed reported by Barton (2, p. 7-13) the life span of seeds varied in accordance with the species. Seed of wild plant species maintained their germinability better than those of cultivated plant species.

Growing conditions. The conditions under which a plant grows has an effect on the seed quality, weight and the chemical composition of seed. These conditions may be environmental (such as temperature, moisture, photoperiodism) or cultural (planting methods and date, fertilizer, irrigation, soil condition, pollinators, competition of other crops, weed, disease and insect, herbicides, fungicides, defoliant). An example of growing conditions affecting seed quality was shown by reduced bean yield due to moisture deficits of varying intensity at specific stages in development of this crop. Seeds from dalapon treated plants had markedly reduced germination and seedling vigor and seedlings showed dalopon injury symptoms according to Warden (68) and Behrens (4).

Harvesting and processing methods. The effect of harvesting and processing methods on seed viability was studied by many workers (1; 25; 30; 48; 53; 66, p. 214-225; 68; 71). The proper methods and adjustment of equipment for harvesting and processing are very important in order to obtain high quality seed with good performance in the field (48; 70). The quality of seed is reduced by mechanical damage. Injured kernels are usually very slow to germinate, extremely sensitive to germination conditions, and give rise to poor stands of weak, or frequently abnormal, seedlings. The type and amount of damage to seed are considered responsible for the longevity of seed viability. Damaged seed is very susceptible to disease and deteriorates rapidly in storage. It was also observed that immature seed with a high moisture content is more subject to mechanical damage than mature seed, and there are also species and variety effects on the amount of visible damage received.

Storage conditions. It has been shown that the viability and field performance of any given seed depends largely upon the conditions under which it is stored (2, p. 30-36; 8; 9; 10; 17; 18; 26; 34; 49; 50, p. 4-8; 57; 61; 66). The influence of storage conditions on seed viability are as follows:

(1) Temperature: The effect of temperature on seed germinability and vigor are closely interrelated with the effect of moisture content of seed and atmospheric relative humidity (2, p. 30; 10; 61). In general, the higher the temperature, the more rapid the deterioration occurs at a given moisture level. This indicates that the low temperatures are more desirable than higher temperatures for storing seeds. Barton's (2, p. 30-33) discovery of the superiority of below-freezing temperatures over above-freezing ones for keeping coniferous tree seeds offered a new way for storing seeds. According to Hutchinson (26) temperatures just below the freezing point are superior to any temperature above freezing for the storage of dry seeds. However the advisability of storing seeds at below-freezing temperatures depends on the moisture content of the seed. Those high in moisture content can be damaged by freezing.

(2) Relative humidity and seed moisture: These two factors

have not been studied to any extent as separate factors, because the ultimate result of an increase in relative humidity is to increase the moisture content of seed. The importance of seed moisture content in storage has been reported by many workers (2, p. 14-22; 8; 9; 10; 17; 18; 26; 34, p. 288-303; 49; 50, p. 9-12; 57; 59; 61). They all found that decreasing the moisture content in seed increased the longevity in storage. Prolonged exposure to moderately high relative humidity and high temperature markedly reduced the percent of germination. It was found by Barton (2, p. 22-28) and Toole (66, p. 214-225) that the relative humidity of the atmosphere, rather than the absolute moisture content of the air, controls seed moisture. Each kind of seed will attain a characteristic moisture content at a given relative humidity. This moisture equilibrium varies slightly with temperature. As the relative humidity of the air increases, the seed moisture equilibrium increases. The moisture content of seed stored in open containers changes quite drastically with the season. Hutchinson (26) reported that it is lowest during the winter months and highest during August and September in Maine. In Oregon, the reverse is true (10). This seasonal moisture fluctuation usually results in a shortening of the life span of the seed, indicating the desirability of using sealed containers whenever possible and practical.

Grain stored with a high water content tends to have mold infestation (57; 34, p. 288-303). In addition, a high water content increases seed susceptibility to injurious action from the chemical used in treating the seed. An interesting attempt was recently made by Roberts (57) to express the relationship between temperature, moisture content and viability of cereal seeds by a simple mathematical equation ($\log p = kv - c_1 m\ c_2 t$). Where p is the half-life viability period of the seeds, t is the temperature in ° C, m the moisture as percent, and k and c are constants. These constants were calculated from experiments with wheat, but they appeared to also fit for oats and barley. Then he suggested that it is possible to predict the expected life span of a given cereal seed under almost all storage conditions. Although it appears that dry conditions are essential for retention of viability, in many kinds of seeds viability tends to decline as seeds are dried below two percent moisture content as shown by Nutile (49).

(3) The gaseous phases of storage: According to Roberts (59) and Barton (2, p. 33-36) anaerobic conditions do not appear to be deleterious to the viability of cereal seeds. They found that storage in nitrogen results in a slightly longer period of viability than storage in air. In fact an increase in oxygen concentration tends to decrease the viability period of seeds. The effect of CO_2 in high concentration is not entirely clear, but it is suggested that high CO_2 is not markedly deleterious to viability.

Storage period or seed age. The storage period has a definite effect on seed germination capacity and its performance in the field.

The safe storage period of seed depends entirely upon the type of seed and the conditions under which it is stored. The life span of cereals as predicted by Barton (2, p. 67) and Roberts (58) ranges from a few days up to 123 years. Roberts (58) indicated that his previously suggested equation (57) describing the relationship between temperature, moisture content and viability period of seed tends to underestimate the retention of viability over very long periods of storage.

The causes of seed aging are not clearly understood. According to Harrington (23) the loss of activity of the enzymes involved in protein synthesis or in the ability of ribonucleic acid or deoxyribose nucleic acid to function may be the biochemical area in which aging first occurs in seeds.

Insect and molds. Seeds are a good nutritive source for insects which feed on the seed and lay eggs in it. The amount of damage done by an adult insect is often negligible by comparison to the complete hollowing out of seed by larvae. Therefore, insects have a direct effect on the growing ability of seed. Michelbacher and Swift (42) and Oxley (51, p. 73-77) showed that the rate of development of insects increases with increasing temperature up to about 82.4° F and with increasing humidity up to the highest level. Prolonged exposure to a temperature of 100° F results in the death of most insect species. They also found that the principal insect pests of stored grain are the granary weevil and the rice weevil. The granary weevil is the more harmful of the two. The less moisture stored seed contains, the less attractive it is to insects.

The loss of viability in damp grains after a period of storage has been attributed to a number of factors by earlier workers. The most important of these seems to be mold (11; 12, p. 507-518; 13; 43, p. 23-28; 67). The impact of this factor depends on the magnitude of the influence of accessory factors, such as seed water content (6; 13) and improper seed treatment (34, p. 288-303). It was stated that grain with low germination is often infected by one or more species of fungi. The fungus hyphae have been found in pericarp, embryo, aleurone layer and endosperm tissues. The sound seeds were largely contaminated with *Aternaria* sp. which disappeared when stored under moisture conditions favorable to the proliferation of *Aspergillus*. *Aspergillus* and *Penicillium* predominate in low grade seed. Generally mechanical injuries predispose seed to molding, and invasion of the seed by molds results in its deterioration. The work of Hummel *et al.* (24), Machacek (34, p. 228-303), Qasem and Christensen (55), Roberts (57) have indicated that seed deterioration is influenced greatly by fungi at high humidities but not at low humidities, so the fungi are not usually the main factor which lowers seed viability in storage.

From the discussion above it can be seen that the storage conditions have an important role in seed life. Even though each kind of seed requires different conditions to maintain its high germination and vigor, low moisture content and low temperature are important for safe storage.

Germination conditions. The major conditions affecting germination were stated by Mayer and Poljakoff-Mayber (39, p. 37-58) as (1) water, (2) temperature, (3) gases in the atmosphere, and (4) light. The requirement of seed for these conditions is determinated by age, hereditary factors and the environment in which the seed has developed. Each species and variety of seed apparently has its own germination requirements.

(1) Water: A certain amount of water is essential for the initial germination process which is the hydration of colloidal materials and the activation of enzymes. This is accomplished by imbibition. The extent to which imbibition occurs is determined by three factors; the chemical composition of the seed, the permeability of the seed coat to water, and the availability of water in liquid or gaseous form in the environment. In addition, water is a limiting factor for continuous seedling growth.

(2) Temperature: Each type of seed has a specific temperature range in which it germinates. In this range there is usually an optimal temperature, below and above which germination is delayed but not prevented. In general, at very low and very high temperatures the germination of all kinds of seeds is prevented. A rise in

temperature does not necessarily cause an increase in either the speed of germination or in its percentage. Some kinds of seeds require an alternating temperature to germinate. The effect of temperature is not independent of other factors. For example, the light requirement of lettuce seed increases with temperatures above 25° C.

(3) Gases: Germination is an energy-requiring process. This energy is obtained by the oxidation of storage material in the seed. Consequently, seed germination is markedly affected by the composition of the atmosphere. In most cases, seeds will show lower germination when the oxygen tension is decreased appreciably below that normally present in the atmosphere. It is known that superoptimal carbon-dioxide and suboptimal oxygen are usually inhibitory.

(4) Light: The importance of light as a germination factor for several kinds of seeds has long been recognized. Thus seed may be divided into those which germinate only in the dark, only in continuous light, only after being given a brief illumination, and those which are not affected by light. In some species a light requirement only exists immediately after harvesting; in other species this requirement persists at least for a year, while in yet other species it develops during storage. Studies on the action spectrum for germination have shown that red and blue lights promote, but far-red light inhibits germination. Whether germination is stimulated or inhibited depends entirely on the exact period of illumination as related to the

beginning of imbibition. Work on the effect of light has been carried out on a limited number of species and lettuce seed in particular. The germination of light-sensitive seed is stimulated by red light and inhibited by far-red light. This process is reversible, and indicates the presence of a photoreversible pigment in seed. The nature of the last illumination determines the germination response. The effect noted depends on both the intensity, energy and duration of illumination. Later it was shown that these effects are more complicated when the irradiation is given for longer periods of time. The inhibitory effect of prolonged illumination is such that the final germination percentage does not only depend on the actual duration of the irradiation, but also on the time interval between stimulating and inhibiting irradiation, and on the interval of time between the onset of inhibition and these irradiations. It is also known that short-wave irradiation such as γ-rays and x-rays can affect germination and development of the seedlings.

The theories formulated to explain the effect of light on germination are all based on the assumption of a photochemical reaction in seeds.

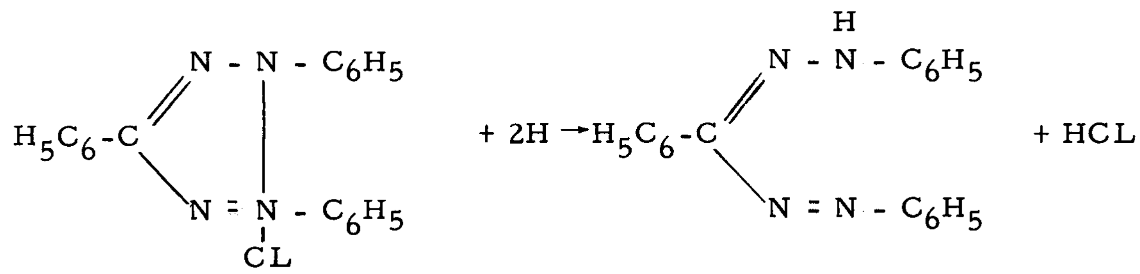
Methods of Evaluating Seed Germination and Vigor

Seed germination capacity has been expressed as the percentage of normal seedlings germinated under favorable laboratory conditions. The germinated seedlings are usually classified as normal and abnormal. Normal seedlings are those which possess essential structures indicative of their ability to produce normal plants under favorable conditions. Abnormal seedlings are either too small or lack normal morphology.

It has been shown that seed may have a lower percent of germination in the field under adverse conditions than the results of seed testing in the laboratory. This indicates the necessity of a vigor test. Although several kinds of vigor tests have been suggested, no standard method to measure the seedling vigor has been developed. Vigor has been expressed as the speed of germination, weight of the seedlings after a definite period of growth (64), the length of seedlings grown in light (56) or in dark (21), percent of seedlings when sown under unfavorable field conditions (29), and recently, the percent of normally stained embryo in the tetrazolium test (46).

Tetrazolium Test

The principle of this test is based on the reduction of colorless water-soluble triphenyl tetrazolium chloride to insoluble red formazan by the action of dehydrogenases present in respiratory system of living tissue.



Triphenyl tetrazolium chloride (colorless) red formazan

This test depends on the function of these enzymes in tissue, and on their associated hydrogen carriers, the pyridine nucleotides and flavoproteins. The test, in effect, measures the distribution within the embryo and the activity of one phase of the respiratory process. TZ substitutes, in part at least, for the natural oxidase systems in the tissue accepting the transferred hydrogen (3, p. 92-93). In order to conduct a reliable TZ test the enzymes should be activated in the seed by soaking prior to the reaction. The TZ solution should be uniformly and rapidly provided to the tissue, the proper reaction time should be allowed for the action of the enzymes, the temperature should be optimum and consistent, and lastly the pH of the solution should be optimum for the dehydrogenase enzymes.

The following are detailed findings observed by various workers regarding the procedures and factors affecting TZ tests. The importance of pre-soaking seed for consistent testing results has been established (15; 45) as well as the soaking temperature of 80 to 90° F (28; 32; 45). After soaking, the commonly-used method for preparing seeds is cutting the seed longitudinally through the embryo

(27; 28; 32; 45). In some seed it is necessary to remove the hulls (20), although in some grasses, puncturing the seed is enough to allow the entrance of the solution (27; 45). Moore (45) also reported that intact staining is especially suitable for some species to avoid cutting injuries. It is known that failure to use a sharp razor blade in cutting will often result in surface incisions through the embryo parts which prevent accurate evaluation (15; 32; 40; 45; 47).

In general the solution concentration used (14; 16; 20; 27; 28; 32; 40; 45; 47) varies with the kind of seed and ranges from one percent for small seeds and cereals to one-half percent for large seeds. Lakon (32) stated that the solution should have a pH value between 6 and 7 in order to be properly reactive. Parker's work (52) indicated that ascorbic acid, cysteine and glutathione do not reduce the salt if the pH value is under 9. Mattson (38) found that any other viable materials such as the fleshy parts of apples and grapes, carrot roots, sweet potatoes, the gill area of mushrooms, the stigmas on ovaries of certain pollinated flowers in addition to the seed, and yeast will reduce TZ at pH 6.9. Further he showed that the reduction of TZ salt is not due to sugars by these materials because reducing sugar forms the red formazan only above pH 11 at 40° C.

Room temperature was found adeuqate for staining by early workers (32). Later the temperature of 20° C or 30° C was suggested (27; 36; 41; 45). The time required to complete the reaction is closely related to the temperature, and sufficient time must be allowed for the reaction to take place. A high temperature, longer period of staining, and high concentrations of solutions are associated with the formation of red substances as pointed out by several workers (16; 20; 27; 45).

TZ solution is known to be light sensitive. It was reduced rapidly by strong light in Parker's experiment (52), if the temperature was up to 40° C.

Heat damage to seed resulting from a high temperature during drying and storage may not be detected in TZ tests. Misleading results were obtained by many workers (21; 27; 33; 36). These indicate that the dehydrogenase system responsible for reduction of the TZ solution may stay active after the seed loses its power of germination.

Seed stored following freezing give a TZ reading higher than germination according to Isley (29). Furthermore, Isley (26) and Goodsell (22) found that in freshly frozen, immature seed the dead kernels tended to be more intense in color; thus classification for viability was difficult and uncertain with TZ tests. Moore (45) was able to detect freezing injury at an early stage in seeds by blurred, flaccid tissue of deep red color during TZ tests.

Generally it was found by Moore (45) and Rice (56) that injured seed shows a dark red color which is due to an increase in reducing activity and tissue degeneration. At more advanced stages of deterioration the color of the tissue will change from red to colorless.

Copeland (15), Macleod (36), Metzer (41), found that the presence of fungi is not revealed by TZ tests.

The seed which had been made non-viable by methyl bromide, orgono mercuric or other fungicides gave a positive red stain in TZ (14; 15; 16; 41). Cobb (14) stated that embryo protein is responsible for the reduction of TZ by non-viable embryos after fumigation. Metzer (41) also indicated that fumigation in some manner kills or inhibits the growth mechanism, but apparently produces some substances capable of changing TZ solution to a red stain.

