LABORATORY AND FIELD EVALUATIONS OF SEED VIGOR

by

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TABLE OF CONTENTS

INTRODUCTION

The basic method of evaluating agricultural seed is the official laboratory germination by which the maximum potential germination of a seed lot is ascertained. Although germination potential is valuable information, it represents only one aspect of seed quality. In many instances, particularly under unfavorable field conditions, the level of vigor of the live seeds may be as important as the number of live seeds present.

This study, in which alfalfa and brome were employed as experimental material, was concerned with the following problems relating to seed vigor. First, does the official laboratory germination in any way reflect the vigor of seeds? Secondly, for the seed kinds concerned, is the development of supplementary laboratory tests for vigor feasible? Finally, to what extent can fungicide treatment compensate for the lack of vigor?

The specific objectives of the research were: (a) to clarify, for the subject crop kinds, the relationship of laboratory germination with field emergence, (b) to ascertain if this relationship can be interpreted more clearly with germination levels of identical genetical material than with different genetic stocks, (c) to explore the peculiarities of seed germination responses throughout the range of tempera-

tures in which germination is possible, (d) to determine if techniques employing controlled temperature levels can be used to measure the differential ability of seed lots to produce stands in the field, and (e) to ascertain the effect of fungicide treatment on seeds of different germination and vigor levels.

LITERATURE REVIEW

Numerous workers have studied methods for attaining maximum germination of seed in the laboratory. Many students have investigated the effect of specific factors on seed germination. Fewer individuals have been interested in the relationship between laboratory germination and field emergence.

The following includes studies of seed germination, seed vigor, and investigations of factors affecting field stand -- which are pertinent to the present investigation.

Factors Affecting Seed Germination

Several factors which influence seed germination are considered separately in the following discussion. Obviously no single factor operates independently. The mode of treatment is one of convenience.

Temperature

Many investigations concern the determination of temperatures for germinating seeds. The temperatures listed for agricultural and vegetable kinds in the Rules for Testing Seeds published by the Association of Official Seed Analysts (4) may be considered as a summary of much of this work.

Toole and Hollowell (61) tested the effect of different temperatures ranging in 5°C increments from 5° to 35°C on several winter annual species of Trifolium. They found that all the species tested responded similarly in that seedling production increased as temperatures approached 20°C from either direction. Interim responses to temperature were not recorded during the 28 day duration of the tests. Andersen (3) found that higher average germination results were obtained at 15°C than at 20°C with 57 lots of suckling clover (Trifolium dubium) seed. (Suckling clover was not included in Toole and Hollowell's work). In another study, Andersen (2) germinated seeds of red, crimson, subterranean, white and sweet clover, and birdsfoot trefoil and alfalfa at 20°, 15°, 10°C constant temperatures and 10°-20° and 20°-30°C alternating temperatures. She obtained no differential response to the various temperatures used. With specific reference to subterranean clover, her findings are contrary to those of Toole and Hollowell.

Coffman (13) placed seeds of several species in an ice box between blotters at about 0°C. Twenty four per cent of an alfalfa seed lot emerged (the radicle broke the seed coat) at this low temperature. The germination of other seed kinds was lower, but some emergence was obtained from spring wheat, winter rye and radish. Coffman obtained the best results with alfalfa germination in the 58°-63°F and 50°-60°F ranges. He

did not use temperatures approximating the official 68°F (20°C) temperature recommended by the Rules for Testing Seeds (4).

Samples of a lot of Black Eyebrow soybeans were incubated by Edwards (19) on 0.5 per cent agar plates at five temperatures (24.5°, 28.5°, 33.0°, 36.5° and 40.0°C). The number of seeds germinating within a series of 2 hour intervals were recorded until 80 per cent of all the seeds in each test had germinated. A seed was declared germinated when the radicle had just penetrated the seed coat. In the temperature environments of 33.0° and 36.5°C Edwards obtained what he believed to be optimum germination. Within this range (a) the least time was required for the appearance of any given germination percentage, (b) the greatest percentage of seeds germinated during any of the incubation periods tested and (c) the mean incubation time was shortest.

Delouche (15) used temperature of 20°, 25°, 30° and 35°C as well as alternating temperature of 20°-30° and 30°-20°C (first temperature was held for 16 hours and the second for 18) to test the germination response of corn, soybeans and watermelon. In general, as the temperature increased the rapidity of germination also increased. Maximum germination in the minimum time for corn and soybeans was 30° while for watermelon through use of the 20°-30°C combination. The

AOSA[1] rules recommends 20°-30° and 25°C for all three.

Grabe (25) concluded that constant temperatures of 25°, 30°C, or a prechill period (5 days) followed by constant 30°C were the most favorable for bromegrass seed germination. He was unable to germinate bromegrass at 40°, 35°, 5° and 2°C.

Dickson (17) worked with Turkey and Marquis wheat seed planted in soil in metal containers. He used temperatures ranging from 8° to 36°C in 4° intervals. Emergence increased gradually with the rise of soil temperature up to 28°C. Then emergence decreased rapidly associated with a noticeable irregularity in emergence. At temperatures lower than 28°C, emergence was correspondingly slower until at 8°C, 10 to 12 days were needed for emergence. At temperatures above 28°C, shoot growth was favored, whereas root development was favored at lower temperatures.

Moisture

In order to germinate, seeds must take up moisture. Burch and Delouche (8) indicate that kinds of seed vary in moisture content required for germination. Cotton, soybean, castor bean and oat seed had to reach moisture contents of about 52, 50, 32 and 34 per cent respectively for germination

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[1]For convenience purposes the name Association of Official Seed Analysts will be abbreviated as AOSA.

of at least half of the seeds. However, Eslick and Vogel (23) used 5 different soil moisture levels ranging from 22.0 per cent (field capacity for that particular type of soil) down to 7.5 per cent and recorded no effect due to moisture levels for either Lincoln bromegrass or Ladak alfalfa. Presumably, seeds have a considerable capacity to draw moisture from the soil.

Burch and Delouche (8) reported an interaction between temperature and water absorption, indicating that water absorption proceeds more rapidly at higher temperatures. Fayemi (24), working with various small seeded legumes reached similar conclusions.

Dormancy

Whitcomb (62) studied the effect of hard seed in normal and discolored alfalfa seed. If the per cent hard seed was added to the laboratory germination per cent of the normal colored seed, a low correlation coefficient (laboratory to field) of .230 $\pm$ .064, was obtained. However, if the hard seeds were not considered, the correlation coefficient was .493 $\pm$ .051. Hard seed from discolored seed had the opposite effect. Since discolored seed was found to have only about 1/2 the crop producing value as normal seed, this opposite correlation probably means that hard seed content alone may correlate with field stands but colored seed alone would not.

Erickson and Porter (21) observed that if lots of small-seeded legumes with the same germination were compared, the lots with the higher hard seed content ranked higher in the field than those with a lower hard seed content.

Toole and Hollowell (61) reported a possible secondary type of dormancy when seeds of various Trifolium were germinated at 30° and 35°C. After 21 days at these temperatures, the swollen non-germinated seeds were transferred to the 20° or 10°C where germination in most instances was rapid and complete.

Shuck (54) working with lettuce seed stated that "...often false evaluation of lettuce seed germination in the field is done because the seed is sown dry and covered with soil thereby excluding light. Some lettuce seeds must be exposed to light after they have been soaked for 5 minutes or more in order to initiate germination."

Seed Germination in the Field

Seed laboratory germination tests are carried out under conditions considered to be optimum. Field conditions are usually much less than optimum.

Soil

Heydecker (31), noting differences in emergence of seed from the same lot planted in different soils, explains: "What

matters to seed is apparently not so much the texture of a soil, as characterized by its mechanical analysis, but its structure, i.e., the special relationship of its physical ingredients, conditioned by the nature of its humus and its cementing agents...different soils can respond very differently to the same weather in terms of seedling emergence."

Sylwester (60) presented data collected from plantings of red clover, alfalfa and sweet clover in several Iowa locations over a period of 3 years. He indicated that the number of plants produced by the same amount of seed was diverse between localities. Murphy and Arny (46) found that in 5 different soil types, average field emergences varied from 52 to 82 per cent. Moore (43) noted that differences in soil type influenced the ability of seedlings to emerge from various depths of planting.

Larsen (41) planted alfalfa, brome and red clover seeds in 4 widely separated areas of Iowa. The overall response to various treatments was significantly different between locations but the relationship of the treatments to each other within locations was much the same. For this reason both location and treatment effects were significant but the interaction between them was not.

Rainfall and soil temperature

Environmental factors, such as rainfall and temperature may vary considerably between seasons at the same location.

Schoorel (50) determined a correlation coefficient between laboratory germination and field emergence for garden beans of 0.95 in good weather. The figure dropped to 0.81 in dry weather. Similar data were obtained for peas.

Swanson and Hunter (59) showed that sorghum seed planted on June 15 emerged 45 to 61 per cent better in the field than June 1 plantings. The difference was attributed to higher soil temperatures at the later planting dates.

Toole and Hollowell (61) pointed out that ecological factors such as moisture, soil type, and the amount of solar energy are either directly or indirectly related to soil temperatures. Furthermore, under natural conditions, diurnal variation in surface soil temperature is often very great.

Relation of laboratory germination and field emergence

Whitcomb (62), reporting on four year's data, obtained a 24 and 21 per cent emergence in the field for alfalfa and red clover seed that germinated 71 and 59 per cent respectively in the laboratory. Erickson and Porter (21) in a similar manner found a reduction from laboratory to field germination in alfalfa and red clover from 71 to 40 per cent and 79 to 42 per cent.

Working with garden peas, Munn (44) noted field stands to be 35 per cent lower than laboratory germination. On the contrary Hay (28), in Wisconsin, in one instance, obtained slightly better emergence with garden peas in the field than in the laboratory. Justice and Marks (35), experiencing similar results with rough peas (Lathyrus hirsutus L.) commented that perhaps more favorable moisture and air relationship existed in the soil than between blotters. Stahl (57) observed a 21 and 26 per cent reduction (laboratory to field) for cabbage and cauliflower respectively. Sherf (52) reported the field germination for untreated watermelon, cantaloupe and cucumber to be 21, 25 and 20 per cent lower respectively than laboratory germination.

Larsen (41), comparing laboratory germinations with field stands of red clover, alfalfa, and brome, found differences of 24.6, 19.7 and 16.2 per cent respectively. In other words, field emergence of red clover was lowest with respect to laboratory germination, and brome the highest.

Drake and Haferkamp (18) recorded 47 per cent lower results with radish seed in the field than in either the laboratory or greenhouse. They also noted many abnormal seedlings in the laboratory; however these were eliminated in the field.

Ratios

The "emergence ratio" of a seed lot[1], as the term is used in the experiments reported in this paper, is the percentage field emergence of the lot divided by its laboratory germination percentage. The ratio provides a mathematical means of comparing the ability of the viable seed of different lots to produce a field stand.

Stahl (56, 57) used a concept very similar to the emergence ratio. His objective was to find a method of testing the seed in the laboratory which would produce a constant ratio with the field emergence. If the ratios did not change to a considerable degree from one lot to another, then the laboratory method was said to be a good relative evaluation of the field emergence capacity. For example, he found that the ratio of germination speed to field emergence was more consistent between various lots of bromegrass seed than the ratio of total germination to field emergence.

Clark, (11) working with onion seed, used the ratio to determine the validity of laboratory germination tests as compared with the field results. He stated that "...the number of plants produced in the field per 100 normal seedlings observed in the laboratory seems to be a more satisfactory

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[1]A lot is a relatively homogeneous quantity of seed whose identity is distinct from that of other seed lots.

index of field value than the actual field germination test." Clark noted that the ratio of laboratory germination to field emergence was fairly constant throughout the entire range of onion seed germinations tested, and that there was less deviation from the average ratio among the lower germinations.

Larsen (41) reported that ratios for alfalfa and red clover increased with laboratory germination whereas bromegrass ratio generally remained the same.

Vigor in Seeds

The term "vigor" is difficult to define. It has been defined by Isely (32) as relating to the general state of the seed's health or their susceptibility to unfavorable growing conditions for either inherent or acquired reasons. Seed can manifest vigor in several different ways. Some of these are seedling size, penetration power, disease resistance and speed of germination. Not only are these characteristics diverse among seeds of one kind, but they differ strikingly between kinds.

Delouche, et al. (16) listed common factors causing loss of seed vigor including: (a) unfavorable weather conditions during ripening and harvesting, (b) mechanical injury during harvesting, (c) heating of high moisture seed, (d) heat injury during artificial drying, (e) storage conditions, (f) chemical

injury, and (g) seed borne organisms.

As to the importance of vigor with respect to field emergence of ornamental species, Heit (30) states that "...seed vitality was found to be an equally or sometimes a more important factor in field seedling stand and eventual flower performance than actual percentage of laboratory germination."

Size of seed and depth of planting

A seed size separation may be made on the basis of differential weight. Such a procedure was used by Rogler (48) for chaffy grasses. Seed size differentiation may also be made by the separation of the seeds by passing them through various sizes of round or rectangular hole screens.

With respect to the first method, Rogler (48) reported that, in general, field emergence dropped for all weights of seed as the depth of planting was increased. However, the heavier seed survived the deeper planting depths better than the lighter seed. Kneebone and Cremer (38) used screen separations for caryopses of a number of native grasses. Their experiments indicated that the larger the seeds, the more vigorous were the seedlings. Seedlings from larger seeds emerged faster and grew at a more rapid rate in the field. On the other hand "...seed size had little effect on (laboratory) germination except in switchgrass. Small seeds of

switchgrass germinated poorly in all tests."

Kalton et al. (36) working with alfalfa, bromegrass, red clover, birdsfoot trefoil and timothy, summarized that planting depth proved to be a major factor in the expression of seedling vigor of all species studied. Generally they said that rate and amount of emergence decreased as planting depth increased from 1/2 to 1 to 1-1/2 inches. With particular reference to bromegrass, they also said that larger-seeded strains generally were superior to small-seeded strains in seedling vigor attributes at all depths. This superiority was greatest at the greater depths of planting in both greenhouse and field.

Beveridge (6) using round hole screens, graded lots of three alfalfa seed varieties into 3 sizes. Size did not greatly affect laboratory germination. The responses of seedling emergence in the field in relation to seed size were unpredictable. The author generalized that although "...a seeding of selected large seed would not produce a greater number of plants as compared to normal seeding, a more vigorous young sward with better survival value would result."

Contrariwise, Erickson (22) using rectangular hole screens found a distinct correlation between seed size and laboratory germination. He states: "The theory that small seeds will produce more plants per acre if planted at the same rate in pounds is not substantiated by this study. Quite the

opposite is true. The seeds in the smallest seed-size separation had only one-tenth the germination value of those in the largest seed size. The average weight of the largest seeds was only twice that of the smallest. To be of equal value, on a weight basis, the small seeds should have produced at least one-half as many plants per square foot. These data definitely indicate that seed quality is of greater importance than the amount of seed sown per acre in the procurement of alfalfa stands."

Working with crimson clover, Moore (43) concludes that "the emergence of seedlings from either the extra large or extremely small crimson clover seeds was reduced to a greater extent by the deeper planting depths than was the medium-sized seed." Hay (28) as well as Kidd and West (37), both working with large-seeded legumes found that larger seeds gave rise to more vigorous plants and better yields.

Larsen (41) screened lots of red clover seeds into various sizes using rectangular screens. He found that the germinating capacity increased with size in the three size classes used.

In an effort to explain seed size in relation to seedling emergence and establishment, Black (7) said the importance of seed size was two-fold: first, seed size determines the depth from which emergence can occur as affected by reserve food supply; secondly, seed size determines the initial area of

cotyledons; presumably the initial larger photosynthetic area would give a larger seed an advantage over a smaller seed after emergence.

Temperature and speed of germination

Temperature and the speed at which a seed germinates are interrelated. Grabe (25) points out that warmer temperatures (within those used in his work) favor "speed of germination."

The determination of germination speed, often termed "germinating energy" requires no additional equipment beyond that used for the regular germination test. Such a test may be made by making a series of counts of the number of germinated seedlings prior to completion of germination. A test, based on a measurement of germination speed can be regarded as a measurement of seed vigor on the assumption that less vigorous seeds germinate more slowly. In order to assess speed of germination numerically, Kotowski (39) proposed the following formula:

$$\text{Coefficient of velocity} = \frac{A_1 + A_2 + \cdots + A_x}{A_1T_1 + A_2T_2 + \cdots + A_xT_x}$$

where A is the number of seedlings counted and T is the number of days after planting.

Chilton (10) and Grabe (25) used a formula developed by Isely (in lit.) which is similar in function but different in concept from that used by Kotowski. This formula is described

in the Materials and Method section. Edwards (19) employed still another concept which he termed "mean incubation time." Edward's formula is as follows:

$$\text{Mean incubation time} = \frac{A_1T_1 + A_2T_2 + \cdots + A_xT_x}{\text{Total germination}}$$

where again A is the number of seedlings counted and T is the number of days after planting. This formula is essentially the reciprocal of that used by Kotowski.

Edwards (op. cit.) studied the patterns of interim germination counts under a variety of temperature conditions. Using Black Eyebrow soybeans he made germination counts at two hour intervals. The seeds were declared germinated and removed when the radicle just penetrated the seed coat. He found that regardless of temperature used, the numbers of seedlings removed in the first three productive counts were remarkably similar. The third count, regardless of the germination temperature, produced the largest number of seedlings.

Stahl (56) compared both germinating capacity (total germination) and germinating-speed with field emergence. He found the germinating capacity of perennial ryegrass correlated best with field emergence. It was his opinion that germinating speed underestimated the field emergence of lots with lower viability. For field bromegrass, swedes and turnips, germinating speed provided the best estimate of field

performance.

A very practical application of a modified speed of germination test is discussed by Schwass (51) for ryegrass in New Zealand. Germination quality of seed lots varies widely from year to year owing to the degree of incidence of blind seed disease. Frequently there are not sufficient supplies of good seed (as measured by total germination) to meet the demand. Schwass found that seed lots with a low germination but with a high interim count in relation to the final germination produced good stands. The interim count is included on the seed tag as a vigor index.

Differential response to temperature in the laboratory can reveal vigor differences in seed lots. Wilson (63) found that soybeans of high viability germinated equally well at 10°, 15°, 20°, 25° and 30°C. Soybeans of low viability germinated best at 25°C. Caldwell (9) used various combinations of temperature and moisture for germinating garden peas. A laboratory test at 30°C in sterile sand at a moisture level of 70 per cent of saturation provided a reasonable selection of seed lots most apt to succeed in the field. Caldwell advocated this type of a test because: (a) good correlation was obtained between laboratory and field emergence, (b) the test is a physiological test and is not subject to the inconsistencies of pathological tests conducted in soil, (c) the test is sufficiently severe to detect weak seed lots, and (d)

the use of sterile conditions and controlled environment in the test makes it adaptable for standardization among its users.

Disease resistance

Field responses are usually affected by the presence of disease organisms. The extent of stand reduction is mediated by the number, kinds and activity of micro-organisms present, the tolerance of seeds and seedlings to attack by organisms, and the presence or absence of seed treatment.