Methods of Evaluating the TZ Tests

Interpretation of the TZ test has been made according to the absence and presence of red color in the embryo of the seeds (15; 21; 27; 28; 32; 33; 36; 40). Seed showing a bright red color were considered viable; if they remained colorless after treatment, they were non-viable. In addition, seeds in which only a part of the embryo was stained were evaluated by the amount and location of the non-stained portion. It was first found by Lakon (32) and later Copeland (15) that if dead unstained areas included mesocotyl and adventitious root tips adjacent to the precambium and more than half of the scutellum in monocotyledenous seeds, the seedling could not develop. Injured or dead coleorhiza was not considered important. Cottrell (16) modified

Lakon's classification of cereals except in corn. He indicated that the secondary root arises in the region immediately below the scuteller node approximately where the embryo is attached to the scutellum, and as long as this portion of the embryo, the shoot, and the scutellum were stained, a normal seedling could develop. He and Goodsell (22) also pointed out that, in general, normal germination took place even though a portion of the scutellum was necrotic. Hyde (27), Isley (28), Porter (54), Metzer (40) followed almost the same principle used by Cottrell. In the study on dicotyledonous seed by Metzer (40) seed was considered capable of germination if the primary root, hypocotyl and point of epicotyl attachment showed a positive red stain, and as weak if one fourth or more of the cotyledonous area remained colorless.

Moore (45, p. 45-51) suggested a new approach to evaluation of the TZ test, indicating that color is only one of many factors. Turgor of tissue, presence of critically located fractures, bruises, and insect cavities must also be taken into account when interpreting a TZ test.

Correlation of the TZ Test with Germination

A close correlation of TZ test results and laboratory germination tests was shown by several workers (14; 19, p. 243-258; 22; 27; 32; 33). It was also found that TZ gave a higher estimate of germinability (65) and particularly in badly injured seeds (29; 56; 69).

Although quite good agreement may be found for highly viable seed, occasionally erratic results for poor seeds may be noted (16; 19, p. 243-258; 28; 36; 65). Porter (53) found a significant agreement between these two tests in samples of corn, wheat, oats, barley, rice, soybeans, peas, but not in vetch and sorghum.

The two tests do not correlate if the seed is infested by mold (36; 40). Metzer (40) suggested that if seedling development is slow during germination, mold will grow and prevent the seedling from developing into countable size. Although vital embryo structures may show sufficient stain to be considered as viable by TZ test, Cobb (14) stated that fumigation damage, dormancy, presence of fungi and perhaps other situations are not always revealed by TZ test. Therefore, variation may be expected in germination and TZ tests even though a close correlation of results from the two methods can be obtained. Cobb (14) and Moore (45) observed that the result of the TZ test and the standard germination test may not always agree, but each can be used to check the results of the other test.

In general, a significant correlation exists between the test results of TZ and laboratory germination. The correlation coefficient values varied from 0. 94-0. 99 (22; 24; 36).

MATERIALS AND METHODS

Seed of the spring wheat variety, Marfed-Merit Sel. 28, produced at the Hyslop Research Farm in 1962, was used for this study. Prior to the experiment, the seed was stored for six months in order to overcome post harvest dormancy. The seed had a germination capacity of 95.5 percent and 9.21 percent moisture content when the experiment was started. The average weight of one hundred seeds was 3.53 grams. The seed lot was divided into two portions, one of which was subjected to the treatment of mechanical damage and the other was untreated. Mechanical damage was induced by threshing once at high speed in a head thresher. After the treatment, all the visibly damaged seeds were eliminated by hand separation.

Cloth bags were prepared to store treated and untreated seeds. Each bag of 40 grams of seed represented a sample. The outside and inside of the bags were labeled with their treatments, storage conditions and sampling number. One half of the treated and untreated seed was inoculated with granary weevils (*Sitophilus granarius* (L.) obtained from the Entomology Department of Oregon State University) before storage in the following manner.

One hundred active adult weevils were put into each sample bag which contained 40 grams seeds. The weevils were picked up and counted by the aid of a vacuum counter so that they were not injured.

The group of cloth bags which contained the insect-inoculated samples was put into fine, brass mesh (80) bags to prevent the escape of the weevils. Then all the bags were closed tightly and placed in wire containers that permitted proper aeration of the samples.

Storage conditions. Seed samples were put into storage on April 16, 1963 in the four conditioning rooms at Oregon Forest Research Laboratory. The four conditions were:

1. (hot-wet) with high temperature of 90° F and high relative humidity (RH) of 90 percent, simulating tropical and poor storage conditions,

2. (hot-dry) with high temperature (90° F) and low humidity (40 percent RH), representing desert conditions and dry storage in warm areas,

3. (cold-wet) with temperature of 35° F and 85 percent RH, simulating conditions of the Arctic zone and wet seed stored in cold areas,

4. (standard condition) 70° F and 66 percent RH, representing average conditions for wheat production and normal storage areas.

The size of the conditioning room was 6 x 10 x 8 feet and humidity was maintained by steam or dehumidifying.

Samples were taken at weekly intervals from the hot-wet condition until the germination percentage of the seed dropped to zero or near zero. From the other conditioning rooms, seed was sampled monthly for a period of six months.

Germination test. Two replications of 100 seeds were taken without discrimination from each sample. By using a vacuum counter for proper spacing, the seed was planted between moist towels. They were then rolled up and placed in a dark germinator at 20° C.

The counts of seedlings were made on the fourth and sixth days after planting. In the final count, the length of the shoots were measured in centimeters.

Germination capacity was expressed as the total percentage of normal seedlings obtained in six days. Seedlings were classified as normal and abnormal, which were further categorized into different kinds.

Seedling vigor was measured by length of the shoot in centimeters.

Greenhouse test. This test was conducted under controlled temperature (night--65° F, day--75° F). Wooden flats, 33-1/2 x 21 x 2-1/8 inches, were filled with unsterilized soil composed of 50 percent sand and peat moss and 50 percent loam. One hundred seeds from each sample were planted in two rows, 1-1/2 inches deep. The flats were watered every day. The determination of percent germination and the measurement of seedling lengths were made one week and two weeks after planting. The vigor of the seedlings was expressed as the shoot length of seedlings in centimeters.

Moisture content determination. The moisture content of each sample was determined by oven-drying at 105° C overnight. The weight lost was used as the basis of percent moisture calculation.

TZ test. The tetrazolium chloride solution was prepared by dissolving one gram of 2, 3, 5-triphenyl tetrazolium chloride in 100 cc distilled water. It was kept in a brown bottle under refrigeration. This salt was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

Clear solution (Lactophenal) was made of 20 cc lactic acid, 20 cc solubilized phenol, 40 cc glycerin and 20 cc distilled water.

Two replications of one-hundred seeds were examined from each sample. The seeds were soaked in 20 cc of distilled water at 20° C for four hours. The soaking of seeds accelerated respiration and was a necessary step to obtain color reaction when tetrazolium chloride was used as the substrate to detect the enzymatic activity. In addition, the softened seed was more easily sectioned. It was found that the seed from the hot-dry condition required a longer period of soaking, i. e. five hours to reach the same degree of softness and enzymatic activity. The soaking period is critical; over-soaked seed can easily be smashed during cutting. However, with hard, unsoaked seed, it is difficult to cut through to the embryo. After soaking, seeds were bisected longitudinally through the embryo with a single edge razor blade. Care was exercised to make the cut through the center of the embryo, so that both plumule and radicle would be seen for a correct evaluation. As each seed was cut, one-half was immersed in a sufficient amount of TZ solution to cover the seed. They were then placed in a 30° C dark germinator to protect from deterioration of the solution. After one and one-half hours, samples were removed from the germinator, and the tetrazolium solution was carefully poured off. The excess solution was absorbed with a blotter and a few drops of clear solution were added to each sample. By using a clear solution the staining process was stopped and the color which had developed was maintained for observation.

The stained embryos were evaluated under the microscope at 24X magnification. The presence or absence of stained embryo parts, the intensity of coloring and the condition of the root shoot axis were the basis for the separation of seeds into ten categories. These were:

A. Brightly-stained embryo--the entire part of the embryo was in good condition and stained a bright red color.

B. Pink shoot--the entire embryo was stained bright red except for the shoot which was pink.

C. Pink root--only the root was pink.

D. Pink shoot and root.

E. Part of scutellum was pink.

F. A little part of the scutellum was colorless.

G. A little part of the root was colorless.

H. Entire embryo showed pink color.

I. Damaged root cap.

J. Colorless in entire embryo or in a large portion of the embryo, excessively darkly stained, visibly cracked, grey and friable embryos.

Categories from A to I were considered as viable and J as non-viable. In plate (1) examples of viable and non-viable seeds may be noted.

Plate 1. Viable seeds (upper row except the fourth one) and various kinds of non-viable seeds (lower row) as revealed by the TZ test.

RESULTS AND DISCUSSION

Changes in Moisture Content and Viability of Seed Under Different Conditions

Changes in seed moisture content. The changes in moisture content with time in wheat seed stored under different conditions are presented in Figure 1. The initial moisture content was 9.21 percent. It can be observed from the graph that there was a rapid increase in the moisture content of both control and treated seed under the hot-wet condition. After one week's storage, the percent moisture was 16.55-17.55 and the maximum percent moisture was observed in the second week's sample of treated seeds. There was no significant difference in the percent moisture in the four treatments.

The moisture content of seed increased until an equilibrium was reached between the moisture content of the grain and the relative humidity of this conditioning room. The change of moisture content in insect infested and mechanically damaged-insect infested seed followed each other closely. There was also a close relationship in the moisture percentage of mechanically damaged and control seed. Almost the same rapid equilibrium was reached in a month under cold-wet condition with relative humidity of 85 percent and temperature of 35° F. However, there was a small but continuous increase in moisture of wheat seed from the second month to the sixth month. This

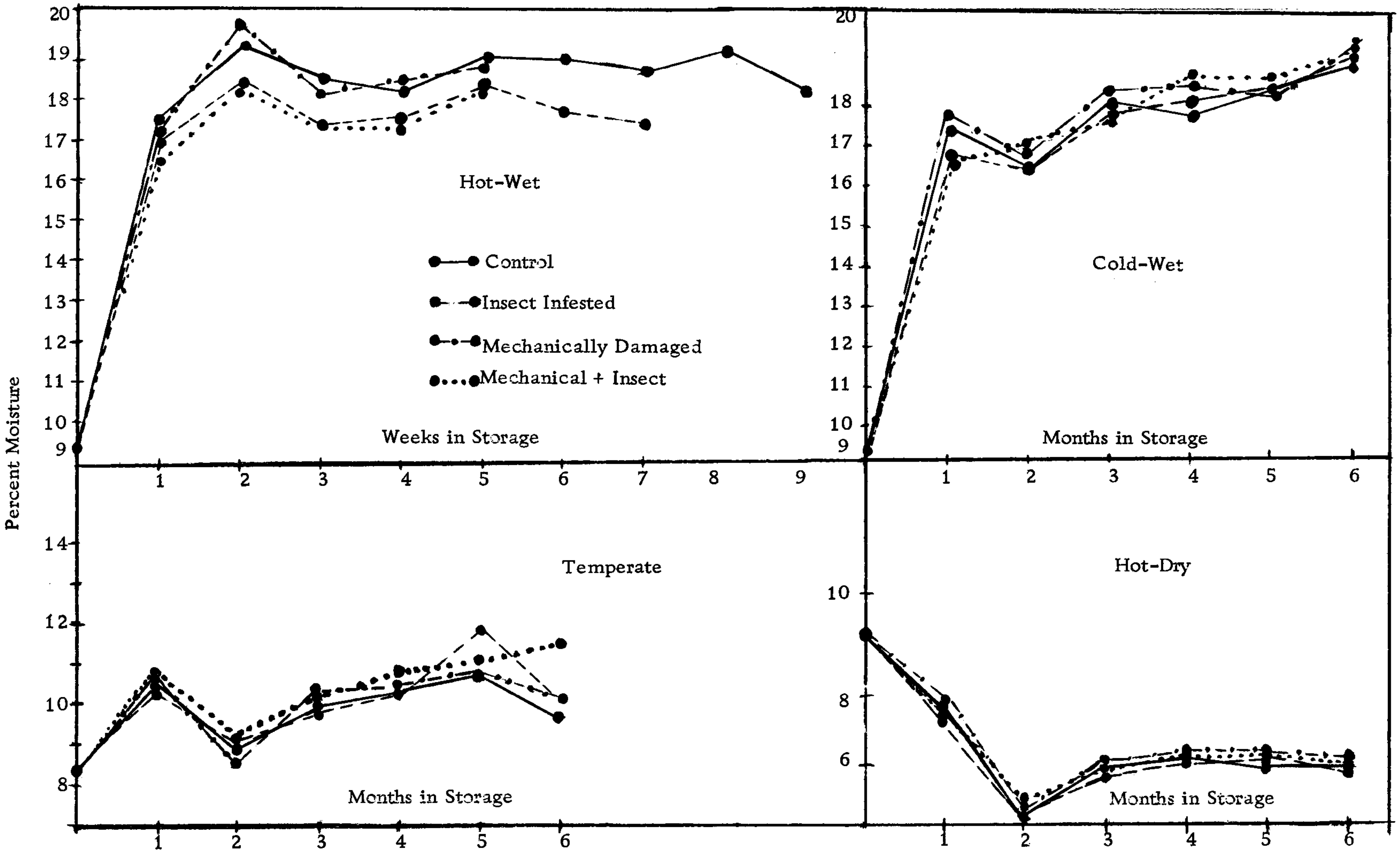


Figure 1. Change in wheat seed moisture content under four different storage conditions.

indicated that there was an accumulation in seed moisture during prolonged storage, although the difference among treatments was less than one percent.

Seed moisture content increased from 9.21 to 11.5 percent after one month of storage in temperate conditions with a relative humidity of 66 percent and a temperature of 70° F. Moisture content stabilized at around 11 to 12 percent during six months storage, varying somewhat from sample to sample. A maximum moisture percent was obtained in the fifth month's sampling of insect infested seed. There were no significant differences in changes of moisture content between the treatments and the control.

In the hot-dry condition in which the relative humidity was low (40 percent) and the temperature high (90° F), seed moisture declined to 4.97 percent, at the end of second month. Moisture content increased to about six percent in the third month, and no further changed occurred in the following months. The results for moisture changes in treated and non-treated seeds closely followed each other in every sampling.

The results obtained from the four conditions above indicate that the seed moisture content changed according to the relative humidity of storage atmosphere until an equilibrium was reached between these two variables. However, a slight continuous increase in moisture of seed occurred under the cold-wet and temperate conditions. Insect activity affected seed moisture content only under hot-wet and temperate conditions. Under hot-dry and cold-wet conditions, all weevils were found dead at the end of the first month. Moisture content in mechanically damaged wheat was slightly higher in the four conditions, but in general it seems that mechanical injury and insect infestation did not have an accelerating effect on the absorption of moisture by seed, after the equilibrium was obtained. However, they might have had an effect on the rate of moisture absorption by seed before the equilibrium was developed.

Changes of seed germination as conducted in the laboratory and greenhouse. The germination results for wheat seed subjected to various treatments stored under four different conditions for various lengths of time are shown in Figures 2, 3, 4 and 5. The seed viability decreased very rapidly under the hot-wet condition and complete loss of viability was observed at the end of second or third week in mechanically damaged and mechanically damaged plus insect infested seeds. In the insect infested seed and control, the germinability was reduced to 30-40 percent by the end of the second week. After that viability of the seed declined gradually until the germinability of grain was entirely lost at the end of the seventh and ninth week respectively.

The germination results were similar for mechanically damaged plus insect infested seeds and the mechanically damaged

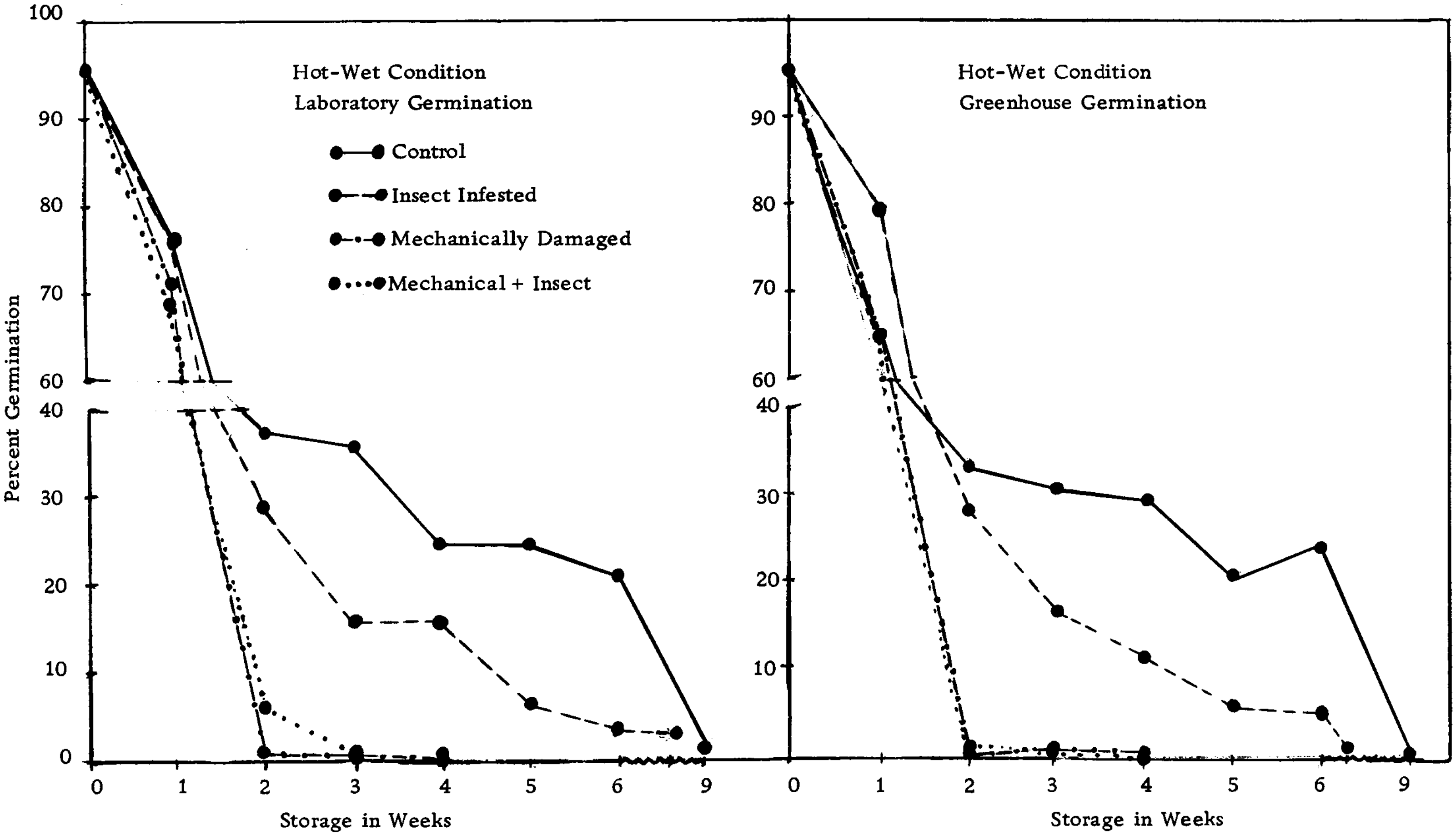


Figure 2. Changes in the percent germination of wheat seed subjected to various treatments and stored under the hot-wet condition as determined by laboratory and greenhouse tests.

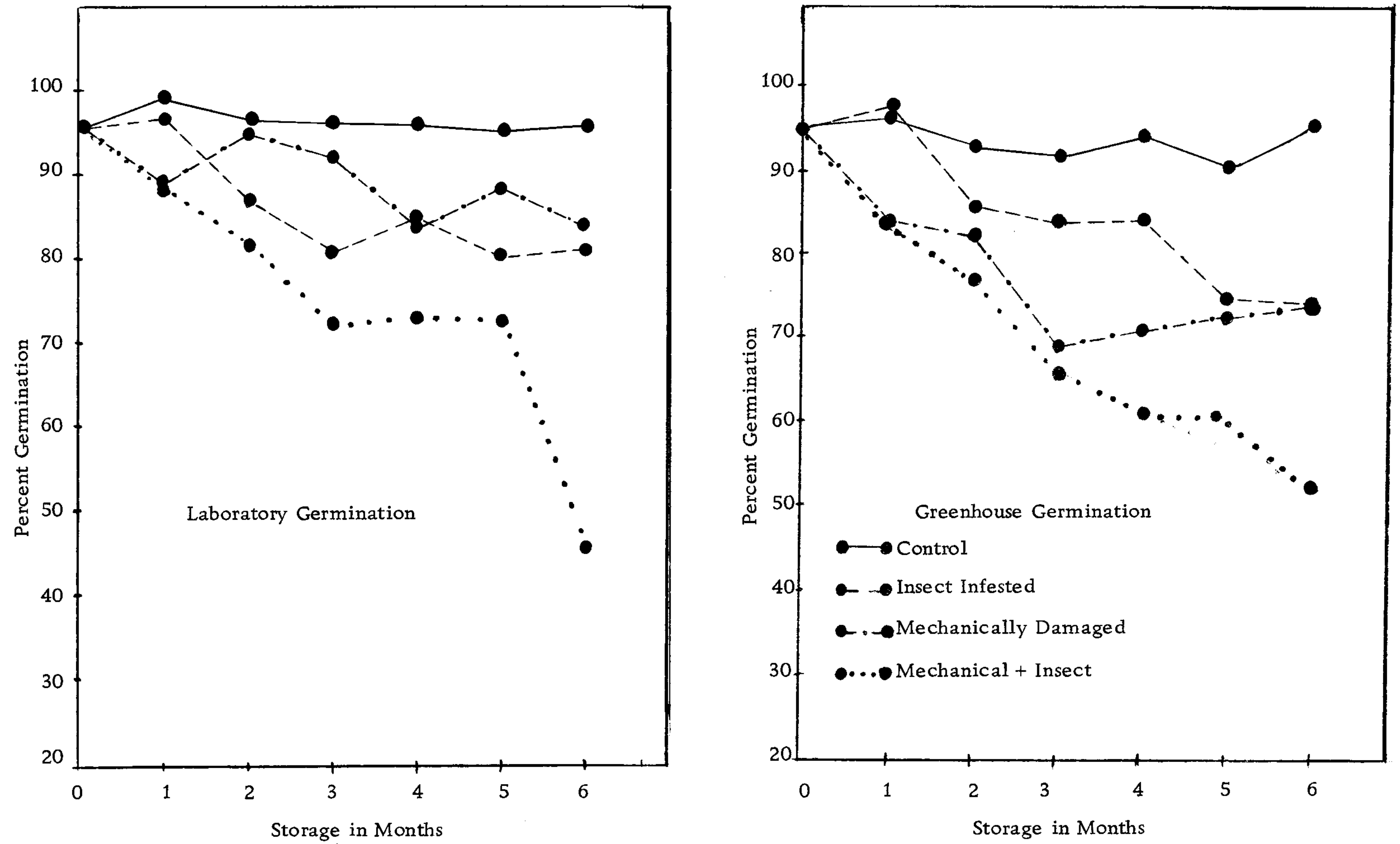


Figure 3. Changes in the percent germination of wheat seed subjected to various treatments and stored under the temperate condition as determined by laboratory and greenhouse tests.

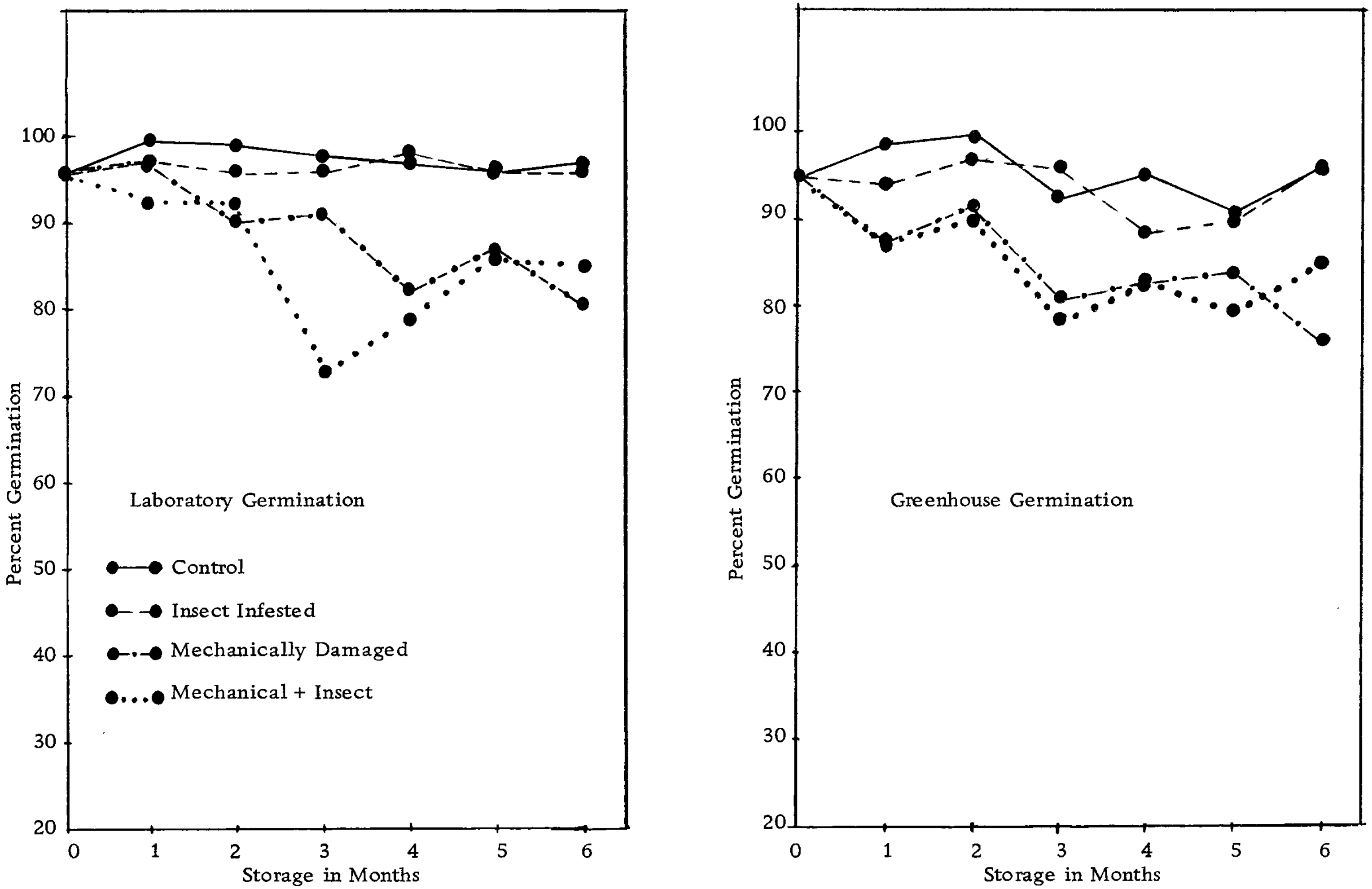


Figure 4. Changes in the percent germination of wheat seed subjected to various treatments and stored under the hot-dry condition as determined by laboratory and greenhouse tests.

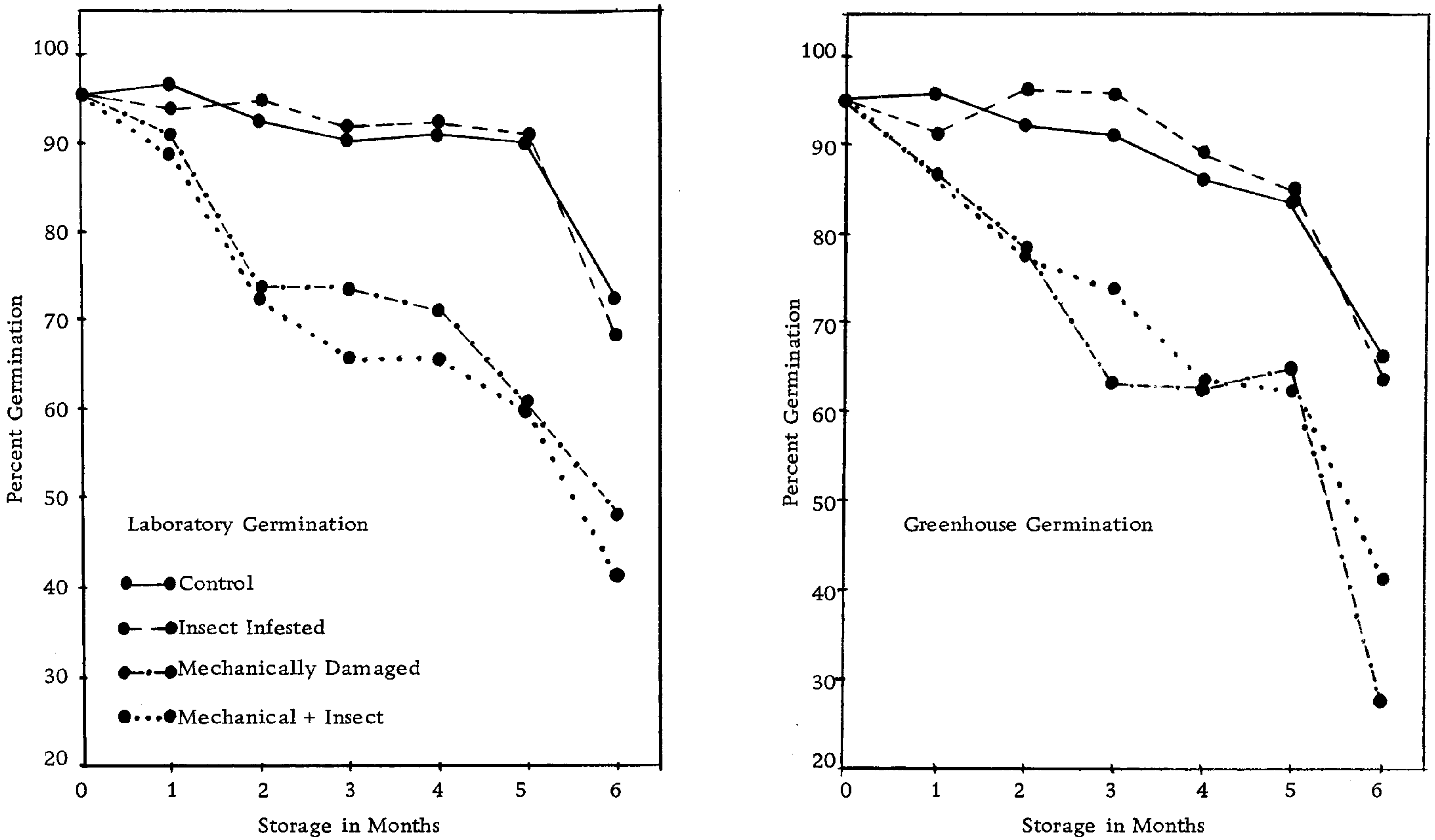


Figure 5. Changes in the percent germination of wheat seed subjected to various treatments and stored under the cold-wet condition as determined by laboratory and greenhouse tests.

seeds. Mechanical damage apparently reduced seed viability more than the insect injury.

The complete deterioration of control seed in nine weeks was attributed to the high moisture, high relative humidity and growth of fungi in seed. In fact, the samples were covered with fungi after two weeks storage and especially infection was noted in seed embryos. In addition to these factors, mechanical and insect injuries accelerated the deterioration of seed. A consistent but slightly lower percent of germination was observed in the greenhouse tests as compared to the laboratory results on the same samples.

Under the temperate condition, the control seed retained full viability for a six month period, and in the last sampling, the observed germination capacity was 96 percent in the laboratory, 95.5 percent in the greenhouse test. Therefore, it appears that quality of seed was maintained for six months or more without any reduction in germination capacity. When seed was contaminated with weevils, viability decreased gradually as shown in Figure 3. It seemed that a hundred adult weevils in 40 grams of seed did not cause much damage during a month's storage. However in the second month, when a new generation of weevil was produced, the reduction in viability was considerable.

Mechanical damage resulted in reduced seed viability even after one month of storage. A marked decrease in germination occurred in mechanically damaged and insect infested samples every month. The total reduction of germination was 44 percent in the greenhouse test and 52 percent in the laboratory test at the end of the sixth month of storage.