Schoorel (50) states that poor seedling emergence in soil is usually correlated with the activity of undesirable soil-borne organisms. Munn (44) likewise, has taken the position that seed diseases are an important factor in field results. Differences in tolerance of different varieties of sorghums to disease attack was reported by Swanson and Hunter (59). They found that Feterita (a soft seeded variety) often molds and rots, and consequently fails to germinate when planted in cold, wet soil. However, better stands usually are obtained with harder seeded varieties when planted in the same soil conditions.

With respect to the location and growth of fungi in injured sudan grass caryopses, Jones et al. (34) reported that presence of a fungus infection in or near a rupture over the embryo usually resulted in germination failure. The initial

site of development of mycelia was usually at the radicle tip. Tetrazolium staining tests indicated that the necrosis originated at the radicle and progressed upward. The last tissue to succumb usually was the upper part of the scutellum. The importance of an intact seed coat was also stressed by Hay (29). Working with Great Northern beans, he found molds did not seriously reduce field emergence below the laboratory germination except in lots in which numerous seeds possessed injured seed coats. Hanson et al. (27) stated that damaged seeds usually benefit more from treatment than sound seeds.

Although saprophytic seed-borne organisms can be detrimental to seed germination under certain conditions, Munn (45) hypothesized that the soil may act as a deterrent to the development of saprophytic types provided no active parasites are resident in the soil. His reasoning was based on the results he obtained with 50 lots of watermelon seeds. The field germination of untreated seeds correlated very well with the laboratory germination of treated seeds. Germination of untreated seed in the laboratory was low because of attack by saprophytic fungi.

Seed-borne diseases may drastically reduce seedling emergence (i.e. Munn, 45). However, evaluation of seeds with respect to disease organisms is complicated by the fact that "clean" seed may be attacked by universally present soil-borne organisms. Tests such as the cold test for corn measure dif-

ferential degrees of susceptibility to such organisms, particularly Pythium. A cold testing procedure for corn is described by Svien and Isely (58). Their substrate consists of unsterilized soil mixed with river sand. This mixture is placed in a plastic box and moistened to 60 per cent of its water holding capacity and the seed planted. The box is then covered and placed in a 10°C chamber for 7 days. Subsequently the container, cover removed, is transferred to a germinating room (20° to 30°C) for an additional 7 days at which time the emergence is determined. Seed corn lots with less resistance to attack by the organisms in the soil usually demonstrate a marked reduction in emergence as compared to tests employing standard testing procedure. Clark and Kline (12) have used a similar procedure for onions.

The standardization of cold test presents considerable difficulties. Schoorel (50) points out: "...from a technical point of view, it is hardly feasible to standardize experiments in non-sterile soil." He goes on to say that "...it seems doubtful to promote the insertion of vigor tests in seed laws as long as little uniformity in results is attained."

Predicting field stands

The objective of any germination test is to obtain information concerning a seed lots ability to perform in the field. However, a test carried out under favorable germinat-

ing conditions does not subject the seed to the stresses it may encounter in the field.

Clark and Kline (12) found that the best laboratory-field correlations (0.906) for onions were achieved by using an average of the cold and standard tests. They said that the cold test provided a good basis for selecting lots of seed with high planting values. However, it did not provide a good basis for adjusting planting rates because field stands often were better. Porter (47) stated that when field conditions were below optimum, the cold test provided a fair index of field response with respect to both treated and untreated vegetable seeds.

Larsen (41) found that live seeds from high germinating lots of red clover and alfalfa, in general, emerge in higher proportions than those from low germinating lots. Nevertheless, the assumption of a direct relationship between the official laboratory test and field emergence is unwise. For example, Schoorel (50) observed that correlation coefficients between germinating capacity and field emergence of certain seed kinds are frequently very low. The laboratory germination provides the performance potential of the seeds. The extent to which this potential is achieved depends on field conditions which are diverse.

Is a vigor test then a test for field emergence? Isely (32) using the cold test for corn as an example, wrote:

"Vigor tests do not predict field stand. The cold test for corn is no more a test for field conditions than the favorable laboratory test. Whether one or the other will most closely correlate with stand achieved when the seed is planted in a particular field at a particular time will depend upon planting conditions then incident." But the vigor test gives further information concerning seed lots which can be used, in conjunction with the favorable test, for choosing seed lots most apt to succeed in the field.

Seed Treatment

The effect seed treatment may have on field stand establishment is varied. Sherf (52) reports that Arasan treatment did not greatly increase germination in either the laboratory or field when used on curcurbit seed. Clark (11) found less than 5 per cent average increase in onion seed field emergence due to the addition of a chemical treatment called 1205FF. Contrariwise Clark reported an increase of 15 to 20 per cent due to seed treatment when weaker lots were involved. Likewise, Schoorel (50) stated that seed treatment increased the field emergence of low vigor or disease-infected lots of Summer wheat.

Sherf (53) found little increase (4 per cent) of treated soybean seed over non-treated soybean seed when planted in the

field. Crosier (14) ran laboratory and field tests with Vicland oats treated with N. I. Ceresan and 1452F and found little difference between treated and non-treated seed in the laboratory. However treated seed emerged 1 to 13 per cent better in the field. Porter (47) reported nearly a 70 per cent increase in field emergence of beet seed due to treatment with 5 per cent ethyl mercury phosphate. He also worked with Arasan, Cersan and Spergon and found them beneficial in promoting field emergence especially if field conditions were below optimum. However, Porter, as Crosier, found no significant benefit from seed treatments in the laboratory tests. Allison and Torrie (1) reported significant increases in the germination of alfalfa seeds treated with Ceresan, Arasan and Spergon over untreated seed when planted in Pythium-infested soil in the greenhouse. However, field plantings revealed no important beneficial effect from these various fungicides. Schmitthenner and Parsons (49) found no difference between thiram (Arasan 50) treated and untreated alfalfa seed in either the greenhouse or field. Johnson (33) noted no significant difference in emergence or hay yields as a result of treating alfalfa seed with Arasan, Spergon or Vancide 51ZW. Hanson et al. (27) reported that small-seeded forage legumes, such as alfalfa, clovers, sweet clover, vetches, lespedezas and trefoils, usually do not respond to seed treatment under field conditions. Although increases in

stand were occasionally obtained, they were not reflected in increased forage yields. These workers felt that treatment was most helpful on Pythium-infested muck soils and under certain other conditions where stand establishment is difficult.

Contrary to some of the above results, Kreitlow (40) observed that alfalfa stands were increased 5 to 17 per cent when the seeds were dusted with a fungicide termed yellow cuprocide. Spergon and DuBay 1205FF likewise increased field stands. Kreitlow said that usually a cold, wet seed bed is most conducive to widespread damping-off; however, beneficial results from seed treatment were noted under a wide range of conditions at time of planting. Mead (42) demonstrated that most seed treatments improved field stands of alfalfa. Brome, however, was benefited to a lesser extent. Athow (5) compared seedling stands from Arasan-treated and untreated seed of 64 lots of alfalfa and 61 lots of red clover in field plantings. He found that field stands of approximately 14 per cent of the alfalfa lots and five per cent of the red clover lots were significantly increased by seed treatment. The poor germinating lots were benefited no more than the better ones. He found no treatment effects in subsequent hay yields.

Excessive use of certain fungicides can be detrimental to seed germination. Dickson (17) pointed out that, at temperatures above 8°C, excessive use of mercuric chloride or

formaldehyde reduced germination of wheat. Hanson et al. (27) emphasized that copper and mercurial fungicides may cause severe seed injury. Mead (42) said that the newer fungicides, based on Captan, were less toxic than others.

MATERIALS AND METHODS

These investigations consisted of field work conducted over a three year period supplemented by two series of laboratory experiments.

The crop kinds used were alfalfa (Medicago sativa cv. Ranger) and smooth bromegrass (Bromus inermis cv. Lincoln). The variables involved were: high, medium and low germination levels of genetically similar lots vs. genetically diverse lots; seeds treated with fungicide as opposed to those not treated; planting sites; and laboratory germination temperatures.

Preparation of Seed

The bromegrass and alfalfa seed used in these experiments was high in purity. The alfalfa was low in hard seed content. The seed lots were obtained in part from the Iowa State University Seed Laboratory files, and in part from seedsmen who were kind enough to contribute material meeting the proper specifications.

Nine lots of seed (each of alfalfa and bromegrass) were employed for one set of experimental material (designated as the ABC series in subsequent discussion). Three lots (designated as A) were relatively high (approximately 90-95 per

cent) in germination, three (B) were medium (approximately 75-95 per cent), and three (C) were (approximately 50-70 per cent) low. These three germination groups thus differed in both germination and genetic origin.

The XYZ series was derived from three high-germinating seed lots for each seed kind. Approximately one-third of the seed was stored in a 10°C chamber with low humidity so that the viability could be maintained. The remainder was placed in a 20°C germinator in which the relative humidity was at or near 100 per cent. Weekly germination checks were taken to monitor the drop in viability which ensued. After the germination had dropped to the intermediate level (as defined in the previous paragraph), half of the seed was transferred to dry conditions in the 10°C chamber to maintain its condition. The remainder was left in the 20°C germinator until it reached the third (lowest) germination level. By this procedure, three germination levels were derived, each of which contained three lots of seed which had genetically identical mates in the other two levels.

Separate lot acquisitions were made of both the ABC and XYZ series in 1961 and 1962. The seed acquired in 1962 was also used in the 1963 field and laboratory work.

1961 and 1962 Field Work

The stand producing qualities of the live seeds in seed lots of different viability levels were investigated. Equal numbers (approximately) of live seed were planted regardless of the germination of the parent lots.

To calculate seed weights containing equal numbers of live seeds, four samples of 100 seeds each (pure seed) of each lot were weighed and the average weight of 100 seeds computed. This weight for each lot was then divided by the laboratory germination and pure seed percentages to determine the weight in grams of seed containing 100 live seeds.

One thousand live seeds (sample weight containing 100 live seeds multiplied by 10) were planted in the field for each treatment combination. Thirty six of these thousand-live-seed samples constituted a replication for each of the two seed kinds. The 36 treatment combinations were derived from nine lots (three lots at each of three viability levels) times two series (ABC and XYZ series as previously described) times two (treated versus untreated). There were three replications at each planting site and two planting sites. The treatment combinations were completely randomized within each replication.

Planting sites for the tests were near Kanawha in north-central Iowa and Beaconsfield in the south-central area. The

soil at Beaconsfield is Grundy-Shelby, and that at Kanawha, Clarion-Webster. The areas used had been in corn the preceding years and the debris was plowed under preceding planting. Excellent seed beds were obtained. Plots were seeded by drill to oats prior to planting. At Beaconsfield, the bromegrass and alfalfa were planted on April 28th in 1961 and April 13th in 1962; at Kanawha, April 26th, both years.

The seeds were planted in the following manner. The packet containing the 1000 live seed was divided into four approximately equal packets. The contents of each of the subdivided packets were planted in a row 10 feet long. Since the rows were placed 9 inches apart, an area of 10 feet by 3 feet was used for each plot. No extra space was alloted between plots within a replication but 3 feet separated the replications themselves.

The seed was planted with a Planet Jr. seeder which had its conventional planter box replaced by a funnel. The contents of each seed packet were poured into the funnel by one person as the second person pushed the seeder ahead. The seeder placed the seed approximately 1/2 inch below the soil surface. This method of planting simulated that probably attained with a commercial grain drill.

Seedling counts were made 5 weeks after planting in one of the two center rows in each plot. The outside rows were excluded to eliminate possible border effect from adjoining

plots.

1963 Field Work

Only the XYZ alfalfa series was used in the 1963 field work. Plantings were made at two locations in the Ames area. One was at the Botany farm on Ash Avenue and the other at the Old Agronomy farm south of Ames on State Street.

During the year the treated seed (treated in 1962) had dropped in viability. It was, therefore, discarded. The untreated seed was divided in half and one-half treated in the spring of 1963. Prior to the 1963 field work, the laboratory germinations of the XYZ alfalfa lots were redetermined.

Two planting rates were employed. One rate ("correction I") involved the same correction employed the previous years. Equal numbers (100) of live seeds were planted in each row ("row" is equivalent to plot in 1961 and 1962). The formula for correction I is as follows:

$$\frac{100}{\text{laboratory germination}} = \text{seeds to be planted}$$

where laboratory germination is used as a decimal figure rather than per cent. The second planting rate ("correction II") provided corrections for vigor based on the differential field response of the lots in the 1962 experiments as well as

laboratory germination. Vigor ratings were derived from Figure 7 in the Experimental Results section. Correction II was used with the objective of obtaining (or expecting) equal numbers of seedlings from each lot. The formula for correction II is as follows:

$$\frac{100}{\text{(laboratory germination)} \times \text{(live seed field emergence)}} = \text{seeds to be planted.}$$

Again the terms are used in decimal form.

A randomized block design was utilized. There were three lots within each set of three germination levels, two planting rates, and treated vs. untreated seed. Each replicate contained 36 rows. The rows were four feet long and 12 inches apart. Thus an area of 35 feet by four feet was employed for each replication. The four replications were separated by a three foot boundary on all sides.

The seeds were hand-planted in freshly prepared soil on April 25th at the Ash Avenue site and on May 1st at the Old Agronomy farm. Five week counts were made on May 30th at Ash Avenue and June 4th at the Old Agronomy farm.

The soil at the Ash Avenue site tended to be moist and heavy. At the Old Agronomy farm, the soil was sandy and drier. A four inch rain fell during the period of seedling emergence. The Ash Avenue plots were flooded. However, hay

from a freshly cut meadow above the plantings washed down over the plots and protected the soil from excessive washing and packing. The hay was raked off after the area dried and little damage was done. The effect of the rain on the Old Agronomy farm was more severe, especially in regard to subsequent crusting of the soil.

Laboratory Work

The thermogradient plate

In order to test seed germination response to a spectrum of temperatures, a "thermogradient plate" was constructed. This device was a modification of an apparatus used by Halldal and French (26) to study the temperature response of certain blue-green algae. A similar contrivance was later used by Elliott and French (20) to investigate lettuce seed germination.

The apparatus used in this study was constructed as follows: an aluminum plate, three by five feet, 5/8 inch thick, was fitted with water jackets on both of the five foot sides. This was done by placing a five foot stainless steel channel on the upper and lower surfaces of the edges. After fitting the channels with proper gaskets, holes were drilled through the extending flanges of the channels, gaskets and plate. Bolts were then inserted to hold the channels in position.

The channels were attached so that water could pass into the lower channel, travel the length of the plate, pass to the upper channel via holes in the plate at the end of the channel, and back across the plate through the upper channel. Warm water (48°C) was passed through the channel on one edge and cold water (2°C) through the other. Thus a gradient of temperatures were established on the plate between the two extreme temperatures of the opposing edges.

The water passing through the warm side was heated in a 10 gallon supply tank by a 500 watt calrod heating element and the temperature controlled by a bimetallic thermoregulator. The warm water was moved by a 1/20 horsepower recirculating pump. The temperature of the cold water was maintained by a Coplematic compressor in conjunction with a White-Roger temperature control. The cold water was circulated through the heat exchanger, storage tank and channels by a 1/8 horsepower circulating pump.

A 1-1/4 inch high aluminum alloy frame was erected around the edge of the plate to support a Plexiglass cover for the plate as a moisture control feature. A foam rubber gasket provided a moisture seal between the Plexiglass cover and the frame. Longitudinal vertical dams were attached to the under-surface of the Plexiglass to prevent excessive movement of evaporated moisture. The entire plate with its Plexiglass cover was placed in an insulated box with an insulated fiber-

Figure 1. Thermogradient plate showing moist, blotter-covered surface planted with eight columns of alfalfa seed. Each column has 35 rows with ten seeds in each row. The columns are the experimental units and are labeled with reference to the particular treatment combination they contain

Figure 2. Thermogradient plate with the plexoglass moisture-control cover in position

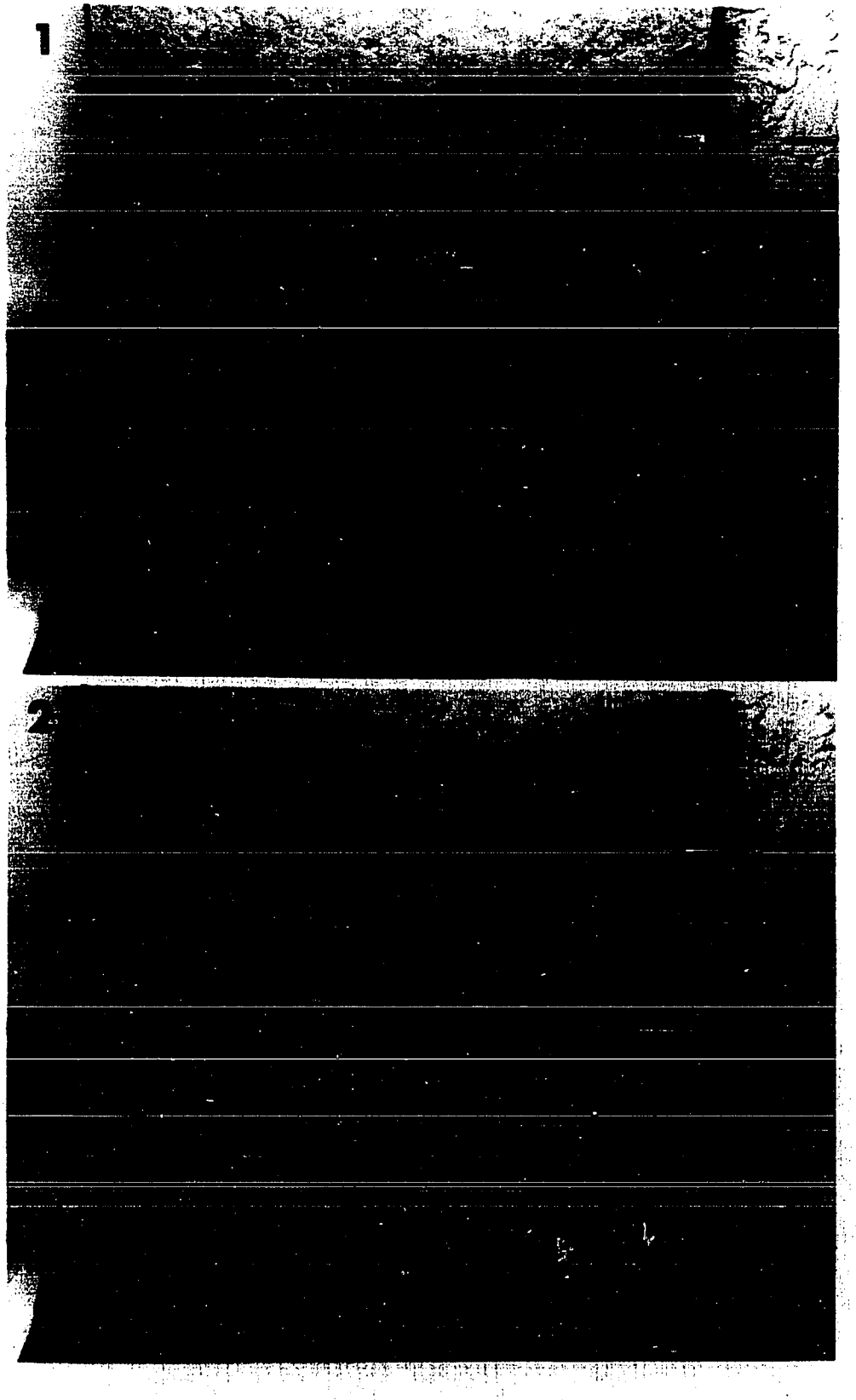


Figure 3. Detail of cold water channels (a) on the edge of 5/8 inch thick plate (b). Gaskets (c) prevent moisture leakage. The same arrangement is found on the opposite edge for warm water. The 1-1/4 inch high frame (d) supports the plexoglass moisture cover