Laboratory and greenhouse germination results followed each other closely except in the seed damaged mechanically. Lower greenhouse germination was obtained for mechanically damaged seed. This indicated that the seed which was mechanically damaged and stored might develop normal seedlings in the laboratory germination test but not in greenhouse germination tests.

It can be observed from the graphs that the treatment effects of mechanical damage and insect infestation are additive, because in every case lower germination results were found for mechanically damaged plus insect infested wheat seed.

The fact that the results of laboratory tests in some samples were lower than the greenhouse test results could be attributed to the growth of fungi under laboratory conditions.

In the hot-dry condition, the control samples maintained their high germination capacity during the six month's storage period, whereas the mechanically damaged seed showed a reduction in percent germination. The rate of the reduction was slower in comparison to mechanically damaged seed under other storage conditions. In general, there was only a slight difference between germination values obtained in the greenhouse and laboratory. However, in the third and fourth sampling, percent germination of mechanically damaged plus insect infested seeds was lower in laboratory tests than the samples which were germinated in the greenhouse. This was related to the mold growth during the six days of laboratory germination.

Under the cold-wet condition, the germination capacity of the control sample declined slowly for four or five months, after which there was a rather rapid deterioration measured in both greenhouse and laboratory germination tests. Even so, mechanically damaged seed deteriorated at a slower rate than those in hot-wet conditions. The difference between the relative humidities of these two conditions was only five percent, thus suggesting the deleterious effect of high temperature on seed viability. The viability of mechanically damaged seeds decreased from the first month through the sixth month. The lowest value in the laboratory germination was 48 percent while the lowest value in the greenhouse was 27.5 percent. Comparison between the results of the laboratory and greenhouse tests showed slightly higher values for the laboratory germination.

Under both hot-dry and cold-wet conditions, weevils were not able to continue their life, dying soon after being placed in storage. Therefore, the insect effect on seed viability cannot be evaluated under these conditions.

The results point out that high-quality seed without any treatment may remain fully viable under temperate and hot-dry conditions, but viability decreases in hot-wet and cold-wet conditions. Therefore, the relative humidity and temperature of storage conditions plays an important role in maintaining seed viability. While the germination capacity of wheat stored in hot-wet condition was completely lost in nine weeks, there was a much slower reduction in viability of seed stored under cold-wet condition up to five months.

The effect of insect infestation on seed viability cannot be considered under cold-wet and hot-dry conditions because in both instances none of the weevils were found alive. However, in hot-wet and temperate conditions weevils continued their life and caused considerable reduction in viability of wheat seed.

The deleterious effect of non-visible mechanical damage on maintenance of viability of grain was noted under all storage conditions. However, the rate of this reduction was closely related to the temperature and relative humidity of the storage condition and the growth of fungi during the storage period.

Changes in Percent Abnormal Seedlings

Figure 6 shows the changes in percent abnormal seedlings for the laboratory germination of seeds which had different treatments and stored under hot-wet and temperate conditions for various length of time. The percent abnormal seedlings is expressed as the percent

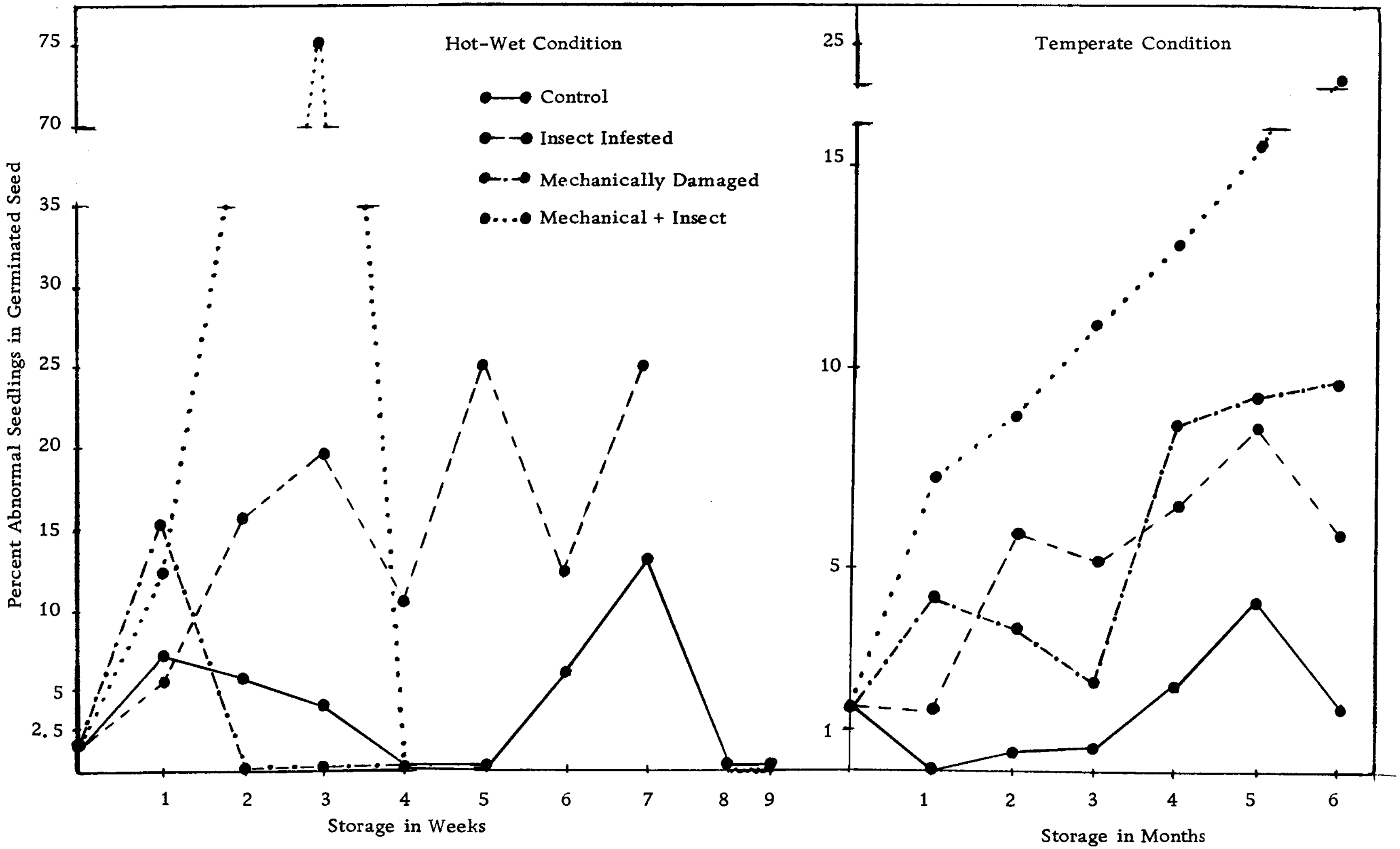


Figure 6. Changes in the percent abnormal seedlings in the laboratory germination of wheat seed subjected to various treatments and stored under the hot-wet and the temperate conditions.

of germinated seeds. The seed originally had 1.5 percent abnormal seedlings prior to the treatment and storage. Under the hot-wet condition, after one week's storage, all the samples showed an increase in abnormal seedlings. In the following weeks, the number of abnormal seedlings from insect infested-mechanically damaged materials showed a maximum percent of 75 after three week's storage, then a disappearance of abnormal seedlings with rapid deterioration of the seed at the end of the fourth week in storage. The percent of abnormal seedlings in the control samples varied from time to time with the maximum amount of abnormal seedlings obtained after seven weeks storage. This was followed by a decline in abnormality accompanying seed deterioration. The percent of abnormal seedlings in samples stored under temperate condition showed an increase with time. There was a fluctuation from sample to sample, except for mechanically damaged-insect infested samples, which increased steadily to 24 percent after six month's storage. It is clearly shown that under the hot-wet condition, the rate of increase in percent of abnormal seedlings and the amount of abnormal seedlings prior to death were higher than under the temperate condition.

Changes in number of abnormal seedlings in the laboratory germination tests on wheat seed which had different treatment and stored under the hot-dry and the cold-wet conditions are given in Figure 7. Under these conditions abnormalities caused by insect infestation

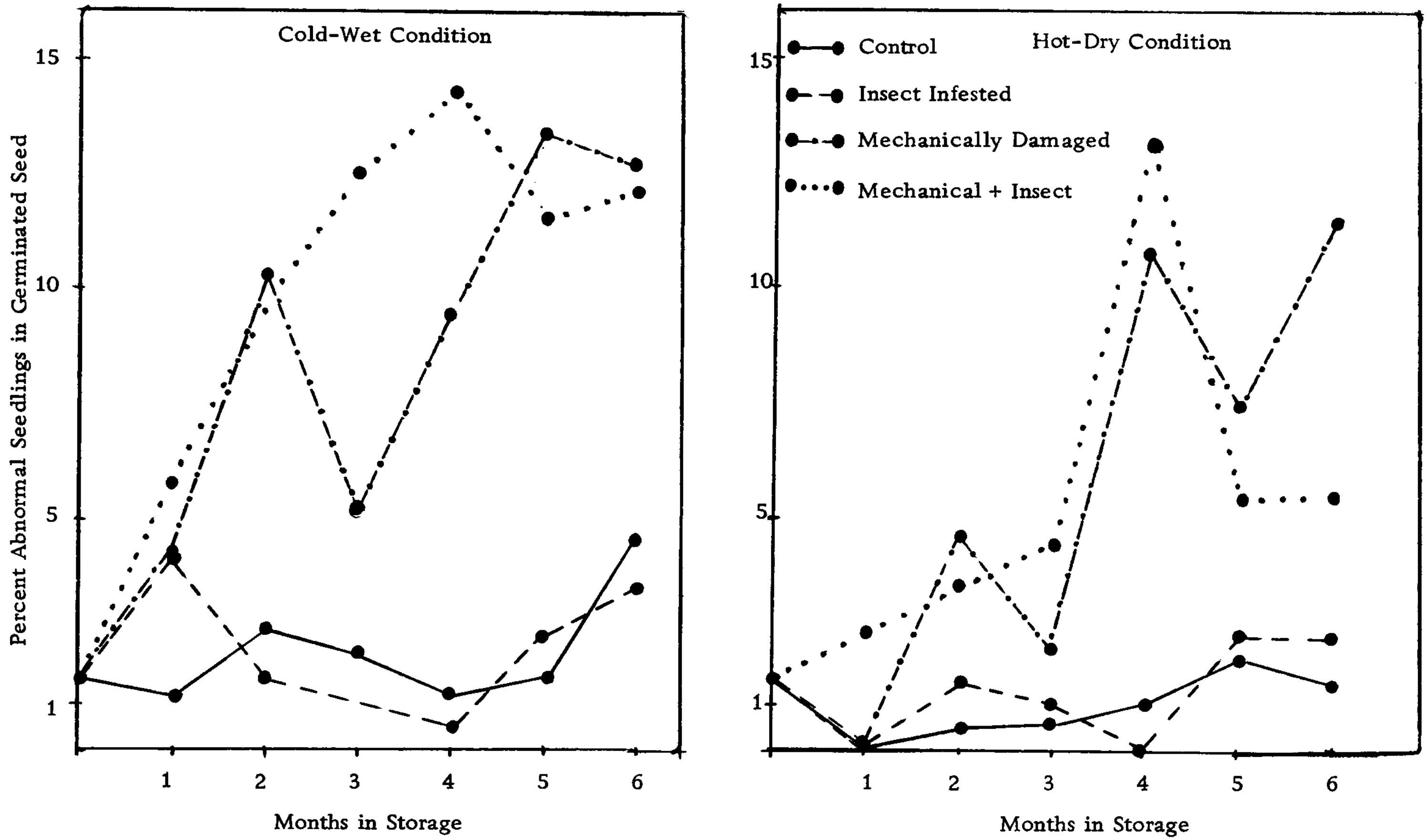


Figure 7. Changes in the percent abnormal seedlings in the laboratory germination of wheat seed subjected to various treatments and stored under the hot-dry and the cold-wet conditions for six months.

were not expected because of the low temperature or the low humidity which interferred with weevil activity. The highest percent of abnormal seedlings was observed in the mechanically injured samples. The rate of increase in abnormal seedlings from cold-wet samples was higher than for the samples stored under the hot-dry condition. The largest number of abnormal seedlings was counted from the fourth month sampling of the hot-dry and cold-wet storage conditions. The values were 13. 18 percent and 14. 28 percent respectively.

In addition to recording the percent of abnormal seedlings in the germination tests, the kinds of abnormalities were also evaluated. Each treatment showed the same types of abnormality, such as: seedlings without shoots or roots (neither primary nor secondary), seedlings with longitudinally split coleoptile or with colorless coleoptiles but no green leaves within, the seedlings with decayed shoots. In most cases mechanically damaged samples gave seedlings which were without well-developed primary roots. However, some of these seedlings were able to develop strong secondary roots in due time. It was observed that in the mechanically injured samples, the amount of rootless seedlings was higher than the amount of shootless seedlings.

Under adverse conditions the rate of increase of abnormal seedlings was very rapid with the maximum number of abnormal seedlings observed prior to complete loss of seed viability.

Mechanical damage and insect injury often resulted in the same type of seedling abnormality. This made it difficult to discern the relationship between cause and effect in the formation of abnormal seedlings in germination tests.

Change in Seedling Vigor

The shoot length of seedlings was measured as an index of seedling vigor; the six-day old seedling used for the laboratory germination test and the 14-day old seedling for the greenhouse tests. The average shoot lengths from all the samples are summarized in Tables 1 and 2 for the laboratory and germination tests respectively. The fluctuation in growth rate among different samplings is clearly shown by these results. This fluctuation could be attributed to the environmental and cultural effects, e. g. moisture availability, planting depth, temperature, light, etc. However, a trend in reduction of vigor with storage time is shown for all the treatments stored under the hot-wet condition. In general, diminution of vigor was observed in mechanically damaged and insect infested samples stored under all conditions.

The Changes in External and Internal Damages

The percents of change in external damage and internal embryo damage revealed by the TZ test in wheat seed are presented in

Table 1. Average shoot length (cm) of seedlings grown in the laboratory from seeds with or without mechanical damage and insect infestation stored under four conditions for various lengths of time.

Condition	Treatment	1	2	3	4	5	6	7	8	9
					Weeks in storage					
HOT-WET	NO	3.5	5.2	6.3	4.0	7.2	2.4	2.9	4.5	0.6
	NI	3.8	4.8	5.6	3.7	6.5	1.3	2.1		
	MO	3.5	1.6	2.8	0.0					
	MI	3.4	1.3	0.7	0.5					
					Months in storage					
COLD-WET	NO	6.1	3.6	7.2	5.6	6.6	6.1			
	NI	4.9	3.9	7.4	5.2	6.4	5.8			
	MO	5.4	2.4	6.3	3.7	4.4	4.9			
	MI	5.2	2.7	5.2	3.1	4.6	4.8			
HOT-DRY	NO	5.2	3.5	6.9	5.2	7.0	6.4			
	NI	4.6	4.0	7.4	5.8	6.6	6.7			
	MO	4.4	3.2	5.7	3.9	5.2	4.4			
	MI	4.2	3.4	4.4	3.6	4.5	4.4			
TEMPERATE	NO	6.0	4.0	7.6	4.9	6.2	5.5			
	NI	4.2	3.7	7.7	4.9	6.2	4.8			
	MO	5.2	3.2	5.8	3.6	3.9	4.4			
	MI	3.5	3.1	3.9	4.0	4.3	3.7			

NO = Control
NI = Insect infested
MO = Mechanically damaged
MI = Damaged and infested

Table 2. Average shoot length (cm) of seedlings grown in the greenhouse from seeds with or without mechanical damage and insect infestation stored under four conditions for various lengths of time.