Figure 4. Left side of thermogradient plate showing temperature monitoring potentiometer (a) and twelve uniformly spaced thermocouples (b)

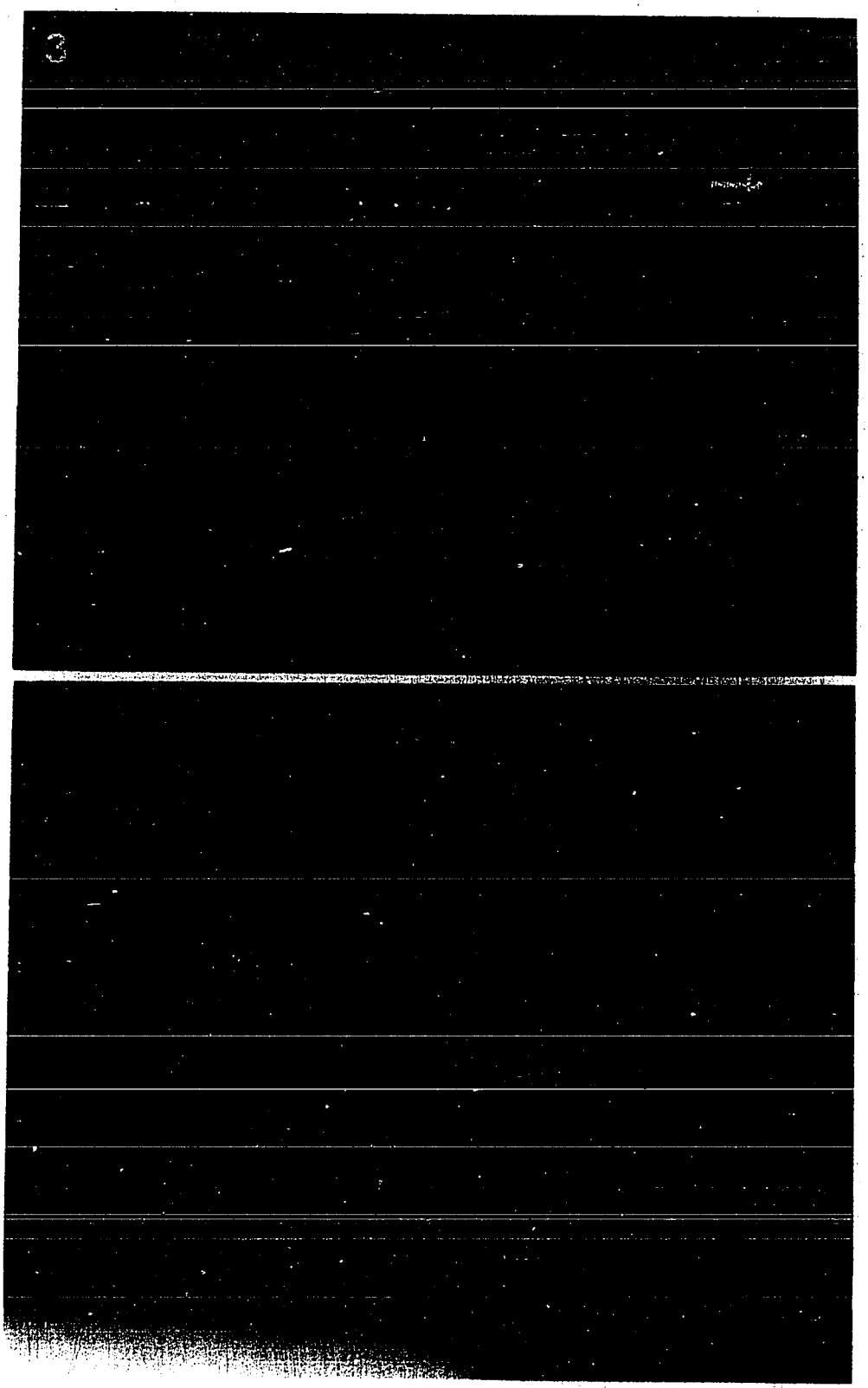
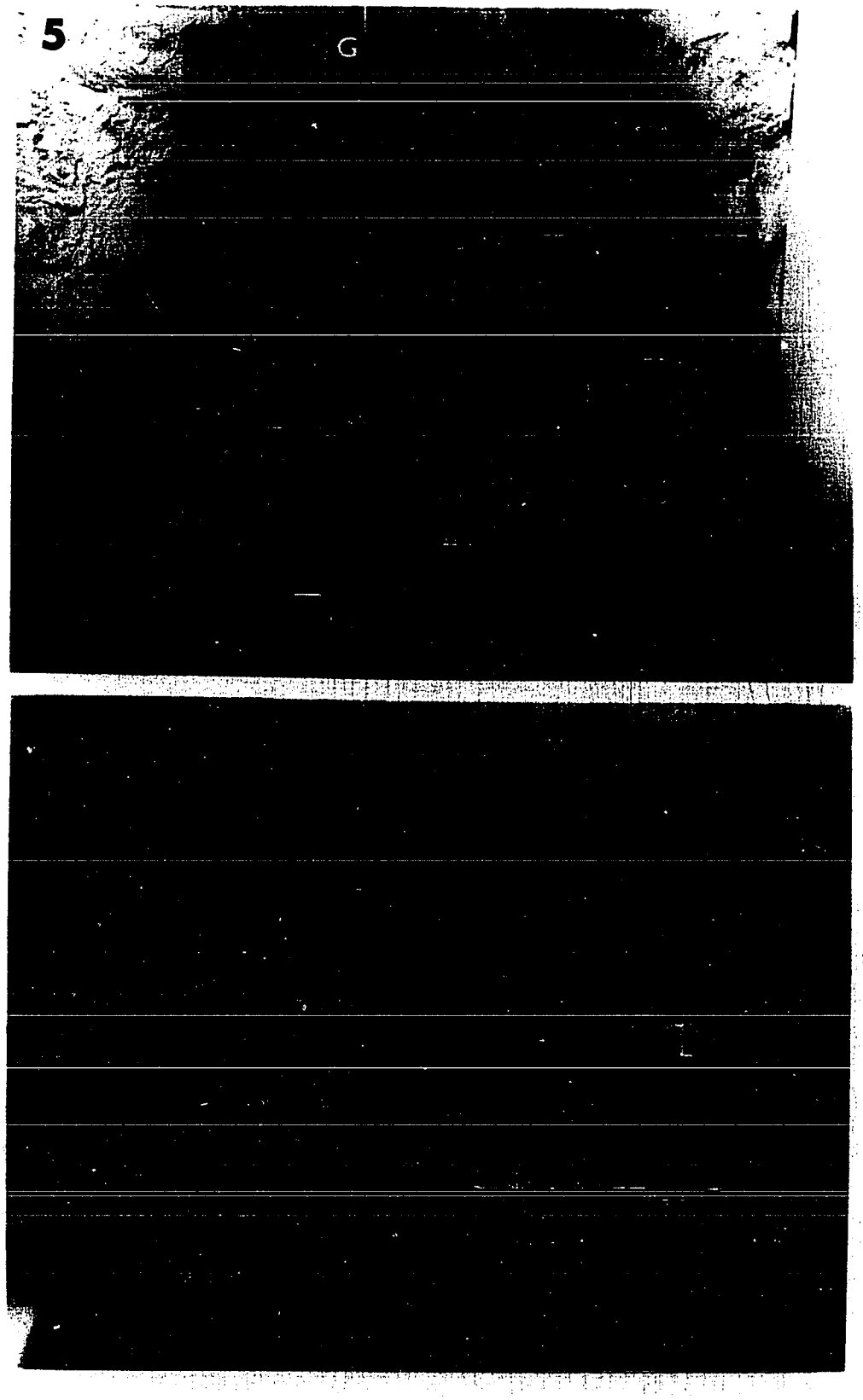


Figure 5. Temperature control equipment on right side of thermogradient plate. Thermoregulator (a) controlled the calrod heating element (not shown) inserted into warm water tank (c). Water in cold water tank (d) was cooled by Coplematic compressor (e). The compressor was activated by a White-Roger temperature control (g). Water was circulated through channels by pumps (b and f)

Figure 6. Thermogradient plate with fiberglass insulated cover in position. On the cover is the equipment used in planting seeds


5
G

board cover to prevent possible influence of outside temperature.

Precise temperature checks of the gradient were made by means of 12 thermocouples located at regular intervals along the gradient on one side of the plate. The temperature on the plate ranged from 9° to 41°C. The nature of the temperature gradient and degree of uniformity of operation is presented in the Experimental Results section.

In preparation for the planting of the seeds, the plate surface was covered with moistened blue germination blotters. Seeds were then planted with an Ames Powercount vacuum seed planter. The seeds were placed 10 in a row and a column of 35 rows was planted in the direction of the temperature gradient. Eight columns were needed to fill the plate. A column was the basic testing unit in this investigation.

The ABC and the XYZ series alfalfa were planted on the plate in three replicates of randomized block design. As the plate held only eight of the 18 treatment combinations per run,[1] two full runs and a part of the third were necessary for each replication. Partially filled plates were completed by the necessary number of randomly selected combinations from the next replication.

Only the ABC series of bromegrass was planted. Six of

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[1]The time interval between planting and the final counting of seed on the thermogradient plate constitutes a run.

the 18 treatment combinations were planted in each run. In this way, three full runs were needed for each replicate. Since four completely randomized replicates were used, a total of 12 runs were needed to complete the test. The remaining two positions, one on each end of the plate, were used for the uniformity tests discussed below.

A test of the plate's uniformity was desirable to check: (a) the homogeneity of temperatures across the length of the plate perpendicular to the temperature gradient, and (b) the uniformity of performance from run to run. For the uniformity tests, a randomly selected sample of Lincoln Bromegrass was taken from the Iowa State University Seed Laboratory files. As these tests were run concurrently with the ABC series of bromegrass, 12 replicates were obtained. However, one replicate was discarded due to irregularities in data recording.

Daily counts for alfalfa were initiated on the second day after planting. Alfalfa seedlings were counted and removed when their hypocotyl and radicle length totaled one cm. The tests ran for six days; thus a total of five counts were made.

The first bromegrass counts were made on the third day and the test was terminated on the eighth day. Thus a total of six counts were made. The criteria for germination and the recording procedures were the same as for the alfalfa.

After each count, additional moisture was added to the substrata.

Laboratory time-and-temperature experiment

The ABC and XYZ alfalfa series untreated seeds were used. The experiment was based on a factorial arrangement of three temperatures (16°, 20°C and 24°C); four counting dates of two, four, six and eight days; three germination levels; and three lots within each germination level. All treatment combinations were completely randomized within replicates within three different controlled-temperature germinators.

Each replicate, within a germinator, filled a single germinator tray. Since three replicates were used, three trays were allocated to each germinator. One hundred seeds for each treatment combination in each replicate were planted between blue germination blotters. They were considered germinated when their radicles reached one cm in length. Additional moisture was added to the blotter substrata after each count was made.

The data were analyzed three different ways. First, the raw data were analyzed. Secondly, the "germination-speed index" was calculated from the data using the following formula:

$$\text{Germination-speed index} = \frac{A_1}{T_1} + \frac{A_2}{T_2} + \cdots + \frac{A_x}{T_x}$$

where A is the number of seedlings removed per count and T is the number of days from initiation of test to time of a

particular count. Third, the "speed index" was calculated from the data using the following formula:

$$\text{Speed index} = \frac{P_1}{T_1} + \frac{P_2}{T_2} + \cdots + \frac{P_x}{T_x}$$

where P is the per cent of the total germination removed per count and T is the number of days from initiation of test to time of a particular count. These formulae were developed by Isely (in lit.) and previously employed by Chilton (10) and Grabe (25).

Statistical Analyses

Statistical procedures in all the above experiments were developed in consultation with members of the Iowa State University Statistics Department staff.

EXPERIMENTAL RESULTS

1961 Field Work

ABC alfalfa

The official laboratory germination of the nine lots are listed in Table 1.

Table 1. Official laboratory germination of the ABC alfalfa seed series (no genetically identical mates between germination level), 1961

Germ. level	A			B			C		
Lot	1	2	3	4	5	6	7	8	9
Germ. %	94	91	94	81	72	77	61	70	59

The analysis of variance of the five week seedling field count data obtained from the two sites used follow in Table 2. (The raw data are in Table 36 of the Appendix.)

The most interesting aspect of the analysis of variance table is the significance of germination levels. As equal numbers of live seed were planted in each case, this indicates a differential emergence of the live seed at the different germination levels. That a higher proportion of the live seed from the higher germinating lots emerged is shown by Table 3.

The superiority of live seed emergence of higher germi-

Table 2. ABC alfalfa, analysis of variance of five weeks seedling count data taken at two sites, 1961

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replications	2	14,583.02**	11,152.80**
Treatments	17	2,561.00	3,800.12*
Germ. levels	2	16,578.36**	14,235.80**
Fungicides	1	2,153.35	3.13
Lots	2	193.41	7,607.58*
GF	2	806.90	226.90
GL	4	560.40	410.46
FL	2	121.18	620.35
GFL	4	935.58	4,393.96*
Error	34	1,795.08	1,639.72
Total	53		

*Significant at the 5% level.

**Significant at the 1% level.

Table 3. Field response of ABC alfalfa compared within fungicide treatment and within lots at two locations, 1961 data represents seedling emergence five weeks after planting

Germ. level	Beaconsfield				Kanawha			
	A	B	C	Total	A	B	C	Total
Fungicide								
None	1357[a]	774	812	2943	1544	1278	1076	3898
Captan	1349	1007	928	3284	1548	1373	1004	3885
Lot								
1	873[b]	657	605	2135	1115	1062	830	3007
2	953	517	547	2017	945	734	613	2292
3	880	607	588	2075	1032	815	637	2484
Total	2706	1781	1740		3092	2611	2080	

[a]Emergence from 2250 live seeds.
[b]Emergence from 1500 live seed.

nating lots over lower ones is quite pronounced. This effect is reasonably consistent at both locations and within fungicide treatment and lots. Some irregularities can be noted in germination level effects within lots, e.g. the emergence of lot B5 was quite low. But the laboratory germination of B5 was also low (Table 1).

In Table 2 it is revealed that lots were significant at Kanawha. Table 3 confirms this, indicating that lots were more variable at Kanawha than at Beaconsfield. Also at Kanawha the analysis of variance indicates that the three-way interaction of germination level, fungicide treatment and lots is significant. An investigation of this three-way interaction revealed no plausible explanation for its significance.

There was no significant effect due to fungicide treatment.

XYZ alfalfa

This series was made of three lots of high germinating alfalfa seed of which portions of each were synthetically reduced to medium and low germination levels.

The official laboratory germinations of these lots are presented in Table 4. Since the investigator was able to control the germination levels to some extent in preparing these lots, there was less variation within germination levels than in the ABC series. The similarity of the germination of lots

under Z is indicative of this control, but the fact that they were exactly the same was coincidence.

Table 4. Official laboratory germination of the XYZ alfalfa seed series, (genetic identity between germination levels), 1961

Germ. level	X			Y			Z		
Lot	1	2	3	1	2	3	1	2	3
Germ. %	93	90	96	78	78	84	71	71	71

The analysis of variance of the five week seedling field count data obtained from the two sites is included in Table 5. (Raw data are in Table 37 in Appendix.)

Table 5. XYZ alfalfa analysis of variance of five week seedling count data taken at two sites, 1961

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replications	2	2,889.58	9,078.13**
Treatments	17	1,124.68	5,613.34
Germ. levels	2	5,013.46*	33,156.68**
Fungicide	1	1,968.07	3,097.80
Lots	2	882.80	1,514.46
GF	2	803.91	3,649.68
GL	4	541.08	1,202.07
FL	2	444.58	1,303.57
GFL	4	174.41	2,067.96
Error	34	714.36	1,421.29
Total	53		

*Significant at the 5% level.

**Significant at the 1% level.

Significant differences were largely limited to germination levels. A closer look at the germination level effect is afforded by Table 6. Again superiority of higher germinating seed with respect to emergence is in general indicated. However, there was a reversal of field response between the Y and Z levels at Beaconsfield.

No significant effect was achieved as a result of using the fungicide Captan 75.

Table 6. Field response of XYZ alfalfa compared within fungicide treatment and within lots at two locations, 1961 data represents seedling emergence five weeks after planting

Germ. level	Beaconsfield				Kanawha			
	X	Y	Z	Total	X	Y	Z	Total
Fungicide								
None	1072[a]	674	760	2506	1565	807	766	3138
Captan	1042	859	931	2832	1512	1235	800	3547
Lot								
1	652[b]	477	640	1733	1033	646	635	2314
2	694	449	540	1683	932	629	477	2038
3	768	607	547	1922	1112	767	454	2333
Total	2114	1533	1691		3077	2042	1566	6685

[a]Emergence from 2250 live seeds.

[b]Emergence from 1500 live seeds.

ABC bromegrass

Again this series is made up of nine distinct lots. The germinations of these lots are presented in Table 7.

Table 7. Official laboratory germination of the ABC bromegrass seed series (no genetically identical mates between levels), 1961

Germ. level	A			B			C		
Lot	1	2	3	4	5	6	7	8	9
Germ. %	92	90	90	78	75	74	53	52	51

There was greater uniformity between lots within germination levels in this series than for the ABC alfalfa.

The analysis of variance of the five week seedling field count from the two sites used are included in Table 8. (Raw data, Table 38 in Appendix.)

Unlike the alfalfa, there are no significant differences due to germination levels. The germination levels times lots interaction at Beaconsfield was significant largely due to a high field response of lot C7.

XYZ bromegrass

The official laboratory germination of these lots are listed in Table 9.

Table 8. ABC bromegrass analysis of variance of five weeks seedling count data taken at two sites, 1961

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replication	2	3,740.66	5,452.06
Treatments	17	2,984.05*	2,455.88
Germ. levels	2	3,593.56	3,997.56
Fungicide	1	104.16	4,125.63
Lots	2	4,580.06	99.38
GF	2	2,204.22	24.52
GL	4	5,959.28**	5,059.78
FL	2	1,023.39	466.91
GFL	4	996.28	2,052.13
Error	34	1,493.55	2,895.56
Total	53		

*Significant at the 5% level.