Conditions	Treatment	1	2	3	4	5	6	7	8	9
					Weeks of storage					
HOT-WET	NO	12.0	14.2	17.5	17.3	13.9	14.8	14.4	13.9	4
	NI	12.94	13.6	17.3	14.1	10.2	7.5	9.4		
	MO	10.1	3.6	9.8	1.9					
	MI	11.3	8.6	6.2	0.0					
					Months of storage					
COLD-WET	NO	17.2	17.1	17.3	17.9	17.9	15.1			
	NI	18.4	17.0	17.1	18.0	17.3	9.5			
	MO	15.6	14.4	15.1	16.5	15.0	7.1			
	MI	14.9	14.4	14.5	15.8	15.9	10.0			
HOT-DRY	NO	18.9	17.3	17.5	19.2	21.0	14.0			
	NI	17.9	17.1	16.6	18.8	21.5	13.8			
	MO	14.8	14.4	14.7	17.1	17.8	10.0			
	MI	15.1	15.1	14.1	16.9	17.9	9.6			
TEMPERATE	NO	17.6	16.7	16.9	18.0	17.2	13.1			
	NI	16.6	16.0	15.7	16.9	17.1	11.4			
	MO	15.4	13.2	13.0	14.0	15.1	10.0			
	MI	14.6	13.5	13.9	12.5	14.5	6.9			

NO = Control MO = Mechanically damaged
NI = Insect infested MI = Damaged and infested

Figures 8, 9, 10 and 11. The visibly mechanically damaged seed was eliminated by hand separation before storage; therefore, external injuries were examined only in the seed which were subjected to insect attack. Seed with seed coat injuries and/or missing parts were counted as externally injuried.

As the results indicate (Figures 8 and 9), the external damage to seed caused by weevils was substantial. The percent of external damages was higher in seed which was mechanically damaged and insect infested. It seems that the weevils attacked and worked with ease on the mechanically damaged seed and caused much more external damage.

As was previously mentioned, cold-wet and hot-dry conditions were not conducive to survival of the weevils. Therefore, the percent of external damages caused by weevils was negligible as shown in Figures 10 and 11.

In Plates 2 and 3, the internal embryo damage is shown by the embryo with missing parts, tissue distortion and cracks. The wheat initially had 5.5 percent internal damages to the embryo as revealed by TZ test before storage. As the external damages increased in the insect infested samples from hot-wet and temperate conditions, the percent of internal damages also increased, but at a slower rate. After two months under the temperate condition the weevils emerged and the rate of damage increased. Under the hot-wet and temperate

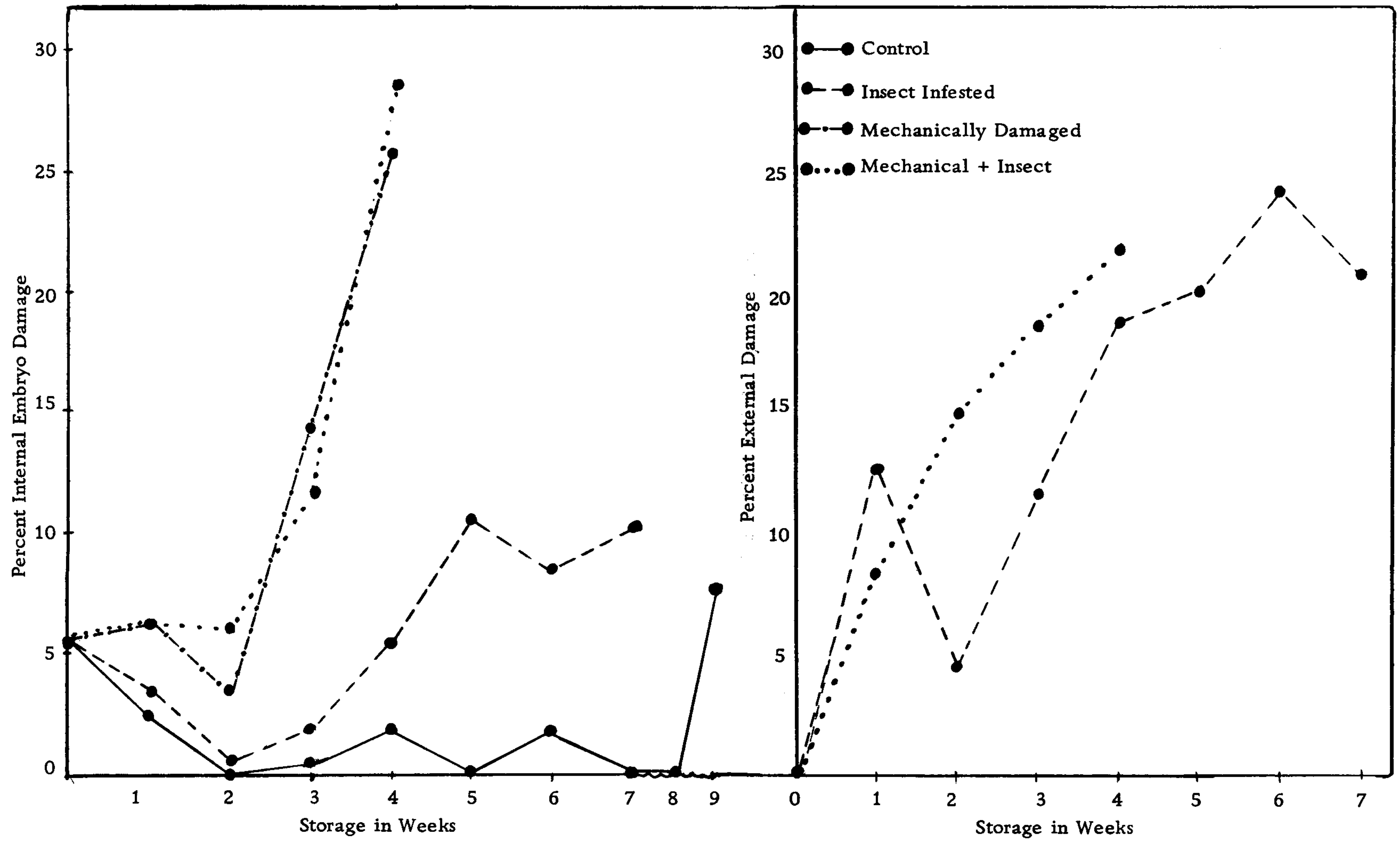


Figure 8. Changes in internal embryo damages revealed by TZ and external damages to wheat seed subjected to various treatments stored under the hot-wet condition.

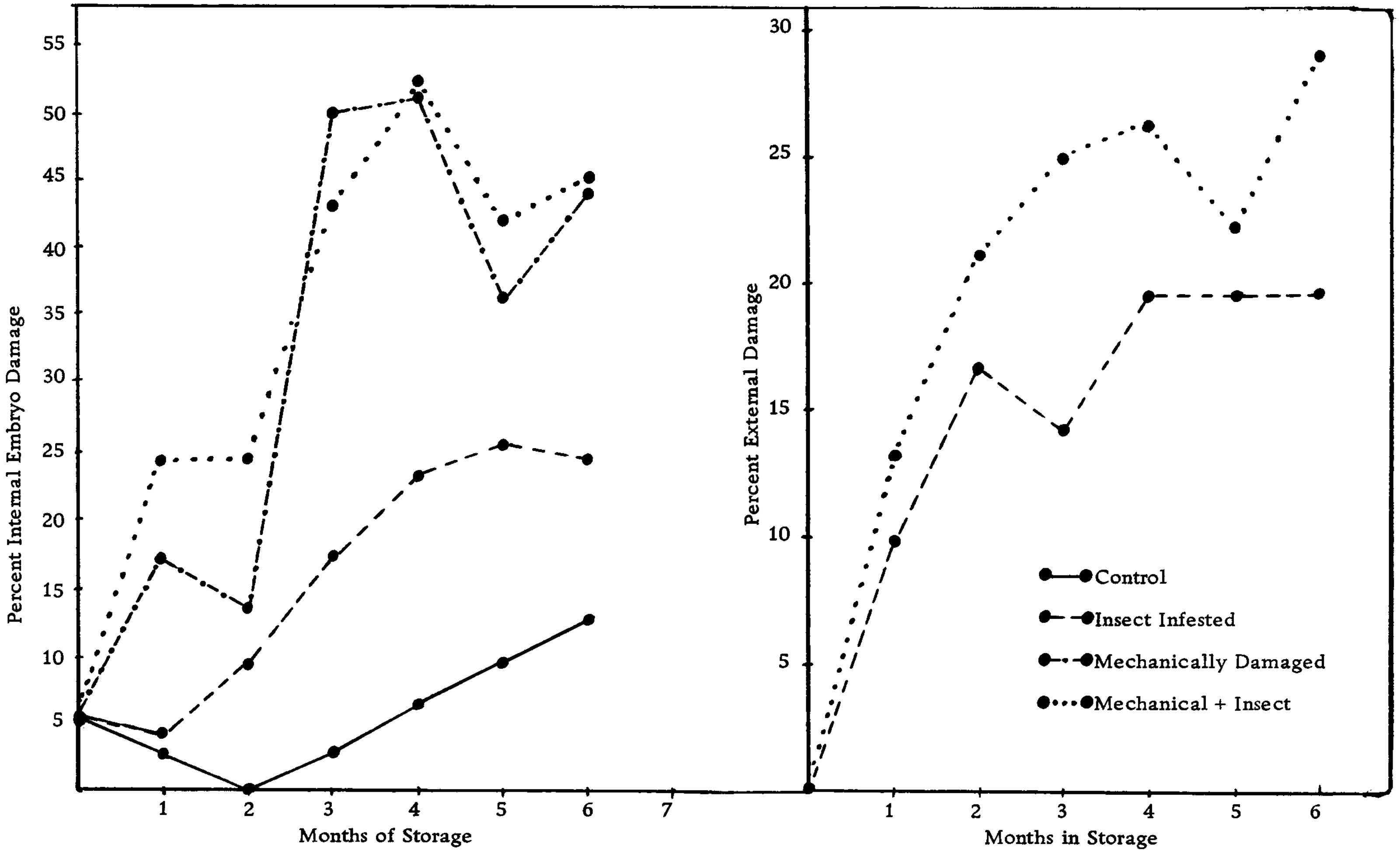


Figure 9. Changes in internal embryo damages revealed by TZ and external damages to wheat seed subjected to various treatments stored under the temperate conditions.

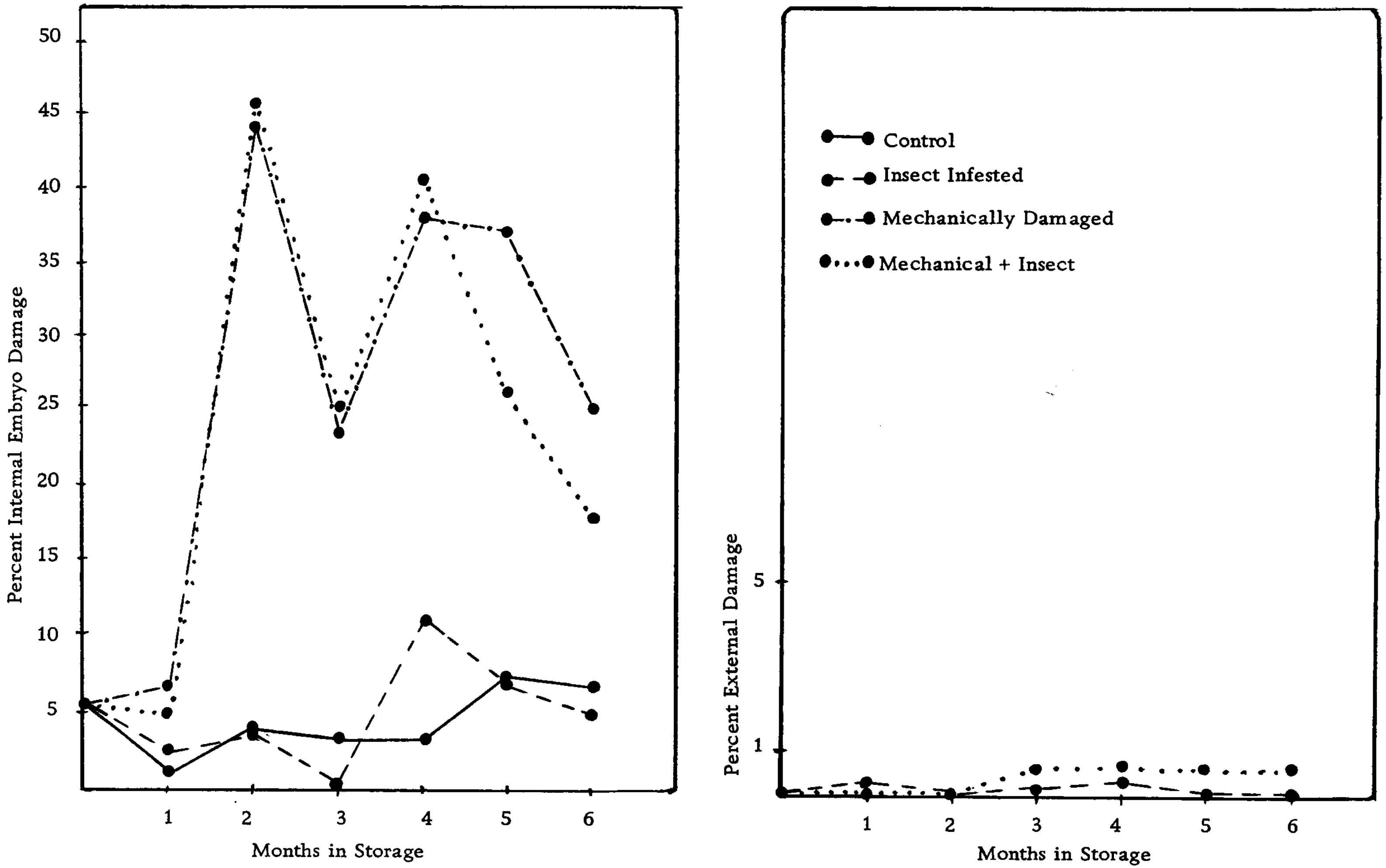


Figure 10. Changes in internal embryo damages revealed by TZ and external damages to wheat seed subjected to various treatments stored under the hot-dry condition.

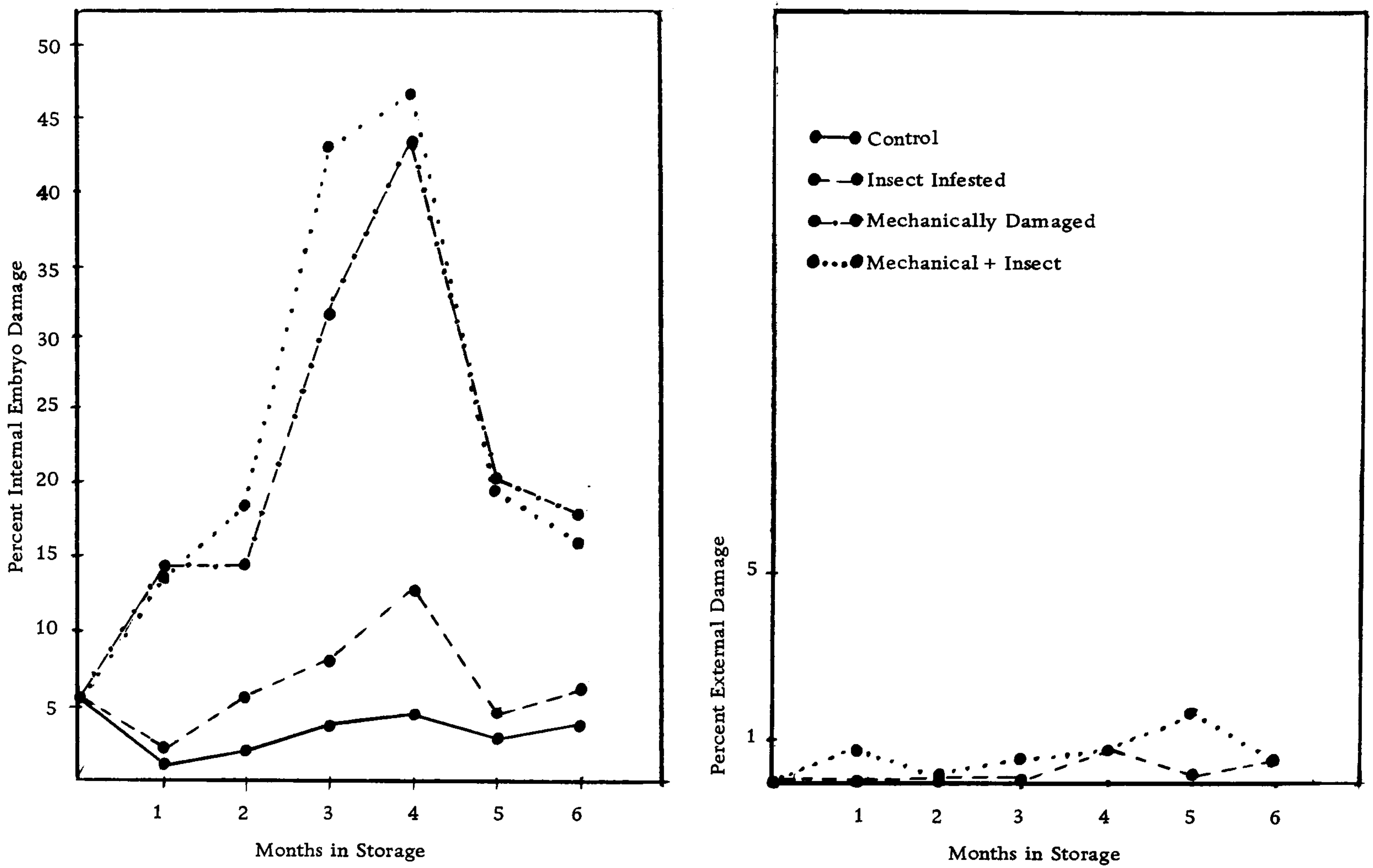


Figure 11. Changes in internal embryo damages revealed by TZ and external damages to wheat seed subjected to various treatments stored under the cold-wet condition.

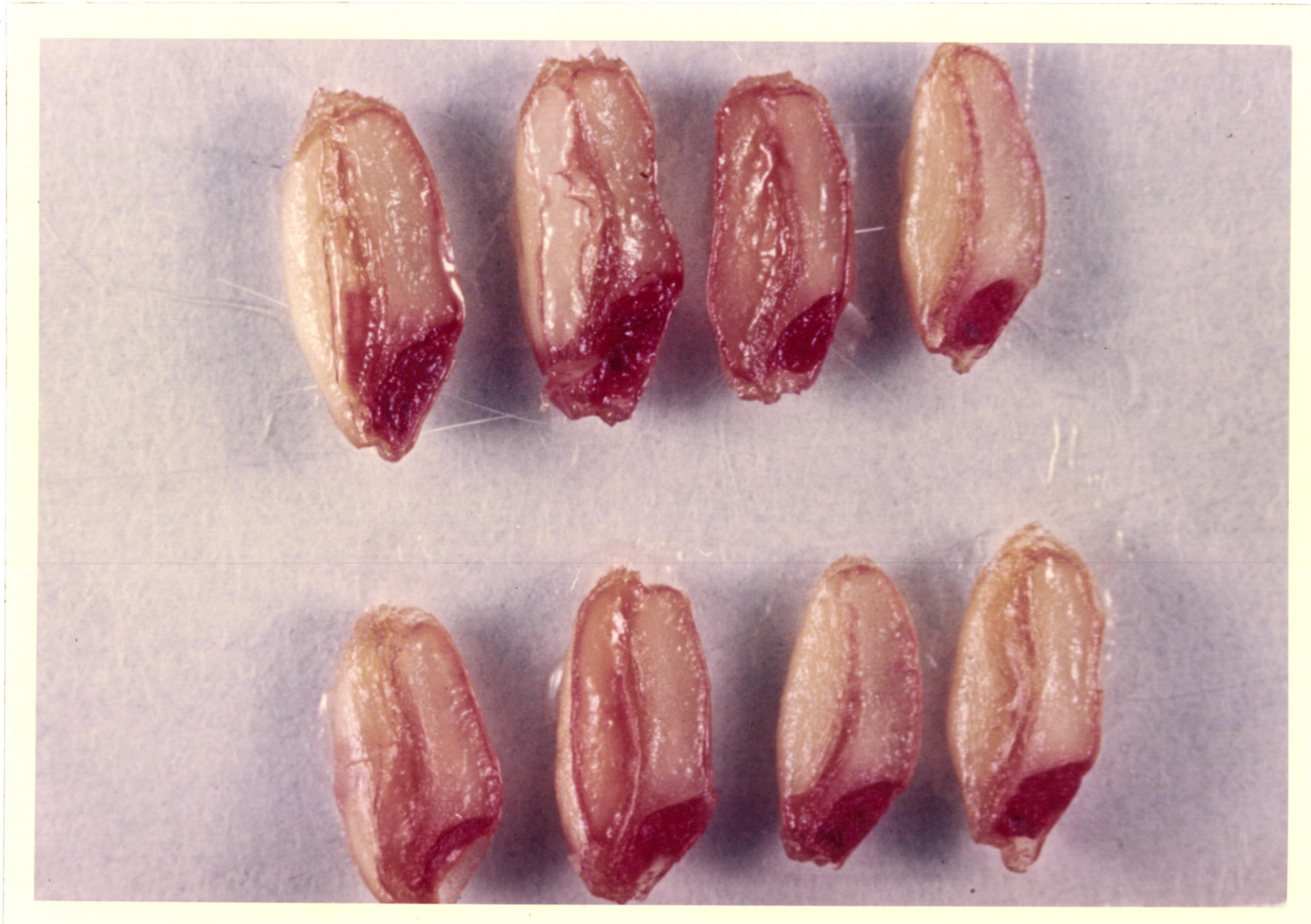


Plate 2. TZ test results for a normal seed (upper left) and seeds with mechanical damage.

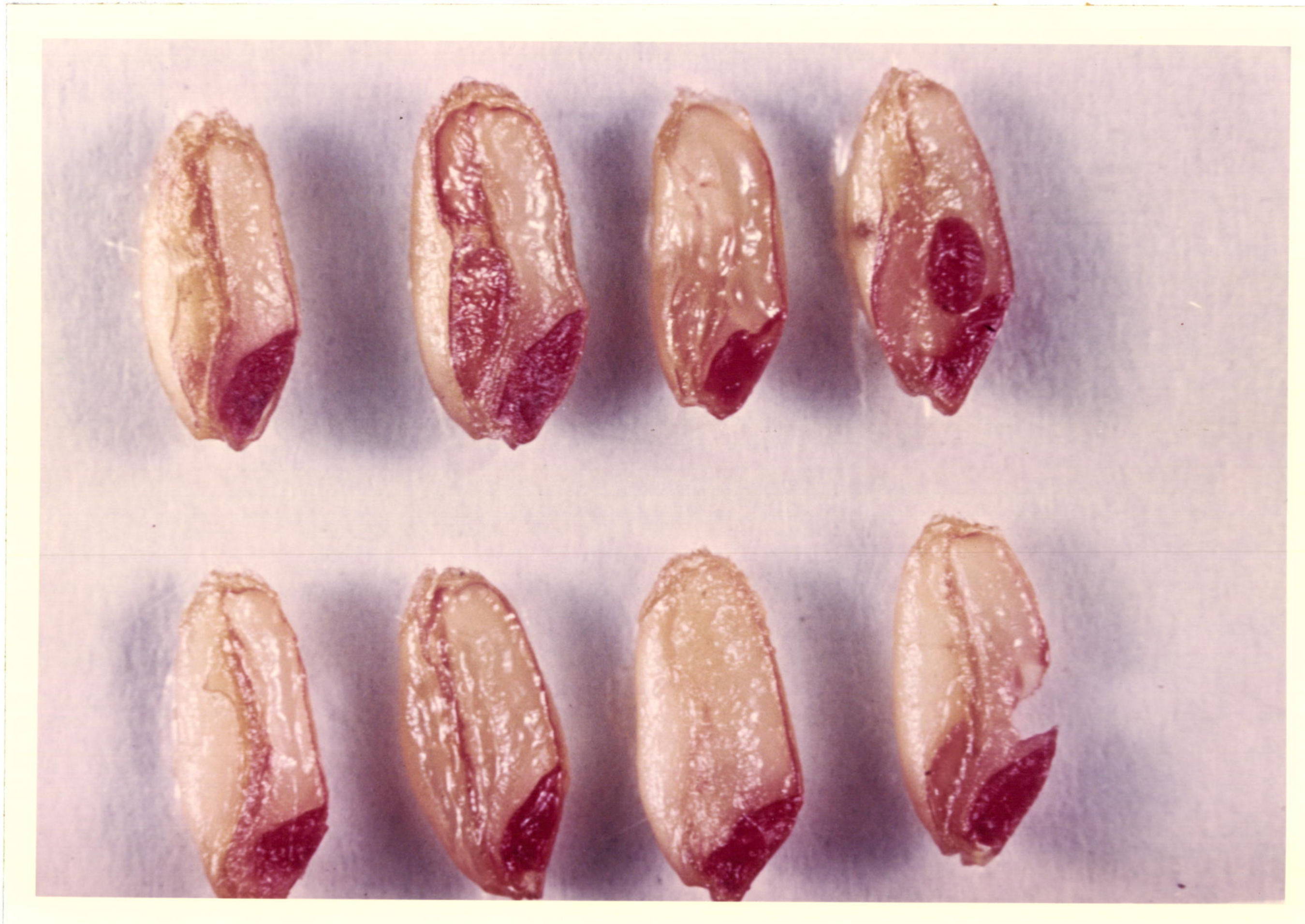


Plate 3. TZ test results for a normal seed (upper left) and seeds with weevil injury.

conditions the results of internal damage tests for mechanically damaged and mechanically damaged-insect infested samples followed each other closely. This probably indicates that the internal embryo damages due to the weevil activity were negligible when the samples was already damaged.

Under each condition, mechanical injury resulted in a large amount of internal embryo damages as expected. Unfavorable storage conditions (high relative humidity) had an accelerating effect on the rate of increase in internal injury. Under the temperate condition, internal embryo injury due to mechanical damages increased gradually with time. In the cold-wet condition, after four months storage, there was an unexplainable decrease in the injury both for the mechanically damaged and mechanically damaged-insect infested samples. Under the hot-dry condition, an increase in the internal embryo damages appeared after two months storage.

Control samples originally containing five percent internal damages showed a slight increase in internal damage under hot-wet and temperate conditions.

It may be concluded that the high speed of the threshing machine caused a considerable amount of internal embryo damage which could only be revealed by sectioning the seed longitudinally through the embryo and applying appropriate tests. The percent internal damage for samples from the hot-wet condition was the lowest of all.

This indicates that a prolonged storage accentuates internal embryo damages.

The Correlation of the TZ Test Results with Germination Tests

The relationship between germination capacity as determined by laboratory and greenhouse tests and the percentage of viability by TZ tests for wheat seed with different treatments stored under four conditions are shown in the scatter diagrams (1, 2, 3, 4, 5, 6, 7, 8). Regression equations for percent germination (Y) and percent viability determined by the TZ (X) were calculated and correlation coefficients were determined for each condition and for each treatments are given below.

	Regression Equation		Correlation Coefficient (r)		
Conditions	Laboratory	Greenhouse	Laboratory	Greenhouse	d.f.
Hot-Wet	Y = -0.185 + 0.892X	Y = -0.069 + 0.841X	+0.990	+0.980	22
Temperate	Y = 18.135 + 0.829X	Y = 0.300 + 0.968X	+0.886	+0.888	22
Hot-Dry	Y = 34.507 + 0.646X	Y = 26.949 + 0.704X	+0.732	+0.838	22
Cold-Wet	Y = 3.877 + 0.931X	Y = -7.555 + 1.046X	+0.935	+0.949	22

Treatments	Regression Equation Laboratory	Regression Equation Greenhouse	Correlation Coefficient(r) Laboratory	Correlation Coefficient(r) Greenhouse	d. f.
Control	Y = -1.359+(1.01) X	Y = -1.476+(0.981)X	r = +0.993	r = +0.986	25
Insect	Y = -2.452+(1.046)X	Y = -3.358+(1.037)X	r = +0.991	r = +0.994	23
Mechanical damage	Y = -0.905+(1.053)X	Y = -4.635+(0.994)X	r = +0.962	r = +0.961	20
Mechanical damage-insect infestation	Y = 1.672+(0.977)X	Y = 0.826+(0.971)X	r = +0.970	r = +0.982	20
		Significant level of r =	5 percent	1 percent	
		DF = 20	0.423	0.537	
		DF = 25	0.381	0.487	

In all cases, significant positive correlations existed between percent germination and the TZ tests at the one percent level. Slightly higher correlations were obtained between the results of greenhouse germination and TZ tests under cold-wet, temperate and hot-dry conditions. However for seed stored under hot-wet conditions an inverse relationship was observed. The fact that correlation coefficient values varied with different conditions indicates that the storage conditions affect the TZ reaction differently from the overall growth of the seed. However, the different treatments applied to seed, e.g. mechanical damage or insect infestation, did not cause additional influences on the relationship of results obtained from germination and TZ tests. This can easily be seen from the correlation coefficient values above, in which there is no significant difference between the correlation coefficient values for different treatments.

The regression equations listed in the table above would be used in actual seed testing if the history of seed lots were known. For the seed lots with unknown history, as is the case for seed testing a regression equation could be calculated in the same manner based on a large number of samples.

Careful standardization of each step in the TZ test might be helpful in establishing the optimum conditions for TZ reaction. By doing so the actual viability will be revealed by the staining techniques. However, any standardized method of TZ test will not give a correlation coefficient of 1 with germination test, as biological variation is an accepted fact. If a regression equation on the basis of a large sample size could be developed the rapid TZ test will be more useful for seed testing than at present.

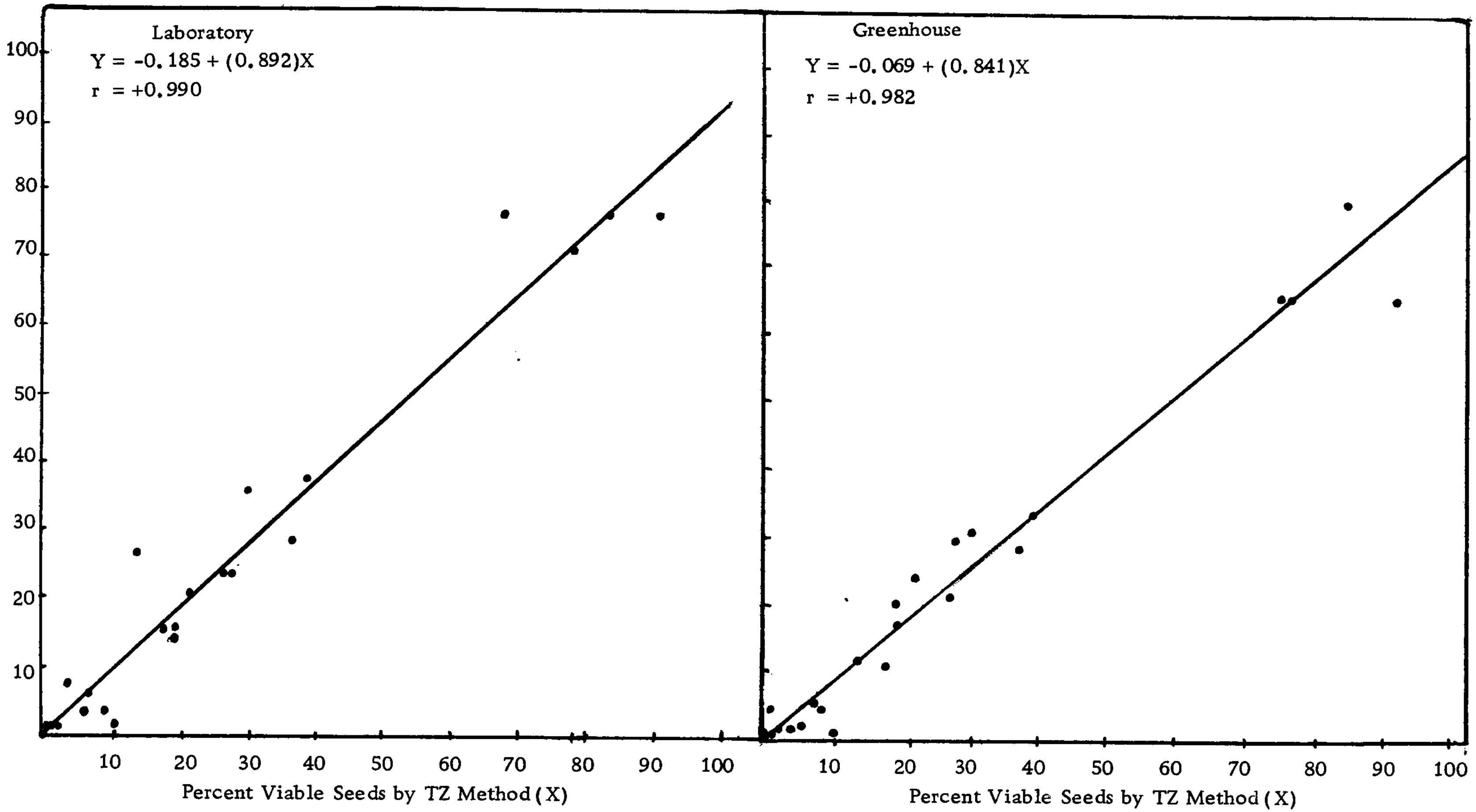


Figure 12. Scatter diagram (1) showing the relation between germinating capacity (Y) determined by laboratory and greenhouse tests and percentage viability by TZ (X) for wheat seed with different treatments stored under the hot-wet condition.