**Significant at the 1% level.

Table 9. Official laboratory germination of the XYZ bromegrass seed series (genetic identity between germination level), 1961

Germ. level	X			Y			Z		
Lot	1	2	3	1	2	3	1	2	3
Germ. %	85	92	87	75	87	77	72	85	69

The analysis of variance of the five week seedling field counts from two sites are included in Table 10. (The raw data appears in Table 39 of the Appendix.) Not a single significant effect was encountered. Again, this contrasts with the

Table 10. XYZ bromegrass analysis of variance of five weeks seedling count data taken at two sites, 1961

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replications	2	1,584.50	1,963.68
Treatments	17	860.59	2,023.98
Germ. levels	2	17.38	1,810.80
Fungicide	1	101.40	75.85
Lots	2	2,195.38	3,093.46
GF	2	64.02	627.36
GL	4	1,828.12	4,529.96
FL	2	135.02	1,044.90
GFL	4	598.12	764.74
Error	34	1,091.13	2,688.98
Total	53		

alfalfa in that no differences due to germination level were discernable. Perhaps this is in part due to the fact that the germination levels as per laboratory germination (Table 9, above) were not nearly as discrete as for the alfalfa (Table 4).

1962 Field Work

ABC alfalfa

The official laboratory germinations of the nine distinct lots (no genetic identity between germination levels) are listed in Table 11.

The analysis of variance of the five week field seedling

Table 11. Official laboratory germination of the ABC alfalfa seed series (no genetic identity between germination levels), 1962

Germ. level	A			B			C		
Lot	1	2	3	4	5	6	7	8	9
Germ. %	95	94	93	83	74	76	69	71	57

Table 12. ABC alfalfa, analysis of variance of five weeks seedling count data taken at two sites, 1962

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replications	2	4,907.06	395.36
Treatments	17	3,402.60	1,403.13
Germ. levels	2	10,423.50	2,698.74
Lots	2	7,080.50	1,140.36
Fungicide	1	4,537.50	3,935.58
GL	4	1,878.67	877.04
GF	2	324.06	987.63
LF	2	918.39	1,058.90
GLF	4	2,074.78	1,159.55
Error	34	2,414.09	1,511.11
Total	53		

count from the two locations are recorded in Table 12. (The raw data appears in Table 40 of the Appendix.) No significant effect due to any of the variables were obtained. However, (Table 13), all the A lots consistently emerged the best. Lack of significance is probably due to the discrepancy in the relationship of B and C lots.

There seem to be beneficial effects from fungicide

Table 13. Field response of ABC alfalfa compared within fungicide treatment and within lots at two locations, 1962, data represents seedling emergence five weeks after planting

Germ. level	Beaconsfield				Kanawha			
	A	B	C	Total	A	B	C	Total
Fungicide								
None	1476[a]	986	1111	3573	1741	1401	1598	4740
Captan	1554	1207	1307	4068	1806	1708	1687	5201
Lot								
1	1184[b]	853	867	2904	1132	1004	1079	3215
2	885	820	842	2547	1175	1162	1141	3478
3	961	520	709	2190	1240	943	1065	3248
Total	3030	2193	2418		3547	3109	3285	

[a]Emergence from 2250 live seeds.

[b]Emergence from 150 live seeds.

treatment within germination levels at both locations as shown on Table 13. The lack of significance between fungicide treated seed and untreated seeds is probably due to the lack of sensitivity of the F test. Fungicide with only one degree of freedom was tested against the fungicide time lot interaction with only two degrees of freedom. Therefore the F number would have to be quite large before significance would be attained.

XYZ alfalfa

The official laboratory germination of the lots used in this study are given in Table 14.

Table 14. Official laboratory germination of the XYZ alfalfa seed series (genetic identity between germination levels), 1962

Germ. level	X			Y			Z		
Lot	1	2	3	1	2	3	1	2	3
Germ. %	88	92	92	77	72	78	55	58	61

The analysis of variance of the field data is recorded in Table 15 (raw data, Table 41 in Appendix).

Germination levels were highly significant in this series. An examination of Table 16 shows that in all instances the higher germination levels performed better with respect to emergence. Fungicide treatment was beneficial, although only at Beaconsfield was the effect significant. The mean square for fungicide was the largest of all at the Kanawha location but still was not significant. The fungicide effect was tested by the interaction of lots and fungicide. The ratio between the mean squares of fungicide and the lots-fungicide interaction at Beaconsfield illustrates how large the difference must be for significance at even the 5% level.

Table 15. XYZ alfalfa analysis of variance of five week seedling count data taken at two sites, 1962

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replications	2	1,579.80	1,308.74*
Treatments	17	5,151.85**	1,300.03**
Germ. levels	2	20,118.46**	5,671.35**
Lots	2	370.91	160.91
Fungicide	1	38,720.67*	7,632.67
GL	4	626.52	198.52
GF	2	340.06	271.72
LF	2	444.38	542.38
GLF	4	951.78	95.28
Error	34	1,424.62	386.98
Total	53		

*Significant at the 5% level.

**Significant at the 1% level.

Table 16. Field response of XYZ alfalfa compared within fungicide treatment and within lots at two locations, 1962; data represents seedling emergence five weeks after planting

Germ. level	Beaconsfield				Kanawha			
	X	Y	Z	Total	X	Y	Z	Total
Fungicide								
None	1239[a]	975	568	2782	1709	1499	1323	4531
Captan	1636	1473	1119	4228	1844	1738	1591	5173
Lots								
1	955[b]	836	497	2288	1146	1088	955	3189
2	911	838	542	2291	1187	1092	942	3221
3	1009	774	648	2431	1220	1057	1017	3294
Total	2875	2448	1687		3553	3237	2914	

[a]Emergence from 2250 live seeds.

[b]Emergence from 1500 live seeds.

ABC bromegrass

The laboratory germinations of the ABC lots are recorded in Table 17.

Table 17. Official laboratory germination of the ABC bromegrass seed series (no genetic identity between germination level), 1962

Germ. level	A			B			C		
Lots	1	2	3	4	5	6	7	8	9
Germ. %	88	87	88	65	68	71	55	58	47

The analysis of variance for the five week field seedling count for both locations is recorded in Table 18 (raw data, Table 42 in Appendix).

Table 18. ABC bromegrass analysis of variance of five weeks seedling count at two sites, 1962

Source of variation	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replications	2	10,069.58*	4,016.08*
Treatments	17	3,084.12	1,217.08
Germ. level	2	11,424.02	236.08
Lots	2	2,607.46	1,219.02
Fungicide	1	5,240.02	6,534.00
GL	4	2,218.83	1,167.96
GF	2	2,016.80	866.00
LF	2	1,966.91	487.38
GLF	4	571.10	966.89
Error	34	2,451.73	807.05
Total	53		

*Significant at the 5% level.

The analysis of variance indicates that none of these treatment effects were significant. However, both within germination levels and within lots, fungicide treatment responses were higher than non-treated responses.

XYZ bromegrass

The official laboratory germinations of the XYZ lots are recorded in Table 19. (Raw data is in Table 43 in Appendix.)

Table 19. Official laboratory germination of the XYZ bromegrass seed series (genetic identity between germination levels), 1962

Germ. level	X			Y			Z		
Lot	1	2	3	1	2	3	1	2	3
Germ. %	85	77	77	78	68	72	74	56	73

The particular lots chosen to prepare the three germination levels did not respond consistently to pretreatment to reduce germination, particularly lot 3. Y3 is lower in germination than Z3. Rather than switching these lots, it was felt best to leave them in position. Z3 was exposed to the pretreatment longer than Y3 and there was a possibility that this longer treatment would have affected the vigor of the seed even if not the total germination.

The analysis of variance of the field data taken at the

two sites is recorded in Table 20. The only significant effect was the interaction between germination levels and lots. This interaction was studied and it was concluded that significance possibly resulted from chance. The germination level effects for both locations were extremely variable. There was no indication of superiority of live seed from one germination level over those of another.

Seed treated with Captan 75 emerged in greater numbers in most instances at both locations and within germination levels and within lots (Table 21). In spite of the fact that the differences were not significant, the uniformity with which their differences occurred is noteworthy.

Table 20. XYZ bromegrass analysis of variance of five weeks seedling count data taken at two sites, 1962

Source of variance	Degrees of freedom	Mean squares	
		Beaconsfield	Kanawha
Replicates	2	6,807.46	457.41
Treatments	17	1,923.80	1,543.05
Germ. levels	2	6,515.24	120.13
Lots	2	2,511.57	195.80
Fungicide	1	988.16	1,980.17
GL	4	1,534.52	4,184.13**
GF	2	193.17	243.39
FL	2	384.06	378.38
GFL	4	1,592.55	1,409.94
Error	34	3,123.52	848.11
Total	53		

**Significant at the 1% level.

Table 21. Fungicide treatment responses within germination levels and within lots for XYZ bromegrass seed planted in the field; 1962 data represents emergence from 1500 live seeds, five weeks after planting

Fung. Trt.	Germ. level			Lot			Total
	X	Y	Z	1	2	3	
(Beaconsfield)							
None	1643	1899	1619	1590	1845	1726	5161
Captan	1652	2013	1727	1686	1831	1875	5392
(Kanawha)							
None	1454	1433	1488	1497	1483	1395	4375
Captan	1518	1618	1566	1517	1607	1578	4702

1963 Field Work

The 1963 field work was based entirely upon the XYZ alfalfa seed used in 1962. There were several reasons for this. First no germination level effects were derived from bromegrass either in 1961 or 1962 - hence bromegrass was excluded as it seemed that vigor effects were not present or were not detected by the methods of investigation used. Secondly, the germination level effects of the 1962 ABC alfalfa were erratic possibly due to the confounding effects of genetic diversity between germination levels. This erratic response made the ABC alfalfa series undesirable for experimental work of the nature planned for 1963. On the other hand, the behavior of the XYZ alfalfa was consistent, and the

information obtained in 1962 was available for making further planting rate corrections in 1963. A comparison of Table 22 with Table 14 indicates that germination of the XYZ alfalfa lots had dropped somewhat during the year.

Table 22. Official laboratory germination of the XYZ alfalfa seed series (genetic identity between germination levels), 1963

Germ. level	X			Y			Z		
Lot	1	2	3	1	2	3	1	2	3
Germ. %	82	88	92	77	70	78	48	57	54

Seeds were planted in the field at two different rates as described in the Materials and Methods section. The first rate (correction I) was corrected for laboratory germination alone. The second rate (correction II) was corrected for both laboratory germination and live seed emergence in the field.

Figure 7 (from which correction II seed planting numbers were calculated) shows the relationship between 1962 live seed field emergence and laboratory germination. The laboratory germinations of the lots used were plotted on the horizontal axis. The reciprocal of the product of laboratory germinations and the corresponding field germinations of live seed (in decimal form), as portrayed by the diagonal line, were multiplied by 100. The figures derived would then represent

the theoretical number of seeds needed to produce 100 seedlings per row in the 1963 field plantings.

The total number of seeds and the live seeds planted in each row for the 1963 field experiments are listed in Table 23.

Table 23. Total seeds and numbers of live seeds planted per row in XYZ alfalfa seed series, 1963

Germ. level	X			Y			Z		
Lot	1	2	3	1	2	3	1	2	3
Total seeds planted per row									
Corr. I	122	114	109	130	144	129	211	176	185
Corr. II	184	163	151	205	244	200	467	341	375
Live seeds planted per row									
Corr. I	100	100	100	100	100	100	100	100	100
Corr. II	151	143	139	158	169	155	221	194	203

Correction I results

The analysis of variance for the two field plantings involving correction I are in Table 24 (raw data, Table 44 in Appendix). The germination level effects within lots and fungicide treatment are shown for both locations in Table 25. Germination levels were significant at both sites. In general a greater proportion of the live seeds from the high germinating lots produced seedlings; however at the Old Agronomy farm, lots 1 and 2 produced more seedlings at the Z level than at

Figure 7. Relationship between emergence of live seed on the field and total germination in the laboratory (XYZ alfalfa, 1963)

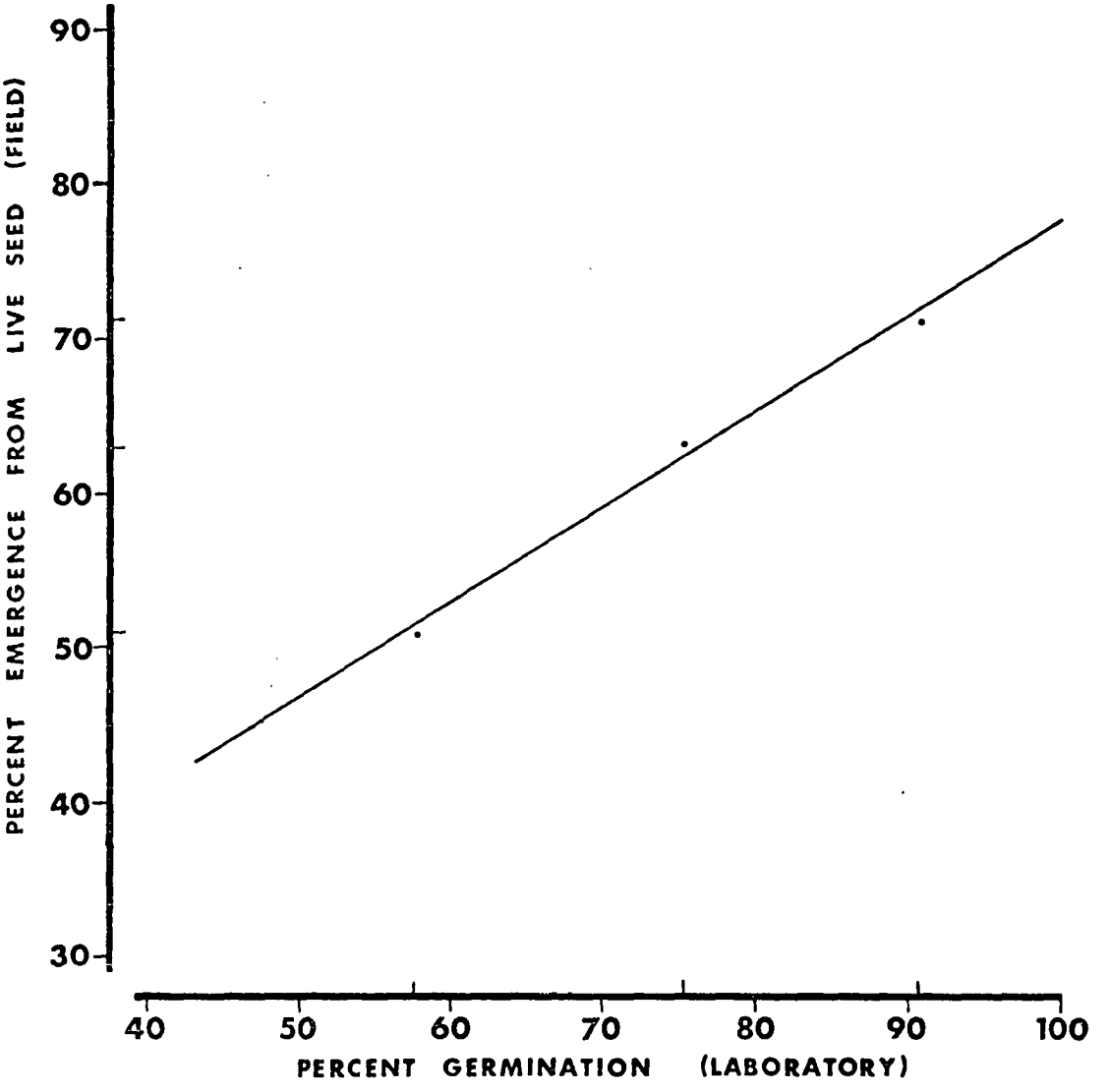

90
80
70
60
50
40
30
PERCENT EMERGENCE FROM LIVE SEED (FIELD)
40
50
60
70
80
90
100
PERCENT GERMINATION (LABORATORY)

Table 24. XYZ alfalfa analysis of variance of five week seedling count data taken at two sites, correction I, 1963

Source of variation	Degrees of freedom	Mean squares	
		Ash Ave. farm	Old Agron. farm
Replications	3	217.72	3,018.54**
Treatments	17	471.02**	564.09**
Germ. levels	2	2,618.60**	1,341.16*
Lots	2	272.22	13.16
Fungicide	1	885.04	2,640.22
GL	4	74.19	166.46
GF	2	63.00	501.56
LF	2	64.88	352.89
GLF	4	197.05	466.47*
Error	51	108.12	173.85
Total	71		

*Significant at the 5% level.

**Significant at the 1% level.

the Y.

Emergence was not significantly improved by fungicide treatment. However the majority of the treated lots at both locations produced a substantially greater number of seedlings than their untreated counterparts.

There are two possible reasons for the significant GLF interaction at the Old Agronomy farm. Lot 3 at the X germination level produced a greater number of seedlings when untreated than treated whereas the other eight lots produced more seedling from treated seed. Further, the treated seed of lots 2 and 3 were inverted at the Y level with respect to the

Table 25. Field response of XYZ alfalfa compared within fungicide, 1963 treatment and within lots at two locations, correction I, 1963; data represents seedling emergence five weeks after planting

Germ. level	Ash Ave. farm X	Y	Z	Total	Old Agron. farm X	Y	Z	Total
Fungicide								
None	456[a]	418	225	1099	372	125	122	619
Captan	567	480	315	1362	392	349	314	1055
Lots								
1	349[b]	326	192	867	228	171	173	572
2	301	289	137	727	260	122	156	538
3	373	283	211	867	276	181	107	564
Total	1023	898	540		764	474	436	

[a]Emergence from 1200 live seeds.

[b]Emergence from 800 live seeds.

positions held in the X and Z level (see Table 44 in Appendix).

Correction II results

The analysis of variance for the two field plantings involving correction II are included in Table 26 (raw data in Table 45 in Appendix).

At the Ash Avenue farm, germination levels are not significant. This presumably is to be expected as a consequence of correction II in the planting rate. However, germination levels are significant at the Old Agronomy farm; possibly, this is due to the added effect of soil crusting. This will

Table 26. XYZ alfalfa analysis of variance of five weeks seedling count data taken at two sites, correction II, 1963

Source of variation	Degrees of freedom	Mean squares	
		Ash Ave. farm	Old Agron. farm
Replications	3	526.76	8,260.27**
Treatments	17	1,171.41**	1,028.52**
Germ. levels	2	109.50	864.06*
Lots	2	1,282.54	197.34
Fungicide	1	9,293.39*	11,425.68*
GL	4	857.79	123.62
GF	2	1,170.72	275.06
LF	2	230.18	213.43
GLF	4	400.89	616.24
Error	51	444.18	291.31
Total	71		

*Significant at the 5% level.

**Significant at the 1% level.

be further considered in the Discussion section following.

Fungicide treated seed significantly outproduced untreated seed at both locations.

The germination level effects within lots and within fungicide treatment are shown in Table 27. Since the numbers of seeds planted for each lot were not equal in numbers of total seeds or live seeds, the basic number of units had to be put on a "seedling expected" basis. The numbers obtained were considerably less than expected on the basis of the 1962 data. Field conditions were much more critical in 1963 than in 1962.

Table 27. Field response of XYZ alfalfa compared within fungicide treatment and within lots at locations, correction II, 1963; data represents seedling emergence five weeks after planting

Germ. level	Ash Ave. farm				Old Agron. farm			
	X	Y	Z	Total	X	Y	Z	Total
Fungicide								
None	563[a]	551	406	1520	257	265	75	597
Captan	789	687	862	2338	552	490	462	1504
Lots								
1	456[b]	430	585	1471	245	279	220	744
2	390	408	324	1122	295	268	173	736
3	506	400	359	1265	269	208	144	621
Total	1352	1238	1268		809	755	537	

[a]Emergence from 1200 seedlings expected.