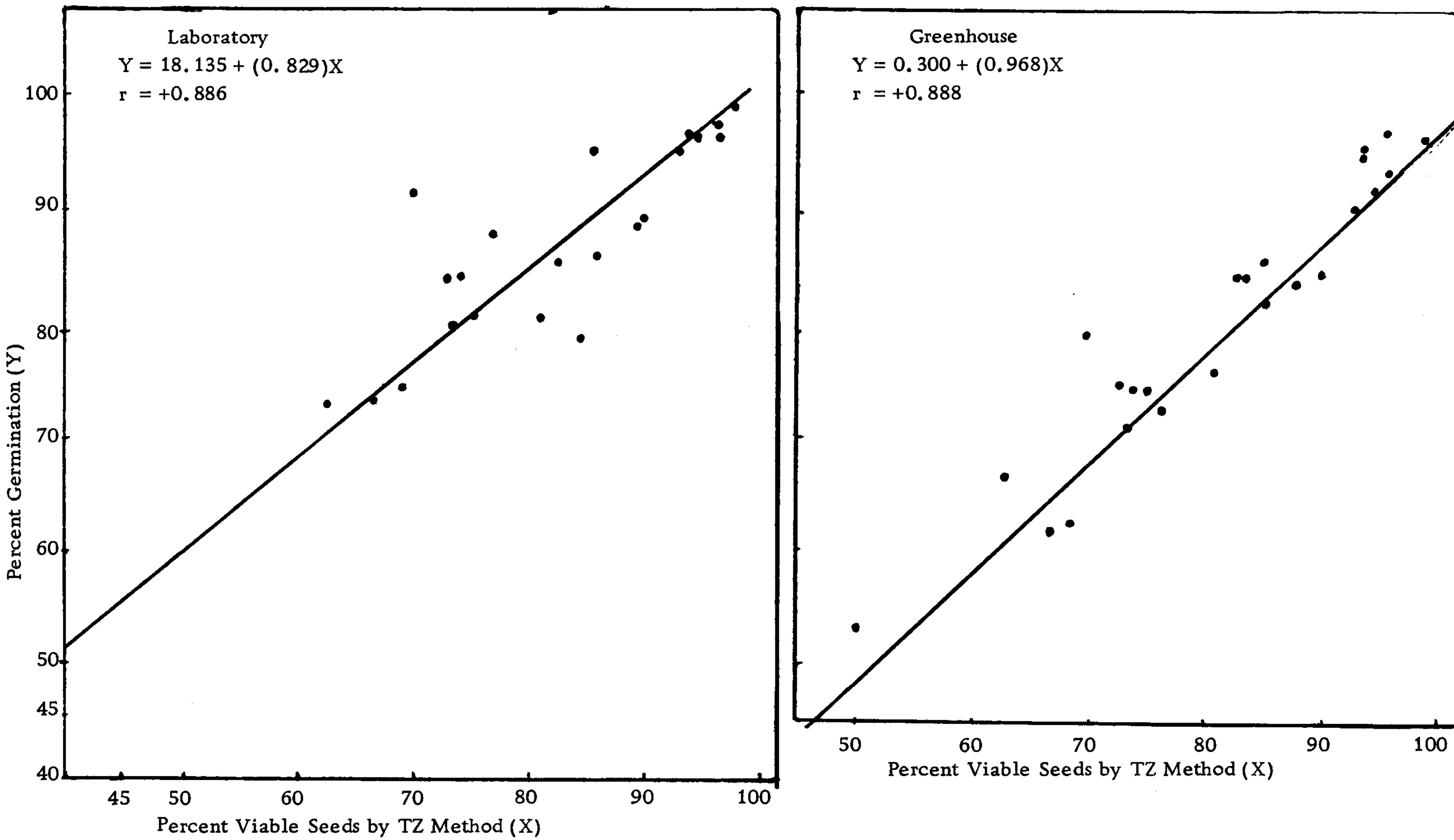


Figure 13. Scatter diagram (2) showing the relation between germinating capacity (Y) determined by laboratory and greenhouse tests and percentage viability (X) by TZ for wheat seed with different treatments stored under the temperate condition.

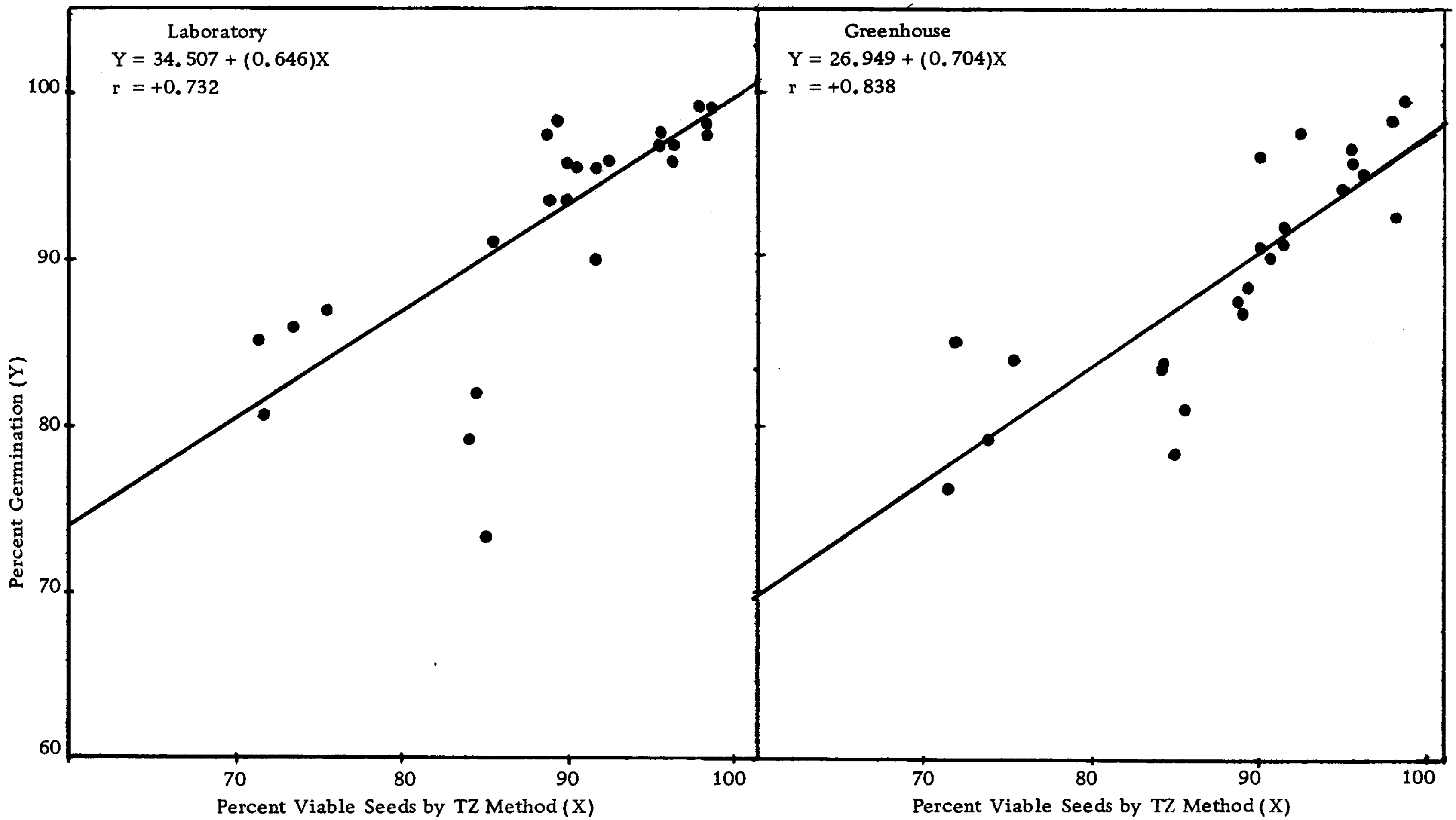


Figure 14. Scatter diagram (3) showing the relation between germinating capacity determined by laboratory and greenhouse tests and percentage viability by TZ for wheat seed with different treatments stored under the hot-dry condition.

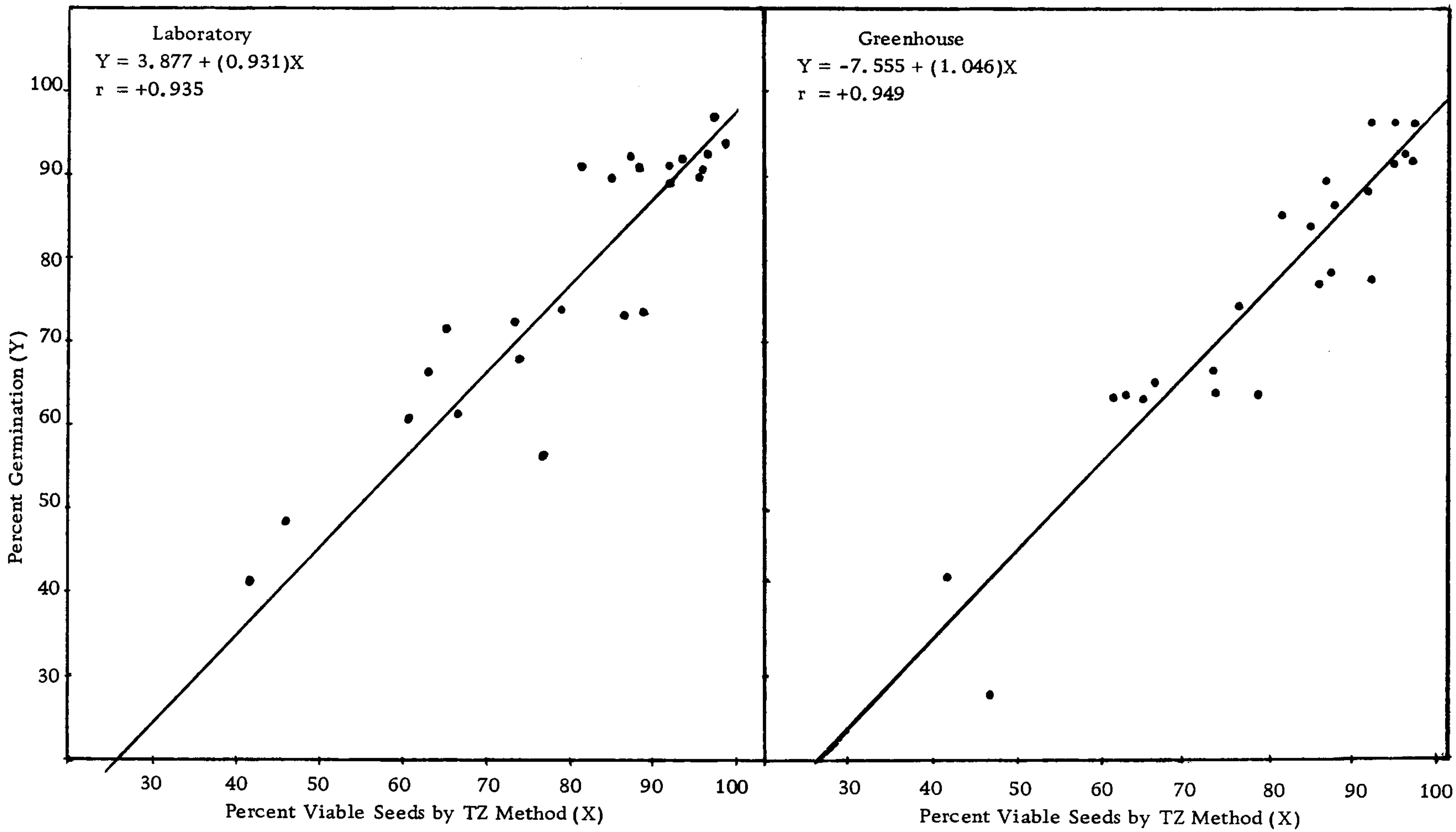


Figure 15. Scatter diagram (4) showing the relation between germinating capacity (Y) determined by laboratory and greenhouse tests and percentage viability (X) by TZ, for wheat seed with different treatments stored under the cold-wet condition.

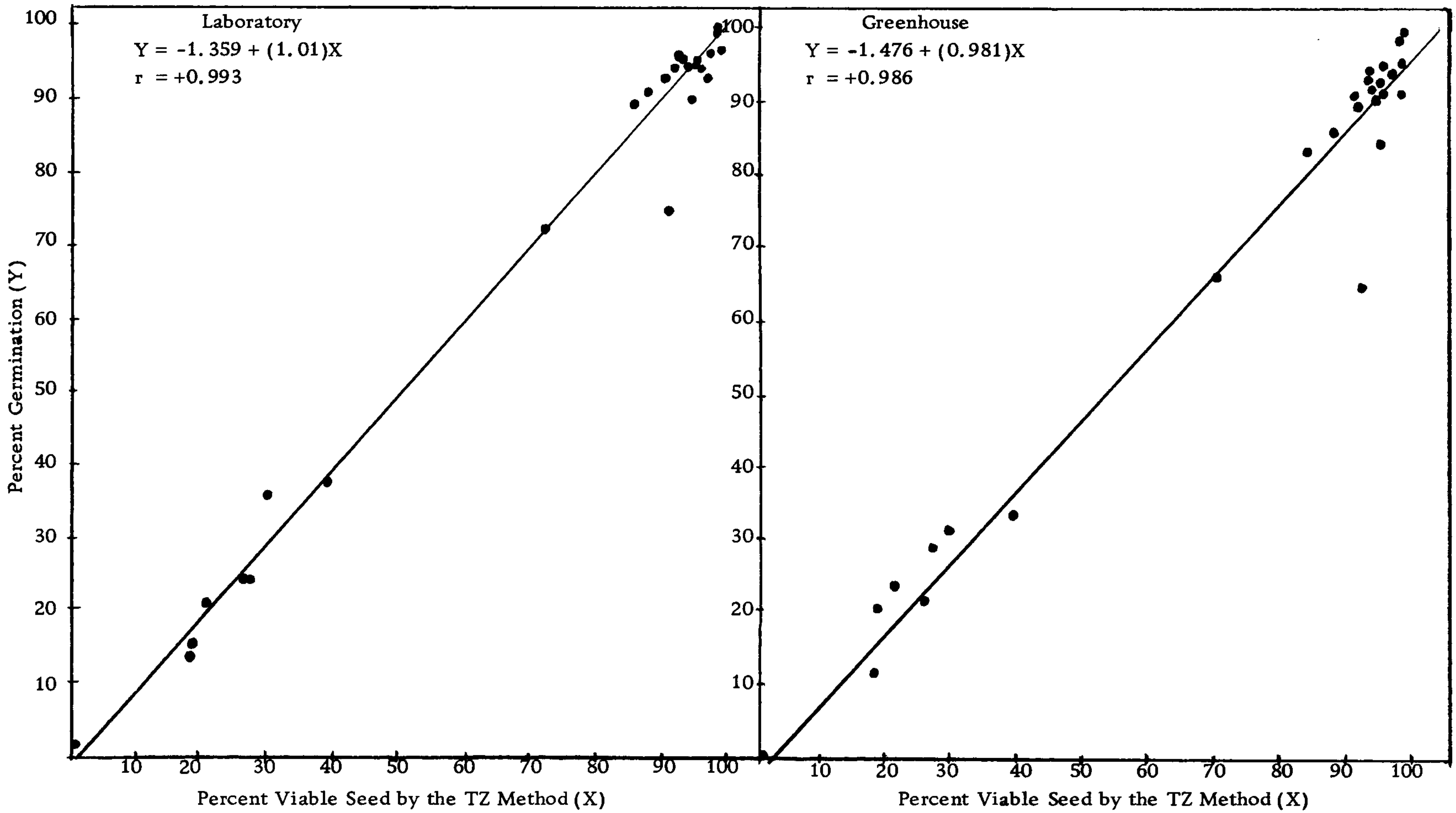


Figure 16. Scatter diagram (5) showing the relation between germination capacity (Y) determined by laboratory and greenhouse tests and percentage viability (X) by TZ for wheat seed stored under four conditions without treatment of any kind.

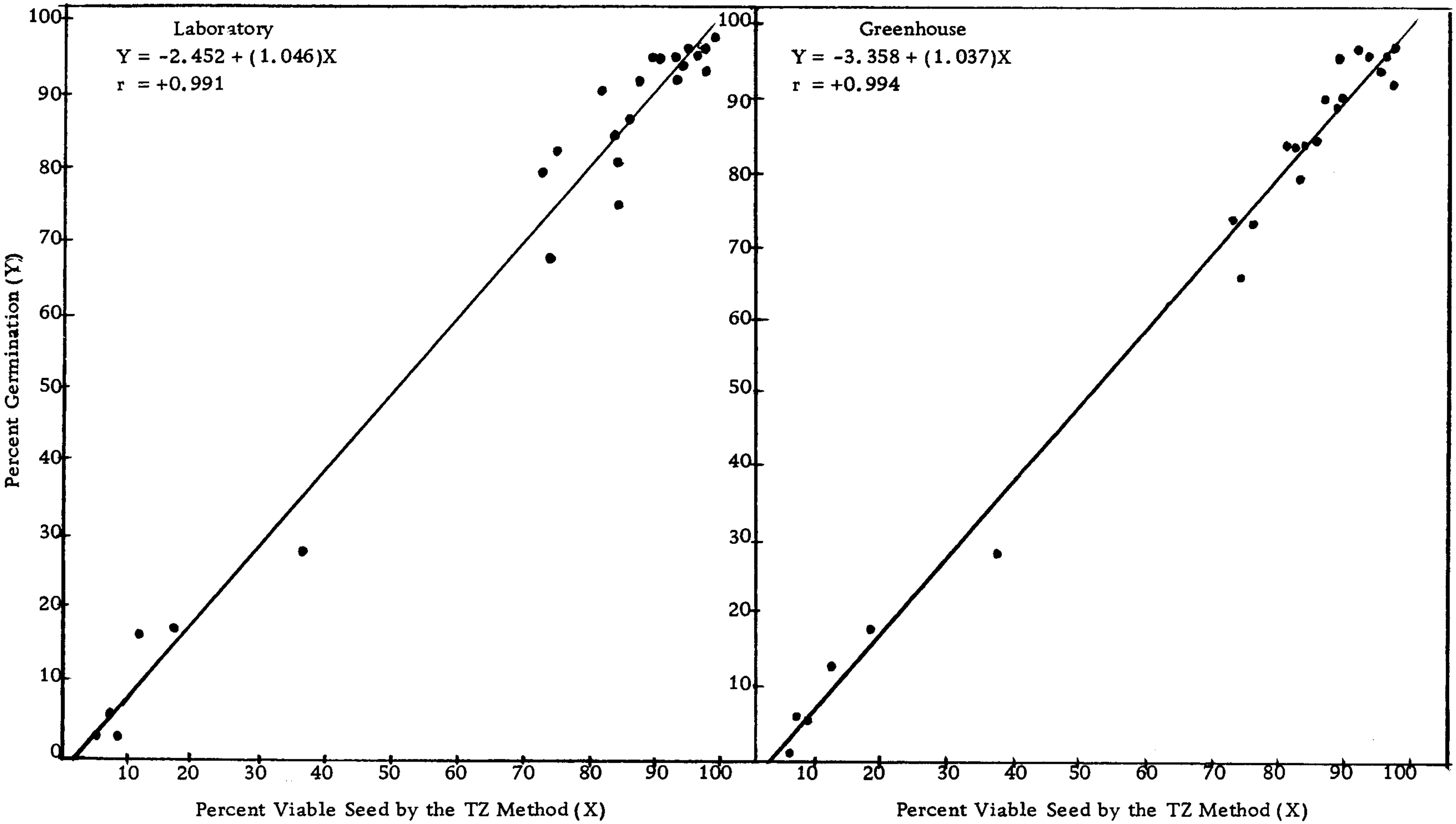


Figure 17. Scatter diagram (6) showing the relation between germination capacity (Y) determined by laboratory and greenhouse tests and percentage viability (X) by TZ for wheat seed subjected to insect infestation treatment stored under four conditions.

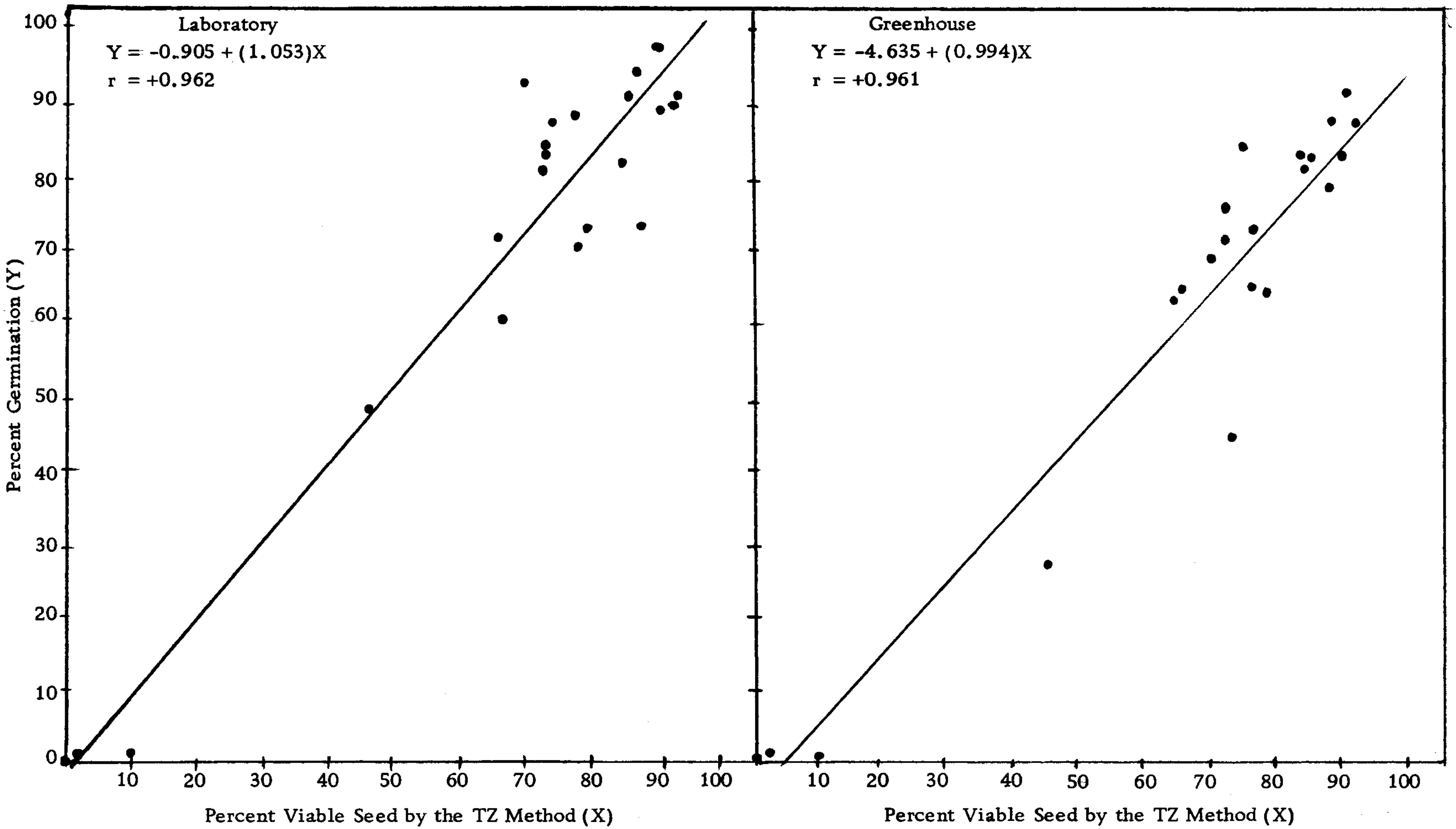


Figure 18. Scatter diagram (7) showing the relation between germination capacity (Y) determined by laboratory and greenhouse tests and percentage viability (X) by TZ for wheat seed subjected to mechanical damage stored under four conditions.

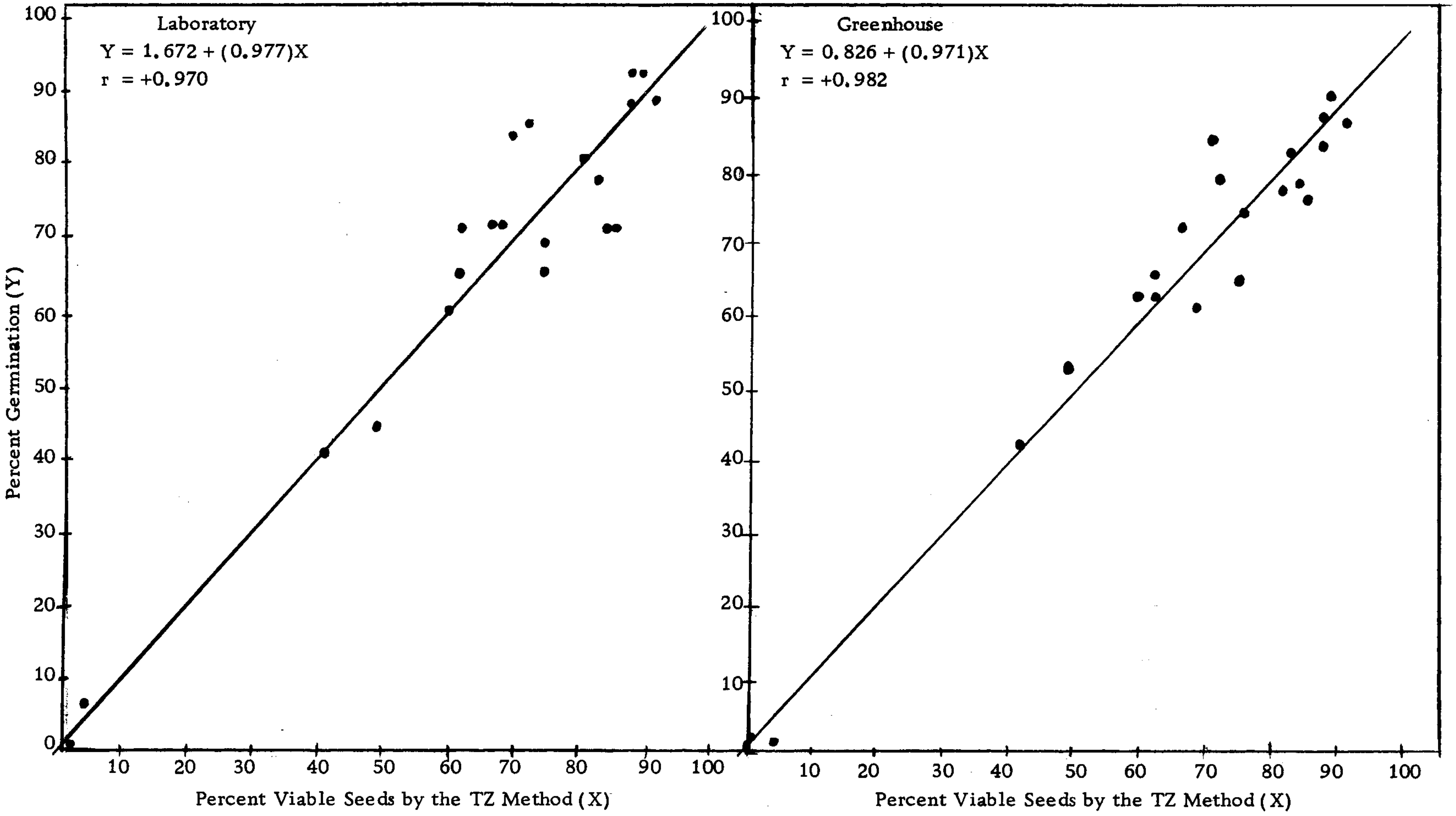


Figure 19. Scatter diagram (8) showing the relation between germinating capacity (Y) determined by laboratory and greenhouse tests and percentage viability (X) by TZ for wheat seed subjected to the mechanical damage-insect infestation treatment stored under four conditions.

SUMMARY AND CONCLUSIONS

The effects of mechanical damage, insect infestation and storage conditions on the viability of spring wheat (variety Malfed-Merit Sel. 28) with a germination capacity of 95.5 percent and 9.21 percent moisture content were studied.

The seed lot was divided into two portions, one of which was subjected to the treatment of mechanical damage and the other was untreated. After externally-damaged seed was eliminated by hand, 60 bags of seed samples each containing 40 grams of wheat were prepared for each treatment. One-half of the treated and untreated seed samples was inoculated with granary weevils. The seeds were then stored under four different conditions (hot-wet, temperate, cold-wet, hot-dry). Samples were taken at weekly intervals from the hot-wet condition until complete loss in viability was observed. For the other conditions, the experiment was conducted for six months and samples were taken monthly.

For each sample externally damaged seeds caused by weevils during storage were counted and percent moisture content was determined. The germination capacity of seeds was measured by three tests: standard laboratory germination, greenhouse growth tests and tetrazolium tests. The standard germination and growth test results were correlated with TZ tests to determine the usefulness of TZ tests.

Internal embryo damages as revealed in TZ test were also recorded. In the laboratory germination test the total germination percent, kind of abnormal seedlings and the shoot length of six-day-old seedlings were observed. In the greenhouse test, the total germination and the shoot length from 14 day old seedlings were recorded.

The results of this study may be summarized as follows:

1. The seed moisture content changed according to the relative humidity of storage atmosphere until an equilibrium was reached between the moisture content of the seed and the relative humidity of the storage atmosphere in each condition. However, a slight continuous increase in moisture content occurred under the cold-wet and temperate conditions. In general the mechanical injury and insect infestation did not increase the moisture content of seed further after the equilibrium was reached under all the conditions.

2. The wheat without treatment remained fully viable under temperate and hot-dry conditions for six months but not in either cold-wet or hot-wet conditions. The rate of reduction in viability was very rapid under the hot-wet condition. Therefore the relative humidity and temperature of these conditions played an important role in maintaining seed viability. In addition, the grain which was contaminated with weevils or mechanically damaged showed a very rapid decline in viability with time.

3. The rate of increase of abnormal seedlings was very rapid under

adverse storage conditions, and the maximum number of abnormal seedlings was observed prior to complete loss in viability of the seed. From every condition the highest percent of abnormal seedlings was found in the mechanically damaged samples. Mechanical damage and insect injuries often caused the same type of abnormalities in germinated seedlings. This made it difficult to discern possible relationships of cause and effect in the formation of abnormal seedlings in germination tests. It was observed that in mechanically injured samples the amount of rootless seedlings was higher than the amount of shootless seedlings.

4. In general a diminution of vigor was found in mechanically damaged and insect infested samples from each of the conditions (fluctuation of growth rate from sample to sample). There was also a trend toward reduction in vigor with storage time in all the treatments stored under the hot-wet condition.

5. The high speed of the threshing machine caused substantial amount of internal embryo damage. When seed was infested with weevils, internal embryo injuries occurred as well as external damages. However, the degree and the location of this internal injury in the embryo played an important role in the development of damaged seed into normal seedlings. The prolonged storage of six months magnified internal damage. The relative humidity and temperature of the storage further affected the rate of increase in observable internal damages.

6. The laboratory and greenhouse germination results were significantly positively correlated with the TZ tests. The correlation coefficient values varied from 0.732 to 0.994 with different storage conditions and seed treatments. However, there was no significant variations among the correlation coefficient values from different treatments. This indicated that the storage conditions affect the TZ reaction more than seed treatments. Regression equations for predicting germination results from TZ tests were calculated for each storage condition and for each treatment. If a regression equation based on large sample size could be developed and the method of TZ testing could be standardized for each species or variety, the TZ test would be a very useful tool for seed testing.

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