[b]Emergence from 800 seedlings expected.

Laboratory Thermogradient Plate Investigations

The seeds were planted on 35 positions along the temperature gradient on the plate. Thermocouples, placed at regular intervals, recorded temperatures at 12 of these positions (Figure 8). The straight line indicates a linear temperature gradient across the plate.

Seeds planted on the thermogradient plate were taken from the ABC and XYZ alfalfa series, and the ABC bromegrass series. The XYZ alfalfa results are presented in Figures 9, 10, and 11.

Figure 8. Temperatures recorded at 12 of the 35 available positions along the temperature gradient on the thermogradient plate

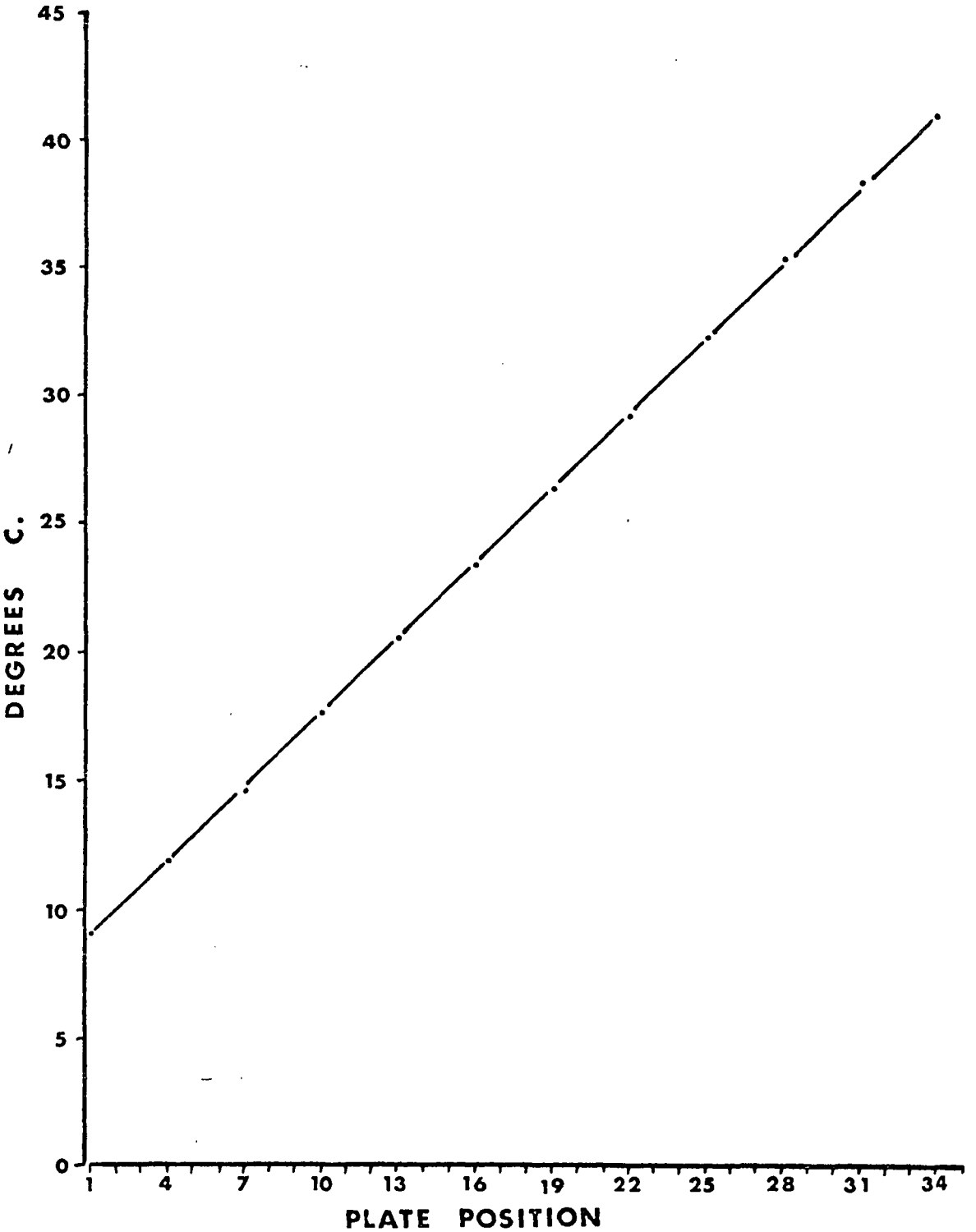

45
40
35
30
25
20
15
10
5
0
DEGREES C.
1
4
7
10
13
16
19
22
25
28
31
34
PLATE POSITION

Figure 9. Germination response to temperature gradient of XYZ alfalfa seed at indicated days after planting. Each point represents per cent germination of 540 seeds

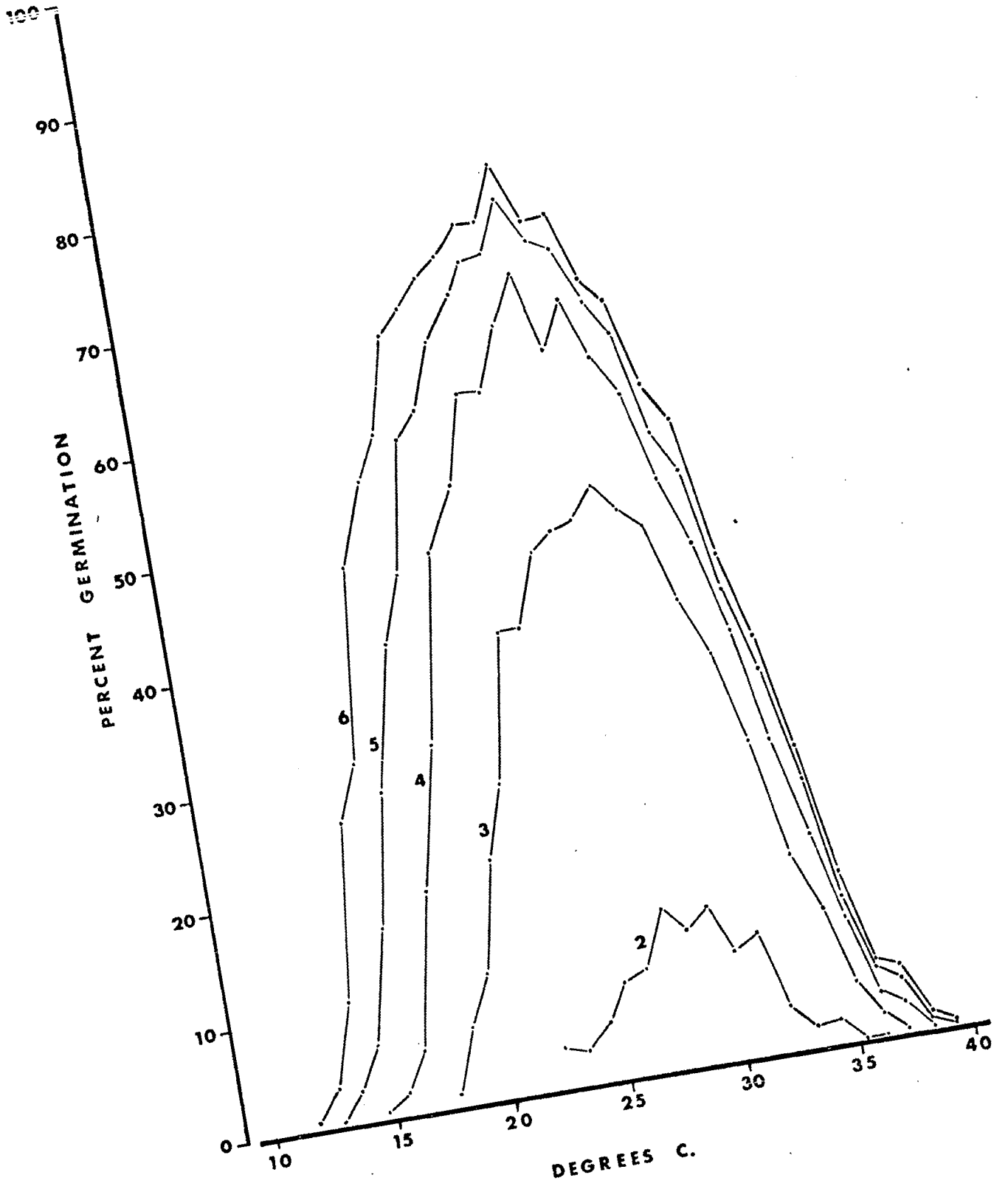

100
90
80
70
60
50
40
30
20
10
0
PERCENT GERMINATION
6
5
4
3
2
10
15
20
25
30
35
40
DEGREES C.

Figure 10. Germination responses to temperature gradient of high (x), medium (y) and low (z) germinating alfalfa seed six days after planting. Each point represents per cent germination of 180 seeds

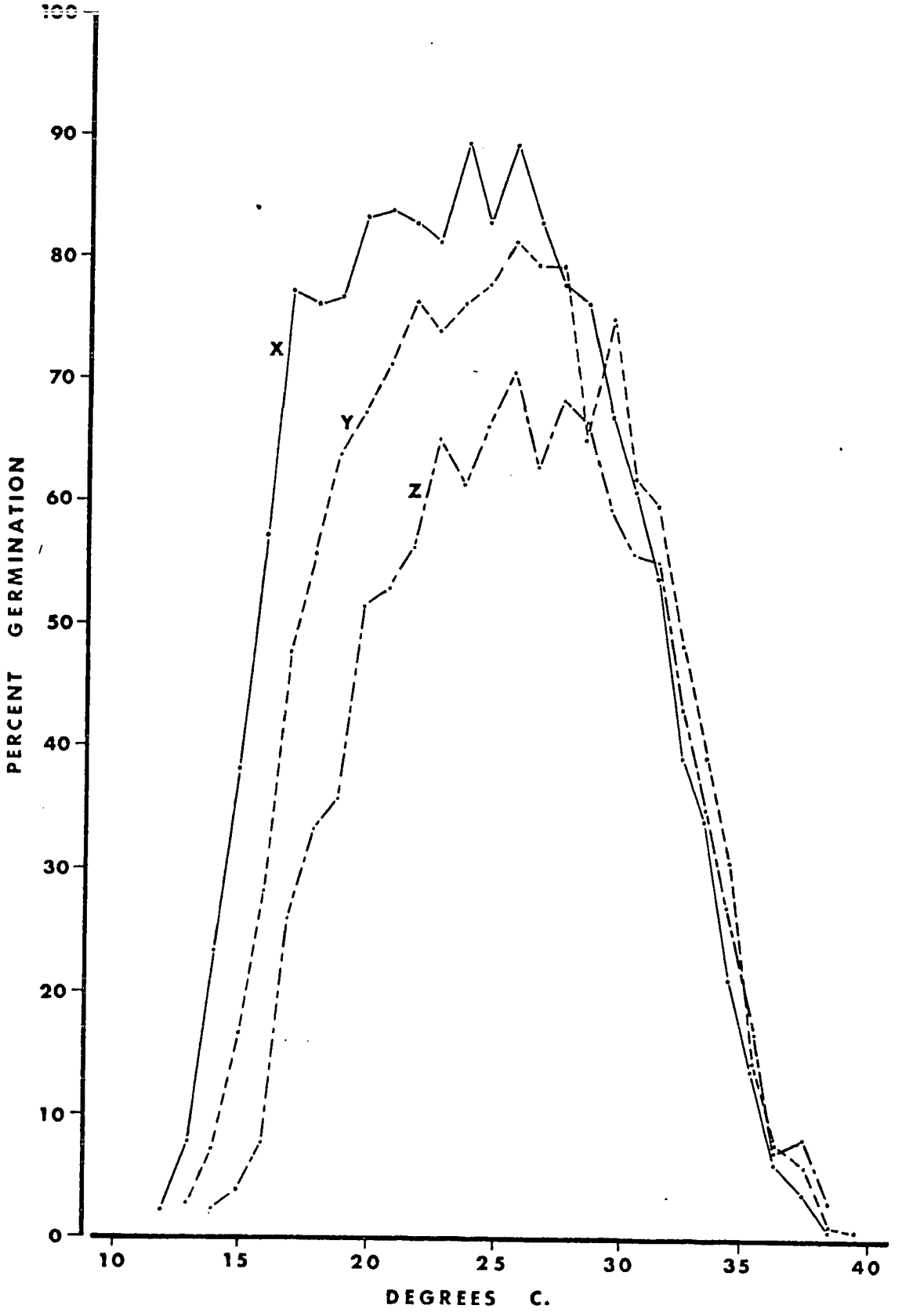

100
90
80
70
60
50
40
30
20
10
0
PERCENT GERMINATION
X
Y
Z
10
15
20
25
30
35
40
DEGREES C.

Figure 11. Germination response to temperature gradient of Captan 75 treated (T) and not-treated (N) alfalfa seeds of XYZ series six days after planting. Each point represents per cent germination of 270 seeds

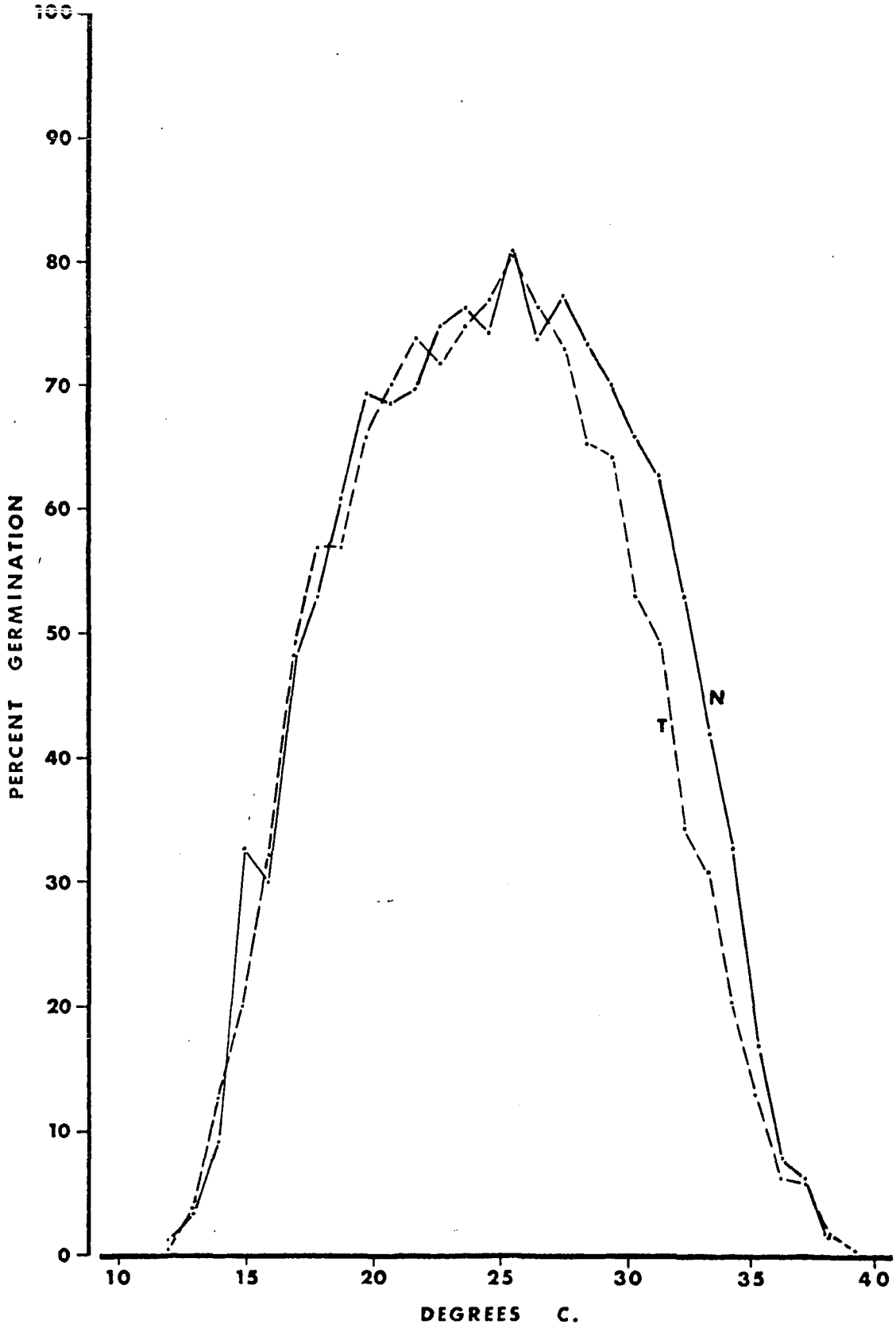

100
90
80
70
60
50
40
30
20
10
0
PERCENT GERMINATION
10
15
20
25
30
35
40
DEGREES C.
N
T

Figure 9 shows the daily progress of the overall pooled germination counts (XYZ alfalfa) starting with the second day through the sixth and final day. The maximum germination on the second day was obtained at approximately 28°C. On each subsequent day, the maximum germination shifted to a slightly lower temperature until at the final day it was obtained at approximately 25°C. This effect was also observed with the ABC alfalfa and bromegrass. However, optimum temperature for the ABC alfalfa was 2 or 3 degrees lower than XYZ alfalfa while the ABC bromegrass was characterized by a broader span of temperatures of maximum germination. The maximum and minimum temperatures at which seeds would germinate were approximately the same for all three series.

Figure 10 shows the temperature gradient effect on the three different germination levels of the XYZ alfalfa. The X germination level curve had a broader base as a result of its ability to germinate at a slightly lower temperature. The Y germination curve was intermediate in this respect and the Z had the narrowest base. All three levels had approximately the same germination limit with respect to maximum temperature (38°C). From 28°C to 38°C all three curves were concurrent. But the right sides of the curves were well separated, the higher germinating seed expressing the greater low temperature tolerance. The temperatures for maximum germination for the X, Y and Z levels were approximately 24°, 25° and 26°C

respectively. As expected, the X germination level produced the highest curve, Y intermediate and Z the lowest.

The major difference between the ABC series alfalfa and bromegrass, when compared with the XYZ alfalfa was that the lower germination curves were more directly under the higher ones. Also the bases of the three curves in each of the ABC series were about the same.

The overall relationship between Captan 75 treated seed and untreated seed is shown in Figure 11. The germination curve for the treated and untreated seeds were concurrent from the low temperature limit until maximum germination occurred at around 25°C. At temperatures above 25°C, the treated seed curve dropped off more rapidly than that of the untreated seed. This was true for all the series tested as well as for all the germination levels within these series.

Some of the seeds planted on the warmer side of the plate did not immediately germinate. They went into a quiescent state which was associated with a darkening of the seed coat color. Limited subsequent emergence resulted in seedlings with abnormally short, stubby radicles. When these quiescent seeds were moved to a cooler temperature, they promptly germinated.

Thermogradient Plate Uniformity Test

The value of the thermogradient plate as a research tool depends on the uniformity of its operation. Of particular concern is its ability to maintain the same temperature gradient across the entire plate. For example, if the temperature gradient on the right side of the plate is (or becomes) different from that on the left side, data obtained may be misleading. This experiment was set up to test the uniformity of left to right variation as well as run to run variation.

The raw data from 11 runs appears in the Appendix in Table 46. The analysis of variance in Table 28 shows runs to be significant. This variation is not necessarily an inherent fault of the plate but more likely of the temperature controls, particularly of the thermoregulator controlling the temperature of the warm water. The thermoregulator was adequately sensitive but had been used for some time prior to the performance of these tests. As a result its contact points had corroded to some extent causing increasingly frequent sticking. The effect was a gradual increasing of temperature on the warm side of the plate over the several months this test was in operation. A careful examination of the data in Table 46 in the Appendix will indicate that the germination response pattern moved gradually toward the cold side of the plate. This defect can be corrected by replacing the thermo-

Table 28. Analysis of variance for thermogradient plate uniformity test, ABC bromegrass, 8th day count, data transformed by $\sqrt{x + 0.5}$, 1963

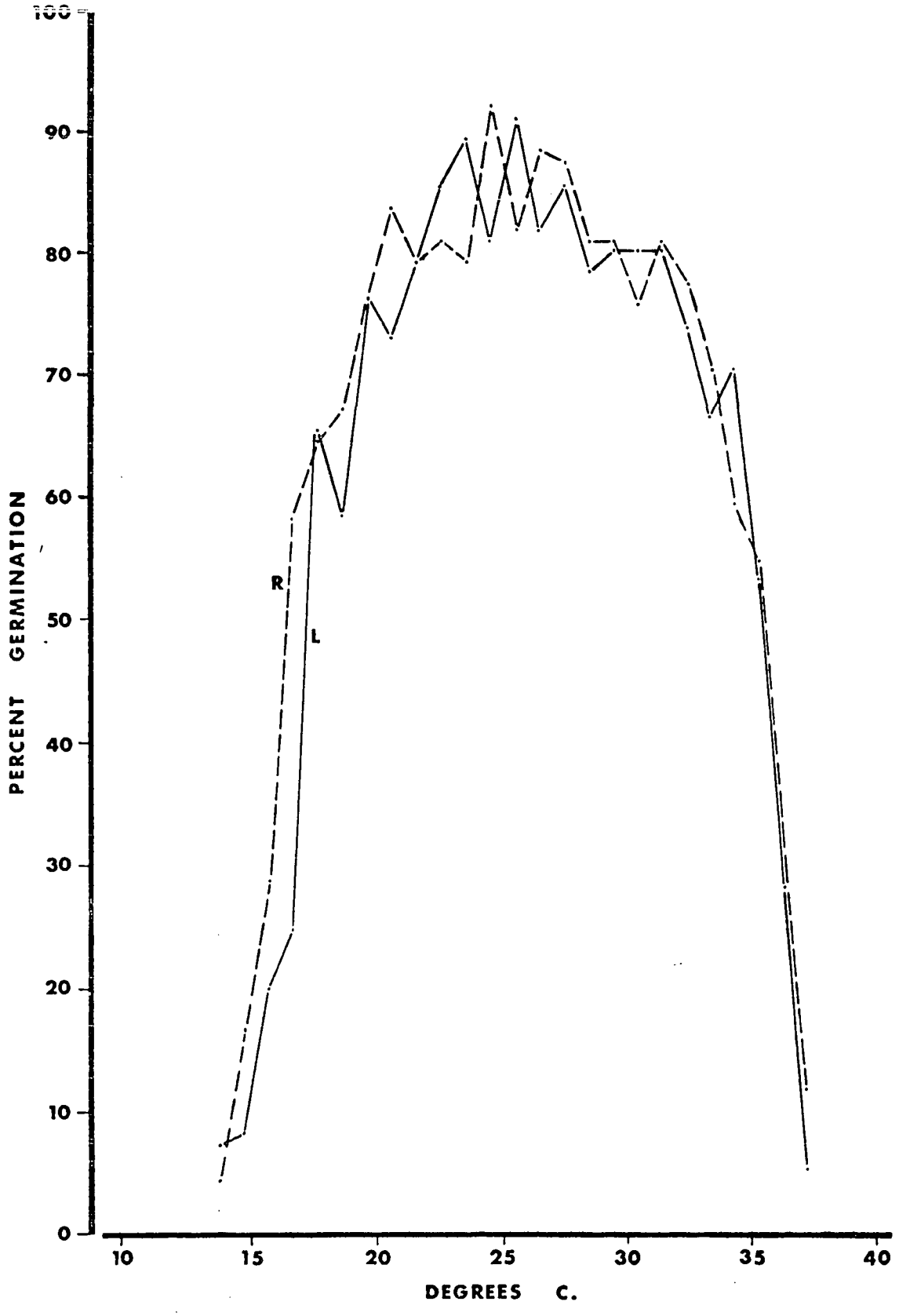
Source of variation	Degrees of freedom	Mean squares
Runs	10	0.427**
Temperatures	24	9.496**
Side	1	0.254
RT	240	0.152**
RS	10	0.111
TS	24	0.120
Error	240	0.082

**Significant at the 1% level.

regulator.

It was expected that the temperature effect would be highly significant as Figures 8, 9, 10, 11 and 12 indicate. The significant interaction between runs and temperatures, however, was not expected. This interaction was investigated and it was concluded that the small number of seeds used to compare a single temperature position between runs was responsible. Comparisons had to be made on the germination response of 20 seeds (temperature positions of left and right side pooled with 10 seeds per position per side). With respect to percentage, the failure or success of two or three seeds to germinate can have a considerable effect on variability when low numbers are used. This lack of testing sensitivity was not inherent in the other variables.

Figure 12. Germination responses to temperature gradient of bromegrass seed at the extreme right (R) and left (L) sides of the thermogradient plate eight days after planting. Each point represents per cent germination of 110 seeds


100
90
80
70
60
50
40
30
20
10
0
PERCENT GERMINATION
R
L
10
15
20
25
30
35
40
DEGREES C.

The most important factor in the analysis of variance table was the lack of significance of the side (left vs. right of plate) effects. Had this effect been significant an inherent fault in the plates design would have been indicated. Figure 12, however reveals how closely the germination response curves for both left and right sides correspond with each other.

Laboratory Time-and-Temperature Experiment

The analysis of variance for the time-and-temperature experiment follows in Table 29. (The raw data are in Table 47 of the Appendix.)

Since the raw data were composed of whole numbers that fluctuated between the limits of zero and 100, an arc sine transformation was made and the transformed data analysed. The analysis of variance of the transformed data was essentially the same as that of the raw data; therefore, the raw data analysis was used.

The grand means in terms of per cent seed germination for the three temperature effects were: 41.5 for 16°, 54.4 for 20° and 65.7 for 24°C. The grand means for germination levels were: 69.6 for the X level, 56.9 for Y and 35.0 for Z. With respect to speed of germination, the means were 19.3 two days following planting, 58.0 for four days, 67.6 for six

Table 29. XYZ alfalfa, analysis of variance of the laboratory time-and-temperature experiment, raw data, 1963

Source of variation	Degrees of freedom	Mean squares
Temperature (T)	2	15,934.8**
Reps./temps. (Error A)	6	40.6
Germination levels (G)	2	33,169.2**
Lots (L)	2	516.0**
GL	4	362.4**
TG	4	486.4**
TL	4	31.9
TLG	8	42.2
Error B	48	62.4
Days (D)	3	45,171.0**
TD	6	2,530.0**
GD	6	406.2**
LD	6	69.5*
GLD	12	25.3
TGD	12	1,352.0**
TLD	12	8.3
TGLD	24	30.6*
Error C	162	19.0

*Significant at the 5% level.

**Significant at the 1% level.

and 70.5 for eight. For lots the means were: 52.4 for lot 1, 52.8 for lot 2 and 56.4 for lot 3. The differences found within all of these effects were highly significant.

Several two-way interactions were also significant. The interaction between germination levels and lots was characterized by lot 3 germinating somewhat better at all three germination levels followed by lot 2 then by lot 1. Lots 1 and 2 were reversed in position at the Y germination level.

Generally there was no great differences between lots within any germination level.

The significant interaction between germination levels and temperatures indicates that the difference between the three germination levels diminished with increasing temperature. On the other hand the difference between temperature effects diminished with higher germination levels.

The significant interaction between temperature and days indicates that with increasing time, there is a diminishing difference between germination levels resulting from the different temperatures used and vice versa.

The general aspect of the interaction between germination levels and days is the rapid progress of germination during the first time interval with a tendency to level off in the final periods. The germination pattern for the higher germination levels indicated faster germination in the initial time periods thereby approaching maximum germination at an earlier time.

Lot 3 was superior in germination at all three levels and lot 2 was higher than lot 1 at the X and Z levels. This difference was magnified with progressing days. As a result, the interaction between lots and days was significant.

The significant three-way interaction between temperatures, germination levels and days is more complex. In general this interaction suggests that both higher tempera-

tures (within those used) and higher germination levels result in faster germination.

The four-way interaction was also significant. It essentially indicates that the statement regarding the three-way interaction could be extended to include lots within germination levels.

The analysis of variance for the germination-speed index data is shown in Table 30. (The raw data are in Table 48 in the Appendix.)

The main effects for temperature, germination levels and lots were highly significant. The grand means for the temperature effect germination-speed indices were: 14.0 at 16°C, 20.3 at 20°C and 29.3 at 24°C. The indices for germination

Table 30. XYZ alfalfa, analysis of variance of the laboratory time-and-temperature germination-speed index data, 1963

Source of variation	Degrees of freedom	Mean squares
Temperature (T)	2	1,587.8**
Reps./temps. (Error A)	6	2.1
Germination levels (G)	2	1,777.5**
TG	4	30.4**
Error B	12	5.2
Lots (L)	2	15.1**
TL	4	1.2
GL	4	19.9**
TGL	8	2.9
Error C	36	2.7

**Significant at the 1% level.

levels were: 28.7 for the X level, 22.4 for the Y level and 12.6 for the Z level. The grand mean indices for lots were: 20.8 for lot 1, 20.8 for lot 2 and 22.1 for lot 3.

The significant interaction between temperatures and germination levels is illustrated in Table 31. The factor most responsible for the significance of this interaction was the disproportionately high index of Y within the 24°C temperature.

The grand means of the germination-speed indices for lots indicate that lots 1 and 2 are the same. However, an examination of the two-way interaction between germination levels and lots reveals that lot 2 was superior to lot 1 at the X and Z germination level but at the Y level lot 1 had a higher index.

The analysis of variance for the speed index data is shown in Table 32. (The raw data are in Table 49 in the Appendix.)

Table 31. XYZ alfalfa, means, germination-speed index by temperatures and germination levels, 1963

Germ. levels[a]	Temperatures (°C)		
	16	20	24
X	20.5	28.1	37.4
Y	14.8	20.3	32.0
Z	6.8	12.6	18.4

[a]X stands for high germinating seed, Y for medium and Z for low.

Table 32. XYZ alfalfa, analysis of variance of the laboratory time-and-temperature, speed index data, 1963

Source of variation	Degrees of freedom	Mean squares
Temperature (T)	2	2,422.1**
Reps./temp. (Error A)	6	1.6
Germination levels (G)	2	875.4**
TG	4	51.8**
Error B	12	5.5
Lots (L)	2	7.2
TL	4	1.0
GL	4	10.7**
TGL	8	1.4
Error C	36	2.4

**Significant at the 1% level.

The grand means for the temperature effect with respect to speed indices were: 20.2 for 16°C, 28.2 for 20°C and 39.0 for 24°C. Those for the germination levels were: 34.5 for the X level, 29.8 for the Y and 23.1 for Z. These two main effects were highly significant. The grand means for the lot indices were all near 29.0. The speed indices were higher numerically than the germination-speed indices.

The interaction between temperatures and germination levels was significant primarily because of the disproportionately high speed index of the Y level within the 24°C temperature (as indicated in Table 33). This is the same effect that was noted for the same interaction in the germination speed index data.

Table 33. XYZ alfalfa, means, speed index by temperatures and germination levels, 1963

Germ. levels[a]	Temperatures (°C)		
	16	20	24
X	24.0	34.6	45.3
Y	20.2	27.5	41.6
Z	16.3	22.9	30.2

[a]X stands for high germinating seeds, Y for medium and Z for low.

An examination of the significant interaction between germination levels and lots, indicates a disproportionately high speed index of lot 1 at the Y germination levels throughout the various temperatures. A combined consideration of these two interactions would suggest that the speed index of lot 1 at the Y germination level at the 24°C temperature was the one single factor most responsible for significance of the interactions.

Table 34 tabulates the mean responses of several laboratory treatments and field plantings of the XYZ alfalfa seed lots. Certain laboratory treatments were excluded due to their similarity to others included.

To determine which of the laboratory treatment responses agree best with field results, correlation coefficients were computed between all possible laboratory effects and field effects. These are listed in Table 35.

Table 34. Mean responses, percentage germination or emergence of XYZ alfalfa seed lots to several laboratory and field treatments, 1963

Treatment	X[a]			Y			Z		
	1[b]	2	3	1	2	3	1	2	3
Field[c] (pooled)[d]	35.7[e]	33.0	42.8	31.3	25.1	27.4	11.3	9.7	14.2
Field (Captan)	36.3	38.6	49.1	33.3	29.0	27.1	14.3	8.0	18.6
Field (not-treat.)	35.2	27.4	36.5	29.4	21.2	27.7	8.4	11.5	9.9
16° (4 days)	66.7	78.3	85.0	43.0	22.0	35.7	5.7	8.0	2.3
16° (6 days)	79.7	82.3	89.0	70.3	61.7	70.7	27.3	31.7	26.7
16° (8 days)	83.0	85.7	89.3	74.0	69.3	77.7	40.3	43.0	42.0
20° (2 days)	31.7	31.0	31.7	13.0	7.0	6.3	0.0	1.0	0.0
20° (4 days)	76.7	78.0	83.7	70.7	64.7	74.7	41.0	41.3	44.0
20° (6 days)[f]	79.7	80.7	85.3	72.7	71.0	77.0	47.3	54.7	56.7
20° (7 days)	81.8	87.5	91.5	76.8	69.5	77.8	47.5	56.8	54.0
24° (2 days)	60.0	73.7	73.7	59.3	45.7	49.7	14.7	14.0	13.3
24° (4 days)	74.7	82.3	86.0	75.0	72.3	81.0	55.0	56.7	60.0
G-S[g] 16°	19.1	20.6	21.9	15.8	12.9	15.5	6.5	7.3	6.5
G-S 20°	27.6	27.7	29.1	21.2	19.0	20.6	11.3	13.3	13.2
G-S 24°	34.0	38.3	40.0	33.8	29.5	32.7	17.7	18.4	19.1
S[h] 16°	23.2	24.1	24.6	21.6	18.8	20.2	19.3	17.0	15.6
S 20°	34.4	34.1	34.0	29.2	26.5	26.8	23.6	22.4	22.7
S 24°	44.0	46.0	45.9	44.1	40.6	40.2	31.1	29.9	29.5

[a]X, Y and Z refer to high, medium and low germinating seeds respectively.

[b]Lots.

[c]1963 field experiments involving correction I.

[d]Treated and not-treated pooled mean response.

[e]Mean per cent germination (excluding six final rows which are indices).

[f]Official laboratory procedure.

[g]Germination-speed index.

[h]Speed index.

Table 35. XYZ alfalfa, correlation coefficients between several laboratory mean responses and field results, 1963

Field[a] (pooled)[b]			Field (Captan)			Field (not-treat.)		
Rank[c]	Lab. treat.	Corr. coef.	Rank	Lab. treat.	Corr. coef.	Rank	Lab. treat.	Corr. coef.
1	G-S[d] 16°	.973	2	G-S 24°	.959	3	20° (4 days)	.968
2	G-S 24°	.972	5	24° (2 days)	.947	1	G-S 16°	.957
3	20° (4 days)	.972	1	G-S 16°	.945	8	20° (6 days)	.956
4	16° (8 days)	.967	6	G-S 20°	.939	7	20° (7 days)	.954
5	24° (2 days)	.965	3	20° (4 days)	.935	2	G-S 24°	.949
6	G-S 20°	.964	9	S 24°[f]	.931	6	G-S 20°	.946
7	20° (7 days)[e]	.961	13	16° (6 days)	.931	9	S 24°	.944
8	20° (6 days)	.959	4	16° (8 days)	.928	5	24° (2 days)	.938
9	S[f] 24°	.957	10	16° (4 days)	.924	10	16° (4 days)	.916
10	16° (4 days)	.937	8	20° (6 days)	.922	11	24° (4 days)	.913
11	24° (4 days)	.935	12	S 20°	.920	12	S 20°	.904
12	S 20°	.934	7	20° (7 days)	.914	13	16° (6 days)	.888
13	16° (6 days)	.909	11	24° (4 days)	.914	4	16° (8 days)	.879
14	S 16°	.891	15	20° (2 days)	.881	14	S 16°	.864
15	20° (2 days)	.888	14	S 16°	.875	15	20° (2 days)	.853

[a]1963 field experiments involving correction I.

[b]Captan 75 treated and not-treated pooled.

[c]Correlation coefficients are listed in the order of highest numerical values. The last two rank columns show relationship of second and third ranking with the first.

[d]Germination-speed index.

[e]Official laboratory conditions.

[f]Speed index.

DISCUSSION

Genetic and Physiological Nature of Experimental Material

Little research has been done comparing germination levels of material of the same genetic stock. Use of such material was undertaken in the present investigations (the XYZ series). Through the employment of genetically identical seed lots across germination levels, the confounding effect of genetic diversity on germination levels was avoided.

The ABC series seed lots, on the other hand, were various genetically. They were probably also diverse physiologically within germination levels. Some of lower germinating lots (B and C) may have been relatively homogenous and "naturally" derived in much the same way as the Y and Z seed (i.e. through physiological deterioration). However, others may have represented a blend of high and low vigor seeds. Or low vigor may have been a consequence of production environment rather than a secondary result of unfavorable storage conditions.

Interpretive consideration of the data, as further elaborated in the following sections, confirms the desirability of employing these two types of experimental material in investigations of this type. The XYZ series demonstrates specifically what happens to seedling vigor, (it falls

precipitously) upon loss of germination, and without the confounding effects of genetic diversity. The vigor picture emerges less clearly in the ABC results due to the interference of other variable factors. Nevertheless, vigor is demonstrated to be of sufficient importance to indicate that, in a general sense, germination level may be employed to prognosticate probable vigor -- regardless of genetic origin and physiological history.

The Germination Level Effect in the Field

The alfalfa XYZ series planted in 1961, 1962 and the correction I plots of 1963 were all comparable in that equal numbers of live seeds were planted. Without exception, there was a significant difference in the emergence of the live seeds between germination levels favoring the higher levels. This presents fairly conclusive evidence that among the alfalfa lots used, higher levels of emergence vigor of the live seeds are associated with higher laboratory germinations. As an alfalfa lot decreases in its viability potential, one can expect a diminishing return in established seedlings from equal planting rates of live seed. The vigor of the live seeds then recedes in connection with a reduction in the percentage of living seeds.

The germination level effect for the 1961 ABC alfalfa

series was similarly significant and verified earlier work by Larsen (41). The A germination level was superior but some inconsistencies occurred between the B and C levels. This was not entirely unexpected as inconsistencies in vigor may be anticipated between genetically and physiologically diverse lots.

The results of these experiments, augmenting previous studies by Larsen (41), indicate, in a general sense, that one can expect better live seed emergence in the field from higher germinating alfalfa seed lots than from low ones. Therefore, if equal numbers of seedlings in the field are desired from two lots of different germination levels, a planting rate adjusted for germination differences alone is inadequate. Consequently, an additional adjustment (correction II) was made in 1963.

The correction II adjustment in 1963 was based on the differential field response in the 1962 plantings. The same seed lots were used. On the basis of this correction, and if weather conditions in 1963 were similar to those in 1962, it would not have been unreasonable to anticipate equal number of seedlings from all seed lots planted. This was essentially borne out at the Ash Avenue plots. However, severe soil crusting during the emergence period was encountered at the Old Agronomy farm plots. Probably seedling emergence was terminated by this crusting. The effect was twofold: first,

total numbers of seedlings were approximately half of those emerged at the Ash Avenue farm, and secondly, a distinct difference in emergence was noted for the different germination levels -- favoring the higher levels over the lower ones.

A possible explanation for this significant difference in spite of the correction II adjustments is offered by Edwards (19). He felt that seed lots usually were not homogenous with respect to vigor, but that seed lots contained seeds representing several vigor levels. He suggested that these levels might be detected (or distinguished) by the speed in which they germinate in the laboratory (this effect probably holds in the field also). Presumably, the seeds with higher vigor would germinate first and would be followed by the successively less vigorous seeds. If this is true, the differential results between germination levels at the Old Agronomy farm might be explained on the basis that the time from planting to soil crusting was not adequate for the lots with a preponderance of low vigor seeds to reach their seedling emergence potential. Only the quick germinating, high vigor seeds had time to emerge and these were found in greater proportions in the high germinating lots.

These results point up the possible merit of laboratory speed-of-germination tests as a supplementary means of identifying seed lots most apt to succeed in the field. They also suggest the desirability of research to determine whether such

tests might be applicable to other kinds of small-seeded legumes.

The relationship between laboratory germination and field live seed emergence of the bromegrass bore no relationship to that of the alfalfa. There were no significant differences in live seed emergence which correlated with germination levels. This is consistent with the earlier findings of Larsen (41). On the other hand Stahl (56) found that live seed of higher germinating bromegrass seed lots germinated somewhat better in the field than seeds of lots possessing lower viability. The differential vigor of live seeds was not, however, as striking as it was for red clover. From the present work, extending over five years (including Larsen (41)), it would seem: (a) that vigor in bromegrass seed is rather constant between germinative levels, or (b) the investigational methods were not adequate to detect vigor differences. Since a variety of experimental methods were employed over several years, the first alternative seems more reasonable. Thus the physiological characteristics of bromegrass and alfalfa seeds with respect to deterioration seem to be rather different. It has not been possible to date to formulate a hypothesis which will reasonably account for this difference.

If equivalent field stands of bromegrass derived from lots of different germination levels are to be achieved, presumably adjustments in planting rates based on laboratory

germination alone would be adequate.

Testing for Vigor in the Laboratory

In 1962 the graph in Figure 7 was studied as a possible guide for the assessment of seed vigor. If derived from a greater number of seed lots, it should properly convey the general relationship of laboratory germination to live seed emergence in the field. However, individual seed lots may be expected to deviate from the average. Thus the relationship cannot be critically applied on an individual seed lot basis. To obtain specific information in regard to the seedling vigor characteristics of individual seed lots, one must turn to supplementary vigor tests.

Isely (32) said that "...vigor may be determined in the laboratory by essentially one of two methods: (a) indirectly, by measuring certain physiological attributes or by observation of certain structural characteristics of the seed stock, (b) directly, by imitating the field conditions one wishes to assess." An indirect method of determining seed vigor involving the use of temperature was chosen for investigation in the present study. This choice was based on several considerations. Employing an indirect method, the investigator can restrict himself to a single variable, whereas if he is trying to imitate field conditions (direct method), he will become

involved with the numerous variables present in the field. A direct test, as for example the cold test for corn, is exceedingly difficult to standardize. Assuming then, an indirect approach, one has the choice between several factors which affect seed emergence. Temperature was selected for the present exploratory investigations because it is the easiest to manipulate and control. The problem was to ascertain if there are temperature conditions, supplemented by appropriate seedling evaluation techniques, that might result in seedling selection equivalent to unfavorable field conditions.

It was decided that the temperatures studied should not necessarily be restricted to those found in the field. Toole and Hollowell (61) stated that soil temperatures at or near the surface of the soil were highly variable anyway. In addition there is the possibility that other temperatures may reveal differential vigor effects as well as those found in the field. For this reason preliminary tests were made with seed using the thermogradient plate which was designed to study seed germination across the entire range of temperatures within which germination is possible.

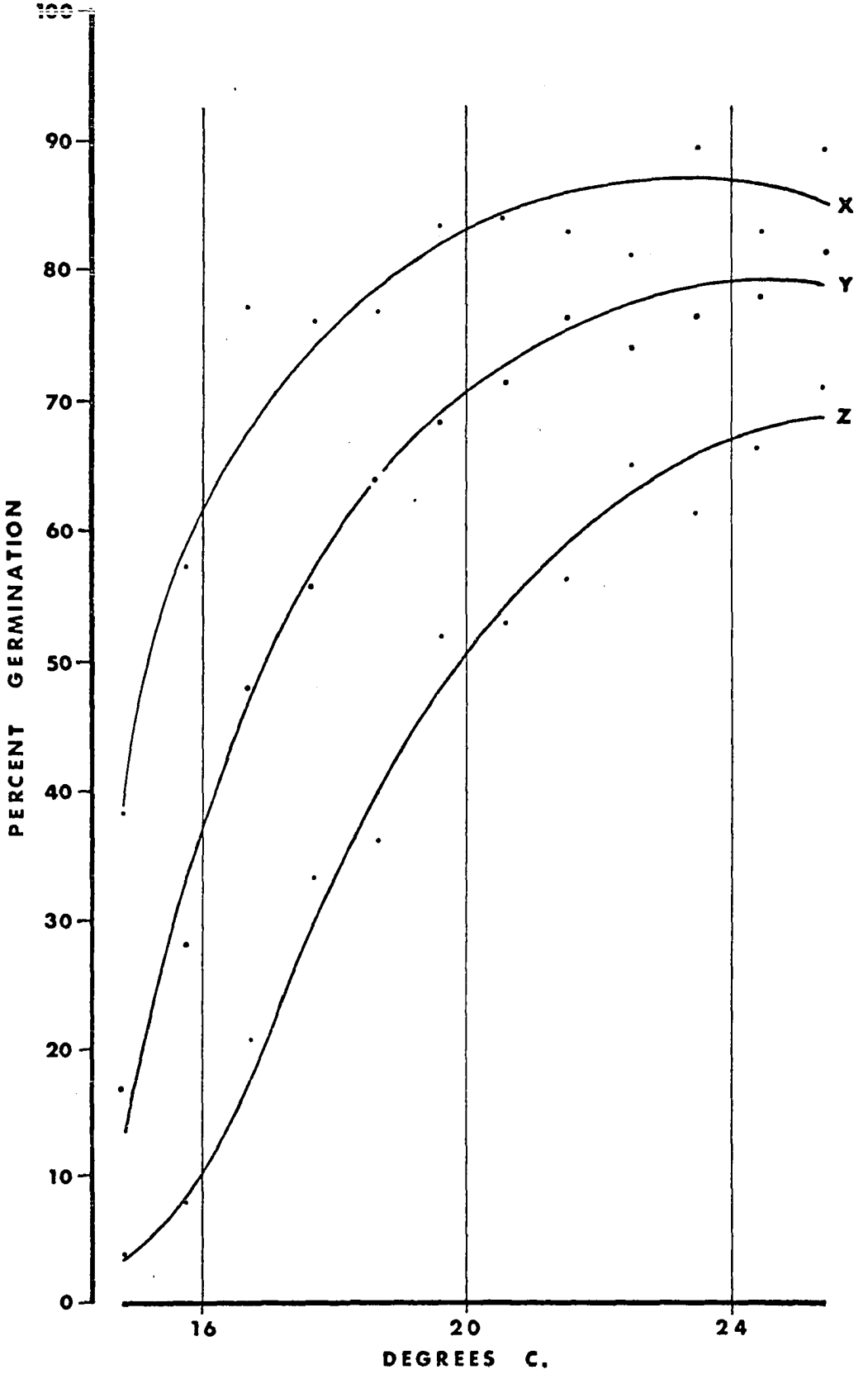
One of the objectives in the use of the thermogradient plate was the determination of a temperature that would give a differential response between germination levels as found in the field. For the XYZ alfalfa series, the portion of the gradient from 27°C up seemed to be of little significance

because there was essentially was no difference between levels in that range (Figure 10). However, differential temperature effects were noted at lower temperatures. Figure 13 presents segments of the curves. The data points are fitted with smooth curves and vertical lines drawn at the 16°, 20° and 24°C levels to facilitate comparisons. It is evident that the segments of the lines between the three curves are longer at the lower temperatures. A subsequent experiment was designed to further test differential emergence at these three temperatures ("time-and-temperature experiment").

In the time-and-temperature experiment the direct effects of germination levels, lots, temperatures and days were highly significant. However, the most interesting aspects were brought out by the significant interactions. In general, the higher germinating lots initiated germination at an earlier time and consequently reached maximum germination earlier. Subsequently, the difference between the germination levels diminished. Also emergence differences were less at the higher temperatures.

Rapidity of germination is presumably an indication of level of vigor. Another suggestion of seed vigor may be the ability of high germinating seed to germinate at cooler temperatures. However, this was not demonstrated with the ABC alfalfa or bromegrass series in the thermogradient plate studies.

Figure 13. X, Y and Z alfalfa seed germination curve segments showing relationships at three specific temperatures (16°, 20° and 24°C). Each point represents per cent germination of 180 seeds


100
90
80
70
60
50
40
30
20
10
0
PERCENT GERMINATION
16
20
24
DEGREES C.
X
Y
Z

There were some discrepancies between the thermogradient plate results and the time-and-temperature experiment. With specific reference to the 16°C temperature, the germination percentages were somewhat higher in the later study than in the first. Further work will be necessary to determine the reason for this discrepancy.

Since the laboratory studies demonstrated a definite differential in speed of germination between the various germination levels, germination-speed and speed indices were computed. The germination-speed concept included both differential germination and speed. The speed indices were corrected for differential germination and thus contrasted lots on the basis of speed only. These indices, as well as total emergence for the three germination levels and three temperatures were compared with the field emergence responses of the 1963 correction I experiment (Tables 34 and 35). The correlation coefficients of the comparisons between the laboratory tests and the pooled field results favored the following three tests: germination-speed index at 16°C, germination-speed index at 24°C and total germination at 20°C at four days. In this ranking series, the presently standard laboratory procedure (20°C for seven days) placed seventh.

This ranking sequence of test procedures fluctuated to some extent when the Captan treated and not-treated seeds were considered independently. These fluctuations were minor when

only the above enumerated top three tests were considered. However, they were considerable for some of the lower ones. For example, the correlation coefficient of the 20°C temperature at seven days (standard laboratory testing procedure) ranked seventh when the field results were pooled, twelve with treated seeds and fourth with untreated seeds.

The germination-speed indices, in general, correlated well with the field results; the speed indices did not. This suggests that tests involving vigor considerations alone may be inadequate in determining the field emergence value of seeds.

In summary, these data intimate that a test involving time and a selected temperature may give a better picture of a field potential of alfalfa seed lots than the traditional laboratory test. Further investigation is needed to substantiate this hypothesis. In such work, careful consideration must be given to time intervals in which the readings are made and to the amount of growth exhibited by the seedlings removed at the various counts.

Fungicide Effect

Some workers (1, 14, 27, 49, 52 and 53) have found no beneficial effect in the field as a result of treating alfalfa seed with fungicides. Others (11, 50) have reported increased

emergence from treated seed under specific conditions, for example, in muck soils heavily populated with seed attacking microorganisms. Still others (5, 40, 42 and 47) have reported general benefit from seed treatment. Generally, limited beneficial effect of fungicide treatment on forage grass seed has been reported.

In the present investigations, significant beneficial effects from fungicide treatment of alfalfa seed were obtained in three out of twelve experiments in 1962 and 1963. Higher seedling emergence of treated seed was also obtained in the other nine tests but the differences were not significant. In 1961 there was less consistency in this effect. In no case was harmful effects from fungicide treatment noted.

The total evidence from the three years research suggests the probability that fungicide treatment of alfalfa seeds may often provide protection to seeds between planting and emergence. Considering that the cost of the fungicide is nominal, treatment may represent worthwhile insurance against unfavorable emergence conditions.

The 1961 and 1963 field plantings of bromegrass seeds indicated no consistent beneficial effect to seed emergence from fungicide treatment.

An interesting effect of seed treatment was noted in the thermogradient plate results. Generally treated seed emerged somewhat more slowly on the warm half of the plate (25°C

upward). On the cooler half, treated and untreated seed emerged at the same rate. Perhaps at the warmer temperatures, the fungicide had an inhibiting or toxic effect on the seeds under the germination conditions on the plate. Such an effect may not exist in the field due to the buffering or dilution influence of the soil on the fungicide.

SUMMARY AND CONCLUSIONS

The purpose of this study was to assess the vigor differences between germination levels of Ranger alfalfa and Lincoln bromegrass seed lots in the field and laboratory.

For each crop kind, two types of experimental material were used: different germination levels also differing genetically (ABC series); and germination levels which were genetically identical (XYZ series). The ABC series tested vigor differences between different lots of various laboratory germinations; the XYZ set tested loss of vigor associated with loss of viability within a lot.

Field Studies

In 1961 and 1962, the seed lots were planted in the field on an equal live seed basis. For the two alfalfa series, live seed from higher germinating lots generally produced more seedlings in the field. This effect was more consistent with the XYZ material than with the ABC. The erratic response of the ABC material may have been due to the confounding effect of genetic diversity.

In 1963, two plantings of the 1962 XYZ alfalfa seed lots were made. The first planting was corrected for equal numbers of live seed (correction I); the second was further corrected

for the differential ability of the seed lots to produce seedlings in the field as demonstrated in 1962 (correction II). Emergence of correction I plantings was essentially as in previous years. The correction II procedure at one planting location resulted in equal numbers of seedlings (within statistical limits) from the different germination levels; thus the prediction value of the correction was in this instance validated. However, at the other location, a differential response favoring the higher germinating levels occurred (despite the corrections). Possibly this was due to early termination of emergence following soil crusting, the result being a distinct stand advantage in favor of the rapid germinating seed lots.

In general, as an alfalfa seed lot decreases in viability, one can expect a diminishing return in established seedlings in the field from equal plantings of live seed. This statement can be extended in a general sense to comparisons between two lots of different laboratory germinations.

In the bromegrass field tests, no noteworthy difference in field seedling emergence of live seeds from lots of different laboratory germination levels was found.

Laboratory Studies

Temperature and time were the variables investigated in the laboratory studies. An assay was made of germination responses from 12-38°C employing the thermogradient plate. From these studies the temperatures of 16°, 20° and 24°C were selected for a more critical investigation using standard seed germinators.

This subsequent study (time-and-temperature experiment) involved the XYZ series alfalfa seed lots germinated at the three temperatures, emergence determined at four time intervals. In general, the higher germinating lots initiated germination earlier and achieved total germination more quickly. However, the differences between the germination levels diminished with both increasing time and temperature.

Germination-speed and speed indices at the various temperatures used were calculated. These indices and the raw germinations data were compared with the field emergence of the same lots. Correlation coefficients indicated that tests which corresponded best with field emergence were: the germination-speed index at 16°C and the germination-speed index at 24°C. Tests of alfalfa using either of these procedures should provide considerably more significant information concerning field value than the standard laboratory evaluation. Time-temperature procedures may be applicable to other crop

kinds.

Fungicide Effects

The experiments indicated that, in alfalfa seed, fungicide treatment may provide added protection to the seed between planting and emergence in the field. There were no consistent beneficial effects on seedling emergence of bromegrass from fungicide treatment.

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APPENDIX

Table 36. ABC alfalfa, five week field seedling counts at Beaconsfield and Kanawha, 1961

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
A	1	N	77[d]	125	233	435	259	153	206	618
		T	148	160	130	438	173	162	162	497
	2	N	185	80	164	429	198	146	148	492
		T	128	154	242	524	147	108	198	453
	3	N	231	89	173	493	144	132	158	434
		T	213	88	86	387	196	133	269	598
B	4	N	172	40	83	295	171	189	180	540
		T	111	148	103	362	104	156	262	522
	5	N	105	52	70	227	123	93	61	277
		T	114	66	110	290	172	87	198	457
	6	N	137	50	65	252	188	145	128	461
		T	141	102	112	355	155	93	106	354
C	7	N	83	46	147	276	133	103	133	369
		T	139	60	130	329	125	75	261	461
	8	N	94	46	135	275	59	119	141	319
		T	137	39	96	272	109	53	132	294
	9	N	77	65	119	261	95	110	183	388
		T	168	84	75	327	69	77	103	249

[a]A, B and C designates high, medium and low germinating seed respectively.

[b]No genetic identity between the nine lots used.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 37. XYZ alfalfa, five week field seedling counts at Beaconsfield and Kanawha, 1961

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
X	1	N	139[d]	96	99	334	201	182	217	600
		T	99	107	112	318	168	110	155	433
	2	N	173	93	81	347	159	71	174	404
		T	129	132	86	347	288	64	176	528
	3	N	160	75	156	391	170	110	281	561
		T	97	140	140	377	173	163	215	551
Y	1	N	81	58	90	229	126	65	72	263
		T	107	65	76	248	140	99	144	383
	2	N	103	46	31	180	88	67	135	290
		T	84	65	120	269	91	162	86	339
	3	N	86	92	87	265	76	93	85	254
		T	171	113	58	342	179	163	171	513
Z	1	N	128	100	71	299	132	94	86	312
		T	91	94	120	305	138	77	108	323
	2	N	79	61	107	247	67	63	101	231
		T	89	96	108	293	96	68	82	246
	3	N	87	46	81	214	74	65	84	223
		T	120	92	121	333	78	46	107	231

[a]X, Y and Z designates high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 38. ABC bromegrass, five week field seedling counts at Beaconsfield and Kanawha, 1961

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
A	1	N	115[d]	176	217	508	158	108	209	475
		T	169	183	188	540	246	121	156	523
	2	N	83	166	151	400	167	211	171	549
		T	162	158	204	524	199	147	95	441
	3	N	192	142	173	507	284	116	175	575
		T	97	178	201	476	168	94	237	499
B	4	N	145	193	137	475	119	138	101	358
		T	89	114	132	335	54	98	133	285
	5	N	197	158	134	489	227	116	135	478
		T	89	141	161	391	120	234	147	501
	6	N	149	155	186	490	170	201	137	508
		T	111	282	84	477	177	112	111	400
C	7	N	148	193	229	570	176	168	263	607
		T	171	195	222	588	140	152	241	533
	8	N	96	104	89	289	140	107	289	536
		T	124	117	153	394	119	125	114	358
	9	N	145	70	125	340	81	136	157	374
		T	107	84	77	268	143	89	216	448

[a]A, B and C designates high, medium and low germinating seed respectively.

[b]No genetic identity between the nine lots used.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 39. XYZ bromegrass, five week field seedling counts at Beaconsfield and Kanawha, 1961

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
X	1	N	120[d]	158	139	417	144	130	227	501
		T	137	113	139	389	151	105	171	427
	2	N	165	143	104	412	189	78	197	464
		T	54	155	129	338	185	109	130	424
	3	N	136	174	146	456	160	200	187	547
		T	173	175	155	503	137	200	183	520
Y	1	N	179	140	101	420	193	150	148	491
		T	128	154	144	426	175	221	216	612
	2	N	171	114	148	433	178	144	100	422
		T	93	174	209	476	203	106	38	347
	3	N	153	157	106	416	109	106	153	368
		T	178	115	86	379	133	92	112	337
Z	1	N	119	129	155	403	215	144	103	462
		T	112	110	120	342	140	265	147	552
	2	N	127	146	125	398	136	178	215	529
		T	185	130	71	386	102	124	315	541
	3	N	194	162	127	483	162	132	132	426
		T	209	181	135	525	112	97	177	386

[a]X, Y and Z designates high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 40. ABC alfalfa, five week field seedling counts at Beaconsfield and Kanawha, 1962

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
A	1	N	185[d]	145	205	535	213	193	188	594
		T	246	266	137	649	158	187	193	538
	2	N	223	138	119	480	179	161	200	540
		T	132	240	33	405	191	212	232	635
	3	N	151	196	114	461	185	219	203	607
		T	189	210	101	500	218	189	226	633
B	4	N	113	194	150	457	179	178	145	502
		T	134	95	167	396	178	207	117	502
	5	N	95	175	84	354	187	184	170	541
		T	101	161	204	466	205	185	231	621
	6	N	65	48	62	175	137	92	129	358
		T	94	153	98	345	184	190	211	585
C	7	N	190	100	155	445	175	162	170	507
		T	197	175	50	422	185	187	200	572
	8	N	122	70	158	350	220	147	207	574
		T	171	149	172	492	178	203	186	567
	9	N	138	89	89	316	158	151	208	517
		T	163	124	106	393	204	174	170	548

[a]A, B and C designates high, medium and low germinating seed respectively.

[b]No genetic identity between the nine lots used.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 41. XYZ alfalfa, five week field seedling counts at Beaconsfield and Kanawha, 1962

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
X	1	N	192[d]	100	82	374	164	161	207	532
		T	188	267	126	581	207	212	195	614
	2	N	111	146	146	403	172	180	212	564
		T	144	168	196	508	175	217	231	623
	3	N	116	162	184	462	192	220	201	613
		T	223	137	187	547	201	214	192	607
Y	1	N	101	170	107	378	140	173	168	481
		T	153	170	135	458	216	194	197	607
	2	N	97	110	83	290	179	206	137	522
		T	201	231	116	548	199	186	185	570
	3	N	135	97	75	307	163	168	165	496
		T	200	85	182	467	196	180	185	561
Z	1	N	64	51	65	180	95	176	152	423
		T	137	92	88	317	168	167	197	532
	2	N	42	79	39	160	123	167	132	422
		T	121	111	150	382	167	192	161	520
	3	N	98	67	63	228	142	148	188	478
		T	128	173	119	420	161	187	191	539

[a]X, Y and Z designates high, medium and low germinating seeds respectively.

[b]Similarly numbered lots of various germination levels are genetically identical.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 42. ABC bromegrass, five week field seedling counts at Beaconsfield and Kanawha, 1962

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield				Kanawha			
			1	2	3	Total	1	2	3	Total
A	1	N	285[d]	264	105	654	175	168	129	472
		T	199	232	135	566	179	231	211	621
	2	N	224	179	175	578	164	187	129	480
		T	247	168	210	625	123	228	146	497
	3	N	184	221	190	595	176	144	104	424
		T	253	274	193	720	170	162	202	534
B	4	N	192	203	152	547	181	138	147	466
		T	289	176	208	673	168	219	167	554
	5	N	160	214	202	576	134	147	115	396
		T	292	231	140	663	159	193	162	514
	6	N	245	301	104	650	168	170	165	503
		T	312	209	311	832	218	158	185	561
C	7	N	119	233	223	575	227	157	122	506
		T	228	154	213	595	183	164	179	526
	8	N	168	170	143	481	135	155	162	452
		T	135	156	142	433	207	217	140	564
	9	N	152	185	113	450	171	190	105	466
		T	160	246	134	540	120	164	104	388

[a]A, B and C designates high, medium and low germinating seed respectively.

[b]No genetic identity between the nine lots used.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 43. XYZ bromegrass, five week field seedling counts at Beaconsfield and Kanawha, 1962

Germ. level[a]	Lot[b]	Fung. trt.[c]	Beaconsfield 1	2	3	Total	Kanawha 1	2	3	Total
X	1	N	139[d]	171	110	420	140	93	133	366
		T	226	115	155	496	143	159	130	432
	2	N	205	229	216	650	195	184	178	557
		T	143	226	201	570	156	212	148	516
	3	N	179	264	130	573	140	194	197	531
		T	209	247	130	586	213	167	190	570
Y	1	N	135	303	152	590	199	220	177	596
		T	265	174	212	651	216	179	143	538
	2	N	236	284	204	724	177	107	145	429
		T	230	209	224	663	156	163	186	505
	3	N	159	166	260	585	123	84	201	408
		T	331	215	153	699	184	236	155	575
Z	1	N	263	155	162	580	197	173	165	535
		T	280	132	127	539	191	186	170	547
	2	N	153	185	133	471	161	141	195	497
		T	143	302	153	598	187	210	189	586
	3	N	142	204	222	568	182	152	122	456
		T	189	219	182	590	169	129	135	433

[a]X, Y and Z designates high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 250 live seeds planted.

Table 44. XYZ alfalfa, five week field seedling counts at two sites, Correction I, 1963

Germ. level[a]	Lot[b]	Fung. trt.[c]	Old Agron. farm					Ash Ave. farm				
			1	2	3	4	Total	1	2	3	4	Total
X	1	N	68[d]	17	8	9	102	32	48	53	39	172
		T	52	30	10	34	126	28	40	50	59	177
	2	N	37	7	19	22	85	30	25	37	33	125
		T	56	64	26	29	175	28	38	48	62	176
	3	N	93	33	45	14	185	39	32	34	54	159
		T	35	4	19	33	91	54	54	44	62	214
Y	1	N	22	0	23	6	51	34	30	52	37	153
		T	48	32	22	18	120	58	35	45	35	173
	2	N	15	17	1	3	36	15	36	35	36	122
		T	32	29	3	22	86	44	46	47	30	167
	3	N	25	0	2	11	38	15	40	47	41	143
		T	71	48	11	13	143	28	42	19	51	140
Z	1	N	18	10	26	5	59	17	19	12	23	71
		T	50	21	11	32	114	37	26	33	25	121
	2	N	9	3	13	5	30	5	10	27	39	81
		T	61	31	4	30	126	17	25	13	1	56
	3	N	23	1	7	2	33	28	10	31	4	73
		T	49	5	17	3	74	32	30	44	32	138

[a]X, Y and Z designates high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent numbers of seedlings emerged from 100 live seeds planted.

Table 45. XYZ alfalfa, five week field seedling counts at two sites, Correction II, 1963

Germ. level[a]	Lot[b]	Fung. trt.[c]	Old Agron. farm 1	2	3	4	Total	Ash Ave. farm 1	2	3	4	Total
X	1	N	50[d]	14	44	9	117	65	59	81	28	233
		T	68	10	43	7	128	64	73	49	37	223
	2	N	49	2	5	18	74	38	39	21	36	134
		T	89	49	42	41	221	54	65	53	84	256
	3	N	11	18	24	13	66	17	62	58	59	196
		T	88	26	73	16	203	88	73	65	84	310
Y	1	N	27	13	25	13	78	33	70	67	45	215
		T	112	44	39	6	201	63	76	34	42	215
	2	N	43	22	12	7	84	35	70	55	9	169
		T	96	21	38	29	184	81	28	63	67	239
	3	N	56	31	13	3	103	38	28	53	48	167
		T	67	21	3	14	105	84	60	11	78	233
Z	1	N	16	3	2	3	24	16	35	56	87	194
		T	117	5	49	25	196	87	127	59	118	391
	2	N	12	10	1	1	24	10	19	8	61	98
		T	84	44	18	3	149	35	52	53	86	226
	3	N	24	1	1	1	27	39	39	21	15	114
		T	78	16	12	11	117	48	51	62	84	245

[a]X, Y and Z designates high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]N indicates seeds not treated and T indicates seeds treated with Captan 75.

[d]Entries represent actual number of seedlings from 100 expected.

Table 46. Thermogradient plate uniformity test using bromegrass seed, data taken at 35 loci at extreme left and right side of plate, eight day count, 1963

Plate position	Left												Right											
	1[a]	2	3	4	5	6	7	8	9	10	11	Total	1	2	3	4	5	6	7	8	9	10	11	Total
1[b]-5 no germination																								
6	0[c]	0	0	0	0	0	1	0	0	4	3	8	0	0	0	0	0	0	0	0	0	2	3	5
7	1	0	0	1	1	0	1	1	1	3	0	9	0	0	0	3	2	1	2	3	1	1	5	18
8	3	1	2	2	1	0	2	1	0	4	6	22	2	2	1	2	4	1	2	1	3	7	6	31
9	4	1	2	4	3	7	5	3	5	7	6	47	3	4	6	5	8	6	3	6	6	10	7	64
10	6	4	6	9	8	4	6	7	8	7	7	72	4	6	7	7	8	5	8	4	6	9	7	71
11	5	6	5	5	8	4	5	8	3	7	8	64	4	8	8	7	6	7	4	7	7	8	8	74
12	9	8	10	7	7	6	9	7	8	8	5	84	9	7	5	8	8	7	8	8	9	6	9	84
13	5	6	8	9	7	8	9	7	7	8	6	80	9	6	10	9	8	9	8	8	8	10	7	92
14	6	8	10	10	8	8	7	7	7	7	9	87	6	9	10	9	9	8	9	6	7	7	7	87
15	9	8	9	9	6	7	8	10	8	10	10	94	9	9	9	7	8	8	10	9	6	9	5	89
16	8	10	9	10	10	9	9	9	9	8	7	98	8	8	8	9	7	7	7	8	10	7	8	87
17	9	8	8	6	7	7	9	8	10	8	9	89	10	8	10	9	9	9	9	10	9	8	10	101
18	10	10	10	9	8	9	9	9	9	8	9	100	10	7	8	9	9	10	8	7	7	8	7	90
19	7	6	8	9	10	9	9	8	9	9	6	90	8	10	10	8	8	10	10	9	7	8	9	97
20	10	9	10	8	7	9	7	9	9	9	7	94	8	10	9	9	9	9	10	7	8	8	9	96
21	8	6	9	8	8	6	8	9	9	8	7	86	10	8	5	10	9	7	7	8	9	9	7	89

[a]Numbers indicate replicates, i.e. "runs" extending over the period from Feb. 8 to June 24, 1963.

[b]The number "1" position possesses the lowest temperature (9°C) on the cool side of the plate; number "35" possesses the warmest temperature (42°C) on the opposing side.

[c]Entries represent seeds germinated from 10 planted.

Table 46 (Continued).

Plate position	Left												Right											
	1[a]	2	3	4	5	6	7	8	9	10	11	Total	1	2	3	4	5	6	7	8	9	10	11	Total
22	9	8	7	8	9	9	6	10	9	6	7	88	8	8	8	10	7	6	8	8	9	9	8	89
23	7	6	10	8	10	8	8	10	8	6	7	88	6	10	9	9	7	9	6	3	10	7	7	83
24	9	8	9	8	10	3	8	8	10	8	7	88	3	8	9	6	10	10	10	7	9	8	9	89
25	7	9	9	8	10	3	6	8	7	5	9	81	6	7	10	9	8	8	9	7	7	7	7	85
26	5	7	7	5	8	8	7	6	7	6	7	73	5	6	9	9	8	7	5	8	6	8	6	77
27	7	8	8	8	8	7	5	6	7	7	6	77	6	2	7	5	8	6	5	6	9	5	6	65
28	3	5	7	4	7	6	7	5	2	7	6	59	2	3	8	7	5	8	4	7	7	5	4	60
29	1	2	4	2	5	3	1	4	3	0	6	31	0	0	7	1	6	3	0	4	7	1	2	31
30	0	0	1	0	0	0	0	1	4	0	0	6	0	0	5	0	0	1	0	2	5	0	0	13
31-35	no germination																							

Table 47. XYZ alfalfa, laboratory time-and-temperature experiment data, 1963

Germ. level[a]	Lot[b]	Rep.	16°C				20°C				24°C			
			2[c]	4	6	8	2	4	6	8	2	4	6	8
X	1	1	0[d]	73	81	83	34	81	84	85	61	71	74	74
		2	0	51	78	81	31	75	79	80	65	79	79	80
		3	0	76	80	85	30	74	76	76	54	74	75	78
	2	1	0	76	80	86	22	76	77	77	67	82	83	83
		2	0	75	82	86	25	77	81	82	78	87	87	87
		3	0	84	85	85	46	81	84	84	66	78	80	80
	3	1	0	83	87	87	34	80	82	82	76	85	86	87
		2	0	83	91	92	26	86	88	89	69	88	90	90
		3	0	89	89	89	35	85	86	87	76	85	86	86
Germ. level means			0.0	76.7	83.7	86.0	31.5	79.4	81.9	82.4	69.1	81.0	82.2	82.8
Y	1	1	0	46	69	71	10	74	75	75	50	71	74	74
		2	0	37	78	81	16	67	69	69	67	76	76	76
		3	0	53	64	70	13	71	74	75	61	78	80	80

[a]X, Y and Z designates high, medium and low germinating seeds.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]Numbers indicate days after planting.

[d]Entries represent cumulative numbers of seeds germinated from 100 planted.

Table 47 (Continued).

Germ. level[a]	Lot[b]	Rep.	16°C				20°C				24°C			
			2[c]	4	6	8	2	4	6	8	2	4	6	8
	2	1	0	40	67	70	12	67	75	76	43	74	75	75
		2	0	15	61	76	3	63	68	69	45	75	76	76
		3	0	11	57	66	6	64	70	70	49	68	68	68
	3	1	0	42	71	76	5	75	78	78	40	76	76	77
		2	0	54	73	76	8	74	76	76	49	85	85	85
		3	0	11	68	81	6	75	77	77	60	82	82	82
Germ. level means			0.0	23.6	67.6	73.7	8.7	70.0	73.6	73.9	51.6	76.1	76.9	77.0
Z	1	1	0	1	15	31	0	43	47	48	20	52	54	54
		2	0	9	34	45	0	39	46	48	10	56	60	60
		3	0	7	33	45	0	41	49	50	14	57	58	58
	2	1	0	3	24	36	1	51	61	66	20	65	70	70
		2	0	11	28	39	1	37	54	57	9	50	55	56
		3	0	10	43	54	1	36	49	56	13	55	58	58
	3	1	0	0	20	37	0	43	56	58	11	53	59	59
		2	0	5	34	39	0	42	56	59	11	60	66	66
		3	0	2	26	50	0	46	58	59	18	67	69	69
Germ. level means			0.0	5.3	28.5	41.8	0.3	42.1	52.9	55.7	14.0	57.2	61.0	61.1

Table 48. XYZ alfalfa, laboratory time-and-temperature experiment germination-speed index data, 1963

Temp.	Germ. level[a]	Lot[b]	Replicates 1	2	3	Lot total	Germ. total
16°C	X	1	19.7[c]	17.5	20.2	57.4	
		2	20.3	20.3	21.1	61.7	
		3	21.3	22.1	22.2	65.6	184.7
	Y	1	15.5	16.3	15.7	47.5	
		2	14.8	12.6	11.4	38.8	
		3	15.9	16.9	13.8	46.6	132.9
	Z	1	4.5	7.6	7.5	19.6	
		2	5.7	6.8	9.3	21.8	
		3	5.4	6.6	7.5	19.5	60.9
20°C	X	1	29.3	27.2	26.3	82.8	
		2	24.6	26.2	32.2	83.0	
		3	28.8	28.4	30.2	87.4	253.2
	Y	1	21.1	21.0	21.6	63.7	
		2	21.1	17.4	18.5	57.0	
		3	20.5	20.8	20.5	61.8	182.5
	Z	1	11.4	11.0	11.6	34.0	
		2	15.2	12.6	12.1	39.9	
		3	13.0	13.1	13.6	39.7	113.6
24°C	X	1	33.5	36.1	32.4	102.0	
		2	37.3	41.2	36.3	114.8	
		3	40.3	39.5	40.3	120.1	336.9
	Y	1	30.7	35.7	35.0	101.4	
		2	29.3	30.1	29.2	88.6	
		3	29.1	33.5	35.5	98.1	288.1
	Z	1	18.3	17.1	17.8	53.2	
		2	22.0	15.6	17.5	55.1	
		3	17.0	18.7	21.5	57.2	165.5

[a]X, Y and Z designate high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]Entries are derived from data in Table 47 using germi-

Table 49. XYZ alfalfa, laboratory time-and-temperature experiment speed index data, 1963

Temp.	level[a]	Lot[b]	Replicates 1	2	3	Lot total	Germ. total
16°C	X	1	23.9[c]	21.8	23.9	69.6	
		2	23.7	23.7	24.9	72.3	
		3	24.6	24.1	25.0	73.7	215.6
	Y	1	21.9	20.3	22.6	64.8	
		2	21.2	17.7	17.5	56.4	
		3	21.0	22.4	17.1	60.5	181.7
	Z	1	14.8	17.3	16.8	48.9	
		2	16.0	17.8	17.3	51.1	
		3	14.7	17.2	15.0	46.9	146.9
20°C	X	1	34.5	34.1	34.6	103.2	
		2	32.0	32.0	38.4	102.4	
		3	35.2	32.0	34.8	102.0	307.6
	Y	1	28.2	30.5	28.8	87.5	
		2	27.9	25.3	26.4	79.6	
		3	26.3	27.4	26.7	80.4	247.5
	Z	1	24.0	23.3	23.4	70.7	
		2	23.1	22.3	21.9	67.3	
		3	22.7	22.4	23.1	68.2	206.2
24°C	X	1	45.2	45.1	41.7	132.0	
		2	45.1	47.4	45.4	137.9	
		3	46.6	44.0	47.0	137.6	407.5
	Y	1	41.5	47.0	43.8	132.3	
		2	39.2	39.7	43.0	121.9	
		3	37.8	39.4	43.3	120.5	374.7
	Z	1	33.9	28.6	30.9	93.4	
		2	31.5	28.0	30.2	89.7	
		3	28.8	28.4	31.3	88.5	271.6

[a]X, Y and Z designate high, medium and low germinating seeds respectively.

[b]Similarly numbered lots at various germination levels are genetically identical.

[c]Entries are derived from data in Table 47 using speed index formula indicated in Materials and Methods section of text.